The Effects of Canola Oil on Metabolic Syndrome Parameters in Diet-Induced Obese Rats

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ABSTRACT

The metabolic syndrome is characterized by obesity, insulin resistance, hypertension and dyslipidemia. It has also been suggested that hepatic steatosis and inflammation should be added to the classical components of the metabolic syndrome. Previous research on obesity and insulin resistance has examined the effects of many different fats and oils, however, the effects of canola oil on metabolic syndrome parameters have yet to be investigated.

It is hypothesized that in a diet induced obese (DIO) rodent model, the combination of canola and flax oils will reduce the severity of metabolic syndrome parameters and favorably alter hepatic phospholipid (PL) and triacylglycerol fatty acid composition. The objective of the study was to investigate the biological effects of a 12 week dietary intervention with high fat diets based on various vegetable oils (high oleic canola, canola, canola/flax (3:1; C/F), safflower and soybean) and lard in 6 week old obese prone rats with regards to obesity, insulin resistance, lipidemia, hypertension, inflammation, hepatic steatosis, hepatic fatty acid composition and markers of hepatic fatty acid oxidation and synthesis.

Overall the C/F diet attenuated more of the components of the metabolic syndrome, including obesity, glycemia, lipidemia, inflammation and hepatic steatosis, than the other high fat diets in DIO rats. However, each of the dietary treatments attenuated various components of the metabolic syndrome suggesting that all dietary fats and oils have their role in the prevention of different components of the metabolic syndrome. Additionally, the C/F diet led to increased eicosapentaenoic acid and

docosahexaenoic acid concentrations in hepatic PL suggesting that α-linolenic acid can be efficiently converted to its very long chain derivatives in DIO rats. Thus, the addition of flax oil to conventional canola oil, in the C/F diet, appeared to enhance the beneficial effects of canola oil on metabolic syndrome parameters.

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LIST OF ABBREVIATIONS

AA	arachidonic acid
ACC	acetyl-CoA carboxylase
ACO	acyl-CoA oxidase
AI	adequate intake
ALA	alpha-linolenic acid
ANOVA	one-way analysis of variance
apoB	apolipoprotein B
BMI	body mass index
C	canola oil
C/F	canola/flax oil blend
CRP	C-reactive protein
DHA	docosahexaenoic acid
DIO	diet-induced obesity
DM2	type 2 diabetes mellitus
DPA	docosapentaenoic acid
EAR	estimated adequate requirement
eEF2	eukaryotic elongation factor 2
ELISA	enzyme-linked immunosorbent assay
en	energy
EPA	eicosapentaenoic acid
FAO	Food and Agriculture Organization
FFA	free fatty acid
FIB	fibronectin
GLP-1	glucagon-like peptide-1
GLUT-4	glucose transporter protein-4

HC	high oleic canola oil
HDL	high density lipoprotein
HOMA	homeostatic assessment model
HTN	hypertension
IL	interleukin
ITT	insulin tolerance test
L	lard
LA	linoleic acid
LDL	low density lipoprotein
LPL	lipoprotein lipase
MUFA	monounsaturated fatty acid
n-3	omega-3
n-6	omega-6
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NHANES	National Health and Nutrition Examination Survey
OCT	optimal cutting temperature
OGTT	oral glucose tolerance test
OP	obese prone
OP-CD	obese prone-Charles River Sprague-Dawley
OR	obese resistant
PL	phospholipid
PPAR	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acid
PVDF	polyvinylidene difluoride
RDA	recommended dietary allowance
RT-PCR	real time polymerase chain reaction

SAA	serum amyloid A
SAS	Statistical Analysis Software
SB	soybean oil
SF	safflower oil
SFA	saturated fatty acid
SDS-PAGE	sodium dodecylsulfate-polyacrylamide gel electrophoresis
SRD	sucrose rich diet
SREBP-1c	sterol regulatory element binding protein-1c
TAG	triacylglyceride
TEMED	N, N, N', N'-Tetramethylethylenediamine
TNF-α	tumor necrosis factor-alpha
USDA	United States Department of Agriculture
VLDL	very low density lipoprotein
VPR	volume pressure recording
WHO	World Health Organization
WM	weight matched

LITERATURE REVIEW

Introduction

Obesity has become a worldwide epidemic that affects adults and children alike (World Health Organization; WHO, 2006). Obesity does not occur in isolation in the body, but is typically accompanied by insulin resistance, hyperlipidemia, hypertension (HTN), inflammation and non-alcoholic fatty liver disease (NAFLD), which are all components of the metabolic syndrome (American Heart Association, 2010; Third Report of the National Cholesterol Education Program [NCEP] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults [Adult Treatment Panel III] Final Report, 2002; Marchesini et al., 2001). Given the current obesity epidemic and the wide consumption of fats in the diet, there is an urgent need to identify dietary fats and oils that can prevent the onset of the metabolic syndrome.

In the past, most of the research that has examined the effects of dietary fatty acids on obesity and related conditions has focused on the effects of saturated fatty acids (SFAs), polyunsaturated fatty acids (PUFAs) and very long chain omega 3 (n-3) PUFAs found in marine sources, while not considering the possible benefit of diets high in monounsaturated fatty acids (MUFAs) and the plant based n-3 PUFA alpha-linolenic acid (ALA). Therefore, it is important to note that the majority of studies cited in this thesis are not based on the long chain n-3 PUFA ALA, but on the very long chain n-3 PUFAs found in marine oils, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with limited mention of MUFAs. Although there is an abundance of literature that has examined dietary fatty acids and insulin resistance in genetically or pharmacologically induced obesity models, the focus of this thesis is on a diet-induced

obesity (DIO) rodent model, which more closely mimics the phenotype and pathogenesis of human obesity. Thus, the overall goal of this research study was to investigate the effects of the fatty acid profiles of various vegetable oils and lard on the prevention of the metabolic syndrome in a DIO model.

Metabolic Syndrome

The metabolic syndrome is characterized by obesity, insulin resistance, hypertension and dyslipidemia (reviewed by Buettner et al., 2006). The prevalence of the metabolic syndrome is on the rise. A sample of 1276 multi-ethnic Canadians revealed that the prevalence of the metabolic syndrome in Canada was 25.8% (Anand et al., 2003). The prevalence of the metabolic syndrome was highest in First Nations (41.6%), followed by South Asians (25.9%), Europeans (22%), and Chinese (11%). The implications of having the metabolic syndrome are severe as it increases the risk of coronary heart disease and stroke by threefold compared with individuals with normal glucose tolerance (reviewed by Nugent, 2004). The rising prevalence of the metabolic syndrome is likely due to the rapid increase in obesity rates. It has also been suggested that NAFLD and inflammation should be added to the classical components of the metabolic syndrome (Haffner, 2006; Marchesini et al., 2001).

Hepatic steatosis is the first stage of NAFLD and is associated with obesity, type 2 diabetes mellitus (DM2), and hyperlipidemia. It is estimated that among patients with hepatic steatosis, 30% to 100% are obese, 10% to 75% have DM2, and 20% to 92% have dyslipidemia (reviewed by Delarue, 2004). In the NHANES III population study, patients with NAFLD had two times higher risk of developing DM2 compared to those without NAFLD (reviewed by Delarue et al., 2004). Obesity and DM2 are now considered low-

grade chronic inflammatory diseases (reviewed by Pérez-Echarri et al., 2008; Faintuch et al., 2007). In addition, obesity is associated with increased adipose mass and enlarged adipocytes leading to adipocyte dysfunction. Dysfunctional adipocytes also contribute to inflammation by increasing circulating pro-inflammatory adipokines and cytokines (reviewed by DeClercq et al., 2008), thus altered adipose function and inflammation are important features of the metabolic syndrome. Each of the components of the metabolic syndrome will be discussed in more detail in the next section.

a) Insulin Resistance

The prevalence of DM2 has increased dramatically in North America over the past 20 years due to a number of factors, such as escalating obesity rates, the aging population and increasingly sedentary lifestyles (reviewed by Delarue, 2004). DM2 is characterized by insulin resistance and hyperglycemia, and is strongly related to obesity, hypertension and the metabolic syndrome. Insulin resistance is characterized by a normal to high production of insulin, which exerts a less than normal biological response and, thus, leads to hyperglycemia (De Vries et al., 1989). Insulin resistance occurs in skeletal muscle, adipose tissue and the liver. It is widely accepted that diet and exercise can effectively prevent or postpone the onset of insulin resistance and DM2 largely by decreasing visceral obesity and improving membrane fluidity and, thus, improving insulin sensitivity (McCarty, 2000). Realistically, however, such lifestyle changes often require more discipline than many people are willing to give (McCarty, 2000). This has led to the search for new compounds to improve glucose uptake and metabolism, even when diet and exercise habits remain suboptimal.

b) Obesity

Obesity has become a worldwide epidemic affecting both adults and children alike. It is defined by the WHO (2006) as a body mass index (BMI) of 30 kg/m^2 or greater and is characterized by excessive fat accumulation, which can negatively affect health. Obesity increases an individual's risk of developing various diseases such as cardiovascular disease, DM2, certain types of cancer and osteoarthritis (WHO, 2006). In most instances, obesity is caused by an imbalance between calories consumed and calories expended resulting in a positive energy balance. A positive energy balance is often obtained when energy dense or empty calorie foods, such as fats and sugars, are over-consumed and when physical activity is lacking (WHO, 2006). Therefore, it should come as no surprise that exercise and diet are at the center of prevention and treatment strategies for obesity.

c) Hyperlipidemia

Hyperlipidemia is simply excess lipids in the blood including triacylglycerides (TAG), phospholipids (PL), free fatty acids (FFA) and cholesterol and can lead to cardiovascular disease and hepatic steatosis (AHA, 2009). Insulin acts to promote free fatty acid uptake into muscle cells and adipocytes by lipoprotein lipase (LPL), resulting in TAG storage. However, in a state of insulin resistance, there is less stimulation of LPL for breakdown of TAG to FFA for uptake by the cells leading to hyperlipidemia. The metabolic syndrome is strongly associated with dyslipidemia. The lipid profile that is characteristic of the metabolic syndrome consists of decreased high density lipoprotein (HDL) and increased apolipoprotein B (apoB), plasma TAG, and intermediate density

lipoprotein levels (reviewed by Nugent, 2004). Low density lipoprotein (LDL) cholesterol levels are usually normal or slightly increased in individuals with the metabolic syndrome.

d) Hypertension

HTN refers to chronically elevated blood pressure in the arteries and is a major risk factor for cardiovascular disease, including heart attack, heart failure and stroke, as well as kidney disease (Chobanian et al., 2003). HTN is diagnosed when systolic blood pressure and/or diastolic blood pressure are above 140 mmHg and/or 90 mmHg, respectively (Chobanian et al., 2003). The incidence of diabetes and high serum cholesterol increase the risk of developing HTN, thus, an individual who has the metabolic syndrome commonly has HTN as one of the components (reviewed by Chobanian et al., 2003).

e) Inflammation and Adipose Function

Inflammation is simply the body’s response to injury and can be classified as acute or chronic (reviewed by Feghali et al., 1997). Chronic inflammation, the type associated with obesity and insulin resistance, is characterized by the development of specific hormonal and cellular immune responses (reviewed by Feghali et al., 1997).

When adipocytes become enlarged, namely due to obesity, their endocrine and metabolic functions are altered, resulting in adipocyte dysfunction (reviewed by DeClercq et al., 2008; reviewed by Weisberg et al., 2003). White adipose tissue and/or infiltrated macrophages not only secrete adipokine hormones such as pro-inflammatory leptin and

anti-inflammatory adiponectin, but also secrete pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and synthesize acute phase proteins like haptoglobin and C-reactive protein (CRP; reviewed by DeClercq et al., 2008; Pérez-Echarri et al., 2008; Ahima et al., 2000). The synthesis and secretion of these pro- and anti-inflammatory markers are altered by changes in adipocyte size and number (Guerre-Millo, 2003). Larger adipocytes are more insulin resistant and produce more pro-inflammatory mediators and less anti-inflammatory mediators (reviewed by Weisberg et al., 2003).

f) Hepatic Steatosis and Hepatic Fatty Acid Composition

NAFLD consists of a continuum of conditions ranging from fat accumulation in the liver (hepatic steatosis), to inflammation and necrosis of hepatocytes (non-alcoholic steatohepatitis [NASH]), to fibrosis due to further inflammatory damage, cirrhosis, and ultimately liver failure (reviewed by Alwayn et al., 2005). Although the etiology of NAFLD is currently unknown, it is associated with hyperlipidemia, obesity and DM2, with insulin resistance being the most important pathogenic factor (reviewed by Mehta et al., 2002; reviewed by Marchesini et al., 2001). NAFLD is diagnosed when fat in the liver exceeds 5% to 10% by weight (Neuschwander-Tetri et al., 2003). The prevalence of NAFLD is high among North Americans, affecting 25% of the general population (reviewed by Svegliati-Baroni et al., 2006). The liver is the major organ of endogenous glucose production; therefore, in addition to hepatic fat accumulation, hepatic steatosis is also associated with hepatic insulin resistance due to an impairment of insulin's ability to lower liver glucose output (Buettner et al., 2007).

Dietary Fatty Acids

There are 3 main classes of dietary fatty acids; SFA, MUFA, and PUFA. Each group of fatty acids has important functions in the body and differs in terms of essentiality. Fatty acids vary in chain length and are designated short (< C6), medium (C6-C12), long (longer than C12 and up to C22) and very long (>C22).

a) Saturated Fatty Acids (SFAs)

Dietary SFAs are abundantly present in animal products, but can also be found in plant products. The major SFAs in the diet include caprylic acid (C8:0), caproic acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0; National Academy of Sciences et al., 2005). SFAs have 2 primary functions in the body: 1) they serve as a source of energy and 2) are structural components of cell membranes (National Academy of Sciences et al., 2005). SFAs are not essential in the diet as they can be synthesized as needed from other fuel sources. In addition, SFAs are not associated with a beneficial role in the prevention of chronic disease and, therefore, neither an adequate intake (AI) nor an estimated average requirement (EAR) and recommended dietary allowance (RDA) have been set for SFA intake (National Academy of Sciences et al., 2005).

b) Monounsaturated Fatty Acids (MUFAs)

MUFAs refer to a family of fatty acids that are characterized as having one double bond. MUFAs in food have the double bond located either at 7 (n-7) or 9 (n-9) carbon atoms from the methyl end. The MUFAs in the diet include myristoleic acid (C14:1n-7),

palmitoleic acid (C16:1n-7), vaccenic acid (C18:1n-7), oleic acid (C18:1n-9), eicosenoic acid (C20:1n-9) and erucic acid (C22:1n-9; National Academy of Sciences et al., 2005). The body uses MUFAs to generate energy and as an important lipid in structural membranes, particularly nervous tissue myelin (National Academy of Sciences et al., 2005). MUFAs are not essential in the diet as they can be biosynthesized from other fuel sources. In addition, MUFAs have no known independent role in the prevention of chronic disease ((National Academy of Sciences et al., 2005). Thus, no AI, EAR or RDA have been set for MUFAs.

c) Polyunsaturated Fatty Acids (PUFAs)

PUFAs consist of a group of fatty acids that have 2 or more double bonds. The first double bond can begin either at 6 carbons from the methyl end (n-6) or 3 carbons from the methyl end (n-3).

i) N-6 PUFAs

Dietary n-6 PUFAs include the fatty acids linoleic acid (LA; C18:2n-6), γ-linolenic acid (C18:3n-6), dihomo-γ-linolenic acid (C20:3n-6), arachidonic acid (AA; C20:4n-6), adrenic acid (C22:4n-6) and n-6 docosapentaenoic acid (C22:5n-6; National Academy of Sciences et al., 2005). Humans cannot synthesize LA, thus, LA is an essential fatty acid and a substrate for synthesizing very long chain n-6 fatty acids (Figure 1). Without dietary LA, deficiency occurs, producing adverse clinical symptoms, including reduced growth and a scaly rash (National Academy of Sciences et al., 2005).

In the body, n-6 PUFAs function as a component of structural cell membranes and are involved in cell signaling pathways. LA is also desaturated and elongated to AA and dihomo-γ-linolenic acid, which are substrates for eicosanoid production (National Academy of Sciences et al., 2005). Eicosanoids, such as prostaglandins, thromboxanes and leukotrienes, are signaling molecules and are important to human health because they exert complex control over many bodily systems (Simopoulos, 1999). A diet rich in n-6 PUFAs increases n-6 eicosanoid production, and this shifts the physiological state to one that is more pro-thrombotic, pro-constrictive and pro-inflammatory (reviewed by Simopoulos, 2002). Thus, it has been shown that the eicosanoids produced from AA contribute to the formation of thrombus, atheroma, allergic and inflammatory disorders, as well as the proliferation of cells (reviewed by Simopoulos, 2002). The AI of LA for men between the ages of 19-50 years is 17 g/day and for men greater than 50 years is 14 g/day. The AI of LA for women between the ages of 19-50 years is 12 g/day and for women over the age of 50, 11 g of LA/day is recommended (National Academy of Sciences et al., 2005). Although n-6 PUFAs are essential to the diet, the typical North American population consumes excessive amounts of n-6 PUFAs (Simopoulos, 2002).

ii) N-3 PUFAs

N-3 PUFAs include ALA, which is an essential fatty acid that cannot be synthesized by humans. ALA is used in the body to synthesize other very long chain fatty acids (Figure 1). A deficiency in ALA leads to adverse clinical symptoms including neurological abnormalities and reduced growth (National Academy of Sciences et al., 2005). Common n-3 PUFAs in the diet include ALA (C18:3n-3), EPA (C20:5n-3),

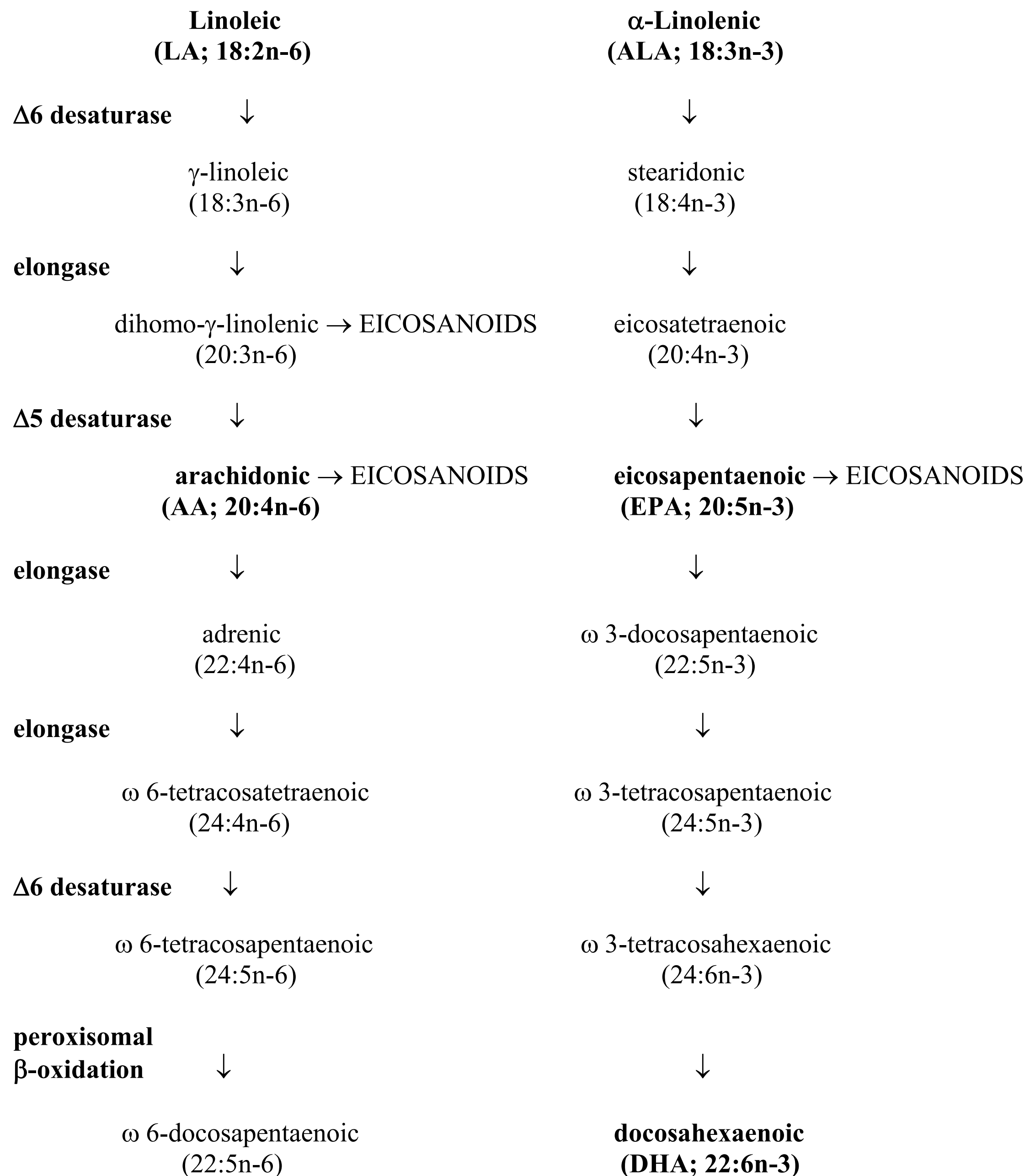


Figure 1: Biosynthesis of Very Long-Chain Polyunsaturated Fatty Acids.
Adapted from Sprecher (2000)

docosapentaenoic acid (DPA; C22:5n-3) and DHA (C22:6n-3; National Academy of Sciences et al., 2005).

ALA is the most common n-3 PUFA in the diet. The body uses the parent fatty acid, ALA, mainly as a source of energy and carbon, but also as a precursor for all n-3 very long chain PUFA derivatives including EPA and DHA (Thomas, 2002). The amount of conversion of dietary ALA to EPA and DHA by humans is not conclusive but is estimated to be around 15% for EPA and 0.05% to 5% for DHA, and strongly depends on the concentration of n-6 PUFAs in the diet (Thomas, 2002). In Fischer-344 rats, Gao et al. (2009) demonstrated that the liver was capable of maintaining brain DHA homeostasis, via ALA conversion, when DHA was absent from the diet and ALA was intravenously infused via the femoral vein.

EPA, which is desaturated and elongated from ALA, is a precursor for n-3 eicosanoids. In contrast to n-6 eicosanoids, eicosanoids produced by n-3 PUFAs are anti-inflammatory, anti-thrombotic, anti-arrhythmic, hypolipidemic and vasodilatory (reviewed by Simopoulos, 2002). Thus, they play an important role in the prevention and treatment of coronary heart disease, hypertension, diabetes, cancer, arthritis and other inflammatory and autoimmune disorders (reviewed by Simopoulos, 1999). The AI of ALA for male and female adults is 1.6 g/day and 1.1 g/day, respectively (National Academy of Sciences et al., 2005).

There is much controversy around the optimal dietary ratio of n-6 to n-3 PUFAs. Increased dietary consumption of n-6 PUFAs has been shown to decrease the production of n-3 eicosanoids, and vice versa (reviewed by Simopoulos, 2002). This is due to competition of ALA and LA for the elongation and desaturation enzymes that are involved in the synthesis of long chain PUFAs (Thomas, 2002). Humans evolved on a

diet in which the n-6 to n-3 ratio was about 1, however, over the last 50-100 years there has been rapid changes in the human diet (Simopoulos, 2002). Currently, the typical North American diet is rich in n-6 PUFAs, which has resulted in an n-6 to n-3 ratio ranging from 15-16.7:1 (Simopoulos, 2002). Intakes of n-3 PUFAs are much lower and intakes of n-6 PUFAs are much higher than in the past because of the decrease in fish consumption and the increase in consumption of both oils that are rich in n-6, such as soybean and safflower oil, and cereal grains that have a high n-6/n-3 ratio (Simopoulos, 1999). Also, the industrial production of animal feed rich in grains containing n-6 PUFAs has led to the production of meat with a high n-6 PUFA content, further offsetting the ratio of n-6 to n-3 (Simopoulos, 1999). Based on limited studies that have been done on animals, children and adults, the Food and Agriculture Organization (FAO) and WHO (1995) recommend that adults consume a LA:ALA ratio of 5-10:1 to maintain health. Many researchers recommend a ratio of 5:1, or less, to reduce the risk of many chronic diseases (Simopoulos, 2002). However, the use/consumption of ALA rich oils in the food industry and in the home has declined largely due to the fact that n-3 PUFAs readily oxidize and, thus, are unstable.

There are many different types of fats and vegetable oils containing different amounts of SFA, MUFA, PUFA, n-6 and n-3 that are available to the food industry and consumers alike. The types of fats and oils that are available are largely driven by the food industry and there is increased demand for oils that have high stability for use in processing and frying and have a long shelf life. Due to advancements in agricultural production and plant breeding, it is now possible to modify the fatty acid composition of crops and, thus, the oils that are made from them. For example, this strategy has been

used to produce higher stability oils with lower n-3 and high MUFA content. However, this has led to a decrease in n-3 PUFAs in the food supply.

In the past, dietary fats were merely seen as a source of calories, whereas today, it is known that dietary fats and oils have an important role in health. Dietary fats and oils not only supply the essential fatty acids (ALA and LA), which are incorporated into membrane structures and can produce important eicosanoids, but also serve as carriers for fat-soluble vitamins, influence cell function, affect immune function and are associated both positively and negatively with many diseases (Emken, 1984). In the 1970's, hydrogenated fats that contain trans fatty acids were widely produced; today it is known that trans fatty acids and SFA have detrimental effects on health. The AHA recommends reducing SFA intake to less than 7% of total kcals and increasing n-3 PUFA content to reduce the risk of coronary heart disease (Krauss et al., 1996). MUFAs are considered to have less of a negative impact on health than SFA and may even be beneficial to people with DM2 (American Diabetes Association, 2009). PUFAs are considered to be the most "healthy" types of fatty acids, however, in the last 50 years dietary consumption of n-6 PUFAs has greatly increased whereas consumption of n-3 PUFAs has decreased. The imbalance between n-6 and n-3 PUFAs can negatively affect health in many ways such as by increasing the production of n-6 eicosanoids and by limiting the incorporation of n-3 PUFAs into cell membranes.

Therefore, in light of what is currently known about the various types of fatty acids, vegetable oils and fats that are low in SFA, have moderate amounts of MUFA, and have a low n-6/n-3 ratio may be especially important for the prevention and treatment of many diseases. The fats described in the next section of this thesis represent the types of fats and vegetable oils that were used as dietary treatments for this research study.

Dietary Fats and Oils

a) Lard

Consumption of lard has declined over the past 30 years (Sanders, 2000). The use of lard as a cooking fat has likely decreased due to health concerns associated with its high saturated fat content. Although lard is high in SFA (43% of total fatty acids) it actually contains higher amounts of MUFAs (47% of total fatty acids; Canola Council of Canada, 2008). Lard contains minimal PUFAs (10% of total fatty acids) with LA representing 9% of total fatty acids and ALA only representing 1% of total fatty acids (Canola Council of Canada, 2008). This means that lard has an n-6 to n-3 ratio of 9:1.

b) High Linoleic Safflower Oil

High linoleic safflower oil is not a vegetable oil that is popularly consumed and therefore is not on the United States' Department of Agriculture's (USDA; 2007) list of the top 10 most consumed vegetable oils in the world. High linoleic safflower oil does, however, have a fatty acid profile similar to sunflower oil, which is the fourth most consumed vegetable oil in the world. High linoleic safflower oil contains high levels of the n-6 PUFA LA (76% of total fatty acids), which makes it unstable for cooking purposes (Canola Council of Canada, 2008). In addition, it is low in SFA (10% of total fatty acids) and MUFA (14% of total fatty acids), and very low in the n-3 PUFA ALA (1 % of total fatty acids; Canola Council of Canada, 2008). The high amounts of LA and low amounts of ALA present in high linoleic safflower oil results in a very high n-6 to n-3 ratio of 75:1.

c) Soybean Oil

Soybean oil is one of the top 2 most consumed vegetable oils in the world (USDA, 2007). The United States is the world's largest producer of soybeans, and soybean oil exports represent a significant net contribution to the United States agricultural trade. Due to climate, Canada is not a major producer of soybean. Currently, in Canada, soybeans are primarily grown near the United States border of the eastern provinces Ontario and Quebec as well as in the Red River Valley of Manitoba. However, varieties are being developed that can grow on the Canadian Prairies. Throughout the world, soybean oil is used extensively in both the food industry and directly by consumers. Many cooking oils sold as "vegetable oil" are actually soybean oil.

Soybean oil is very high in PUFAs while being relatively low in SFA and MUFA. PUFAs represent 62% of total fatty acids in soybean oil, whereas SFA and MUFAs only represent 15% and 23%, respectively (Canola Council of Canada, 2008). Although soybean oil contains 8% of total fatty acids as ALA, which is higher than many other popular vegetable oils, it contains 54% of total fatty acids as LA, resulting in an n-6 to n-3 ratio of 7:1 (Canola Council of Canada, 2008).

d) Canola Oil

Canola is abundantly grown in Canada, as it is a crop that is suited to the Canadian climate. It was developed in the 1970's by crop breeding to reduce the erucic acid content in rapeseed, and the resulting product was called canola (Shahidi, 1990). It is an important crop to the Canadian economy as it annually adds 13.8 billion dollars in economic activity and brings back 2.8 billion dollars in revenue (Canola Council of Canada, 2008). According to the USDA (2007), canola oil was the third most consumed

vegetable oil in the USA in 2007. Although canola meal, which is usually used in animal feed, is an excellent source of protein, canola oil is the most valuable part of the canola seed and is considered to be a "healthy" fat compared to other popular vegetable oils on the market (Canola Council of Canada, 2008).

In the United States, the Food and Drug Association (2006) has approved a health claim for canola oil stating that canola is high in unsaturated fats and consuming 1.5 tablespoons of canola oil per day may reduce the risk of coronary heart disease. Thus, the potential health benefits of canola oil are largely due to its fatty acid composition. Canola oil predominantly contains the MUFA, oleic acid (61% of total fatty acids), and has low levels of SFA (7% of total fatty acids). Canola oil is also a good source of the two essential PUFAs, ALA (11% of total fatty acids), an n-3 PUFA, and LA (21% of total fatty acids), an n-6 PUFA (Canola Council of Canada, 2008). This means that canola oil has an excellent n-6 to n-3 ratio of 2:1.

e) High Oleic Canola Oil

High oleic canola oil is more heat resistant and has a longer shelf life than conventional canola oil and, thus, is most commonly used in commercial food production and service (Canola Council of Canada, 2008). Additionally, high oleic canola oil is commonly used in the industry due to the elimination of the use of hydrogenated oils containing trans fatty acids. High oleic canola oil is more heat stable than conventional canola oil because it contains less PUFAs, mainly ALA. In addition, as its name implies, high oleic canola oil contains more oleic acid (a MUFA) than conventional canola oil. High oleic canola oil has a fatty acid profile that consists of 7% SFA, 70% MUFA, and 23% PUFA; n-3 PUFAs make up only 3% of total fatty acids, whereas n-6 PUFAs

compose 20% (Canola Council of Canada, 2008). Thus, high oleic canola oil contains 8% less ALA and only 1% less LA than conventional canola oil, resulting in a higher n-6 to n-3 ratio of about 7:1.

f) Flax Oil

In Canada, flax is mainly grown in the cool, northern climate of the western prairies. About 42% of the flaxseed is oil, and although flax oil has an excellent fatty acid profile, it is not among the top 10 most commonly consumed vegetable oils (USDA, 2007). Flax is not commonly consumed because it contains high amounts of PUFAs and, thus, has poor heat stability, increased susceptibility to oxidation and rancidity, and a short shelf life. Flax oil is low in SFA (9% of total fatty acids) and MUFA (21% of total fatty acids) and mainly contains PUFA (70% of total fatty acids), primarily as ALA (Francois et al., 2003). N-3 PUFAs make up 54% of total fatty acids whereas n-6 PUFAs make up 16%, resulting in an n-6 to n-3 ratio of 0.3:1 (Francois et al., 2003).

MUFAs/N-3 PUFAs and the Metabolic Syndrome

There is limited research on the ability of canola oil to prevent and treat components of the metabolic syndrome. Canola oil is high in MUFA and ALA, thus the focus of this literature review is on the effects of MUFA and ALA on metabolic syndrome components including insulin resistance, obesity, lipidemia, hypertention, inflammation and hepatic steatosis. The animal studies cited in the literature review used male rats, unless otherwise indicated.

a) MUFAs/N-3 PUFAs and Insulin Resistance

Individuals with DM2 are recommended to consume diets rich in MUFAs and n-3 PUFAs, fatty acids found in canola oil (American Diabetes Association, 2009). MUFAs have been shown to improve blood lipids and glucose homeostasis, and n-3 PUFAs have been shown to decrease the risk of vascular disorders in high-risk individuals (reviewed by Mustad et al., 2006). However, experimental designs (e.g. types of n-3 PUFAs, model, dose, duration, procedures for evaluation of insulin sensitivity) and results show considerable variation. In addition, the effects of MUFAs and n-3 PUFAs have typically been tested independently; therefore, it is important to determine the effects of MUFA and n-3 PUFA together on glucose metabolism and insulin resistance.

i) MUFAs and Insulin Resistance

Several population studies have displayed positive effects of MUFAs on insulin resistance. The Pizarra study from Spain showed less insulin resistance, measured by homeostatic assessment model (HOMA), in individuals who reported consumption of a high MUFA (olive oil) diet compared to those who consumed a diet rich in sunflower oil (Soriguer et al., 2006). In addition, the subjects who consumed high MUFA diets also had the highest levels of oleic acid in plasma phospholipids compared to those individuals who consumed less MUFAs. Similarly, Louheranta et al. (2002) documented that a high MUFA diet increased the proportions of both oleic acid and ALA in plasma phospholipids, and both of these fatty acids were found to be associated with lower fasting plasma glucose concentrations in human subjects with impaired glucose tolerance. However, in this study, no effect on insulin sensitivity (assessed by the frequently sampled intravenous-glucose-tolerance test) was documented in subjects consuming the

diet rich in MUFAs. In addition, a meta-analysis by Garg (1998) revealed that in patients with DM2, a high MUFA diet significantly reduced fasting plasma glucose concentrations compared to a high carbohydrate diet, however, no improvement was seen in insulin sensitivity in either of the groups when the euglycemic-hyperinsulinemic clamp technique was used. Thus, more studies in humans are needed to confirm whether high MUFA diets can improve insulin sensitivity in patients with the metabolic syndrome.

There are very few animal studies that have examined the effects of MUFAs on insulin resistance. A study by Buettner et al. (2006) examined the effect of standard chow versus various high fat diets (42% of energy [42% en]) based on lard, olive oil, coconut oil or fish oil on insulin sensitivity in Wistar rats. The high fat olive oil diet (high in MUFA) and lard diet (high in SFA and MUFA) led to the most pronounced manifestation of insulin resistance (assessed by the insulin tolerance test [ITT]) compared to the standard chow and other high fat diets. In another study, Mustad et al. (2006), determined the effects of high fat diets (43% to 45% en) rich in MUFA alone (containing 85% high-oleic safflower oil, 10% canola oil, and 5% lecithin), or MUFA plus 11% to 14% of kcals from ALA, EPA or DHA on insulin resistance in *ob/ob* mice. After 29 days, only animals consuming the MUFA control and ALA MUFA blend diets showed a trend to lower non-fasting glucose concentrations. Additionally, fasting plasma glucose values were significantly elevated in the animals consuming the EPA and DHA MUFA blends, but not in the ALA MUFA blend or MUFA control group. However, when insulin sensitivity was assessed with the ITT, the MUFA diet did not reduce plasma glucose after the insulin injection. On the other hand, interestingly, the ALA MUFA blend was the only dietary group that had significantly improved insulin sensitivity. Although a genetic mouse model was used in this study, it is the only study, to our

knowledge, that has evaluated the effect of MUFA and ALA in a high fat diet.

ii) Very Long Chain N-3 PUFAs and Insulin Resistance

Most of the research that has examined the effects of n-3 PUFAs on insulin resistance has used the marine very long chain n-3 fatty acids EPA and DHA. Storlien et al. (1987) were the pioneers in determining the effects of marine n-3 PUFAs on insulin resistance in a DIO rat model. In this study, Wistar rats were fed a high fat diet (59% en) containing safflower oil or the same diet with 20% of safflower oil replaced with tuna oil. After 31 days, the development of insulin resistance (assessed by the euglycemic-hyperinsulinemic clamp technique) was completely prevented in the fish oil group. The fish oil diet in this study only replaced approximately 6% of the safflower fatty acids with EPA and DHA (very long chain n-3 PUFAs). However, the effects of ALA, the plant based n-3 PUFA, on insulin resistance were not investigated. Another study, using high fat diets and n-3 PUFAs from fish oil, showed that after 12 weeks on a high fat diet (42% en) insulin sensitivity, assessed with the ITT, was close to normal in high fat-fed Wistar rats fed fish oil or coconut oil compared to rats fed lard and olive oil (Buettner et al., 2006). Like canola oil, olive oil is high in oleic acid, however, olive oil contains less ALA than canola oil. Additionally, dietary n-3 PUFAs from fish oil improved insulin sensitivity, via decreasing adipocyte size, in Wistar rats that were fed a sucrose rich diet (SRD) with added fish oil compared to control rats fed the sucrose rich diet alone (Lombardo et al., 2007). Like high fat diets, high sucrose diets also induce obesity and insulin resistance. There are many other research studies that have shown that insulin resistance can be improved, delayed or prevented by marine n-3 PUFAs, however, other types of animal models (e.g. genetic models) have been used to demonstrate these effects

(Lombardo et al., 2006).

A couple of human studies have also documented improved insulin resistance from consumption of very long chain n-3 PUFAs. In a paired human study, in which a subject in one group was paired based on certain criteria with a subject from another group, a high n-3 PUFA diet from fish significantly increased insulin sensitivity, assessed using octreotide insulin suppression testing, compared to a control diet (Tsitouras et al., 2008). In a cross-sectional study, Rasic-Milutinovic et al. (2007) examined the effects of fish oil on 35 patients with chronic renal failure who were on hemodialysis, had insulin resistance and consumed a high n-6 PUFA diet. When fish oil supplements were added to the patients' usual diet for 8 weeks, their insulin sensitivity, assessed by HOMA, was significantly improved.

iii) Long Chain N-3 PUFAs and Insulin Resistance

Few studies have been conducted on the effects of the plant-based n-3 PUFA ALA on insulin resistance. In a cross-sectional study of 3383 Japanese workers, it was found that ALA intake was inversely associated with prevalence of insulin resistance, assessed by HOMA (Muramatsu et al., 2010). Interestingly, EPA and DHA had no association with insulin resistance in this study.

In Wistar rats, Storlien et al. (1991) documented that insulin's actions were normalized (assessed by the euglycemic clamp method) when 11% of fatty acids in a high fat PUFA diet (59% en) was replaced with fish oil for 30 ± 1 days. ALA was also tested in this study and results showed that substituting 11% of the PUFA diet with ALA (flax oil) had no effect on insulin sensitivity, likely due to competition with n-6 PUFAs (namely, LA) that were present in the high PUFA diet. However, when ALA was

substituted in a SFA diet, insulin resistance was completely prevented compared to the SFA diet alone. These results demonstrate the importance of a low n-6 to n-3 ratio for the beneficial effects of ALA to be observed.

In another study, Sprague Dawley rats were fed a low fat safflower oil diet (4% en) or a high fat (40% en) safflower oil, fish oil or perilla oil (containing the n-3 PUFA, ALA) diet for 21 days (Takahashi et al., 2000). At the end of the study, the safflower oil group had significantly higher serum glucose concentrations than the perilla or fish oil groups and there was no significant difference in serum glucose concentrations among the animals fed the low fat diet and the animals fed the high fat perilla or fish oil diets. This suggests that ALA may be as effective as EPA and DHA from fish oil for preventing hyperglycemia, an important characteristic of insulin resistance.

As previously mentioned, Mustad et al. (2006) documented no improvement in insulin sensitivity after 29 days of treatment in *ob/ob* mice that were fed high fat (43-45% en) MUFA diets (containing 85% high-oleic safflower oil, 10% canola oil and 5% lecithin) that were replaced with 11% to 14% of energy with either of the marine fatty acids EPA or DHA. However, in this study, *ob/ob* mice that were fed the high fat MUFA diet in which ALA replaced 11-14% energy showed significantly improved insulin sensitivity assessed by the ITT. Although a DIO rodent model was not used in this study, it is important to point out that these results suggest that it may be the combination of both MUFA and ALA in the diet that allows for more efficient use of insulin and transport of glucose. In another study, with Wistar rats, the effects of an 8 week diet comprised of canola oil as a fat source and supplemented with 0.5 g of either ALA, EPA or DHA/kg body weight was investigated (Anderson et al., 2008). Conversely, this study showed that EPA and DHA lowered fasting serum glucose and insulin levels by 35% and

38%, respectively, compared to the ALA group. Additionally, the HOMA score was 60% lower in the EPA and DHA groups compared to the ALA group. These data suggest that ALA does not have as great an effect on insulin sensitivity as EPA and DHA.

Research on the effects of the long chain n-3 PUFA ALA on insulin resistance is limited and few studies have compared and contrasted the effects of different types of high fat diets with or without the addition of ALA; thus, more research in this area is warranted.

b) MUFAs/N-3 PUFAs and Obesity

There are few studies that have examined the ability of MUFAs to prevent obesity in non-genetic rodent models, and studies that have been done are not very promising. For example, Wistar rats that were fed high fat diets (42% en) based on olive oil (high in MUFA), coconut oil or lard had the highest weight gain after 12 weeks compared to rats fed a high fat diet based on fish oil or controls fed standard chow (Buettner et al., 2006). However, this study did not determine the effects of the various high fat diets on adipose mass. Thus, more research is needed on the ability of MUFA alone as well as MUFA with ALA to prevent obesity.

There are 5 relevant research studies that demonstrate the ability of marine n-3 PUFAs to reduce obesity in DIO rat models. First, in a 12 week study of single oil comparisons, Wistar rats that were fed a high fat diet (42% en) based on fish oil had significantly lower final body weight than animals fed a high fat diet based on lard, olive oil or coconut oil, and even lower than rats fed standard chow (11% en; Buettner et al., 2006). In a second study, Wistar rats were fed a SRD for 6 months to induce insulin resistance, adiposity and dyslipidemia (Lombardo et al., 2007). After 6 months, control

rats remained on the SRD and treatment rats had fish oil (7 g/100 g) added to their SRD for 2 months. At the end of the study, the fish oil supplemented group had reduced fat pad mass and less hypertrophy of fat cells. There was also a trend for reduced body weight; however, it was not significant compared to control rats. Third, in a study with Fisher rats fed either high fat diets (40% en) based on fish oil or corn oil for 6 weeks, the epididymal fat pads of rats fed the fish oil diet weighed 25% less than those of the corn oil-fed rats (Baillie et al., 1999). Fourth, Wistar rats that were fed a high fat diet (62% en) plus 1 g/kg of the n-3 PUFA EPA for 35 days displayed partial prevention of obesity based on body weight (P = 0.06) compared to high fat-fed control rats (Pérez-Echarri et al., 2008). Fifth, Takahashi et al. (2000) found that Sprague Dawley rats that consumed high fat diets (40% en) based on perilla oil (containing ALA) or fish oil for 21 days had significantly less epididymal and perirenal fat than high fat safflower oil-fed rats.

To our knowledge only one study has explored the effects of canola oil on obesity in a rodent model. Bell et al. (1997) found that when mice were fed a high fat diet (41% en) based on either lard or canola oil, the canola oil fed mice had significantly lower final body weight and fat expressed as g/100 g body weight. Additionally, the groups did not differ significantly in terms of energy intake.

c) MUFAs/N-3 PUFAs and Hyperlipidemia

Current research indicates that MUFA and ALA can improve hyperlipidemia. In human subjects MUFAs have been shown to improve dyslipidemia. A meta analysis by Garg (1998) demonstrated that in patients with DM2 a high MUFA diet (22-33% en from MUFA) significantly reduced fasting plasma TAG by 19% and very low density lipoprotein cholesterol (VLDL-cholesterol) by 22% compared to a high carbohydrate diet.

The high MUFA diet also modestly increased HDL-cholesterol by 4% compared to a high carbohydrate diet.

N-3 PUFAs have also been found to have TAG lowering effects in human subjects (Mckenney et al., 2007). Studies indicate that EPA and DHA are responsible for the lipid lowering properties of n-3 PUFAs; ALA can be converted to EPA and DHA *in vivo*, however, conversion is limited and research on the hypolipidemic effect of ALA is scarce. A meta analysis by Friedberg et al. (1998) revealed a profound TAG lowering effect of fish oil by about 30% in humans with diabetes. Although fish oil reduced plasma TAG concentrations, a small but significant increase was seen in LDL cholesterol due to consumption of fish oil, however, no changes in total and HDL cholesterol levels were observed. Similarly, in an epidemiological study conducted in Italy, total plasma n-3 PUFAs were negatively correlated with TAG and positively correlated with HDL cholesterol (Ferrucci et al. 2006).

In animal models, high fat diets based on animal and plant fats increase plasma TAG and cholesterol concentrations, however, high fat diets based on n-3 PUFAs from fish oil tend to do the opposite (reviewed by Buettner et al., 2007). A study by Takahashi et al. (2000) revealed that Sprague Dawley rats fed high fat diets (40% en) rich in perilla (ALA) or fish oil for 21 days had significantly lower fasting serum TAG, PL and cholesterol concentrations than rats fed a low fat diet. Similarly, Buettner et al. (2006) demonstrated that in Wistar rats fed various types of high fat diets for 12 weeks, the plasma lipids of the high fat-fed fish oil group did not differ from standard chow controls, however, the lard, olive oil (rich in MUFA) and coconut oil high fat fed rats had elevated plasma TAG and FFA. In Sprague Dawley rats fed a SRD for 6 weeks to induce obesity, the addition of 30% fish oil significantly reduced plasma TAG, cholesterol and PL

concentrations compared to control fed rats (Luo et al., 1996).

d) MUFAs/n-3 PUFAs and Hypertension

It was once generally accepted that SFA increase blood pressure whereas MUFAs and PUFAs decrease blood pressure, however, further research has demonstrated that the effects of fatty acids on hypertension are not so clear cut.

The only feeding trial with canola oil in humans was by Mutanen et al. (1992). In this study, 60 subjects were first fed a baseline diet high in SFA (36% en) for 2 weeks. After the 2 weeks, in a cross over design, 30 subjects were fed a diet high in PUFA (sunflower oil, 38% en) for 3.5 weeks followed by a diet high in MUFA (canola oil, 38% en) for 3.5 weeks. This study did not show any significant effect on systolic blood pressure when the diet was enriched with canola or sunflower oil. There have been numerous epidemiological and feeding trials done in humans regarding the effect of MUFA on HTN. However, the source of the MUFA used in these studies was olive oil and results show either no effect or a slightly positive effect of olive oil on lowering blood pressure (reviewed by Alonso et al., 2005).

In an animal study, Wistar rats were fed diets based on canola oil, soybean oil, lard plus egg yolk and lard plus canola oil from the age of 21 days to 12 months (Aguila, 2001). The results indicated that the lard and egg yolk group had the highest blood pressure, assessed with the tail cuff method, and, more specifically, they had 18-21% higher blood pressure than the canola, soybean or lard plus canola oil groups. This study indicates that the addition of the canola oil to the lard diet rescued the blood pressure. Similarly, in another study, 3 month old spontaneously hypertensive rats were fed 1.5 g/kg/day of fish, canola, palm, olive or soybean oil via gavage for 13 weeks (Aguila et al.,

2004). Systolic blood pressure was measured weekly with the tail cuff method and results showed increases in blood pressure were significantly attenuated in the rats fed canola and palm oil compared to the rats fed olive oil and soybean oil. Conversely, Naito et al. (2003) fed 4 week old spontaneously hypertensive rats powdered AIN-93 diet without fat, and provided canola or soybean oil via gavage at 10% of consumed food, once a day for 4 weeks. At the beginning of the study and at the last week of oil administration, blood pressure was measured with the tail cuff method. Results indicated that neither of the oils attenuated the increase in systolic blood pressure in the spontaneously hypertensive rats.

e) MUFAs/N-3 PUFAs, Inflammation and Adipose Function

N-3 PUFAs are natural antagonists of the inflammatory response. An epidemiological study from Tuscany, Italy, revealed that there is a negative association between dietary intake of ALA and pro-inflammatory markers such as serum CRP and IL-1ra (Ferrucci et al., 2006). In another study, 24 obese subjects with elevated inflammatory markers were involved in a prospective, double blind crossover study and randomized into two groups, a flaxseed flour group receiving 5 g ALA/day and a placebo group. After 2 weeks, the flaxseed group had a significant reduction in serum CRP, serum amyloid A (SAA) and fibronectin (FIB), whereas these inflammatory markers were unchanged in the placebo group (Faintuch et al., 2007).

N-3 PUFAs also improve inflammatory markers in DIO animal models. Pérez-Echarri et al. (2008) documented that after 35 days of treatment Wistar rats that were fed a high fat diet (62% en) plus 1 g/kg of the n-3 PUFA EPA had a significant decrease in IL-6 mRNA in adipose tissue compared to high fat-fed control rats. In Wistar rats fed a

SRD, both plasma leptin and adiponectin levels were significantly decreased, however, when fish oil was added to the SRD, adiponectin levels were increased to normal values, similar to those found in rats fed a control diet (Lombardo et al., 2007). Similarly, Sprague Dawley rats that were fed a low fat diet or high fat diets (40% en) based on fish oil or perilla oil (rich in ALA) for 21 days had significantly lower leptin mRNA levels in white adipose tissue compared to rats fed a high fat diet (40% en) based on safflower oil (Takahashi et al., 2000). Additionally, in a genetic model of obesity and insulin resistance, our lab has demonstrated that an ALA-rich flaxseed oil diet (42.5 g of ALA/kg diet) decreases adipocyte size, an indication of adipose dysfunction, but does not alter cytokine or adipokine production by splenocytes and adipocytes, respectively, in adult obese Zucker rats (Baranowski, 2008).

There is limited research available on the effects of MUFAs on inflammatory markers and adipose function. A cross-sectional study evaluated the effects of various components of the Mediterranean diet on inflammatory markers in 339 men and 433 women with type 2 diabetes and showed that subjects with the highest consumption of nuts and olive oil (rich in MUFAs) had the lowest serum concentrations of IL-6 and CRP (Salas-Salvadó et al., 2008). It is important to note that in this study the main source of MUFA was olive oil and like olives, olive oil contains considerable amounts of phenolic compounds, which may have anti-inflammatory properties. Thus, it is yet to be determined if canola oil, which is also high in MUFA, will have similar anti-inflammatory properties. In a clinical trial, insulin resistant patients who consumed MUFA-rich diets containing 47% carbohydrate and 38% fat (9% SFA; 23% MUFA, 75% of which was provided as extra virgin olive oil; and 6% PUFA) had decreased adiponectin gene expression in biopsied peripheral adipose tissue from the upper quadrant

of the buttocks compared to subjects who consumed SFA or carbohydrate rich diets (Paniagua et al., 2007). Given this lack of research, studies on the effects of MUFAs and ALA on inflammatory markers and adipose function in a DIO rat model are warranted.

f) MUFAs/N-3 PUFAs, Hepatic Steatosis and Hepatic Fatty Acid Composition

The study of hepatic steatosis and hepatic fatty acid composition as an important part of the metabolic syndrome is relatively new; therefore, literature on the effects of MUFA and n-3 PUFAs on hepatic steatosis, especially using a DIO model, is scarce.

A study using DIO Wistar rats showed that a 12 week treatment with the very long chain n-3 PUFAs from fish oil significantly increased the size of the liver compared to rats fed high fat diets based on coconut oil, lard and olive oil (Buettner et al., 2006). Although liver size was larger than that of the other dietary groups, the fish oil diet did not have significantly different liver TAG, a marker of hepatic steatosis, than controls fed a low fat diet. On the other hand, rats fed the high fat coconut oil, lard and olive oil diets displayed significantly elevated hepatic TAG content compared to rats fed a low fat control diet.

Studies that have examined the ability of ALA to favorably alter hepatic fatty acid composition of TAG and PL have primarily used genetic models of obesity. In *ob/ob* mice, Mustad et al. (2006) demonstrated that when 11-14% of fat in a high fat diet (43-45% en) is replaced with ALA (flaxseed oil), hepatic lipid content is not decreased and that the hepatic fatty acid profile reflects the diet composition. The ALA diet significantly increased the amount of ALA and EPA, but not DHA, in the liver PL. In our lab, we have shown that a high ALA diet (flax oil plus soybean oil) did not reduce hepatic steatosis in *fa/fa* Zucker rats compared to a control diet (soybean oil), however,

there were a number of differences in hepatic fatty acid composition (Durston et al., 2008). The flax oil group had significantly more total n-3 PUFAs, and in particular EPA, DPA and DHA, in their hepatic PL compared to *fa/fa* controls. In addition, the flax oil group also had significantly higher total n-3 PUFAs, and in particular EPA and DPA in hepatic TAG compared to *fa/fa* controls. Although there is controversy over how much ALA can be converted to its long chain derivatives, a study by Andersen et al. (2008) demonstrated that EPA and DHA levels in the liver are elevated when Wistar rats are fed 0.5 g of EPA methylesters per kg body weight for 8 weeks. Additionally, removal of ALA for 29 days in the diets of Long Evans rats resulted in a decline in DHA content in the liver from 7.6% to 4% of total fatty acids (Moriguchi et al., 2004).

In humans, it was shown that a diet high in ALA could increase red blood cell ALA, EPA and DHA concentrations (Legrand et al., 2010). In this study, 160 participants were fed either an experimental diet that had a low PUFA/SFA ratio and n-6/n-3 ratio or a control diet with less animal fat and a higher PUFA/SFA and n-6/n-3 ratio for 90 days. The experimental diet contained food products from animals fed flaxseed rich diets. Both diets excluded seafood. The results indicated that the experimental diet led to significant increases in ALA and slight increases in the EPA and DHA concentrations of red blood cells. On the other hand, the control diet led to reductions in red blood cell EPA and DHA concentrations.

Although more research is warranted, the current literature suggests that ALA, EPA and DHA may prevent or decrease hepatic steatosis, and may also favorably alter the hepatic fatty acid composition of TAG and PL.

Suggested Mechanisms of Action for Hepatic Steatosis

The progression of NAFLD from hepatic steatosis to NASH is described best by a two-hit theory (reviewed by Metha et al., 2002). In the fist-hit, lipid accumulates in the liver due to insulin resistance. Insulin resistance causes hyperinsulinemia and lypolysis, which is the breakdown of TAG to FFA. Lypolysis triggers FFA to be released from peripheral tissue and results in FFA accumulation in the liver. Hyperisulinemia also causes fat to accumulate in the liver in 2 ways. First, hyperinsulinemia causes hepatic TAG accumulation by decreasing the ability of the liver to re-esterify TAG and package them for transport out of the liver for storage in adipose tissue. Second, hyperisulinemia induces glycolysis and increases the synthesis of FA while simultaneously decreasing mitochondrial β-oxidation. In the second-hit of the two-hit theory, the mitochondria attempt to oxidize the abundance of fatty acids in the liver causing oxidative stress due to the leakage of reactive oxygen species. The second hit leads to steatohepatitis (reviewed by Mehta et al., 2002).

The most established way in which omega-3 fatty acids, and in particular fish oils, have been suggested to prevent hepatic steatosis and, thus, prevent the progression to NASH, is by increasing hepatic fatty acid oxidation via peroxisome proliferator activated receptor α (PPARα) and decreasing hepatic fatty acid synthesis via sterol regulatory element-binding protein-1c (SREBP-1c). This mechanism will be discussed in detail in the next section and will be the mechanism that is examined in this research study.

a) PPARα

PPARs are ligand-activated nuclear transcription factors that belong to the steroid hormone nuclear receptor family (Sampath et al., 2005). There are three PPAR isoforms: PPARα, PPARβ, and PPARγ. PPARα is the major isoform found in hepatocytes and contributes to lipid homeostasis by regulating genes encoding lipid metabolism enzymes, lipid transporters, apolipoproteins and the Δ5, Δ6 and Δ9 desaturase enzymes (Sampath et al., 2005; Jump, 2002; Costet et al., 1998). PPARs regulate genes involved in long chain fatty acid oxidation including fatty acyl-CoA synthase, acyl-CoA oxidase (ACO), 2-enoyl-CoA hydratase and 3-hydroxy acyl-CoA dehydrogenase (reviewed by Clarke, 2000). ACO is the most widely used marker of peroxisome proliferator action and its activity increases during peroxisomal proliferation (Tugwood et al., 1992).

PUFAs are naturally occurring PPARα ligands, which are hypothesized to activate fatty acid oxidation. Although n-6 PUFAs can activate PPARα, n-3 PUFAs are more potent PPARα activators *in vivo* (reviewed by Schmitz et al., 2008). Among n-3 PUFAs, EPA is the strongest PPARα activator while ALA and DHA are weak activators but can be converted, *in vivo*, to EPA (Jump, 2008). Interestingly, eicosanoids are even stronger activators of PPARα than EPA (reviewed by Schmitz et al., 2008).

b) SREBP-1c

Dietary PUFAs also favorably alter lipid metabolism by suppressing lipogenic genes and, thus, reducing fat synthesis. SREBP-1c is a nuclear transcription factor in the liver that is required for the insulin-mediated induction of hepatic fatty acid and TAG synthesis. The expression and nuclear localization of SREBP-1c is suppressed by dietary

PUFAs (Xu et al., 1999; Worgall et al., 1998). In contrast to PUFAs, MUFAs and SFAs have no effect on SREBP-1c (reviewed by Schmitz et al., 2008).

The SREBP transcription factors are located in the bilayer of the endoplasmic reticulum as a helical hairpin (125 kDa), and must undergo 2 proteolytic cleavages to generate an active cytosolic fragment that enters the nucleus (68 kDa; Brown et al., 2000). There are 3 isoforms of SREBP: SREBP-1a, SREBP-1c and SREBP-2. The SREBP-1c isoform is the most prominent in rodent and human liver, and is the key regulator of fatty acid synthesis (reviewed by Price et al., 2000). Acetyl-CoA carboxylase (ACC) and fatty acid synthase are 2 important enzymes that are regulated by SREBP-1c; ACC is the rate-limiting enzyme in long chain fatty acid synthesis (Hardie, 1989; Horton, 2002). Other genes that are regulated by SREBP include stearoyl-CoA carboxylase and glycerol 3-phosphate acyltransferase, which are enzymes involved in TAG production (Horton, 2002). SREBP-1c is primarily regulated by n-3 PUFAs and, in particular, marine EPA (reviewed by Jump, 2002).

The ability of n-3 PUFAs to increase fatty acid oxidation through activation of PPARα and decrease fatty acid synthesis through suppression of SREBP-1c may explain why n-3 PUFAs have a favorable impact on insulin resistance, obesity, fat storage, inflammation and hepatic steatosis.

Diet-Induced Obesity (DIO) Model

In humans, one of the primary causes of obesity is diet. A DIO animal model is more representative of the human situation in which a positive energy balance and multiple genes contribute to obesity versus an animal model in which obesity has been induced by a single gene mutation (reviewed by Madsen et al., 2010; Buettner et al.,

2007). DIO is commonly produced in rodents by feeding them high fat or high sucrose diets. Sprague Dawley, Wistar, Long Evans or Lewis rats are the strains most often used in DIO studies. However, it is important to note that, due to genetic variability, some rats within the same strain are obese resistant (OR) while some are obese prone (OP). Numerous studies indicate that high fat diets not only promote obesity in OP rats, but also cause characteristics of obesity that are common in humans, such as whole body insulin resistance, hypertriglyceridemia, elevated insulin and resistin levels, as well as reduced adiponectin levels (reviewed by Buettner et al., 2007). It is somewhat less clear as to whether or not high fat feeding causes hypertension in OP rats. Some studies have shown an increase in systolic blood pressure, assessed with the tail cuff method, when OP rats are fed a high fat diet (32% en) for 10 weeks (Dobrian et al., 2000). Other studies have shown no difference between OP and OR rats in terms of blood pressure, assessed with telemetry transmitters, when fed high fat diets (32% en) for 12 weeks (Carroll et al., 2006).

High fat feeding also produces organ-specific effects that are also seen in human obesity, including impairment of insulin stimulated glucose uptake in skeletal muscle, changes in adipocyte morphology and metabolism, hepatic steatosis and enlargement of pancreatic β-cells (reviewed by Buettner et al., 2007). In addition, the pro-inflammatory state that accompanies human obesity is also seen in DIO models due to an up-regulation of inflammatory genes in adipocytes (reviewed by Buettner et al., 2007).

Although high fat diets are used to induce obesity in OP rats, neither the exact fat content or fatty acid composition used in previous studies has been standardized; this has led to considerable variability in the results reported (reviewed by Buettner et al., 2006).

High fat diets that have been used typically employ between 20% and 60% total energy as fat and use fat formulations that vary between animal-derived fats such as lard or beef tallow and plant oils such as coconut, corn or safflower. In addition, many of the studies that have tested high fat diets only contrast one high fat formula with standard chow. However, from the limited research that has compared different high fat diets with respect to their metabolic effects, it is clear that the development of obesity and insulin resistance is dependent on the fatty acid composition of the dietary fat. Diets based on SFAs, MUFAs, and n-6 PUFAs tend to induce obesity and its many symptoms, whereas diets containing n-3 PUFAs, particularly from marine oils, have beneficial effects on body composition and insulin action (reviewed by Buettner et al., 2007; Storlien et al., 1991; 1987). However, the effects of ALA and MUFAs on the metabolic parameters of the DIO model remains to be investigated (reviewed by Buettner et al., 2006).

STUDY RATIONALE

Limitations of Current Knowledge

There are many limitations in the current literature regarding the effects fatty acids on the metabolic syndrome characteristics. First, studies that have looked at fat intake, and in particular n-3 PUFAs, and insulin resistance in humans have used various types of studies (from epidemiological to well-controlled clinical trials) and the results from these studies are inconsistent. Second, it is important to note that very few of the studies cited in this literature review have actually tested the effects of canola oil, which is high in both MUFAs and ALA, on metabolic syndrome parameters. Most of the studies cited have used the marine fatty acids EPA and DHA as the n-3 PUFAs in their diet formulations and not the long chain n-3 PUFA ALA. Additionally, there is a lack of comprehensive comparison of various fats and oils that represent different SFA/MUFA/PUFA and n-6/n-3 ratios. Third, in the past, genetically or pharmacologically-induced obese animals have mostly been used to study obesity and insulin resistance instead of DIO animal models which more closely mimic the etiology of human obesity. Fourth, not much research has been done to investigate the effects of MUFAs and n-3 PUFAs on hepatic steatosis, especially not in a DIO model. In the past, research on the metabolic syndrome and DM2 has focused on how dietary lipids affect hyperlipidemia, insulin signaling in the muscle tissue or muscle fatty acid composition. However, hepatic steatosis, inflammation and adipose function are beginning to be recognized as important parts of the metabolic syndrome. Thus, the focus needs to shift from muscle tissue to hepatic and adipose tissue to get a better overall picture of how different dietary interventions influence all components of the metabolic syndrome.

Significance of Research

There is limited research on the ability of canola oil, which contains high amounts of MUFA and the long chain n-3 PUFA ALA, to prevent components of the metabolic syndrome, namely obesity, glycemia, lipidemia, hypertension, inflammation and hepatic steatosis. There is even less literature available on the effects of dietary MUFA and ALA in a DIO model. Since there is a worldwide obesity epidemic in adults and children alike, it is important to determine a dietary intervention with a fatty acid profile for the prevention of obesity and its related complications.

HYPOTHESIS

It is hypothesized that in a DIO model, the combination of canola and flax oils will slow the onset or reduce the severity of weight or fat gain and hypertension while improving insulin sensitivity and enhancing adipocyte functionality, thus, favorably modifying hepatic steatosis, lipidemia and circulating markers of inflammation. In addition, it is hypothesized that the canola/flax oil diet, which is high in both MUFA and ALA, will favorably alter hepatic PL and TAG composition by enabling the conversion of ALA to its very long chain derivatives, EPA and DHA.

OBJECTIVES

To investigate the biological effect of dietary vegetable oils and lard that contain various fatty acid compositions (representing different compositions of SFA, MUFA, PUFA and ALA) in OP rats, the following objectives were defined:

1. To determine the effects of a 12 week dietary intervention with various dietary fats and oils in OP rats on various characteristics of the metabolic syndrome:

   a) Glycemia and insulinemia (fasting serum glucose and insulin concentrations);

   b) Insulin sensitivity (ITT, oral glucose tolerances test [OGTT], HOMA and glucose/insulin ratio);

   c) Obesity (body weights, weight gain, fat pad mass and fat pad/body weight ratios);

   d) Lipidemia (fasting serum FFA, TAG and cholesterol);

e) Hypertension (tail cuff measurements); and

f) Inflammation (fasting serum haptoglobin and adiponectin, an anti-inflammatory adipokine).

2. To examine the effects of various dietary fats and oils on hepatic steatosis and hepatic fatty acid composition of TAG and PL in OP-CD rats.

3. To determine if various dietary fatty acid compositions alter molecular markers of:

a) hepatic fatty acid oxidation (PPARα and ACO protein levels), and

b) hepatic fatty acid synthesis (SREBP-1 and ACC protein levels).

RESEARCH DESIGN and METHODOLOGY

Animals and Diets

Six week old male OP-CD (Charles River Sprague-Dawley) rats were used in this study (Charles River Laboratories, St Constant, PQ). The OP-CD rats were developed from a line of Crl:CD (Sprague Dawley) rats. When fed a high fat diet the OP-CD rats develop obesity, even though they have a fully functional leptin receptor and, thus, are an ideal model of diet-induced obesity and insulin resistance.

Following a 12-17 day acclimatization period, 70 OP-CD rats were randomly assigned to one of 7 dietary treatments (n=10) for 12 weeks (Figure 2):

1. High Oleic Canola Oil (**HC**; high MUFA and minimal ALA)

2. Conventional Canola Oil (**C**; high MUFA and ALA)

3. Conventional Canola/Flax Oil Blend (3:1 ratio; **C/F**; high MUFA and high ALA)

4. High Linoleic Safflower Oil (**SF**; high n-6 PUFA)

5. Soybean Oil (**SB**; high n-6 PUFA and ALA)

6. Lard and Soybean Oil (**L**; high SFA + MUFA)

7. Weight-matched group fed Lard and Soybean Oil (**WM**; high SFA + MUFA)

Originally, the study design included a control group consisting of obese resistant (OR) rats fed the L diet. However, when serum biochemistry of the OP rats was analyzed, they had abnormal values on many of the parameters, and, were therefore

removed from the study (see Appendix 1 for these data). The diet formulations and expected fatty acid compositions are shown in Table 1. HC oil was chosen because it is what is commonly used in the food industry and because it contains MUFA with minimal ALA and, therefore, could be compared to the C oil (MUFA and moderate ALA) and C/F oil (MUFA and high ALA). A 3:1 ratio of canola and flax oil was chosen for the C/F diet for a number of different reasons. First, a 3:1 ratio keeps the LA content of the C/F oil diet similar to the HC and C diets (19-21% of total fatty acids). Second, the 3:1 ratio was chosen for the C/F diet to increase the ALA content of the canola oil diets from 3% total fatty acids in the HC diet to 11% in the C diet and 21% in the C/F diet. Third, the 3:1 ratio of the C/F oil diet also made for a nice decrease in MUFA content of the canola oil diets from 70% MUFA in the HC diet, to 61% in the C diet and 51% in the C/F diet. The SF and SB oil diets were chosen to determine the effects of a high PUFA diet (SF) and a high PUFA plus ALA diet (SB) on metabolic syndrome parameters. The lard diet was chosen because it is the most common diet used to develop DIO in the OP rats. A small amount of soybean oil was added to the L diet to prevent deficient intakes of essential fatty acids.

All 7 high fat diets contained 55% of total energy as fat, 30% as carbohydrate and 15% as protein. In the literature, high fat diets are diets that contain between 20-60% en from fat. Storlien et al. (1987) found that when Wistar rats consumed a diet that consisted of 59% of total energy as fat for 31 days, insulin resistance (assessed by the eugylcemic clamp method) occurred in the high fat safflower oil fed rats but was prevented in the high fat fish oil fed rats. The present study was 12 week long. This is an optimal length of time because it enabled us to take multiple fasting blood samples throughout the study. Additionally, Buettner et al. (2006) conducted a study on Wistar fats that were fed high

fat diets based various types of fats and oils, for 12 weeks, and found that it was a sufficient amount of time to see changes in body weight, glucose, insulin, as well as many other parameters. Ten kg batches of the oil diets and 5 kg batches of the lard diet were prepared as needed and stored at 4°C while being used. Rats were fed 3 times per week and feed consumption (corrected for spillage) as well as weekly body weights were recorded. All rats had free access to feed except the weight matched group, which were fed restricted amounts of feed at week 11 and week 12 of the study to match their body weight to the group that was gaining the least amount of weight during the study. During the study, the rats were routinely held in order accustom them to being handled. This was important so that measures of glucose and insulin were not confounded due to stress during the ITT and OGTT.

		Dietary Groups						
	Week	HC	C	C/F	SF	SB	L	WM
Fasting Blood	0							
	1							
	2							
	3							
Fasting Blood	4							
	5							
	6	n=10	n=10	n=10	n=10	n=10	n=10	n=10
	7							
Fasting Blood	8							
	9							
Blood Pressure & ITT	10							
OGTT	11							
Fasting Blood & Tissue Analysis	12							

Figure 2: Study Design. HC = high oleic canola, C = conventional canola, C/F = canola/flax blend, SF = safflower, SB = soybean, L = lard, WM = weight matched, ITT = insulin tolerance test, OGTT = oral glucose tolerance test.

Table 1: Experimental Diets.

Treatment groups by oil type	HC	C	C/F	SF[7]	SB	L
g per kg:						
Cornstarch[1]	209	209	209	209	209	209
Maltodextrin[1]	69.4	69.4	69.4	69.4	69.4	69.4
Sucrose[1]	100	100	100	100	100	100
Cellulose[1]	63.8	63.8	63.8	63.8	63.8	63.8
Casein[1]	186.2	186.2	186.2	186.2	186.2	186.2
High Oleic Canola Oil[2]	308.3	0	0	0	0	0
Canola Oil[2]	0	308.3	231.2	0	0	0
Flax Oil[3]	0	0	77.1	0	0	0
High Linoleic Safflower Oil[4]	0	0	0	308.3	0	0
Soybean Oil[2]	0	0	0	0	308.3	28.5
Lard[2]	0	0	0	0	0	279.8
Mineral Mix AIN-93G[1]	44.6	44.6	44.6	44.6	44.6	44.6
Vitamin Mix AIN-93G[1]	12.7	12.7	12.7	12.7	12.7	12.7
L-cystine[1]	3	3	3	3	3	3
Choline Bitartrate[1]	3.2	3.2	3.2	3.2	3.2	3.2
t-Butylhydroquinone[5]	0.006	0.006	0.006	0.006	0.006	0.006

Table 1: Experimental Diets (continued).

	HC	C	C/F	SF[7]	SB	L
Macronutrients (% en):						
Carbohydrate	30	30	30	30	30	30
Protein	15	15	15	15	15	15
Fat	55	55	55	55	55	55
Fatty Acid Composition (% Fat)[6]:						
SFA	7	7	7.5	12	15	40.5
MUFA	70	61	51	16	23	44.8
PUFA	23	32	41	72	62	14.7
C18:2n6 (LA)	20	21	19	71	54	13.1
C18:3n3 (ALA)	3	11	22	1	8	1.6
LA/ALA	7:1	2:1	0.8:1	71:1	7:1	8:1

[1] Dyets Inc., Bethlehem, Pennsylvania
[2] Bunge Canada, Oakville, Ontario
[3] Omega Nutrition, Vancouver, British Columbia
[4] Alnoroil Company, Inc., Valley Stream, New York
[5] Sigma-Aldrich, St. Louis, Missouri
[6] Expected fatty acid composition of oils and lard based on composition information from Canola Council of Canada (2008)
[7] The fatty acid composition of the high linoleic SF diet was estimated using the fatty acid composition of sunflower oil shown on the Canola Council of Canada fact sheet (Canola Council of Canada, 2008).

Blood Pressure

At week 10.5, blood pressure was assessed by the tail cuff method using a CODA^{TM} multi-channel, computerized, non-invasive blood pressure system for mice and rats (Kent Scientific, Torrington, CT). Approximately one week prior to blood pressure readings, the rats were acclimatized to the procedure. The animals were restrained in a rodent holder containing a darkened nose cone to limit their view and reduce the level of stress, and a spacer at the rear of the holder to allow the rat's tail to be free. The procedure room was kept at 26 ºC and warming devices including a heating platform and a heat lamp were used to maintain the animal's surface body temperature at 30ºC. An occlusion cuff was then placed close to the base of the tail followed by a volume pressure recording (VPR) sensor. Prior to obtaining blood pressure measurements, the rat was left in the holder with the tail cuff on for 10 to 15 minutes to allow it time to get used to its surroundings. Nine acclimation blood pressure readings (9 cycles) were taken to help the rat get accustomed to the procedure, then 15 blood pressure readings (15 cycles) were taken and used to calculate an average blood pressure reading. During one blood pressure cycle the occlusion tail cuff was inflated to impede blood flow to the tail, then the occlusion cuff was deflated and the VPR sensor measured the tail swelling as a result of the arterial pulsations from the blood flow. All equipment was automatically computer controlled. Systolic blood pressure (when the tail first begins to swell) and diastolic blood pressure (when the increased rate of swelling in the tail ceases) was determined using CODA^{TM} Software (Kent Scientific, Torrington, CT).

ITT

At week 10.5, an ITT was conducted to assess how responsive the animal was to insulin, thus, reflecting insulin sensitivity. After a 5 hour fast (8:30-13:30), blood glucose (t=0) was determined with an AlphaTRAK blood glucose monitor (Abbott Laboratories, Inc., Chicago, IL) after pricking the tail with an AlphaTRAK 25 gauge sterile lancet and collecting the drop of blood on an AlphaTRAK test strip. All glucose monitors were calibrated with AlphaTRAK control solution prior to testing. Next, Novolin®ge Toronto human insulin (100 IU/mL) was injected peritoneally (0.75 U/kg body weight insulin; Novo Nordisk A/s, Bagsvaerd, Denmark). Insulin was prepared under sterile conditions in a cell culture hood to provide a solution of 15 μL insulin/10 mL sterile phosphate buffered saline. Blood glucose was measured with the blood glucose monitor every 15 minutes for 60 minutes (t=15, t=30, t=45, and t=60). After the last glucose reading (t=60), 1 mL of 50 % dextrose was given orally to each rat to increase their blood glucose and acclimatize them to oral ingestion of glucose prior to OGTT.

OGTT

At week 11.5, an OGTT was conducted to measure how quickly a glucose load is cleared from the blood, thus, reflecting *in vivo* glucose handling. After a 5 hour fast (8:30-13:30), an initial blood sample was collected (t=0) via the saphenous vein into a microvette tube. Next, an oral glucose load (1 g glucose/kg body weight, provided as 0.00143 mL/g body weight of 50% dextrose solution) was orally administered followed by additional blood collection at t=15, t=30, t=60, and t=120 minutes post-glucose administration. Two hundred μL of blood was collected at each time point. Blood

samples were stored on ice until centrifuged at 1500 rpm for 15 min at 4°C with a 5804R centrifuge (Eppendorf, Mississauga, ON). The serum layer was then stored at -80°C for future analyses.

Blood Collection

On day 0 and week 4 and 8 of the study, after a 5-hour fast, fasting blood samples were obtained via the jugular vein. Fifteen percent of the total blood volume was taken at each time point. The total blood volume was estimated as 6% of the animal's body weight. Blood samples were stored on ice until centrifuged at 1500 rpm for 15 min at 4°C. The serum layer was aliquoted and stored at -80°C for future analyses.

Tissue Collection

At week 12, rats were fasted overnight (from 21:00) and then euthanized by carbon dioxide asphyxiation and cervical dislocation according to the Canadian Council on Animal Care Guidelines (Olfert et al., 1993). Each rat was weighed following asphyxiation. Trunk blood was collected immediately after cervical dislocation and was placed on ice until centrifuged at 2000 rpm for 10 min at 4°C to separate the serum, which was stored in aliquots at -80°C. Tissue was weighed, immediately frozen in liquid nitrogen and stored at -80°C for subsequent analyses.

Serum Biochemistry

Fasting serum, obtained upon termination (week 12), was analyzed for glucose, haptoglobin, cholesterol, TAG, FFA, adiponectin and insulin. If results from the 12 week

time point were significantly different for any of the parameters, week 8 serum was analyzed for that parameter. Similarly, if data were significantly different at the week 8 time point, week 4 serum was analyzed for that particular parameter and, if significantly different, baseline (week 0) serum was analyzed. This was done in order to determine when the animals in particular groups began to develop abnormal serum biochemistry and if the parameter worsened or improved over the course of the study.

a) Enzymatic Colourimetric Endpoint Spectrophotometric Assays

Enzymatic colourimetric kits were used to quantify OGTT, termination and week 8 fasting serum glucose (Cat. # 220-32, Genzyme Diagnostics P.E.I. Inc., Charlottetown, PE), termination, week 8, week 4 and baseline fasting serum haptoglobin (Cat. # TP 801, Tri-Delta Diagnostics, Inc., Wicklow, Ireland), TAG (Cat. # 236-60, Genzyme Diagnostics P.E.I. Inc., Charlottetown, PE Canada), and termination fasting serum cholesterol (Cat. # 234-60, Genzyme Diagnostics P.E.I. Inc., Charlottetown, PE) and FFA (Cat. #11 383 175 001, Roche Diagnostics, Penzberg, Germany).

All assays produced a coloured end product, and the intensity of which was proportional to the amount of compound being measured in the sample. The coloured end product was detected at a designated wavelength with a FLUOstar Omega microplate reader (BMG LABTECH, Thermo Fisher Scientific, Offenburg, Germany) using Omega Control Software (Version 1.0, BMG LABTECH, Thermo Fisher Scientific, Offenburg, Germany). The final serum concentration of the compound being measured was obtained by multiplying the mean result for a sample by the dilution factor. Samples with coefficients of variation greater than 10% were re-assayed and the standard curve of all assays had a correlation coefficient of 0.9 or greater.

i) Glucose

The principle of the assay is that in the presence of glucose oxidase, glucose, oxygen and H_2O are converted to gluconic acid and hydrogen peroxide (H_2O_2). Next, H_2O_2, hydroxybenzoate and 4-aminoantipyrine react with peroxidase to form quinoneimine dye and H_2O.

The reagents used in the assay include a glucose colour reagent, which is a solution containing a buffer (pH 7.25 at 25°C), 0.25 mmol/L 4-aminoantipyrine, 20 mmol/L p-hydroxybenzoate, >40, 000 U/L glucose oxidase (microbial, >2000 U/L peroxidase (botanical) and preservatives. A glucose calibrator was also used, which is a solution containing 5 mmol/L glucose and preservatives that is serially diluted to produce 3 additional standards (0.625, 1.25, and 2.5 mmol/L).

Serum from OP rats was diluted 5× with deionized water (ddH_2O). Five μL of blank (ddH_2O), standard, quality control and sample were pipetted, in triplicate, into the wells of a Nunc 96-well polystyrene plate (Roskilde, Denmark). Next, 200 μL of reconstituted glucose colour reagent was added to each well. The plate was incubated at room temperature for 10 minutes after which the absorbance of the colour in each well was read at 505 nm in a microplate reader.

ii) Haptoglobin

The principle of the assay is a one step process where haptoglobin, haemoglobin, H_2O_2 and chromogen produce a coloured dye in the presence of peroxidase. Haptoglobin in the serum combines with haemoglobin and preserves its peroxidase activity. When

hydrogen peroxide and chromogen are added, the haemoglobin peroxidase reacts with the hydrogen peroxide to produce a coloured dye.

The reagents used in this procedure include reagent 1, which contains equal volumes of haemoglobin and haemoglobin diluent, and reagent 2, which contains chromogen and substrate containing H_2O_2 in a 9:5 ratio. Other components used include a sample/standard diluent and a haptoglobin standard containing 2 mg/mL haptoglobin, which was serially diluted with sample/standard diluent to produce 4 additional standards (0.125, 0.25, 0.5, and 1.0 mg/mL).

Termination serum was diluted 2× with sample/standard diluent. Samples and standards (7.5 μL) were pipetted, in triplicate, into the wells of a Nunc 96-well plate. Next, 100 μL of reagent 1 was added to each well and the plate was mixed gently. Then 140 μL of reagent 2 was added to each well and the plate was incubated for 5 minutes at room temperature. The absorbance of the colour produced in each well was read at 630 nm with a microplate reader.

iii) TAG

In this assay, 4 enzymatic reactions occur. First, serum TAGs are hydrolyzed with lipase to form glycerol and fatty acids. Second, in the presence of glycerol kinase and Mg^{2+}, glycerol and ATP are converted to glycerol-1-phosphate and ADP. Third, in the presence of glycerol phosphate oxidase, glycerol-1-phosphate and O_2 form H_2O_2 and dihydroxyacetone phosphate. Finally, H_2O_2, p-chlorophenol and 4-aminoantipyrine produce quinoneimine dye, and H_2O in the presence of peroxidase.

The reagents used in this assay include a TAG reagent, which is a buffered solution containing 0.4 mmol/L 4-aminoantipyrine, 2.6 mmol/L adenosine triphosphate, 3.0 mmol/L p-chlorophenol, >2400 U/L glycerol phosphate oxidase (*Pediococcus sp.*), >1000 U/L lipoprotein lipase (*Pseudomonas sp.*), >540 U/L peroxidase (horseradish), >400 U/L glycerol kinase (*Cellulosmonos sp.*), stabilizers and preservatives. The standard used in this reaction was a human based, lyophilized serum calibrator (DC-Cal Calibrator, Cat. # SE-035, Genzyme Diagnostics P.E.I. Inc., Charlottetown, PE). The calibrator contained 1.95 mmol/L TAG and was serially diluted to produce 4 additional standards (0.975, 0.4875, 0.244, and 0 mmol/L).

Serum was diluted 4× with ddH_2O and 10 μL of standard, sample, blank (ddH_2O) and quality control were pipetted in triplicate into the wells of a Nunc 96-well plate. Next, 200 μL of TAG reagent were added to each well and the plate was allowed to incubate for 15 minutes at 37°C. After the incubation, the absorbance of the colour in each well was read at 520 nm with a microplate reader.

iv) Cholesterol

The kit employs a 3-step reaction wherein cholesterol esters are first converted to cholesterol plus free fatty acids in the presence of cholesterol esterase. Next, cholesterol and oxygen are converted to cholest-4-ene-3-one plus H_2O_2 in the presence of cholesterol oxidase. Finally, with the aid of the enzyme peroxidase, H_2O_2, 4-aminoantipyrine and p-hydroxybenzoate are converted to quinoneimine and 4 H_2O.

The kit has a cholesterol reagent which contains a buffered solution of 0.5 mmol/L 4-aminoantipyrine, 25 mmol/L p-hydroxybenzoic acid, > 240 U/L cholesterol esterase, >

150 U/L cholesterol oxidase (nocardia), > 1600 U/L peroxidase (horseradish), surfactants, stabilizers and preservatives. The standard used in this reaction was a human based, lyophilized serum calibrator (DC-Cal Calibrator, Cat. # SE-035, Genzyme Diagnostics P.E.I. Inc., Charlottetown, PE). The calibrator contained 4.6 mmol/L cholesterol and was serially diluted to produce 3 additional standards (2.3, 1.15, and 0.575 mmol/L).

Serum was diluted 2× with ddH_2O. Ten μL of standard, sample, blank (ddH_2O), and quality control were pipetted in triplicate into the wells of a Nunc 96-well plate. Next, 200 μL of cholesterol reagent was added to each well and the plate was incubated for 5 minutes at 37°C. The absorbance of the colour in each well was read at 505 nm with a microplate reader.

v) FFA

The principle of the assay follows a 3-step process. First, Acyl-CoA synthetase converts FFA, ATP and CoA to acyl-CoA, AMP and pyrophosphate. Next, acyl-CoA reacts with oxygen to form 2, 3-enoyl-CoA and H_2O_2 in the presence of acyl-CoA oxidase. Third, H_2O_2, 4-aminoantipyrine and 2, 4, 6-tribromo-3-hydoxy-benzoic acid (TBHB) are converted to 2H_2O, HBr and red dye in the presence of peroxidase.

Three reagents were used in this reaction. Reaction mixture A contained 1 tablet A (ATP, CoA, acyl-CoA synthetase, peroxidase, ascorbate oxidase, 4-aminoantipyrine and stabilizers) and was dissolved in 11 mL potassium phosphate buffer pH 7.8. Reaction mixture B contained 1 tablet B (acyl-CoA-oxidase and stabilizers) and was dissolved in 0.6 mL acyl-CoA oxidase dilution solution and stabilizers. Lastly, solution C contained aqueous N-ethyl-maleinimide solution and stabilizers.

First, 200 µL of reaction mixture A was pipetted into the wells of a Nunc 96-well plate. Next, 10 µL of a blank (ddH_2O), quality control and serum samples (undiluted) were plated in triplicate. The plate was then mixed for 30 seconds and incubated at 22°C for 10 minutes. After the 10 minute incubation period, 10 µL of solution C was added to each well and the plate was mixed for 30 seconds in a microplate reader and read at 546 nm. This absorbance was labeled A_1. Next, 10 µL of reaction mixture B was added to each well and the plate was mixed for 20 seconds and incubated at 22°C for 20 minutes. After the incubation period, the plate was mixed for 30 seconds in a microplate reader and once again the absorbance was read at 545 nm. This absorbance was labeled A_2. Serum FFA concentrations were then calculated using the following formula:

$$C\ (\text{mmol/L}) = \frac{V}{\varepsilon \times d \times v} \times \Delta A$$

Where:

C = concentration of FFA in the sample

V = final sample volume in mL (0.230 mL)

v = sample volume in mL (0.010 mL)

d = light path in cm (0.53326 cm)

ε = absorption coefficient at 546 nm [$19.3 \text{ x } (1 \text{ x mmol}^{-1} \times \text{cm}^{-1})^3$]

ΔA = change in absorbance of the sample minus the change in absorbance of the blank, $(A_2 - A_1)_{sample} - (A_2 - A_1)_{blank}$

b) Enzyme Immunoassay

An enzyme-linked immunosorbent kit was used to quantify termination fasting serum adiponectin (Cat. # 44-ADPRT-E01, Alpco Diagnostics, Salem, NH). An insulin ultrasensitive enzyme immunosorbent kit was used to quantify OGTT and termination fasting serum insulin (Cat. # 80-INSRTU-E10, Alpco Diagnostics, Salem, NH).

Both assays produced a coloured end product and the colour was proportional to the amount of compound being measured in the sample. The coloured end product was detected at a designated wavelength with a FLUOstar Omega microplate reader (BMG, LABTECH, Thermo Fisher Scientific, Offenburg, Germany) using Omega Control Software (Version 1.0, BMG LABTECH, Thermo Fisher Scientific, Offenburg, Germany). The final serum concentration of the compound being measured was obtained by multiplying the mean result for a sample by the dilution factor. Samples with coefficients of variation greater than 10% were re-assayed and the standard curve of all assays had a correlation coefficient of 0.9 or greater.

i) Adiponectin

The components used in this procedure include a polyclonal anti-rat adiponectin antibody coated 96-well plate, a wash buffer, consisting of a buffered detergent solution, and a diluent. A secondary antibody was also used that contained monoclonal anti-rat adiponectin, 100× detector, containing HRP conjugated mouse IgG, and a standard, which was recombinant rat adiponectin at a concentration of 48 ng/vial and was serially diluted to produce 7 additional standards (24, 12, 6, 3, 1.5, 0.75, 0.375, and 0 ng/mL). Other components used in this assay included a quality control serum, which is a positive

control of rat serum adiponectin, substrates 1 and 2 , which are chromogenic reagents, and a stop solution, consisting of 1 M H_3PO_4.

All samples and kit components were brought to room temperature. Termination serum was diluted 2000× and 100 µL of diluted sample, standards and quality control were pipetted into the wells of the polyclonal anti-rat adiponectin antibody coated 96-well plate. The plate was incubated at 37°C for 1 hour. After the incubation, the solution was removed and the plate was washed 3 times with 250 µL of wash solution with a Wellwash AC (Thermo Fisher Scientific, Offenburg, Germany). Next, 100 µL of secondary antibody was added to each well and the plate was incubated for 1 hour at 37°C. After the incubation, the solution was removed and the plate was once again washed 3 times with 250 µL of wash solution. The 100× detector was diluted with the 1× diluent to produce a 1× detector and 100 µL was added to each well and incubated for 1 hour at 37°C. The solution was removed and the plate was washed 5× with 250 µL of wash solution. Next, 100 µL of the substrate solution was added to each well and the plate was incubated at room temperature for 20 minutes protected from light. Next, 100 µL of stop solution was added to each well and the absorbance was read at 450 nm with a microplate reader.

ii) Insulin

The components used in this procedure include a horseradish peroxidase enzyme labeled monoclonal anti-insulin coated plate, insulin standards at concentrations of 0, 0.15, 0.4, 1, 3, and 5.5 ng/mL, lyophilized mammalian insulin controls (low and high), which were diluted with 0.6 mL of ultra pure water to yield a concentration of 0.72

ng/mL (0.45 – 0.88 ng/mL) for the low control and 3.89 ng/mL (2.70 – 4.79 ng/mL) for the high control. An 11× enzyme conjugate solution was also used and was diluted with 10 parts enzyme conjugate buffer. Likewise, a 21× wash buffer was diluted with 20 parts ultra pure water. Lastly, a substrate solution and a stop solution were also used.

All samples and kit components were brought to room temperature. Termination serum was not diluted and OGTT serum was diluted 2-4× with ultra pure water. Five μL of diluted sample, standards and quality controls were pipetted into the wells of the 96-well monoclonal anti-insulin coated plate. Seventy-five μL of working strength enzyme conjugate was then added to each well and the plate was incubated for 2 hours while shaking on an orbital microplate shaker at room temperature. After the incubation, the solution was removed and the plate was washed 6 times with 250 μL of working strength wash solution. Next, 100 μL of substrate was added to each well and the plate was incubated for 30 minutes while shaking on an orbital microplate shaker at room temperature. After incubation, 100 μL of stop solution was added to each well and the plate was gently mixed to stop the reaction. Finally, the absorbance was read at 450 nm with a reference wavelength of 620 using a microplate reader.

iii) Insulin Sensitivity Calculations

A HOMA score was calculated as an indirect measure of the action of insulin on glucose disposal (fasting serum insulin [uU/mL] × fasting plasma glucose [mmol/L]/22.5; Matthews et al., 1985). A glucose to insulin ratio was also calculated, as it is a simple screening test for insulin resistance (Legro et al., 1998).

Additionally, area under the curve (AUC) was calculated for both glucose and insulin for serum collected during the OGTT as well as glucose readings from the ITT using this equation (Brouns et al., 2005):

$$AUC = \left[\frac{(t15 + t0)}{2} \times 15(\text{min})\right] + \left[\frac{(t30 + t15)}{2} \times 15(\text{min})\right] + \left[\frac{(t60 + t30)}{2} \times 30(\text{min})\right] + \left[\frac{(t120 + t60)}{2}\ 60(\text{min})\right]$$

Extraction and Analysis of Fatty Acids in Diet

a) Fat Extraction

Two grams of diet were placed in a 100 mL beaker containing 40 mL of 2:1 chloroform:methanol with 0.01% BHT (Optima grade reagents) and homogenized using a PT-MR Polytron 1600E (Kinematica, Lucerne, Switzerland) for 30 seconds. Whatman #1 filter paper was used to filter the homogenate and it was decanted into a 100 mL graduated cylinder. Next, 10 mL of 2:1 chloroform:methanol was used to rinse both the filter paper and the funnel. The volume in the graduated cylinder was recorded and 25% of the volume was added as ddH_20. Next, the graduated cylinder was stoppered and inverted 5 times to mix the solutions. The mixture was allowed to separate overnight.

The next day, the top layer was removed as waste and a calculated volume of the chloroform/fat layer was set aside for fatty acid analysis.

b) Fatty Acid Analysis

For the fatty acid profile of the diet to be determined, the lipids in the chloroform layer first needed to be methylated. The volume of the cholorform layer that was needed for fatty acid analysis was calculated as follows:

2 g (original amount of sample used) × 31% (weight of fat in diet) = 0.62 g or 620 mg fat in 2 g sample of diet

$$\frac{10 \text{ mg} \times \text{total volume (mL) of chloroform layer}}{620 \text{ mg}} = \text{volume (mL) of chloroform layer needed for fatty acid analysis}$$

The calculated volume of the chloroform layer was placed in an 8 mL screw top test tube and was heated at 35°C in a water bath under nitrogen to evaporate the solvent. Once the lipid was dried, 1 mL toluene (Optima grade) was added to the tube and vortexed for 10 seconds. Next 1.2 mL of 3N methanolic hydrochloric acid (Cat # 33050-U, Supelco Analytical, Bellefonte, PA) was added to the sample to methylate the lipid and the tubes were tightly capped, vortexed for 10 seconds and placed in an 80°C oven for 1 hour. The tubes were then removed from the oven and allowed to cool. Once cooled, 1 mL of ddH_2O and 1 mL of hexane (Optima grade) were added to the samples and the tubes were vortexed for 20 seconds and centrifuged at 2000 rpm for 4 minutes. The top layer, containing the lipid and the hexane, was transferred to a clean 8 mL tube and 2 mL of ddH_2O was added. The samples were once again vortexed for 20 minutes and centrifuged at 2000 rpm for 4 minutes. The top (lipid and hexane) layer was placed into GC vials for fatty acid analysis. Lastly the samples were run on a Varian 450-GC Gas Chromatograph with a flame ionization detector (FID; Varian, Lake Forest, CA) and a GC capillary column, 100 m × 0.25 mm diameter and 0.25 μm film thickness (Cat # CP7420, Varian, Lake Forest, CA). The temperature program was: 70°C hold × 2 minutes, 180°C at 30°C/minute, 200°C at 10°C/minute × 2 minutes, 220°C at 2°C/minute × 10 minutes, 240°C at 20°C/minute × 5 minutes. Total run time was 36.67 minutes. All samples were run with 10:1 split ratio.

Total Hepatic Fat

Total liver lipid was determined by a modified Folch method (Folch et al., 1956) and liver lipid extraction for fatty acid analysis used a modified Bligh and Dyer method (Bligh et al., 1959). First, 1.5 g of liver was weighed and homogenized in 32 mL of 2:1 chloroform:methanol with 0.01% butylated hydroxytoluene (BHT) for 60 seconds with a PT-MR Polytron 1600E (Kinematica, Lucerne, Switzerland). Twenty-two mL of the homogenate was filtered through a #1 Whatman filter paper into a 25 mL graduated cylinder for total hepatic lipid analysis and 6 mL of the homogenate was filtered through a # 1 Whatman filter paper into a 15 mL test tube for fatty acid analysis. Next, 0.73% NaCl was added at a volume of 20% of the volume of the homogenate in the graduated cylinder. The cylinder was stoppered and the mixture was inverted 5 times and left to separate overnight. Additionally, 20 mL glass scintillation vials were placed in the dessicator for use the next day.

The following day, the volume of the lower chloroform/lipid layer was recorded and 10 mL of this layer were placed in dried and weighed 20 mL glass vails. The cholorform was evaporated under nitrogen at 30°C with a N-EVAP III Nitrogen Evaporator (Organomation Associates, Inc., Berlin, MA). Next, the 20 mL glass vials containing the lipid were dried in the dessicator overnight.

The next day the vials were weighed and the lipid content was calculated as follows:

$$\text{\% lipid} = \frac{(\text{dry weight vial + lipid}) - (\text{dry weight vial})}{\text{10 mL chloroform used}} \times \text{volume of chloroform layer} \times 100$$

Hepatic Fatty Acid Composition

a) Extraction

Lipids were extracted using a modified Bligh and Dyer extraction procedure (Bligh & Dyer, 1959). To the 6 mL of filtered homogenate, 2.3 mL of 0.73% sodium chloride was added. Next, the mixture was vortexed for 30 seconds and centrifuged with a Sorvall ST 16R centrifuge for 10 minutes at 1500 rpm (Thermo Fisher Scientific, Offenburg, Germany). Once complete, the top layer, which consisted of methanol and water, was removed from each sample and discarded, and the remaining bottom layer was rinsed twice with 1-2 mL of TUP (3:48:47 chloroform: methanol: water) with the top layer removed and discarded each time. The sample was then transferred to a clean 8 mL test tube and evaporated under nitrogen to dryness in a 30°C water bath. Once dry, the samples were diluted with 2 mL of 2:1 chloroform: methanol with 0.01% BHT. The PL and TAG were then isolated by thin layer chromatography.

b) Thin Layer Chromatography (TLC)

A Whatman K8 Silica Gel 80A plate was scored to create lanes and prevent samples from merging. Next, the plate was activated by heating in a preheated 120°C oven for 30 min and cooled in a dessicator for 30 min. Once cool, the plate was spotted with the appropriate lipid sample and the PL and TAG standards were applied to the same plate.

Two hundred µL of lipid extract was combined with both 100 µL of PL internal standard (1.4 mg/mL 1,2-dipentadecanoyl-sn-glycero-3-phosphocholine; Avanti, Alabaster, AL) and 5 µL of TAG internal standard (5 mg/mL triheptadecanoin; Nu-

Check, Elasian, MN). The combined lipid extract, plus standards, were dried at 30°C with a nitrogen evaporator, reconstituted with 50 µL 2:1 chloroform: methanol and then spotted onto a TLC plate in 4 cm bands. Before and between each sample, the application needle was rinsed 5 times with 2:1 chloroform: methanol to avoid cross contamination. Once the plate was spotted, it was placed into a pre-prepared chromatography tank containing 80:20:1 petroleum ether: ethyl ether: acetic acid (v/v/v) and the tank was closed. The plate stayed in the chamber for 25-35 minutes, until the solvent advanced to about half an inch from the top of the plate. The plate was removed from the tank, allowed to air dry, and sprayed with 0.1% 8-anilino-1-napthalene sulfonic acid. The bands showing the different lipid classes were illuminated under UV light, and marked lightly with a pencil. Using a razor blade, the PL and TAG bands were scraped from the plate onto weigh paper and transported to a clean 8 mL test tube containing 1 mL toluene with 0.01% BHT. Lastly, the test tubes were flushed with nitrogen and capped.

c) Methylation

To each sample, 1.2 mL of 0.3 N methanolic HCl (Cat # 33050-U, Supelco Analytical, Bellefonte, PA) was added. The test tubes were capped tightly, vortexed for 30 seconds and placed in a pre-heated 80°C oven for 1 hour. The tubes were allowed to cool for 10-15 minutes. One mL of deionized water was added to each sample, the tubes were capped, vortexed for 30 seconds and centrifuged for 10 minutes at 1500-2000 rpm. The top layer was transferred to a clean 8 mL screw top test tube. To the bottom layer, 1 mL of petroleum ether was added, the tube was capped, vortexed for 15 seconds and centrifuged for 10 minutes at 1500-2000 rpm. The top layer was added to the previously

removed top layer, and 2 mL of deionized water was added to the combined top layers. The tubes were capped, vortexed for 15 seconds and centrifuged for 5 minutes at 1500-2000 rpm. The top layer was transferred to a conical GC vial with care not to include any of the bottom layer. The samples were then dried under at 30°C with a nitrogen evaporator. Once evaporated, 100 μL of hexane was added and vials were capped and analyzed by gas chromatography.

d) Gas Chromatography

Lastly the samples were run on a Varian 450-GC Gas Chromatograph with FID detector (Varian, Lake Forest, CA) and a GC capillary column, 100 m × 0.25 mm diameter and 0.25 μm film thickness (Cat # CP7420, Varian, Lake Forest, CA). The temperature program was: 70°C hold × 2 minutes, 180°C at 30°C/minute, 200°C at 10°C/minute × 2 minutes, 220°C at 2°C/minute × 10 minutes, 240°C at 20°C/minute × 5 minutes. Total run time was 36.67 minutes. All samples were run with 10:1 split ratio.

Western Immunoblotting

Western blotting was used to determine the relative amount of several proteins of interest in the sample. First, protein is extracted from the tissue of interest and is then separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Next, the samples are transferred to polyvinylidene difluoride (PVDF) membrane. Non-specific binding sites on the membrane are then blocked with bovine serum albumin and a primary antibody is applied which binds to the protein of interest. The secondary antibody, which is coupled to horseradish peroxidase, is added and tags the antigen-

antibody complex. The location of the protein of interest on the membrane can then be illuminated using a chemiluminescent substrate.

a) Hepatic Protein Extraction

To quantify the amount of protein in the liver and make it available for Western blot analysis, the protein must be released from the selected tissue. To accomplish this, 40 mg of liver was ground to a fine powder under liquid nitrogen with a mortar and pestle. Next 30 μL of 3× sample buffer (0.2 M Tris-HCl, 3% SDS and 30% glycerol) per mg of liver was mixed with the powdered tissue. The mixture was allowed to sit for 15 minutes at room temperature to permit lysis of the hepatocytes. Next the mixture was pipetted into 1.5 mL microcentrifuge tubes and centrifuged for 20 minutes at 13 000 rpm. The supernatant containing the protein was removed and sonicated (Sonic dismembrator, Model 100, Thermo Fisher Scientific Inc., Ottawa, ON) for 10 seconds, and stored at -80°C until used.

b) Protein Assay

The Pierce bicinchoninic acid (BCA) assay kit (Cat. #23225, Pierce, Rockford, IL) was used to quantify the protein concentration of the hepatic protein extract. The principle of this assay is as follows: Cu^{2+} is reduced to Cu^{1+} in an alkaline environment and the Cu^{1+} combines with BCA to produce a purple complex. The intensity of the coloured end product can be measured by spectrometric analysis at 550 nm and is proportional to the concentration of protein in the sample.

The reagents used in this procedure include a protein standard containing 2 mg/mL BSA (Cat # 23209, Thermo Scientific, Rockford, IL), which was diluted with ddH_2O to produce 6 additional standards (1, 0.8, 0.6, 0.4, 0.2, and 0 mg/mL). Reagent A (Cat # 23223, Thermo Scientific, Rockford, IL) containing sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.2 M sodium hydroxide and reagent B (Cat # 23224, Thermo Scientific, Rockford, IL) containing 4% cupric sulphate were also used.

The hepatic protein extracts were diluted 5× with deionized water. Ten μL each of standards and samples were pipetted in triplicate into a Nunc 96-well plate. Fifty parts of Reagent A and 1 part of Reagent B were mixed and 200 μL of the Reagent mixture was added to each well and gently mixed for 30 seconds. Next, the plate was incubated for 30 minutes at 37°C. After incubation, the plate was allowed to cool to room temperature and then the absorbance was read at 550 nm with a FLUOstar Omega microplate reader (BMG, LABTECH, Thermo Fisher Scientific, Offenburg, Germany) using Omega Control Software (Version 1.0, BMG LABTECH, Thermo Fisher Scientific, Offenburg, Germany). The mean result for each sample was multiplied by the dilution factor to obtain the final protein concentration of the liver samples. Samples with coefficients of variation greater than 10% were re-assayed and the standard curve of all assays had a correlation coefficient of 0.9 or greater.

c) Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

After quantification of the protein in the sample, SDS-PAGE was used to separate the proteins according to molecular mass. This is done by placing the sample into a polyacrylamide gel and exposing the gel to an electric current which draws the proteins

through the porous gel, with higher molecular weight proteins migrating slower through the gel than those with a lower molecular mass.

Reagents used:

- 5 – 10% separating gel consisting of 20% acrylamide, 10% SDS, 1.5 M Tris-HCl pH 8.8, 10% ammonium persulfate, N, N, N', N'-Tetramethylethylenediamine (TEMED) and ddH_2O.
- 5% stacking gel consisting of 20% acrylamide, 10% SDS, 0.5 M Tris-HCl pH 6.8. 10% ammonium persulfate, TEMED and ddH_2O.
- H_2O-saturated butanol
- 10% bromophenol blue
- 2-mercaptoethanol
- SDS-PAGE electrode buffer (0.125 M Tris, 0.96 M glycine, 0.5% SDS)

One-mm spacers were set up between 2 glass plates. The plates were put into a sandwich clamp assembly and placed into a casting stand. Components for the 5-10% gels were mixed and poured between the glass plates. H_2O-saturated butanol was pipetted over top of the gel to ensure that the top of the gel was even. After the separating gel had polymerized completely (about 1 hour), the H_2O-saturated butanol was poured off the gel and the gel was rinsed with ddH_2O. Next, the components of the 5% stacking gel were mixed and poured over the separating gel. A 15-well comb was inserted into the stacking gel and the gel was left to polymerize (about 15 minutes).

The sandwich clamp assembly was transferred to the electrophoresis apparatus and placed in a transparent buffer tank. The SDS-PAGE buffer was poured over the middle of the electrophoresis apparatus and the rest poured into the buffer tank.

Using the concentration of protein in the sample from the protein assay, the volume of protein sample required to load 5 μg of protein per well was calculated. Each protein sample was mixed with 10 μL of 3× sample buffer and 1 μL of a 1:1 solution of bromophenol blue and 2-mercaptoethanol. The samples were heated in a 90°C water bath for 3 minutes to denature the proteins. Samples, an internal loading control (a sample that is loaded on all gels to control for different intensities on different gels) and a molecular mass marker were loaded onto the gel using a glass syringe. The electrophoresis apparatus was then connected to a power supply and electrophoresis was conduced at 20 mA per gel for about 1 hour, until the bromophenol blue reached the bottom of the gel.

d) Gel Transfer

After proteins were separated based on molecular mass they were electrophoretically transferred to a 6.8 cm × 8 cm polyvinylidene difluoride membrane. Reagents used:

- Transfer buffer (20% methanol, 0.25 mM Tris, 130 mM glycine and ddH_2O)
- 5× Tris-buffered saline in Tween-20 (TBST): 0.1 M Tris-HCl pH 7.4, 0.25% Tween-20
- 1× TBST: 1 part 5× TBST and 4 parts ddH_2O
- methanol

The glass plates from the sandwich assembly were separated and the stacking gel discarded. The gel was removed from the glass plate by adhering to a piece of blotting paper. A PVDF membrane, which was soaked in methanol and then equilibrated in the transfer buffer for 5 minutes, was placed on top of the gel. The membrane was covered with another piece of blotting paper and both sides were covered with a fiber pad and placed inside a transfer cassette. The transfer cassette was placed inside the electrode module with the negative side of the cassette facing the negative side of the module. An ice pack and a stir bar were placed inside the transfer tank with the cassettes and the transfer buffer was poured over the entire apparatus. The apparatus was placed on a magnetic stirrer set at 200 rpm, and the protein transfer was achieved by electrophoresis at 100 V for 1 hour. Membranes containing the transferred proteins were placed in a container with 1× TBST and stored at 4°C.

e) Identification of Proteins

Western blotting uses antibodies to detect a protein of interest on a PVDF membrane by complexing them with a primary antibody and then a secondary antibody that is conjugated to horseradish peroxidase. When exposed to a chemiluminescent agent, the horseradish peroxidase catalyzes the production of a luminescent compound detectable upon exposure to autoradiographic film. The intensity of the light produced is proportional to the amount of the protein of interest that is present in the sample.
Reagents used:

- 3% BSA in TBST
- 1% BSA in TBST

- 1× TBST
- Primary antibody
- Horseradish peroxidase-linked secondary antibody
- ECL Plus Western Blotting Detection System (Cat # RPN2132, GE Healthcare, Buckinghamshire, UK)

All incubation steps were done on an orbital shaker. First, the PVDF membrane was placed in 12 mL of 3% BSA in TBST for 1 hour. The primary antibody was then added to the 3% BSA in TBST at a specific dilution ratio (Table 2) and incubated for 1 hour. Next, the primary antibody was removed and the membrane was rinsed 4 times with 1× TBST for 5 minutes each rinse. A horseradish peroxidase-linked secondary antibody, specific to the animal source of the primary antibody, was added to 1% BSA in TBST at a specific dilution ratio (Table 2) and incubated for another hour. Following the incubation, the secondary antibody was removed and the membranes were once again rinsed 4 times with 1× TBST for 5 minutes each rinse. Membranes were then immersed in chemiluminescent reagent, placed between acetate sheets and exposed to clear blue x-ray film (Cat # 34091, Thermo Scientific, Rockford, IL).

The intensity of the bands was quantified with a densitometer (trace analysis with a GS 800 Imaging Densitometer, Bio-Rad Laboratories, Hercules, CA) using Quantity One software (Version 4.5.0, Bio-Rad Laboratories, Hercules, CA). Data for each protein were calculated by determining the ratio of the trace analysis value of the protein of interest to the trace analysis value of β-tubulin or eEF2 (loading controls immunoblotted on the same membrane), then dividing this value by the ratio of the trace analysis of the

internal control to the trace analysis of β-tubulin or eEF2. The data were expressed as arbitrary units.

g) Stripping of Membrane Blots

Membranes were stripped after Western blotting to allow the membrane to be probed with other antibodies.

Reagents used:

- Stripping buffer (10% SDS, 0.5 M Tris HCl pH 6.8 and ddH_2O)
- 2-mercaptoethanol
- 1× TBST
- 10% bleach

The membrane was covered with 25 mL of stripping buffer and, in the fume hood, 200 µL 2-mercaptoethanol was added. The container was closed and placed into a larger container, which was also closed to help reduce odor and placed on the orbital shaker overnight. The next day, the stripping buffer solution was poured down the sink in the fume hood and the membranes were placed in a new container and covered with 1× TBST. The membranes were then rinsed 4 times with 1× TBST for 5 minutes each rinse. Once rinsed the membranes were stored for subsequent use in a container containing 1× TBST. The old containers were rinsed with 10% bleach and left in the fume hood until the odor dissipated.

Table 2: Antibodies used in Western Blotting Analysis.

Primary Antibody	Source	Primary Dilution	Secondary Antibody	Secondary dilution	Molecular Weight (kDa)	% Separating Gel
SREBP-1 (c20)[1]	Santa Cruz Cat # sc-366	1:1000	anti-rabbit horseradish peroxidase	1:10 000	125 (inactive) 68 (active)	7.5
ACC-1[2]	Santa Cruz Cat # sc-490S	1:1000	anti-rabbit horseradish peroxidase	1:20 000	265	5
PPARα (s12)[3]	Abcam Cat # 3484-100	1:2000	anti-rabbit horseradish peroxidase	1:20 000	52	10
PPARα (s21)[3]	Abcam Cat # 3485-100	1:2000	anti-rabbit horseradish peroxidase	1:20 000	52	10
ACOX1 (h140)[4]	Santa Cruz Cat # sc-98499	1:1000	anti-rabbit horseradish peroxidase	1:10 000	72	7.5
β-Tubulin (loading control)	Cell Signaling Cat # 2146	1:1000	anti-rabbit horseradish peroxidase	1:10 000	55	7.5–10
eEF2 (loading control)[5]	Cell Signaling Cat # 2332	1:1000	anti-rabbit horseradish peroxidase	1:10 000	95	5

[1] sterol regulatory element binding protein-1c (SREBP-1c)
[2] acetyl-CoA oxidase (ACC-1)
[3] peroxisome proliferator-activated receptor (PPAR)
[4] acyl-CoA oxidase (ACOX1)
[5] eukaryotic elongation factor 2 (eEF2)

Statistical Analysis

Statistical analysis was performed using Statistical Analysis Software (SAS; Version 9.1.3, SAS Institute Inc., Cary, NC). Time course and end-point data were analyzed by repeated measures ANOVA and one-way ANOVA, respectively. Significant differences among means were determined using Duncan's multiple range test. Data that did not follow a normal distribution or was not homogenous were analyzed by Kruskal-Wallis, a non-parametric test, followed by least significant differences post hoc testing using SAS. Data are shown as the mean ± standard error of the mean (SEM) and the level of significance was set at $P < 0.05$.

RESULTS

Fatty Acid Analysis of Diet

When analyzed with GC (Table 3), most of the diets were relatively similar to the estimated values for SFA indicated on the Comparison of Dietary Fats fact sheet (Canola Council of Canada, 2008; Table 1, *pg 44*), however, the L diet had more SFA (49% vs. 41%) and the SF diet had less SFA (10% vs. 12%) than what was predicted. Regarding MUFA, the HC, C and C/F diets had more MUFA (78%, 66% and 54%, respectively) compared to the fact sheet (70%, 61% and 51%, respectively). Conversely, the SB and L diets had slightly less MUFA (21% and 42%, respectively) than what was estimated on the Canola Council fact sheet (23% and 45%, respectively). The SF diet had similar amounts of MUFA to what was expected. In terms of total PUFA, LA and ALA content, most of the diets, except the SB diet, had less total PUFA, LA and ALA than what was indicated on the Canola Council fact sheet. The HC, C, C/F and L diets all had less total PUFA (-3% to -7%). The HC, C and L diets had less LA (-2% to -6%) and the HC, C, C/F, L and SF diets had less ALA (-1% to -3%).

When comparing the analyzed LA/ALA ratio of the diet to what was indicated on the fact sheet, the HC, C, C/F, SB and L diets had a very similar ratio of 7, 2, 1, 6 and 8, respectively. The fact sheet indicated that the LA/ALA ratio of SF would be 71, however, the SF diet actually had a much greater LA/ALA ratio (365). The analyzed and predicted ALA content of the SF diet was similar (0.2% vs 1%). However, due to the fact that there is so much LA in the SF diet, when ALA was expressed as an LA/ALA ratio, the small difference between the analyzed and predicted ALA content became magnified. When comparing the analyzed total n-6/n-3 ratio to the analyzed LA/ALA ratio, only the

SF diet differed. The analyzed n-6/n-3 ratio of the SF diet was 183, whereas the analyzed LA/ALA ratio was 365, however, this ratio was still a lot higher than the LA/ALA ratio predicted on the fact sheet. The fact sheet did not estimate total n-6/n-3 ratios. The amount of total n-3 PUFAs in the analyzed SF diet was higher than the ALA content of the diet due to a small amount of EPA that was present in the diet.

Table 3: Analyzed Fatty Acid Composition of Diets.

Fatty Acid[1]	HC	C	C/F	SF	SB	L
Total SFA	**7**	**7**	**8**	**10**	**15**	**49**
C16:0	4	4	4	6	10	24
C18:0	2	2	2	3	4	21
Total MUFA	**78**	**66**	**54**	**17**	**21**	**42**
C18:1	76	64	53	16	20	39
Total PUFA	**16**	**27**	**38**	**73**	**63**	**9**
LA	14	19	18	73	54	8
ALA	2	8	20	0.2	9	1
LA/ALA	7	2	1	365	6	8
Total n-6	14	19	19	73	54	8
Total n-3	2	8	20	0.4	9	1
n-6/n-3	7	2	1	183	6	7

[1] g/100 g fatty acids
HC = high oleic canola, C = conventional canola, C/F = canola/flax blend, SF = safflower, SB = soybean, L = lard.

Feed Intake, Body Weight and Obesity

In terms of weekly feed intake, there were no differences among groups from baseline to week 10. However, at week 11 and 12, the WM group had their feed intake restricted in order to match their body weight to the group that was gaining the least amount of weight during the study and, therefore, ate significantly less feed than all groups except the L group which ate similar amounts of feed as all groups (Figure 3). During most weeks of the study the SB and L groups had a higher weekly body weight than the HC, C and C/F groups, whereas the SF and WM groups had intermediate weekly body weights (Figure 4). For complete data (means ± SEM) on weekly body weight see Appendix 2. Regarding total feed intake, there were no differences among groups (Figure 5a). The HC, C and C/F oil groups all gained the least amount of weight during the study and had the lowest final body weight (Figure 5b and 5c, respectively). The SB and L groups gained the most amount of weight and had the highest final body weight. The SF and WM groups had an intermediate amount of weight gain and final body weight.

As in indicator of how well food mass was converted into body mass, a feed efficiency ratio was calculated (total weight gain [g]/total feed intake [g]). The L group had the highest feed efficiency ratio and the C/F group had the lowest (Figure 5d). All other groups had intermediate feed intake ratios.

Fat pads as a percent body weight were used as an indicator of obesity. The SB group had the highest mesenteric fat as a percent body weight compared to all other groups (Figure 6a). Epididymal, peri-renal and visceral fat as a percent body weight was similar among groups (Figure 6b, c and d, respectively). For absolute fat pad mass see Appendix 3.

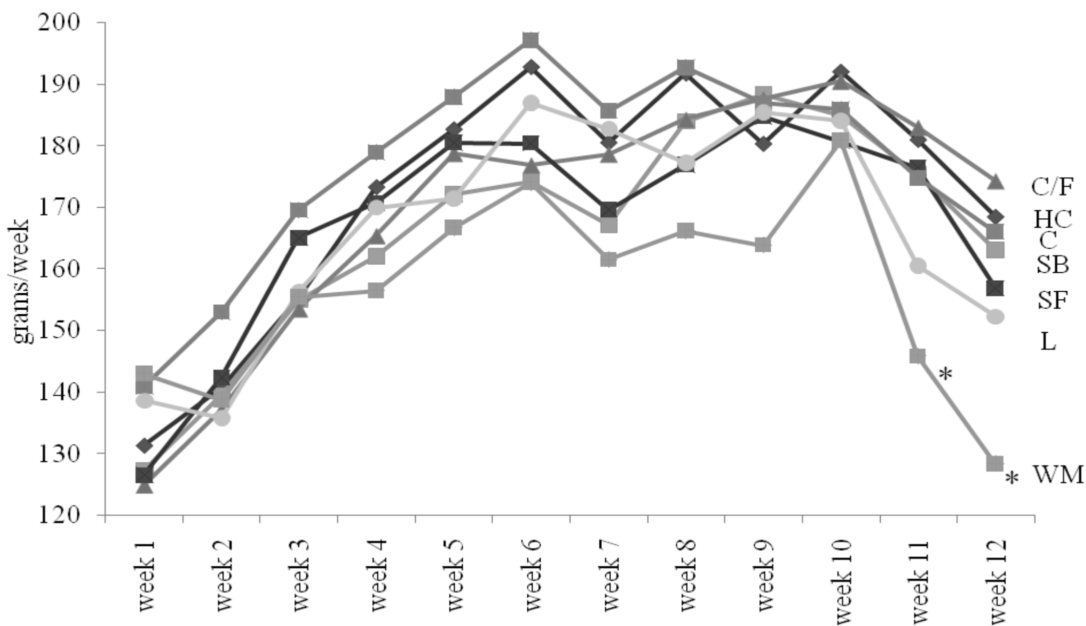


Figure 3: Weekly Feed Intake. Weekly feed intake data are presented as means. SEMs were ± 8, 7, 7, 7, 8, 9 and 6 at week 1; ± 7, 5, 7, 6, 5, 8 and 6 at week 2; ± 10, 5, 7, 6, 7, 9 and 6 at week 3; ± 11, 7, 7, 6, 6, 1 and 7 at week 4; 11, 9, 6, 10, 6, 12 and 7 at week 5; 10, 6, 7, 9, 8, 11 and 8 at week 6; 15, 9, 11, 8, 11, 13 and 9 at week 7; 16, 9, 10, 8, 6, 10 and 8 at week 8; 11, 8, 14, 9, 4, 11 and 10 at week 9; 15, 10, 12, 6, 5, 13 and 21 at week 10; 13, 9, 6, 8, 5, 6 and 7 at week 11; and 10, 8, 10, 7, 8, 11 and 7 at week 12 for the HC, C, C/F, SF, SB, L and WM groups respectively (n = 9-10/group). The WM group was fed restricted amounts of the lard diet during week 11 and 12. Statistical differences among means are indicated as * $p<0.05$ vs. HC, C, C/F, SF and SB, but not L.

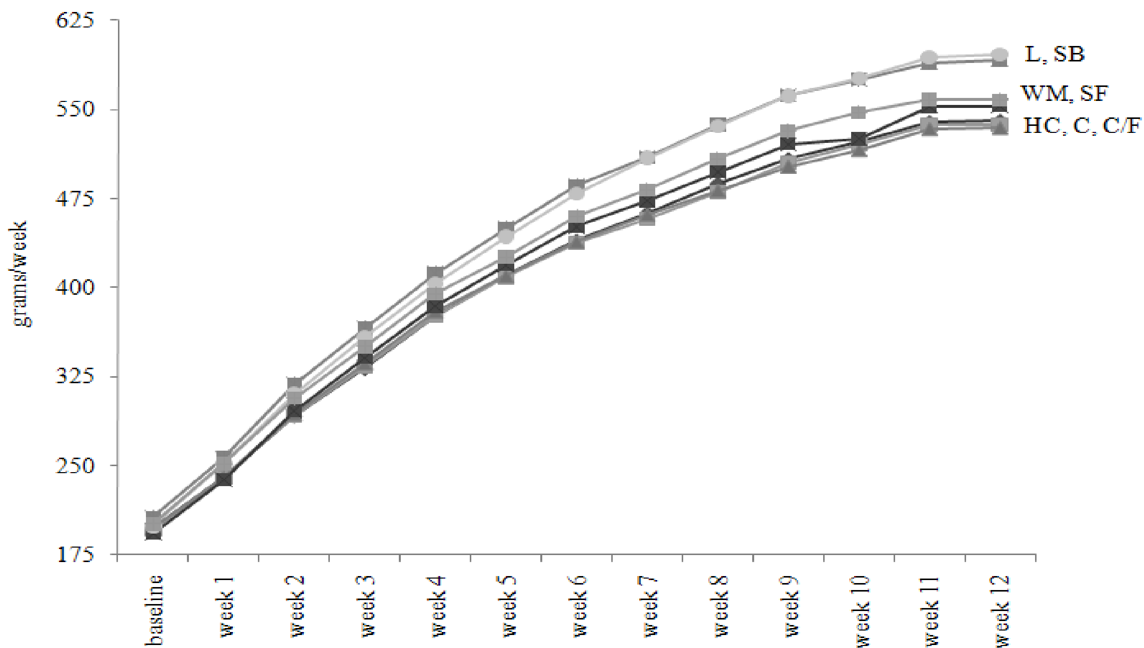


Figure 4: Weekly Body Weight. Weekly body weight data are presented as means.

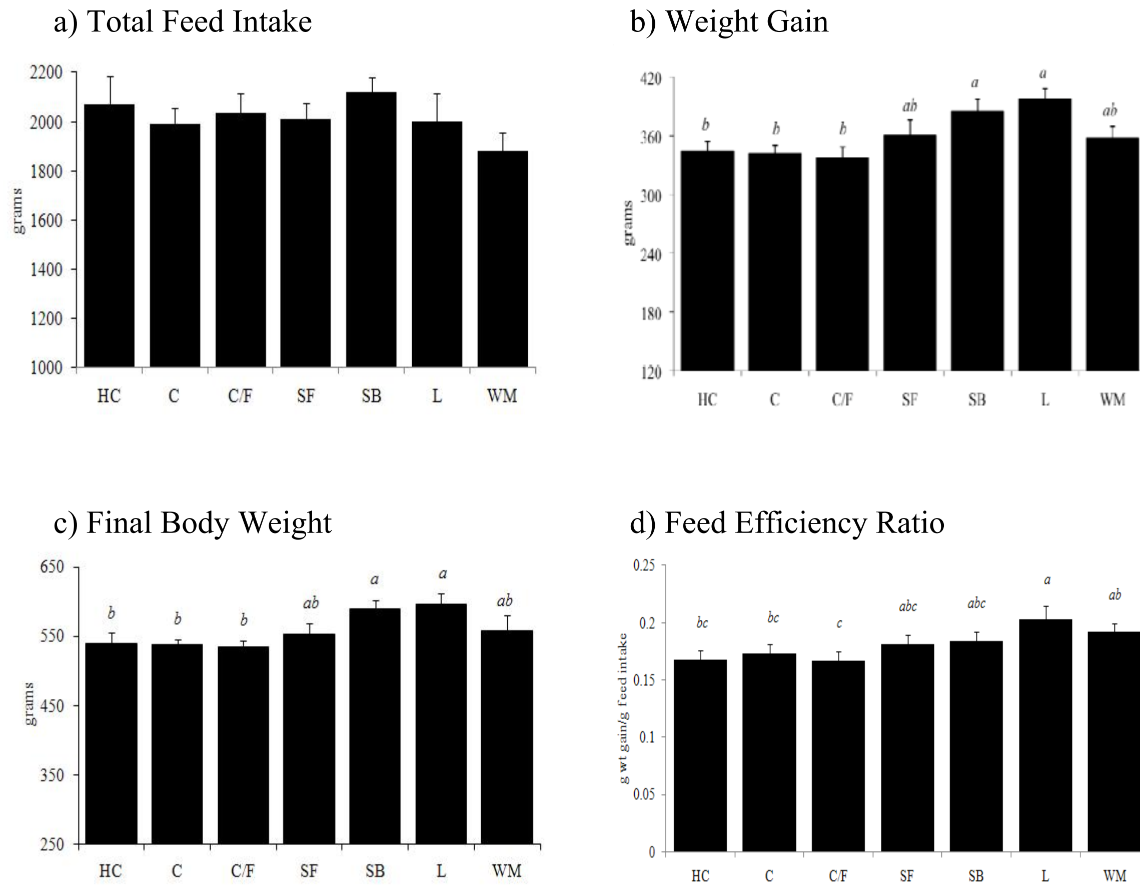


Figure 5: Feed Intake and Body Weight. Total feed intake (a), weight gain (b), final body weight (c) and feed efficiency ratio (d) are presented as means ± SEM (n = 9-10/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different.

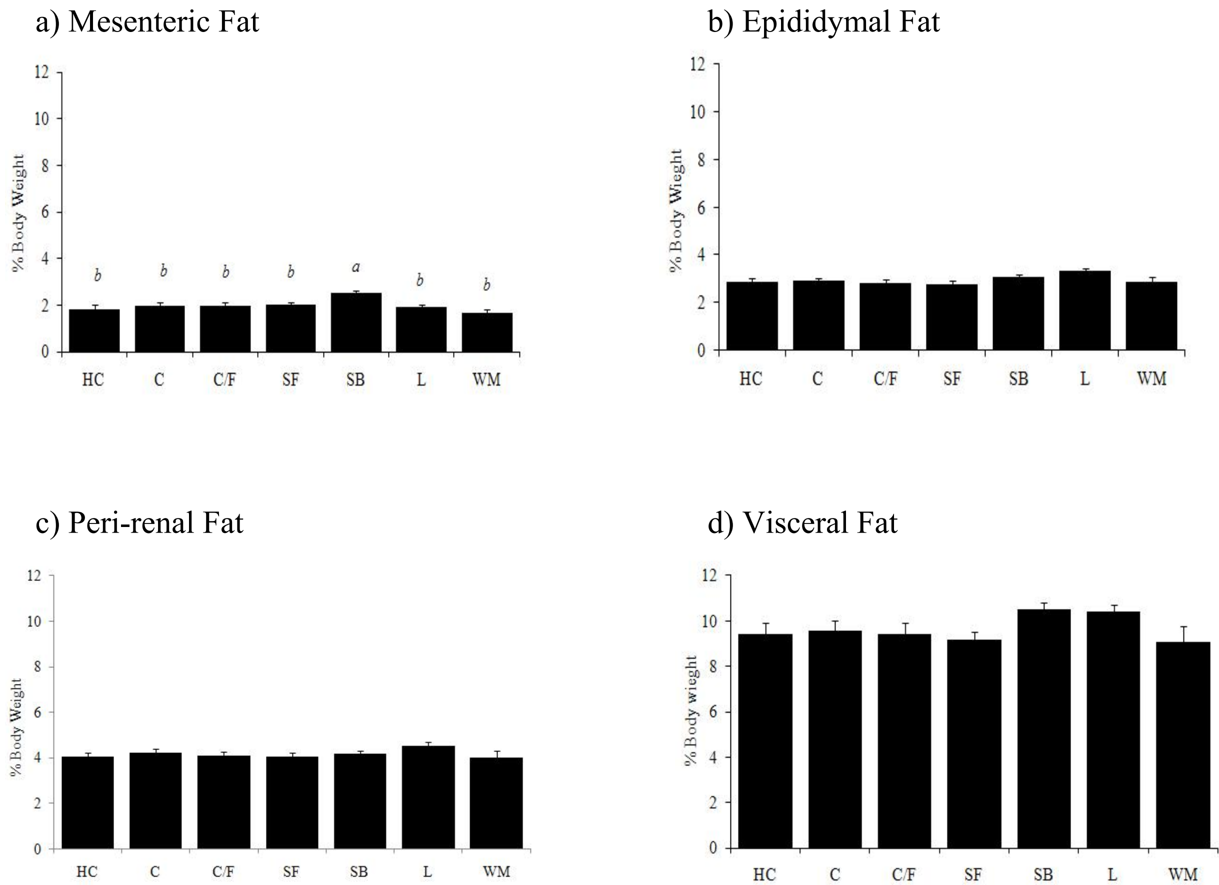


Figure 6: Adiposity. Mesenteric (a), epididymal (b), peri-renal (c) and visceral (d) fat as a percent body weight are presented as means ± SEM (n = 8-10/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different. Visceral fat includes mesenteric, epididymal, and peri-renal fat pads.

Insulin Resistance

The HC, C, C/F and SF groups had the lowest fasting serum glucose concentrations at the end of the study, whereas the SB and WM groups had intermediate levels, and the lard group had the highest fasting serum glucose at termination (Figure 7). However, groups did not differ in terms of fasting serum glucose at week 8 (Figure 7). Additionally, groups did not differ in terms of fasting serum insulin concentrations at termination (Figure 8) or differ in terms of pancreas as a percent of body weight (Figure 9). For absolute pancreas weight see Appendix 3. The HC, C and SF groups had a lower HOMA score than the L group (Figure 10). However, the L, WM, SB and C/F groups did not differ from each other in regards to HOMA score. When insulin and glucose were expressed as a ratio to determine insulin sensitivity, groups did not differ (Table 4).

The ITT and OGTT results indicated that groups did not differ in serum glucose in either test (group, $p = 0.2809$ and group × time, $p = 0.8075$ for ITT; group, $p = 0.9117$ and group × time, $p = 0.708$ for OGTT; Table 4). However, as expected, all groups differed in serum glucose over the time course of the ITT and OGTT (time, $p < 0.0001$ for ITT and OGTT). Three different monitors were used during the ITT; however, the monitor did not have a significant effect on the glucose readings ($p = 0.4313$). Likewise, groups did not differ in serum insulin during the OGTT (group $p = 0.0649$ and group x time $p = 0.3728$), however, as expected, all groups differed in serum insulin over the time course of the ITT (time, $p < 0.0001$). Area under the curve (AUC) indicated no difference among groups for AUC glucose or AUC insulin during the OGTT as well as for AUC glucose during the ITT (Table 4).

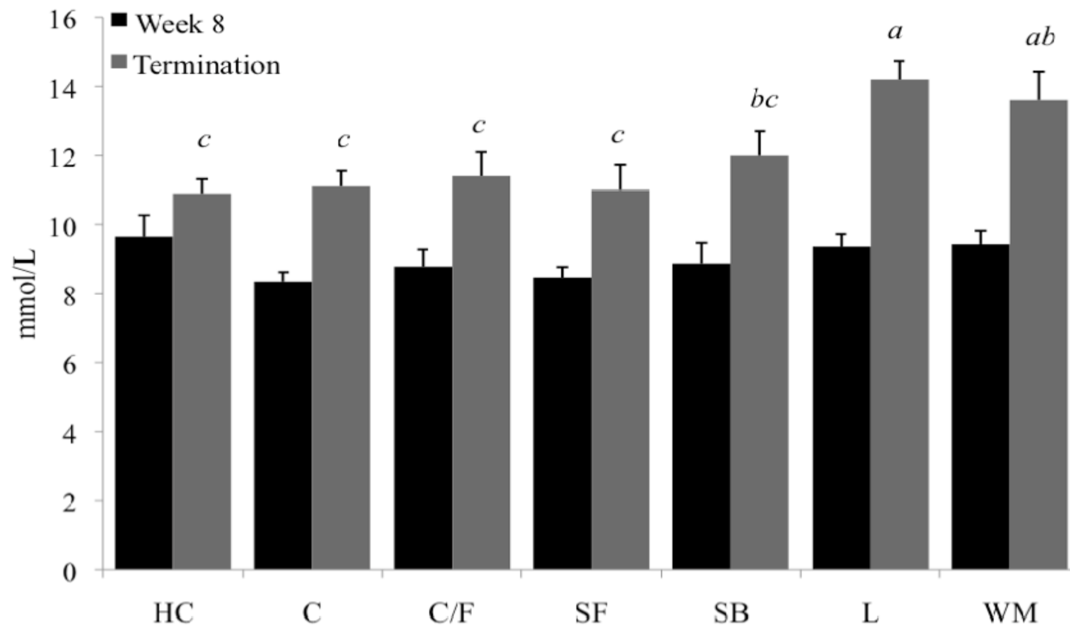


Figure 7: Serum Glucose. Termination and week 8 fasting serum glucose concentrations are presented as means ± SEM (n = 9-10/group). Statistical differences among means (p<0.05) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different. Log transformed data were analyzed.

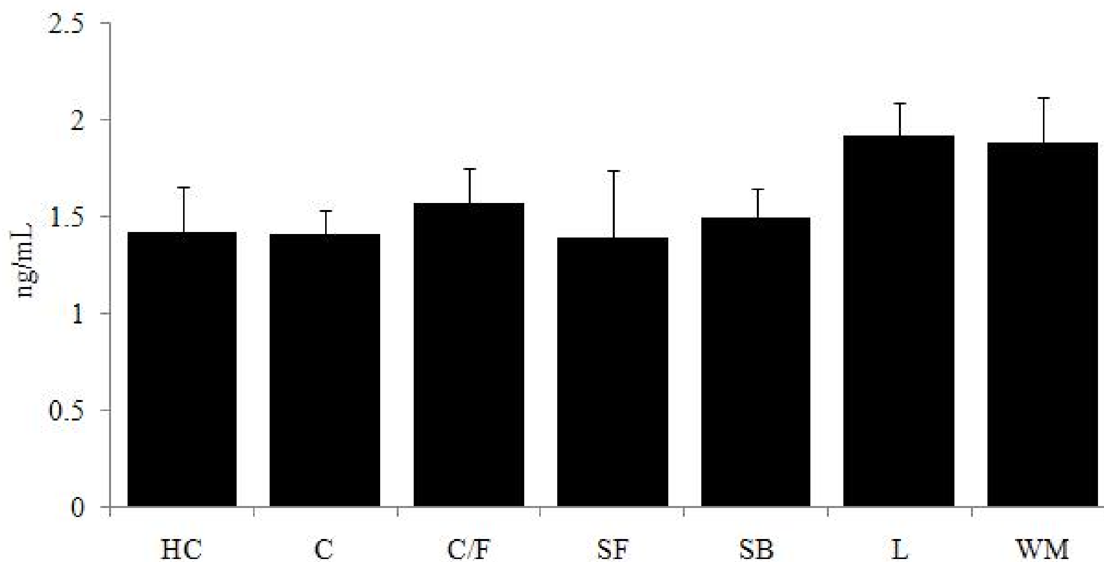


Figure 8: Serum Insulin. Termination serum insulin is presented as means ± SEM (n = 9-10/group). An absence of letters indicates that means are not statistically different.

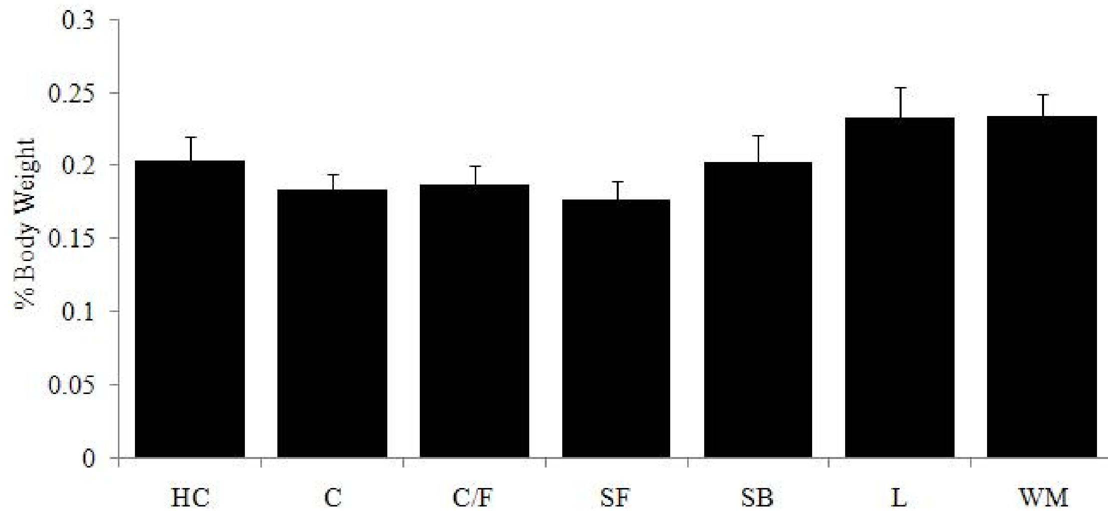


Figure 9: Pancreas Weight. Pancreas as a percent body weight is presented as means ± SEM (n = 9-10/group). An absence of letters indicates that means are not statistically different.

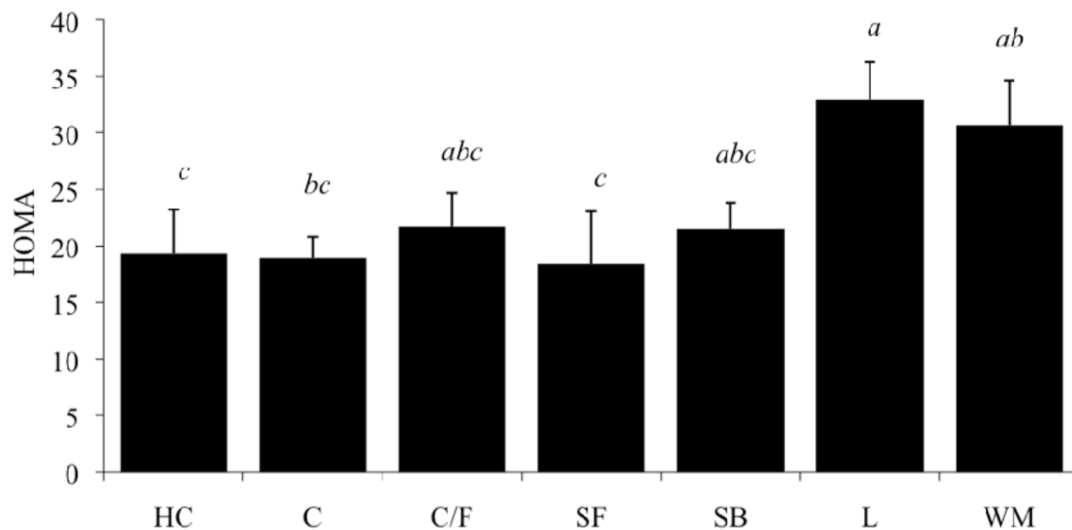


Figure 10: Homeostasis Model Assessment. Homeostasis model assessment (HOMA) was calculated using fasting termination serum insulin and glucose. Data are presented as means ± SEM (n = 9-10/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. Log transformed data were analyzed.

Table 4: Assessment of Insulin Resistance

	HO	C	C/F	SF	SB	L	WM	P- value
Insulin:Glucose (pmol/L:mmol/L)	20.7 ± 3.0	20.8 ± 2.0	22.8 ± 2.7	15.8 ± 1.7	20.8 ± 2.3	22.1 ± 2.3	23.4 ± 3.2	0.4734
ITT Glucose (mmol/L)								
T=0 min	7.14 ± 0.41	6.99 ± 0.28	7.23 ± 0.36	6.97 ± 0.31	7.84 ± 0.17	7.35 ± 0.16	7.38 ± 0.20	
T=15 min	7.49 ± 0.25	7.08 ± 0.31	6.91 ± 0.47	7.12 ± 0.450	7.82 ± 0.36	7.15 ± 0.28	7.97 ± 0.45	
T=30 min	6.58 ± 0.32	5.82 ± 0.35	6.17 ± 0.432	5.87 ± 0.47	6.29 ± 0.18	6.01 ± 0.25	6.50 ± 0.19	
T=45 min	6.28 ± 0.48	5.10 ± 0.24	5.39 ± 0.29	5.71 ± 0.52	6.46 ± 0.52	5.15 ± 0.16	5.93 ± 0.21	
T=60 min	5.83 ± 0.37	4.74 ± 0.45	5.44 ± 0.35	5.34 ± 0.41	6.01 ± 0.35	5.28 ± 0.51	5.99 ± 0.47	
AUC Glucose (for ITT)	456 ± 21	410 ± 15	426 ± 22	429 ± 27	458 ± 10	429 ± 11	462 ± 13	0.3142

Table 4: Assessment of Insulin Resistance (continued).

	HO	C	C/F	SF	SB	L	WM	P- value
OGTT Glucose (mmol/L)								
T=0 min	7.57 ± 0.26	7.52 ± 0.23	7.62 ± 0.27	7.56 ± 0.31	7.59 ± 0.25	7.35 ± 0.28	7.55 ± 0.20	
T=15 min	9.92 ± 0.34	9.40 ± 0.33	9.20 ± 0.42	8.91 ± 0.40	9.27 ± 0.51	8.80 ± 0.44	8.87 ± 0.32	
T=30 min	9.22 ± 0.25	9.16 ± 0.19	9.02 ± 0.45	8.71 ± 0.35	9.29 ± 0.89	8.61 ± 0.40	8.86 ± 0.19	
T=60 min	8.92 ± 0.36	8.98 ± 0.30	8.48 ± 0.54	8.90 ± 0.71	8.39 ± 0.39	8.64 ± 0.36	9.21 ± 0.28	
T=120 min	8.74 ± 0.36	8.51 ± 0.25	7.65 ± 0.37	7.87 ± 0.38	8.06 ± 0.32	7.83 ± 0.304	8.19 ± 0.53	
AUC Glucose (for OGTT)	171 ± 28	162 ± 24	117 ± 34	129 ± 37	117 ± 29	122 ± 20	149 ± 19	0.696

Table 4: Assessment of Insulin Resistance (continued)

	HO	C	C/F	SF	SB	L	WM	P- value
OGTT Insulin (ng/mL)								
T=0 min	3.01 ± 0.19	3.09 ± 0.29	2.86 ± 0.32	3.46 ± 0.45	3.83 ± 0.35	3.83 ± 0.43	2.74 ± 0.49	
T=15 min	4.25 ± 0.41	3.09 ± 0.29	5.07 ± 0.49	5.22 ± 0.78	6.89 ± 0.84	6.73 ± 1.13	4.66 ± 0.84	
T=30 min	3.21 ± 0.38	3.00 ± 0.51	3.91 ± 0.38	3.40 ± 0.39	4.92 ± 0.41	4.46 ± 0.38	4.14 ± 0.76	
T=60 min	3.00 ± 0.22	3.12 ± 0.22	3.19 ± 0.33	3.54 ± 0.70	4.57 ± 0.62	3.55 ± 0.40	3.05 ± 0.47	
T=120 min	2.71 ± 0.23	3.01 ± 0.42	2.84 ± 0.47	2.65 ± 0.56	3.62 ± 0.21	3.43 ± 0.31	2.96 ± 0.62	
AUC Insulin (for OGTT)	44.9 ± 10.2	48.0 ± 11.1	74.3 ± 23.5	55.0 ± 17.2	108 ± 23	94.2 ± 13.9	84.1 ± 24.9	0.0987

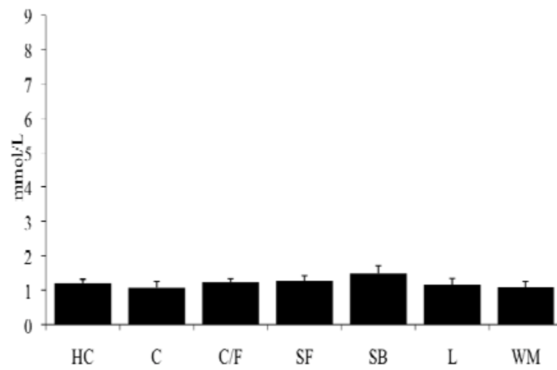
Data are presented as means ± SEM (n = 8-10/group). An absence of letters indicates that means are not statistically different.

Lipidemia

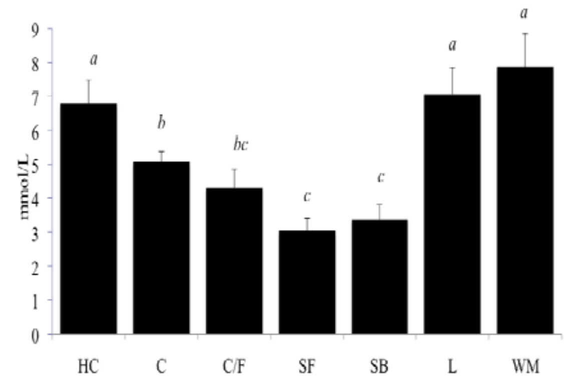
Lipidemia can be characterized by measuring fasting serum TAG, cholesterol and FFA concentrations. There was no difference among groups in terms of fasting serum TAG at baseline (Figure 11a). At week 4, the SF and SB groups had the lowest fasting serum TAG, whereas the HC, L and WM groups had the highest (Figure 11b). The C/F group had similar concentrations of TAG to the SF and SB groups, but also similar concentrations as the C group. At week 8, the SF and SB groups had the lowest fasting serum TAG. The C/F oil group had similar concentrations of TAG to the SF and SB groups but also similar concentrations to the C group (Figure 11c). The WM group had the highest fasting serum TAG at week 8, whereas the HC group had the second highest and the L group had similar TAG concentrations to both the WM and the HC groups. At termination, the C/F, SF and SB oil groups had the lowest fasting serum TAG, the HC, L and WM groups had the highest serum TAG and the C group was not different from any group (Figure 11d). Repeated measures statistics indicated that not only was there differences in serum TAG among groups (group, $p < 0.0001$), but there was also a time effect (time, $p < 0.0001$) and a group × time effect (group × time, $p < 0.0001$). Statistical analysis of how fasting serum TAG changed over the course of the study in each group is provided in Appendix 4.

There was a trend for differences in fasting serum cholesterol at termination ($p = 0.0612$; Table 5). The trend indicated that the WM group had the lowest cholesterol, the HC and SB groups had the highest cholesterol and all other groups had intermediate amounts of cholesterol. No differences were seen among groups for fasting serum FFA at termination (Table 5).

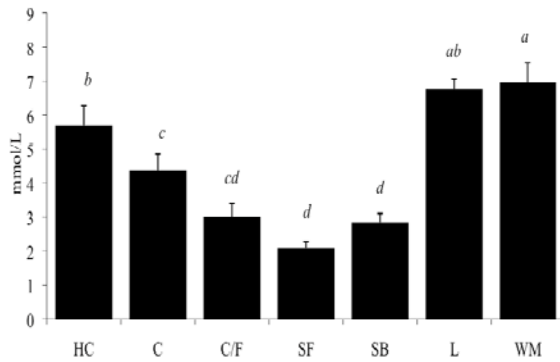
a) Baseline serum TAG



b) Week 4 Serum TAG



c) Week 8 Serum TAG



d) Termination Serum TAG

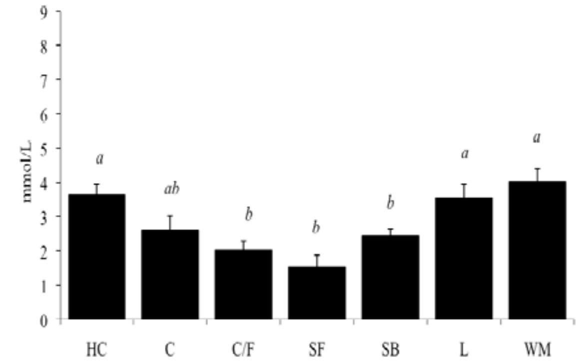


Figure 11: Serum TAG. Baseline (a), week 4 (b), week 8 (c), and termination (d) fasting serum TAG are presented as means ± SEM (n = 8-10/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different.

Table 5: Lipidemia.

	HO	C	C/F	SF	SB	L	WM	P- value
Term Cholesterol (mmol/L)	4.66 ± 0.34 *a*	4.20 ± 0.35 *ab*	3.96 ± 0.45 *ab*	4.19 ± 0.29 *ab*	4.73 ± 0.32 *a*	3.77 ± 0.27 *ab*	3.42 ± 0.23 *b*	0.0612
Term FFA (mmol/L)	0.232 ± 0.034	0.202 ± 0.025	0.288 ± 0.047	0.278 ± 0.039	0.232 ± 0.034	0.231 ± 0.036	0.192 ± 0.030	0.2353

Data are presented as means ± SEM (n = 9-10/group). An absence of letters indicates that means were not statistically different.

Hypertension

Systolic and diastolic blood pressures were both similar among groups at week 10.5 (Table 6).

Table 6: Systolic and Diastolic Blood Pressure.

	HO	C	C/F	SF	SB	L	WM	P-value
Systolic mmHg	154 ± 6	155 ± 4	159 ± 5	150 ± 4	159 ± 3	159 ± 6	162 ± 7	0.4270
Diastolic mmHg	110 ± 5	109 ± 3	114 ± 5	106 ± 6	113 ± 3	116 ± 7	120 ± 6	0.5032

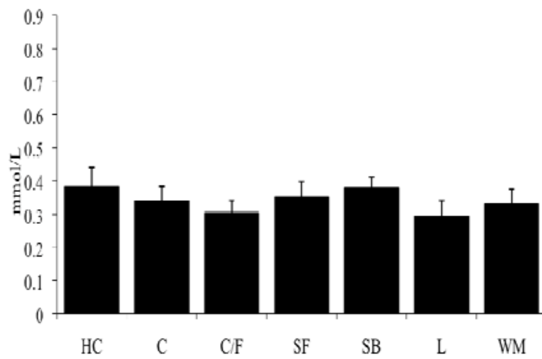
Data are presented as means ± SEM (n = 9-10/group). An absence of letters indicates that means are not statistically different.

Inflammation

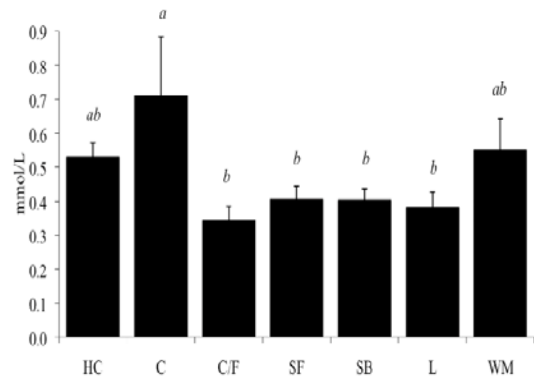
Haptoglobin is an acute phase protein that can be used as a marker of inflammation. Groups did not differ in fasting serum haptoglobin at baseline (Figure 12a). At week 4, the C/F, SF, SB and L groups had the lowest fasting haptoglobin, whereas the C group had the highest, and the HC and WM groups had intermediate levels (Figure 12b). At week 8, the C/F, SF, SB, L and WM groups had the lowest fasting haptoglobin, the HC group had the highest, and the C group had intermediate levels (Figure 12c). The C/F, L and WM groups had the lowest fasting serum haptoglobin at termination, whereas the HC group had the highest, and the C, SF and SB groups had intermediate levels (Figure 12d). Repeated measures statistics indicated that there was a group and time effect ($p < 0.0002$, and 0.002, respectively), but no group × time effect ($p < 0.2984$). Statistical analysis of how fasting serum haptoglobin changed over the course of the study in each group is provided in Appendix 5.

At termination, groups had similar concentrations of fasting serum adiponectin, which is an anti-inflammatory adipokine (Figure 13).

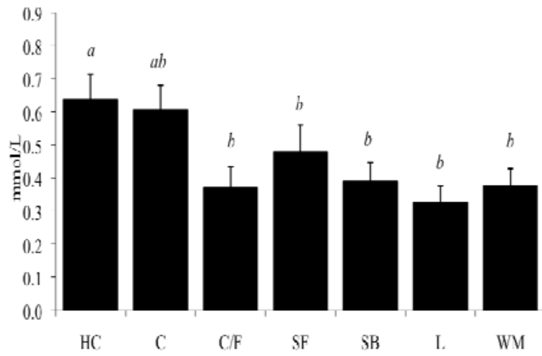
a) Baseline Serum Haptoglobin



b) Week 4 Serum Haptoglobin



c) Week 8 Serum Haptoglobin



d) Termination Serum Haptoglobin

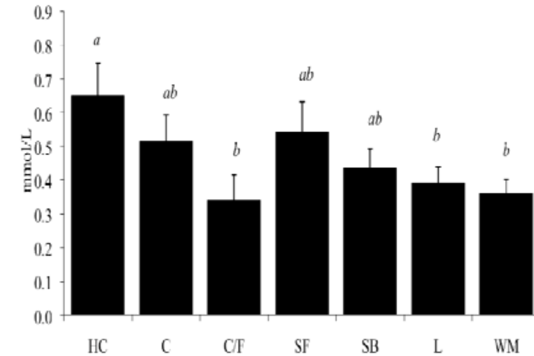


Figure 12: Serum Haptoglobin. Baseline (a), week 4 (b), week 8 (c) and termination (d) fasting serum haptoglobin are presented as means ± SEM (n = 8-10/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different.

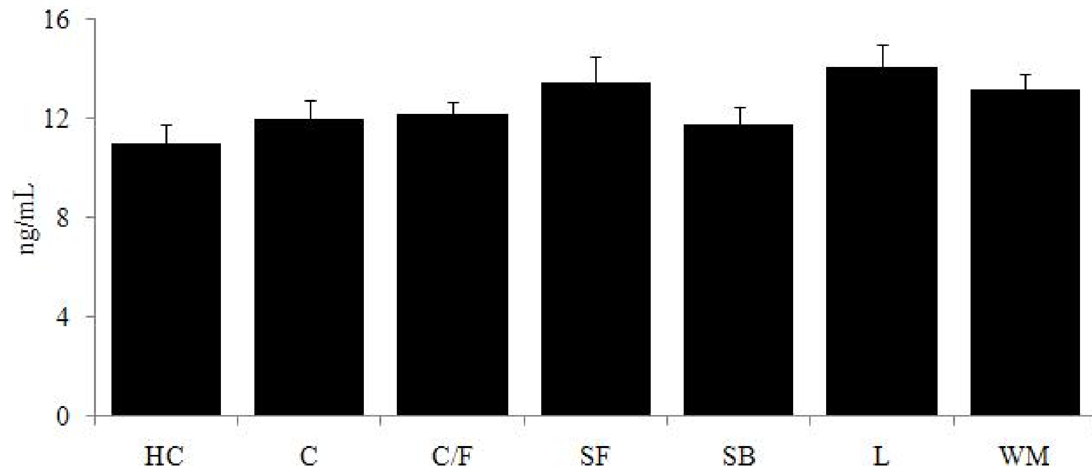


Figure 13: Serum Adiponectin. Termination serum adiponectin is presented as means ± SEM (n = 9/group). An absence of letters indicates that means are not statistically different.

Liver Weight and Hepatic Steatosis

Liver as a percent body weight was highest in the HC group compared to all of the other dietary treatment groups (Figure 14). For absolute liver weight see Appendix 3. The C/F and WM groups had the lowest percent liver lipid compared with all other groups (Figure 15).

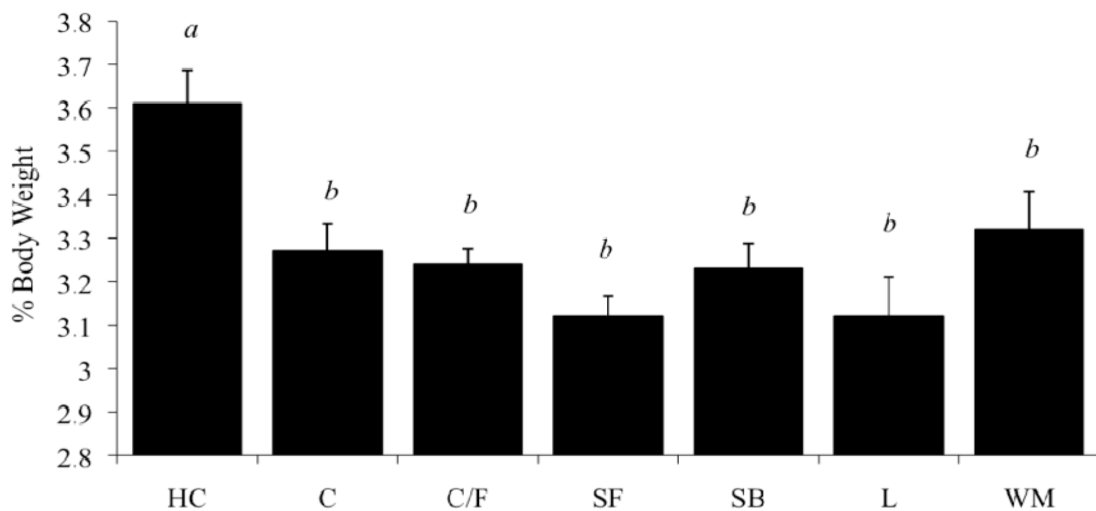


Figure 14: Liver Weight. Liver as a percent of body weight is presented as means ± SEM (n = 9-10/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters.

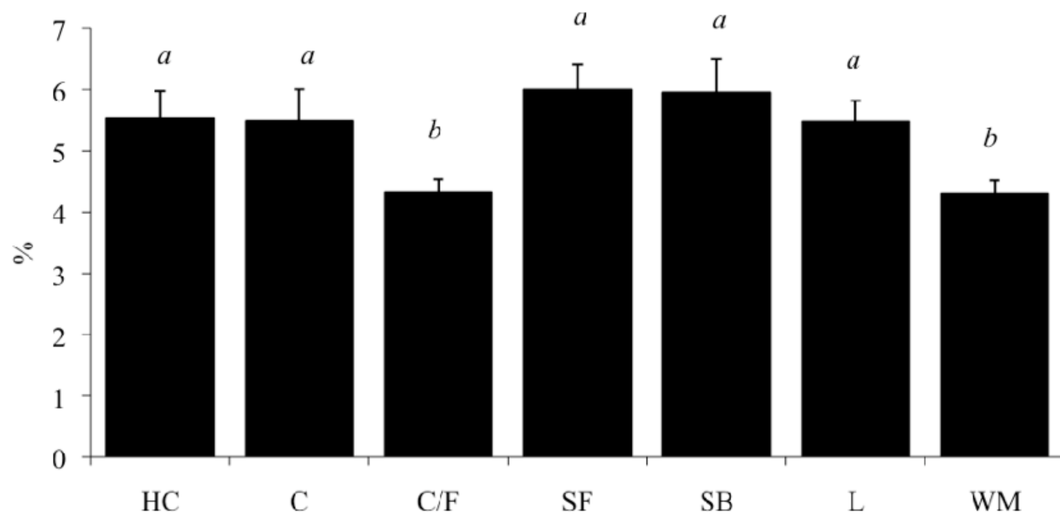


Figure 15: Hepatic Lipid. Percent hepatic lipid is presented as means ± SEM (n = 8/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. Log transformed data were analyzed.

Hepatic Fatty Acid Composition

a) TAG

A summary of the hepatic TAG fatty acid classes and fatty acid ratios are shown in Table 7. In general, the fatty acid profile of the diet is reflected in the fatty acid profile of the hepatic TAG. For example, the L and WM groups had significantly higher total SFA than all other diets. In terms of total MUFA, the HC groups had the highest amount followed by the C group. The C/F, L and WM groups had the next highest total MUFA. The SF group had the highest total PUFA, followed by the SB group. The C/F had the highest total n-3 in hepatic TAG, whereas the SF group had the highest total n-6 and the HC group had the highest total n-9.

The SF oil group had the highest n-6/n-3 ratio, followed by the SB group. All other groups had lower n-6/n-3 ratios. The HC group had the highest n-9/n-6 ratio and the highest Δ9 desaturase index (MUFA/[MUFA + SFA] and C18:1n-9/C18:0). The HC, L and WM groups had the highest Δ5 desaturase index (C20:4n-6/C20:3n-6).

Figure 16 highlights the individual fatty acids of interest in hepatic TAG. For a complete fatty acid profile of hepatic TAG, refer to Appendix6. The L and WM groups had the most C16:0, compared to all other groups (Figure 16a). The HC group had the highest C18:1n-9 (53.0%) followed by the C group (44.5%) and then the C/F, L and WM groups (36.8% - 40.2%; Figure 16b). The SF and SB groups had the lowest C18:1n-9 (12.4% - 15.0%). On the contrary, the SF and SB groups had the highest C18:2n-6 (Figure 16c). Regarding ALA (C18:3n-3), the C/F group had the highest ALA in the hepatic TAG (4.25%), followed by the SB group (3.40%) and then the C group (2.01%; Figure 16d). All other groups (HC, SF, L and WM) had lower ALA (0.32%-0.50%). The

SF group had the highest AA (3.30%; C20:4n-6) and all of the canola groups (HC, C and C/F) had the lowest AA (0.23% - 0.38%; Figure 16e). The SB group had the highest EPA (0.45%; C20:5n-3) followed by the C/F group (0.28%; Figure 16f). All other groups had lower EPA in hepatic TAG (0.02% - 0.15%). Groups did not differ in terms of DHA (C22:6n-3; Figure 16g).

Table 7: Hepatic TAG.

TOTALS[1]	HC	C	C/F	SF	SB	L	WM	P-Value
Total SFA *	23.4 ± 2.2 *b*	25.6 ± 0.7 *b*	24.6 ± 1.3 *b*	24.6 ± 1.3 *b*	24.0 ± 0.7 *b*	35.7 ± 0.8 *a*	35.5 ± 1.4 *a*	0.001
Total MUFA *	56.2 ± 1.5 *a*	47.9 ± 0.8 *b*	44.9 ± 0.9 *bc*	14.9 ± 0.6 *e*	20.3 ± 0.7 *d*	44.4 ± 1.6 *bc*	43.1 ± 3.0 *c*	<0.0001
Total PUFA *	18.8 ± 1.3 *e*	25.1 ± 0.7 *d*	30.1 ± 1.0 *c*	59.1 ± 1.0 *a*	55.2 ± 1.3 *b*	18.9 ± 1.1 *e*	20.3 ± 1.8 *e*	<0.0001
Total n-3	10.4 ± 1.0 *bc*	12.3 ± 1.1 *b*	17.0 ± 0.4 *a*	8.20 ± 1.55 *c*	12.5 ± 0.5 *b*	9.25 ± 1.02 *bc*	10.5 ± 1.98 *bc*	0.0005
Total n-6 *	8.46 ± 0.85 *d*	12.8 ± 0.5 *c*	13.1 ± 0.8 *c*	51.4 ± 2.6 *a*	43.0 ± 1.3 *b*	9.69 ± 0.25 c*d*	9.81 ± 0.31 c*d*	<0.0001
Total n-9 *	54.2 ± 1.5 *a*	45.9 ± 1.3 *b*	37.9 ± 1.9 *c*	12.8 ± 0.6 *d*	15.5 ± 0.5 *d*	41.4 ± 1.4 *bc*	39.6 ± 2.9 *c*	<0.0001

Table 7: Hepatic TAG (continued)

RATIOS	HC	C	C/F	SF	SB	L	WM	P-Value
n-6/n-3 *	0.850 ± 0.136 *c*	1.09 ± 0.14 *c*	0.772 ± 0.042 *c*	7.46 ± 1.59 *a*	3.47 ± 0.18 *b*	1.10 ± 0.13 *c*	1.08 ± 0.20 *c*	0.0004
n-9/n-6 *	6.64 ± 0.61 *a*	3.60 ± 0.12 *bc*	2.90 ± 0.009 *c*	0.252 ± 0.013 *d*	0.362 ± 0.011 *d*	4.28 ± 0.16 *b*	4.04. ± 0.27 *b*	<0.0001
Δ9[1]	0.704 ± 0.025 *a*	0.652 ± 0.007 *b*	0.646 ± 0.015 *b*	0.376 ± 0.018 *e*	0.460 ± 0.003 *d*	0.554 ± 0.015 *c*	0.546 ± 0.027 *c*	<0.0001
Δ9[2] *	40.7 ± 3.7 *a*	26.4 ± 2.3 *b*	15.8 ± 0.4 *c*	6.18 ± 0.64 *d*	6.67 ± 0.55 *d*	10.6 ± 0.9 *d*	9.95 ± 1.07 *d*	<0.0001
Δ5[3]	5.19 ± 1.35 *a*	2.05 ± 0.84 *c*	2.67 ± 0.72 *bc*	4.50 ± 0.19 *ab*	2.59 ± 0.05 *bc*	6.30 ± 0.51 *a*	5.92 ± 0.85 *a*	0.0011
Δ5[4]	9.92 ± 1.00	9.68 ± 1.20	12.3 ± 0.7	7.61 ± 1.61	8.63 ± 0.51	8.64 ± 1.04	9.66 ± 1.79	0.2087

Data are presented as means ± SEM (n = 8-10/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different. * indicates data analyzed by non-parametric statistics.

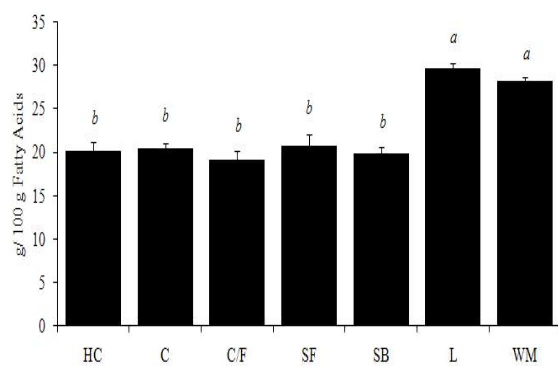
[1] MUFA/(MUFA + SFA)

[2] C18:1n9/C18:0

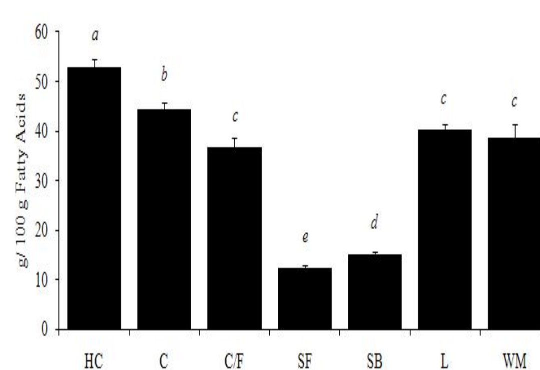
[3] C20:4n-6 /C20:3n-6

[4] C20:5n-3 +C22:6n-3

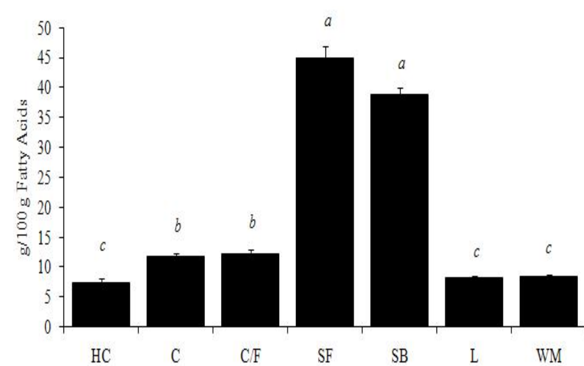
a) C16:0



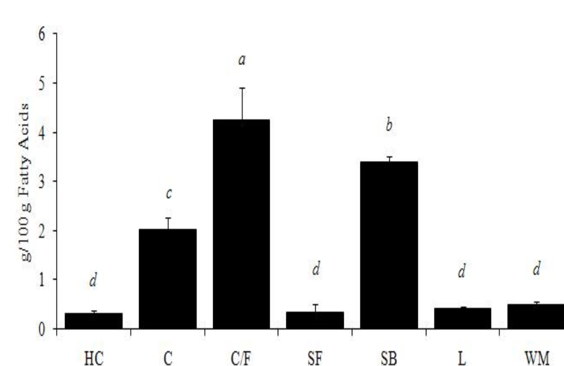
b) C18:1n-9



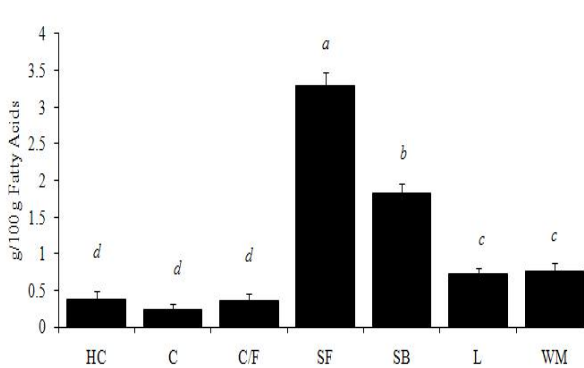
c) C18:2n-6 (LA)



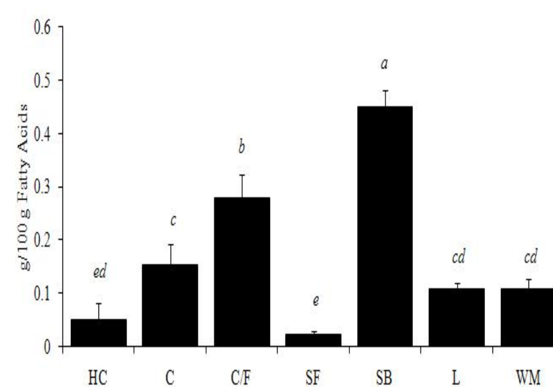
d) C18:3n-3 (ALA)



e) C20:4n-6 (AA)



f) C20:5n-3 (EPA)



g) C22:6n-3 (DHA)

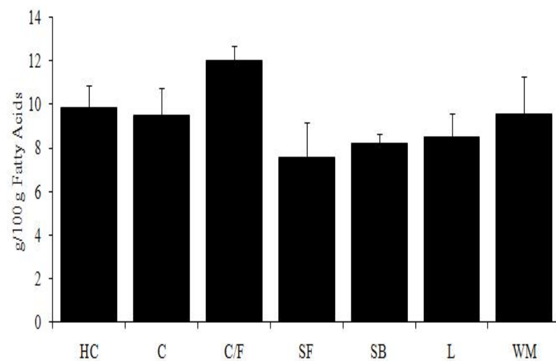


Figure 16: Hepatic TAG. C16:0 (a), C18:1n-9 (b), C18:2n-6 (c), C18:3n-3 (d), C20:4n-6 (e), C20:5n-3 (f) and C22:6n-3 (g) as g/100 g fatty acids are presented as means ± SEM (n = 5/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different. Data was analyzed by non-parametric statistics except for g) DHA.

b) PL

A summary of the hepatic PL fatty acid classes and fatty acid ratios is shown in Table 8. Total SFA was lowest in the HC group, whereas total MUFA was highest in the HC group compared tot eh other groups. No difference was seen among groups in terms of total PUFA. The HC group had the highest total n-9, the SF group had the highest total n-6 and the C/F group had the highest total n-3.

The SF group had the highest n-6/n-3 ratio and the HC group had the highest n-9/n-6 ratio. In terms of desaturase indices, the HC group had the highest Δ9 desaturase index (MUFA/[MUFA + SFA] and C18:1n-9/C18:0) and Δ6 desaturase index (C18:3n-6/C18:2n-6). The Δ5 desaturase indices were highest in the SB group (C20:4n-6/C20:3n-6) and C/F group (C20:5n-3+C22:6n-3).

Figure 17 highlights the individual fatty acids of interest in hepatic PL. For a complete fatty acid profile of hepatic PL, refer to Appendix 7. The SB group had the highest C16:0 and the L group had the highest C18:0 (Figure 17a and 17b, respectively). C18:1n-9 and C18:1n-7 was highest in the HC group (7.19% and 2.65%, respectively) and lowest in the SB (2.10% and 1.22%, respectively) and SF groups (1.86% and 1.27%, respectively; Figure 17c and 17d, respectively). The C/F, SF and SB groups had the highest C18:2n-6 (10.1% - 10.6%) and the HC group had the lowest (6.02%; Figure 17e). There was no difference among groups in terms of ALA (C18:3n-3; Figure 17f). The HC, SF, L and WM groups had the highest AA (26.7% - 28.8%; C20:4n-6) and the C, C/F and SB groups had the lowest AA in the hepatic PL (22.4% - 24.9%; Figure 17g). Additionally, the C/F group had the highest EPA (0.83%; C20:5n-3; Figure 17h). The C group had the next highest EPA (0.32%) and all other groups had lower EPA (0.01% -

0.09%). The C/F and C groups also had the highest DHA (9.36% and 8.67, respectively; C22:6n-3; Figure 17i). The SF group had the lowest DHA (2.94%) and all other groups had between 7.89% and 8.52% DHA in hepatic PL.

Table 8: Hepatic PL.

TOTALS (g/100 g fatty acids)	HC	C	C/F	SF	SB	L	WM	P-Value
Total SFA	42.2 ± 0.9 *b*	46.0 ± 1.8 *a*	45.1 ± 0.6 *a*	45.4 ± 1.1 *a*	47.8 ± 0.6 *a*	47.4 ± 0.6 *a*	45.6 ± 0.7 *a*	0.011
Total MUFA	11.7 ± 0.8 *a*	9.09 ± 0.42 *b*	7.95 ± 0.48 *b*	4.92 ± 0.33 *c*	5.49 ± 0.80 *c*	7.30 ± 0.24 *b*	7.83 ± 0.18 *b*	<0.0001
Total PUFA	45.1 ± 0.8	44.7 ± 1.6	46.8 ± 0.9	45.5 ± 1.3	46.0 ± 1.4	45.0 ± 0.8	46.1 ± 1.1	0.8709
Total n-3 *	8.54 ± 0.16 *d*	9.88 ± 0.30 *b*	11.1 ± 0.32 *a*	3.45 ± 0.26 *e*	9.28 ± 0.17 *bc*	9.07 ± 0.16 *cd*	8.99 ± 0.23 *cd*	0.0001
Total n-6	36.6 ± 0.76 *b*	34.8 ± 1.6 *b*	35.0 ± 1.1 *b*	41.5 ± 1.3 *a*	36.7 ± 1.2 *b*	35.9 ± 0.77 *b*	37.1 ± 1.3 *b*	0.008
Total n-9 *	7.43 ± 0.53 *a*	5.17 ± 0.21 *b*	4.66 ± 0.22 *b*	2.22 ± 0.21 *c*	1.98 ± 0.09 *c*	4.76 ± 0.03 *b*	5.31 ± 0.25 *b*	<0.0001

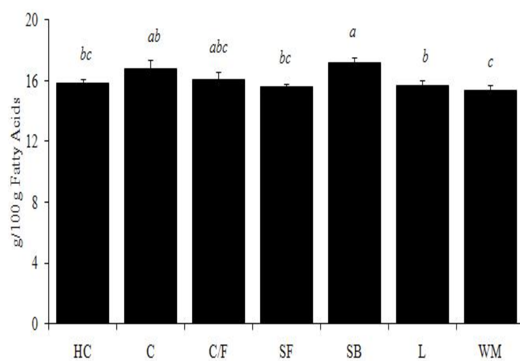
Table 8: Hepatic PL (continued).

RATIOS	HC	C	C/F	SF	SB	L	WM	P-Value
n-6/n-3 *	4.29 ± 0.10 *b*	3.55 ± 0.22 *b*	3.16 ± 0.08 *b*	12.4 ± 1.2 *a*	3.96 ± 0.08 *b*	3.97 ± 0.11 *b*	4.15 ± 0.23 *b*	0.0003
n-9/n-6 *	0.202 ± 0.014 *a*	0.148 ± 0.004 *b*	0.134 ± 0.008 *b*	0.054 ± 0.004 *c*	0.054 ± 0.002 *c*	0.132 ± 0.004 *b*	0.146 ± 0.008 *b*	<0.0001
Δ9[1] *	0.216 ± 0.014 *a*	0.164 ± 0.011 *b*	0.150 ± 0.006 *bc*	0.098 ± 0.005 *d*	0.104 ± 0.013 *d*	0.134 ± 0.002 *c*	0.146 ± 0.004 *bc*	0.0002
Δ9[2] *	0.300 ± 0.027 *a*	0.190 ± 0.013 *b*	0.170 ± 0.009 *b*	0.078 ± 0.009 *c*	0.070 ± 0.005 *c*	0.154 ± 0.004 *b*	0.184 ± 0.012 *b*	<0.0001
Δ6[3] *	0.038 ± 0.004 *a*	0.022 ± 0.004 *b*	0.018 ± 0.004 *b*	0.026 ± 0.002 *b*	0.018 ± 0.002 *b*	0.024 ± 0.002 *b*	0.022 ± 0.002 *b*	0.0144
Δ5[4] *	41.1 ± 8.9 *b*	18.1 ± 1.6 *b*	13.1 ± 1.0 *b*	45.9 ± 1.8 *b*	202.9 ± 109.7 *a*	53.4 ± 29.4 *b*	20.9 ± 2.0 *b*	0.0001
Δ5[5] *	7.93 ± 0.20 *d*	8.99 ± 0.23 *b*	10.2 ± 0.3 *a*	2.94 ± 0.12 *e*	8.61 ± 0.08 *bc*	8.22 ± 0.24 *cd*	8.08 ± 0.20 *cd*	0.0001
Δ5[6] *	4.31 ± 4.30	5.27 ± 3.23	1.93 ± 1.92	0.010 ± 0.001	5.54 ± 3.39	6.42 ± 3.93	75.1 ± 39.6	0.0948

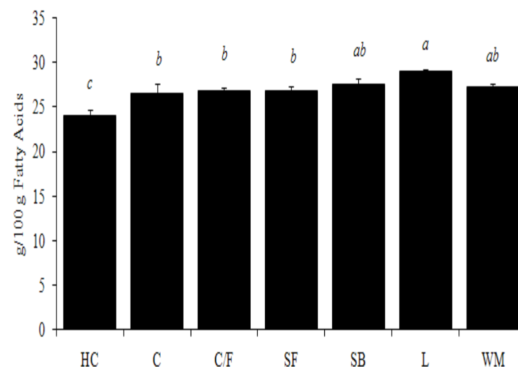
Data are presented as means ± SEM (n = 8-10/group). Statistical differences among means (p<0.05) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different. * indicates log non-parametric data were analyzed.

[1] MUFA/(MUFA + SFA), [2] C18:1n-9/C18:0, [3] C18:3n-6 /C18:2n-6,
[4] C20:4n-6 /C20:3n-6, [5] C20:5n-3+C22:6n-3, [6] C22:6n-3 /C22:5n-3

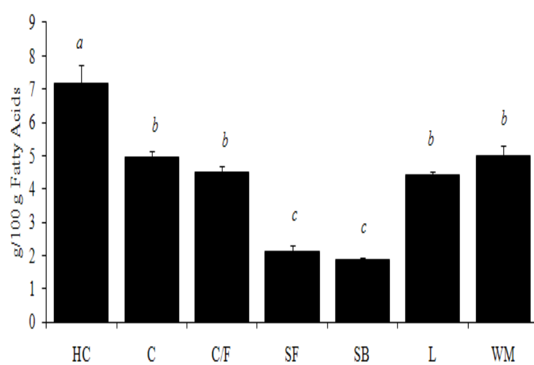
a) C16:0



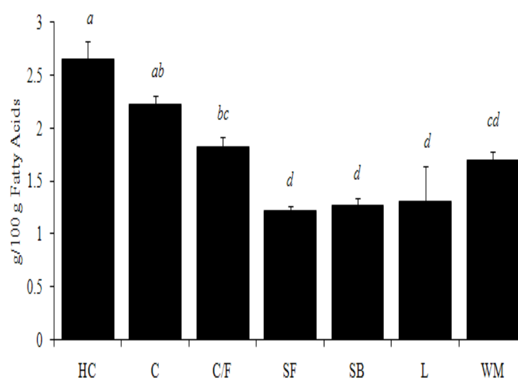
b) C18:0



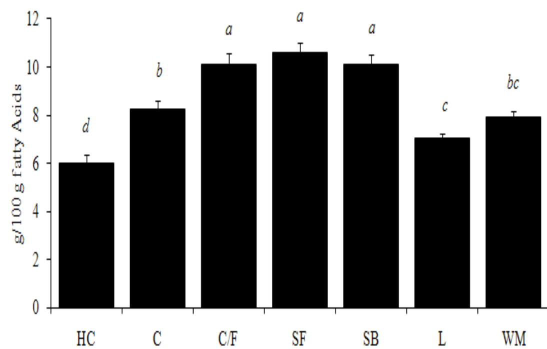
c) C18:1n-9



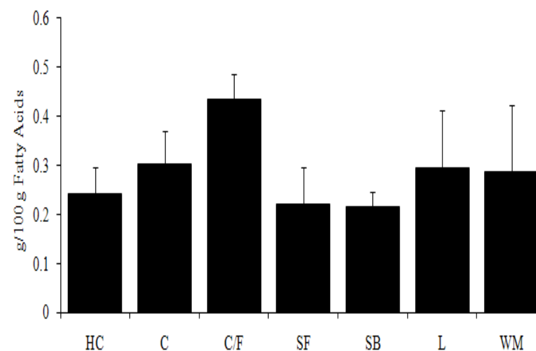
d) C18:1n-7



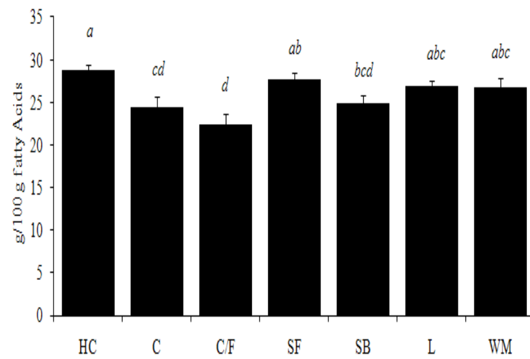
e) C18:2n-6 (LA)



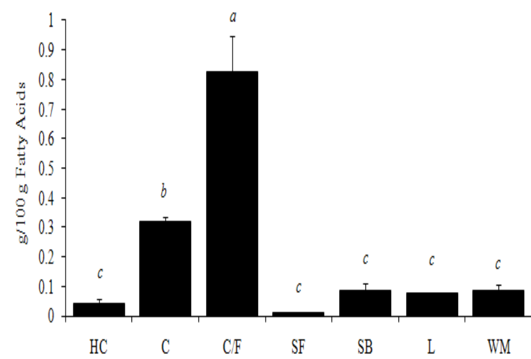
f) C18:3n-3 (ALA)



g) C20:4n-6 (AA)



h) C20:5n-3 (EPA)



i) C22:6n-3 (DHA)

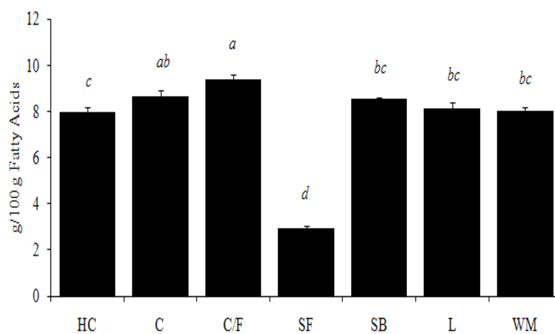


Figure 17: Hepatic PL. C16:0 (a), C18:0 (b), C18:1n-9 (c), C18:1n-7 (d), C18:2n-6 (e), C18:3n-3 (f), C20:4n-6 (g), C20:5n-3 (h) and C22:6n-3 (i) as g/100 g fatty acids are presented as means ± SEM (n = 5/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different. Data were analyzed by non-parametric statistics except for g) DHA.

Molecular Markers of Hepatic Fatty Acid Oxidation and Synthesis

No difference was seen among groups in terms of molecular markers for fatty acid oxidation, and in particular protein levels of PPARα (both s12 and s21) and ACO (Table 9 and Figure 18). Similarly, no differences were seen among groups in terms of markers for fatty acid synthesis and in particular, protein levels of SREBP-1c (68 and 125 kDa), calculated activation state of SREBP-1c (68 kDa/[68 kDa + 125 kDa]), which indicates how much active SREBP is in the nucleus, and ACC (Table 9).

Table 9: Molecular Markers of Hepatic Fatty Acid Oxidation and Synthesis.

	HO	C	C/F	SF	SB	L	WM	P- value
PPARα (s12)	1.09 ± 0.19	1.31 ± 0.11	1.24 ± 0.06	1.27 ± 0.28	1.28 ± 0.34	0.809 ± 0.189	1.59 ± 0.31	0.4745
PPARα (s21)	1.65 ± 0.60	3.22 ± 1.96	3.37 ± 2.22	2.25 ± 1.25	1.71 ± 0.26	2.02 ± 0.57	2.66 ± 1.58	0.9477
ACOX1 (h140)	0.534 ± 0.177	0.642 ± 0.177	0.957 ± 0.328	1.32 ± 0.34	1.06 ± 0.28	0.657 ± 0.123	0.852 ± 0.390	0.3583
SREBP-1(c20) 125 kDa	0.760 ± 0.256	0.949 ± 0.166	1.56 ± 0.59	1.59 ± 0.41	1.57 ± 0.84	1.39 ± 0.44	0.723 ± 0.157	0.7577
SREBP-1(c20) 68 kDa	0.393 ± 0.095	0.484 ± 0.131	0.594 ± 0.226	0.881 ± 0.258	0.676 ± 0.221	0.685 ± 0.154	0.441 ± 0.182	0.6075
SREBP1(c20) Activation[1]	0.367 ± 0.105	0.332 ± 0.073	0.295 ± 0.086	0.367 ± 0.101	0.361 ± 0.115	0.355 ± 0.100	0.376 ± 0.148	0.9979
ACC-1	0.517 ± 0.067	0.512 ± 0.232	0.311 ± 0.106	0.427 ± 0.087	0.629 ± 0.111	1.04 ± 0.34	0.625 ± 0.328	0.2347

[1] SREBP-1 (68 kDa)/SREBP-1 (68 kDa) + SREBP-1 (125 kDa)

Data are presented as means (arbitrary units) ± SEM (n = 2-4/group). An absence of letters indicates that means are not statistically different.

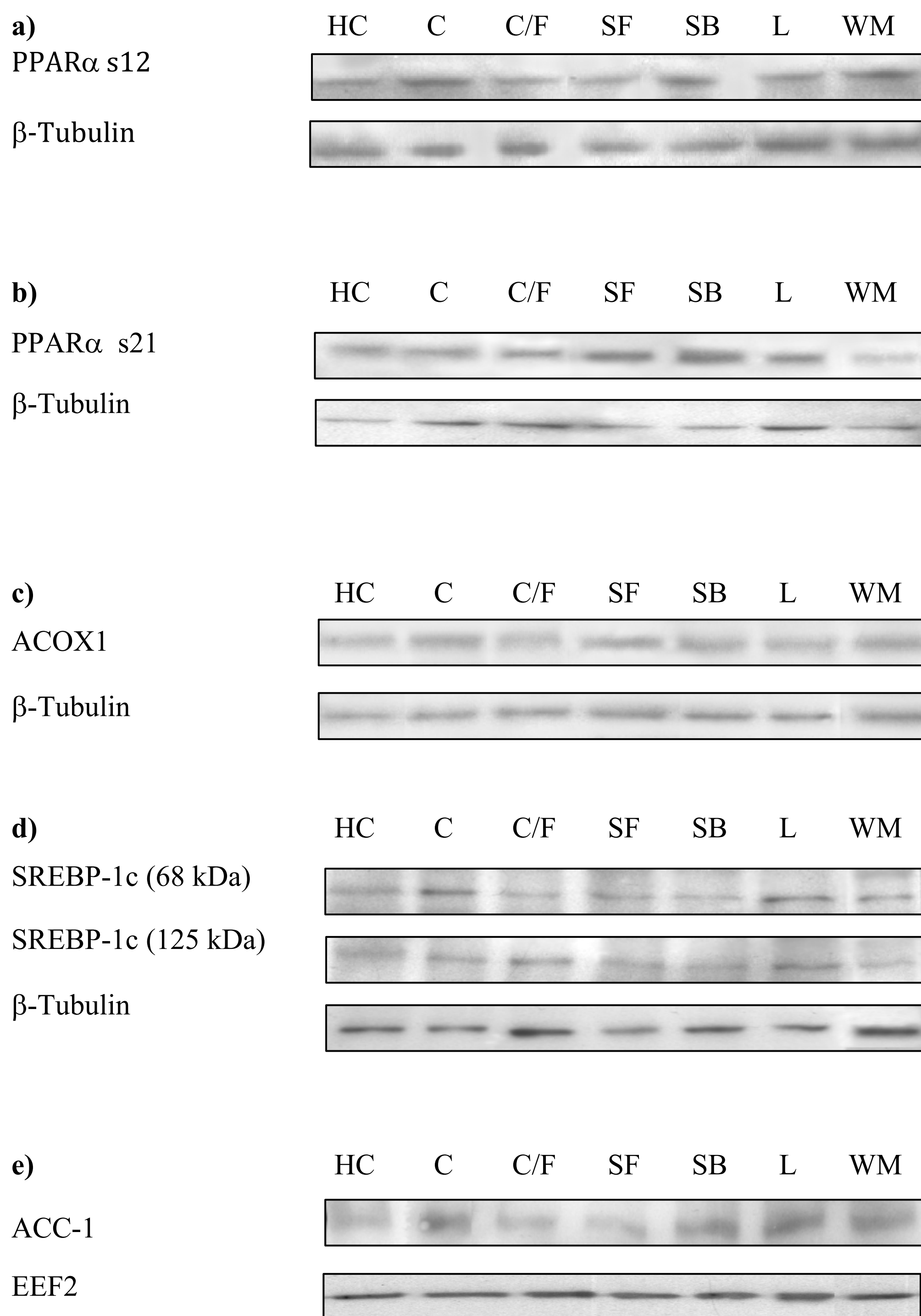


Figure 18: Representative Blots. Relative levels of PPARα s12 (a), PPARα s21 (b), ACOX-1 (c), SREBP-1c (d) and ACC-1 (e) were determined by Western blotting of liver tissue samples.

DISCUSSION

Summary of Major Findings

A summary of how each of the high fat diets affected the metabolic syndrome parameters is presented in Table 10. In general, the canola diets (HC, C and C/F) seemed to attenuate obesity the most based on body weight and weight gain, but not on fat pad mass. The SF diet improved insulin resistance the most, with all of the canola groups following closely behind. The SF and SB diets seemed to reduce lipidemia the most, whereas the C/F and L diets had the best effect on reducing inflammation. No effect of diet was seen on hypertension. Lastly, in terms of hepatic steatosis, the C/F diet and the feed restriction group (WM) had the most positive effect for attenuating liver lipid accumulation. These findings will be discussed in more detail in the following sections.

Fatty Acid Analysis of Diet

In general, the analyzed fatty acid profile of the diet (Table 3) was similar to what was expected based on the Comparison of Dietary Fats fact sheet (Canola Council of Canada, 2008; Table 1). However, likely due to company, batch, storage and purity, a few discrepancies existed between what was predicted and what the fatty acid profile of the diet was when it was analyzed. The LA/ALA ratio of the safflower oil had the biggest discrepancy. It must be noted that high LA safflower oil was used in the study and it was expected that the fatty acid composition of the high LA safflower oil would be similar to sunflower oil. Therefore, the analyzed fatty acid composition of the SF diet was compared to the estimated fatty acid composition of sunflower oil, instead of safflower oil, shown on the Canola Council fact sheet. This may account for some of the

Table 10: Summary of Effects of Diets on Metabolic Syndrome Parameters.

Best	Intermediate	Worst	No Change

	HC	C	C/F	SF	SB	L	WM
Obesity:							
Weight Gain	Best	Best	Best	Intermediate	Worst	Worst	Intermediate
Final BW	Best	Best	Best	Intermediate	Worst	Worst	Intermediate
Mesenteric Fat % BW	Best	Best	Best	Best	Worst	Best	Best
Epididymal Fat % BW							
Peri-renal Fat % BW							
Visceral Fat % BW							
Insulin Resistance:							
Termination Glucose	Best	Best	Best	Best	Intermediate	Worst	Intermediate
Termination Insulin							
Pancreas % BW							
HOMA	Best	Intermediate	Intermediate	Best	Intermediate	Worst	Intermediate
Lipidemia:							
TAG (week 4)	Worst	Intermediate	Intermediate	Best	Best	Worst	Worst
(week 8)	Intermediate	Intermediate	Intermediate	Best	Best	Intermediate	Worst
(week 12)	Worst	Intermediate	Best	Best	Best	Worst	Worst
Cholesterol	Worst	Intermediate	Intermediate	Intermediate	Worst	Intermediate	Best
FFA							
Hypertension:							
Systolic Blood Pressure							
Diastolic Blood Pressure							
Inflammation:							
Haptoglobin (week 4)	Intermediate	Worst	Best	Best	Best	Best	Intermediate
(week 8)	Worst	Intermediate	Best	Best	Best	Best	Best
(week 12)	Worst	Intermediate	Best	Intermediate	Intermediate	Best	Best
Adiponectin							
Hepatic Steatosis							
Liver % Body Wt	Worst	Best	Best	Best	Best	Best	Best
% hepatic fat	Worst	Worst	Best	Worst	Worst	Worst	Best

differences between the analyzed fatty acid composition of the high LA safflower oil diet and the estimated fatty acid composition on the Canola Council fact sheet.

Obesity

In the present study, rats in all dietary treatment groups did not differ in terms of total feed intake (Figure 5a). However, the HC, C and C/F groups all gained the least amount of weight during the study and had the lowest final body weight (Figure 5b and c, respectively), suggesting that the diet was able to attenuate weight gain in these groups. In terms of weekly feed intake, there was no difference among groups from baseline to week 10 (Figure 3). However, at week 11 and 12, the WM group had their feed intake restricted in order to match their body weight to the C/F group, which was the group that was gaining the least amount of weight during the study. Thus, the WM animals ate significantly less feed than all groups except the L group which ate intermediate amounts of feed. The C/F group also had the lowest feed efficiency ratio (Figure 5d). Similarly, Buettner et al. (2006) found that DIO rats that ate high fat diets high in n-3 (as fish oil) had lower final body weight than all groups, including the standard chow fed rats, indicating that it was the high n-3 diet that led to less weight gain. However, the n-3 group (fish oil) also ate less feed than all other high fat fed rats and similar amounts of feed as the standard chow fed rats, whereas in the present study all groups at similar amounts of feed.

Another way to determine obesity is measuring fat pads as a percent body weight. In the present study, it was only the SB group that had elevated mesenteric fat as a

percent body weight (Figure 6a). Epididymal, peri-renal and visceral fat as a percent body weight were similar among groups (Figure 6b, c and d, respectively). There are a very limited number of studies that have looked at the effects of high fat diets on fat pad mass in DIO rats. One study by Takahashi et al. (2000) found that Sprague Dawley rats that consumed high fat diets based on perilla oil (containing ALA) or fish oil had white adipose tissue mass comparable to rats fed a low fat diet. All groups gained similar amounts of weight during the study, but fat pad as a percent body weight was not determined. Additionally, both the perilla and fish oil groups had significantly lower epididymal and peri-renal fat weights compared to the safflower oil group. Other studies that have looked at the effects of n-3 PUFAs on obesity have mainly used the marine n-3 PUFAs, EPA and DHA. Results from these studies indicate that a high fat fish oil diet can decrease weight gain and/or adiposity in DIO rat models (Pérez-Echarri et al., 2008; Lombardo et al., 2007; Buettner et al., 2006; Baillie et al., 1999).

Dietary fats and oils differ in their ability to attenuate obesity and the mechanism by which they work is not fully understood. Several possible explanations have been suggested. First, there are studies that suggest that high MUFA diets decrease weight and fat mass through increased diet-induced thermogenesis (reviewed by Moussavi et al., 2008). Next, it has been suggested that n-3 PUFAs reduce lipid accumulation via suppressing production of VLDL (Kinsella et al., 1990). The TAG in VLDL is an energy source for peripheral tissue but it can also lead to increased lipid storage in adipocytes. Another possible mechanism is explained by the oxidation of dietary fats. Rats tend to oxidize oleic acid the most followed by ALA, LA, palmitic acid and stearic acid (reviewed by Bell et al., 1997). Thus, it is suggested that rats fed diets high in oleic acid and ALA, as seen in the HC, C and C/F groups, oxidize more dietary fatty acids, whereas

rats fed diets high in SFA, as seen in the L group, store more fatty acids. Additionally, it has been demonstrated in rats that a lard diet, which is high in SFA, decreases whole-body oxygen consumption compared to an isocaloric diet high in MUFAs (Bell et al., 1997). Dietary fats are incorporated into cell membranes; thus, they are able to alter cell membrane function and metabolic rate. It is suggested that metabolic rate is decreased when SFA are incorporated into cell membranes (reviewed by Bell et al., 1997). It is relatively well accepted that diets high in n-3 PUFAs attenuate weight gain in rats, and it is suggested that the amount of n-3 PUFAs incorporated into the tissue is directly related to weight gain (Buettner, et al., 2006; reviewed by Bell et al., 1997). However, when high n-6 PUFAs are present in the diet, there is competition with n-3 PUFAs resulting in n-3 PUFA oxidation and ultimately less n-3 PUFAs incorporated into tissues. For example, in the present study, the SB and L groups had similar amounts of EPA and DHA in their hepatic PL regardless of the fact that the SB oil diet contained 9 times more ALA than the L diet. The SB diet in the present study contained moderate amounts of ALA, but also very high amounts LA. This may explain why the SB group gained as much weight as the L fed rats and had the highest amount of mesenteric fat as a percent body weight.

Insulin Resistance

This study showed that after a 12 week dietary intervention with different types of fats and oils that the HC, C, C/F and SF groups had the lowest fasting serum glucose at termination (Figure 7) and that the HC and SF groups had the lowest HOMA score (Figure 10), an indirect measure of the action of insulin on glucose disposal. There were no differences between groups in terms of fasting serum insulin (Figure 8), ITT and

OGTT results (Table 4), AUC for glucose and insulin (Table 4), and pancreas as a percent body weight (Figure 9). Thus, the diets that seemed to have the best effect on serum glucose, HOMA and pancreas weight were that HC and SF, followed by the C and C/F.

The HC group is high in MUFA and the other canola groups are high in both MUFA and ALA. Literature on human subjects suggests that diets high in MUFAs can decrease fasting glucose and/or insulin resistance (Sroiguer et al., 2006; Lauheranta et al., 2002; Garg, 1998). However, studies that have been done on rats show both positive (Mustad et al., 2006) and negative effects (Buettner et al., 2006) of MUFAs on glucose and insulin resistance. One mechanism by which MUFAs may improve insulin resistance is through the stimulation of glucagon-like peptide-1 (GLP-1; Rocca 2001). GLP-1 is a hormone that is secreted by the intestinal tract in the presence of carbohydrates, and acts to increase insulin secretion (reviewed by Rocca et al., 2001).

Studies that have investigated the effects of ALA or other n-3 PUFAs, with or without a high MUFA diet, generally show an improvement in insulin sensitivity in rodent models (Buettner et al, 2006; Takahashi et al., 2000). Interestingly, in Wistar rats, Storlien et al. (1991) documented that a high fat safflower oil diet induced insulin resistance and that insulin sensitivity could not be improved when ALA was added to the safflower oil diet, likely due to competition with n-6 PUFAs (namely, LA) that were present in the high fat safflower oil diet. However, when ALA was substituted in a SFA diet containing lard and safflower oil, insulin resistance was completely prevented compared to the SFA diet alone. Thus, it would be expected that a high ALA diet in combination with a high MUFA diet, as seen in the C and C/F diets, would improve insulin resistance, however, no difference was seen among groups for the ITT and OGTT in the present study.

It seems as though diets high in MUFA and ALA improve insulin resistance by increasing the proportion of MUFA and ALA in phospholipids. Increased oleic acid and ALA in phospholipids is associated with a decrease in fasting plasma glucose (Louheranta et al., 2002). It is suggested that MUFA and ALA can positively alter the structure and functioning of the cell membrane phospholipids, which in turn can positively affect the insulin cascade and allow for a more efficient use of insulin and transport of glucose (Mustad et al., 2006; Louheranta et al., 2002). Additionally, one study indicates that n-3 PUFAs can increase GLUT-4 gene expression in white adipose tissue and, thus, improve glucose metabolism (Takahashi et al., 2000).

The SF diet used in the present study was very high in n-6 PUFAs and the literature indicates that n-6 PUFAs have a protective role against insulin resistance and that n-6 PUFA tissue content is negatively correlated with HOMA index (Hernández et al., 2008). However, studies in DIO rats that have used high fat diets based on safflower oil show a deleterious effect of safflower oil on insulin resistance (Takahashi et al, 2000; Storlien et al., 1991; Storlien et al., 1987). It must be noted that the SF diet used in the present study was high LA safflower oil, which is very high in PUFA and has very little ALA. The literature are not always to clear as to the type of safflower oil that is being used. Thus, it is hard to compare and contrast the effects of the high LA SF diet used in the present study to the effects of the safflower used in other studies.

Increased pancreas size can be a result of pancreatic β-cell hyperplasia, which may occur due to increased insulin secretion if insulin sensitivity is impaired (Bonner-Weir, 2000). However, in the present study no difference was seen among groups in terms of pancreas as a percent body weight (Figure 9) and all groups had similar results in the ITT

and OGTT (Table 4).

Although OGTT and ITT can be used as a measure of insulin resistance, the euglycemic-hyperinsulinemic clamp is the gold standard (González et al., 2008). The OGTT and ITT have a few key limitations. First, insulin sensitivity changes in pathological situations and in normal physiology (González et al., 2008). Also, changes in insulin, after an oral glucose load, are not entirely dependent on glucose but also on factors such as gut hormones and neural stimulation, which can confound the results (González et al., 2008). The limitations of the euglycemic-hyperinsulinemic clamp technique are that it is expensive, labor intensive and complex to perform (González et al., 2008). Thus, although ITT and OGTT results tend to correlate well with the clamp method, the euglycemic-hyperinsulinemic clamp technique is still the gold standard and the outcome of the insulin sensitivity results in the present study may have been different if this method was employed (González et al., 2008).

Lipidemia

The SF and SB diets tended to attenuate serum TAG the best, followed by the C/F and then the C diet (Figure 11). There was a trend to lower fasting serum cholesterol in the WM group, with the HC and SB groups having the highest fasting serum cholesterol ($P = 0.0612$; Table 5). No differences were seen among groups in terms of fasting serum FFA (Table 5). Both the C/F and SB diets are high in n-3 PUFAs and research indicates that n-3 PUFAs decrease plasma TAG by about 25% in normolipidemic subjects and about 50% in hypertriglyceridemic subject, but n-3 PUFAs have a very negligible effect on cholesterol (Zuliani et al., 2009). The effects of n-3 PUFAs on cholesterol have been

documented to slightly increase HDL, and slightly decrease LDL and total cholesterol (Zuliani et al., 2009). In the present study only total cholesterol was measured and not lipoproteins; this may account for the fact that total cholesterol did not significantly differ among dietary treatment groups.

The results from the present study showing lower serum TAG in the SB, C/F and C diets, which contain 9%, 20% and 8% ALA, respectively, is consistent with other studies in which DIO rats are fed high n-3 PUFA diets (Buettner et al., 2006; Takahashi et al., 2000). McKenney et al. (2007) demonstrated that the direct effect of n-3 PUFAs on lipidemia seems to be due to the marine n-3 PUFAs EPA and DHA. Research on the hypolipidemic effect of ALA is limited, and results are inconsistent. However, it is likely that ALA indirectly improves hyperlipidemia because it is a precursor for EPA and DHA synthesis. For example, in the present study the SB oil group had the highest EPA in hepatic TAG followed by the C/F group and then the C group, suggesting that conversion of ALA to EPA in these groups may be involved in lowering serum TAG (Figure 16f). Many other mechanisms for explaining how n-3 PUFAs improve hyperlipidemia have been suggested (McKenney et al., 2007). First, n-3 PUFAs have been shown to inhibit enzymes involved in TAG biosynthesis. Second, n-3 PUFAs seem to decrease the rate of hepatic VLDL production in rodents, which has been linked to a faster rate of hepatic fatty acid oxidation. Third, n-3 PUFAs may enhance TAG clearance by increasing LPL activity. And fourth, n-3 PUFAs may improve lipidemia by limiting the production of n-6 eicosanoids and enhancing the production of n-3 eicosanoids, which are hypolipidemic.

The SF diet used in the present study contains high linoleic safflower oil, and therefore is very high in n-6 PUFAs. Studies indicate that plasma n-6 PUFAs are

negatively associated with fasting serum and plasma TAG in human subjects (Motoyama et al., 2009; Hernández-Morante et al., 2008). It is expected that hepatic fatty acids reflect plasma fatty acids and in the present study the SF group had among the highest C18:2n-6 in hepatic PL and TAG (Figure 16c and 17e). It is suggested that n-6 PUFAs have TAG lowering effects via the activation of PPAR and subsequent activation of genes involved in fatty acid oxidation (Hernández-Morante et al., 2008).

Interestingly, all of the treatment groups had a substantial increase (2.4-7.2 fold; Figure 11) in fasting serum TAG at week 4 of the study, followed by a slow reduction in fasting serum TAG from week 4 to termination. This pattern suggests that as the animal ages its serum TAG declines, or that the animal adapts to the high fat diet over time. The latter is more likely as results from week 4 indicate that the SF and SB groups did not increase serum TAG as much as other groups, suggesting that these diets tended to have a rapid serum TAG lowering effect. Another factor that may explain why TAG concentrations decreased throughout the study may be the physiological changes in the liver leading to increased accumulation of TAG in the liver. The SF and SB groups exhibited low serum TAG throughout the study but had increased amounts of hepatic lipid at termination (Figure 15). However, the C/F group had equally low serum TAG at termination, but had the least amount of hepatic lipid, suggesting that the C/F diet my lower serum TAG by one of the mechanisms mentioned above and not by redirecting TAG to the liver.

Termination fasting serum cholesterol showed a trend among groups ($p = 0.0612$) with the WM group having the lowest serum cholesterol and the HC and SB groups having the highest serum cholesterol at termination. Therefore, the serum from 1-2

animals from each group was tested for cholesterol concentrations at all time points (baseline, week 4, and week 8) to see if the cholesterol results followed the same pattern as the TAG results. No changes were detected with this analysis (Appendix 8).

Hypertension

No difference was seen among groups in terms of blood pressure (Table 6). However, the rats in the present study had elevated blood pressure as compared to normal values for a rat, 129 mmHg for systolic blood pressure and 91 mmHg for diastolic blood pressure (Kent Scientific Corporation, 2008). These results suggest that there may have been no effect of diet on blood pressure, however, it is generally well accepted that dietary fats affect blood pressure differently. Diets high in SFA tend to increase blood pressure, whereas diets high in MUFA and PUFA tend to decrease blood pressure (Pietinen, 1994; Iacono et al., 1990). Thus, it is possible that no effect was seen in terms of blood pressure in this study because all of the diets used were high in MUFA and/or PUFA (even the L diet had considerable amounts of MUFA). Additionally, based on the limited literature, it has been suggested that n-3 PUFA, from fish and canola oil attenuate blood pressure (Aguila et al., 2004; Aguila, 2001). The mechanism by which n-3 PUFAs are suggested to attenuate blood pressure is by enhancing endothelium-dependent relaxation (Aguila et al., 2004). N-3 PUFAs increase endothelium relaxation because eicosanoids produced by n-3 PUFAs are considered to be vasodialatory and because n-3 PUFAs decrease endogenous AA levels, which in turn suppress the release of vasoconstrictor prostaglandins from the endothelium.

Inflammation

An interesting pattern was seen in terms of fasting serum haptoglobin in this study. The HC and C groups generally had the highest haptoglobin throughout the study, whereas the C/F and L groups consistently had the lowest haptoglobin at all weeks of the study (Figure 12). A mechanism by which n-3 PUFAs may attenuate inflammation is via the production of eicosanoids, which are anti-inflammatory (reviewed by Simopoulos, 2002). Increased consumption of dietary ALA increases the production of n-3 eicosanoids and decreases the production of n-6 eicosanoids produced from LA, which are pro-inflammatory, and vice versa. In the present study, the C/F diet, which contains the highest amount of ALA, led to the most EPA and DHA and the least AA in hepatic PL (Figure 17g, h, and i). The C/F group also had the highest total n-3 PUFAs in hepatic TAG and PL (Table 7 and 8). Thus, it makes sense that the C/F group, which is high in n-3 PUFAs and has little competition with n-6 PUFAs would lower serum haptoglobin as it is well accepted that n-3 PUFAs are independently correlated with lower levels of pro-inflammatory markers (Ferrucci et al., 2006). N-3 PUFAs have also been shown to have anti-inflammatory effects in both healthy populations and animal models of chronic inflammatory conditions, including DIO rodent models (Pérez-Echarri et al., 2008; Faintuch et al., 2007; Lombardo et al., 2007; Takahashi et al., 2000). However, little literature exists on the ability of MUFA to decrease inflammation, suggesting that it was likely the ALA in the C/F diet that improved serum haptoglobin, as the C and HC groups had higher MUFA and less ALA in the diet than the C/F group and failed to improve inflammation. Additionally, the C/F group had the lowest total liver lipids (Figure 15). Haptoglobin is predominately produced by the liver and these data may indicate that the

C/F group had less liver damage and/or inflammation. Interestingly, the HC group had the highest liver weight, liver as a percent body weight (Figure 14), and had among the highest amounts of serum haptoglobin at all weeks of the study, suggesting that haptoglobin concentrations and liver physiology may be related.

The reason that the L diet, which is high in SFA and MUFA, was able to attenuate haptoglobin in the present study is a little more difficult to explain. Additionally, very few studies on DIO rats have explored the effects of high fat diets on markers of inflammation. One study by Buettner et al. (2006) indicated that serum adiponectin was significantly lower in lard and olive oil fed rats compared to rats fed coconut, fish oil or standard chow. However, in this same study, liver ALT, a marker of hepatic damage and/or inflammation was significantly reduced in the lard and coconut groups compared to the olive oil, fish oil and standard chow fed rats. Additionally, Buettner et al. (2006) documented that histological examination of the liver did not reveal signs of inflammation in the lard fed rats. On the other hand, lard fed rats did develop hepatic steatosis. A potential mechanism by which a diet based on lard can attenuate markers of inflammation has not been reported.

No difference was seen among groups in terms of serum adiponectin concentrations at termination (Figure 13). This result conflicts with other studies, which suggest that a diet high in n-3 PUFAs (from fish oil) increases adiponectin concentrations in DIO rats (Lombardo et al., 2007; Buettner et al., 2006).

Hepatic Steatosis

The present study showed that liver as a percent of body weight was highest in the HC group compared to all of the other dietary treatment groups (Figure 14). However, it

was only the C/F and WM groups that had the lowest amount of hepatic fat (Figure 15). Although it would be easy to explain that the lower liver lipid was due to the lower body weight gain in these 2 groups, it is important to point out the HC and C groups also gained the same amount of weight during the study as the C/F and WM groups. Thus, there must be a biologically active component in the C/F diet (beyond what is found in the HC and C diets) that was able to attenuate hepatic lipid accumulation. Additionally, because the L and WM groups ate the same lard diet, the less hepatic fat accumulation in the WM group is likely due to their lower body weight. It is well known that a reduction in body weight can quickly reduce the amount of fat stored in the liver (reviewed by Angulo, 2002). In the present study we induced rapid weight loss in the WM group by restricting feed intake during week 11 and 12 and, thus, likely caused the reduction in liver lipids in the WM group.

The C/F diet was different from the HC and C diets in that it had less total MUFA, more ALA and a lower n-6/n-3 ratio. Another study in DIO rats demonstrated that a diet high in n-3 PUFAs (from fish oil) leads to increased liver size, but similar amounts of liver TAG as control rats fed a low fat diet (Buettner et al., 2006). Additionally, Buettner et al. (2006) found that rats fed diets high in lard and olive oil (high MUFA) had significantly elevated liver TAG compared to rats fed the low fat control diet; similarly, the present study showed that diets high in MUFA (the HC, C and L groups) had significantly elevated total liver lipids.

Although we do not yet have any histological data on the progression of NAFLD in this study, we were able to demonstrate that haptoglobin, which is a marker of chronic inflammation and which is secreted predominately by the liver, was lower in the C/F group throughout the study. The attenuated haptoglobin and hepatic lipid accumulation

in the C/F group suggests that the C/F group would be less likely to have NASH and cirrhosis compared to other groups.

It is important to point out that serum TAG did not reflect the level of hepatic steatosis in the present study. At termination, the C/F, SF and SB groups had the lowest fasting TAG, however, hepatic steatosis was only attenuated in the C/F group, whereas the SF and SB groups had elevated hepatic lipid accumulation. Thus, this data suggests that the liver may not be properly packaging and exporting excess lipid into the circulation and other tissues. In a study by Buettner et al. (2006), it was found that DIO rats fed high fat n-3 PUFA diet (from fish oil) and rats fed a low fat standard chow diet had the lowest hepatic TAG and the lowest plasma TAG. Interestingly, the DIO rats fed high fat lard and olive oil diets had significantly higher liver TAG than the fish and standard chow fed rats, but similar amounts of plasma TAG. It was only the DIO rats that were fed coconut oil that had significantly elevated TAG in liver and plasma. These data suggest that dietary fats and oils differ in their ability to attenuate hepatic lipid accumulation and serum TAG concentrations, and that hepatic lipid and serum TAG concentrations are not as strongly related as once believed (Buettner et al., 2006).

Additionally, the liver is the major organ of endogenous glucose production; therefore, in addition to hepatic fat accumulation, hepatic steatosis is commonly associated with hepatic insulin resistance due to an impairment of insulin's ability to lower liver glucose output (Buettner et al., 2007). However, this pattern was not consistently seen in the present study, as the C/F and WM groups had the lowest hepatic lipid accumulation, but the HC, C, C/F and SF groups had the lowest serum glucose at termination. Additionally, no difference was seen among groups on the ITT and OGTT, which are considered reliable methods to assess insulin resistance.

Hepatic Fatty Acid Composition

The study of hepatic steatosis and hepatic fatty acid composition, as an important part of the metabolic syndrome, is relatively new; therefore, literature on the effects of various fats and oils on hepatic steatosis and hepatic fatty acid composition, especially using a DIO model, is scarce. Studies that have investigated the hepatic lipid composition of patients with NAFLD reveal a depletion of n-3 and n-6 PUFAs in hepatic TAG, decreased n-3 PUFAs in hepatic PL and an increase in the n-6 to n-3 ratio in hepatic tissue (Araya et al., 2004). Thus, it has been suggested that n-3 PUFAs may play an important role in the treatment or prevention of hepatic steatosis.

The results of the fatty acid analysis provided information that was used to calculate the desaturase index, which is often used as an indicator of the relative activity of the desaturase enzymes. There are, however, a number of limitations to using a calculation to determine desaturase index. In particular, the diet may contain large amounts of a fatty acid that is a product of one of the desaturases, while at the same time containing very little of the fatty acid that serves as the substrate. As a result, the fatty acid analysis will show that there are high amounts of product fatty acid and low amounts of substrate fatty acid in the liver. Consequently, when these values are used to calculate the desaturase index, it will appear as if the diet had caused an increase in the desaturase index. The limitation of this calculation should thus be recognized when viewing the results.

a) TAG

As expected, in general, the hepatic fatty acid composition of hepatic TAG mimicked the diet fatty acid composition. For example, the L and WM groups had the

highest total SFA, in particular C16:0 (Table 7 and Figure 16a). The HC group had the highest n-9/n-6 ratio, Δ9 desaturase index (MUFA/[MUFA + SFA] and C18:1n-9/C18:0), total MUFA, total n-9 and C18:1n-9 concentrations (Table 7 and Figure 16b). The SF group had the highest n-6/n-3 ratio, total PUFA, total n-6 and AA concentrations (Table 7 and Figure 16e). Both the SF and SB groups had the highest LA concentrations (Figure 16c). The C/F group had the highest total n-3 and ALA concentrations (Figure 16d). Interestingly, the SB group had the highest EPA concentrations, and no differences were seen among groups in terms of DHA concentrations (Figure 16f and g, respectively). The HC, L and WM groups had the highest Δ5 desaturase index (C20:4n-6/C20:3n-6; Table 7).

What was most surprising in terms of hepatic TAG in the present study was that, although the C/F diet had the highest ALA, the SB group had the highest hepatic EPA concentrations and there were no differences in hepatic DHA concentrations. In our lab we have shown that a diet high in ALA (flax oil plus soybean oil) leads to significantly higher total n-3 PUFAs, in particular, EPA and DPA in hepatic TAG in *fa/fa* Zucker rats compared to *fa/fa* controls (Durston et al., 2008). In the present study, the C/F diet, which was highest in ALA, increased total n-3 PUFAs in the liver, and in particular ALA (Figure 16d), however, no increase was seen in EPA (Figure 16f) or DPA in hepatic TAG. It was the SB diet that elevated EPA concentrations the most (Figure 16f). It is surprising that the SB diet would lead to higher EPA in hepatic TAG than the C/F group because the SB diet has a much higher analyzed n-6 to n-3 ratio compared to the C/F diet (7 vs 1). Thus, it would be expected that the SB group would have increased competition for elongation and desaturation enzymes resulting in less production of EPA. However,

the C/F diet had the second highest EPA, containing 62% as much EPA as the SB group (Figure 16f). Interestingly, regarding all of the canola oil diets, there was a relatively direct relationship between the amount of ALA present in the diet and the amount of ALA and EPA present in hepatic TAG (Figure 16d and f). Additionally, all of the canola oil groups had among the lowest amounts of LA and AA in hepatic TAG (Figure 16c and e). In the present study, DPA was not consistently present in detectable amounts and no differences were seen in DHA concentrations in hepatic TAG, whereas it would be expected that a high ALA diet would increase the concentrations of both EPA and DHA.

Also surprising, was that the HC, L, and WM groups had the highest Δ5 desaturase index. Delta 5 desaturase index was elevated in the HC, L and WM groups, all of which are low in dietary PUFA. It has been suggested that dietary PUFA may suppress Δ5, Δ6 and Δ9 desaturase activity (Nakamura et al., 2004; Sekiya et al., 2003; Table 7). Delta 5 desaturase catalyzes the conversion of dihomo-γ-linolenic acid to AA in the n-6 series, and eicosatetraenoic acid to EPA in the n-3 series. The HC, L, WM and SF group had the lowest EPA in hepatic TAG (Figure 16f). However, the SF group had the highest AA (Figure 16e). Therefore, Δ5 desaturase activity may have been increased in the HC, L, and WM groups to increase EPA content in the hepatic TAG, but not increased in the SF group because the SF diet is very low in ALA, and thus, has very little substrate to convert to EPA. Additionally, the SF group already had the most AA in the hepatic TAG (Figure 16e).

There is only one study that has examined the effects of various types of fats and oils on fatty acid composition in DIO rats. In a study by Buettner et al. (2006), plasma free fatty acid profiles were determined in Wistar rats that were fed high fat diets based

on different types fats and oils for 12 weeks. It was found that the coconut oil diet, which was high in SFA, lead to the highest total SFA concentrations in plasma FFA, followed by the lard diet. Similarly, the olive oil group, which is high in MUFA, had the highest total MUFA in plasma FFA. Interestingly, it was the standard chow fed rats in the study that had the highest total PUFA. Unfortunately, a diet high in ALA was not included in the study by Buettner et al. (2006).

b) PL

The proportions of lipid classes in hepatic PL remained remarkably stable despite vast differences in SFA, MUFA and PUFA composition of the fats and oils consumed by the rats. No differences were seen among groups in terms of total PUFA, although the SF diet had very high amounts of PUFA, whereas the L diet had very little total PUFA. The PUFA content of hepatic PL represented between 44-47 g/100 g fatty acids (Table 8). The HC group had the highest total MUFA, however, considering the fact that the HC diet had very high amounts of MUFA and the SF diet had very little, total MUFA did not differ much and represented between 5-12 g/100 g fatty acids (Table 8). Total SFA represented between 42-48 g/100 g fatty acids (Table 8). Interestingly, the HC group had lower total SFA than all other groups, although the total SFA content of the HC diet was essentially the same as the SFA content of the C and C/F diet. These results indicate that the HC diet increased total MUFA in PL at the expense of total SFA. However, the HC group had less total SFA compared to all of the other groups (42.2% vs 45.1% - 47.8%). Mammalian cells require specific amounts of SFA, MUFA and PUFA to maintain cell fluidity and for optimal cellular functions, which explains why the composition of SFA, MUFA and PUFA in PL is kept within a very tight range (Nakamura et al., 2004).

Regarding individual fatty acids, the HC group had the highest C18:1n-9 and C18:1n-7, which was to be expected due to the high MUFA content of the HC diet (Figure 17c and d, respectively). The C/F, SF and SB groups had the highest LA, and the HC, SF, L and WM groups had the highest AA (Figure 17e and g, respectively). There was no difference among groups in terms of C18:3n-3 (Figure 17f), however, the C/F group had the highest C20:5n-3 and one of the highest C22:6n-3 (Figure 17h and i, respectively).

These results suggest that the proportion of SFA, MUFA and PUFA in PL are very resistant to change even in the presence of hepatic steatosis. However, the individual fatty acids within the fatty acid classes can be more easily influenced by diet. Similar results were seen in our lab when *fa/fa* Zucker rats were fed a control diet or various CLA diets (Noto, 2004).

In our lab, a high ALA diet (flax oil and soybean oil) has been shown to increase the composition of n-3 very long chain PUFAs in hepatic PL of *fa/fa* Zucker rats (Durston et al., 2008). The *fa/fa* rats fed the high ALA diet had significantly more total n-3 PUFAs, in particular EPA, DPA and DHA, in their hepatic PL compared to *fa/fa* controls, however, no increase in ALA was seen in hepatic PL. Similarly, in the present study, the C/F group, which had the highest dietary ALA, had similar amounts of ALA in PL as the other dietary treatments (Figure 17f) but had the highest EPA and among the highest DHA (Figure 17h and i, respectively). Conversely, there was a negligible amount of DPA present in hepatic PL in animals from all dietary treatment groups. The SF group consumed the least amount of dietary ALA and had among the lowest EPA and the lowest DHA in hepatic PL. In *ob/ob* mice, Mustad et al. (2006) demonstrated that when 11-14% of fat in a high fat diet (43-45% en) was replaced with ALA, the amount of ALA

and EPA in liver PL was significantly increased, but not DHA. Although there is controversy over if or how much ALA can be converted to its very long chain derivatives, Legrand et al. (2010) demonstrated that in Long Evans rats the removal of ALA from the diet for 29 days resulted in a decline of DHA in the liver from 8% to 4% (Moriguchi et al., 2004). In humans, it was shown that a diet high in ALA and absent in seafood could increase red blood cell ALA, EPA and DHA content (Legrand et al., 2010). Very long chain PUFAs tend to be readily incorporated into PL and contribute to membrane fluidity, eicosanoid signaling, pinocytosis, ion channel modualtion and regulation of gene expression (Nakamura et al., 2004). Additionally, an increase in very long chain n-3 PUFAs in hepatic PL may also improve inflammation associated with hepatic steatosis by increasing the biosynthesis of anti-inflammatory eicosanoids. In the present study, the C/F group, which had the most n-3 PUFAs in hepatic PL, had the lowest fasting serum haptoglobin concentrations at all time points, however, the L group also had decreased haptoglobin throughout the study, regardless of low n-3 PUFA in liver PL. Clearly, more research is needed to determine if the increase in very long chain n-3 PUFAs in hepatic PL is correlated with an increase in anti-inflammatory eicosanoid production as well as to determine the other effects the increased hepatic PL content of very long chain n-3 PUFA may have on health.

Interestingly, the C/F, SF and SB groups had the highest LA in hepatic PL (Figure 17e). In terms of the canola oil groups (HC, C and C/F) there was an increase of LA in hepatic PL as the amount of dietary ALA was increased and the amount of dietary MUFA was decreased, resulting in the HC group having less LA than the C group which had less LA than the C/F group. Both the SF and the SB diets are high in LA; however, the C/F diet is low in LA. Although the C/F group had increased amounts of LA in hepatic PL,

this did not lead to an increase in hepatic PL AA, as the C/F group contained one of the lowest AA (Figure 17g). This is consistent with another study that showed that a diet high in flaxseed oil (high in ALA) decreased AA content in hepatic microsomal membrane lipids compared to a lard diet (Garg et al, 1988). Thus, it is expected that the decreased AA present in hepatic PL of the C/F group would in turn limit the AA cascade. On the other hand, the HC, SF, L and WM groups had the highest AA, although it would be anticipated that the only the SF or SB diets, which are high in LA, would lead to higher AA in hepatic PL (Figure 17g). In addition to having one of the highest AA, the HC group also had the highest Δ6 desaturase index (Table 8). Delta 6 desaturase catalyzes the conversion of C18:2n-6 to C18:3n-6, which is the rate limiting step in the conversion of AA from C18:2n-6 (Garg et al., 1988). Thus, an increase in the Δ6 desaturase index indicates an increase in AA (Garg et al., 1988).

It came as no surprise that the HC group had the highest total n-9, the SF group had the highest total n-6 and the C/F group had the highest total n-3 (Table 8). In terms of fatty acid ratios, the SF group had the highest n-6/n-3 ratio and the HC group had the highest n-9/n-6 ratio (Table 8). The HC group had the highest Δ9 (C18:1n-9/C18:0) as well as Δ6 desaturase indices (C18:3n-6/C18:2n-6), whereas the SB and C/F group had the highest Δ5 desaturase indices (C20:4n-6/C20:3n-6 and C20:5n-3 + C22:6n-3, respectively; Table 8). As mentioned, PUFAs suppress Δ9, Δ6 and Δ5 desaturase activity; therefore, it makes sense that the HC group, which is very high in MUFA and low in PUFA, would have elevated Δ9 and Δ6 activity. However, the C/F and SB groups had increased Δ5 desaturase index suggesting that the long chain PUFAs present in these diets are being actively converted to very long chain derivatives, which is evidenced by

the increase in EPA and DHA concentrations in the C/F group. However, the SB oil group did not have elevated amounts of AA, EPA or DHA in the hepatic PL compared to most other groups. The SB diet has high amounts of PUFA and the C/F diets has high amounts of the PUFA ALA. It is generally recognized that PUFAs decrease Δ9, Δ6 and Δ5 desaturase activity; however, the effect of ALA on desaturase activity is less clear. A study by Morise et al. (2004) found no increase in Δ5 and Δ6 desaturase activity in hamsters when they were fed ALA in various amounts (from 1.1% total fatty acids to 41.3% of total fatty acids). However, in this study, the amount of EPA in red blood cell PL was strictly linear and proportional to dietary ALA even though Δ5 and Δ6 desaturase activities were identical among groups. Similarly, in the present study, the amount of EPA in hepatic PL was linear to the amount of dietary ALA in the canola oil groups (C/F>C>HC for EPA). However, the C/F diet increased the Δ5 desaturase index in the present study. Therefore, it is unclear as to whether or not ALA regulates its own desaturation. It has been found that impairment in Δ5 and Δ6 desaturase activity is associated with NAFLD (Allard et al., 2008; Burdge et al., 2002). A reduction in Δ5 desaturase activity is also associated with insulin resistance (reviewed by Vessby 2000) and obesity (Pan, Hulbert & Storlien, 1994).

Molecular Markers of Hepatic Fatty Acid Oxidation and Synthesis

No differences were seen among groups in terms of molecular markers of fatty acid oxidation, in particular protein levels of PPARα and ACO (Table 9). Additionally, no differences were seen among groups in terms of molecular markers fatty acid synthesis, specifically protein levels of SREBP1-c and ACC (Table 9). It is generally

well accepted that n-3 PUFAs activate PPARα (Neschen et al., 2002) and suppress SREBP1-c (reviewed by Lombardo et al., 2007). Among n-3 PUFAs, EPA is the strongest PPARα activator and DHA is the most potent fatty acid regulator of SREBP, however, ALA can be converted, *in vivo*, to EPA and DHA (Jump, 2008). Literature on the ability of ALA to impact fatty acid synthesis and oxidation via its conversion to EPA and DHA is scarce.

The results from the present study are in contrast to other studies that have found that diets high in n-3 PUFAs from fish oils can prevent or attenuate obesity, adiposity and high fat diet-induced insulin resistance by acting in a hepatic PPARα-dependent manner (Neschen et al., 2007; Buettner et al., 2006; Neschen et al., 2002; Baillie et al., 1999). Additionally, these changes in the fish oil fed rats were associated with significantly lower hepatic lipid content (Buettner et al., 2006; Neschen et al., 2002).

To summarize, previous studies in DIO rats document that marine n-3 PUFAs can prevent or treat obesity, insulin resistance and hepatic steatosis. Similarly, in the present study, the C/F group, which is high in n-3 PUFAs, had among the lowest body weight gain, termination glucose and hepatic lipid accumulation. However, activation of PPARα and suppression of SREBP1-c is not a viable mechanism by which the C/F diet had its physiological effect as no difference was seen among groups in the levels of proteins associated with fatty acid oxidation and synthesis. However, it is to be noted that the C/F diet used in the present study was high in the plant based n-3 PUFA, ALA, which has not been well documented to increase fatty acid oxidation and fatty acid synthesis, even though ALA can be elongated and desaturated to the very long chain n-3 PUFAs EPA and DHA, which have been shown to have these physiological effects.

SUMMARY and CONCLUSIONS

Obesity

The HC, C and C/F diets attenuated weight gain in DIO rats fed high fat diets. Similarly, the HC, C and C/F groups had the lowest final body weight. The HC, C, C/F, SF, L and WM groups had the lowest mesenteric fat as a percent body weight. Epididymal, peri-renal and visceral fat as a percent of body weight were similar among groups.

Insulin Resistance

The HC, C, C/F and SF diets attenuated fasting serum glucose at termination and the HC and SF groups had the lowest HOMA score. No differences were seen among groups in terms of pancreas weight as a percent body weight. Additionally, groups did not differ in terms of fasting serum insulin, glucose/insulin ratio, and ITT and OGTT results.

Lipidemia

At all time points (week 4, 8 and 12), the SF and SB groups had the lowest fasting serum TAG. The C/F group was also among the lowest in terms of serum TAG at termination. The type of dietary fat or oil had no significant effect on fasting serum cholesterol or FFA.

Hypertension

No effect of diet was seen on systolic or diastolic blood pressure in DIO rats fed high fat diets for 12 weeks. However, it is unclear as to whether or not OP rats develop hypertension when fed high fat diets.

Inflammation

The C/F and L groups had the lowest fasting serum haptoglobin at all time points of the study (week 4, 8, and 12). Additionally, fasting serum adiponectin concentrations were similar among groups.

Hepatic Steatosis

Liver as a percent of body weight was highest in the HC group compared to all other groups. The C/F and WM groups had the lowest percent liver lipid.

Hepatic Fatty Acid Composition

a) TAG

In terms of lipid classes in hepatic TAG, the L and WM groups had the highest total SFA, the HC group had the highest total MUFA, and the SF group had the highest total PUFA. The HC group had the highest total n-9, the SF group had the highest total n-6, and the C/F group had the highest total n-3.

In terms of fatty acid ratios, the SF had the highest n-6/n-3 ratio, the HC group had the highest n-9/n-6 ratio and the highest Δ9 desaturase index, and the HC, L and WM groups had the highest Δ5 desaturase index.

Regarding individual fatty acids, the L and WM groups had the highest C16:0, the HC group had the highest C18:1n-9, the SF and SB groups had the highest C18:2n-6, the C/F group had the highest C18:3n-3, the SF group had the highest C20:4n-6, the SB group had the highest C20:5n-3, and groups did not differ in terms of C22:6n-3.

b) PL

In terms of lipid classes of hepatic PL, all groups had higher total SFA than the HC group, the HC group had the highest total MUFA, and no differences were seen among groups for total PUFA. The HC group had the highest total n-9, the SF group had the highest total n-6 and the C/F group had the highest total n-3.

In terms of fatty acid ratios, the SF group had the highest n-6/n-3 ratio and the HC group had the highest n-9/n-6 ratio. The HC group had the highest Δ9 and Δ6 desaturase index, whereas the SB and C/F group had the highest Δ5 desaturase index.

Regarding individual fatty acids, the SB group had the highest C16:0, the L group had the highest C18:0, the HC group had the highest C18:1n-9 and C18:1n-7, and the C/F, SF and SB groups had the highest C18:2n-6. There were no differences among groups in terms of C18:3n-3, the HC, SF, L and WM groups had the highest C20:4n-6, the C/F group had the highest C20:5n-3, and the C/F and C groups had the highest C22:6n-3.

Molecular Markers of Hepatic Fatty Acid Oxidation and Synthesis

The type of fat or oil did not affect hepatic protein levels of PPARα and ACO, which are markers of fatty acid oxidation. Likewise, diets did not affect hepatic protein levels of SREBP1 and ACC, which are markers of fatty acid synthesis.

Overall

Overall the C/F diet attenuated more of the components of the metabolic syndrome, including obesity, glycemia, lipidemia, inflammation and hepatic steatosis, than the other oil sources in DIO rats. Thus, conventional canola oil plus additional ALA from the flax oil in the C/F diet appeared to enhance the beneficial effects of canola oil on metabolic syndrome parameters. However, it should be noted that all of the dietary oils attenuated some components of the metabolic syndrome. Results from the present study suggest that all dietary fats and oils have their role in the prevention of different components of the metabolic syndrome, and if prevention of a particular disease or condition is desired the diet may need to be altered in terms of fats and oils consumed to achieve this goal. Humans generally consume a variety of dietary fats, and not just a single source of oil and results from the present study indicate that a mixed diet may be advantageous to health. However, the best combination of mixed dietary fats is yet to be determined.

Additionally, the C/F group lower AA concentrations and higher EPA and DHA concentrations in hepatic PL, suggesting that ALA can be efficiently converted to its very long chain derivatives in DIO rats. These results also suggest a mechanism by which the C/F diet may have reduced hepatic fat accumulation and inflammation.

STRENGTHS

- The first study to determine the effects of various types of fats and oils, representing different amounts of SFA, MUFA and PUFA, and different n-6/n-3 ratios, on metabolic syndrome parameters in DIO rats.

- The rats in the study were growing and the aim of the study was prevention, therefore, the results of the study may reflect strategies to prevent the metabolic syndrome in growing children or adolescents.

- The use of a weight-matched group was useful in determining if changes in parameters were due to decreased body weight or due to the diet.

- The diets were formulated to contain the same amount of fat (55% en).

- All components of the metabolic syndrome were assessed: insulin resistance, obesity, lipidemia, hypertension, inflammation and hepatic steatosis.

- Multiple tools were used to assess insulin resistance: ITT, OGTT and HOMA score.

LIMITATIONS

- The biologically active components of the diets were not identified, therefore, there is no way to be sure that the effects were due to the fatty acids in the diets or other components present in the oils and fats.
- The rats in this study were growing and therefore, not in energy balance, which needs to be considered before any comparisons to adult human disease conditions can be made.
- No lean control animals were used in the study; therefore, it is hard to determine if attenuation/improvement of parameters is similar to lean control normal values.
- The flax oil was not tested independently, therefore, it is not known if a flax oil diet alone would be more effective at attenuating metabolic syndrome components than the C/F diet, or if it is the combination of the canola and flax oil that is important.
- Different ratios of the canola and flax oil diet were not tested.
- The amount of lipid fed to the animals per kilogram basis was much higher than the amount of lipid a person would consume on a daily basis.
- Only total cholesterol was analyzed and not lipoprotein subfractions (HDL, LDL, and VLDL).
- Calculations estimating desaturase activity are not corroborated with data on protein expression or enzyme activity.

- Results indicating reduced hepatic lipid accumulation and inflammation (i.e. haptoglobin) are not backed up by histological data, which would indicate the progression of NAFLD.
- Data on protein levels are not backed up with data on enzyme activity.

FUTURE RESEARCH

- Determine the biologically active components of the diets.
- Test a high fat flax oil diet on its own as well as various combinations of canola and flax oil.
- Determine an appropriate lean control, to deduce whether the parameters analyzed in this study were attenuated to normal levels.
- Establish the lipoprotein subfractions of cholesterol in the fasting serum.
- Measure Δ9, Δ6 and Δ5 desaturase protein levels and/or mRNA levels.
- Investigate the mechanism by which certain high fat diets improve serum TAG, and, at the same time, worsen liver lipid accumulation.
- Perform histological examination of the liver to quantify lipid droplet size and numbers to verify hepatic steatosis.
- Determine if the increase in very long chain n-3 PUFA hepatic PL content is associated with an increase in anti-inflammatory eicosanoid production and decreased haptoglobin concentrations.

IMPLICATIONS

Importantly, we have provided a comprehensive examination of how various fats and oils affect metabolic syndrome parameters in DIO rats. Presently, there is an absence of research in which canola oil has been a dietary intervention. Our study investigated the effect of 3 different types of canola oil diets (among other fats and oils) on metabolic syndrome parameters and, thus, has added valuable information to this gap in knowledge. Our research has shown that all of the fats and oils that were used in the study were able to attenuate various components of the metabolic syndrome. Moreover, dramatic changes were seen in the hepatic composition of TAG and PL due to dietary intervention. In particular, it was found that the C/F diet increased EPA and DHA hepatic PL content. Thus, our data suggests that ALA is converted to EPA and DHA *in vivo* and more research is required to fully understand the ramifications of the altered hepatic fatty acid composition on health.

REFERENCES

Aguila, M. B. & Mandarim-de-Lacerda, C. A. (2001). Blood pressure, ventricular volume and number of cardiomyocyte nuclei in rats fed for 12 months on diets differing in fat composition. *Mechanisms of Ageing and Development, 122,* 77 88.

Aguila, M. B., Sa Silva, S. P., Pinheiro, A. R., & Mandarim-de-Lacerda, C. A. (2004). Effects of long-term intake of edible-oils on hypertension and myocardial and aortic remodeling in spontaneously hypertensive rats. *Journal of Hypertension, 22,* 921-929.

Ahima, R. S. & Flier, J. S. (2000). Adipose tissue as an endocrine organ. *Trends in Endocrinology and Metabolism, 11(8),* 327-332.

Allard, J. P., Aghdassi, E., Mohammed, S., Ramn, M., Avand, G., Arendt, B. M., Jalali, P., Kandasamy, T., Prayitno, N., Guindi, M. M., Ma, D. W. L., & Heathcote, J. E. (2008). Nutritional assessment and hepatic fatty acid composition in non-alcoholic fatty liver disease (NAFLD): A cross-sectional study. *Journal of Hepatology, 48,* 300-307.

Alwayn, I. P. J., Gura, K., Nose′, V., Zausche, B., Javid, P., Garza, J., Verbessey, J., Voss, S., Ollero, M., Anderson, C., Bistrian, B., Folkman, J., & Puder, M. (2005). Omega-3 fatty acid supplementation prevents hepatic steatosis in a marine model of nonalcoholic fatty liver disease. *Pediatric Research, 57,* 445-452.

American Diabetes Association (2009). *Fats and diabetes.* Retrieved April 2, 2009, from http://www.diabetes.org/food-nutrition-ifestyle/nutrition/mealplanning/fatand diabetes.jsp

American Heart Association. (2010). *Metabolic Syndrome.* Retrieved July 7, 2010, from http://www.americanheart.org/presenter.jhtml?identifier=4756

American Heart Association. (2009). *Hyperlipidemia.* Retrieved April 24, 2009, from http://www.americanheart.org/presenter.jhtml?identifier=4600

Anand, S. S., Yi, Q., Gerstein, H., Lonn, E., Jacobs, R., Vuksan, V., Teo, K., Davis, B., Montague, P., & DPhil, S. Y. (2003). Relationship of metabolic syndrome and fibrinolytic dysfunction to cardiovascular disease. *Circulation, 108,* 420-425.

Anderson, G., Harnack, K., Erbersdobler, H. F., & Somoza, V. (2008). Dietary eicosapentaenoic acid and docosahexaenoic acid are more effective than alpa-linolenic acid in improb=ving insulin sensitivity in rats. *Annals of Nutrition and Metabolism, 52,* 250-256.

Angulo, P. (2002). Nonalcoholic fatty liver disease. *New England Journal of Medicine, 346(16),* 1221-1231.

Araya, J., Rodrigo, R., Videla, L. A., Thielemann, L., Orellana, M., Pettinelli, P., & Poniachik, J. (2004). Increase in long-chain polyunsaturated fatty acid n-6/n-3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clinical Science, 106,* 635-643.

Baillie, R. A., Takada, R., Nakamura, M., & Clarke, S. D. (1999). Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fish oil: a mechanism for decreased body fat deposition. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 60(5&6),* 351-356.

Baranowski, M. (2008). *The effect of dietary alpha-linolenic acid-rich flaxseed oil intervention on immune and adipose tissue function in obese zucker rats.* University of Manitoba, MSc dissertation.

Bensi, G., Raugeu, G., Klefenz, H., & Cortese, R. (1985). Structure and Expression of human haptoglobin locus. *European Molecular Biology Organization, 4(1),* 119 126.

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37,* 911-917.

Bonner-Weir, S. (2000). Islet growth and development in the adult. *Journal of Molecular Endocrinology, 24,* 297-302.

Brouns, F., Bjorck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G., & Wolever, T. M. S. (2005). Glycaemic index methodology. *Research Reviews, 18,* 145-171.

Brown, M. S., Parhofer, K. G., Rawsn, R. B., & Goldstein, J. L. (2000). Regulated intramembrane proteolysis: A review a control mechanism conserved from bacteria to humans. *Cell, 100,* 391-398.

Buettner, R., Parhofer, K. G., Woenckhaus, M., Wrede, C. E., Kunz-Schughart, L. A., Scholmerich, J., & Bollheimer, L. C. (2006). Defining high-fat diet rat models: Metabolic and molecular effects of different fat types. *Journal of Molecular Endocrinology, 36,* 485-501.

Buettner, R., Scholmerich, J., & Bollheimer, L. C. (2007). High-fat diets: Modeling the metabolic disorders of human obesity in rodents. *Obesity, 15(4),* 798-808.

Burdge, G. C., Jones, A. E., & Wootton, S. A. (2002). Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *British Journal of Nutrition, 88,* 355-363.

Canola Council of Canada. (2008). *Comparison of Dietary Fats*. Retrieved December 16, 2008, from http://www.canola-council.org/

Carroll, J. F., Zenebe, W. J., & Strange, T. B. (2006). Cardiovascular function in a rat model of diet-induced obesity. *Hypertension, 48,* 65-72.

Charles River Research animal models. Retrieved February 1, 2009, from US/PRODSERV/BYTYPE/RESMODOVER/RESMOD/Pages/ObeseResistantRa .aspx

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L. Jones, Jr., D, W., Materson, B., J., Oparil, S., Wright, J. T., Roccella, E. J., & The National Blood Pressure Education Program Coordinating Committee. (2003). Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension, 42,* 1206-1252.

Clarke, S. D. (2000). Polyunsaturated fatty acid regulation of gene transcription: A mechanism to improved energy balance and insulin resistance. *British Journal of Nutrition, 83(1),* 59-66.

Costet, P., Legendre, C., More′, J., Edgar, A., Galtier, P., & Pineau, T. (1998). Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *The Journal of Biological Chemistry, 273(45),* 29577-29585.

DeClercq, V., Taylor, C., & Zahradka, P. (2008). Adipose tissue: The link between obesity and cardiovascular disease. *Cardiovascular & Haematological Disorders-Drug Targets, 8,* 228-237.

Delarue, J., LeFoll, C., Corporeau, C., & Lucas, D. (2004). N-3 long chain polyunsaturated fatty acids: A nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity? *Reproduction Nutrition Development, 44, 289-299.*

De Vries, C. P., Van Haeften, T. W., Wieringa, T. J., & Van der Veen, E. A. (1989). The insulin receptor. *Diabetes Research 11,* 155-165.

Dobrian, A. D., Davies, M. J., Prewitt, R. L., & Lauterio, T. J. (2000). Development of hypertension in a rat model of diet-induced obesity. *Hypertension, 35,* 1009-1025.

Durston, D., Zahradka, P., & Taylor, C. (2008). *Dietary flax oil favorably alters fatty acid composition in fa/fa Zucker rats with hepatic steatosis.* Natural Science and Engineering Research Council of Canada, University of Manitoba Undergraduate Poster Competition.

Emken, E. A. (1984). Nutrition and biochemistry of trans and positional fatty acid isomers in hydrogenated oils. *Annual Review of Nutrition, 4,* 339-376.

Faintuch, J., Horie, L. M., Barbeiro, H. V., Barbeiro, D. F., Soriano, F. G., Ishida, R. K., & Cecconello, I. (2007). Systemic inflammation in morbidly obese subjects: Response to oral supplementation with alpha-linolenic acid. *Obesity Surgery, 17,* 341-347.

Feghali, C. A. & Wright, T. M. (1997). Cytokines in acute and chronic inflammation. *Frontiers in Bioscience, 2,* 12-26.

Ferrucci, L., Cherubini, A., Bandinelli, S., Bartali, B., Corsi, A., Lauretani, F., Martin, A., Andres-Lacueva, C., Senin, U., & Guralnik, J. M. (2006). Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *The Journal of Clinical Endocrinology and Metabolism, 91(2),* 439-436.

Fickova, M., Hubert, P., Cre′mel, G., & Leray, C. (1998). Dietary (n-3) and (n-6) polyunsaturated fatty acids rapidly modify fatty acid composition and insulin effects in rat adipocytes. *Journal of Nutrition, 128,* 512-519.

Folch, J., Lees, M., & Sloane-Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry, 226,* 497-509.

Food and Agricultural Organization/World Health Organization. (1995). WHO and FAO joint consultation: Fats and oils in human nutrition. *Nutrition Reviews, 53(7),* 202-205.

Food and Drug Administration. (2006). Unsaturated Fatty Acids from Canola Oil and Reduced Risk of Coronary Heart Disease. Retrieved December 16, 2008, from http://www.fda.gov/Food/LabelingNutrition/LabelClaims/QualifiedHealthClaims ucm073992.htm#canola

Francois, C. A., Connor, S. L., Bolewicz, L. C., & Connor, W. E. (2003). Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. *American Journal of Clinical Nutrition, 77,* 226-233.

Friedberg, C. E., Janssen, M. J., Heine, R. J., & Grobbee, D. E. (1998). Fish oil and glycemic control in diabetes. *Diabetes Care, 21(4),* 494-500.

Gao, F., Kiesewetter, D., Chang, L., Ma, K., Bell, J. M., Rapoport, S. I., & Igarashi, M. (2009). Whole-body synthesis-secretion rates of long-chain n-3 PUFAs from circulating unesterified a-linolenic acid in unanesthetized rats. *Journal of Lipid Research, 50,* 749-758.

Garg, M. L., Sebokova, E., Thomson, A. B. R., & Clandinin, M. (1988). Δ6 Desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/ore ω3 fatty acids. *Biochemical Journal, 249,* 351-356.

Garg, A. (1998). High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. *The American Journal of Clinical* Nutrition*, 67,* 577-582.

Ghosh, S., Novak, E. M., & Innis, S. M. (2007). Cardiac proinflammatory pathways are altered with different dietary n-6 linoleic to n-3 α-linolenic acid ratios in normal, fat-fed pigs. *American Journal of Physiology Heart and Circulatory Physiology, 293,* 2919-2927.

Giffen, P. S., Turton, J., Andrews, C. M., Barrett, P., Clarke, C. J., Fung, K. W., Munday, M. R., Roman, I. F., Smyth, R., Walshe, K., & York, M. J. (2003). Markers of experimental acute inflammation in the wistar han rat with particular reference to haptoglobin and C-reactive protein. *Archives of Toxicology, 77,* 392-402.

Gingras, A-A., White, P. J., Chouinard, P. Y., Julien, P., Davis, T. A., Dombrowski, L., Couture, Y., Dubreuil, P., Myre, A., Bergeron, K., Marette, A., & Thivierge, M. C. (2007). Long-chain omega-3 fatty acids regulate bovine whole-body protein metabolism by promoting muscle insulin signaling to the Akt-mTOR-S6K1 pathway and insulin sensitivity. *Journal of Physiology, 579.1,* 269-284.

Goa, F., Kiesewetter, D., Chang, L., Ma, K., Bell, J. M., Rapoport, S. I., & Igarashi, M. (2009). Whole-body synthesis-secretion rates of long-chain n-3 PUFAs from circulating unesterified a-linolenic acid in unanesthetized rats. *Journal of Lipid Research, 50,* 749-758.

González, J. A., Gómez-Urbano, A., & Berral de la Rossa, F. J. (2008). Euglycemic hyperinsulinemic clamp. *Diabetologia*, *24(4)*, 305-311.

Guerre-Millo, M. (2003). Extending the glucose/fatty acid cycle: A glucose/adipose tissue cycle. *Biochemical Society Transactions, 31(6),* 1161-1164.

Haffner, S. M. (2006). The metabolic syndrome: inflammation, diabetes mellitus, and cardiovascular disease. *American Journal of Cardiology, 97,* 3A-11A.

Hardie, D. G. (1989). Regulation of fatty acid synthesis via phosphorylation of acetyl CoA carboxylase. *Progress in Lipid Research, 28,* 117-146.

Harper, C. R., Edwards, M. J., DeFilipis, A. P., & Jacobson, T. A. (2007). Flaxseed oil increases the plasma concentrations of cardioprotective. *Journal of Nutrition, 136,* 83-87.

Heikkinen, S. S. (2007). Evaluation of glucose homeostasis. *Current Protocols in Molecular Biology,* 29B.3.1-29B.3.22.

Hernández-Morante, J. J., Larque′, E., Luján, J. A., Zamora, S., & Garaulet, M. (2008). N-6 from different sources protect form metabolic alterations to obese patients: a factor analysis. *Obesity, 17,* 452-459.

Horton, J. D. (2002). Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis. *Biochemical Society Transactions, 30(6),* 1091-1095.

Iacono, J.M. & Dougherty, R.M. (1990). Blood pressure and fat intake. In: Laragh, J., Brener, B.M. (Eds.), *Hypertension, Physiology, Diagnosis and Treatment.* Raven Press, New York, pp. 257–276.

Innis, S. M. (2008). Dietary omega-3 fatty acids and the developing brain. *Brain Research 1237,* 35-43.

Ji, H. & Friedman, M. I. (2008). Reduced hepatocyte fatty acid oxidation in outbred rats prescreened for susceptibility to diet-induced. *International Journal of Obesity, 32,* 1331-1334.

Jump, D. B. (2002). The biochemistry of n-3 polyunsaturated fatty acids. *The Journal of Biochemical Chemistry, 277(11),* 8755-8758.

Jump, D. B. (2008). N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. *Current Opinion in Lipidology, 19,* 242-247.

Kent Scientific Corporation. (2008). Instruction Manual: *CODA multi-channel, computerized, non-invasive blood pressure system for mice and rats.*

Kinsella, J. E., Lokesh, B., & Stone, R. A. (1990). Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: Possible mechanisms. *The American Journal of Nutrition, 52,* 1-28.

Kliewer, S. A., Sundseth, S. S., Jones, S. A., Brown, P. J., Wisel, G. B., Kobles, C. S., Devchand, P., Wahli, W., Willson, T. M., Lenhard, J. M., & Lehmann, J. M. (1997). Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. *Proceedings of the National Academy of Science, 94,* 4318-4323.

Krauss, R. M., Deckelbaum, R. J., Ernst, N., Fisher, E., Howard, B. V., Knopp, R. H., Kotchen, T., Lichtenstein, A. H., McGill, H. C., Pearson, T. A., Prewitt, T. E., Stone, N. J., Van Horn, L., & Weinberg, R. (1996). Dietary guidelines for healthy American adults. A statement for health professionals from the nutrition committee, American heart association. *Circulation, 94,* 1795-1800.

Kris-Etherton, P. M. (1999). Monounsaturated fatty acids and risk of cardiovascular disease. *Circulation, 100,* 1253-1258.

Larter, C. Z., Yeh, M. M., Cheng, J., Williams, J., Brown, S., Pena, A. D., Bell-Anderson, K. S., & Farrell, G. C. (2007). Activation of peroxisome proliferator-activated receptor α by dietary fish oil attenuates steatosis, but does not prevent experimental steatohepatitis because of hepatic lipoperoxide accumulation. *Journal of Gastroenterology and Hepatology, 23,* 267-275.

Legrand, P., Schmitt, B., Mourot, J., Catheline, D., Chesneau, G., Mireaux, M., Kerhoas, N., & Weill, P. (2010). The consumption of food products from linseed-fed

animals maintains erythrocyte omega-3 fatty acids in obese humans. *Lipids, 45,* 11-19.

Legro, R. S., Finegood, D., & Dunaif, A. (1998). A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. *Journal of Clincal Endocrinology and Metabolism, 83,* 2694-2698.

Lombardo, Y. B. & Chicco, A. G. (2006). Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans: a review. *Journal of Nutritional Biochemistry, 17,* 1-13.

Lombardo, Y. B., Hein, G., & Chicco, A. (2007). Metabolic syndrome: Effects of n-3 PUFAs on a model of dyslipidemia, insulin resistance and adiposity. *Lipids, 42,* 427-437.

Louheranta, A. M., Sarkkinen, E. S., Vidgren, H. M., Schwab, U. S., & Uusitupa, M. I. J. (2002). Association of the fatty acid profile of serum lipids with glucose and insulin metabolism during 2 fat-modified diets in subjects with impaired glucose tolerance. *The American Journal of Clinical Nutrition, 76,* 331-337.

Luo, J., Rizkalla, S. W., Boillot, J., Alamowitch, C., Chaib, H., Bruzzo, F., Desplanque, N., Dalix, A-M., Durand, G., & Slama, G. (1996). Dietary (n-3) polyunsaturated fatty acids improve adipocyte insulin action and glucose metabolism in insulin resistant rats: Relation to membrane fatty acids. *The American Journal of Nutrition, 126,* 1951-1958.

Madsen, A. N., Hansen, G., Paulsen, S. J., Lykkegaard, K., Tang-Christensen, M., Hansen, H. S., Levin, B. E., Larsen, P. J., Knudsen, L. B., Fosgerau, K., & Vrang, N. (2010). Long-term characterization of the diet-induced resistant rat model: A polygenetic rat model mimicking the human obesity syndrome. *Journal of Endocrinology,* May 2, 2010 [Epub ahead of print].

Marchesini, G., Brizi, M., Bianchi, G., Tomassetti, S., Bugianesi, E., Lenzi, M., McCullough, A. J., Natale, S., Forlani, G., & Melchionda, N. (2001). Nonalcoholic fatty liver disease: A feature of the metabolic syndrome. *Diabetes, 50,* 1844-1850.

Matthews, D. R., Hosker, J. R., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and fl-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28,* 412-419.

McCarty, M. F. (2000). Towards practical prevention of type two diabetes. *Medical Hypotheses, 54(5),* 786-793.

McKenney, J. M. & Sica, D. (2007). Role of prescription omega-3 fatty acids in the treatment of hypertriglyceridemia. *Pharmacotherapy, 27(5),* 715-728.

Mehta, K., Van Thiel, D. H., Shah, N., & Mobarhan, S. (2002). Nonalcoholic fatty liver disease: Pathogenesis and the role of antioxidants. *Nutrition Reviews, 60(9),* 289 293.

Montalto, M. B. & Bensadoun, A. (1993). Lipoprotein lipase synthesis and secretion: Effects of concentration and type of fatty acids in adipocyte cell culture. *Journal of Lipid research, 34,* 397-407.

Moriguchi, T., Lim, S-Y., Greiner, R., Lefkowitz, B., Loewke, J., Hoshiba, J., Salamen, Jr., N. (2004). Effects of n-3 deficient diet on brain, retina and liver fatty acyl composition in artificially reared rats. *Journal of Lipid Research, 45(8),* 1437-1445.

Morise, A., Combe, N., Boue′, C., Legrand, P., Catheline, D., Delplanque, B., Fénart, E., Weill, P. & Hermier, D. (2004). Dose effect of α-linolenic acid on PUFA conversion, bioavailability, and storage in the hamster. *Lipids, 39,* 325-334.

Moussavi, N., Gavino, V., & Receveur, O. (2008). Could the quality of dietary fat, and not just the quantity, be related to risk of obesity. *Obesity, 16,* 7-15.

Mustad, V. A., DeMichele, S., Huang, Y-S, Mika, A., Lubbers, N., Berthiaume, N., Polakowski, J., & Zinker, B. (2006). Differential effects of n-3 polyunsaturated fatty acids on metabolic control and vascular reactivity in the type 2 diabetic ob/ob mouse. *Metabolism Clinical and Experimental, 55,* 1365-1374.

National Academy of Sciences. Institute of Medicine. Food and Nutrition Board. (2005). *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients).* Retrieved February 9, 2009, from http://www.nal.usda.gov/fnic/DRI//DRI_Energy/422-541.pdf

Neschen, S., Moore, I., Regittnig, W., Yu, C. L., Wang, Y., Pypaert, M., Petersen, K. F., & Shulman, G. I. (2002). Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. *The American Journal of Physiology, Endocrinology and Metabolism, 282,* 395-401.

Neschen, S., Morino, K., Dong, J., Wang-Fischer, Y., Cline, G. W., Romanelli, A. J., Rossbacher, J. C., Moore, I. K., Regittnig, W., Munoz, D. S., Kim, J. H., & Shulman, G. I. (2007). N-3 fatty acids preserve insulin sensitivity *in vivo* in a peroxisome proliferator-activated receptor-α-dependent manner. *Diabetes, 56,* 1034-1041.

Neuschwander-Tetri, B. A. & Caldwell, S. H. (2003). Nonalcoholic steatohepatitis: Summary of an ASLD single topic conference. *Hepatology, 37(5),* 1202-1219.

Noto, A. (2004). *The effects of conjugated linoleic acid (CLA) on insulin resistance, adiposity and hepatic steatosis in fa/fa Zucker rats.* University of Manitoba, MSc dissertation.

Nugent, A. P. (2004). The metabolic syndrome. *British Nutrition Foundation Nutrition Bulletin, 29,* 36-43.

Olfert, E. D., Cross, B. M., & McWilliam, A. A. (1993). *Guide to the care and use of experimental animals* (2nd ed.). Ottawa: Canadian Council on Animal Care.

Pan, D. A., Hulbert, A. J., & Storlien, L. H. (1994). Dietary fats, membrane phospholipids and obesity. *Journal of Nutrition, 124,* 1555-1565.

Paniagua, J. A., Gallego De La Sacristana, A., Romero, I., Vidal-Puig, A., Sanchez, E., Perez-Martinez, P., Lopez-Miranda, J., & Perez-Jimenez, F. (2007). Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin resistance subjects. *Diabetes Care, 30,* 1717-1723.

Pérez-Echarri, N., Pérez Matute, P., Marcos-Gómez, B., Baena, M. J., Marti, A., Martinez, J. A., & Moreno-Aliaga, M. J. (2008). Differential inflammatory status in rats susceptible or resistant to diet-induced obesity: Effects of EPA ethyl ester treatment. *European Journal of Nutrition, 47,* 380-386.

Pietinen, P. (1994). Dietary fat and blood pressure. *Annals of Internal Medicine, 26,* 465–468.

Price, P. T., Nelson, C. M., & Clarke, S. D. (2000). Omega-3 polyunsaturated fatty acid regulation of gene expression. *Current Opinion in Lipidology, 11,* 3-7.

Rasic-Milutinovic, Z., Perunicic, G., Pljesa, S., Gluvic, Z., Sobajic, S., Djuric, I., & Ristic, D. (2007). Effects of n-3 PUFAs supplementation on insulin resistance and inflammatory biomarkers in hemodialysis patients. *Renal Failure, 29,* 321 329.

Salas-Salvado, J., Garcia-Arellano, A., Estruch, R., Marquez-Sandoval, F., Corella, D., Fiol, M., Gómez-Gracia, E., Vinoles, E., Arós, F., Herrera, C., Lahoz, J., Perona, J. S., Munoz-Aguado, D., Martínez-González, M. A., & Ros, E. (2008). Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. *European Journal of Clinical Nutrition, 62,* 651-659.

Sampath, H. & Ntambi, J. M. (2005). Polyunsaturated fatty acid regulation of genes in lipid metabolism. *Annual Review of Nutrition, 25,* 317-40.

Sanders, T. A. B. (2000). Polyunsaturated fatty acids in the food chain in Europe. *American Journal of Clinical Nutrition, 71,* 176-178.

Schmitz, G. & Ecker, J. (2008). The opposing effects of n-3 and n-6 fatty acids. *Progress in Clinical Research, 47,* 147-155.

Sekiya, M., Yahagi, N., Matsuzaka, T., Najima, Y., Nakakuki, M., Nagai, R., Ishibashi, S., Osuga, J., Yamada, N., & Shiman, H. (2003). Polyunsaturated fatty acids

ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. *Hepatology, 38,* 1529-1539.

Shahidi, F. (1990). *Canola and rapeseed: production, chemistry, nutrition, and processing technology.* New York, NY: Springer.

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *American Journal of Clinical Nutrition, 70,* 560-569.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 fatty acids. *Biomedicine and Pharmacotherapy, 56,* 365-379.

Soriguer, F., Morcillo, S., Cardona, F., Rojo-Martiʹnez, G., Almaraʹz, M. C., Adana, M. S. R., Olveira, G., Tinahones, F., & Esteva, I. (2006). Pro12Ala polymorphism of the PPARG2 gene is associated with type 2 diabetes mellitus and peripheral insulin sensitivity in a population with a high intake of oleic acid. *Journal of Nutrition, 136,* 2325-2330.

Sprecher, H. (2000). Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochimica et Biophysica Acta, 1486,* 219-231.

Storlien, L. H., Kraegen, E. W., Chisholm, D. J., Ford, G. L., Bruce, D. G., & Pascoe, W. S. (1987). Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science, 237(4817),* 885-888.

Storlien, L. H., Jenkins, A. B., Chisholm, D. J., Pascoe, W. S., Khouri, S., & Kraegen, E. W. (1991). Influence of dietary fat on development of insulin resistance in rats: Relationship to muscle triglyceride and n-3 fatty acids in muscle phospholipid. *Diabetes, 40(2),* 280-289.

Storlien, L. H., Pan, D. A., Kriketos, A. D., O'Connor, J., Caterson, I. D., Cooney, G. J., Jenkins, A. B., & Baur, L. A. (1996). Skeletal muscle membrane lipids and insulin resistance. *Lipids, 31,* 261-265.

Svegliati-Baroni, G., Candelaresi, C., Saccomanno, S., Ferretti, G., Bachetti, T., Marzioni, M., De Minicis, S., Bobili, L., Salzano, R., Omenetti, A., Pacetti, D., Sigmund, S., Benedetti, A., & Casini, A. (2006). A model of insulin resistance and nonalcoholic steatohepatitis in rats: Role of peroxisome proliferator-activated receptor-α and n-3 polyunsaturated fatty acid treatment on liver injury. *The American Journal of Pathology, 169(3),* 846-860.

Takahashi, Y. & Ide, T. (2000). Dietary n-3 fatty acids affect mRNA level of brown adipose tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat. *British Journal of Nutrition, 84,* 175-184.

Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. (2002). *Circulation, 106,* 3143-3421

Thomas, B. J. (2002). Efficiency of conversion of [alpha]-linolenic acid to long chain n 3 fatty acids in man. *Lipid Metabolism and Therapy, 5(2),* 127-132.

Tsitouras, P. D., Gucciardo, F., Salbe, A. D., Heward, C., & Harman, S. M. (2008). High omega-3 fat intake improves insulin sensitivity and reduces CRP and IL6, but does not affect other endocrine axes in healthy older adults. *Hormone and Metabolic Research, 40(3),* 199-205.

Tugwood, J. D., Issemann, I., Anderson, R. G., Bundel, K. R., McPheat, W. L., & Green, S. (1992). The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. *The EMBO Journal, 11(2),* 433-439.

United States Department of Agriculture. (2007). World statistics: World vegetable oil consumption 2007. Retrieved April 1, 2009, from http://www.soystats.com/2008/page_35.htm

Vessby, B. (2000). Dietary fat and insulin action in humans. *British Journal of Nutrition, 83,* 96-91.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *The Journal of Clinical Investigation, 111(12),* 1796-1808.

Wellen, K. E. & Hotamisligil. G. S. (2003). Obesity-induced inflammatory changes in adipose tissue. *The Journal of Clinical Investigation, 112(12),* 1785-1788.

Wilson, M. D., Blake, W. L., Salati, L. M., & Clarke, S. D. (1990). Potency of polyunsaturated and saturated fats as short-term inhibitors of hepatic lipogenesis in rats. *Journal of Nutrition, 120,* 544-552.

World Health Organization. (2006). *Obesity and Overweight*. Retrieved February 1, 2009, from http://www.who.int/mediacentre/factsheets/fs311/en/index.html

Worgall, T. S., Sturley, S. L., Seo, T., Osborne, T. F., & Deckelbaum, R. J. (1998). Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element binding protein. *The Journal of Biological Chemistry, 273(40),* 25537-25540.

Xu, J., Nakamura, M. T., Cho, H. P., & Clarke, S. D. (1999). Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. *The Journal of Biological Chemistry, 274(33),* 23577-23583.

Zuliani, G., Galvani, M., Leitersdorf, E., Volpato, E., Cavalieri, M., Fellin, R. (2009). The role of polyunsaturated fatty acids (PUFA) in the treatment of dyslipidemias. *Current Pharmaceutical Design, 15(36),* 4087-4093.

APPENDICES

Appendix 1: Obese Resistant Serum Biochemistry

	OR rats
Final Body Weight (g)	439 ± 12
Weight Gain (g)	261 ± 9
Glucose (mmol/L)	
Week 8	8.78 ± 0.25
Week 12	11.3 ± 0.8
Insulin (ng/mL)	0.95 ± 0.18
HOMA	12.8 ± 2.2
Insulin:Glucose (pmol/L:mmol/L)	14.1 ±2.8
TAG (mmol/L)	
Baseline	0.49 ± 0.05
Week 0	2.93 ± 0.61
Week 8	3.31 ± 0.61
Week 12	1.71 ± 0.29
Cholesterol (mmol/L)	4.46 ±0.54
FFA (mmol/L)	0.33 ± 0.04
Haptoglobin (mmol/L)	
Week 4	0.35 ± 0.03
Week 8	0.22 ± 0.04
Week 12	0.36 ± 0.09
Adiponectin (ng/mL)	7.19 ± 0.33
% liver lipid	4.42 ± 0.44

Data are presented as means ± SEM (n = 8-9).

Appendix 2: Weekly Body Weight.

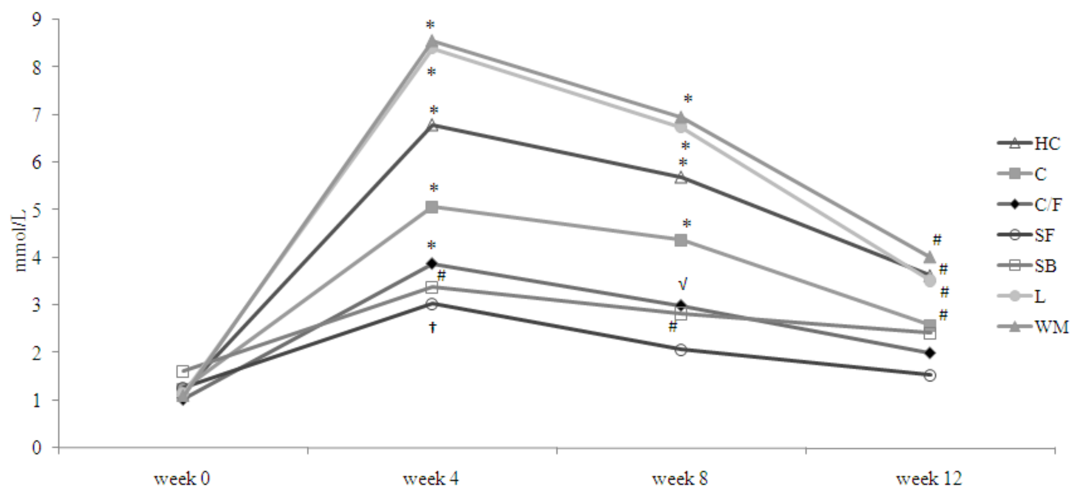
	HC	C	C/F	SF	SB	L	WM
Baseline	197 ± 7	196 ± 6	198 ± 5	193 ± 4	206 ± 6	199 ± 6	201 ± 10
Week 1	240 ± 8	239 ± 7	240 ± 5	238 ± 4	256 ± 7	252 ± 8	252 ± 11
Week 2	292 ± 11	292 ± 6	293 ± 7	296 ± 3	318 ± 8	310 ± 9	306 ± 14
Week 3	332 ± 10 *b*	333 ± 5 *b*	336 ± 7 *ab*	341 ± 4 *ab*	366 ± 9 *a*	358 ± 10 *ab*	350 ± 13 *ab*
Week 4	376 ± 11	376 ± 6	380 ± 8	384 ± 5	412 ± 10	403 ± 11	395 ± 15
Week 5	410 ± 11 *b*	409 ± 6 *b*	410 ± 7 *b*	419 ± 6 *ab*	450 ± 10 *a*	443 ± 11 *ab*	426 ± 16 *ab*
Week 6	440 ± 12 *b*	438 ± 6 *b*	439 ± 7 *b*	451 ± 7 *ab*	486 ± 12 *a*	479 ± 12 *a*	460 ± 16 *ab*
Week 7	462 ± 12 *b*	458 ± 5 *b*	461 ± 8 *b*	473 ± 8 *ab*	510 ± 11 *a*	509 ± 11 *a*	482 ± 19 *ab*
Week 8	487 ± 13 *b*	480 ± 6 *b*	482 ± 8 *b*	497 ± 10 *ab*	537 ± 12 *a*	536 ± 13 *a*	508 ± 18 *ab*
Week 9	508 ± 13 *b*	505 ± 5 *b*	502 ± 9 *b*	521 ± 12 *ab*	562 ± 12 *a*	561 ± 13 *a*	532 ± 19 *ab*
Week 10	523 ± 13 *b*	520 ± 6 *b*	516 ± 9 *b*	525 ± 15 *b*	575 ± 13 *a*	576 ± 14 *a*	547 ± 19 *ab*
Week 11	539 ± 14 *b*	537 ± 6 *b*	533 ± 8 *b*	553 ± 14 *ab*	589 ± 12 *a*	594 ± 14 *a*	558 ± 19 *ab*
Week 12	540 ± 13 *b*	537 ± 7 *b*	534 ± 8 *b*	553 ± 15 *ab*	592 ± 13 *a*	596 ± 14 *a*	558 ± 20 *ab*

Data are presented as means ± SEM (n = 9-10/group) for each week. Statistical differences among means ($p<0.05$) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different.

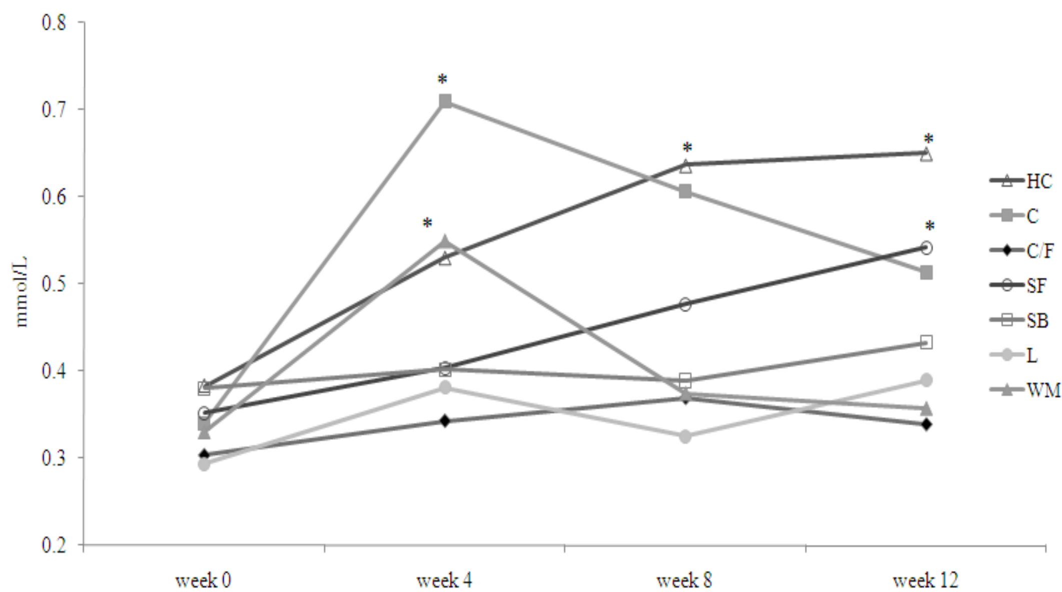
Appendix 3: Absolute Tissue Weights.

	HO	C	C/F	SF	SB	L	WM	P- value
Mesenteric fat (g)	10.2 ± 1.2 *b*	10.6 ± 0.7 *b*	10.4 ± 0.9 *b*	10.8 ± 0.9 *b*	15.1 ± 1.0 *a*	11.8 ± 0.7 *a*	9.50 ± 1.27 *b*	0.0055
Epididymal fat (g)	2.84 ± 0.16	2.90 ± 0.112	2.82 ± 0.12	2.77 ± 0.16	3.04 ± 0.12	3.34 ± 0.10	2.86 ± 0.20	0.1032
Peri-renal fat (g)	22.0 ± 1.5	22.8 ± 1.3	22.0 ± 1.3	22.5 ± 1.7	24.8 ± 1.1	27.1 ± 1.5	22.9 ± 2.5	0.2431
Visceral fat (g)	51.7 ± 4.4 *ab*	51.3 ± 2.8 *ab*	50.2 ± 3.3 *b*	49.4 ± 2.3 *b*	62.9 ± 3.1 *a*	63.0 ± 3.3 *a*	52.1 ± 5.9 *ab*	0.0346
Pancreas (g)	1.11 ± 0.09 *ab*	0.983 ± 0.065 *b*	0.994 ± 0.064 *b*	0.970 ± 0.056 *b*	1.20 ± 0.10 *ab*	1.32 ± 0.13 *a*	1.29 ± 0.09 *a*	0.0293
Liver (g)	19.5 ± 0.6 *a*	17.6 ± 0.4 *abc*	17.3 ± 0.4 *bc*	16.8 ± 0.7 *c*	19.1 ± 0.7 *ab*	18.6 ± 0.6 *abc*	18.5 ± 0.6 *abc*	0.0271

Data are presented as means ± SEM (n = 9-10/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different.



Appendix 4: Serum TAG. Data are presented as means (n=8-10/group). * indicates the time point is different from week 0. An absence of symbols indicates that the time point is not statistically different from the other time points in the group.



Appendix 5: Serum Haptoglobin. Data are presented as means (n=8-10/group). * indicates the time point is different from week 0 and 12. # indicates the time point is different from week 0. √ indicates the time point is different from week 0 and 4. † indicates the time point is different from week 0, 8 and 12. An absence of symbols indicates that the time point is not statistically different from the other time points in the group.

Appendix 6: Hepatic TAG.

FATTY ACIDS (g/100 g fatty acids)	HC	C	C/F	SF	SB	L	WM	P-Value
C8:0	0.164 ± 0.056	0.592 ± 0.193	0.432 ± 0.235	0.170 ± 0.069	0.230 ± 0.091	0.150 ± 0.031	0250 ± 0.112	0.1874
C10:0	0.128 ± 0.041	0.524 ± 0.416	0.288 ± 0.165	0.262 ± 0.137	0.402 ± 0.146	0.282 ± 0.118	0.306 ± 0.228	0.9075
C14:0 *	0.458 ± 0.032 *b*	0.388 ± 0.030 *bcd*	0.422 ± 0.084 *bc*	0.288 ± 0.035 *d*	0.302 ± 0.019 *cd*	0.796 ± 0.044 *a*	0.762 ± 0.030 *a*	0.0002
C15:0	0.426 ± 0.203	0.944 ± 0.370	0.818 ± 0.547	0.240 ± 0.044	0.216 ± 0.031	0.598 ± 0.183	1.12 ± 0.69	0.530
Unidentified Peak	1.29 ± 1.11	0.898 ± 0.570	5.19 ± 2.66	0.808 ± 0.385	3.07 ± 1.11	0.990 ± 0.614	1.47 ± 0.87	0.1714
C16:1n-9 *	1.18 ± 0.08 *ab*	1.38 ± 0.25 *a*	1.02 ± 0.03 *b*	0.428 ± 0.068 *c*	0.518 ± 0.019 *c*	1.20 ± 0.11 *ab*	1.08 ± 0.06 *ab*	0.0006
C16:1n-7	0.288 ± 0.172	0.126 ± 0.116	0.114 ± 0.104	0.010 ± 0.001	0.112 ± 0.102	0.630 ± 0.380	0.854 ± 0.348	0.0823
C17:1 *	0.164 ± 0.023 *b*	0.170 ± 0.027 *b*	0.266 ± 0.085 *b*	0.176 ± 0.100 *b*	0.118 ± 0.018 *b*	0.486 ± 0.041 *a*	0.482 ± 0.046 *a*	0.0042
C18:0 *	1.34 ± 0.10 *d*	1.73 ± 0.13 *cd*	2.33 ± 0.13 *b*	2.08 ± 0.21 *bc*	2.29 ± 0.12 *b*	3.85 ± 0.24 *a*	3.94 ± 0.16 *a*	<0.0001

Appendix 6: Hepatic TAG (continued).

FATTY ACIDS (g/100 g fatty acids)	HC	C	C/F	SF	SB	L	WM	P-Value
C18:3n-6 *	0.162 ± 0.017 *cd*	0.138 ± 0.036 *cd*	0.206 ± 0.010 *c*	0.956 ± 0.038 *a*	0.638 ± 0.011 *b*	0.178 ± 0.009 *cd*	0.114 ± 0.043 *d*	0.0004
C20:1	0.400 ± 0.102	0.430 ± 0.119	0.340 ± 0.062	0.310 ± 0.124	0.240 ± 0.016	0.248 ± 0.034	0.345 ± 0.059	0.6219
C20:2n-6 *	0.184 ± 0.071 *c*	0.110 ± 0.015 *c*	0.108 ± 0.015 *c*	0.712 ± 0.072 *a*	0.532 ± 0.049 *b*	0.166 ± 0.024 *c*	0.142 ± 0.032 *c*	0.0010
C20:3n-6 *	0.160 ± 0.088 *b*	0.250 ± 0.090 *b*	0.178 ± 0.044 *b*	0.742 ± 0.062 *a*	0.708 ± 0.052 *a*	0.116 ± 0.009 *b*	0.132 ± 0.010 *b*	0.0008
C22:4n-6 *	0.180 ± 0.052 *c*	0.262 ± 0.098 *bc*	0.174 ± 0.030 *c*	0.890 ± 0.223 *a*	0.512 ± 0.045 *b*	0.232 ± 0.021 *bc*	0.318 ± 0.102 *bc*	0.0408

Data are presented as means ± SEM (n = 5/group). Statistical differences among means ($p<0.05$) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different. * Data were analyzed by non-parametric statistics. Only fatty acids with an overall mean >0.25% are presented.

Appendix 7: Hepatic PL.

FATTY ACIDS (g/100 g fatty acids)	HC	C	C/F	SF	SB	L	WM	P-Value
C8:0	0.286 ± 0.097	0.298 ± 0.059	0.192 ± 0.049	0.306 ± 0.152	0.372 ± 0.076	0.298 ± 0.081	0.268 ± 0.095	0.9121
C10:0	0.264 ± 0.129	0.672 ± 0.126	0.456 ± 0.138	0.780 ± 0.483	0.758 ± 0.131	0.610 ± 0.145	0.584 ± 0.211	0.7114
Unidentified Peak	0.504 ± 0.494	0.552 ± 0.273	0.538 ± 0.160	0.852 ± 0302	1.70 ± 0.83	0.442 ± 0.197	0.308 ± 0.162	0.287
C17:0 *	0.464 ± 0.029 *b*	0.340 ± 0.023 *cd*	0.304 ± 0.019 *d*	0.332 ± 0.017 *cd*	0.384 ± 0.017 *c*	0.632 ± 0.014 *a*	0.616 ± 0.013 *a*	<0.0001
C20:0	0.348 ± 0.054	0.382 ± 0.084	0.258 ± 0.040	0.366 ± 0.105	0.230 ± 0.053	0.364 ± 0.128	0.354 ± 0.198	0.9284
C20:2n-6 *	0.250 ± 0.063 *c*	0.366 ± 0.021 *c*	0.344 ± 0.007 *c*	1.25 ± 0.079 *a*	0.704 ± 0.036 *b*	0.308 ± 0.010 *c*	0.328 ± 0.012 *c*	0.0003
C20:3n-6	0.786 ± 0.100 *cd*	1.37 ± 0.07 *b*	1.75 ± 0.13 *a*	0.606 ± 0.029 *cd*	0.492 ± 0.178 *d*	0.930 ± 0.195 *c*	1.31 ± 0.12 *b*	<0.0001
C20:3n-3	0.294 ± 0.060	0.332 ± 0.092	0.252 ± 0.059	0.288 ± 0.102	0.208 ± 0.038	0.354 ± 0.115	0.404 ± 0.200	0.8923
C22:0	0.316 ± 0.055	0.338 ± 0.092	0.272 ± 0.085	0.362 ± 0.100	0.312 ± 0.071	0.316 ± 0.079	0.500 ± 0.195	0.8130

Appendix 7: Hepatic PL (continued).

FATTY ACIDS (g/100 g fatty acids)	HC	C	C/F	SF	SB	L	WM	P-Value
C24:0	0.434 ± 0.152	0.564 ± 0.031	0.468 ± 0.085	0.552 ± 0.051	0.564 ± 0.081	0.490 ± 0.013	0.574 ± 0.076	0.8223
C24:1 *	0.396 ± 0.051 *a*	0.482 ± 0.061 *a*	0.378 ± 0.052 *ab*	0.250 ± 0.014 *c*	0.124 ± 0.047 *d*	0.248 ± 0.011 *c*	0.260 ± 0.013 *bc*	0.0011

Data are presented as means ± SEM (n = 5/group). Statistical differences among means (p<0.05) are indicated by different lower case letters. An absence of letters indicates that means are not statistically different. * Data were analyzed by non-parametric statistics. Only fatty acids with an overall mean >0.25% are presented.

Appendix 8: Serum Cholesterol

Group and animal #	Baseline (mmol/L)	Week 4 (mmol/L)	Week 8 (mmol/L)	Termination (mmol/L)
HC				
1	2.54	4.82	4.27	5.03
9	2.26	2.91	3.5	4.56
C				
2	2.17	3.11	4.18	5.09
10	n.a.	2.94	3.65	4.64
C/F				
3	2.58	3.73	3.92	4.95
SF				
4	2.51	3.52	4.31	5.04
SB				
5	2.53	3.15	3.49	4
L				
6	2.09	8.8	4.23	2.56
WM				
7	1.96	5.58	5.24	3.55

Baseline, Week 4, Week 8 and Termination fasting serum cholesterol were determined in 1-2 animals from each group.