THE RELATIONSHIP BETWEEN HIGH FAT FEEDING, INSUILN RESISTANCE, AND TNF-α GENE EXPRESSION IN GROWING RATS

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LIST OF ABBREVIATIONS

BMI	Body Mass Index
BW	Body weight
%BF	Percentage body fat
DM	Diabetes mellitus
DXA	Dual X-Ray Absorptiometry
FFA	Free Fatty Acid
GLUT4	Glucose Transporter 4
HF	High fat diet
HFS	High fat soy bean oil diet
HFT	High fat tallow diet
IR	Insulin resistance/insulin receptor
IRS-1	Insulin receptor substrate 1
MFS	Moderate fat soy bean oil diet
LPL	Lipoprotein Lipase
OGTT	Oral glucose tolerance test/ Oral glucose challenge
RT-PCR	Real Time Polymerase Chain Reaction
SAS	Statistical Analysis System
TNF-α	Tumor Necrosis Factor - alpha

CHAPTER I

INTRODUCTION

Although obesity has been identified as an epidemic in the United States for nearly 2 decades, the number of obese individuals continues to grow to this day (1). Between 1971 and 2000, the prevalence of obesity in the U.S. increased from 14.5% to 30.9% (2). Healthy People 2010 (3) estimate that by 2010 more than half of the U.S. adult population will be overweight or obese. Currently, about 65% of U.S. adults are overweight (1). Obesity is associated with unhealthy eating habits and sedentary lifestyles. These nutritional and environmental factors have been shown to be the primary contributors to mortality related to obesity and the development of chronic disease in the U.S. (3).

Overweight and obesity are associated with an increased risk of serious health conditions and diseases such as: heart disease, high blood pressure, high blood cholesterol concentrations, certain types of cancer, and type II diabetes. Excess body fat, predominantly visceral adiposity, is linked to insulin resistance which plays a major role the development of type II diabetes (2).

Insulin resistance is defined as reduced tissue sensitivity or responsiveness to insulin leading to decreased cellular uptake of glucose and impaired glucose tolerance. A common indicator of insulin resistance is higher than normal insulin concentrations in the

blood in response to a glucose challenge or food intake. In the early stage of the insulin resistant state, hyperglycemia is not observed due to the hypersecretion of insulin by the beta cells of the pancreas which results in normal blood glucose concentrations. Insulin resistance is characterized by an abnormally high blood insulin concentration in proportion to the glucose concentration in the blood. Uncorrected insulin resistance eventually leads to hyperglycemia which is a diagnostic criterion for type II diabetes. The pathogenesis of type II diabetes involves the development of insulin resistance leading to relative insulin deficiency and impaired glucose tolerance (4). Left unchecked, overt diabetes, its related clinical symptoms and associated chronic disorders occur.

Currently, the US population consumes an average dietary fat intake of 39%, which is above the U.S. government's (5) and the American Diabetes Association's (6) recommendation of a total fat intake of no more than 30% of total daily energy intake (2). High fat (HF) diets in humans and rats have been found to increase body weight, percent body fat, impair glucose tolerance (7,8), and increase tumor necrosis factor –α (TNF-α) mRNA expression related to insulin resistance (9).

TNF-α, a cytokine produced primarily by macrophages and adipocytes, affects body glucose and lipid metabolism (10,11), is elevated in rodent models of obesity (12-16) and in human subjects (9), and, is associated with hyperinsulinemia (7,9). TNF-α inhibits insulin receptor signaling by causing serine phosphorylation which interrupts the insulin signaling cascade (15-17). The inhibition of insulin action also results in disruption of glucose tranporter-4 (GLUT-4) expression and mRNA stability, a main glucose transport system in insulin sensitive cells (17, 18-21).

TNF-α also increases leptin protein levels within adipocytes (22). The synthesis

of TNF-α from adipocytes is directly related to metabolism in adipocytes and regulation of adipose tissue mass (23). Obese subjects expressed 2.5-fold higher TNF-α mRNA levels in adipose tissue compared to lean subjects (9). Weight reduction in obese individuals improves insulin sensitivity and has been reported to decrease TNF-α mRNA expression in adipose tissue (16).

Research performed a study in mature rodents (14, 24) and in humans (25) demonstrate the association of HF diets, obesity, insulin resistance, and TNF-α expression. To our knowledge, research examining the relationship of high fat feeding, insulin resistance and TNF-α expression in pubertal female rats has not been reported. Results of this research will contribute to our understanding of the involvement of TNF-α gene expression in insulin sensitive tissues in the early development of insulin resistance due to weight and body fat gain in young animals fed high-fat diets of differing fatty acid composition.

Therefore the following research hypotheses were developed:

1. Rats fed high fat tallow (HFT) diets will have greater body weight and adiposity compared to rats fed high fat soybean oil (HFS) and moderately high fat soybean oil (MFS) diets.
2. Rats fed HFT diets will develop more overt insulin resistance compared to HFS and MFS fed rats as demonstrated by serum insulin, glucose, and leptin concentrations at fasting and in response to oral glucose tolerance testing.
3. TNF-α expression levels within liver, muscle and adipose tissues will be significantly greater in rats fed HFT diets compared to rats fed HFS and MFS dietary treatments.

Based on these hypotheses, the following research objectives were developed:

1. To examine the effects of dietary fatty acid composition and concentrations on growth (body weight) and adiposity (% body fat) in pubertal female rats.
2. To assess the effects of dietary fat treatments on insulin resistance by measuring serum concentrations of insulin, glucose, and leptin at fasting and in response to an oral glucose challenge.
3. To determine the relationship between TNF-α expression, insulin resistance, body weight, adiposity and dietary fat content and fatty acid composition in the diet.

Limitations

This study was designed to examine the effects of a HFT, HFS oil, and MFS oil diet treatments during rapid growth in female rats. Some limitations of this study were due to our interest in early onset insulin resistance. The study was limited to a 10 week feeding period to examine the parameters of interest in the animals. This study is also limited to young female rats in an effort to simulate the most common developmental stage and gender in which early symptoms of Type II diabetes occurs (26) These results cannot be directly applied to humans although findings in rodent models have been the most widely used and accepted venue for science based human research. Therefore, rodent studies of male rats as well as human research are warranted to further substantiate these findings.

Thesis Format

This thesis in composed of five chapters: the introduction, literature review, methodology, results in the form of a journal article, and a summary, conclusion, and recommendation section. The bibliography and journal article were written in the format designed by Diabetes, the journal of the American Diabetes Association.

CHAPTER II

REIVEW OF LITERATURE

Obesity and Type II diabetes Mellitus

The prevalence of obesity and type II diabetes mellitus are increasing rapidly within the United States as well as throughout the world. In the year 2000, 65% of adults in the U.S. were overweight with 31% being obese (2). The percent of overweight children and adolescents in the U.S. is also rapidly increasing. Between 1980 and 2000, the prevalence of overweight among children and adolescents in the U.S. doubled, reaching 15% (26). The rising rate of type II diabetes in children and adolescents seems to follow behind this rising rate of childhood obesity (27). Type II diabetes is among the most common endocrine disorders in the western worlds and according to the World Health Organization (WHO), it is estimated that the prevalence of type II diabetes will double from 135 million in 1995 to 300 million by the year 2025 (28).

Diabetes is the 5th leading cause of death in women and the 6th leading cause of death in men in the U S (3). Diabetes is associated with life-threatening conditions such as: heart disease, stroke, hypertension, blindness, kidney disease, nervous system disorders and amputations. The rate of stroke and heart disease death is 2 to 4 times higher in adults with diabetes. Nearly 70% of individuals with diabetes have high blood pressure and mild to severe forms of nervous system damage such as diabetic retinopathy

and impaired sensations in the hands and feet which is a major contributing factor in amputations (28).

Overweight and obesity substantially increase the risk of developing type II diabetes, heart disease, hypertension, and some types of cancers. The majority of individuals with insulin resistance and type II diabetes are overweight and nearly 80% are obese (28-30). In the US, obesity is the 7th leading cause of death with an estimated 280,000 deaths in adults and children per year as a direct result of obesity (31).

The criteria and classification for overweight and obese are based on body mass index (BMI), which is defined as weight in kg divided by height in m² (BMI=kg/m²). According to the WHO, a BMI ≥25.0 indicates overweight whereas a BMI ≥30.0 indicates obese (28). Obesity indicates excess adipose tissue while overweight indicates excess weight for height, regardless of the percent of adiposity. Obesity is characterized by an abundant accumulation of excess body fat resulting from an imbalance between energy intake and energy expenditure.

The continuous over consumption of calories above daily dietary needs produces weight gain which may lead to obesity. The autonomic nervous system and several circulating hormones such as insulin, cortisol, growth hormone, and leptin are involved in the metabolic response to food intake (32). Under normal circumstances, these signals, triggered by food intake, prompt adjustments not only in nutrient intake, but also in energy and nutrient metabolism. Therefore, the control of body weight and composition depends upon food intake, nutrient thermogenesis, and body fat stores (32). The outcome of this equilibrium has a direct influence on fat deposition. Weight gain may also depend on the distribution of dietary energy substrates, which have different impacts on

metabolism and food intake (32). Physical inactivity is also an independent risk factor for insulin resistance and type II diabetes (33).

Diabetes mellitus is subdivided into two categories (28,29): Type I and Type II diabetes. Type I diabetes, which accounts for 5% to 10% of all diabetes cases, results from the autoimmune destruction of pancreatic β cells resulting in absolute deficiency of insulin secretion. Type II diabetes on the other hand is caused by defects in insulin action resulting in insulin resistance and hyperglycemia. Impaired insulin action results from defects at one or more points in the complex pathways initiated by insulin binding to its receptor in insulin sensitive tissues. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient. It is often unclear which abnormality is the primary cause of the hyperglycemia. Type II diabetes is also characterized by elevated free fatty acids (FFA) and glucose levels, which is even more evident with visceral adiposity (34,35).

Current diagnostic criteria designated by the World Health Organization (28) and the American Diabetes Association (30) for type II diabetes are:

Fasting glucose ≥ 126 mg/dL (7.0 mmol/L)

Blood glucose concentration ≥ 200 mg/dL (7.8 mmol/L)

Oral glucose tolerance test with 2 h postload value ≥ 200 mg/dL (7.8 mmol/L)

All findings must be confirmed on a subsequent day.

Many individuals with type II diabetes are obese, and obesity itself has the potential to lead to insulin resistance. A study composed of 85 obese males with normal glucose levels was evaluated in order to determine any relationship between the degree of obesity and insulin action (34). From hyperinsulinemic, euglycemic clamp studies, the

investigators discovered that there was a significant inverse correlation between the level of adiposity and insulin action.

Obesity is characterized by a chronic inflammatory response characterized by abnormal elevations in cytokine production and the activation of signaling pathways involved in the inflammatory process. It has been suggested that this inflammatory response is due to interaction between adipocytes and macrophages within adipose tissue. There is a large molecular overlap between the functions of adipocytes and macrophages and studies examining this overlap have observed that there is a significant increase in the number of macrophages infiltrating adipose tissue as obesity develops (35,36).

When macrophage activation and infiltration occur due to signaling pathways, it is believed to mediate inflammatory response and lead to impaired insulin response in adipocytes (35) Macrophages are also capable of secreting numerous cytokines including TNF-α, IL-1, and IL-6 which are known to impair insulin sensitivity (13,37). Increased secretion of leptin may also contribute to the elevation of macrophages by increasing their transport to adipose tissue. Regardless of the exact mechanism stimulating macrophage accumulation, the presence of macrophages within adipose tissue results in a chronic inflammatory condition due to increases in pro-inflammatory cytokines.

Insulin resistance is defined as the inability of insulin to stimulate glucose uptake in insulin sensitive tissues. Insulin is a potent anabolic hormone which stimulates the uptake of glucose, amino acids, and fatty acids in muscle and fat tissue making it a key regulator of blood glucose concentrations. Insulin acts via a complex signaling process that involves multiple pathways and cascades (38). The inefficient uptake and utilization

of glucose in response to insulin stimulation is thought to be due in part to abnormalities in the insulin signaling cascade following insulin receptor binding (39). Development of insulin resistance results in hyperinsulinemia, a compensatory increase in insulin to overcome cellular resistance and hyperglycemia. Symptoms of hyperglycemia include polyuria, polydypsia, weight loss and blurred vision (30). Hyperglycemia is partly due to impaired β cell function. Beta cell impairment leads to an overproduction of glucose by the liver and underutilization of glucose by tissue resulting in hyperglycemia (39).

The prediabetic phase, which often develops before the diagnosis of type II diabetes, results in two common conditions of insulin resistance: impaired glucose tolerance and elevated fasting insulin levels. Several techniques have been developed to accurately detect the presence of insulin resistance such as: euglycemic hyperinsulinemic clamp tests, oral glucose tolerance tests, and measurements of plasma insulin concentrations. Collectively, these tests are reliable in detecting insulin resistance before clinical signs for type II diabetes appear (30).

The American Diabetes Association (30) and World Health Organization (27) criteria for diagnosis of impaired glucose tolerance consists of fasting glucose values of >109 mg/dL to ≤ 126 mg/dL (6.1 to 6.9 mmol/L) or 2-h postprandial blood glucose values of ≥ 140 mg/dL to < 200 mg/dL (7.8 and 11.1 mmol/L) after a standard 75 g oral glucose load (30). Abnormalities in glucose tolerance are often asymptomatic and defective glucose tolerance may be present before the appearance of overt diabetes (42). Early detection of impaired glucose tolerance allows intense diet and exercise modification, which in many instances has proven effective in normalizing postprandial glucose and inhibiting the progression of overt diabetes.

Type II diabetes is a complex disease but proper diet and exercise play important roles in the development of the disease. This was evident in a Finnish Diabetes Prevention Study of 522 adult subjects with impaired glucose tolerance who where randomly assigned to an intervention (diet and exercise) or control group (43). In a 4-year follow-up, significant decreases in weight and waist circumference were observed in the intervention group when compared to the control subjects. The intervention group also had increased insulin sensitivity and significantly decreased fasting insulin levels in comparison to controls.

Their results indicate that changes in insulin resistance are strongly correlated with changes in body weight and that it is possible to achieve a sustained improvement in insulin sensitivity by moderate weight loss and healthy lifestyle. Intervention studies have also observed that exercise training significantly reduces the risk of developing insulin resistance by improving glucose tolerance and insulin action in humans predisposed to developing type II diabetes (44).

The complex relationship between insulin resistance and obesity has yet to be fully defined. The infiltration of macrophages into adipose tissue during obesity creates a concentrated site of pro-inflammatory cytokine action. The inflammatory response produced during obesity has been established as one of the many mechanisms involved in the development of insulin resistance and type II diabetes (35).

The ability of insulin to stimulate glucose uptake to insulin sensitive tissues is dependent on several processes. Insulin signaling, glucose transport, and proper function of pancreatic β cells are all needed for proper insulin action. Due to the increasing number of obese individuals with type II diabetes, further research is needed to identify

the exact mechanisms involved in the development of this disease.

High fat diet and insulin resistance

High fat diets have been found to increase the risk of life threatening diseases such as cardiovascular disease and cancer (44). Studies have also investigated the effects of high dietary fat on insulin sensitivity.

A study of African-American and Caucasian women compared the effects of an isocaloric HF (50% kcals from fat) and LF (20% kcals from fat) diet treatment. For 3 weeks of consuming the experimental diet and found that increased dietary fat intake reduced insulin sensitivity by 6%, independent of race. While the LF diet treatment increased insulin sensitivity by 20% (45). Several other studies have observed an association between HF diets and increases in fasting insulin concentrations (44,46,47). A study of 544 non-diabetic twin women who participated in the Kaiser Permanente Women Twins Study found an association between fasting insulin levels and high dietary fat intakes and a 20 g increase in fat a day increased fasting insulin levels by 9% (46). A similar study of 782 healthy adult men and women investigated the relationship between dietary fat intake and insulin levels (47). Subjects where administered a 24 h food recall and participated in an 8 h overnight fast. Fasting blood samples were later taken and oral glucose tolerance tests of 75 g glucose were performed. Results of the study found that the percent of dietary fat consumed was strongly associated with insulin resistance. Collectively, these results suggest that a high fat intake decreases insulin sensitivity and fasting insulin concentrations contributing to the development of insulin resistance.

Most rodent studies have found a significant relationship between high fat feeding and insulin resistance (7,8,48-51). Weanling rats fed a HF (31% kcals from fat) diet gained significantly more weight and had higher glucose, insulin, and triglyceride levels compared to standard laboratory chow fed rats (48). Studies have also been conducted to investigate the effect of HF feeding on tissue specific insulin sensitivity. Rats fed a HF (60% kcals from fat) diet compared to rats fed a high carbohydrate (61% kcals from CHO) diet had significantly decreased insulin response to OGTT. This decrease was thought to de be due to reduced insulin binding and action within the adipocytes (49).

Similarly, investigators reported that rats fed an isocaloric high fat (59% kcal from fat) diet and high carbohydrate (70% kcals from CHO) diet and given euglycemic clamp tests resulted in whole-body insulin resistance due to decreased glucose utilization in muscle and fat tissues (50). Thus it is clear that tissue specific alterations produced by HF feeding contribute to abnormalities in insulin action as well as glucose utilization.

In rat models, HF diet feeding with equal calorie intake result in increased body fat accumulation and percent body fat (7,8,14,48). A study compared 20 female rats fed HF (39% kcal from fat) and MF (22% kcal from fat) diets to identify any correlations between fat content of the diet and insulin resistance. It was discovered that HF fed rats had an increased percent body fat and body fat mass when compared to their MF fed counterparts. In addition, (OGTT) were also performed and researchers found that the HF treatment group had significantly higher insulin levels as well as serum leptin concentrations than the MF group (48). Collectively, results of these

studies suggest that the percent of energy intake from fat is an independent predictor of weight gain and is positively correlated with insulin resistance.

Fatty acid composition and insulin resistance

Dietary fatty acid composition has been shown to influence the development of insulin resistance and also the incidence of type II diabetes mellitus. This was evident in the Health Professionals Follow-Up study where increases in total fat and saturated fat were associated with a higher risk of developing type II diabetes (52). Many epidemiological studies have observed that a higher intake of saturated fat is associated with impaired insulin sensitivity and glucose intolerance, while higher amounts of unsaturated fat in the diet have been associated with increases in insulin sensitivity (53). These results indicate that the quality of dietary fat consumed has some influence on the risk of developing type II diabetes.

In much the same way, intervention studies have been used to establish a relationship between the type of dietary fat and insulin resistance. A study performed on 162 healthy women and men found that a diet high in saturated fat (18% of kcals) decreased insulin sensitivity compared to a diet high in monounsaturated fat (21% of kcals) with equal levels of total fat content (54). However, when total fat intake was greater than 37% of total calories, these beneficial effects on insulin sensitivity where not observed. Another human study (58) compared obese, non-obese, and type II diabetes mellitus patients and found that a moderate substitution of unsaturated fat in the diet significantly improved insulin resistance. Still, several studies (56-58) have found that changes in dietary fat composition were unable to significantly affect insulin sensitivity.

These latter studies were conducted in a shorter period of time and with fewer subjects, which could explain the absence of any correlation between types of dietary fat and insulin resistance.

Animal studies have been useful in determining the influence of dietary variables on insulin action (59,60). Many animal studies have found a relationship between high fat intake and insulin resistance. Similarly, researchers have observed that the type and amount of dietary fat has an affect on insulin sensitivity. For example, rats fed a HF (59% kcal from fat) diet had decreased insulin sensitivity compared to those fed a MF (12% kcal from fat) diet. In addition, feeding rats for periods of 3-4 weeks with differing fatty acid compositions was sufficient to provoke insulin resistance in some HF fed rats (59). A rat study compared diets with increased levels of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) with equal (39%) total calories from fat (60). These researchers found that diets high in PUFA had significantly lower fasting plasma insulin levels and increased peripheral glucose utilization compared to rats fed MUFA and SFA enriched diets. Whereas, a study by Storlien and colleagues (59) revealed that MUFA diets increased insulin sensitivity compared to SFA and PUFA enriched diets. Collectively, these results indicate that the presence of unsaturated fatty acids in the diet does improve the overall level of insulin sensitivity.

High fat diets and body fat content

The role of dietary fat in obesity has been addressed by a number of studies in animal (7,8,48,61) as well as in humans (62-67). All indicate that a high intake of dietary

fat is positively correlated with increases in adiposity. It has also been shown that there is a significant positive correlation between mean dietary fat intake, BMI, and the incidence of obesity (67,68).

One hundred and twenty-eight male subjects, who participated in the Quebec Family Study (65), were studied to determine of any association between adiposity and total dietary fat intake could be identified. A significant positive correlation was discovered between the percentage of dietary energy as total fat and body fatness. Similarly, a study of 782 adult men and women found that the percent of energy consumed from dietary fat was positively associated with weight gain over time (47). This association was still evident after adjustments were made for total calories in the diet. These results suggest that a habitual intake of high dietary fat without a significant increase in total calories increases body weight and percent body fat in humans.

Several rat models have examined the effects of increased dietary fat levels on body weight and body composition (7,8,48,61). Adult rats were fed 1 of 4 diet treatments (12%, 24%, 36%, and 48% kcal from fat) with equal calories of energy in order to identify any relationship to body fat accumulation (61). After 6 weeks of treatment, researchers found that total body fat and adipose deposits increased as the level of fat increased in the diet. Rats fed a 48% kcal from fat diet had significantly greater total body fat when compared to control groups yet no significant increases in body weight were observed. Both human and rat studies indicate that increases in dietary fat increase body weight as well as percent body fat.

Introduction to TNF-α

In 1980, Rouzer and Cerami (69) observed rabbits with a Trypanosome parasitic infection became anorexic, emaciated, and developed hypertriglyceridemia. Similarly, another study found that mice, like trypanosome infected rabbits, developed hypertriglyceridemia and had deficient expression of lipoprotein lipase (LPL) (70). The metabolic changes in LPL were shown to be regulated by a serum factor, cachectin. Cachectin was also showed to decrease the mRNA expression of specific molecules in mature adipocytes (71). Researchers observed an increase in the release of glycerol from adipose tissue which suggested that cachectin may have a role in wasting during chronic diseases (72).

In 1975, Caswell et al. (73) observed a cytotoxic effect on tumor cells in bacterial endotoxin-injected mice and termed this effect tumor necrosis factor (TNF). Both cachectin and TNF were found to decrease LPL activity. The two proteins were later found to be identical when mouse TNF-α cDNA had exactly the same amino acid sequence as cachectin (74). Consequently, TNF was subdivided into TNF-α (TNF/cachectin) and TNF-β (lymphotoxin) due to their abilities to cause hemorrhagic necrosis (75). A study using a euglycemic, hyperinsulinemic clamp on male Sprague-Dawley rats found that TNF-α produced hepatic and peripheral insulin resistance (76). Many years later, researchers discovered that TNF-α contributed to insulin resistance by inhibiting insulin receptor signaling and glucose transportation (16,18).

TNF- α Secretion and Actions

TNF-α is involved in many processes of the body, including growth promotion and inhibition, metabolism, immune and inflammatory responses, and cytotoxicity (77,78). This cytokine is primarily derived from macrophages where it is synthesized as a 26 kDa transmembrane precursor protein. TNF-α undergoes proteolytic cleavage to the 17 kDa soluble TNF- α molecule, which is present in circulation. TNF-α is produced and released from numerous cells including mammalian, including human, adipocytes. Cytokines secretions from adipose tissue is elevated in obese rats when compared to lean controls, and an attenuation of the elevated levels are observed after weight loss (12).

TNF-α has been found to impair insulin receptor signaling and glucose transport with in insulin-sensitive cells (16,18). Binding of insulin to its insulin receptor (IR) causes the autophosphorylation of IR and the activation of tyrosine kinase on insulin substrate-1 (IRS-1). This cascading action is necessary for insulin's action and TNF-α exposure results in the inhibition of insulin-stimulated glucose uptake. Qi and Pekala (17) describe the exposure of TNF-α within adipocytes causes an increase in serine phosphorylation inhibiting tyrosine kinase activity between the insulin receptor and IRS-1, which compromises insulin signaling to cells. Additional studies (19,20,79) have discovered that treatment of adipocytes with TNF-α produces a decrease in insulin stimulated insulin receptor autophosphorylation and tyrosine phosphorylation of IRS-1 blocking the action of insulin. The actual mechanism of TNF-α inhibitory effect on tyrosine phosphorylation remains unknown, however a recent study found that the over expression of inhibitor k-β kinase (IkkB) is thought to be involved (50). Investigations on the ability of TNF-α to disrupt insulin signaling has been further strengthened by

creating TNF-α knockout animal models. Mice without the TNF- α gene showed increased insulin receptor signaling and improvements in insulin sensitivity (17,80). Combined, these studies demonstrate that TNF-α impairs insulin receptor signaling through increases in the serine phosphorylation of IRS-1 and the attenuation of tyrosine phosphorylation.

TNF –α has also been shown to down-regulate gene expression of the insulin-stimulated glucose transporter, GLUT4, in rodent and human adipocytes (79,81). A study was performed on both human and murine 3T3-L1 adipocytes, cells found exclusively in adipose tissue, to examine the actions of GLUT4 and to identify any correlations in the development of TNF-α induced insulin resistance (81). Fully differentiated 3T3-L1 adipocytes were treated for 1, 24, 48, 72, and 96 h with 250 pM of TNF-α and were probed by Western blotting with the anti-phosphotyrosine monoclonal antibody, or 4G10, and exhibited a significant decrease in insulin-stimulated glucose uptake compared to untreated cells. Additionally, there was a significant decrease in GLUT4 over time.

Furthermore, TNF- α decreases the stability of GLUT4 mRNA inhibiting glucose transport within insulin sensitive tissues (17,21,78,79). Researchers exposed 3T3-L1 human adipocytes to 5 nM of TNF-α to determine if the exposure of TNF-α attenuates GLUT4 action (21). Through Western blot analysis, investigators discovered a total depletion of GLUT4 protein in cells treated with TNF-α. In addition, with continued exposure to TNF-α, GLUT4 mRNA content decreased by 85-90% compared to controls. These studies suggest that TNF- α impairs glucose transport by inhibiting GLUT4 gene expression and protein secretion.

TNF-α has been found to inhibit adipocyte differentiation as well as induce adipocyte and pre-adipocyte apoptosis (79). If TNF-α is over expressed, it acts in a paracrine fashion to inhibit the hypertrophy of adipose tissue (77), and attenuates lipogenesis, LPL activity and increase lipolysis. LPL activity is an indicator of the cells capacity to take up triglycerides from the medium (81). Researchers examined human adipose tissue to determine the effects of TNF-α on LFL activity. Following a 20 h incubation of the adipocytes in TNF-α containing medium, cells exhibited a dose dependent inhibition of LPL activity. RNA extraction and Northern blotting also found that TNF-α decreased LPL mRNA levels. Thus, TNF-α is also a potent inhibitor of LPL gene expression and activity in human adipose tissue, and may act as an adipostat, yet many other molecules maybe involved in the control of fat mass.

TNF-α has been shown to be involved in the actions of various other proteins and hormones. Adipose tissue releases a variety of signaling molecules as well as produces and secretes a number of hormones resulting in influential effects on energy balance and metabolism. Increased production of non-esterified fatty acids (NEFA), leptin, TNF-α, as well as several other cytokines and peptides from adipose tissue have been discovered to contribute to the changes in systemic metabolism of obese subjects (82). Increased production of NEFA, leptin, and TNF-α cause changes in the metabolism of obese subjects and contribute to insulin resistance (83). Elevated concentrations of NEFA have been found to contribute to impaired insulin sensitivity by impairing insulin-stimulated glucose uptake and glycogen synthase activity in skeletal muscle. A recent study (82) discovered that increased concentrations of NEFA help in the development of insulin resistance in muscle and liver tissues, which may lead to hyperinsulinemia. The presence

of hyperinsulinemia is commonly seen in obese subjects.

TNF-α has been found to regulate leptin, a cytokine-like molecule produced by adipocytes (22,24,78,82). Leptin and TNF-α, which are both secreted from adipocytes, play an important role in regulating fat mass. Elevated leptin expression and secretion are associated with obesity. Kirchgessner and colleagues (22) reported that obese mice with a null mutation of the TNF-α gene in the presence of a high fat and high kcal diet (50% kcal from fat with 5,286 kcal/kg) had lower leptin protein levels in comparison to obese mice with a functional TNF-α gene. Additionally, circulating leptin levels were lower in animals with no functional copy of TNF-α compared to the control group. These results suggest that TNF-α may play a potential role in the regulation of leptin expression as well as secretion from adipose tissue and contribute to insulin resistance. Both leptin and TNF-α have also been shown to be involved in the impairment of insulin action in hepatic and muscle tissues by decreasing glucose uptake (82).

TNF-α has also been found to decrease the gene expression of adiponectin (84). Adiponectin, also referred to as Acrp 30, is a peptide produced specifically within adipocytes and has been found to decrease insulin resistance and plasma FFA levels in mice (73). A study investigating these effects by incubating 3T3-L1 cells in 1.0 nmol/L of TNF-α for 24 h (84) followed by Northern blot analysis revealed a decrease of 79% in Acrp 30 mRNA levels. In addition, 12 genes were induced by 24 h of TNF-α treatment. Through RT-PCR analysis, TNF-α was found to suppress hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), and acyl-CoA synthetase. These results suggest that TNF-α induces changes in adipocytes gene expression and suppresses hormones essential for the metabolic functions of adipose tissue which could potentially lead to insulin

resistance.

TNF-α and Obesity

The relationship of insulin resistance and adipose tissue expression of TNF-α in obesity was demonstrated in a study of obese women (9). TNF-α mRNA levels within adipose tissue were found to be 2.5-fold greater ($p<0.001$) in the obese subjects compared to the lean controls. Additionally, a positive correlation was found between TNF-α mRNA expression levels and fasting plasma insulin ($r = 0.82$ $p <0.001$), BMI ($r = 0.70$ $p<0.001$) and hyperinsulinemia ($p <0.001$). The direct correlation between a HF diet and obesity as previously discussed supports the corollary that TNF-α plays a major role in the mechanism by which fat intake affects insulin sensitivity in obesity.

In many studies of obesity in rats, TNF-α expression and production have been found to be elevated within adipose tissue (11-14). All these were studies performed in older rats simulating adulthood. Morin and colleagues (14) demonstrated that HF diets (45% kcals from fat) in older rats increased TNF-α expression in adipose tissue as well as increased adiposity as the rats aged. However, TNF-α protein concentrations were found to be decreased.

Other studies have found a decrease in the content of TNF-α within adipose tissue during the onset of insulin resistance (7,85). A study comparing tissue responses to glucose tolerance tests performed following a 24 h fast in 5 and 12 month old male rats found that insulin resistance was associated with decreased TNF-α protein content in visceral and subcutaneous fat (85). These results indicate that TNF-α protein may decrease with age whereas possibly its bioactivity increases.

In summary, the growing number of individuals with type II diabetes and obesity are of increasing concern in the US as well as throughout the westernized world. Excess consumption of dietary fat has lead to increases in body weight and percent body fat (1). High fat diets have also contributed to insulin resistance and other chronic diseases in both human and rodent studies (14,44,45,63). Although, several studies (7,8) clearly indicate that HF diets increase insulin resistance, a more in-depth look at the fatty acid composition of the diet is needed.

With the growing population of persons being diagnosed with type II diabetes mellitus, there is an increasing need for further studies into the exact molecular mechanisms involved in the progression of the insulin resistance. Several studies have been conducted to identify TNF-α as a major component of insulin resistance. TNF-α is positively associated with insulin resistance, insulin stimulated glucose uptake, and obesity (76,80). However, whether composition or type of fat influences this association has not yet been determined. In addition, whether TNF-α gene expression in non-adipose insulin sensitive tissues such as liver and muscle tissues is altered by high saturated fat feeding is unknown. Obese individuals have been shown to overexpress TNF-α mRNA 2.5-fold more than their lean counterparts and studies have shown that this is due to a high dietary fat intake. Therefore, the present study examines the expression of TNF-α mRNA in muscle, liver, and fat tissues of female rats fed different types of HF diets during growth.

CHAPTER III

MATERIALS AND METHODS

Animals

This research project and all protocols were approved by the Oklahoma State University Animal Care and Use Committee of Oklahoma State University, Stillwater, under protocol number HE 01-16 (Appendix A).

Thirty weanling Sprague-Dawley female rats were randomly assigned to one of three diet treatments in an incomplete 2 x 2 factorial design. Animals were housed separately in hanging steel cages. On arrival and throughout the duration of the study which was 10 weeks, the animals were fed their assigned diet. All animals were allowed free access to deionized water and were kept on a 12:12 light: dark cycle.

Diet Treatments

Diet composition and energy values are presented in Table 1. Carbohydrate and fat content were adjusted by weight to create the treatment diets. The Moderate high fat soy bean oil (MFS) diet contained 10% fat by weight, the High fat soy bean oil (HFS) and High fat tallow (HFT) diet treatments contained 20% fat by weight. According to the Subcommittee on Laboratory Animal Nutrition Committee on Animal Nutrition Board of

Agriculture National Research Council (86) the recommended fat concentration of dietary lipid for growing male and female rats and for adult female rats during reproduction and lactation is 5% by weight. Thus, the diet containing 10% fat by weight is labeled moderate, MFS, and the diets containing 20% fat are labeled high, HF. All rats consumed an average of 59 kcals/d with equal proportions of vitamins, and minerals per unit of energy.

Glucose Tolerance Tests

After 9 weeks on the dietary treatments, the rats were fasted overnight after which an oral glucose tolerance test (OGTT) was performed. On the day of the OGTT, animals were weighed and 1 mL of fasting blood was collected from the tail vein from each animal. The animals were then gavaged with 2 g glucose/kg BW. After the glucose load was administered, blood was collected at the 30, 60 and 120 minute time points. Blood glucose was determined using a DEX glucometer (Bayer Corporation, Elkhart, IN). Blood samples were placed in polypropylene tubes and kept on ice or frozen until analyzed. All blood samples were analyzed for glucose, insulin, and leptin concentrations.

Insulin resistance was estimated with the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) equation developed by Mathews et al (87).

HOMA-IR = (FI x FG)/22.5. (FI = fasting insulin, FG = fasting glucose.)

Insulin sensitivity was calculated utilizing the $ISI_{0,120}$ equation as stated by Gutt et. al. (88) where the insulin sensitivity index is equal to the glucose clearance rate (MCR) divided by the log of the mean serum insulin concentration (MSI) as follows:

ISI = MCR/log MSI

Dual X-ray Absorptiometry and Tissue Collection

One week following the OGTT, all rats were anesthetized to evaluate components of body mass measured with a Dual X-ray Absorptiometry machine, model 4500 Elite (DXA, Hologic, Waltham, MA). After the DXA scan, animals were sacrificed and blood, liver, soleus muscle, and fat samples were harvested. Tissue samples were placed in labeled cryovials, frozen in liquid nitrogen, and stored at -80° C. All blood samples were analyzed for glucose, insulin, and leptin concentrations and tissue analyzed for TNF-α gene expression.

mRNA extraction

Adipose surrounding the kidneys, soleus muscle, and liver tissues were thawed and homogenized in 5 ml Trizol reagent (Life Technologies, Inc., Faithersburg, MD). Chloroform 1 mL (Sigma Chemical Co. St. Louis, Mo) was added to each sample and vortexed for 15 sec. After 3 min of incubation at 22° C, samples were centrifuged (3500 x g) for 30 min at 4° C. The upper aqueous phase and RNA precipitate were placed in new polypropylene tubes with 2.5 mL isopropanol (Pierce Chemical Co. Rockford, IL) and incubated for 10 min at room temperature. The supernatant was removed and samples were centrifuged (3500 x g) 10 min at 4° C forming a RNA pellet. The RNA pellet was twice washed with 5 mL 75% ethanol and centrifuged once again for 10 min. The ethanol supernatant was then removed and the RNA pellet was dissolved in 100 ul TE buffer (10mM Tris-Cl, 1nM EDTA; pH 7.4), aliquoted and stored at -80° C. RNA concentrations were determined using Ribogreen nucleic acid staining kit (Molecular Probes, Inc. Eugene, OR). Prior to use, samples were thawed on ice for 3 to 5 min.

Primer and Probe design

Primers and probes for the RT-PCR procedure were developed using ABS Primer Express™ software (Applied Biosystems, Foster City, CA). TNF-α forward and reverse primers were constructed from bp 434 to bp 452 with a sequence of GACAAGGCTGCCCCGACTA and from bp 501 to bp 479 with a sequence of CTCCTGGTATGAAGTGGCAAATC, respectively. The probe for TNF-α was constructed between bp 455 to 477 with a sequence of TGCTCCTCACCCACAC CGTCAGC. High resolution gel electrophoresis and sequence analysis were used to confirm the desired PCR product was TNF-α.

Quantitative RT-PCR

Real-time quantitative PCR was used to determine mRNA expression levels for TNF-α in adipocytes, muscle, and liver tissue. Taqman Gold RT-PCR kit (Applied Biosystems, Branchburg, MA) was used to quantify TNF-α mRNA expression levels. The thermal cycling conditions, assay design, and optimization procedure was performed according to the guidelines developed by the manufacturer. The Taqman probe contained a 5' reporter dye (TET) and 3' quencher dye (TAMRA). Cleavage of the probe after annealing to the chosen TNF-α target by the 5' endogenous nuclease activity of Amplitaq Gold DNA polymerase that resulted in the increase of florescence of the reporter dye once the quencher was released and was quantified at each PCR cycle. A total reaction volume of 25 ul consisted of 200 nM forward primer, 200 nM reverse primer, 100 nM fluorescent probe for TNF-α. The reaction mix consisted of 12.5 ul Taqman Master Mix, 0.625 ul Multiscribe and RNase inhibitor mix (Applied Biosystems, Foster City, CA),

and 100 ng of total RNA aliquoted with DEPC water. Two milliliters of each RNA sample was combined with 23 ul reaction mix in each well. The One-step RT-PCR amplification was performed in the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA).

After the parallelism of TNF-α was found to be acceptable, the final assay was conducted to identify the fold expression of TNF-α in each sample. One-step RT -PCR amplification was performed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). Thermal cycling parameters were as follows: 30 min at 48° C for reverse transcription, 10 min at 95° C for AmpliTaq Gold Activation, and 40 cycles at 95° C for 15 sec for denaturing and 60° C for 1 min for annealing and extension. Ribosomal 18S rRNA control kit (PE Biosystems, Foster City, CA) was used as a valid house keeping gene to normalize samples for variations in RNA.

Statistical Analysis

The relative quantification of TNF-α was assessed using comparative threshold cycle method (Ct). The ΔCt value was established by subtracting the 18S Ct from the target unknown (TNF-a) Ct value. The highest ΔCt value was subtracted from all other ΔCt values within each experiment determining the ΔΔCt. The fold change in mRNA expression for TNF-α was calculated as $2^{-\Delta\Delta Ct}$. SAS version 8 Statistical Analysis Software for Windows (89) was used for analysis of all data. Data was presented as the least-squared means ± SEM. Outliers were detected by an outlier determination test developed by Ott (90). The significance of experimental effects and differences among treatment groups was determine using the Mixed and Slice procedures (SAS) if

significant treatment effects were detected. Significance levels for all analysis were set at $p < 0.05$.

Table 1. Composition and energy value of experimental diets.

	MFS	HFS	HFT
		g/kg Diet	
Casein	200	200	200
Cornstarch	100	100	100
Sucrose	500	400	400
Cellulose	50	50	50
Soybean oil	100	200	0
Beef tallow	0	0	200
Mineral mix*	35	39.3	39.3
Vitamin mix†	10	11.2	11.2
L-Cysteine	3	3	3
Choline Bitartrate	2	2	2
		% kcal	
CHO	43	58	58
Fat	22	39	39
Protein	20	17	17
		kcal/ g diet	
Energy Density**	4.10	4.57	4.57

* Mineral mix for experimental diets did not have added Chromium.

†Amounts of mineral and vitamin mixes in the high-fat diet were adjusted to equal the amounts per calorie in the low-fat diet.

** kcal/gram diet

CHAPTER IV

THE RELATIONSHIP BETWEEN HIGH FAT FEEDING, INSULIN RESISTANCE AND TNF-α GENE EXPRESSION IN GROWING RATS

Abstract

Tumor Necrosis Factor–α (TNF-α) is a low molecular weight cytokine thought to be involved in the insulin resistance. The exact mechanism of TNF-α involvement is unknown, however, researchers believe it involves the interruption of the insulin cascade and inhibition of GLUT4, which decreases glucose clearance. Studies have observed that TNF-α mRNA overexpression has been observed in adipose tissue of several rodent models fed high fat diets. This study was designed to examine the effects of dietary fat content and fatty acid composition on weight and adiposity, fasting blood, glucose, insulin, and leptin concentrations, and to investigate the relationship between TNF- α fold expression and insulin resistance. At weaning, 30 female Sprague Dawley rats were randomly assigned to either high-fat tallow (HFT, 39% of calories, 20% fat by weight; n = 10), high-fat soybean oil (HFS**,** 39% of calories, 20% fat by weight; n = 10), and moderate-fat soybean oil (MFS**,** 22% of calories, 10% fat by weight; n = 10) diet treatments. All rats consumed an average of 59 calories a day over 10 weeks with equal vitamins and minerals per unit energy. An oral glucose tolerance test, OGTT, was administered after 9 weeks on the feeding trial. At 10 weeks, body composition analysis was conducted by dual X-ray absorptiometry (DXA). The homeostasis assessment model

for insulin resistance (HOMA-IR) and insulin sensitivity index ($ISI_{0,120}$) were calculated to determine insulin resistance as well as insulin sensitivity. Liver, muscle, and fat tissues were collected and mRNA was extracted from each sample. Real Time RT-PCR was used to quantify gene expression of TNF-α in the tissues. HFT and HFS animals gained significantly more weight ($p < 0.05$) and had a greater percentage of body fat (%BF) ($p < 0.0001$) than the MFS animals. Fasting insulin concentrations were significantly elevated in the HFT fed animals ($p<0.05$) compared to HFS which had significantly greater insulin levels than the MFS fed animals ($p<0.05$). Compared to MFS animals, both HFS and HFT animals had significantly greater insulin resistance (HOMA-IR) and lower insulin sensitivity ($ISI_{0,120}$) levels ($p<0.05$). TNF-α gene expression was significantly greater in the liver tissues of HFT fed rats. TNF-α was significantly lower ($p<0.05$) in the adipose tissue of the HFT fed rats compared to the HFS and MFS fed rats. However, there were no significant differences observed in muscle TNF-α mRNA abundance among any of the dietary treatment groupss. There were positive correlations between TNF-α in the liver and insulin resistance ($p< 0.05$), as well as between fasting insulin ($p<0.05$) and fasting glucose ($p < 0.05$). A negative correlation was found between TNF-α liver and insulin sensitivity ($p < 0.05$). High fat tallow diets fed to growing female rats is associated with increased insulin resistance, reduced adipose and increased hepatic levels of TNF-α mRNA.

Introduction

Obesity and type II diabetes have been found to be associated with a chronic inflammatory state and the infiltration of macrophages in adipose tissue (1). The production of such inflammation is a direct result of increased cytokines, one of which has been identified as tumor necrosis factor-α (TNF-α). When macrophages are activated and infiltration occurs, it is believed to mediate inflammatory responses that lead to impaired insulin response in adipocytes (2).

TNF-α is a low molecular weight cytokine primarily produced in macrophages and adipocytes and has been found to regulate leptin and free fatty acid release from adipose tissue (3) There are several potential mechanisms by which TNF-α may induce insulin resistance. These mechanisms include actions on glucose transport, insulin receptor signaling, and FFA levels. It is thought that the mechanism of TNF-α induced insulin resistance involves inhibition of insulin receptor signaling and glucose transport within insulin sensitive cells (4-7). The exposure of adipocytes to TNF- α causes an increase in serine phosphorylation inhibiting tyrosine kinase activity between the insulin receptor and IRS-1 which compromises insulin signaling in insulin sensitive cells (5,8). The mechanism by which TNF- α inhibits tyrosine phosphorylation is unknown, however, the overexpression of inhibitor k-β kinase (Ikk-β) is thought to be involved (7).

TNF-α has long been known to inhibit GLUT 4 mRNA and gene expression. Several studies have reported that increases in TNF-α mRNA levels decrease insulin signaling and down regulate GLUT4 leading to decreased glucose transport to insulin sensitive cells (9-11). The cytokine also contributes to insulin resistance by increasing FFA secretion (12) and by disrupting IR and IRS-1 tyrosine phosphorylation (13,14).

Treatment of adipocytes with TNF-α produced a decrease in insulin-stimulated IR autophosphorylation and tyrosine phosphorylation of IRS-1, which could result in impaired insulin signaling.

Several studies have found that consuming high fat diets decreases insulin sensitivity as body fat increases and contributes to increases in the risk of developing type II diabetes (15). Hotamisligil and colleagues (16,17) reported significant increases in fasting plasma insulin and TNF-α mRNA levels when compared to lean subjects. Neutralization of TNFR1 in animal models was found to increase insulin sensitivity 2 to 3-fold, indicating a greater response to insulin in vivo in the absence of functional TNF-α receptors (17). Similarly, researchers (18) found that body weight, body fat percentages, and serum insulin concentrations of rats fed high fat diets greatly increased compared to rats fed moderate levels of fat.

In obese mice with and without a functional copy of the TNF-α gene, researchers (6,9) found that unaltered TNF-α gene have significantly decreased insulin receptor signaling when compared to mice with a neutralized TNF-α gene. These alterations inhibit glucose transportation in to all insulin sensitive tissues. Moreover, TNF-α receptor 1 (TNFR1) (p55-kDa in rats, p60-kDa in humans) and TNF-α receptor 2 (TNFR2) (p75-kDa in rats, p80-kDa in humans) mediates the inhibition of glucose uptake by cells (14,17). Primarily mediated by TNFR1, the down-regulation of GLUT4 mRNA rapidly decreases insulin stimulated glucose transport (11). Although several studies clearly indicate that HF diets increase insulin resistance associated with TNF- α and its receptors (6-21), the effect of fatty acid composition in high fat diets is warrants further study especially during the postnatal and peripubertal period of growth.

In the present study, we set out to determine the effects of a high fat soybean oil diet (HFS), a high fat tallow diet (HFT), and a moderately high fat soybean oil diet (MFS) on insulin resistance and TNF-α gene expression in adipose, muscle, and liver tissues of growing female rats. Diet treatments were developed to enable a comparison of 40% of total energy consumed from fat, which is more commonly consumed by humans (22), with 20% of total kcals from fat, which is considered a low fat diet for humans. The U.S. dietary guidelines recommendation for fat in the diet is no more than 30% of total daily energy needs from fat (23). Given the current recommendations of the Animal Nutrition Committee of the Agricultural National Research Council, the current recommended fat intake for growing rats is 5% dietary lipid content (24). Thus, this study uses the label of moderately high fat soybean oil (MFS) diet rather than low fat.

The objective of this study was to define the effects of high levels of different sources of fat on TNF-α gene expression and insulin resistance with the hypothesis that rats fed HFT diets would have impaired insulin sensitivity compared to rats fed HFS and MFS diets due to higher levels of TNF-α gene expression in insulin sensitive tissues.

Materials and Methods

Thirty weanling Sprague-Dawley female rats were randomly assigned to one of three diet treatments: a moderately high fat soybean oil diet with 10% of kcals from fat by weight (MFS), a high fat soybean oil diet with 20% fat by weight (HFS), and a high fat tallow diet with 20% fat by weight (HFT) in an incomplete 2 x 2 factorial experimental design (Table 1). All rats consumed an average of 59 kcals/d over 10 weeks of the feeding trial with equal proportions of vitamins and minerals per unit of energy.

After 9 weeks on the feeding treatments, the rats were fasted overnight and an OGTT (2 g glucose/kg BW) was performed. One week following the OGTT, after 10 weeks on diet treatments, the rats were anesthetized to allow body composition analysis by Dual X-ray Absorptiometry (DXA, Hologic, Waltham, MA). Tissue and blood samples were collected and analyzed for glucose, insulin, and leptin concentrations. Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR) equation by Matthews et al. (25) and insulin sensitivity by Gutt et al. ($ISI_{0,120}$) equation (26). Adipose, muscle, and liver tissues were analyzed for TNF-α gene expression using Real Time RT-PCR. All protocols were approved by the Oklahoma State University Animal Care and Use Committee.

Total cellular RNA was isolated from adipocytes, muscle, and liver tissue after exposure to 5 mL of Trizol reagent (Life Technologies, Inc.) according to manufacturer's protocols. Quantification of total RNA was established spectrophotometrically at the 260 nm reading. After determining quantification of total RNA, samples were aliquated and stored at -80° C. Prior to use for quantification for TNF-α mRNA expression, aliquoted samples were thawed on ice for 3 to 5 min.

In each experiment, fluorescent real-time quantitative PCR was used to determine differences in mRNA expression levels for TNF-α between diet treatments within adipocytes, muscle, and liver tissue. Expression levels were quantitated using one-step RT-PCR reaction following specifications provided by the manufacture of Taqman Gold RT-PCR kit (P/N 4309169; Applied Biosystems, Foster City, CA). The Taqman probe for TNF-α contained a 5' reporter dye (TET) and 3' quencher dye (TAMRA). Cleavage of the probe after annealing to the TNF-α target of 5' endogenous nuclease activity of

AmpliTaq Gold DNA polymerase results in an increase in detection of fluorescence of the 5' reporter dye upon release from the 3'quencher dye, which is quantified at each PCR cycle.

A total reaction volume of 25 μL consisted of 200 nM forward primer (TNF- α), 200 nM reverse primer (TNF-α), 100 nM fluorescent probe for TNF-α, 12.5 μL Taqman Master Mix, 0.625 μL Multiscribe and RNase inhibitor mix (Applied Biosystems, Foster City, CA), and 100 ng of total RNA increased to specifically need volume using RNase free water. One-step RT -PCR amplification was performed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). The thermal cycling conditions, assay design, and optimization procedure was performed according to the guidelines developed by the manufacturer.

Thermal cycling parameters were as follows: 30 min at 48° C for reverse transcription, 10 min at 95° C for AmpliTaq Gold Activation, and 40 cycles at 95° C for 15 sec for denaturing and 60° C for 1 min for annealing and extension. Ribosomal 18S rRNA control kit (P/N 4308329 Applied Biosystems, Foster City, CA) was used as a valid house keeping gene to normalize samples for variations in RNA loading. 18S rRNA was verified as a valid housekeeping gene by determining that decreasing amounts of 18S rRNA (500, 100, 50, 10, 5, or 1 pg) were parallel to decreasing amounts of TNF-α (500, 100, 50, 10, 5 or 1 pg).

Quantification of gene expression was attained by setting an approximate threshold through examination of the log view on the TET curve in the RT-PCR amplification plot. The relative quantification of TNF-α mRNA was accomplished using the comparative threshold cycle threshold (Ct) method (2,3). The ΔCt value was

established by subtracting the 18S Ct from the target unknown TNF-α Ct value. For each TNF-α mRNA, the highest ΔCt value was subtracted from all the other ΔCt values within the experiment, thus determining the ΔΔCt The fold change TNF- α mRNA expression was determined as $2^{-\Delta\Delta Ct}$. Data for TNF-α mRNA was expressed as fold of the lowest treatment-group mean fold gene expression within each experiment.

Primers and probes for the RT-PCR procedure were developed using ABS Primer Express software (Applied Biosystems, Foster City, CA). Manufacture restrictions for development include: The temperature melting (Tm) for primers was set at 59-60° C and the probes Tm set at 69° C. The minimum GC bp content for both primers and probes should be 20-80% avoiding identical nucleotides. The minimum nucleotide length of the strands should be at least 9 but not exceeding 40 nucleotides. The sequence for TNF-α was analyzed by the Primer Express Tm program which determined optimal primer and probe locations. All primers and probe sequences met Tm and GC content established by the manufacturer.

TNF-α forward and reverse primers were constructed from bp 434 to 452 with a sequence of GACAAGGCTGCCCCGACTA and from bp 501 to 479 with a sequence of CTCCTGGTATGAAGTGGCAAATC, respectively. The probe for TNF-α was constructed between bp 455 to 477 with a sequence of TGCTCCTCACCCACACCG TCAGC. A blast search (www.ncbi.nlm.nih.gov/BLAST/) was performed to assure that no homologous regions for other proteins were present. Verification that the desired PCR product was achieved by using high resolution gel electrophoresis and further confirmed through sequence analysis.

Each experiment used duplicate samples of extracted TNF-α mRNA from adipose, muscle, and liver tissue. Diet treatment effects were statistically analyzed using the general linear model procedure of SAS (27) for Windows. Primarily, the effects of diet treatment on the dependent variables (glucose, insulin, leptin, HOMA-IR, ISI, ΔCt and TNF-α mRNA levels) were analyzed. Outlier determination test were used to detect any outliers within the PCR data sets (28). Pearson correlations were also determined using SAS. Significance levels for all analyses were set at $p < 0.05$. The data are presented as the least-square means ± S.E.M.

Results

Although rats were fed approximately the same amount of food energy with altered fat content, there was a significant increase in body weight ($p<0.05$) and % body fat ($p<0.0005$) in rats fed HFT and HFS compared to MFS fed rats (Figure 1,2).

Fasting insulin concentrations (Figure 3) were significantly elevated in the HFT fed animals ($p<0.05$) compared to HFS which had significantly greater insulin levels than the MFS fed animals ($p<0.05$). Compared to MFS animals, both HFS and HFT animals had significantly greater insulin resistance (HOMA-IR, Figure 4) and lower insulin sensitivity (ISI, Figure 5) levels ($p<0.05$). No significant differences were observed in fasting glucose or leptin levels between any diet treatments.

Fasting leptin was positively correlated (Table 2) with fasting insulin ($r = 0.413$, $p<0.05$), and insulin resistance ($r= 0.404$, $p<0.05$). As expected, there was a significant correlation found between body weight and % body fat ($r = 0.555$, $p<0.005$). Positive correlations were also observed between body weight and fasting insulin ($r = 0.432$,

$p<0.05$), fasting leptin ($r = 0.414$, $p<0.05$) and insulin resistance ($r = 0.543$, $p<0.005$). Significant negative correlations were found between body weight and insulin sensitivity ($r = -0.688$, $p<0.0005$) as well as %BF and insulin sensitivity ($r = -0.628$, $p<0.001$). Additionally, % BF was positively correlated with fasting insulin ($r = 0.685$, $p<0.001$), fasting leptin ($r = 0.570$, $p < 0.005$), and insulin resistance ($r = 0.616$, $p<0.001$).

Rats fed a HFT diet had significantly less TNF-α mRNA in adipose tissue than rats fed HFS and MFS diets ($p<0.05$, Figure 6) Liver TNF-α mRNA levels were greater ($p < 0.05$) in HFT fed rats compared to HFS and MFS fed rats. TNF-α mRNA levels were 3-fold greater ($p < 0.05$) in liver tissue of rats fed HFT versus MFS fed rats (Figure 7). Positive correlations (Table 2) were observed between liver TNF-α mRNA expression and insulin resistance ($r = 0.545$, $p<0.05$), fasting insulin ($r = 0.492$, $p<0.05$), and fasting glucose ($r = 0.410$, $p<0.05$). In addition, there was a negative correlation between liver TNF-α expression and insulin sensitivity ($r = -0.485$, $p < 0.05$). No significant difference was found in TNF-α mRNA abundance within muscle tissue among any of the treatment groups (Figure 8).

Discussion

Increasing evidence has established that TNF-α is a key mediator of insulin resistance in many different obese rodent models, through its overexpression in adipose tissue (6,10,14). To further explore this phenomenon, we designed diet treatments based on current averages of dietary fat intakes of Americans in order to evaluate TNF-α gene expression during growth and the developmental stages of reproduction.

In the present study TNF-α mRNA abundance in fat, liver, and muscle of young female rats fed HFT, HFS, and MFS diets were analyzed in order to identify relationships between increased TNF-α gene expression and insulin resistance. The results of this study indicate that specific dietary treatments exert alterations in TNF-α expression establishing TNF-α as a mechanism in the development of an extremely complex disease. A major finding of the study is that TNF-α mRNA abundance increased in liver tissue while significantly decreasing in adipose tissue of rats fed a HFT diet. We have also demonstrated that a HF intake regardless of fatty acid composition is able to significantly increase BW, %BF, and insulin resistance.

Previous studies (18,29) found that HF diets increased insulin resistance, body weight, % body fat, and impair glucose tolerance. In the present study, correlations were observed between body weight and body fat mass with fasting insulin, leptin, and insulin sensitivity tests. This demonstrates that body weight and % body fat may affect various hormones involved in energy homeostasis and contribute to decreased insulin sensitivity leading to insulin resistance.

Several studies (16,30) have used HF diets in older rats to determine TNF-α effects on insulin resistance. Morin and colleagues (30) demonstrated that HF diets increased TNF-α expression in adipose tissue as well as increased adiposity as rats aged. In contrast, this study used young female SD rats to establish the relationship between diet, adiposity, TNF-α mRNA abundance and insulin resistance during post natal growth which included peripubertal development.

Storlien et al. (29) has shown that compared to chow fed controls, animals fed a HF diet (59% kcals from fat) had decreased insulin sensitivity. The current study shows

serum insulin was significantly higher in the HFT fed rats compared to the HFS and MFS treatment groups with HFS animals having significantly higher serum insulin levels than MFS animals. This suggests that a diet high in saturated fat may have a substantial effect on serum insulin levels by increasing insulin resistance or by increased insulin secretion by the pancreas but impaired insulin clearance. In addition, this study demonstrates that high fat intake regardless of fatty acid composition elevates serum insulin concentrations.

The ability of insulin to stimulate peripheral glucose utilization and inhibit glucose production has been found to be impaired in TNF-α infused rats (31). Liver insulin receptor knockout mice have severe insulin resistance and glucose tolerance, confirming that hepatic insulin signaling plays a major role in the regulation of glucose levels and insulin sensitivity throughout the body (32). These mice also exhibited hyperinsulinemia caused by increases in insulin secretion and impaired insulin clearance.

Results of the present study found that a HFT diet treatment significantly increased liver TNF-α expression 3-fold compared to HFS and MFS fed rats. Liver TNF-α was also correlated with fasting glucose, fasting insulin, HOMA-IR, ISI. These findings suggest that a high saturated fat diet increases liver TNF-α contributing to decrease insulin sensitivity and increased insulin resistance.

Adipocytes are the predominant source of TNF-α and an important determinant of insulin sensitivity (15,33,34). Increasing evidence has established a definitive link between increased TNF-α mRNA levels within adipocytes and insulin resistance associated with obesity (6,16). Elevated TNF-α mRNA levels in adipose tissue have been observed in both obese animals (4) and human subjects (21). However, during the early onset of insulin resistance, TNF-α expression in adipose tissue is significantly

decreased (35). These findings agree with our results in that a significant decrease in adipose tissue TNF-α mRNA was observed in the peripubertal rats fed HFT diets. This intriguing result would suggest that during a state of rapid growth TNF-α fails to function properly as a modulator of adipose tissue metabolism when high saturated fat diets are consumed during growth affecting glucose tolerance and insulin sensitivity.

TNF-α and its receptors has been discovered as a key component in many metabolic diseases. The effects of diet on TNF-α and TNFR gene expression is crucial in better understanding of the molecular mechanisms by which the insulin cascade is mediated by this cytokine. This would offer targeted intervention for the treatment of metabolic disorders related to high saturated fat diets.

In the light of the current obesity epidemic associated with the increased incidence of metabolic syndrome and type II diabetes, further experiments are needed to determine the exact effects of specific dietary fat treatments on the expression of genes associated with inflammation and insulin related pathways in various tissues and the development of insulin resistance, metabolic syndrome and type II diabetes mellitus in humans.

Table1. Composition and energy values of experimental diets

g/kg	**MFS**	**HFS**	**HFT**
Casein	200	200	200
Cornstarch	100	100	100
Sucrose	500	400	400
Cellulose	50	50	50
Soybean oil	100	200	200
Beef tallow	0	0	0
Mineral Mix•	35	39.3	39.3
Vitamin Mix°	10	11.2	11.2
L-Cysteine	3	3	3
Choline Bitartrate	2	2	2
	% of total energyl		
CHO	58	43	43
Fat	22	39	39
Protein	20	17	17
	Kcals per kilogram		
Energy Density	4.10	4.57	4.57

Dietary compositions of Low fat soy oil (LFS), High fat soy oil (HFS), and High fat tallow (HFT)

- Mineral mix composition, g/kg mix: $CaCo_3$,357; KH_2Po_4,196; $K_3C_6H_5O_7$-H_20, 70.78; NaCl, 74, K_2So_4, 46.6; MgO, 24: FeCl2-6H_2O, 3.6; ZnCL3, 1.65; MnCo3, 0.63; $CuCo_3$, 0.3; KIO $_3$, 0.01; Na_2SeO4, 0.01; NH_4MoO4-H_2O, 0.008; Na_2SiO_2, 1.45; $LiCl_2$, 0.017; H_3Bo_3, 0.08; NaF, 0.064; $NiCo_3$, 0.032; NH_4VO_3, 0.0066

° Vitamin mix obtained from Teklad, Madison, WI, catalog #40060

Table 2. Pearson correlation coefficients among fasting blood parameters, body weight, body fat percentage, insulin sensitivity indexes, and TNF-α mRNA expression levels in rats fed moderate-fat or high-fat diets.

	Insulin	**Glucose**	**Leptin**	**HOMA-IR**	**ISI**
	R value Significance	R value Significance	R value Significance	R value Significance	R value Significance
Body wt.	r = 0.431 p<0.05	r = 0.064 NS	r = 0.414 p<0.05	r = 0.543 p<0.005	r = -0.668 p<0.0005
% BF	r = 0.685 p<0.005	r = -0.040 NS	r = 0.570 p<0.005	r = 0.616 p<0.005	r = - 0.628 p<0.005
TNF-α muscle	r = 0.048 NS	r = 0.040 NS	r = 0.560 p<0.05	r = 0.026 NS	r = -0.088 NS
TNF-α liver	r = 0.492 p < 0.05	r = 0.411 p < 0.005	r = 0.089 NS	r = 0.545 p < 0.05	r = -0.485 p < 0.05
TNF-α fat	r = -0.192 NS	r = 0.262 NS	r = 0.166 NS	r = -0.149 NS	r = 0.019 NS

HOMA-IR, Homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; %BF, percentage body fat

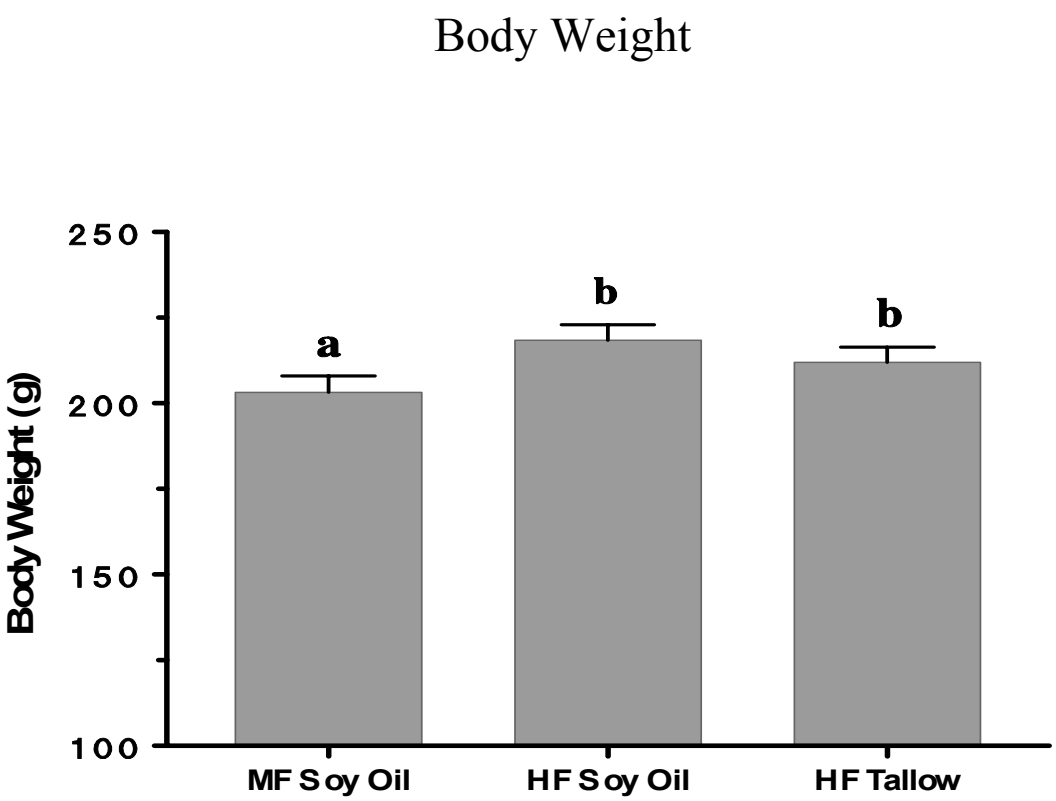

Figure 1. Body weight of female rats fed Moderately high fat soybean oil (MFS), High fat soybean oil (HFS), or High fat tallow (HFT) diets. ($p < 0.005$)

[a b] means without a common letter are not significantly different; n = 10 rats per group

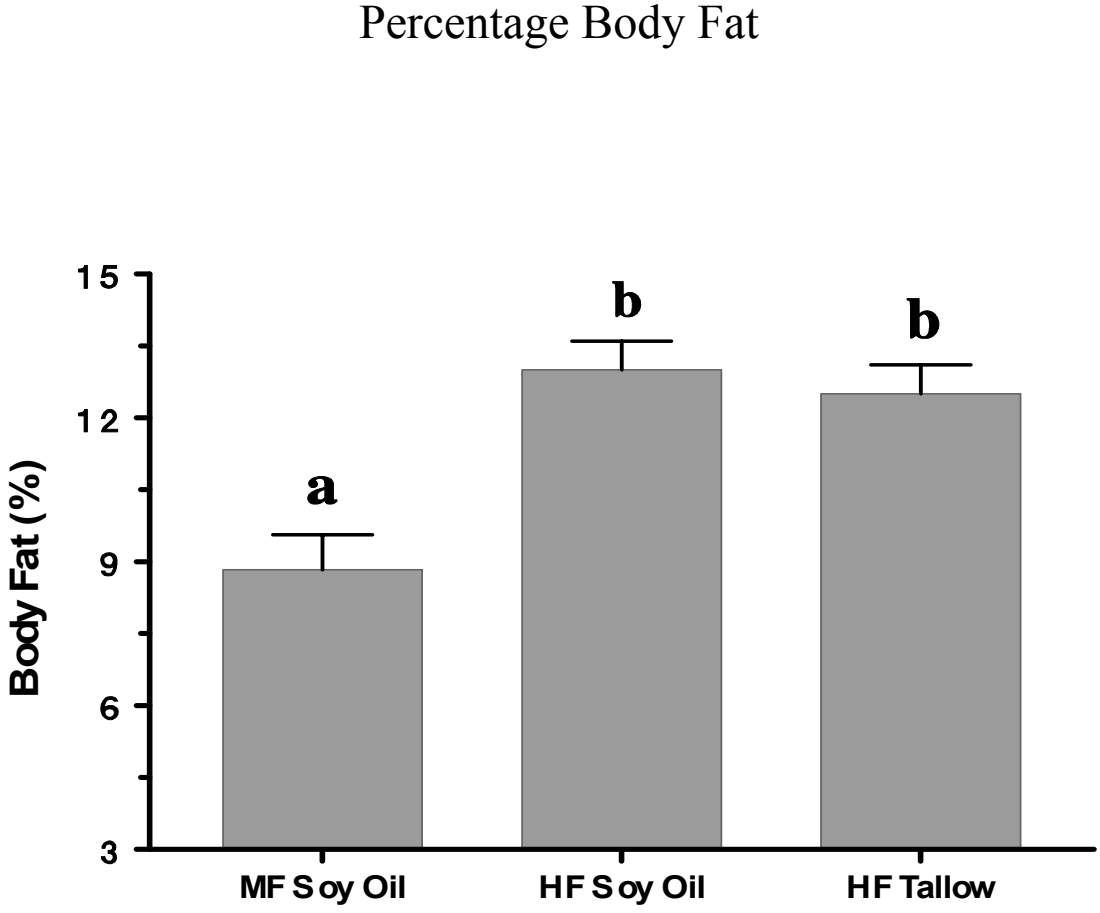

Figure 2. Percentage of body fat in female rats fed Moderately high fat soybean oil (MFS), High fat soybean oil (HFS), or High fat tallow (HFT) diets after 10 weeks of diet treatment, as measured by Dual X-Ray Absorptiometry analysis. ($p < 0.005$)

[a b] means without a common letter are not significantly different; n = 10 rats per group.

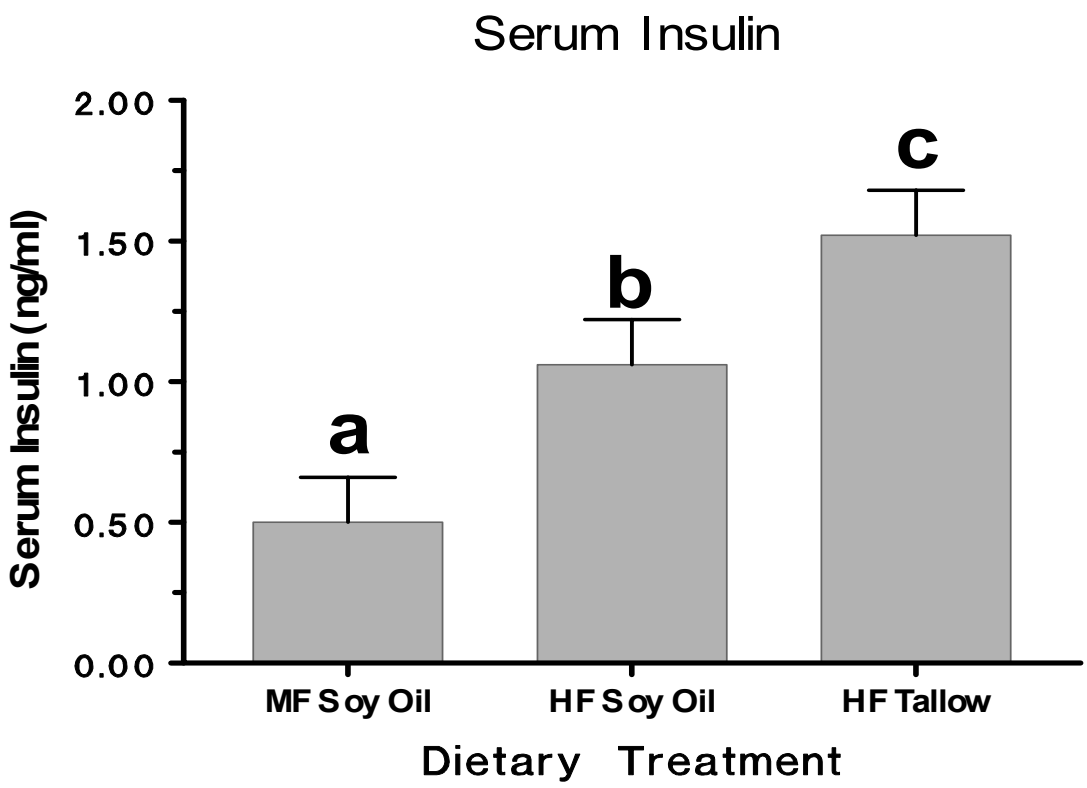

Figure 3. Serum insulin levels of female rats fed Moderately high fat soybean oil (MFS), High fat soybean oil (HFS), or High fat tallow (HFT) diets.

[a b c] Means without a common letter differ ($p < 0.05$); n = 10 rats per group

HOMA-IR

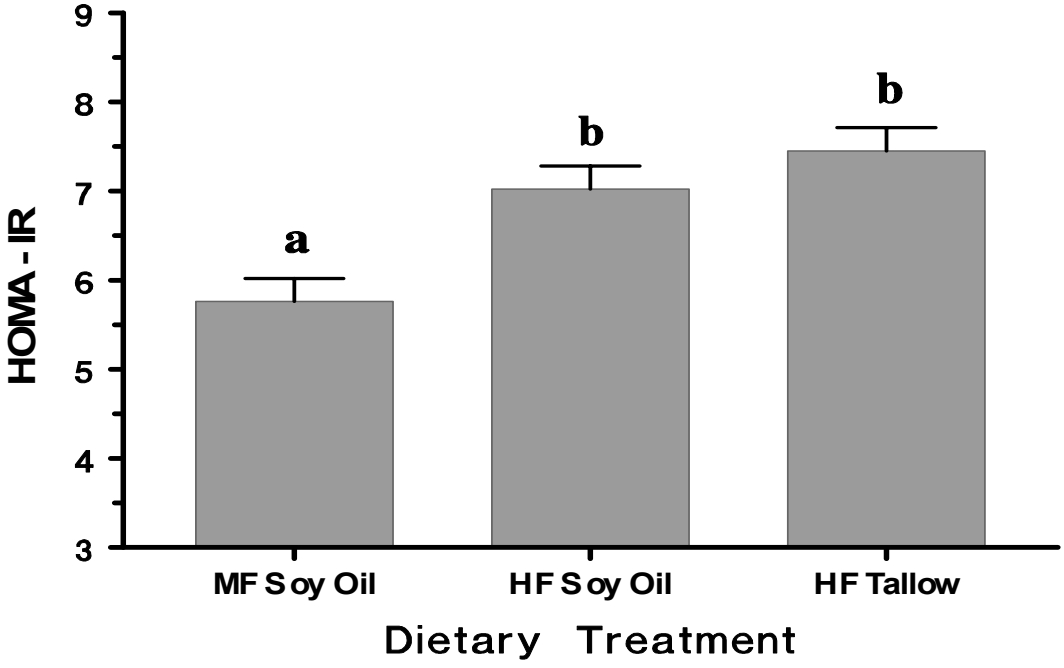

Figure 4. Insulin resistance index of female rats fed Moderately high fat soybean oil (MFS), High fat soybean oil (HFS), or High fat tallow (HFT) diets.

[a b] Means without a common letter differ ($p < 0.005$) ; n = 10 rats per group.

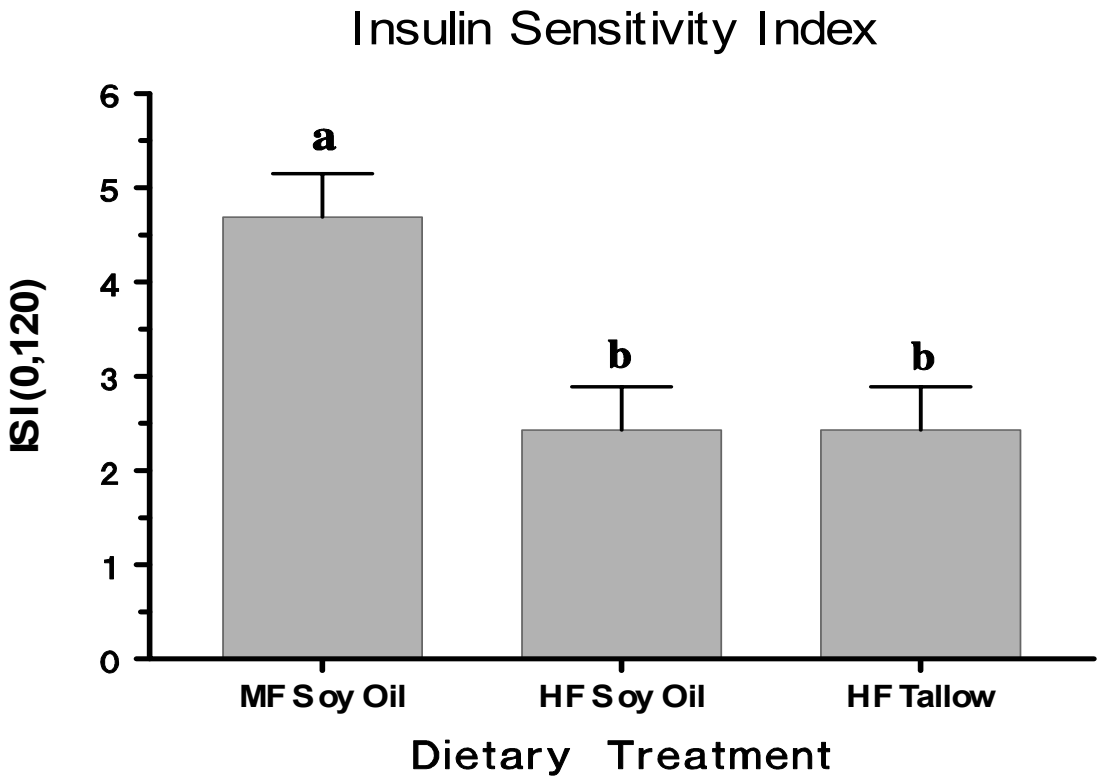

Figure 5. Insulin sensitivity index (ISI) of female rats fed Moderately high fat soybean oil (MFS), High fat soybean oil (HFS), or High fat tallow (HFT) diets.

[a b] Means without a common letter differ ($p < 0.05$); n = 10 rats per group.

Adipose Tissue TNF- α Fold Expression

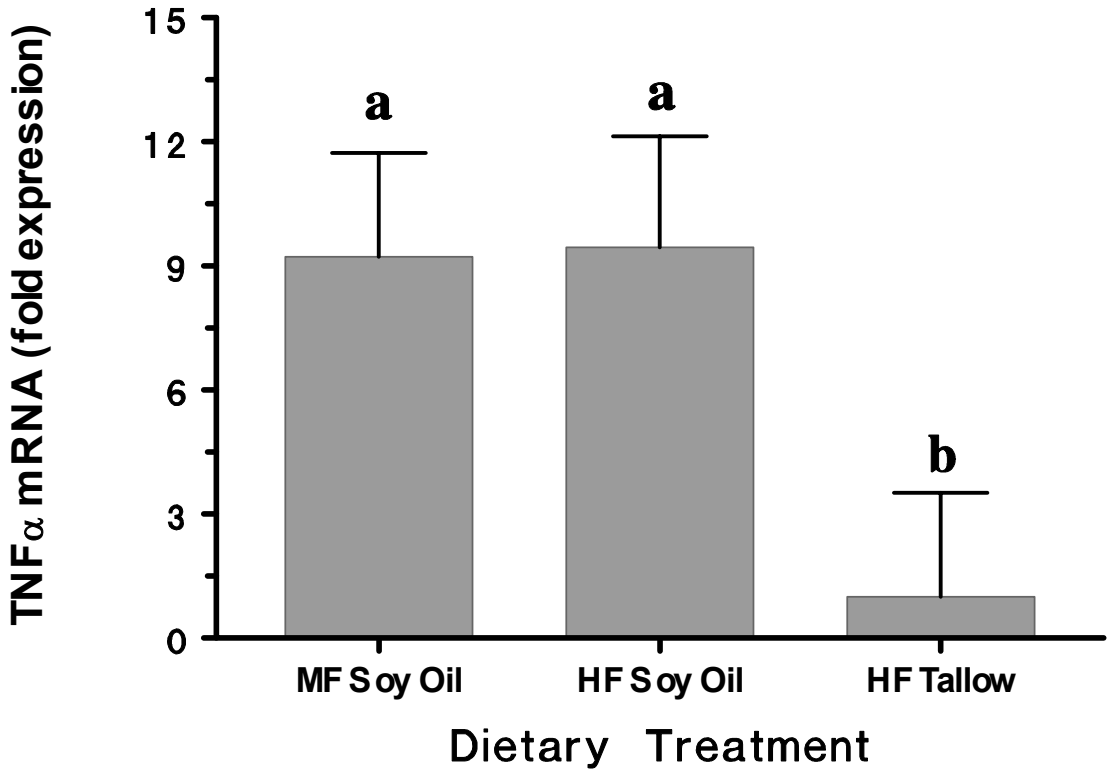

Figure 6. TNF-α mRNA fold expression of female rats fed Moderately high fat soybean oil (MFS), High fat soybean oil (HFS), or High fat tallow (HFT) diets.

[a b] Means without a common letter differ ($p < 0.05$); n = 10 rats per group.

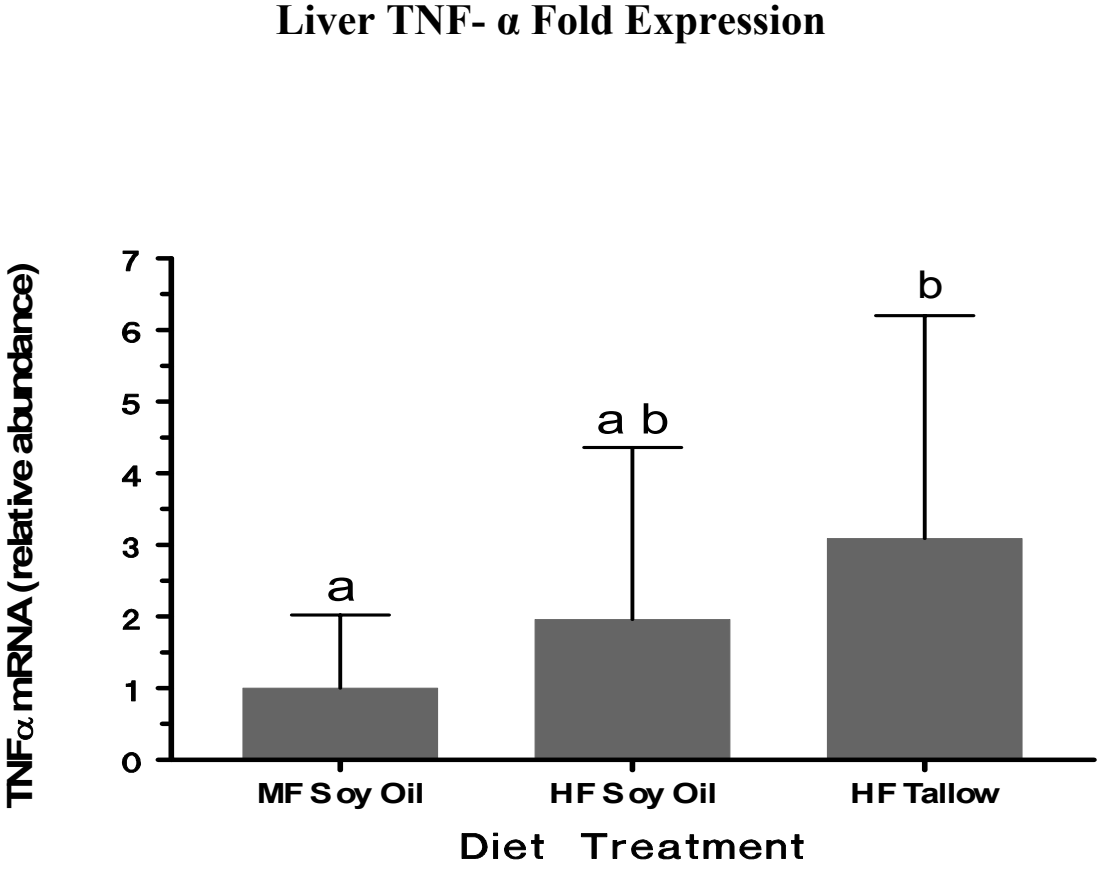

Figure 7. TNF-α mRNA fold expression of female rats fed Moderately high fat soybean oil (MFS), High fat soybean oil (HFS) or High fat tallow (HFT) diets.

[a b] Means without a common letter differ ($p < 0.05$); n = 10 rats per group.

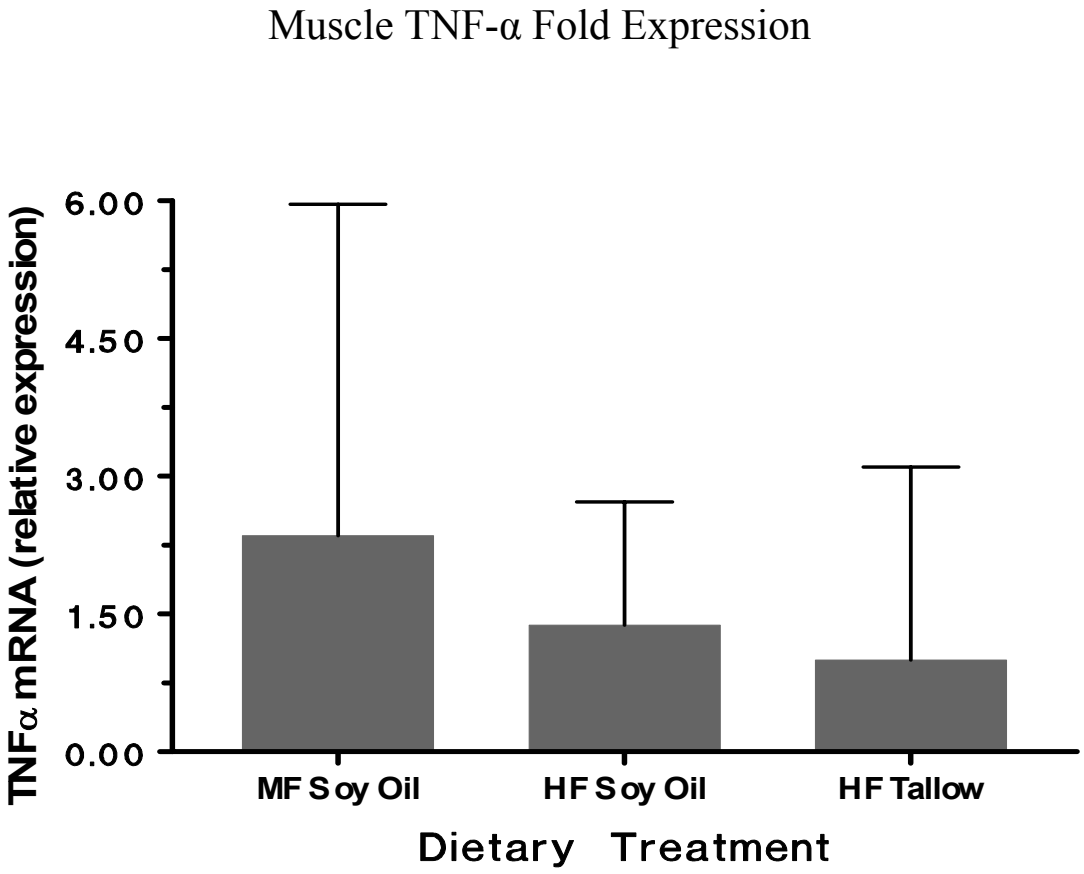

Figure 8. TNF-α mRNA fold expression of female rats fed Moderately high fat soybean oil (MFS), High fat soybean oil (HFS), or High fat tallow (HFT) diets. There were no significant differences in TNF- α fold expression between groups; n = 10 rats per group

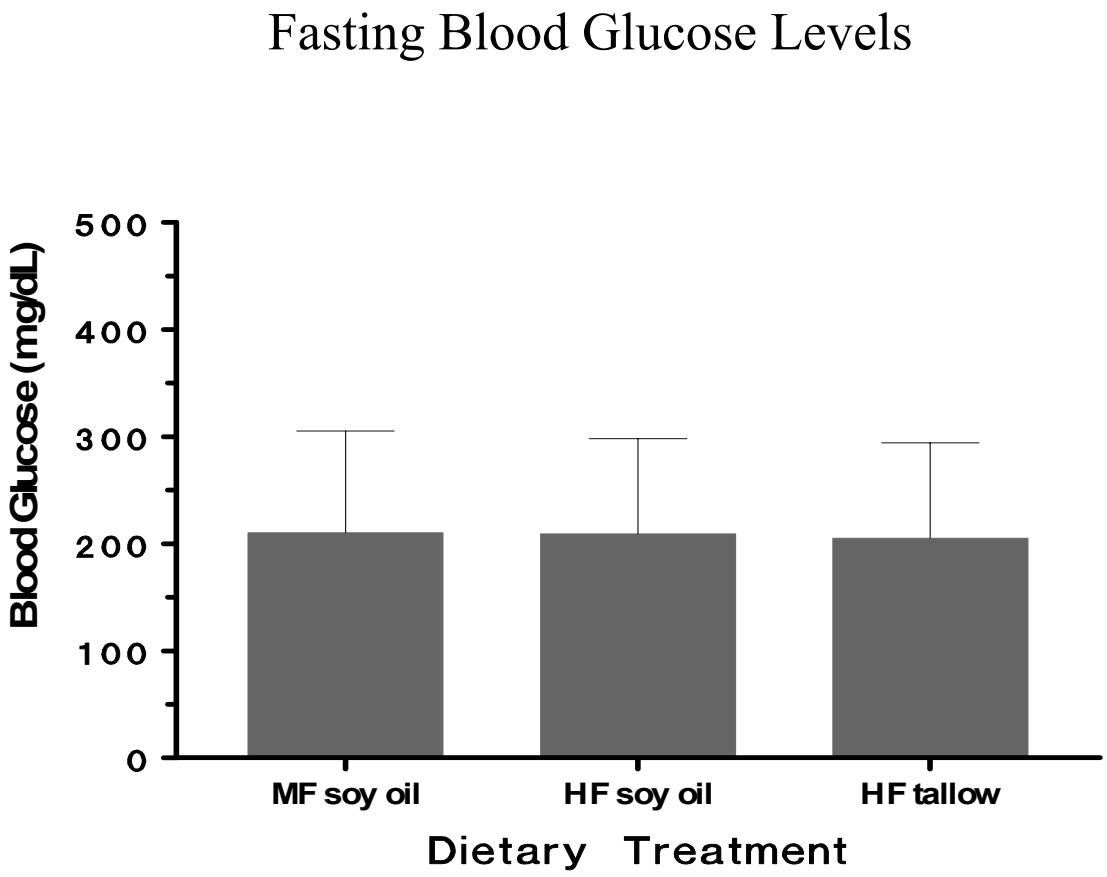

Figure 9. Blood glucose levels of female rats fed Moderately high fat soybean oil (MFS), High fat soybean oil (HFS), or High fat tallow (HFT) diets. There were no significant differences between groups; n = 10 rats per group

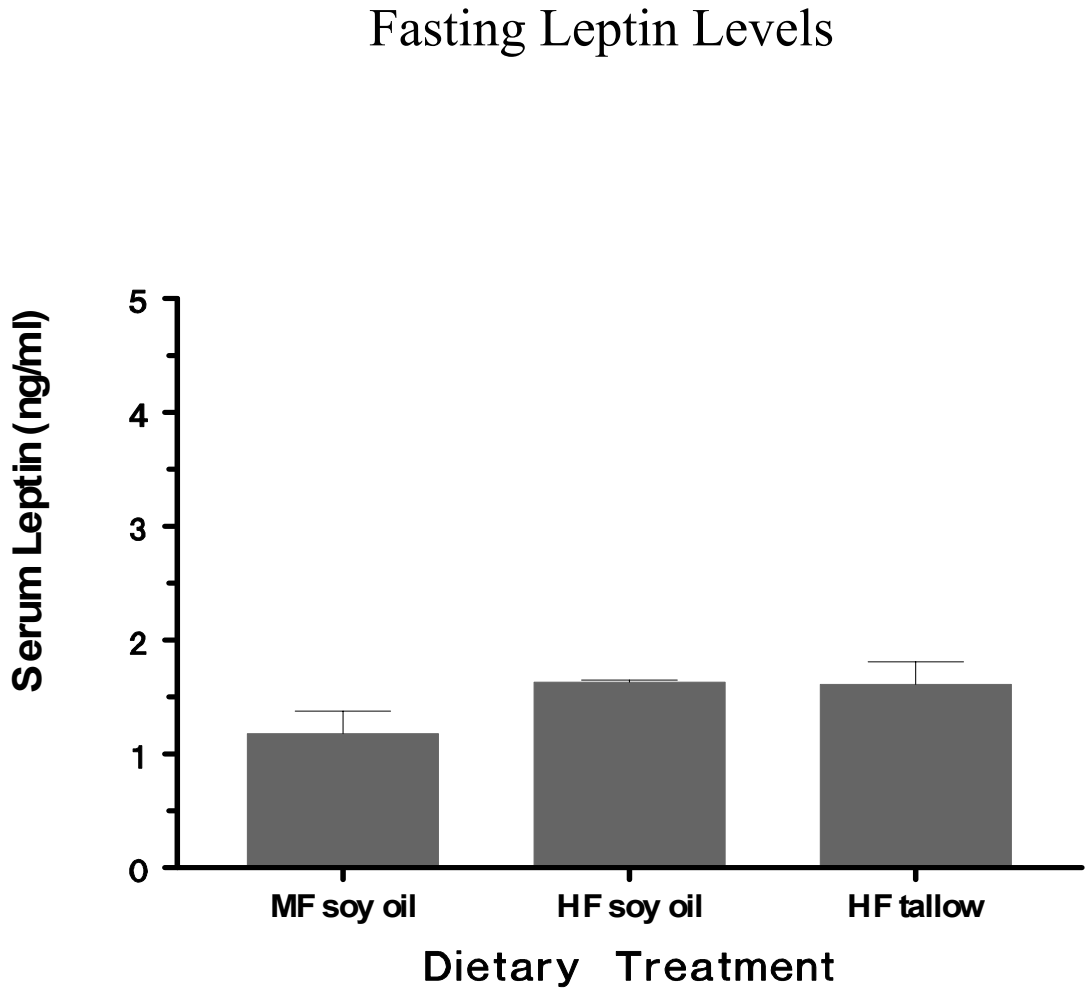

Figure 10. Fasting leptin levels of female rats fed Moderately high fat soybean oil (MFS), High fat soybean oil (HFS), or High fat tallow (HFT) diets. There were no significant differences between groups ; n = 10 rats per group

Sources Cited

1. Wellen KE, Hotamisligil GS: Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest.* 112: 1785-1788, 2003

2. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*. 112: 1821-1830, 2003

3. Bullo-Bonet M., Garcia-Lorda P, Lopez-Soriano F, Argiles J, Salas-Salvado J: Tumor necrosis factor, a key role in obesity. *F.E.S.B.* 451: 215- 219, 1999

4. Hotamisligil GS: The role of TNF-α and TNF receptors in obesity and insulin resistance. *J Int Med* 245: 621-625, 1999

5. Qi C, Pekala PH: Tumor necrosis factor-α-induced insulin resistance in adipocytes. *P.S.E.B.M*. 223: 128-135, 2000

6. Hotamisligil GS: The role of TNF-α and TNF receptors in obesity and insulin resistance. *J Int Med*. 245: 621-625,1999

7. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZM, Karin M: Reversal of obesity and diet-induced insulin resistance with salicylates or targeted disruption of Ikk-β. *Science*. 293: 1673-1677, 2001

8. Liu LS, Spelleken M, Rohrig K, Hauner H, Eckel J: Tumor necrosis factor-α acutely inhibits insulin signaling in human adipocytes: implication of the p80 tumor necrosis factor receptor. *Diabetes* 47: 515-522, 1998

9. Uysal KT, Wiesbrock SM, Hotamisligil GS: Functional analysis of tumor necrosis factor (TNF) receptors in TNF-α -medicated insulin resistance in genetic obesity. *Endocrinology*. 139: 4832-4838, 1998

10. Hotamisligil GS, Budavari A, Muray D, Spiegelman BM: Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. *J Clin Invest.* 94: 1543-1549, 1994

11. Stephens JM, Pekala PH: Transcriptional repression of the GLUT4 & C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-α. *J Biol Chem*. 266: 21839-21845, 1991

12. Ruan H and Lodish HF: Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-α. *Cytokine & Growth Factor Reviews*. 14: 447-455, 2003

13. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM: Tumor necrosis factor-α inhibits signaling from the insulin receptor. *Proc Natl. Acad. Sci*. 91: 4854-4858, 1994

14. Hotamisligil GS, Pascal P, Budavari A, Ellis R, White MF, and Spiegelman BM: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α and obesity-induced insulin resistance. *Science*. 271: 665 668, 1996

15. Hotamisligil GS, Spiegelman BM: TNF-α: a key component in obesity-diabetes link. *Diabetes* 43: 1271-1278, 1994

16. Hotamisligil GS: Mechanism of TNF-α-induced insulin resistance. *Clin Endocrinol Diabetes* 107: 119-125, 1999

17. Mal K, Wiesbrock S, Hotamisligil GS: Functional analysis of tumor necrosis factor (TNF) receptors in TNF-α-mediated insulin resistance in genetic obesity. *Endocrinology* 139: 4832-4838, 1998

18. Brown JL, Spicer MT, Spicer LJ: Effect of high-fat diet on body composition and hormone responses to glucose tolerance tests. *Endocrine* 19(3): 327-332, 2002

19. Lofgren P, van Harmelen V, Reynisdottir S, Naslund E, Ryden M, Rossner S, Arner P: Secretion of tumor necrosis factor-alpha shows a strong relationship to insulin-stimulated glucose transport in human adipose tissue. *Diabetes* 49: 688-692, 2000

20. Hube F, Hauner H: The two tumor necrosis factor receptors mediate opposite effects on differentiation and glucose metabolism in human adipocytes in primary culture. *Endocrinology*. 141: 2582-2588, 2000

21. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. *J Clin Invest*. 95: 2409 – 2415, 1995

22. U.S. Department of Health and Human Services: Healthy People 2010. Washington, D.C., U.S. Government Printing Office, 2000

23. USDA, DHHA: Nutrition and Your Health: Dietary Guidelines for Americans. Fifth Edition, 2000. (http://www.health.gov/dietary guidelines/dga2000/)

24. Subcommittee on Laboratory Animal Nutrition Committee, Animal Nutrition Board of Agriculture National Research Council: Nutrient Requirements of Laboratory Animals, 4th revised edition. 1995, p. 20

25. Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412-419, 1985

26. Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, Schneiderman N, Skyler JS, Marks JB: Validation of the insulin sensitivity index ($ISI_{0,120}$) : comparison with other measures. Diabetes Research and Clinical Practice 47: 177-184, 2000

27. SAS. 1999. *The SAS system for Windows: Version 8*. Cary, NC: Statistical Analysis Systems Institute.

28. Ott, L: *An introduction to statistical methods and data analysis*. Duxbury: North Scituate, MA, pp. 215-216, 1997

29. Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW: Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle TG and omega-3 fatty acids in muscle phospholipids.

Diabetes. 40: 280-289, 1991

30. Morin CL, Eckel RH, MarcelT, Pagliassotti MJ: High fat diets elevate adipose tissue-derived tumor necrosis factor-α activity. *Endocrinology* 138: 4665-4671, 1997

31. Lang CH, Dobrescu C, Bagby GJ: Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. *Endocrinology*. 130: 43-52, 1992

32. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA.: Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatocyte dysfunction. *Mol Cell*. 6: 87-97, 2000

33. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science*. 259: 87 – 90, 1993

34. Kern PH, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB: The expression of tumor necrosis factor in human adipose tissue. *J Clin Invest* 95: 2111- 2119, 1995

35. Borst S, Bagby G: Adipose tumor necrosis factor-α is reduced during onset of insulin resistance in Sprague-Dawley rats. *Cytokine* 26: 217-222, 2004

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This study was designed to investigate the effects of a HF diet on body composition and insulin, leptin, and glucose responses to OGTTs. Thirty female weanling SD rats were randomly assigned to consume either a HFT (39% kcals from fat), HFS (39% from fat) or MFS (22% kcals from fat) diet. Animals were provided isocallorically excess food energy in comparison to their required needs in order to accelerate weight gain. Oral glucose tolerance tests were performed after 10 weeks of diet treatment. Animals were then gavaged with 2 g glucose/kg BW following an overnight fasting period. Blood was sampled following the OGTT for measurement of serum insulin, leptin, and glucose levels. ISI and HOMA-IR values were also calculated. Body mass analysis was assessed using DXA. Animals were sacrificed and liver, muscle, and adipose tissue samples were obtained. Tissues were then homogenized and total RNA was collected. Quantification of gene expression of extracted RNA was attained using Real Time RT-PCR. A housekeeping gene, 18S, was used to normalize values.

Both HF diets induced significantly greater body weight and adiposity when compared to the MFS diet treatment. HFT diet increased serum insulin levels above that of the HFS and MFS diets. HFT and HFS diets decreased insulin sensitivity

while increasing insulin resistance, observed through HOMA-IR analysis. Liver TNF-α mRNA abundance was significantly greater in the HFT diet compared to the other dietary treatments. Adipose TNF-α mRNA abundance was significantly less in HFT compared to both HFS and MFS treatment diets.

Conclusions

In order to test the previously listed hypotheses, the objectives of this study were to determine the effects of dietary fat content on weight gain and adiposity in rats fed isocaloric diets that were composed of either HFT, HFS, or MFS content and to determine the effects of dietary fat content on serum insulin, glucose, and lepin levels as well as TNF-α mRNA abundance. Each hypothesis is addressed below:

H_1 Rats fed HFT diets will have greater body mass and adiposity compared to rats fed isocaloric HFS and MFS diets.

In this study, rats fed both HFT and HFS diets developed greater body mass ($p < 0.05$) compared to MFS fed rats, which was evident at the end of the 10 diet treatment period. There was no significant difference in body weight between rats fed HFT and HFS diets. Percentage of body fat was greater ($p < 0.005$) in both HFT and HFS rats following the 10 week diet treatment. There was no significant difference in adiposity between the HFT and HFS fed rats.

H_2 Rats fed HFT diets will produce significantly higher fasting insulin levels in response to oral glucose tolerance tests compared to rats fed HFS or MFS diets.

Fasting insulin levels of rats fed HFT diets were significantly higher ($p < 0.0005$) compared to HFS and MFS fed rats. HFS diet also had significantly

greater ($p < 0.05$) fasting insulin levels when compared to the MFS diet.

H_3 Rats fed HFT diets will develop greater insulin resistance compare to isocallorically fed HFS and MFS fed rats.

HFT diet significantly increased ($p < 0.0001$) insulin resistance, determined by HOMA-IR analysis, compared to the MFS diet. To a lesser extent, HFS significantly increased ($p < 0.005$) insulin resistance compared to the MFS diet. Both HFT and HFS diets significantly decreased ($p < 0.005$) insulin sensitivity, assessed using ISI equation, compared to the MFS diet.

H_4 TNF-α mRNA levels within liver and adipose tissues will be significantly higher in rats fed HFT diets, compared to rats fed isocaloric HFS and MFS dietary treatments.

Liver TNF-α mRNA levels were significantly increased ($p < 0.05$) in rats fed HFT diets compared to HFS and MFS fed rats. Adipose TNF-α expression levels were significantly decreased ($p < 0.05$) in rats fed HFT diets compared to HFS and MFS fed rats.

Recommendations

This study was designed to evaluate the effects of high fat feeding with differing fatty acid compositions during rapid growth in female rats. There were however some limitations to our studies. One species and gender of rat were used in our study. Future study would benefit from using differing strains and rats of both genders, which would allow of the examination of the effects on a larger scale. A longitudinal study would also be of value, in that, more comprehensive data on the long term effects of a habitual HF diet could provide insight into the mechanisms involved in obesity and TNF- α gene expression.

In this study, TNF-α mRNA levels were measured in order to identify any changes that may occur during differing dietary treatments. More in depth evaluation of these findings may be helpful in identifying the role of TNF-α in obesity. TNF-α is only one of many molecules and pro-inflammatory cytokines involved in obesity related insulin resistance. Therefore, the study of other cytokines involved in adipocyte metabolism and function should also be evaluated.

Well controlled longitudinal study should be performed to determine if high saturated fat feeding in human subjects alters TNF-α production and expression. Ideally, all metabolic parameters, hormones, and TNF- α receptors could be examined to identify any correlations with body composition and high saturated fat feeding. The benefits of identifying many of the mechanisms and molecules involved in insulin resistance and Type II diabetes could some day lead to a therapeutic alternative for those suffering from the disease.

BIBLIOGRAPHY

1. Flegal KM, Carroll MD, Ogden CL, Johnson CL: Prevalence and trends in obesity among US adults, 1999-2000. *JAMA* 288: 1723-1727, 2002

2. U.S. Department of Health and Human Services. The Surgeon General's call to action to prevent and decrease overweight and obesity. Rockville, Maryland: U.S. Department of Health and Human Services, Public Health Service, Office of Surgeon General, 2001

3. U.S. Department of Health and Human Services: Healthy People 2010. Washington, D.C., U.S. Government Printing, 2000

4. Trevisan R, Vedovato M, Tiengo A: The epidemiology of diabetes mellitus. *Nephrol Dial Transplant* 13: S2-S5, 1998

5. USDA, DHHA: Nutrition and Your Health: Dietary Guidelines for Americans. Fifth Edition, 2000. (http://www.health.gov/dietary guidelines/dga2000/)

6. American Diabetes Association: Nutrition principles and recommendations in diabetes. *Diabetes Care* 27: S36-S46, 2004

7. Buettner R, Newgard CB, Rhodes CJ, O'Doherty RM: Correction of diet-induced hyperglycemia, hyperinsulinemia, and skeletal muscle insulin resistance by moderate hyperleptinemia. *Am J Physiol Endocrinol Metab* 278: E563-E569, 2000

8. Commerford SR, Pagliassotti MJ, Melby CL, Wei Y, Gayles EC, Hill JO: Fat oxidation, lipolysis, and free fatty acid cycling in obesity-prone and obesity-resistant rats. *Am J Physiol Endocrinol Metab* 279: E875-E885, 2000

9. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor α in human obesity and insulin resistance. *J Clin Invest*. 95: 2409-2415, 1995

10. Beutler B, Cerami S: The biology of cachectin / TNF-α primary mediator of the host response. *Annu Rev Immunol* 7: 625-655, 1989

11. Hofmann CK, Lorenz SS, Braithwaite BJ, Palazuk GS, Hotamisligil GS, Spielgelman BM: Altered gene expression for tumor necrosis factor-α and its receptors during drug and dietary modulation of insulin resistance. *Endocrinology* 134: 264-270, 1994

12. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science* 259: 87-90, 1993

13. Hotamisligil GS, Spiegelman BM: TNF-α: a key component in obesity-diabetes link. *Diabetes* 43: 1271-1278, 1994

14. Morin C, Eckel R, Marcel T, Pagliassotti M: High fat diets elevate adipose tissue-derived tumor necrosis factor-α activity. *Endocrinology* 138(11): 4665- 4671, 1997

15. Hotamisligil GS: Mechanism of TNF-α-induced insulin resistance. *Clin Endocrinol Diabetes* 107: 119-125, 1999

16. Mal K, Wiesbrock S, Hotamisligil GS: Functional analysis of tumor necrosis factor (TNF) receptors in TNF-α-mediated insulin resistance in genetic obesity. *Endocrinology* 139: 4832-4838, 1998

17. Qi C, Pekala P: Tumor necrosis factor-α-induced insulin resistance in adipocytes. *P.S.E.B.M*. 223:128-135, 2000

18. Lofgren P, van Harmelen V, Reynisdottir S, Naslund E, Ryden M, Rossner S, Arner P: Secretion of tumor necrosis factor-alpha shows a strong relationship to insulin-stimulated glucose transport in human adipose tissue. *Diabetes* 49: 688-692, 2000

19. Hotamisligil GS, Pascal P, Budavari A, Ellis R, White MF, Spiegelmanm BM: IRS-1 mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α and obesity induced insulin resistance. *Science* 271; 665-668, 1996

20. Hotamisligl GS, Murray DL, Choy LN, Spiegelman BM: Tumor necrosis factor-α inhibits signaling from the insulin receptor. *Proc Natl Acad Sci* 91; 4854-4858, 1994

21. Stephens JM, Pekala PH: Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-α. *J Biol Chem* 266 (32): 21839-21845, 1991

22. Kirchgessner TG, Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS: Tumor necrosis factor-α contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes. *J Clin Invest*. 100; 2777-2782, 1997

23. Liu LS, Spelleken M, Rohrig K, Hauner H, Eckel J: Tumor necrosis factor-α acutely inhibits insulin signaling in human adipocytes: implication of the p80 tumor necrosis factor receptor. *Diabetes* 47: 515-522, 1998

24. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS: Protection from obesity-induced insulin resistance in mice lacking TNF-α function. *Nature* 389: 610-614, 1997

25. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB: The expression of tumor necrosis factor in human adipose tissue. *J Clin Invest* 95: 2111-2119, 1995

26. Ogden CL: Prevalence and trends in overweight among US children and adolescents, 1999-2000. *JAMA* 288: 1728-1732, 2002

27. American Diabetes Association: Type II diabetes in children and adolescents. Diabetes Care 23: 381-389, 2000

28. Alberti K, Zimmet P: Definition, diagnosis and classification of diabetes mellitus and its complications, part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539-553, 1998

29. Centers for Disease Control and Prevention: National Diabetes Fact Sheet: general information and national estimates on diabetes in the United States, 2003. Revised edition. Atlanta, GA. Department of Health and Human Services, Centers for Disease Control and Prevention, 2004

30. The Expert Committee on The Diagnosis and Classification of Diabetes Mellitus: Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 20: 1183-1197, 1997

31. Bjorntorp P: Obesity: a chronic disease with alarming prevalence and consequences. *J Inter Medicine* 244: 267-269, 1998

32. Astrup A, Buemann B, Toubro S, Raben A: Defects in substrate oxidation involved in the predisposition to obesity. Proceedings of the Nutrition Society 55: 817-828, 1996

33. Hu FB: Sedentary lifestyle and risk of obesity and type II diabetes. *Lipids* 38: 103-108, 2003

34. Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G: Relationship between degree of obesity and in vivo insulin action in man. *Am J Physiol* 248: E286-E291, 1985

35. Iida, M, Murakami T, Sei M, Kumajima M, Yamada M, Aono T, Shima K: Circulating leptin did not associate with the development of the hyperglycemia accompanied by insulin sensitivity in spontaneous non-insulin dependent diabetes mellitus model Otsuka-Long-Evans-Tokushima-Fatty rats. *Reg Peptide* 77: 141-146, 1998

36. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112: 1821-1830, 2003

37. Weisberg SP, et al: Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112: 1796-1808, 2003

38. Grimble RF: Inflammatory status and insulin resistance. *Curr. Opn. Clin. Nutr. Metab. Care*. 5: 551-559, 2002

39. Saltiel A, Kahn CR: Insulin signaling and the regulation of glucose and lipid metabolism. *Nature* 414: 799-806, 2001

40. Fujimoto, WY: The importance of insulin resistance in the pathogenesis of type II diabetes mellitus. *Am J Med* 108: 9S-14S, 2000

41. Carnevale Schianca GPC, Rossi A, Sainaghi PP, Maduli E, Bartoli E: The significance of impaired fasting glucose versus impaired glucose tolerance. *Diabetes Care* 26: 1333-1337, 2003

42. Uusitupa M, Lindi V, Louheranta A, Salopuro T, Lindstrom J, Tuomilehto J: Long-term improvement in insulin sensitivity by changing lifestyles of people with impaired glucose tolerance. *Diabetes* 52: 2532-2538, 2003

43. Hawley, JA: Exercise as a therapeutic intervention for the prevention and treatment of insulin resistance. *Diabetes Metab Res Rev* 20: 383-393, 2004

44. Law, M: Dietary fat and adult diseases and the implications for childhood nutrition: an epidemiologic approach. *Am J Clin Nutr* 72: 1291S-1296S, 2000

45. Lovejoy JC, Windhauser MM, Rood JC, Bretonne JA: Effects of a controlled high-fat versus low-fat diet on insulin sensitivity and leptin levels in African-American and Caucasian women. *Metabolism* 47: 1520-1524, 1998

46. Mayer EJ, Newman B, Quesenberry CP, Selby JV: Usual dietary fat intakes and insulin concentrations in healthy women twins. *Diabetes Care* 59: 1069- 1074, 1993

47. Mosca CL, Marchall JA, Grunwald GK, Cornier MA, Baxter J: Insulin resistance as a modifier of the relationship between dietary fat intake and weight gain. *Int J Obes* 28: 803-812, 2004

48. Brown JL, Spicer MT, Spicer LJ: Effect of high-fat diet on body composition and hormone responses to glucose tolerance tests. *Endocrine* 19(3): 327-332, 2002

49. Storlien LH, James DE, Burleigh KM, Chisholm DJ, Kraegen EW: Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. *Am J Physiol* 251: E576-E583, 1986

50. Watarai T, Kobayashi M, Takata Y, Sasaoka T, Isasaki M, Shigeta Y: Alteration of insulin-receptor kinase activity by high-fat feeding. *Diabetes* 37: 197-1404, 1988

51. Nagy K, Levy J, Grunberger G: High-fat feeding induces tissue specific alterations in proportion of activated insulin receptors in rats. *Acta Endocrinol* 122: 361-368, 1990

52. Van Dam RM, Willett WC, Rimm EB, Stampfer MJ, Hu FB: Dietary fat and meat intake in relation to risk of type II diabetes in men. *Diabetes Care* 25: 417-424, 2002

53. Hu FB, van Dam RM, Liu S: Diet and risk of type II diabetes: the role of types of fat and carbohydrate. *Diabetologia* 44: 805-817, 2001

54. Vessby B, Uusitupa M, Herman K: Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU study. *Diabetologia* 44: 312-319, 2001

55. Summers LM, Fielding BA, Bradshow HA: Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. *Diabetologia* 45: 369-377, 2002

56. Heine RJ, Mulder C, Popp-Snijders C, van der Meer J, van der Veen EA: Linoleid-acid-enriched diet: long term effects on serum lipoprotein and apolipoprotein concentrations and insulin sensitivity in non insulin-dependent diabetic patients. *Am J Clin Nutr* 49: 448-456, 1989

57. Schawb US, Karhapaa P, Sarkkinen ES, Salminen I, Laakso M, Uusitupa MI: Metabolic effects of diets rich in saturated and 4-6 polyunsaturated fatty acids in healthy young females. *Diabetes Nutr Metab* 10: 35-38, 1997

58. Uusitupa M, Schawb U, Makimattila S: Effects of two high-fat diets with different fatty acid composition on glucose and lipid metabolism in healthy young women. *Am J Clin Nutr* 59: 1310-1316, 1994

59. Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW: Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. *Diabetes* 40: 280-289, 1991

60. Lardinois CK, Starich GH: Polyunsaturated fats enhance peripheral glucose utilization in rats. *J Am Coll Nutr* 10: 340-345, 1991

61. Boozer CN, Schoenbach G, Atkinson RL: Dietary fat and adiposity: a dose-response relationship in adult male rats fed isocalorically. *Am J Physiol* 268: E546 – E550, 1995

62. Tremblay A, Plourde G, Despres JP, Leblanc C, Bouchard C: Impact of dietary fat content and fat oxidation on energy intake in humans. *Am J Clin Nutr* 49: 799 – 805, 1989

63. Heitmann BL, Lissner L, Sorensen TIA, Bengtsson C: Dietary fat intake and weight gain in women genetically predisposed for obesity. *Am J Clin Nutr* 61: 1213 – 1217, 1995

64. Bray GA, Popkin BM: Dietary fat intake dose affect obesity! *Am J Clin Nutr* 68: 1157-1173, 1998

65. Doucet E, Almeras N, White MD, Despres JP, Bouchard C, Tremblay A: Dietary fat composition and human adiposity. *Eur J Clin Nutr* 52: 2-6, 1998

66. Astrup A: Dietary composition, substrate balances and body fat in subjects with a predisposition to obesity. *Int J Obes Relat Metab Disord* 17: S32-S36, 1993

67. Lichtenstein A, Kennedy E, Barrier P: Dietary fat consumption and health. *Nutr Rev* 56: S3-S19, 1998

68. George V., Tremblay A., Despres, J., Leblanc C, Bouchard C: Effects of dietary fat content on total and regional adiposity in man and women. *Int J Obes* 14: 1085-1094, 1990

69. Rouzer CA, Cerami A: Hypertriglyceridemia associated with Trapansoma brucei brucei infection in rabbits: role of defective triglyceride removal. *Mol Biochem Parasitol* 2: 31-38, 1980
70. Kawakami M, Cerami A: Studies of endotoxin-induced decrease in lipoprotein lipase activity. *J Exp Med* 154: 631-639, 1981

71. Torti FM, Dieckmann B, Beutler B, Cerami A, Ringold GM: A macrophage factor inhibits adipocytes gene expression: an in vivo model of cachexia. *Science* 229: 867-869, 1985

72. Pekala PH, Price SR, Horn CA, Hom BE, Moss J, Cerami A: Model for cachexia in chronic disease: secretory products of endotoxin-stimulated macrophages induce a catabolic state in 3T3-L1 adipocytes. *Trans Assoc Am Physicians* 97: 251-259, 1984

73. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B: An exdotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA* 72: 3666-3670, 1975

74. Caput D, Beutler B, Hartog K, Brown-Shimer S, Cerami A: Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammation mediators. *Proc Natl Acad Sci USA* 83: 1670-1674, 1986

75. Desch CE, Dobrina A, Aggarwal BB, Harlan JM: Tumor necrosis factor-α exhibits greater proinflammatory activity than lymphotoxin in vitro. *Blood* 75: 2030-2034, 1990

76. Lang CH, Dobrescu C, Bagby GJ: Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. *Endocrinology* 30: 43-52, 1992

77. Bullo-Bonet M., Garcia-Lorda P, Lopez-Soriano F, Argiles J, Salas-Salvado J: Tumor necrosis factor, a key role in obesity. *F.E.S.B.* 451: 215- 219, 1999

78. Hotamisligil GS: The role of TNF-α and TNF receptors in obesity and insulin resistance. *J Int Med* 245: 621-625, 1999

79. Hotamisligil GS, Budavari A, Muray D, Spiegelman BM: Reudced tyrosine kinase activity of the insulin receptor in obesity-diabetes. *J Clin Invest* 94: 1543-1549, 1994

80. Hotamisligil GS, Peraldi P, Spiegerrnan B: The molecular link between obesity and diabetes. *Curro Opin Endocrino Diabetes* 3: 16-23, 1996

81. Stephens JM, Lee J, Pilch PF: Tumor necrosis factor-α-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without loss of insulin receptor mediated signal transduction. J Biol Chem 272: 971-976,1997

82. Mohamed-Ali V, Pinkney JH, Coppack SW: Adipose tissue as an endocrine and paracrine organ. *Int J Obesity* 22: 1145-1158, 1998

83. Frayn KN: Role of non-esterified fatty acids in the metabolic changes of obesity. *Int J Obesity* 20: S7-S10, 1996

84. Ruan H, Hacohen N, Golub T, Parijs LV, Lodish HF: Tumore necrosis factor-α suppresses adipocytes-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes. *Diabetes*. 51: 1319-1336, 2002

85. Borst S, Bagby G: Adipose tumor necrosis factor-α is reduced during onset of insulin resistance in Sprague-Dawley rats. *Cytokine* 26: 217-222, 2004

86. Subcommittee on Laboratory Animal Nutrition Committee on Animal Nutrition Board of Agriculture National Research Council: Nutrient Requirements of Laboratory Animals, 4th revised edition. 1995, p. 20

87. Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412-419,1985

88. Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, Schneiderman N, Skyler JS, Marks JB: Validation of the insulin sensitivity index ($ISI_{0,120}$) : comparison with other measures. Diabetes Research and Clinical Practice 47: 177-184, 2000

89. SAS. 1999. *The SAS system for Windows: Version 8*. Cary, NC: Statistical Analysis Systems Institute.

90. Ott, L: *An introduction to statistical methods and data analysis*. Duxbury: North Scituate, MA, pp. 215-216, 1997

APPENDICES

APPENDIX A

Oklahoma State University
Institutional Animal Care and Use Committee (IACUC)

Protocol Expires: 9/30/03

Date : Friday, July 27, 2001 Animal Care and Use From (ACUF) No: HE0116

Proposal Title: EFFECTS OF CHROMIUM AND SUCROSE FEEDING ON METABOLIC HORMONES AND SUBSTRATE UTILIZATION

Principal Investigator:

Maria Spicer

Reviewed and Processed as: Full Committee **Modification**

Approval Status Recommended by Reviewer(s) : Approved

Modification approved per memo on July 03, 2001.

Signatures :

Dr. Kent Olson, IACUC Chairperson

Friday, July 27, 2001
Date

Approvals are valid for three calendar years, after which time a request for renewal must be submitted. Any modifications to the research project or course must be submitted for review and approval by the IACUC, prior to initiating any changes in animal use. Modifications do not affect the original approval period. Modification approvals are valid for the duration o the protocol approval (see protocol expiration date). Approved projects are subject to monitoring by the IACUC. OSU is a USDA registered research facility and maintains an Animal Welfare Assurance document with the Public Health Service Office of Laboratory Animal Welfare, Assurance number AA3722-01.

APPENDIX B

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	weight	Zerogluc	gluAUC	zeroins	pctfat	ttnffat	ttnfmusc	ttnfliv
weight		0.06411 0.7557 26	-0.00334 0.9871 26	0.43188 0.0311 25	0.55467 0.0033 26	0.01707 0.9384 23	0.16719 0.4571 22	0.21426 0.3262 23
Zerogluc	0.06411 0.7557 26		0.49245 0.0106 26	0.16222 0.4385 25	-0.04011 0.8457 26	0.26173 0.2277 23	0.04035 0.8585 22	0.41076 0.0515 23
gluAUC	-0.00334 0.9871 26	0.49245 0.0106 26		-0.16188 0.4395 25	-0.10360 0.6145 26	0.22880 0.2937 23	-0.05732 0.8000 22	0.01146 0.9586 23
zeroins	0.43188 0.0311 25	0.16222 0.4385 25	-0.16188 0.4395 25		0.68505 0.0002 25	-0.19217 0.3916 22	0.04812 0.8359 21	0.49197 0.0200 22
pctfat	0.55467 0.0033 26	-0.04011 0.8457 26	-0.10360 0.6145 26	0.68505 0.0002 25		-0.13449 0.5407 23	0.05299 0.8148 22	0.25055 0.2489 23
ttnffat	0.01707 0.9384 23	0.26173 0.2277 23	0.22880 0.2937 23	-0.19217 0.3916 22	-0.13449 0.5407 23		0.46923 0.0276 22	0.10673 0.6452 21
ttnfmusc	0.16719 0.4571 22	0.04035 0.8585 22	-0.05732 0.8000 22	0.04812 0.8359 21	0.05299 0.8148 22	0.46923 0.0276 22		0.07565 0.7512 20
ttnfliv	0.21426 0.3262 23	0.41076 0.0515 23	0.01146 0.9586 23	0.49197 0.0200 22	0.25055 0.2489 23	0.10673 0.6452 21	0.07565 0.7512 20	

The CORR Procedure
Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	aveglu	aveins	ISIcomp	HOMA	isi	homair	zerolep
weight	-0.00259 0.9900 26	0.44583 0.0255 25	-0.62788 0.0008 25	0.43962 0.0279 25	-0.66851 0.0004 24	0.54259 0.0051 25	0.41365 0.0398 25
Zerogluc	0.55936 0.0030 26	0.00066 0.9975 25	-0.30408 0.1395 25	0.29959 0.1457 25	-0.29214 0.1660 24	0.30654 0.1361 25	0.02099 0.9207 25
gluAUC	0.99409 <.0001 26	-0.04409 0.8342 25	-0.11448 0.5858 25	-0.08419 0.6891 25	-0.12840 0.5499 24	-0.00412 0.9844 25	-0.12422 0.5541 25
zeroins	-0.13275 0.5270 25	0.26231 0.2053 25	-0.73965 <.0001 25	0.98674 <.0001 25	-0.73559 <.0001 24	0.92066 <.0001 25	0.41309 0.0448 24
pctfat	-0.10119 0.6228 26	0.22431 0.2811 25	-0.56820 0.0030 25	0.65572 0.0004 25	-0.62771 0.0010 24	0.67929 0.0002 25	0.57030 0.0029 25
ttnffat	0.25302 0.2441 23	0.24557 0.2707 22	-0.01720 0.9395 22	-0.15513 0.4906 22	0.01897 0.9350 21	-0.14963 0.5063 22	0.16633 0.4594 22
ttnfmusc	-0.02320 0.9184 22	0.16462 0.4758 21	-0.08859 0.7026 21	0.01990 0.9318 21	-0.08773 0.7053 21	0.02576 0.9117 21	0.56043 0.0082 21
ttnfliv	0.05376 0.8075 23	0.22705 0.3096 22	-0.49003 0.0206 22	0.51176 0.0149 22	-0.48540 0.0257 21	0.54490 0.0087 22	0.08979 0.6911 22

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	weight	Zerogluc	gluAUC	zeroins	pctfat	ttnffat	ttnfmusc	ttnfliv
aveglu	-0.00259 0.9900 26	0.55936 0.0030 26	0.99409 <.0001 26	-0.13275 0.5270 25	-0.10119 0.6228 26	0.25302 0.2441 23	-0.02320 0.9184 22	0.05376 0.8075 23
aveins	0.44583 0.0255 25	0.00066 0.9975 25	-0.04409 0.8342 25	0.26231 0.2053 25	0.22431 0.2811 25	0.24557 0.2707 22	0.16462 0.4758 21	0.22705 0.3096 22
ISIcomp	-0.62788 0.0008 25	-0.30408 0.1395 25	-0.11448 0.5858 25	-0.73965 <.0001 25	-0.56820 0.0030 25	-0.01720 0.9395 22	-0.08859 0.7026 21	-0.49003 0.0206 22
HOMA	0.43962 0.0279 25	0.29959 0.1457 25	-0.08419 0.6891 25	0.98674 <.0001 25	0.65572 0.0004 25	-0.15513 0.4906 22	0.01990 0.9318 21	0.51176 0.0149 22
isi	-0.66851 0.0004 24	-0.29214 0.1660 24	-0.12840 0.5499 24	-0.73559 <.0001 24	-0.62771 0.0010 24	0.01897 0.9350 21	-0.08773 0.7053 21	-0.48540 0.0257 21
homair	0.54259 0.0051 25	0.30654 0.1361 25	-0.00412 0.9844 25	0.92066 <.0001 25	0.67929 0.0002 25	-0.14963 0.5063 22	0.02576 0.9117 21	0.54490 0.0087 22
zerolep	0.41365 0.0398 25	0.02099 0.9207 25	-0.12422 0.5541 25	0.41309 0.0448 24	0.57030 0.0029 25	0.16633 0.4594 22	0.56043 0.0082 21	0.08979 0.6911 22

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	aveglu	aveins	ISIcomp	HOMA	isi	homair	zerolep
aveglu		-0.04188 0.8425 25	-0.14041 0.5032 25	-0.04523 0.8300 25	-0.15266 0.4764 24	0.03145 0.8814 25	-0.09300 0.6584 25
aveins	-0.04188 0.8425 25		-0.54205 0.0051 25	0.26981 0.1921 25	-0.53841 0.0066 24	0.28467 0.1678 25	0.25085 0.2371 24
ISIcomp	-0.14041 0.5032 25	-0.54205 0.0051 25		-0.75815 <.0001 25	0.99998 <.0001 24	-0.90547 <.0001 25	-0.36743 0.0773 24
HOMA	-0.04523 0.8300 25	0.26981 0.1921 25	-0.75815 <.0001 25		-0.75388 <.0001 24	0.92958 <.0001 25	0.38719 0.0616 24
isi	-0.15266 0.4764 24	-0.53841 0.0066 24	0.99998 <.0001 24	-0.75388 <.0001 24		-0.90449 <.0001 24	-0.45454 0.0293 23
homair	0.03145 0.8814 25	0.28467 0.1678 25	-0.90547 <.0001 25	0.92958 <.0001 25	-0.90449 <.0001 24		0.40459 0.0499 24
zerolep	-0.09300 0.6584 25	0.25085 0.2371 24	-0.36743 0.0773 24	0.38719 0.0616 24	-0.45454 0.0293 23	0.40459 0.0499 24	

APPENDIX C

Real-time PCR analysis of TNF-α liver, muscle, and fat tissues from growing rats

Liver TNF α			Muscle TNF-α			Fat TNF-α		
Ct	18s Ct	ΔCt	Ct	18s Ct	ΔCt	Ct	18s Ct	ΔCt
40.77	21.28	19.49	33.01	24.28	8.73	31.73	23.31	8..42
40.69	21.7	18.99	33.44	24.49	8.95	32.57	23.48	9.09
26.61	16.31	10.30	32.15	23.61	8.54	40	31.05	8.95
26.52	16.27	10.25	32.44	23.97	8.47	40	24.19	15.81
45	15.67	29.33	32.82	25.04	7.78	33.78	23.38	10.40
45	15.56	29.44	32.99	24.27	8.72	33.41	23.92	9.49
31.29	16.03	15.26	32.65	24.34	8.31	32.08	23.23	8.85
30.88	15.70	15.18	32.72	24.57	8.15	31.74	22.12	9.62
36.11	15.96	20.15	33.01	24.61	8.40	33.14	32.26	0.88
36.03	15.77	20.26	33.09	24.45	8.64	32.68	21.63	11.05
33.75	15.64	18.11	33.31	24.81	8.50	31.58	22.08	9.50
34.56	15.41	19.15	33.01	24.17	8.84	31.47	24.71	6.76
35.39	15.63	19.76	32.55	22.13	10.42	32.30	24.35	7.95
33.41	15.65	17.76	32.85	22.84	10.01	32.06	20.57	11.49
33.10	15.95	17.15	31.34	24.33	7.01	40	22.29	17.71
34.58	15.92	18.66	31.48	24.55	6.93	31.61	22.87	8.74
44	15.40	28.60	34.37	23.35	11.02	33.58	22.35	11.23
45	15.30	29.70	34.13	23.42	10.71	32.31	19.49	12.82
34.73	15.79	18.94	36.09	23.2	12.89	31.54	19.41	12.13
33.44	15.88	17.56	34.79	23.38	11.41	31.62	22.69	8.93

42.15	16.35	25.80	35.19	23.1	12.09	32.16	19.89	12.30
39.47	16.30	23.17	38.4	22.95	15.45	31.75	20.41	11.34
33.94	15.84	18.10	35.76	22.87	12.89	32.41	40	7.59
33.22	15.67	17.55	35.37	23.00	12.37	32.71	21.77	10.94
35.43	15.46	19.97	34.38	24.39	9.99	32.05	17.94	14.11
36.54	15.28	21.26	32.79	24.37	8.42	32.30	18.40	13.90
32.05	15.49	16.56	35.02	24.10	10.92	31.43	19.66	11.77
31.33	15.62	15.71	29.51	23.92	5.59	31.02	19.26	11.76
45	15.41	29.59	30.19	23.94	6.25	31.57	19.11	12.46
45	15.32	29.68	30.04	24.35	5.69	31.55	18.73	12.82

33.05	18.17	14.88	31.48	24.75	6.73	30.49	19.20	11.29
33.46	18.25	15.21	31.67	24.79	6.88	40	19.05	20.95
31.08	15.77	15.31	36.03	23.34	12.69	28.99	18.56	10.43
31.11	15.66	15.45	35.62	23.15	12.47	29.09	17.67	11.42
45	15.24	29.76	35.62	24.07	11.55	28.58	19.25	9.33
45	15.19	29.81	35.09	23.89	12.01	28.54	18.75	9.79
45	15.88	29.12	29.03	23.14	5.89	31.35	18.80	12.55
45	15.85	29.15	28.88	23.90	4.98	31.32	20.11	11.21
31.95	15.68	16.27	40	22.49	17.51	34.09	20.53	13.56
31.59	15.80	15.79	40	22.59	17.41	33.63	18.45	15.18
45	16.01	28.99	33.5	23.57	9.93	31.12	19.46	11.66
45	16.05	28.95	33.84	23.21	10.63	31.37	19.66	11.71
45	15.80	29.20	35.26	25.04	10.22	33.44	22.05	11.39

45	15.76	29.24	36.08	25.45	10.63	32.16	19.45	12.71
45	15.88	29.12	36.19	23.70	12.49	31.72	19.61	12.11
45	17.43	27.57	39.92	24.95	14.97	31.48	19.14	12.34

Ct values are those of TNF-α, 18S Ct are those of the housekeeping gene for normalization, ΔCt are the values of the Ct subtracted from the 18S Ct values. Duplicate samples were used for all Real-time PCR quantification.

APPENDIX D

---------- treatment=1 ----------

The MEANS Procedure

Variable	N	Mean	Std Dev	Std Error	Minimum	Maximum
weight	8	203.1787500	15.7337512	5.5627211	171.5500000	216.5300000
Zerogluc	8	107.5000000	15.8113883	5.5901699	88.0000000	134.0000000
thirtgluc	8	184.6250000	37.8905850	13.3963448	155.0000000	246.0000000
sixtgluc	8	187.2500000	23.1686364	8.1913500	165.0000000	235.0000000
one20gluc	8	180.8750000	37.7110726	13.3328776	133.0000000	249.0000000
gluAUC	8	21003.75	3159.67	1117.11	17925.00	27375.00
zeroins	8	0.5312500	0.2659988	0.0940448	0.1300000	0.9700000
thirtins	8	1.4262500	0.7253952	0.2564659	0.3300000	2.3300000
sixtins	8	1.2787500	0.3474165	0.1228303	0.6700000	1.7400000
one20ins	8	1.1087500	0.5669073	0.2004320	0.2700000	2.1100000
pctfat	8	8.8250000	1.1297914	0.3994416	7.5000000	10.1000000
tnffat	7	8701.14	12146.92	4591.11	1.5100000	28024.77
tnfmuscle	7	153.8757143	257.7832816	97.4329222	7.1800000	727.6000000
tnfliver	8	13.4187500	11.3732461	4.0210497	2.2600000	39.5500000
meanglu	8	165.0625000	25.2864835	8.9401220	140.5000000	215.5000000
meanins	8	1.0862500	0.3228860	0.1141574	0.5950000	1.4675000
meanlep	6	3.7204167	0.5610312	0.2290400	2.9675000	4.3225000
ISIcomp	8	117.1650000	48.8497916	17.2710095	67.3200000	217.7900000
HOMA	8	2.5125000	1.2159740	0.4299117	0.6900000	4.2700000
zerolep	8	1.1775000	0.2999881	0.1060618	0.8500000	1.8200000
thirtlep	7	1.1271429	0.1145592	0.0432993	0.9100000	1.2500000
sistlep	8	1.2162500	0.3561275	0.1259101	0.6700000	1.7800000
one20lep	7	1.4071429	0.3268391	0.1235336	0.9900000	1.8400000

---------- treatment=2 ----------

Variable	N	Mean	Std Dev	Std Error	Minimum	Maximum
weight	9	218.3644444	11.1415395	3.7138465	203.5800000	233.1600000
Zerogluc	9	115.4444444	11.8333333	3.9444444	102.0000000	141.0000000
thirtgluc	9	179.5555556	28.6404919	9.5468306	145.0000000	234.0000000
sixtgluc	9	194.7777778	15.5545635	5.1848545	174.0000000	219.0000000
one20gluc	9	169.3333333	41.3642358	13.7880786	107.0000000	220.0000000
gluAUC	9	20963.33	1798.42	599.4743067	18465.00	23475.00
zeroins	8	1.0587500	0.3510978	0.1241318	0.6200000	1.4800000
thirtins	8	1.8837500	0.3563280	0.1259810	1.3100000	2.4800000
sixtins	8	1.4287500	0.5925595	0.2095014	0.4300000	2.1900000
one20ins	8	1.4375000	0.6548010	0.2315071	0.3700000	2.0800000
pctfat	9	13.0333333	2.0266968	0.6755656	9.8000000	15.8000000
tnffat	8	101875.19	265956.65	94029.87	3.1700000	759777.04

---------- treatment=2 ----------

The MEANS Procedure

Variable	N	Mean	Std Dev	Std Error	Minimum	Maximum
tnfmuscle	8	138.3775000	157.2192417	55.5853960	0.2500000	400.8600000
tnfliver	8	43.9487500	67.6924658	23.9329008	2.0400000	196.8900000
meanglu	9	164.7777778	15.2292094	5.0764031	142.7500000	189.2500000
meanins	8	1.4521875	0.3014976	0.1065955	0.9675000	1.9275000
meanlep	9	6.0502778	1.9632568	0.6544189	3.5625000	9.9475000
ISIcomp	8	60.7462500	10.9564057	3.8736744	42.9500000	74.6600000
HOMA	8	5.5400000	1.9595626	0.6928100	3.1700000	8.2100000
zerolep	9	1.6355556	0.4945986	0.1648662	1.0800000	2.6700000
thirtlep	9	1.5944444	0.8592164	0.2864055	0.6300000	2.9300000
sistlep	9	2.1477778	0.6146499	0.2048833	1.2600000	3.5600000
one20lep	9	2.6900000	0.7810410	0.2603470	1.2100000	3.8000000

---------- treatment=3 ----------

Variable	N	Mean	Std Dev	Std Error	Minimum	Maximum
weight	9	211.8611111	13.1161898	4.3720633	181.5100000	226.9700000
Zerogluc	9	114.6666667	11.1691540	3.7230513	98.0000000	131.0000000
thirtgluc	9	173.5555556	27.5549955	9.1849985	133.0000000	217.0000000
sixtgluc	9	184.4444444	27.2172331	9.0724110	134.0000000	235.0000000
one20gluc	9	174.6666667	54.0300842	18.0100281	119.0000000	277.0000000
gluAUC	9	20466.67	2964.32	988.1077595	16170.00	26355.00
zeroins	9	1.5288889	0.6391487	0.2130496	0.5400000	2.1900000
thirtins	8	1.1825000	0.5551255	0.1962665	0.3700000	1.9400000
sixtins	8	1.0237500	0.4741289	0.1676299	0.3400000	1.8300000
one20ins	8	0.9412500	0.3817418	0.1349661	0.2800000	1.3800000
pctfat	9	12.5000000	2.6720778	0.8906926	7.5000000	15.6000000
tnffat	8	826.0687500	1589.72	562.0499554	1.0200000	4127.50
tnfmuscle	7	114.9557143	169.3769134	64.0184558	5.6300000	476.2000000
tnfliver	7	39.7400000	27.4496399	10.3749887	10.6700000	81.5100000
meanglu	9	161.8333333	21.7733352	7.2577784	132.0000000	204.5000000
meanins	8	1.1934375	0.3618700	0.1279404	0.6000000	1.6800000
meanlep	8	6.3246875	2.9038323	1.0266597	2.7900000	10.7775000
ISIcomp	9	64.9888889	27.2740973	9.0913658	40.0100000	103.0900000
HOMA	9	7.8000000	3.3615919	1.1205306	3.1200000	11.3200000
zerolep	8	1.6087500	0.8040334	0.2842687	1.0000000	2.9700000
thirtlep	9	1.9544444	0.7952218	0.2650739	0.8000000	3.2600000
sistlep	9	2.2266667	1.0618498	0.3539499	0.6500000	3.7300000
one20lep	9	2.7544444	1.0217645	0.3405882	1.2000000	4.1900000

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
treatment	3	1 2 3

Number of observations 26

Dependent Variables With Equivalent Missing Value Patterns

Pattern	Obs	Dependent Variables
1	26	weight Zerogluc thirtgluc sixtgluc one20gluc gluAUC pctfat meanglu sistlep
2	25	zeroins ISIcomp HOMA
3	24	thirtins sixtins one20ins meanins
4	23	tnffat
5	22	tnfmuscle
6	23	tnfliver
7	23	meanlep
8	25	zerolep
9	25	thirtlep
10	25	one20lep

NOTE: Variables in each group are consistent with respect to the presence or absence of missing values.

The SAS System
The GLM Procedure

Dependent Variable: weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	979.113386	489.556693	2.74	0.0853
Error	23	4102.203199	178.356661		
Corrected Total	25	5081.316585			

R-Square	Coeff Var	Root MSE	weight Mean
0.192689	6.316201	13.35502	211.4408

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	979.1133860	489.5566930	2.74	0.0853

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	979.1133860	489.5566930	2.74	0.0853

The SAS System
The GLM Procedure

Dependent Variable: Zerogluc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	318.893162	159.446581	0.95	0.4021
Error	23	3868.222222	168.183575		
Corrected Total	25	4187.115385			

R-Square	Coeff Var	Root MSE	Zerogluc Mean
0.076161	11.50401	12.96856	112.7308

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	318.8931624	159.4465812	0.95	0.4021

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	318.8931624	159.4465812	0.95	0.4021

The SAS System

The GLM Procedure

Dependent Variable: thirtgluc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	522.64209	261.32105	0.26	0.7696
Error	23	22686.31944	986.36171		
Corrected Total	25	23208.96154			

R-Square	Coeff Var	Root MSE	thirtgluc Mean
0.022519	17.54170	31.40640	179.0385

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	522.6420940	261.3210470	0.26	0.7696

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	522.6420940	261.3210470	0.26	0.7696

The SAS System

The GLM Procedure

Dependent Variable: thirtgluc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	522.64209	261.32105	0.26	0.7696
Error	23	22686.31944	986.36171		
Corrected Total	25	23208.96154			

R-Square	Coeff Var	Root MSE	thirtgluc Mean
0.022519	17.54170	31.40640	179.0385

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	522.6420940	261.3210470	0.26	0.7696

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	522.6420940	261.3210470	0.26	0.7696

The SAS System

The GLM Procedure

Dependent Variable: one20gluc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	564.24038	282.12019	0.14	0.8718
Error	23	46996.87500	2043.34239		
Corrected Total	25	47561.11538			

R-Square	Coeff Var	Root MSE	one20gluc Mean
0.011863	25.87028	45.20334	174.7308

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	564.2403846	282.1201923	0.14	0.8718

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	564.2403846	282.1201923	0.14	0.8718

The SAS System

The GLM Procedure

Dependent Variable: gluAUC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1571827.9	785913.9	0.11	0.8973
Error	23	166056737.5	7219858.2		
Corrected Total	25	167628565.4			

R-Square	Coeff Var	Root MSE	gluAUC Mean
0.009377	12.91578	2686.979	20803.85

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	1571827.885	785913.942	0.11	0.8973

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	1571827.885	785913.942	0.11	0.8973

The SAS System

The GLM Procedure

Dependent Variable: pctfat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	87.3296154	43.6648077	10.15	0.0007
Error	23	98.9150000	4.3006522		
Corrected Total	25	186.2446154			

R-Square	Coeff Var	Root MSE	pctfat Mean
0.468897	17.94901	2.073801	11.55385

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	87.32961538	43.66480769	10.15	0.0007

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	87.32961538	43.66480769	10.15	0.0007

The SAS System

The GLM Procedure

Dependent Variable: meanglu

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	56.11031	28.05515	0.06	0.9384
Error	23	10123.89931	440.16954		
Corrected Total	25	10180.00962			

R-Square	Coeff Var	Root MSE	meanglu Mean
0.005512	12.80483	20.98022	163.8462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	56.11030983	28.05515491	0.06	0.9384

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	56.11030983	28.05515491	0.06	0.9384

The SAS System

The GLM Procedure

Dependent Variable: sistlep

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	5.24959541	2.62479770	4.67	0.0199
Error	23	12.93034306	0.56218883		
Corrected Total	25	18.17993846			

R-Square	Coeff Var	Root MSE	sistlep Mean
0.288758	39.70388	0.749793	1.888462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	5.24959541	2.62479770	4.67	0.0199

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	5.24959541	2.62479770	4.67	0.0199

The SAS System
The GLM Procedure
Least Squares Means

treatment	weight LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	203.178750	4.721714	<.0001	1
2	218.364444	4.451675	<.0001	2
3	211.861111	4.451675	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: weight

i/j	1	2	3
1		0.0283	0.1940
2	0.0283		0.3123
3	0.1940	0.3123	

treatment	Zerogluc LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	107.500000	4.585079	<.0001	1
2	115.444444	4.322854	<.0001	2
3	114.666667	4.322854	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Zerogluc

i/j	1	2	3
1		0.2201	0.2671
2	0.2201		0.8999
3	0.2671	0.8999	

treatment	thirtgluc LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	184.625000	11.103838	<.0001	1
2	179.555556	10.468799	<.0001	2
3	173.555556	10.468799	<.0001	3

The SAS System

The GLM Procedure
Least Squares Means

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: thirtgluc

i/j	1	2	3
1		0.7428	0.4756
2	0.7428		0.6890
3	0.4756	0.6890	

treatment	sixtgluc LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	187.250000	7.946587	<.0001	1
2	194.777778	7.492114	<.0001	2
3	184.444444	7.492114	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: sixtgluc

i/j	1	2	3
1		0.4976	0.7996
2	0.4976		0.3396
3	0.7996	0.3396	

treatment	one20gluc LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	180.875000	15.981796	<.0001	1
2	169.333333	15.067782	<.0001	2
3	174.666667	15.067782	<.0001	3

The SAS System
The GLM Procedure
Least Squares Means

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: one2Ogluc

i/j	1	2	3
1		0.6043	0.7800
2	0.6043		0.8046
3	0.7800	0.8046	

treatment	gluAUC LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	21003.7500	949.9907	<.0001	1
2	20963.3333	895.6598	<.0001	2
3	20466.6667	895.6598	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: gluAUC

i/j	1	2	3
1		0.9756	0.6846
2	0.9756		0.6986
3	0.6846	0.6986	

treatment	pctfat LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	8.8250000	0.7331995	<.0001	1
2	13.0333333	0.6912671	<.0001	2
3	12.5000000	0.6912671	<.0001	3

The SAS System

The GLM Procedure
Least Squares Means

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: pctfat

i/j	1	2	3
1		0.0004	0.0013
2	0.0004		0.5906
3	0.0013	0.5906	

treatment	meanglu LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	165.062500	7.417627	<.0001	1
2	164.777778	6.993406	<.0001	2
3	161.833333	6.993406	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: meanglu

i/j	1	2	3
1		0.9780	0.7543
2	0.9780		0.7686
3	0.7543	0.7686	

treatment	sistlep LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	1.21625000	0.26509169	0.0001	1
2	2.14777778	0.24993084	<.0001	2
3	2.22666667	0.24993084	<.0001	3

The SAS System

The GLM Procedure
Least Squares Means

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: sistlep

i/j	1	2	3
1		0.0176	0.0108
2	0.0176		0.8254
3	0.0108	0.8254	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The SAS System

The GLM Procedure

Dependent Variable: zeroins

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4.21532011	2.10766006	10.02	0.0008
Error	22	4.62626389	0.21028472		
Corrected Total	24	8.84158400			

R-Square	Coeff Var	Root MSE	zeroins Mean
0.476761	43.29382	0.458568	1.059200

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	4.21532011	2.10766006	10.02	0.0008

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	4.21532011	2.10766006	10.02	0.0008

The SAS System
The GLM Procedure

Dependent Variable: ISIcomp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	16040.87098	8020.43549	7.51	0.0033
Error	22	23495.42588	1067.97390		
Corrected Total	24	39536.29686			

R-Square	Coeff Var	Root MSE	ISIcomp Mean
0.405725	40.68324	32.67987	80.32760

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	16040.87098	8020.43549	7.51	0.0033

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	16040.87098	8020.43549	7.51	0.0033

The SAS System
The GLM Procedure

Dependent Variable: HOMA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	118.6922740	59.3461370	10.23	0.0007
Error	22	127.6317500	5.8014432		
Corrected Total	24	246.3240240			

R-Square	Coeff Var	Root MSE	HOMA Mean
0.481854	44.72995	2.408619	5.384800

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	118.6922740	59.3461370	10.23	0.0007

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	118.6922740	59.3461370	10.23	0.0007

The SAS System
The GLM Procedure
Least Squares Means

treatment	zeroins LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	0.53125000	0.16212831	0.0034	1
2	1.05875000	0.16212831	<.0001	2
3	1.52888889	0.15285604	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: zeroins

i/j	1	2	3
1		0.0313	0.0002
2	0.0313		0.0465
3	0.0002	0.0465	

treatment	ISIcomp LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	117.165000	11.554079	<.0001	1
2	60.746250	11.554079	<.0001	2
3	64.988889	10.893290	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: ISIcomp

i/j	1	2	3
1		0.0023	0.0034
2	0.0023		0.7918
3	0.0034	0.7918	

treatment	HOMA LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	2.51250000	0.85157524	0.0074	1
2	5.54000000	0.85157524	<.0001	2
3	7.80000000	0.80287284	<.0001	3

The SAS System

The GLM Procedure
Least Squares Means

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: HOMA

i/j	1	2	3
1		0.0198	0.0002
2	0.0198		0.0665
3	0.0002	0.0665	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The SAS System

The GLM Procedure

Dependent Variable: thirtins

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2.02792500	1.01396250	3.16	0.0629
Error	21	6.72932500	0.32044405		
Corrected Total	23	8.75725000			

R-Square	Coeff Var	Root MSE	thirtins Mean
0.231571	37.80152	0.566078	1.497500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	2.02792500	1.01396250	3.16	0.0629

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	2.02792500	1.01396250	3.16	0.0629

The SAS System

The GLM Procedure

Dependent Variable: sixtins

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.67080000	0.33540000	1.44	0.2584
Error	21	4.87636250	0.23220774		
Corrected Total	23	5.54716250			

R-Square	Coeff Var	Root MSE	sixtins Mean
0.120927	38.74407	0.481879	1.243750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	0.67080000	0.33540000	1.44	0.2584

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	0.67080000	0.33540000	1.44	0.2584

The SAS System

The GLM Procedure

Dependent Variable: one20ins

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.01972500	0.50986250	1.71	0.2056
Error	21	6.27112500	0.29862500		
Corrected Total	23	7.29085000			

R-Square	Coeff Var	Root MSE	one20ins Mean
0.139864	47.00782	0.546466	1.162500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	1.01972500	0.50986250	1.71	0.2056

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	1.01972500	0.50986250	1.71	0.2056

The SAS System
The GLM Procedure

Dependent Variable: meanins

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.56626927	0.28313464	2.60	0.0976
Error	21	2.28274219	0.10870201		
Corrected Total	23	2.84901146			

R-Square	Coeff Var	Root MSE	meanins Mean
0.198760	26.50409	0.329700	1.243958

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	0.56626927	0.28313464	2.60	0.0976

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	0.56626927	0.28313464	2.60	0.0976

The SAS System

The GLM Procedure
Least Squares Means

treatment	thirtins LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	1.42625000	0.20013872	<.0001	1
2	1.88375000	0.20013872	<.0001	2
3	1.18250000	0.20013872	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: thirtins

i/j	1	2	3
1		0.1209	0.3989
2	0.1209		0.0218
3	0.3989	0.0218	

treatment	sixtins LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	1.27875000	0.17037009	<.0001	1
2	1.42875000	0.17037009	<.0001	2
3	1.02375000	0.17037009	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: sixtins

i/j	1	2	3
1		0.5403	0.3019
2	0.5403		0.1076
3	0.3019	0.1076	

treatment	one20ins LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	1.10875000	0.19320488	<.0001	1
2	1.43750000	0.19320488	<.0001	2
3	0.94125000	0.19320488	<.0001	3

The SAS System
The GLM Procedure
Least Squares Means

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: one2Oins

i/j	1	2	3
1		0.2423	0.5464
2	0.2423		0.0836
3	0.5464	0.0836	

treatment	meanins LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	1.08625000	0.11656651	<.0001	1
2	1.45218750	0.11656651	<.0001	2
3	1.19343750	0.11656651	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: meanins

i/j	1	2	3
1		0.0376	0.5226
2	0.0376		0.1315
3	0.5226	0.1315	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The SAS System

The GLM Procedure

Dependent Variable: tnffat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	49701335458	24850667729	1.00	0.3849
Error	20	496033540705	24801677035		
Corrected Total	22	545734876163			

R-Square	Coeff Var	Root MSE	tnffat Mean
0.091072	410.4354	157485.5	38370.35

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	49701335458	24850667729	1.00	0.3849

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	49701335458	24850667729	1.00	0.3849

The SAS System

The GLM Procedure
Least Squares Means

treatment	tnffat LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	8701.136	59523.917	0.8852	1
2	101875.188	55679.526	0.0822	2
3	826.069	55679.526	0.9883	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: tnffat

i/j	1	2	3
1		0.2665	0.9240
2	0.2665		0.2141
3	0.9240	0.2141	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The SAS System

The GLM Procedure

Dependent Variable: tnfmuscle

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	5381.5880	2690.7940	0.07	0.9338
Error	19	743869.7841	39151.0413		
Corrected Total	21	749251.3721			

R-Square	Coeff Var	Root MSE	tnfmuscle Mean
0.007183	145.6437	197.8662	135.8564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	5381.588016	2690.794008	0.07	0.9338

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	5381.588016	2690.794008	0.07	0.9338

The SAS System

The GLM Procedure
Least Squares Means

treatment	tnfmuscle LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	153.875714	74.786402	0.0536	1
2	138.377500	69.956273	0.0626	2
3	114.955714	74.786402	0.1408	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: tnfmuscle

i/j	1	2	3
1		0.8813	0.7169
2	0.8813		0.8215
3	0.7169	0.8215	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The SAS System

The GLM Procedure

Dependent Variable: tnfliver

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4323.58249	2161.79124	1.15	0.3358
Error	20	37502.24097	1875.11205		
Corrected Total	22	41825.82346			

R-Square	Coeff Var	Root MSE	tnfliver Mean
0.103371	135.1149	43.30256	32.04870

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	4323.582486	2161.791243	1.15	0.3358

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	4323.582486	2161.791243	1.15	0.3358

The SAS System

The GLM Procedure

Dependent Variable: tnfliver

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4323.58249	2161.79124	1.15	0.3358
Error	20	37502.24097	1875.11205		
Corrected Total	22	41825.82346			

R-Square	Coeff Var	Root MSE	tnfliver Mean
0.103371	135.1149	43.30256	32.04870

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	4323.582486	2161.791243	1.15	0.3358

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	4323.582486	2161.791243	1.15	0.3358

The SAS System

The GLM Procedure
Least Squares Means

treatment	tnfliver LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	13.4187500	15.3097683	0.3912	1
2	43.9487500	15.3097683	0.0095	2
3	39.7400000	16.3668308	0.0247	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: tnfliver

i/j	1	2	3
1		0.1739	0.2540
2	0.1739		0.8529
3	0.2540	0.8529	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The SAS System

The GLM Procedure

Dependent Variable: meanlep

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	27.1345294	13.5672647	2.97	0.0744
Error	20	91.4344912	4.5717246		
Corrected Total	22	118.5690207			

R-Square	Coeff Var	Root MSE	meanlep Mean
0.228850	38.60932	2.138159	5.537935

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	27.13452942	13.56726471	2.97	0.0744

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	27.13452942	13.56726471	2.97	0.0744

The SAS System
The GLM Procedure
Least Squares Means

treatment	meanlep LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	3.72041667	0.87289982	0.0004	1
2	6.05027778	0.71271972	<.0001	2
3	6.32468750	0.75595342	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: meanlep

i/j	1	2	3
1		0.0519	0.0355
2	0.0519		0.7944
3	0.0355	0.7944	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The SAS System
The GLM Procedure

Dependent Variable: zerolep

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.08243628	0.54121814	1.67	0.2105
Error	22	7.11225972	0.32328453		
Corrected Total	24	8.19469600			

R-Square	Coeff Var	Root MSE	zerolep Mean
0.132090	38.40727	0.568581	1.480400

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	1.08243628	0.54121814	1.67	0.2105

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	1.08243628	0.54121814	1.67	0.2105

The SAS System

The GLM Procedure
Least Squares Means

treatment	zerolep LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	1.17750000	0.20102380	<.0001	1
2	1.63555556	0.18952705	<.0001	2
3	1.60875000	0.20102380	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: zerolep

i/j	1	2	3
1		0.1115	0.1435
2	0.1115		0.9236
3	0.1435	0.9236	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The SAS System

The GLM Procedure

Dependent Variable: thirtlep

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2.69495670	1.34747835	2.68	0.0905
Error	22	11.04378730	0.50199033		
Corrected Total	24	13.73874400			

R-Square	Coeff Var	Root MSE	thirtlep Mean
0.196157	44.47105	0.708513	1.593200

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	2.69495670	1.34747835	2.68	0.0905

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	2.69495670	1.34747835	2.68	0.0905

The SAS System

The GLM Procedure
Least Squares Means

treatment	thirtlep LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	1.12714286	0.26779265	0.0004	1
2	1.59444444	0.23617092	<.0001	2
3	1.95444444	0.23617092	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: thirtlep

i/j	1	2	3
1		0.2041	0.0302
2	0.2041		0.2928
3	0.0302	0.2928	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The SAS System
The GLM Procedure

Dependent Variable: one2Olep

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	8.73503492	4.36751746	6.93	0.0046
Error	22	13.87316508	0.63059841		
Corrected Total	24	22.60820000			

R-Square	Coeff Var	Root MSE	one2Olep Mean
0.386366	33.73417	0.794102	2.354000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	2	8.73503492	4.36751746	6.93	0.0046

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	2	8.73503492	4.36751746	6.93	0.0046

The SAS System

The GLM Procedure
Least Squares Means

treatment	one20lep LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
1	1.40714286	0.30014245	0.0001	1
2	2.69000000	0.26470076	<.0001	2
3	2.75444444	0.26470076	<.0001	3

Least Squares Means for effect treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: one20lep

i/j	1	2	3
1		0.0041	0.0028
2	0.0041		0.8649
3	0.0028	0.8649	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

23 Variables: weight Zerogluc thirtgluc sixtgluc one20gluc gluAUC zeroins thirtins sixtins one20ins pctfat tnffat tnfmuscle tnfliver meanglu meanins meanlep ISIcomp HOMA zerolep thirtlep sistlep one20lep

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
weight	26	211.44077	14.25667	5497	171.55000	233.16000
Zerogluc	26	112.73077	12.94158	2931	88.00000	141.00000
thirtgluc	26	179.03846	30.46898	4655	133.00000	246.00000
sixtgluc	26	188.88462	22.02785	4911	134.00000	235.00000
one20gluc	26	174.73077	43.61702	4543	107.00000	277.00000
gluAUC	26	20804	2589	540900	16170	27375
zeroins	25	1.05920	0.60696	26.48000	0.13000	2.19000
thirtins	24	1.49750	0.61705	35.94000	0.33000	2.48000
sixtins	24	1.24375	0.49110	29.85000	0.34000	2.19000
one20ins	24	1.16250	0.56302	27.90000	0.27000	2.11000
pctfat	26	11.55385	2.72943	300.40000	7.50000	15.80000
tnffat	23	38370	157500	882518	1.02000	759777
tnfmuscle	22	135.85636	188.88789	2989	0.25000	727.60000
tnfliver	23	32.04870	43.60245	737.12000	2.04000	196.89000
meanglu	26	163.84615	20.17921	4260	132.00000	215.50000
meanins	24	1.24396	0.35195	29.85500	0.59500	1.92750
meanlep	23	5.53793	2.32153	127.37250	2.79000	10.77750
ISIcomp	25	80.32760	40.58751	2008	40.01000	217.79000
HOMA	25	5.38480	3.20367	134.62000	0.69000	11.32000
zerolep	25	1.48040	0.58433	37.01000	0.85000	2.97000
thirtlep	25	1.59320	0.75660	39.83000	0.63000	3.26000
sistlep	26	1.88846	0.85276	49.10000	0.65000	3.73000
one20lep	25	2.35400	0.97057	58.85000	0.99000	4.19000

The SAS System

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	weight	Zerogluc	thirtgluc	sixtgluc	one20gluc	gluAUC	zeroins	thirtins
weight	1.00000	0.06411	-0.18946	0.03045	0.09315	-0.00334	0.43188	0.37179
		0.7557	0.3539	0.8826	0.6508	0.9871	0.0311	0.0736
	26	26	26	26	26	26	25	24
Zerogluc	0.06411	1.00000	0.42121	0.34744	0.26872	0.49245	0.16222	0.09207
	0.7557		0.0321	0.0820	0.1844	0.0106	0.4385	0.6687
	26	26	26	26	26	26	25	24
thirtgluc	-0.18946	0.42121	1.00000	0.44073	0.20895	0.65888	-0.22137	-0.07930
	0.3539	0.0321		0.0242	0.3056	0.0003	0.2876	0.7126
	26	26	26	26	26	26	25	24
sixtgluc	0.03045	0.34744	0.44073	1.00000	0.54698	0.84084	-0.20250	0.14301
	0.8826	0.0820	0.0242		0.0038	<.0001	0.3317	0.5050
	26	26	26	26	26	26	25	24
one20gluc	0.09315	0.26872	0.20895	0.54698	1.00000	0.80862	-0.03532	0.24436
	0.6508	0.1844	0.3056	0.0038		<.0001	0.8669	0.2498
	26	26	26	26	26	26	25	24
gluAUC	-0.00334	0.49245	0.65888	0.84084	0.80862	1.00000	-0.16188	0.15341
	0.9871	0.0106	0.0003	<.0001	<.0001		0.4395	0.4742
	26	26	26	26	26	26	25	24
zeroins	0.43188	0.16222	-0.22137	-0.20250	-0.03532	-0.16188	1.00000	-0.17066
	0.0311	0.4385	0.2876	0.3317	0.8669	0.4395		0.4253
	25	25	25	25	25	25	25	24
thirtins	0.37179	0.09207	-0.07930	0.14301	0.24436	0.15341	-0.17066	1.00000
	0.0736	0.6687	0.7126	0.5050	0.2498	0.4742	0.4253	
	24	24	24	24	24	24	24	24
sixtins	0.18015	-0.30020	0.11451	0.03388	-0.05666	0.00361	-0.17837	0.38402
	0.3996	0.1541	0.5942	0.8751	0.7926	0.9866	0.4043	0.0639
	24	24	24	24	24	24	24	24
one20ins	0.27333	-0.11568	0.07873	-0.00210	-0.06693	-0.01421	-0.14605	0.56555
	0.1963	0.5904	0.7146	0.9922	0.7560	0.9475	0.4959	0.0040
	24	24	24	24	24	24	24	24

The SAS System

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	sixtins	one20ins	pctfat	tnffat	tnfmuscle	tnfliver	meanglu	meanins
weight	0.18015 0.3996 24	0.27333 0.1963 24	0.55467 0.0033 26	0.12376 0.5737 23	0.16111 0.4738 22	0.12416 0.5724 23	-0.00259 0.9900 26	0.54059 0.0064 24
Zerogluc	-0.30020 0.1541 24	-0.11568 0.5904 24	-0.04011 0.8457 26	0.06040 0.7843 23	0.19743 0.3785 22	0.12446 0.5715 23	0.55936 0.0030 26	-0.04592 0.8313 24
thirtgluc	0.11451 0.5942 24	0.07873 0.7146 24	-0.25028 0.2175 26	0.13605 0.5359 23	-0.13719 0.5427 22	-0.01279 0.9538 23	0.67820 0.0001 26	-0.06596 0.7595 24
sixtgluc	0.03388 0.8751 24	-0.00210 0.9922 24	-0.09390 0.6482 26	0.17517 0.4240 23	-0.21385 0.3393 22	-0.06362 0.7731 23	0.79055 <.0001 26	-0.01113 0.9589 24
one20gluc	-0.05666 0.7926 24	-0.06693 0.7560 24	0.04690 0.8200 26	-0.11283 0.6083 23	0.18413 0.4120 22	-0.21902 0.3154 23	0.81160 <.0001 26	0.05960 0.7821 24
gluAUC	0.00361 0.9866 24	-0.01421 0.9475 24	-0.10360 0.6145 26	0.06017 0.7851 23	-0.02622 0.9078 22	-0.12876 0.5582 23	0.99409 <.0001 26	-0.00215 0.9921 24
zeroins	-0.17837 0.4043 24	-0.14605 0.4959 24	0.68505 0.0002 25	-0.08532 0.7058 22	0.04032 0.8622 21	0.27619 0.2134 22	-0.13275 0.5270 25	0.24249 0.2536 24
thirtins	0.38402 0.0639 24	0.56555 0.0040 24	-0.07867 0.7148 24	0.07894 0.7338 21	0.24656 0.2813 21	0.12280 0.5959 21	0.15202 0.4782 24	0.72371 <.0001 24
sixtins	1.00000 24	0.68074 0.0003 24	0.01599 0.9409 24	0.25609 0.2625 21	-0.17020 0.4608 21	-0.08118 0.7265 21	-0.02505 0.9075 24	0.71130 <.0001 24
one20ins	0.68074 0.0003 24	1.00000 24	0.08028 0.7092 24	0.29592 0.1928 21	0.32044 0.1567 21	0.28994 0.2023 21	-0.02427 0.9104 24	0.82132 <.0001 24

The SAS System

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	meanlep	ISIcomp	HOMA	zerolep	thirtlep	sistlep	one20lep
weight	0.51770 0.0114 23	-0.62788 0.0008 25	0.43962 0.0279 25	0.41365 0.0398 25	0.43544 0.0296 25	0.46228 0.0174 26	0.47269 0.0170 25
Zerogluc	0.16097 0.4631 23	-0.30408 0.1395 25	0.29959 0.1457 25	0.02099 0.9207 25	0.20843 0.3174 25	0.17604 0.3897 26	0.31105 0.1302 25
thirtgluc	-0.11617 0.5976 23	-0.07919 0.7067 25	-0.14197 0.4984 25	-0.30043 0.1445 25	-0.02347 0.9113 25	-0.05271 0.7982 26	-0.09148 0.6636 25
sixtgluc	-0.19789 0.3654 23	-0.05259 0.8028 25	-0.15120 0.4706 25	-0.28032 0.1747 25	-0.21647 0.2986 25	-0.18044 0.3777 26	-0.03302 0.8755 25
one20gluc	0.20280 0.3534 23	-0.08617 0.6821 25	0.00359 0.9864 25	0.17277 0.4089 25	0.23553 0.2570 25	0.09176 0.6557 26	0.21699 0.2975 25
gluAUC	0.00273 0.9901 23	-0.11448 0.5858 25	-0.08419 0.6891 25	-0.12422 0.5541 25	0.04605 0.8270 25	-0.02811 0.8916 26	0.08796 0.6759 25
zeroins	0.52528 0.0121 22	-0.73965 <.0001 25	0.98674 <.0001 25	0.41309 0.0448 24	0.51637 0.0098 24	0.57427 0.0027 25	0.61887 0.0013 24
thirtins	0.06997 0.7631 21	-0.24899 0.2407 24	-0.13277 0.5363 24	0.19335 0.3767 23	-0.02334 0.9158 23	0.05739 0.7900 24	0.05167 0.8149 23
sixtins	0.08914 0.7008 21	-0.06359 0.7678 24	-0.19954 0.3499 24	0.18068 0.4094 23	-0.03514 0.8735 23	0.16025 0.4545 24	-0.04898 0.8244 23
one20ins	0.07188 0.7568 21	-0.21461 0.3139 24	-0.14864 0.4882 24	0.17365 0.4281 23	-0.05911 0.7888 23	0.11677 0.5869 24	-0.04600 0.8349 23

The SAS System

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	weight	Zerogluc	thirtgluc	sixtgluc	one20gluc	gluAUC	zeroins	thirtins
pctfat	0.55467	-0.04011	-0.25028	-0.09390	0.04690	-0.10360	0.68505	-0.07867
	0.0033	0.8457	0.2175	0.6482	0.8200	0.6145	0.0002	0.7148
	26	26	26	26	26	26	25	24
tnffat	0.12376	0.06040	0.13605	0.17517	-0.11283	0.06017	-0.08532	0.07894
	0.5737	0.7843	0.5359	0.4240	0.6083	0.7851	0.7058	0.7338
	23	23	23	23	23	23	22	21
tnfmuscle	0.16111	0.19743	-0.13719	-0.21385	0.18413	-0.02622	0.04032	0.24656
	0.4738	0.3785	0.5427	0.3393	0.4120	0.9078	0.8622	0.2813
	22	22	22	22	22	22	21	21
tnfliver	0.12416	0.12446	-0.01279	-0.06362	-0.21902	-0.12876	0.27619	0.12280
	0.5724	0.5715	0.9538	0.7731	0.3154	0.5582	0.2134	0.5959
	23	23	23	23	23	23	22	21
meanglu	-0.00259	0.55936	0.67820	0.79055	0.81160	0.99409	-0.13275	0.15202
	0.9900	0.0030	0.0001	<.0001	<.0001	<.0001	0.5270	0.4782
	26	26	26	26	26	26	25	24
meanins	0.54059	-0.04592	-0.06596	-0.01113	0.05960	-0.00215	0.24249	0.72371
	0.0064	0.8313	0.7595	0.9589	0.7821	0.9921	0.2536	<.0001
	24	24	24	24	24	24	24	24
meanlep	0.51770	0.16097	-0.11617	-0.19789	0.20280	0.00273	0.52528	0.06997
	0.0114	0.4631	0.5976	0.3654	0.3534	0.9901	0.0121	0.7631
	23	23	23	23	23	23	22	21
ISIcomp	-0.62788	-0.30408	-0.07919	-0.05259	-0.08617	-0.11448	-0.73965	-0.24899
	0.0008	0.1395	0.7067	0.8028	0.6821	0.5858	<.0001	0.2407
	25	25	25	25	25	25	25	24
HOMA	0.43962	0.29959	-0.14197	-0.15120	0.00359	-0.08419	0.98674	-0.13277
	0.0279	0.1457	0.4984	0.4706	0.9864	0.6891	<.0001	0.5363
	25	25	25	25	25	25	25	24
zerolep	0.41365	0.02099	-0.30043	-0.28032	0.17277	-0.12422	0.41309	0.19335
	0.0398	0.9207	0.1445	0.1747	0.4089	0.5541	0.0448	0.3767
	25	25	25	25	25	25	24	23

The SAS System

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	sixtins	one20ins	pctfat	tnffat	tnfmuscle	tnfliver	meanglu	meanins
pctfat	0.01599 0.9409 24	0.08028 0.7092 24	1.00000 26	-0.03261 0.8826 23	-0.01175 0.9586 22	0.31222 0.1469 23	-0.10119 0.6228 26	0.33509 0.1095 24
tnffat	0.25609 0.2625 21	0.29592 0.1928 21	-0.03261 0.8826 23	1.00000 23	0.01897 0.9332 22	0.26763 0.2408 21	0.04540 0.8370 23	0.20531 0.3720 21
tnfmuscle	-0.17020 0.4608 21	0.32044 0.1567 21	-0.01175 0.9586 22	0.01897 0.9332 22	1.00000 22	-0.14410 0.5444 20	0.01478 0.9479 22	0.19120 0.4064 21
tnfliver	-0.08118 0.7265 21	0.28994 0.2023 21	0.31222 0.1469 23	0.26763 0.2408 21	-0.14410 0.5444 20	1.00000 23	-0.11986 0.5859 23	0.26013 0.2548 21
meanglu	-0.02505 0.9075 24	-0.02427 0.9104 24	-0.10119 0.6228 26	0.04540 0.8370 23	0.01478 0.9479 22	-0.11986 0.5859 23	1.00000 26	-0.00444 0.9836 24
meanins	0.71130 <.0001 24	0.82132 <.0001 24	0.33509 0.1095 24	0.20531 0.3720 21	0.19120 0.4064 21	0.26013 0.2548 21	-0.00444 0.9836 24	1.00000 24
meanlep	0.08914 0.7008 21	0.07188 0.7568 21	0.69342 0.0002 23	-0.05989 0.8019 20	0.44752 0.0547 19	-0.05543 0.8165 20	0.04325 0.8446 23	0.36802 0.1007 21
ISIcomp	-0.06359 0.7678 24	-0.21461 0.3139 24	-0.56820 0.0030 25	-0.09175 0.6847 22	-0.13640 0.5555 21	-0.30838 0.1626 22	-0.14041 0.5032 25	-0.53994 0.0065 24
HOMA	-0.19954 0.3499 24	-0.14864 0.4882 24	0.65572 0.0004 25	-0.07884 0.7273 22	0.04867 0.8341 21	0.27545 0.2147 22	-0.04523 0.8300 25	0.24489 0.2488 24
zerolep	0.18068 0.4094 23	0.17365 0.4281 23	0.57030 0.0029 25	0.11055 0.6243 22	0.55471 0.0091 21	-0.08469 0.7079 22	-0.09300 0.6584 25	0.43239 0.0393 23

The SAS System

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	meanlep	ISIcomp	HOMA	zerolep	thirtlep	sistlep	one20lep
pctfat	0.69342 0.0002 23	-0.56820 0.0030 25	0.65572 0.0004 25	0.57030 0.0029 25	0.61150 0.0012 25	0.73613 <.0001 26	0.75359 <.0001 25
tnffat	-0.05989 0.8019 20	-0.09175 0.6847 22	-0.07884 0.7273 22	0.11055 0.6243 22	-0.26080 0.2411 22	-0.02241 0.9191 23	0.10970 0.6270 22
tnfmuscle	0.44752 0.0547 19	-0.13640 0.5555 21	0.04867 0.8341 21	0.55471 0.0091 21	0.41898 0.0587 21	0.26382 0.2355 22	0.15905 0.4911 21
tnfliver	-0.05543 0.8165 20	-0.30838 0.1626 22	0.27545 0.2147 22	-0.08469 0.7079 22	-0.19517 0.3841 22	0.17650 0.4205 23	0.30682 0.1649 22
meanglu	0.04325 0.8446 23	-0.14041 0.5032 25	-0.04523 0.8300 25	-0.09300 0.6584 25	0.09642 0.6466 25	0.00867 0.9665 26	0.12346 0.5566 25
meanins	0.36802 0.1007 21	-0.53994 0.0065 24	0.24489 0.2488 24	0.43239 0.0393 23	0.23612 0.2781 23	0.43226 0.0349 24	0.30088 0.1630 23
meanlep	1.00000 23	-0.49007 0.0206 22	0.52699 0.0117 22	0.90144 <.0001 23	0.93167 <.0001 23	0.96789 <.0001 23	0.87354 <.0001 23
ISIcomp	-0.49007 0.0206 22	1.00000 25	-0.75815 <.0001 25	-0.36743 0.0773 24	-0.37235 0.0732 24	-0.46668 0.0187 25	-0.60956 0.0016 24
HOMA	0.52699 0.0117 22	-0.75815 <.0001 25	1.00000 25	0.38719 0.0616 24	0.53425 0.0072 24	0.57814 0.0025 25	0.63928 0.0008 24
zerolep	0.90144 <.0001 23	-0.36743 0.0773 24	0.38719 0.0616 24	1.00000 25	0.77136 <.0001 24	0.81934 <.0001 25	0.73822 <.0001 24

The SAS System

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	weight	Zerogluc	thirtgluc	sixtgluc	one20gluc	gluAUC	zeroins	thirtins
thirtlep	0.43544 0.0296 25	0.20843 0.3174 25	-0.02347 0.9113 25	-0.21647 0.2986 25	0.23553 0.2570 25	0.04605 0.8270 25	0.51637 0.0098 24	-0.02334 0.9158 23
sistlep	0.46228 0.0174 26	0.17604 0.3897 26	-0.05271 0.7982 26	-0.18044 0.3777 26	0.09176 0.6557 26	-0.02811 0.8916 26	0.57427 0.0027 25	0.05739 0.7900 24
one20lep	0.47269 0.0170 25	0.31105 0.1302 25	-0.09148 0.6636 25	-0.03302 0.8755 25	0.21699 0.2975 25	0.08796 0.6759 25	0.61887 0.0013 24	0.05167 0.8149 23

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	sixtins	one20ins	pctfat	tnffat	tnfmuscle	tnfliver	meanglu	meanins
thirtlep	-0.03514 0.8735 23	-0.05911 0.7888 23	0.61150 0.0012 25	-0.26080 0.2411 22	0.41898 0.0587 21	-0.19517 0.3841 22	0.09642 0.6466 25	0.23612 0.2781 23
sistlep	0.16025 0.4545 24	0.11677 0.5869 24	0.73613 <.0001 26	-0.02241 0.9191 23	0.26382 0.2355 22	0.17650 0.4205 23	0.00867 0.9665 26	0.43226 0.0349 24
one20lep	-0.04898 0.8244 23	-0.04600 0.8349 23	0.75359 <.0001 25	0.10970 0.6270 22	0.15905 0.4911 21	0.30682 0.1649 22	0.12346 0.5566 25	0.30088 0.1630 23

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	meanlep	ISIcomp	HOMA	zerolep	thirtlep	sistlep	one20lep
thirtlep	0.93167 <.0001 23	-0.37235 0.0732 24	0.53425 0.0072 24	0.77136 <.0001 24	1.00000 25	0.86503 <.0001 25	0.74620 <.0001 24

The SAS System

The CORR Procedure

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	meanlep	ISIcomp	HOMA	zerolep	thirtlep	sistlep	one20lep
sistlep	0.96789 <.0001 23	-0.46668 0.0187 25	0.57814 0.0025 25	0.81934 <.0001 25	0.86503 <.0001 25	1.00000 26	0.88377 <.0001 25
one20lep	0.87354 <.0001 23	-0.60956 0.0016 24	0.63928 0.0008 24	0.73822 <.0001 24	0.74620 <.0001 24	0.88377 <.0001 25	1.00000 25

VITA

Anne Marie Flanagan

Candidate for the Degree of

Master of Science

Thesis: THE RELATIONSHIP BETWEEN HIGH FAT FEEDING, INSULIN RESISTANCE, AND TNF-α GENE EXPRESSION IN GROWING RATS

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Professional Memberships: American Dietetics Association, Oklahoma Dietetics Association.