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Effects of dietary fat saturation on lipoprotein metabolism in rodents and humans

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**EFFECTS OF DIETARY FAT SATURATION ON LIPOPROTEIN METABOLISM IN
RODENTS AND HUMANS**

by

DEEPINDER KAUR

DISSERTATION

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Chapter 1: Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States and industrialized countries, with Coronary Heart Disease (CHD) as its major manifestation [1]. Despite an approximate 29% decline in death rate between 1996 and 2006, CVD accounted for about 34% deaths in 2006. CHD is a major health concern causing approximately one in every six deaths reported in 2006. It is estimated in the year 2010, 785,000 Americans will have first cardiac arrest and about 470,000 will experience recurrent attacks [2]. CVD can be described as chain of events that is initiated by a cluster of risk factors, progressing through physiological pathways of atherosclerosis, CHD, myocardial infarction, arrhythmia, leading to congestive heart failure, sudden death and development of end stage heart disease [3].

Besides age, gender and genetics that are identified as non-modifiable risk factors, modifiable risk factors can be divided into two main categories : Risk factors that have direct causal association to the development and progression of CHD, namely, dyslipidemia, hypertension, elevated blood glucose levels and tobacco consumption; and risk factors that have significant impact on the direct risk factors predisposing them to development of the disease which include physical inactivity, obesity and atherogenic diet [4, 5]. Emerging risk factors have been identified and are being studied to understand their role in the progression of the disease including LDL and HDL particle sizes, Lp(a), markers of LDL and HDL (Apo B-100 and Apo A1 respectively) and Total and LDL cholesterol to HDL cholesterol ratios [6]. Prospective data shows that clustering of greater than 3 risk factors increases the relative risk 2.4 folds in men and about 6 folds in women for [7]. Comprehensive therapeutic risk factor

modifications need to be made to prevent or delay the onset and progression of CHD. These include dietary modifications, increase physical activity, smoking cessation and weight management.

Plasma Cholesterol is implicated in the pathology of CVD

Elevated plasma cholesterol levels have been established as a key biomarker in categorizing an individual's risk for CVD. High levels of total cholesterol (TC), LDL-cholesterol (LDL-C), small dense LDL particles and triglycerides (TG) along with low levels of HDL-cholesterol (HDL-C) are characteristic to a dyslipidemic profile. The current National Cholesterol Education Program (NCEP) guidelines suggest target levels of TC, LDL-C and HDL-C for determining an individual's risk status and in evaluating the efficacy of lipid lowering therapies [6]. Although most of the nutritional and pharmaceutical research has kept their emphasis primarily on attenuating the risk for CVD by lowering TC and LDL-C, raising HDL-C is becoming an important therapeutic focal point.

Elevated TC and LDL-C is one of the primary risk factors involved in the onset and progression of atherosclerosis. Prospective studies reveal a higher and positive correlation of CHD, CVD and mortality at higher cholesterol levels. Positive relationship between cholesterol levels and first or subsequent attacks of CHD have been observed over a range of LDL-C levels [6, 8, 9]. Also, cholesterol levels in young adults seem to be indicative of development of CHD later in life. In a tri cohort prospective study, Stamler et al concluded that CVD in middle age is positively linked to high levels of baseline TC in young adulthood [10]. The relationship of elevated LDL-C to

development of CHD is mediated by atherosclerosis, a process that begins much earlier in life than the actual disease. Oxidized LDL-C could either initiate the process of atherosclerosis by causing an injury to the endothelial lining or have an impact due to its role in proliferation and formation of fatty atherosclerotic plaques [6, 11]. Due to such strong associations, reduction of TC and LDL-C has been the primary therapeutic targets. Depending on the level and associated risk factors, strategies to lower TC include weight loss, diet modifications and/or pharmaceutical intervention of HMGCoA reductase inhibitors (statins) and bile acid resins [11].

An emerging area of interest is the association of total LDL particle number and LDL particle size with CHD. Studies suggest that small LDL particles and increased LDL particle number are positively associated with CVD endpoints [12, 13]. One of the reasons for increased atherogenic propensity of small LDL particles could be higher susceptibility to oxidation in relation to lipid-poor large particles [14]. Another plausible mechanism suggested for the above relationships is lower affinity for LDL receptors for small LDL particles compared to midsize particles [15]. More research is needed to determine clinical manifestations of size and number of LDL particles before they can be used as routine biomarkers.

HDL-C concentrations have been identified as an independent risk factor for CVD. HDL scavenges cholesterol from the peripheral tissues and delivers it back to the liver for excretion or repackaging via Reverse Cholesterol Transport. Prospective studies suggest inverse relationship between HDL-C concentrations and CVD risk, independent of LDL concentrations. Gordon et al evaluated multiple cohort groups and reinstated the inverse relationship between CHD event rates and HDL-C [16]. Due to

this relationship HDL-C is an important factor to consider while assessing the overall risk for CVD. There is evidence that small HDL particle size may have an atheroprotective effect mediated through prevention of LDL oxidation [17]. HDL therefore has a potential for therapeutic interventions.

LDL-C concentrations have been used as the primary index of CVD risk assessment and target for therapy. However, atherogenic indices, TC:HDL and LDL:HDL cholesterol ratios are thought to be more sensitive in assessing the risk than considering TC, LDL or HDL alone. An increase in the ratio could be indicative of either an increase in TC or LDL or decrease in HDL-C. For CVD risk standpoint, the ratios should be lower for decreased risk. In the following segments in assessing the effects of various fatty acids on CVD risk, TC:HDL-C ratio will be considered for risk assessment.

Modification of an atherogenic diet attenuates the risk for CVD

Modifications in an atherogenic diet pattern is considered cornerstone in the prevention as well as progression of CVD. AHA dietary guidelines suggest that a diet high in fruits, vegetables, whole grains and unsaturated fatty acids have lower associations with CVD risk factors compared to an atherogenic diet. In context of fatty acids, a diet high in saturated fatty acids (SFA), trans fatty acids (tFA) and cholesterol along with low intakes of Unsaturated fatty acids is considered atherogenic [18].

A Fatty acid (FA) is a carboxylic acid with a saturated or unsaturated hydrocarbon chain. Most naturally occurring FA have a chain length between 4 and 28. Saturated fatty acids (SFA) are linear carbon chain molecules fully saturated with hydrogen atoms. Owing to its saturated configuration with no double bonds, they are solid at room

temperature and have a high melting point. The major types of dietary SFA are Lauric acid (12:0), Myristic Acid (14:0), Palmitic Acid (16:0) and Stearic Acid (18:0). In the American diet, full fat dairy and red meats constitute the major sources of SFA with a smaller portion from plant sources such as coconut, palm, cocoa and Shea nut oils. Monounsaturated fatty acids (MUFA) have one point of unsaturation in the hydrocarbon chain. The main dietary sources of MUFA include olive oil, canola oil, avocados, nuts and seeds. Polyunsaturated fatty acids (PUFA) have two or more points of unsaturation on the hydrocarbon chain. The main dietary sources include fish, soybean, sunflower and safflower oil. Trans fatty acids (tFA) are unsaturated fatty acids with at least one double bond in trans configuration. They are present at low levels (~5%) in meats and dairy products formed due to bacterial fermentation in ruminant animals. The independent effects of SFA and tFA in relation to their replacement with unsaturated fatty acids on plasma cholesterol levels and CVD risk factors are considered below.

Consumption of SFA has been the center of controversy for the last 60 years. Consumption of dietary SFA has been linked with elevated concentrations of total cholesterol and LDL-C, markers for increased risk for CVD which led to the basis for the diet-heart hypothesis. The current guidelines for SFA consumption is <7 % of total energy (AHA) and <10% of total energy (USDA) [19-21]. Dietary and policy recommendations often focus on reducing SFA consumption for improving the CVD risk factors based on ecological and animal studies [22]. Most individual prospective cohort studies have not established an independent relationship between SFA consumption and CHD incidence [23, 24]. In an assessment of 11 prospective studies across 3 continents analyzed for 4-10 year follow up, SFA was associated with higher CHD only

in comparison to PUFA. The effects of SFA are studied in relation to other fatty acids or macronutrients. Consumption of CHO and MUFA were also associated with trends towards higher risk for CHD [25]. Replacement of SFA with MUFA or PUFA has been shown to decrease LDL-C as well as HDL-C, with equal effects in reducing TC:HDL ratio in free living young adults. [26]. Interestingly, effect of SFA on LDL-C is mediated by the presence and amount of PUFA in the diet. If PUFA is less than 5% of the total energy, SFA increase LDL-C. Conversely, if PUFA is greater than 5% of total energy, the effect of SFA is negated [27]. In a clinical trial, LDL, TC and apoB were not different between women who consumed diets high or low in SFA with similar P:S ratio [28].

An adverse lipid profile includes elevation of LDL-C specifically small dense LDL-C particles. Interestingly, SFA increases LDL-C but increases the large LDL particles and not the small dense LDL particles [20, 29]. In a meta-analysis by Siri-Tarino et.al, associations of SFA on CHD and CVD were analyzed. In a 5-23 year follow up of 16 cohorts, they concluded that there was no significant effect of dietary SFA on CHD, stroke or CVD [23]. In a meta-analysis of 60 controlled trials by Mensink et al, the effects of various dietary SFA were compared in reference to carbohydrates which revealed that Lauric and Myristic fatty acids tend to increase LDL-C compared with Palmitic acid while Stearic acid is considered neutral [30]. A recent review elucidated the effects of SFA consumption and CVD risk. The authors noted that replacement of SFA with MUFA and PUFA decreases the TC/HDL-C ratio while the ratio increases when SFA are replaced with tFA [22].

In the United States, partially hydrogenated vegetable oil (PHVO) is the main source of tFA in the food supply (~30-50%) [31]. Partial hydrogenation is a process of

transforming liquid oil into a semi solid or solid state. The process involves introduction of hydrogen into the oil in presence of a nickel catalyst. This saturates the double bonds and in the process forms some metastable intermediates which allow the double bonds to rotate and form the trans configuration. Alterations in temperature, pressure and stirring speed can change the melting range, stability and mouth feel of the hydrogenated oil. PHVO in the food supply come from processed food products that require solid fats e.g. margarines, baked goods and shortenings containing 30-50% tFA [32-35].

Although PHVO was being used in small quantities, its production and usage increased after 1960s as a replacement response to health recommendations to reduce SFA in the diet. Initial studies conducted to evaluate the health implications of tFA showed inconclusive results. Mensink et al (1990) conducted a human study with 59 healthy subjects and compared the effects of consumption of 10.9% total energy from either tFA, SFA or MUFA. The results from the study concluded that consumption of tFA and SFA resulted in similar increases in LDL-C compared to MUFA, but HDL-C was lowered in case of tFA compared to SFA and MUFA suggesting a higher cholesterolemic effect of tFA compared to SFA [36]. Judd et al compared the effects of diets containing oleic acid, 3.8% tFA, 6.6% tFA or SFA in 58 healthy subjects. Results from the study showed similar increases in LDL-C for tFA diet groups and SFA compared to oleic acid diet. HDL-C was highest in case of SFA diet and lowest for diet with highest percentage of tFA [37].

Lichtenstein et al compared the effects of diets with increasing percentage (0.9% - 6.7%) of trans fatty acids in margarines to soybean oil and butter diets in 36 subjects

in a randomized double blinded crossover study. The study results revealed that increasing tFA consumption linearly increased LDL-C. HDL-C remained unchanged at lower intakes [32] of tFA, but at highest intake (6.7%), HDL-C was significantly lower compared to butter. TC:HDL ratio increased linearly being least favorable at highest consumption of tFA suggesting higher risk for CHD with increasing amounts of tFA in the diet. Mauger et al conducted a human study to investigate the effect of tFA on LDL-C particle sizes relative to SFA. The results from the study concluded that consumption of tFA increased the harmful small dense LDL-C particles leading to an increased risk for CVD. For the SFA diet, the large LDL-C particles increased as opposed to the small dense particles in tFA diet. The data shows that tFA are more deleterious to health compared to SFA[29].

Results of prospective epidemiological studies also support the changes reflected in the controlled trials. The Nurses' Health study is a longitudinal research initiated in 1976 with 121,700 female registered nurses, to investigate the relation of diet and lifestyle on chronic diseases. Ascertainment of their dietary habits, lifestyle and disease is made every 2 years by mailed questionnaires. In a 8 year follow up by Willett et.al, there was no association observed for CHD risk with the consumption of red meats containing ruminant tFA, as opposed to a strong association with consumption of margarine and cookies in which the primary form was trans elaidic acid predominantly present in PHVO [38]. In a 14 year follow up Hu et.al showed that SFA and MUFA were not significantly related to the risk of CHD, PUFA was inversely related to the risk of CHD from the lowest to the highest consumption and tFA was directly related to the risk for CHD. The analysis also revealed that with 1.6% increase in total energy from tFA the

relative risk for CHD increased by 53% [39]. Also during the 14 year follow up Salmeron et al assessed the impact of dietary fatty acids on risk for Diabetes Mellitus II (DM II). The analyses showed that tFA increased the risk for DM II linearly with increased consumption. Replacing 5% energy from SFA with PUFA decreased the RR by 35% in comparison with replacing 2% energy of tFA with PUFA, decreased the RR by 40% suggesting an increased risk for DM II with tFA consumption [40]. In the most recent meta-analysis of 20 year follow up Oh et al reinstated that PUFA intake was related to decreased risk of CHD and tFA intake was associated with an increased risk for CHD specially in women younger than 65 years [41]. In the Health Professionals study, Ascherio et al also concluded that a positive relationship existed between the consumption of tFA and CHD risk. The risk increased by 40% after adjusting for multivariates [42].

In a recent meta-analysis by Mozaffarian et al, effects of tFA on blood lipids and lipoproteins were analyzed and the association of habitual consumption of tFA with CHD was studied. The analysis showed that compared with equivalent calories from other fats (SFA, MUFA and PUFA), tFA increased TC/HDL-C, Lp(a) levels and fasting triglycerides and lowered HDL-C and ApoA1 levels. The analysis suggested a predictive model showing that replacement of tFA with SFA, MUFA and PUFA decreases TC/HDL-C ratio and total CHD risk with the greatest effects being with replacement by PUFA [43].

Background

In the presence of the experimental and observational data, adverse effects of PHVO containing tFA on lipids and lipoprotein metabolism and its increased risk in the progression of CHD in comparison to SFA is evident. Owing to the adverse health implications of tFA, the Food and Drug Administration (FDA) proposed the labeling of Trans Fats on the food label in 1999 and as of January 1, 2006, it is now mandatory to include Trans Fat on the nutrition label [44, 45]. In the post-trans era, food industry is looking for alternatives that can substitute PHVO in the food products with alternatives that provide similar end products for the food industry without causing adverse health effects. Alternatives that are being sort include using natural sources of saturated fat (tropical oils), interesterified fats, genetically modifying oil seeds, modifying the process of hydrogenation and blended oils.

Modification of the hydrogenation process

Modification of the hydrogenation process involves modifying the conditions during hydrogenation like pressure, temperature and catalyst. Altering of the process would affect the amount of tFA formed, melting point of the resulting fat and the solid fat content. The process would decrease the amount of tFA formed and could also alter the sensory properties of fried food and consumer acceptability in use in baked products [34, 46].

Genetic modification of plant seeds

Genetic modification of plants to alter fatty acid composition can be attained by traditional plant breeding or biotechnological methods. Genetic modification has been attempted in case of high oleic sunflower and canola oils, mid oleic sunflower and

soybean oils and low linolenic canola and soybean oils. The drawback to this method is that it requires sustained efforts by the growers, need a long lead time of one to two years and also incur more cost than traditional oils [34, 46].

Blending Oils

Blended oils are obtained by physically mixing two or more oils. Blends are generally prepared by mixing partially hydrogenated, fully hydrogenated or tropical oil with a liquid vegetable oil. This approach has been used for better thermal stability of the oil. The physical properties of the native oils in the process remain intact, but the blends may not yield the desired physiochemical and nutritional properties consistently. This can be overcome by the process of interesterification [34, 46].

Interesterification (IE)

Interesterification or the process of randomization is an alternative to partial hydrogenation and can generate fats with higher melting points. The process involves fully hydrogenating an oil to make a fully saturated hard stock (either 16:0 or 18:0) followed by randomization of fatty acids to achieve the degree of plasticity required. The two main oils in terms of world supply are palm oil and soybean oil, which can be used in the process of IE, yielding an 18:0:16:0 ratios of 85:15 for soybean oil and 55:45 for palm oil [47]. Randomization can be achieved by

(a) Chemical Process: In chemical IE, an unhydrogenated liquid vegetable oil (85%) is mixed and heated with a fully hydrogenated vegetable oil (15%) in the presence of a chemical catalyst sodium methoxide [46]. The process causes a random arrangement of fatty acids on all the three positions on the glycerol molecule and the composition of the TAG being formed can be controlled to a certain extent by controlling

the relative amounts of fat/oil used for the process [48]. One of the disadvantages of the process is the substantial oil loss due to the formation of fatty acid methyl esters [48].

(b) Enzymatic randomization: A heat stable 1, 3 specific lipase is employed in the enzyme based interesterification resulting in partially randomization instead of complete randomization in case of the chemical process. Enzymatic IE is preferred over chemical IE when a specific TAG is desired and does not lead to unwanted intermediates causing wastage of oils. The drawbacks for this process include long reaction time, higher sensitivity to reaction conditions and high cost.

The advantages of IE are that the process does not change the degree of unsaturation of the fatty acids and maintains the orientation of the isomers in the cis form [49]. The process imparts enhanced oxidative stability as achieved in PHVO [34]. The process leads to the formation of tFA but less than 10% as opposed to 30-50% in PHVO [34, 46, 50]. In the process the physical and nutritional properties of the oils may change leading to altered absorption compared to native oils. The position of the fatty acid on the glycerol molecule and which saturated fatty acid (16:0 or 18:0) should be used in terms of health implications is one of the target areas of research. The absorption and health implications of these interesterified oils are presently under investigation and need to be understood in depth before it becomes the replacement for PHVO [47].

In order to replace PHVO, various alternatives are being considered. The main points of consideration in choosing the alternatives would be cost, availability, oxidative stability, functionality in terms of appearances, texture and flavor and nutrient

composition. Increased dietary fatty acid saturation is one of the inevitable requirements to achieve some of the above targets.

Replacement of Saturated Fatty Acids with Carbohydrates

Limiting intake of SFA requires substitution of other nutrients for isocaloric intakes, generally achieved with carbohydrates. Compared with CHO, SFA intakes raise TC, LDL-C, but lowers TG and raises HDL-C [30]. In a randomized control study, greater reductions in plasma SFA levels were seen in response to a CHO restricted diet with 3 fold SFA compared to a low fat diet [51]. Knopp et al suggested a negating effect of decreasing SFA with increased plasma concentration of palmitate with increase CHO intake [52]. CHO restricted diet with high SFA compared with CHO restricted diet with high unsaturated fatty acids showed no differences in the TC:HDL-C ratio [53]. In a weight loss study with low CHO diets, LDL and TC:HDL-C ratios did not increase despite high intakes of SFA [54]. Substitution of SFA for CHO increased HDL-C with no net effect on TC:HDL-C ratio. PUFA and MUFA substitutions reduced TC:HDL-C ratio compared with SFA. In regards to individual SFA, there is a smaller LDL-C and HDL-C raising effect with substitution for CHO with SFA of increasing chain length with no net effect on TC:HDL-C ratio [30]. Krauss et al studied the effects of moderate CHO restriction on atherogenic dyslipidemia in conjunction with low or high SFA intake. Results from the study revealed that in context of moderate CHO restriction (26%), high SFA (15%) showed an increase in LDL-C compared with low SFA (7-9%) with the LDL increase attributed to increase in mass of large LDL particles [55].

Replacing CHO for SFA pose additional complications with little known about the type of CHO needed for replacement. Effects of high CHO diets, coupled with overweight or obesity caused worsening of atherogenic dyslipidemia. To fully elucidate SFA replacement with CHO, its effects on cholesterol metabolism and regulation need to be evaluated.

Cholesterol Regulation and Homeostasis

Cholesterol, found in the lipid bilayer of the cell membrane, is a steroid based alcohol with both hydrophilic and hydrophobic properties. Being a structural component of the plasma membrane, it provides stability, flexibility and permeability to the cell. It is critical for cell viability, growth, regulation of integral protein function and transcriptional regulation. It plays an essential role in synthesis of steroid hormones, bile acids and provides function to fat soluble vitamins. Cholesterol can be synthesized endogenously in the liver from Acetyl CoA and therefore is not an essential nutrient. In the body, lipid components TG and cholesterol along with phospholipids and protein are packaged and carried in the blood as lipoproteins [11, 56]. The density of the lipoprotein particle is ascertained by the proportion of lipids and protein in the particle; higher the amount of lipids, the less dense is the particle. Density of the lipoprotein particles changes throughout the metabolic pathway. Lipoproteins interact with lipoprotein lipase which act on the circulating particles releasing free fatty acids, thus making the lipoproteins denser. The protein portion of the lipoprotein is called Apolipoprotein (Apo). They provide stability and confer specificity on the lipoprotein complexes allowing them to be recognized by specific receptors on the cell surface. Apo also stimulates certain

enzymatic reactions which in turn regulate lipoprotein metabolic functions. The five main lipoprotein classes are Chylomicrons (CM), Very low density lipoprotein (VLDL), Intermediate density lipoprotein (IDL), Low density lipoprotein (LDL) and High density lipoprotein (HDL).

The primary form of lipoproteins formed from dietary lipids is the CM. The role of the CM is to deliver lipids to adipose and muscle tissues (80%) and to the liver (20%) in the form of CM remnants. CM remnants are removed by the liver by endocytosis following interaction of specific receptors for Apo E or B/E on the cell surface. VLDL have a density of <1.006 g/mL and are synthesized in the liver. They provide the major medium of transport of TG in the fasting state. As the VLDL circulates, lipoprotein lipase cleaves TG giving rise to IDL which is rapidly converted to LDL.

LDL is the catabolic product of VLDL with density of 1.006 - 1.063 g/mL and can be identified by the major protein Apo B-100, the major protein of LDL. LDL contains and transport about 50-70% of total serum cholesterol to the liver and peripheral tissues by interactions with LDL B-100 surface receptors. Once bound to the receptors, LDL is internalized by endocytosis, dissociated in the lysosome into amino acids, free fatty acids and free cholesterol (FC) [57]. FC exerts regulatory functions in the liver by decreasing the activity of HMGCoA reductase, the rate limiting enzyme in the endogenous synthesis of cholesterol and increasing the activity AcylCoA-cholesteryl acyl transferase (ACAT), which converts FC to cholesterol esters (CE). Also, it lowers the concentration of receptor mRNA, suppressing the synthesis of LDL receptors and hence preventing entry of LDL into the cell [11, 57]. High concentrations of LDL in the

plasma have been implicated in the progression of cardiovascular disease and therefore considered the atherogenic lipoprotein.

HDL is the most dense lipoprotein with a density of $1.063 < d < 1.21$ g/mL and can be identified by their major protein ApoA1. HDL is synthesized in the liver and intestine is considered the anti-atherogenic lipoprotein owing to its function of removing free cholesterol from the cells and other lipoproteins and returning the cholesterol to the liver for excretion in the bile.

Reverse Cholesterol Transport: Importance and Regulation

Cellular cholesterol efflux is a critical event for cholesterol homeostasis. In cells of the peripheral tissues, excess cellular cholesterol needs to be removed and transported to the liver for reutilization and excretion. This process is termed Reverse Cholesterol Transport (RCT). HDL serves as the major acceptor for cellular cholesterol. A membrane bound protein ATP-binding cassette (ABC) transporter mediates cholesterol efflux. This process marks the rate limiting step in RCT. ABCA1 utilizes ATP to transfer Phospholipids and cholesterol to the nascent HDL particle. Absence of ABCA1 is associated with an autosomal recessive disorder, Tangier's disease, leading to absence of plasma HDL and HDL mediated efflux. ABCA1 gene mutations lead to low HDL and high TG suggesting its role in the progression of CVD [58]. It is also suggested that mutations in ABCA1 may be responsible for almost 10% cases of low HDL-C in population based studies [59].

HDL is mediated through its Apo-A1 component, the main protein for HDL. ApoA1 is primarily synthesized in the liver and the intestine. Individuals with ApoA1 deficiency

fail to form normal HDL particles. ApoA1 promotes cholesterol efflux and increased ApoA1 expression leads to more cholesterol taken up for excretion. Mutations of ApoA1 have been seen, but one of the targeted mutations is the ApoA1_{Milano}, which is considered atheroprotective and has shown to reduce the size of atheroma in patients with CHD [60]. Although the exact mechanisms are still being studied, it has the potential of being considered as a therapeutic target in elevating the HDL-C levels [59].

ApoA1 stimulates the activity of the enzyme lecithin: cholesterol acyltransferase (LCAT). This enzyme mediates the esterification of free cholesterol. Cholesteryl esters resulting from this reaction can then exchange cholesterol esters for triglycerides readily among plasma lipoproteins mediated by cholesterol ester transfer protein (CETP). CETP activity poses an effect on ApoA and ApoB containing particles, HDL and VLDL and LDL particles respectively. Studies suggest low prevalence of CHD among subjects with CETP deficiency due to increased HDL-C [61]. Over expression of CETP leads to increased VLDL and LDL levels which is pro-atherogenic [62]. The exact role of CETP and whether decrease in CETP is cardio protective is still under scrutiny [59].

Cholesteryl esters can then be selectively taken up by the liver mediated by SR-B1 or indirectly via LDL, following CETP transfer from HDL to LDL. This is mediated by the affinity of the LDL receptors for ApoB and E [11, 63]. SR-B1 plays an important role in the clearance as well as recycling of the HDL particles. Compared to complete internalization of LDL particle, SR-B1 selectively binds to the HDL particle through the ApoA1 component, sequesters cholesterol esters and takes them to the hepatocytes for bile acid synthesis. In this way the HDL particle is quickly recycled and ready for more

cholesterol efflux [62]. SR-B1 deficiency is associated with reduction in the clearance of HDL-C from circulation.

Few studies have been done to understand the underlying mechanisms of effects of dietary fat through hepatic gene expression. Dorfman et al revealed differential effects of dietary fatty acids on expression of RCT genes in hamsters [64]. Hatahet et al also studied the effects of dietary fat on LCAT, ApoA1 and SR-B1 in rats to explore their potential role in the progression of atherosclerosis [65]. In transgenic mice models, Azrolan et al showed that a diet high in SFA increased mRNA levels of hepatic ApoA1 [66]. More research is warranted to see the differential role of individual saturated fatty acids and understanding the underlying mechanisms.

Summary and Specific Aims

Consumption of Saturated fatty acids (SFA) and partially hydrogenated vegetable oils (PHVO) containing trans-fatty acids (tFA) has been associated with higher incidence of coronary heart disease (CHD). Experimental and observational data suggests that consumption of SFA and PHVO containing tFA leads to increased total cholesterol and LDL-cholesterol (LDL-C) but compared to SFA, tFA lowers HDL-cholesterol (HDL-C) leading to an increased risk for CHD. The evidence has prompted health agencies to advocate reduction or complete elimination of PHVO from the food supply. In the post-trans era, alternatives to PHVO are being considered including interesterified (IE) oils, genetically modified oils and blended oils providing increased dietary fat saturation compared to naturally occurring vegetable oils. Since health agencies are seeking alternatives to PHVO, our long term goal is to establish the effects of these alternatives on plasma lipoprotein metabolism. The objective of this PhD proposal is to evaluate the effects of dietary fat saturation on lipoprotein metabolism using – High Linoleic soybean oil (Hi-LO), High Linoleic Low Linolenic Soybean Oil (Hi-LO Low-LN), High Stearic and Linoleic Soybean oil (Hi-SLO), High Oleic Oil Blend (palm, corn & sunflower) (Hi-OL) and a palm oil fraction with an increased content of oleic acid (Hi-PO). The central hypothesis for the present study is that dietary fat saturation will not contribute to an adverse lipid profile. The rationale for the proposed studies is that once the effects of dietary fat saturation on lipoprotein metabolism has been established, it will allow for more rapid elimination of PHVO from the food supply.

To test the above hypothesis, the specific aims of my dissertation research were as follows:

Specific Aim 1: To evaluate the effects of naturally occurring, blended, interesterified and genetically modified oils (with different fatty acid compositions) on lipid and lipoprotein metabolism in Mongolian gerbil and Golden Syrian hamster.

Specific Aim 2: To document the extent to which the amount and type of Saturated Fatty acids affect lipoproteins in Golden Syrian hamster.

Specific Aim 3: To examine postprandial changes in lipids following an oral challenge to test oils with various fatty acid compositions in human subjects.

Chapter 2: Materials and Methods

The **first objective** of the present study was to evaluate the effects of naturally occurring, blended, interesterified and genetically modified oils (with different fatty acid compositions) on lipid and lipoprotein metabolism. Two animal models were used; gerbils - as they are highly sensitive to fatty acid manipulations and hamsters which are widely accepted as appropriate models to depict human lipoprotein metabolism. By employing both animal models, any effects on lipoprotein metabolism could be readily determined.

Model 1: Gerbil

Male Mongolian Gerbils (8 weeks of age, initial weight 60 g) were obtained from Charles River Laboratories (Wilmington, MA). A total of 96 gerbils (21 for Experiment 1 and 75 for Experiment 2) were obtained. Upon arrival, gerbils were housed in individual cages in an isolation room with a 12-h light-dark cycle (lights on 6am, lights off 6pm) and were fed a purified rodent diet (#5001, LabDiet), ad libitum, and allowed free access to water. After a week of acclimatization, the gerbils were randomly assigned to one of the feeding groups.

Experiment 1: CHOW (CHOW, n=3), Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=6), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=6) or Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=6).

Experiment 2: CHOW (CHOW, n=4), Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=13), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=13), Stearic acid

and Linoleic Acid supplemented diet using Interesterified Soybean Oil (Hi-SLO, n=15). Veggie Fruit Oil and Palm Olein were provided by the Nisshin Oillio Group Ltd (Japan), Low Lin Soybean oil was a gift from Cargill (Minneapolis, MN), Coconut oil, and soybean oil were provided by Dyets (Bethlehem, PA). The Interesterified fat was a gift from Dr. Gerald McNeill, Loders Croklaan (Channahon, IL)

Diets were formulated based on current recommendations from the American Society for Nutritional Studies and the nomenclature is based on the predominant and relative fatty acid/s present in the diet. Gerbil diets were obtained in pelleted form from Dyets Inc. (Bethlehem, PA). Sufficient diet was obtained for the entire duration of the study. The diets were kept at -20°C, were removed weekly as needed and refrigerated at 4°C. The final diet (as fed to the animals) comprised ~32% energy from fat and each diet was supplemented with 0.08% (w/w) cholesterol. For comparative purposes, a purified rodent diet (CHOW) was also utilized. The diet compositions are summarized in Tables 2-1 and 2-3, with the final fatty acid composition listed in Tables 2-2 and 2-4. Final Fatty acid (FA) compositions were calculated based on the FA compositions of the oils as provided by the manufacturers.

A flowchart of the study design is shown in Figure 2-1. Briefly, the gerbils were provided a fixed measured amount of diet (approximately 7g of diet per day) and had free access to tap water. Food intake was monitored and was estimated by subtracting the diet spilled in the cage from the amount of diet provided. Body weight was recorded weekly to monitor their growth. All procedures and protocols were approved by the Animal Investigation Committee of Wayne State University.

Blood and Tissue Collection:

After 4 weeks of feeding the test diet, gerbils were fasted overnight and were sacrificed while anaesthetized using 50% CO₂:O₂. Blood was collected by cardiac puncture into EDTA-containing tubes and was spun at 4500 rpm @ 4 degrees for 20 minutes. Sodium azide (10%), gentamycin sulphate (0.01%) and EDTA (10%) were added to plasma samples and were stored at 4°C. Liver was excised and wet weight was recorded prior to flash freezing in liquid nitrogen. Adipose tissue was collected, flash frozen in liquid nitrogen and stored at -80 degrees.

Lipoprotein Characterization:

Plasma lipoproteins were separated by density gradient, discontinuous ultracentrifugation as described by Chapman et.al [67] with one modification, namely, instead of 3 mL of plasma, 4 mL plasma was used. Plasma was pooled from 3-6 gerbils from each group. The density of plasma was raised to 1.210g/mL by addition of solid potassium bromide (0.325 g/mL plasma). Each discontinuous density gradient was then constructed in 16X93 mm ultracentrifuge tubes (Beckman, Palo Alto, CA). 2mL of sodium chloride-potassium bromide (NaCl-KBr) solution of density 1.240g/mL was pipetted into the tube. The following solutions were then sequentially layered: 4 mL of plasma at $d = 1.21 \text{ g/mL}$, 2 mL of $d = 1.063 \text{ g/mL}$, 2.5 mL of $d = 1.019 \text{ g/mL}$ and 3 mL of $d = 1.006 \text{ g/mL}$. Tubes were ultra-centrifuged in a Beckman SW-40.1 Ti rotor at 35000rpm, for 24 hours at 15°C using Optima L-90K ultracentrifuge (Beckman Coulter, Palo Alto, CA). After centrifugation, 25 fractions (500 μL each) were collected sequentially from top into micro centrifuge tubes. For each fraction, total cholesterol and

triglyceride were determined by enzymatic methods using kits purchased from Pointe Scientific Inc. (Canton, MI).

For Experiment 2: In addition to total cholesterol and triglyceride, phospholipids and protein in the fractions were analyzed using enzymatic kit from Wako Chemicals USA (Richmond, VA) and Lowry Assay [68] respectively.

Determination of Liver lipids

Liver lipids were extracted using the Folch method [69]. Briefly, approximately 0.5g of liver samples was taken. Samples were homogenized in 10mL of chloroform-methanol solution 2:1 (v/v) and kept overnight at room temperature in a shaking water bath. Phases were separated by the addition of 3mL 0.5% H₂SO₄. The upper phase was removed and the lower phase was brought to a volume of 10mL. Aliquots of 50μL were removed and dried overnight and reconstituted in 50μL of EtOH. Total cholesterol and free cholesterol were determined using enzymatic kits from Pointe Scientific Inc. (Canton, MI) and Wako Chemicals USA (Richmond, VA) respectively. The difference between total and free cholesterol represent cholesterol esters.

Model 2: Hamster

76 male Golden Syrian Hamsters (6 weeks of age, Initial weight 90g) were obtained from Charles River Laboratories (Wilmington, MA). On arrival, hamsters were housed in individual cages in an isolation room with a 12-h light-dark cycle (lights on 6am, lights off 6pm) and were fed purified rodent diet (#5001, LabDiet) ad libitum and allowed free access to water. After a week of acclimatization, hamsters were randomly

assigned to one of the feeding groups: Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15). For the study, Veggie Fruit Oil and Palm oil were provided by the Nisshin Oillio Group Ltd (Japan), Low Lin Soybean oil was a gift from Cargill (Minneapolis, MN), Coconut oil, flaxseed oil and soybean oil were provided by Dyets (Bethlehem, PA).

Diets were formulated based on current recommendations from the American Society for Nutritional Studies and the nomenclature is based on the predominant and relative fatty acid/s present in the diet. The diet contained 30% en from fat and each diet was supplemented with 0.1% cholesterol. Sufficient diet was obtained for the entire duration of the study. The diets were kept at -20°C, were removed weekly as needed and refrigerated at 4°C. The diet compositions are summarized in Table 2-5 with the final fatty acid composition listed in Tables 2-6.

A flowchart of the study design is shown in Figure 2-2. Briefly, the hamsters were provided a fixed measured amount of diet (approximately 10g of diet per day) and had free access to tap water. Food intake was monitored and was estimated by subtracting the diet spilled in the cage from the amount of diet provided. Body weight was recorded weekly to monitor their growth. All procedures and protocols were approved by the Animal Investigation Committee of Wayne State University.

Blood and Tissue Collection

After 4 weeks of feeding the test diet, 48 hamsters were fasted overnight and were sacrificed while anaesthetized using 50% CO₂:O₂. Blood was collected by cardiac puncture into EDTA-containing tubes and was spun at 4500 rpm @ 4 degrees for 20 minutes. Sodium azide (10%), gentamycin sulphate (0.01%) and EDTA (10%) were added to plasma samples and were stored at 4°C. Liver was excised and wet weight was recorded prior to flash freezing in liquid nitrogen. Adipose tissue was collected, flash frozen in liquid nitrogen and stored at -80 degrees.

The **second objective** of the study was to examine the extent to which the amount and type of Saturated Fatty acids affect lipoproteins. Since hamsters are shown to depict human lipoprotein metabolism, they were chosen as the model of evaluation for the study.

Diets were formulated such that percentage of calories from protein, monounsaturated fatty acids and polyunsaturated fatty acids were kept constant while calories from fat were replaced with calories from carbohydrate. The diet compositions are summarized in Table 2-7, with the final fatty acid composition listed in Table 2-8. The diets were obtained in pelleted form from Dyets Inc. (Bethlehem, PA). Sufficient diet was obtained for the entire duration of the study. The diets were kept at -20°C, were removed weekly as needed and refrigerated at 4°C.

90 male Golden Syrian Hamsters (6 weeks of age, Initial weight 90g) were obtained from Charles River Laboratories (Wilmington, MA). On arrival, hamsters were housed in individual cages in an isolation room with a 12-h light-dark cycle (lights on

6am, lights off 6pm) and were fed purified rodent diet (#5001, LabDiet) ad libitum and allowed free access to water. After a week of acclimatization, hamsters were randomly assigned to one of the six feeding groups: Lauric and Myristic Acid supplemented diet with varying percentage of fat; 60%LM, n=15; 45%LM, n=15; 30%LM, n=15; 21%CON, n=15; and Palmitic Acid supplemented group with varying percentages of fat; 30%PA, n=15; 45%PA, n=15. The nomenclature is based on the percentage of fat present in the diet along with the predominant saturated fatty acid/s. For the study, Palm oil was provided by the Nisshin Oillio Group Ltd (Japan) and Lodders Croklaan (Channahon, IL); Coconut oil was provided by Dyets (Bethlehem, PA).

A flowchart of the study design is shown in Figure 2-3. Briefly, the hamsters were provided a fixed measured amount of diet (approximately 9g of diet per day) and had free access to tap water. Food intake was monitored and was estimated by subtracting the diet spilled in the cage from the amount of diet provided. Body weight was recorded weekly to monitor their growth. All procedures and protocols were approved by the Animal Investigation Committee of Wayne State University.

Blood and Tissue Collection

After 7 weeks of feeding the test diet, 48 hamsters were fasted overnight and were sacrificed while anaesthetized using 50% CO₂:O₂. Blood was collected by cardiac puncture into EDTA-containing tubes and was spun at 4500 rpm @ 4 degrees for 20 minutes. Sodium azide (10%), gentamycin sulphate (0.01%) and EDTA (10%) were added to plasma samples and were stored at 4⁰C. Liver was excised and wet weight

was recorded prior to flash freezing in liquid nitrogen. Adipose tissue was collected, flash frozen in liquid nitrogen and stored at -80 degrees.

For hamsters from Specific Aim 1 and 2, common procedures are detailed below:

Plasma Lipid and Lipoprotein Characterization

Plasma was isolated from fresh blood samples and assayed for Total Cholesterol, HDL-C and Triglycerides using enzymatic kits purchased from Pointe Scientific Inc. (Canton, MI). The plasma lipoproteins were separated by sequential ultracentrifugation. Three lipoprotein fractions were isolated based on human plasma density cuts for lipoproteins, namely, VLDL/IDL (1.006-1.019 g/mL), LDL (1.019-1.055 g/mL) and HDL (1.055-1.21 g/mL). For each fraction, total cholesterol and triglyceride were determined by enzymatic methods using kits purchased from Pointe Scientific Inc. (Canton, MI). Phospholipids and Free Cholesterol in the fractions were analyzed using enzymatic kit from Wako Chemicals USA (Richmond, VA). Proteins in the fractions were analyzed using Lowry Assay[68]. Cholesterol esters were calculated as a difference between Total Cholesterol and Free Cholesterol. Particle size was determined using Van Heek and Zilversmit [70] core to surface volume ratio, according to the formula [71]:

$$R = (\text{nm}) = \frac{1.093(\text{TG}) + 1.044 (\text{CE})}{0.968 (\text{FC}) + 0.97 (\text{PL}) + 0.705 (\text{PR})} \times (3 \times 2.15)$$

$$D (\text{nm}) = 2R + 2(2.15)$$

Determination of Liver lipids

Liver lipids were extracted using the Folch method [69] as detailed for gerbils.

RNA Isolation

Liver sample (0.25-0.30 mg) was excised and was homogenized using polytron homogenizer. Total RNA was isolated from the liver of the hamster using the RNeasy Mini Kit (Qiagen, Valencia, CA) per the manufacturer's protocol. Following isolation, RNA was quantified using a NanoDrop spectrophotometer and samples with a RNA concentration greater than 350ng/ul were used for analysis. Denaturing Agarose gel electrophoresis was performed to check for RNA quality. RNA samples were stored at -80°C until further analysis.

cDNA was synthesized from 1 µg of RNA using random primers (ImProm-II™ reverse transcriptase system, Promega, Madison, WI) and purified with the QIAquick PCR Purification kit (Qiagen). The levels of cDNAs were quantified using a LightCycler real time PCR machine (Stratagene, La Jolla, CA). For the analysis, all cDNA samples were standardized to a concentration of 50ng/ul to ensure equal starting concentrations for every sample. Triplicates for each sample were analyzed. PCR contained 3 µL of purified cDNA, 12.5 µL of PCR master mix (SYBR Green, Applied Biosystems), and 0.5 µmol/liter each of forward and reverse primers (Invitrogen). The primer sequences for ABCA1, ApoA1, CETP, SR-B1 and GAPDH used are summarized in Table 2-9. No template controls were present on each plate to ensure no contamination of primers, reagent or nuclease free water and to check for primer dimers. For all amplifications, PCR conditions consisted of an initial denaturing step of 95 °C for 10 min followed by 40

cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 2 s with a melting curve analysis from 58 to 95 °C to confirm specificity. The level of each transcript was normalized to GAPDH. Results are expressed as mean values from 4-5 animals per experimental group.

PCR Result Analysis

Ct values were normalized using GAPDH as an endogenous control. Relative gene expression was calculated using the equation

$$2^{-(\text{avg. (Ct}_{\text{gene of interest}}) - \text{avg. (Ct}_{\text{Housekeeping gene}}))}$$

The relative expression values were used to determine fold changes in the test diet compared to the control diet.

Human postprandial study

The postprandial state is a reflection of the metabolic state in humans due to regular diet intake. The **third objective** of the study was to evaluate postprandial changes in lipids following an oral challenge to test oils with various fatty acid compositions.

Subjects were administered a test “smoothie” prepared by blending 240g non-fat milk, 140g frozen strawberries, ~55g banana, 24g sugar and 60g test oil (Hi-OL, Hi-PO or Hi-LO). The “smoothie” provided 820kcal with 66% en from fat, 30% en from carbohydrates and 5% en from protein. This test meal represented an extreme dietary oral fat challenge.

A flowchart of the study design is shown in Figure 2-4. Briefly, 9 subjects, aged between 18 and 50 years, generally healthy were recruited for the study. Subjects who volunteered for the study filled out a basic questionnaire for any known preexisting conditions of high BP, TC, TG, diabetes or heart problems. As a prescreen test, fasting blood samples for the subjects were taken and plasma was analyzed for TC, HDL-C and TG using enzymatic kits. Subjects were tested with each of the 3 fat compositions namely Oleic Acid supplemented test oil using Veggie fruit oil (Hi-OL), Palmitic and Oleic Acid supplemented test oil using Palm Olein (Hi-PO) or Linoleic acid supplemented test oil using Soybean oil (Hi-LO) with a wash out period of 2 weeks. The nomenclature is based on the predominant and relative fatty acid/s present in the diet. For the study, Palm oil and Veggie Fruit oil were provided by the Nisshin Oillio Group Ltd (Japan) and Soybean Oil (Brand - Pure Wesson) was purchased from local supermarket. All procedures and protocols were approved by the Human Investigation Committee of Wayne State University.

The first time the subjects participated, they were asked to record their food and beverage intake on the day before the experiment. Subjects reported fasting for 12 hours on the day of the study. They provided a fasting blood sample. They were then administered the test meal smoothie which was consumed within 15 minutes. Additional blood samples were collected at 2, 4 and 8 hours. All blood samples were collected by a trained phlebotomist by venipuncture. On the day of the study, subjects were asked to refrain from any rigorous physical activity and food. They were allowed approximately 300 mL of water or decaffeinated coffee without milk and sugar. On subsequent

occasions subjects were asked to follow the diet they had consumed the evening before the day of the experiment.

Blood Collection:

Blood was collected into EDTA-containing tubes at 0, 2, 4 and 8 hours and was spun at 4500 rpm @ 4 degrees for 20 minutes. Sodium azide (10%), gentamycin sulphate (0.01%) and EDTA (10%) were added to plasma samples which were kept at 4°C.

Plasma Lipid and Lipoprotein Characterization

Plasma was assayed for Total Cholesterol, HDL-C and Triglycerides using enzymatic kits purchased from Pointe Scientific Inc. (Canton, MI). To fully characterize postprandial effects on lipoproteins induced by dietary fat blends, TAG rich fractions (Sf>400, Sf 60-400, Sf 20-60) were isolated from all plasma samples that were obtained at 0, 2, 4 and 8 hours using discontinuous ultracentrifugation [72]. Briefly, plasma was adjusted to $d=1.1$ g/mL. Four mL of this plasma was then successively over layered with 3 mL solutions of density 1.063 g/mL, 1.02 g/mL and 1.006 g/mL. Following ultracentrifugation in a Optima L-90K ultracentrifuge (Beckman Coulter, Palo Alto, CA) for 32 minutes at 15°C using a SW-40 Ti rotor, the Sf>400 fraction was obtained from the top 1mL of the gradient. The centrifuge tube was then refilled with 1 mL of d 1.006 g/mL solution, and the tube was recentrifuged for 3 h 28 min to obtain the Sf 60-400 fraction recovered from the top 1mL. The ultracentrifuge

tube was then refilled and a further 16 h ultracentrifugation yielded the Sf 20-60 fraction [72]. Triglycerides were measured in the TAG rich fractions using an enzymatic kit purchased from Pointe Scientific Inc. (Canton, MI).

CETP activity

CETP is active during triglyceridemic state. CETP activity was measured in plasma samples using CETP Activity Assay kit purchased from BioVision (Mountain View, CA). CETP activity was measured at excitation 465nm and emission of 535nm using microplate reader (Tecan GENios Plus). The activity was observed as an increase in fluorescence intensity and was expressed as pmol/ul plasma/hr.

Statistical Analysis

All statistical analyses were performed using PASW Statistics 18 for Windows® (SPSS Inc. Chicago, IL). Data were analyzed using one-way ANOVA followed using Tukey post-hoc tests. Results are presented as the mean $\pm$ SD and significance was considered at $p \leq 0.05$.

TABLE 2-1. Specific Aim 1: Experiment 1: Composition of purified diets for gerbils (g/kg).

Ingredient	Hi-LM	Hi-OL ^a	Hi-LO
Vitamin Free Casein	105	105	105
Lactalbumin	105	105	105
Dextrose	180	180	180
Cornstarch	250.17	250.17	250.17
Dyetrose	100	100	100
Cellulose	59.5	59.5	59.5
FAT	144	144	144
Coconut Oil	142.6	0	0
Veggie Fruit Oil	0	144	0
Soybean Oil	1.4	0	144
TBHQ	0.029	0.029	0.029
Mineral mix #290003	40	40	40
Vitamin mix #390005	10	10	10
DL-Methionine	3	3	3
Choline Bitartrate	2.5	2.5	2.50
Cholesterol	0.8	0.8	0.8
<i>Caloric content (kcal/kg diet)</i>			
Protein	800 (19.6%) ^b	800 (19.6%)	800 (19.6%)
Carbohydrate	1936 (47.5%)	1936 (47.5%)	1936 (47.5%)
Fat	1296 (31.8%)	1296 (31.8%)	1296 (31.8%)

Diets were prepared and pelleted by Dyets Inc. (Bethlehem, PA).

^a Veggie Fruit Oil was supplied by Nisshin Oillio Group Ltd., Tokyo, Japan.

^b Percent of total calories contributed by each macronutrient.

TABLE 2-2. Specific Aim 1: Experiment 1:Percentage of total dietary calories contributed by each fatty acid.

Fatty acid	Hi-LM	Hi-OL	Hi-LO
% of total calories			
12:0	15.5	0.06	0
14:0	7.08	0.13	0
16:0	3.05	5.47	3.18
18:0	0.71	0.80	1.27
18:1	2.44	14.9	7.95
18:2	0.86	9.01	16.54
18:3	0.02	1.06	2.23
Σ SFAs	28.47	6.47	4.45
Σ MUFAs	2.44	14.9	7.95
Σ PUFAs	0.88	10.07	18.77
P/S ratio	0.031	1.56	4.22

Major FA indicated in bold-face. Fatty Acid composition was calculated based on the FA profile supplied by Nisshin Oillio and Dyets Inc.

TABLE 2-3. Specific Aim 1: Experiment 2: Composition of purified diets for gerbils (g/kg).

Parameter	-----Diet-----				
	Hi-LM	Hi-SLO	Hi-LO Low-LN	Hi-PO	Hi-OL
Vitamin Free Casein	105	105	105	105	105
Lactalbumin	105	105	105	105	105
Dextrose	180	180	180	180	180
Cornstarch	250.17	250.17	250.17	250.17	250.17
Dyetrose	100	100	100	100	100
Cellulose	59.5	59.5	59.5	59.5	59.5
FAT	144	144	144	144	144
Coconut Oil	142.6	0	0	0	0
Interesterified Fat	0	144	0	0	0
Low Lin Soybean Oil	0	0	144	0	0
Palm Olein IV67	0	0	0	144	0
Soybean Oil	1.4	0	0	0	0
Veggie Fruit Oil	0	0	0	0	144
TBHQ	0.029	0.029	0.029	0.029	0.029
Mineral Mix #290003	40	40	40	40	40
Vitamin Mix # 390005	10	10	10	10	10
DL-Methionine	3	3	3	3	3
Choline Bitrtrate	2.5	2.5	2.5	2.5	2.5
Cholesterol	0.8	0.8	0.8	0.8	0.8
<i>Caloric content (kcal/kg diet)</i>					
Protein	----- 800 (19.6%) ^b -----				
Carbohydrate	-----1936 (47.5%) -----				
Fat	-----1296 (31.8%) -----				

Diets were prepared and pelleted by Dyets Inc. (Bethlehem, PA). Palm Olein IV67 and Veggie Fruit Oil was supplied by Nisshin Oillio Group Ltd., Tokyo, Japan. Low linoleic soybean oil was from Cargill, Inc. The Interesterified fat was a gift from Loders Croklaan

^b Percent of total calories contributed by each macronutrient.

TABLE 2-4. Specific Aim 1: Experiment 2: Percentage of total dietary calories contributed by each fatty acid.

Fatty Acid	-----Diet-----				
	Hi-LM	Hi-SLO	Hi-LO Low-LN	Hi-PO	Hi-OL
	-----% of total calories -----				
12:0	15.49	0	0	0.12	0.06
14:0	7.08	0.04	0.03	0.34	0.13
16:0	3.05	3.84	3.43	9.72	5.47
18:0	0.71	8.49	1.34	1.07	0.80
18:1	2.44	5.24	7.57	14.92	14.87
18:2	0.86	12.02	18.09	4.43	9.01
18:3	0.02	1.65	1.02	0.98	1.06
Σ SFA	28.47	12.38	4.80	11.25	6.47
Σ MUFA	2.44	5.24	7.57	14.92	14.87
Σ PUFA	0.88	13.67	19.11	5.41	10.07
P/S ratio	0.03	1.10	3.98	0.48	1.56

Major FA indicated in bold-face. Fatty Acid composition was calculated based on the FA profile supplied by Nisshin Oillio, Cargill Inc., Loders Croklaan and Dyets Inc.

TABLE 2-5. Specific Aim 1: Experiment 3: Composition of purified diets for hamsters(g/kg).

Ingredient	High LM	Hi-LO Low-LN	High PO	High LO	High OL
Casein	110	110	110	110	110
Lactalbumin	110	110	110	110	110
L-arginine	2.5	2.5	2.5	2.5	2.5
L-tryptophan	0.3	0.3	0.3	0.3	0.3
Cornstarch	370.2	370.2	370.2	370.2	370.2
Dyetrose	175	175	175	175	175
Cellulose	44	44	44	44	44
FAT	144	144	144	144	144
Coconut Oil	142.56	0	0	0	0
Flax Oil	0	0	7.2	0	0
Low Lin soybean oil	0	144	0	0	0
Palm Olein (IV68) ^a	0	0	136.8	0	0
Soybean Oil	1.44	0	0	144	0
Veggie Fruit Oil ^a	0	0	0	0	144
Mineral mix #260001	35	35	35	35	35
Vitamin mix #360001	10	10	10	10	10
Choline Bitartrate	2	2	2	2	2
Cholesterol	1	1	1	1	1
Caloric content (kcal/kg diet)					
Protein	-----834 (20.1%) ^b -----				
Carbohydrate	-----1998 (48.1%) -----				
Fat	-----1260 (30.4%) -----				

Diets were prepared and pelleted by Dyets Inc. (Bethlehem, PA).

^aPalm Olein (IV68) and Veggie Fruit Oil were supplied by Nisshin Oillio Group Ltd., Tokyo, Japan. Low linolenic soybean oil was from Cargill, Inc.

^bPercent of total calories contributed by each macronutrient.

TABLE 2-6. Specific Aim 1: Experiment 3: Percentage of total dietary calories contributed by each fatty acid.

Fatty Acid	High LM	Hi-LO Low-LN	High PO	High LO	High OL
% of total calories					
12:0	14.81	0	0.12	0	0.06
14:0	6.78	0.03	0.32	0	0.12
16:0	2.92	3.28	9.29	3.04	5.23
18:0	0.67	1.28	1.02	1.22	0.77
18:1	2.33	7.24	14.26	7.6	14.22
18:2	0.82	17.3	4.24	15.81	8.62
18:3	0.02	0.97	0.94	2.13	1.02
ΣSFAs	25.18	4.59	10.75	4.26	6.18
ΣMUFAs	2.33	7.2	14.26	7.6	14.2
ΣPUFAs	0.84	18.27	5.18	17.94	9.64
P/S ratio	0.03	3.98	0.48	4.21	1.56

Major FA indicated in bold-face. Fatty Acid composition was calculated based on the FA profile supplied by Nisshin Oillio, Cargill Inc., and Dyets Inc.

TABLE 2-7. Specific Aim 2: Composition of purified diets for hamsters (g/kg).

Ingredients	60%LM	45%LM	30%LM	21%CON	30%PA	45%PA
Caesin	554.9	519.1	465.4	436.8	465.4	519.1
Lactalbumin	604.5	565.5	507	475.8	507	565.5
L-Arginine	10	10	10	10	10	10
L-Tryptophan	1.2	1.2	1.2	1.2	1.2	1.2
Total	1170.6	1095.8	983.6	923.8	983.6	1095.8
Pro %	23.6	23.9	23.9	23.9	23.9	23.9
Cornstarch	453	666	1225	1347	1225	666
Dyetrose	317	707	631	745	631	707
Total	770	1373	1856	2092	1856	1373
CHO %	15.5	29.9	45.1	54.0	45.1	29.9
Safflower	505	476	419	410	0	145
Sunflower	327	373	407	410	0	0
Coconut	2138	1221	407	0	0	269
Corn	0	0	0	0	555	414
Palm Olien	0	0	0	0	678	0
Palm Stearin	0	0	0	0	0	1242
Total (kcal)	2970	2070	1233	820	1233	2070
Fat %	59.9	45.1	30.0	21.2	30.0	45.1
Cholesterol	1.2	1.1	1.0	0.94	1.0	1.1
Choline	2	2	2	2	2	2

Diets were prepared and pelleted by Dyets Inc. (Bethlehem, PA).

TABLE 2-8. Specific Aim 2: Percentage of total dietary calories contributed by each nutrient.

	60%LM	45%LM	30%LM	21%CON	30%PA	45%PA
SFA %	40.62	25.73	10.83	1.97	10.12	24.56
CHO %	15.5	29.9	45.1	54	45.1	29.9
MUFA %	9.83	9.81	10.01	9.79	9.86	10.44
PUFA %	9.5	9.39	9.06	9.14	9.87	10
PRO %	23.9	23.9	23.9	23.9	23.9	23.9
P/S ratio	0.23	0.36	0.84	4.64	0.98	0.41

Fatty Acid composition was calculated based on the FA profile supplied by Nisshin Oillio and Dyets Inc.

TABLE 2-9. Reverse Cholesterol Transport Gene Primers

<i>GENE</i>	<i>FORWARD</i>	<i>REVERSE</i>
ABCA1 ^[73]	5'-ATAGCAGGCTCCAACCCTGAC-3'	5'-GGTACTGAAGCATGTTTCGATGTT-3'
ApoA1 ^[74]	5'-ACCGTTCAGGATGAAAAGTGTAG-3'	5'-GTGACTCAGGAGTTCTGGGATAAC-3'
CETP ^[75]	5'-AAGGGTGTCTGTGGTCAGTTCT-3'	5'-ACTGATGATCTCGGGGTTGAT-3'
SR-B1 ^[74]	5'-AAGCCTGCAGGTCTATGAAGC-3'	5'-AGAAACCTTCATTGGGTGGGTA-3'
GAPDH ^a	5'-ACCCAGAAGACTGTGGATGG-3'	5'-CAGTGAGCTTCCCGTTCAG-3'

All primers were provided by Invitrogen. Superscript numbers are the references from which the primers were taken.

^a The primer sequence was a generous gift from Dr. Fazlul Sarkar and Dr. Smiti Gupta (Wayne State University).

Figure 2.1: Specific Aim 1: Flowchart of Study Design for Gerbils (Experiment 1 and Experiment 2)

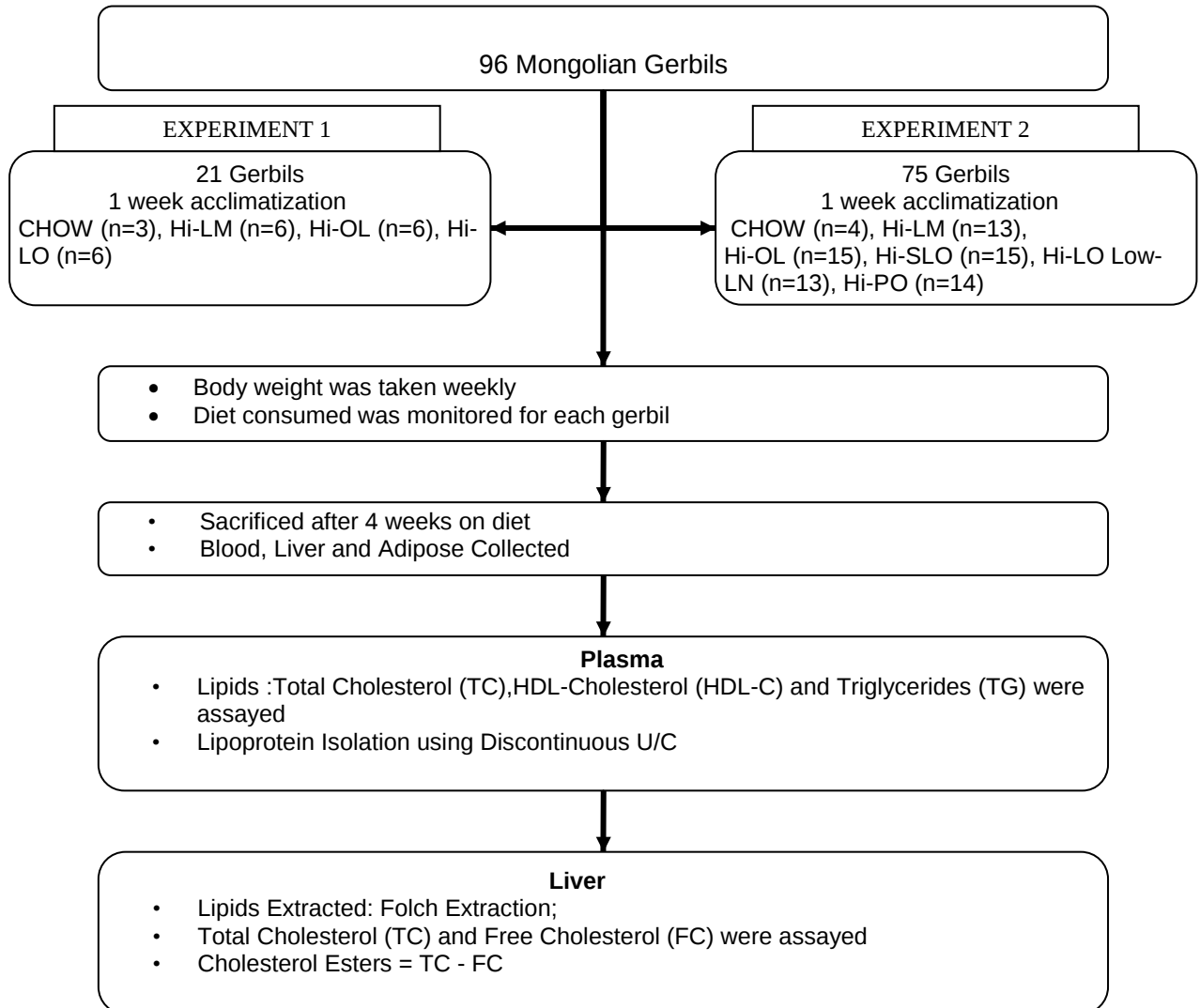


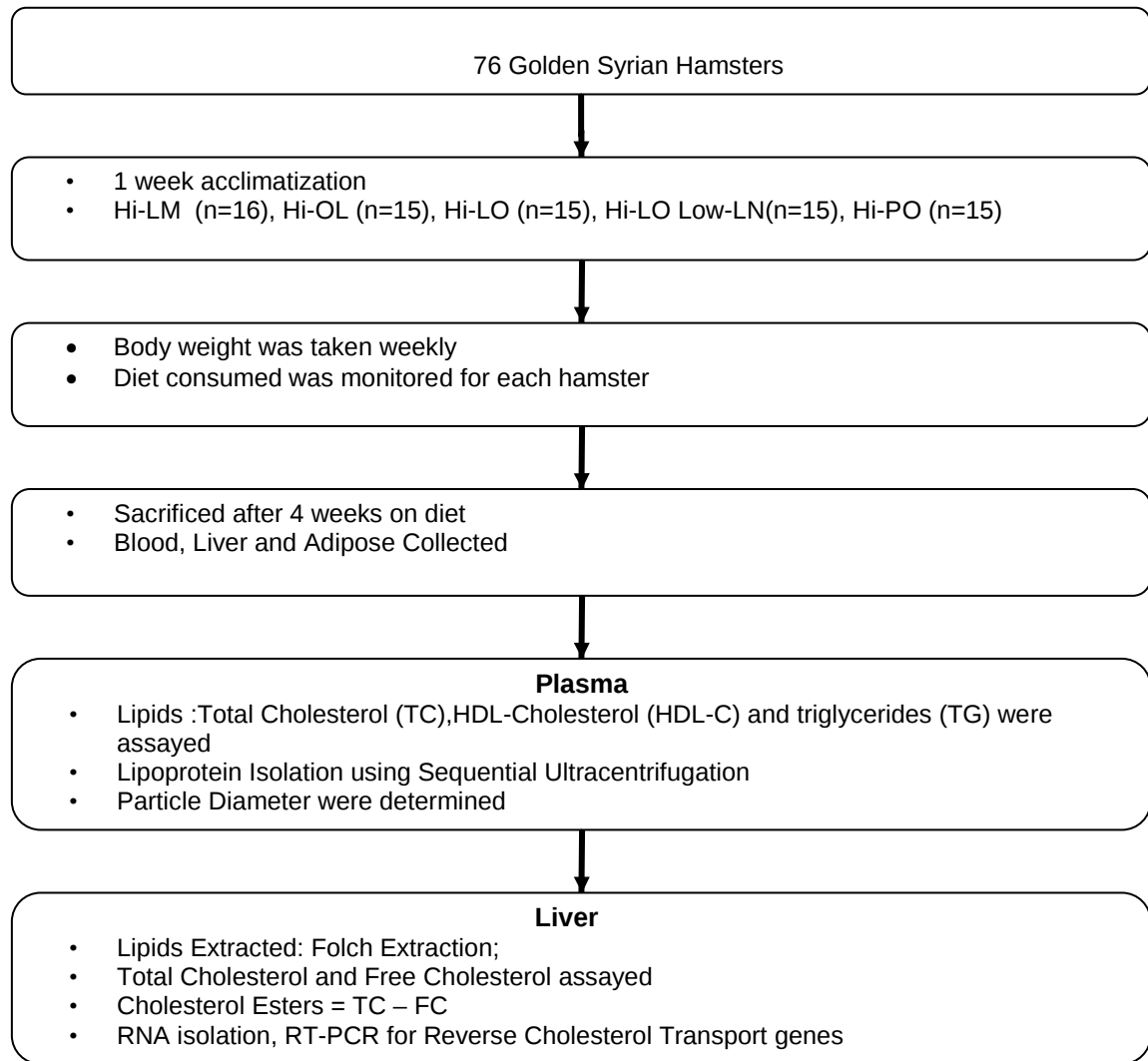
Figure 2.2: Specific Aim 1: Flowchart of Study Design for Hamsters

Figure 2.3: Specific Aim 2: Flowchart of Study Design for Hamsters

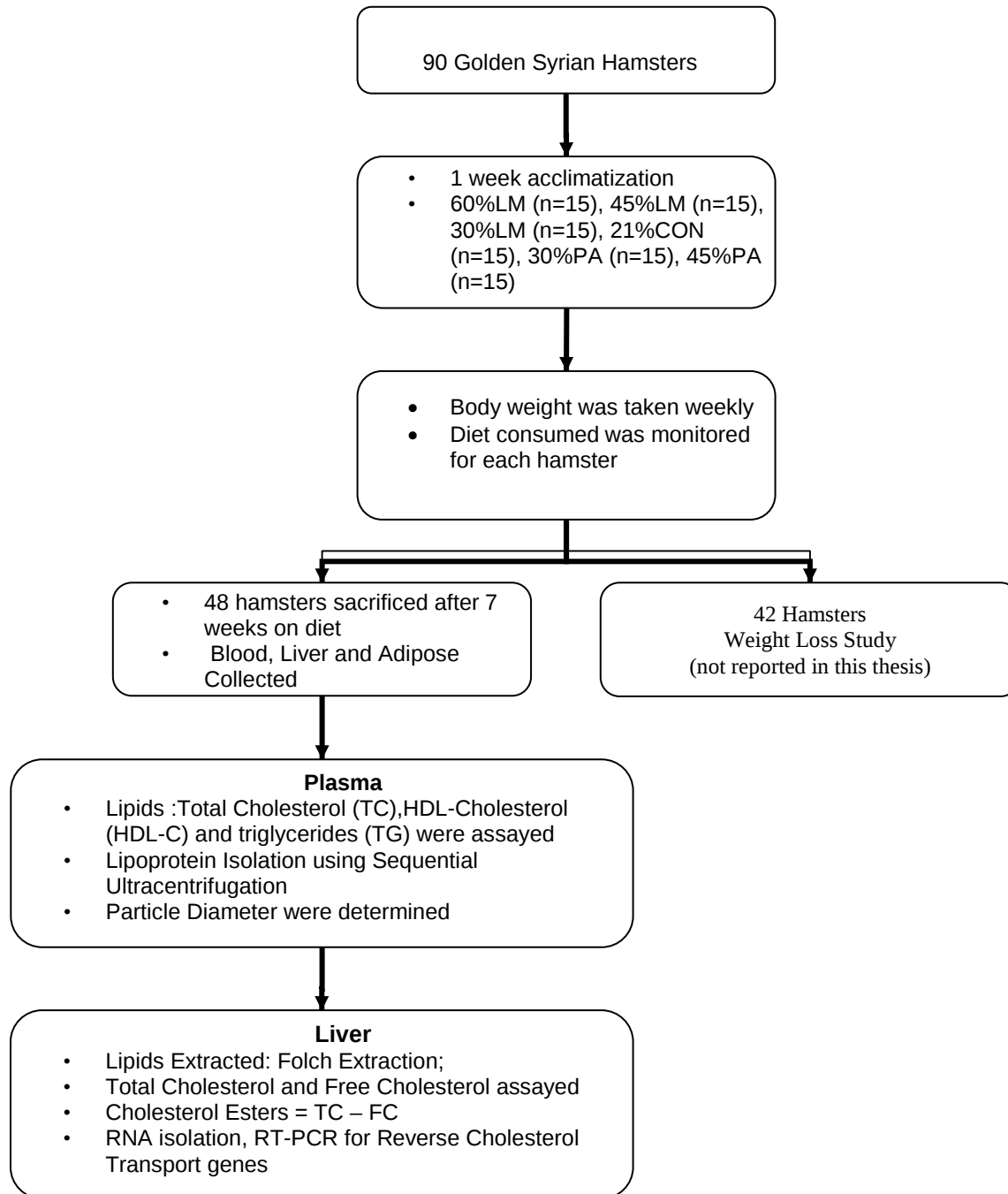
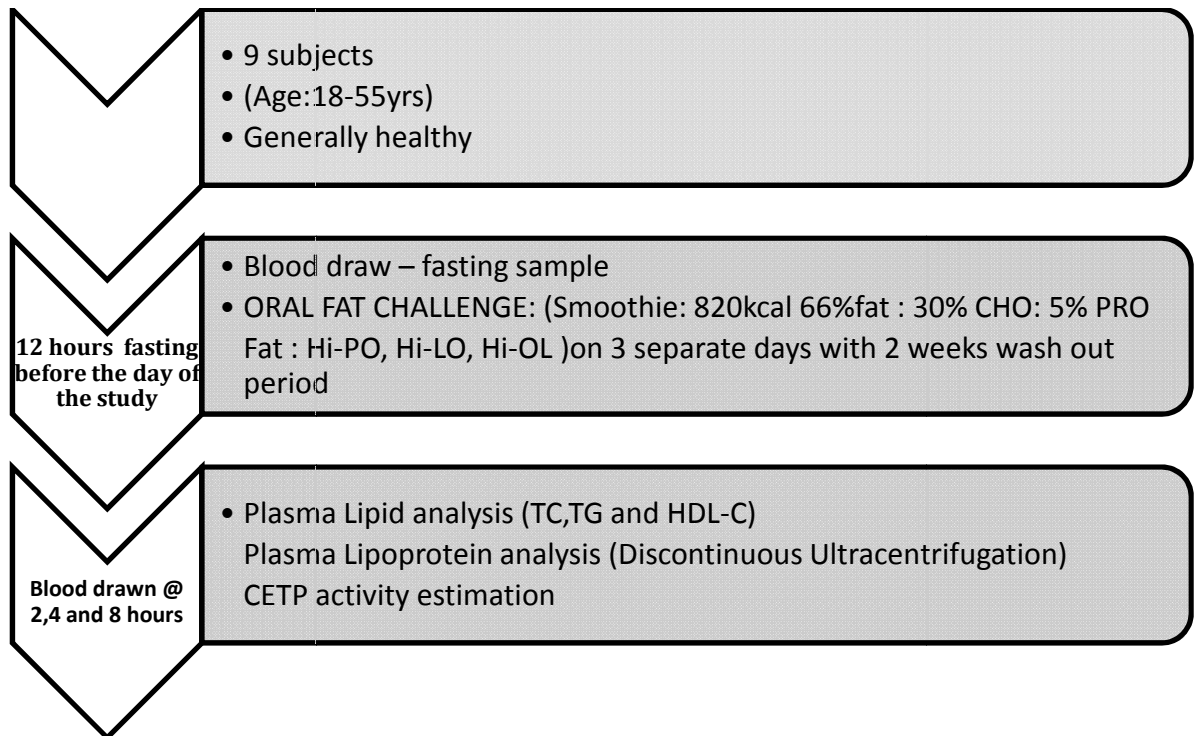


Figure 2.4: Specific Aim 3: Flowchart of Study Design for Postprandial Human Study.



Chapter 3: Results

Specific Aim 1

The **first objective** of the present study was to evaluate the effects of naturally occurring, blended, interesterified and genetically modified oils (with different fatty acid compositions) on lipid and lipoprotein metabolism in two different animal models. Gerbils (Experiment 1 and 2) were used as they are highly sensitive to fatty acid manipulations and Hamsters (Experiment 3) are widely accepted as appropriate models for human lipoprotein metabolism. By employing both animal models, any alterations in lipoprotein metabolism attributed to the dietary fats can be readily determined.

Diet Consumed

Gerbils and hamsters were fed purified pelleted diets. Food intake was monitored every two to three days and was estimated by subtracting the diet spilled in the cage from the amount of diet provided.

Experiment 1- Pilot Gerbil Study

The gerbils were randomly assigned to one of the feeding groups - CHOW (CHOW, n=3), Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=6), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=6) or a Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=6). Over the four week feeding period, there were no significant differences in food intakes between the diet groups (Table 3-1).

Experiment 2- Gerbil Study

The gerbils were randomly assigned to one of the feeding groups - CHOW (CHOW, n=4), Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM,

n=13), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=13), Stearic acid and Linoleic Acid supplemented diet using Interesterified Soybean Oil (Hi-SLO, n=15). Over the four week feeding period, there were no significant differences in food intakes between the diet groups (Table 3-2).

Experiment 3- Hamster Study

The hamsters were randomly assigned to one of the feeding groups - Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15). Over the feeding period, there were no significant differences observed in food intakes between the diet groups (Table 3-3).

Body Weight and Liver Weight

During the four week feeding period, there was a progressive increase in body weight with age in gerbils and hamsters for all diet groups indicating adequacy of the diets.

Experiment 1- Pilot Gerbil Study

There were no significant differences observed for change in body weight and liver weight between the diet groups after the four week feeding period of the study.

Liver weight taken as a percentage of body weight was not significantly different between the diet groups (Table 3-1).

Experiment 2- Gerbil Study

There were no significant differences observed for change in body weight and liver weight between the diet groups after the four week feeding period of the study. When liver weight was taken as a percentage of body weight, the ratio of liver to body weight was significantly lower in the Hi-SLO and Hi-LO Low-LN group compared with Hi-LM and Hi-OL groups (Table 3-2).

Experiment 3- Hamster Study

There were no significant differences in the growth of the hamsters over the 4 week feeding period between the diet groups (Figure 3-1). No significant differences were observed for change in body weight, liver weight and liver weight to body weight between the five diet groups over the 4 weeks of the feeding period (Table 3-3).

Plasma Total Cholesterol, HDL-Cholesterol and Triglycerides

Experiment 1- Pilot Gerbil Study

Analyses of plasma revealed that the mean plasma TC was significantly lower for Hi-OL and Hi-LO diet groups compared to Hi-LM ($p \leq 0.05$). Also, mean plasma HDL-C was significantly lower for Hi-OL and Hi-LO diet groups compared to Hi-LM ($p \leq 0.05$) (Figure 3-2). Lauric acid has the greatest Total cholesterol raising effect and also raises HDL-C most significantly compared to other fatty acids [30, 76]. This is more pronounced in the gerbil model as in gerbils about 70% of TC is transported in HDL [71, 77]. Based on the changes in TC and HDL-C, the TC:HDL-C ratio was

significantly lower in Hi-LM diet groups compared with Hi-OL and Hi-LO groups ($p \leq 0.05$) (Figure 3-3).

Mean plasma triglycerides were significantly higher in the Hi-LM diet group compared with Hi-OL and Hi-LO groups ($p \leq 0.05$) (Figure 3-4). This is in agreement with the effect seen with feeding high Lauric and Myristic acid diet in a gerbil model compared to diet high in PUFA[71].

Experiment 2- Gerbil Study

Analyses of plasma revealed that the mean plasma TC was significantly lower for all diet groups compared to Hi-LM ($p \leq 0.05$). Also, mean plasma HDL-C was significantly lower for all diet groups compared to Hi-LM ($p \leq 0.05$) (Figure 3-5). As mentioned earlier, lauric acid has the greatest total cholesterol raising effect and also raises HDL-C most significantly [30, 76]. Since the increase in TC was proportional to the increase in HDL-C for Hi-LM, the TC:HDL-C ratio was not significantly different between the diet groups (Figure 3-6).

Mean plasma triglycerides were significantly higher in the Hi-LM diet group compared with Hi-OL and Hi-PO groups ($p \leq 0.05$) (Figure 3-7).

Experiment 3- Hamster Study

Analyses of plasma revealed that the mean plasma TC was significantly lower for all diet groups compared to Hi-LM and Hi-LO diets ($p \leq 0.05$). Also, mean plasma HDL-C was significantly lower for Hi-OL and Hi-PO diet groups compared to Hi-LM and Hi-LO diet groups ($p \leq 0.05$) (Figure 3-8). Since the increase in TC was proportional to the increase in HDL-C, the TC:HDL-C ratio was not significantly different between the diet groups (Figure 3-9).

Mean plasma triglycerides (Figure 3-10) were not significantly different between the diet groups.

Lipoprotein Fractions Analyses

Experiment 2- Gerbil Study

Fractions were designated based on plasma density cuts for gerbils; fractions 1-3 ($d < 1.018 \text{ g/mL}$) are denoted as “VLDL”, fractions 4-8 ($1.018 < d < 1.063 \text{ g/mL}$) are denoted as “LDL” and fractions 9-15 ($d > 1.063 \text{ g/mL}$) are denoted as “HDL” (Figure 3-11).

In the VLDL fraction, there were no significant differences observed for total cholesterol, phospholipids and protein. For triglycerides in the VLDL fraction, Hi-LM showed the maximum increase compared to all other diet groups although it did not reach significance ($p=0.16$) (Figure 3-12A). In the LDL fraction, total cholesterol and phospholipids were lower in all diet groups compared with Hi-LM. Also, protein for diets Hi-PO and Hi-OL were significantly lower compared with Hi-LM (Figure 3-12B). In the HDL fraction, total cholesterol, phospholipids and protein was lower in all diet groups compared with Hi-LM (Figure 3-12C). The analyses of the lipoproteins is in consensus with the results seen in plasma, where the triglycerides were higher in the Hi-LM group compared to other diet groups as VLDL is the major carrier of triglycerides. Also, In the LDL and HDL fractions total cholesterol was highest in the Hi-LM group compared to all other diet groups as seen in plasma analyses.

Experiment 3- Hamster Study

CETP transfers cholesterol esters from HDL to VLDL and LDL in exchange for triglycerides. Hamsters show CETP activity similar in humans and therefore can help in

better understanding of the changes for the LDL and HDL fractions in TG and CE. To fully elucidate the changes, plasma lipoproteins were separated using sequential ultracentrifugation. Three lipoprotein fractions were isolated using density cuts of lipoproteins for humans, namely, VLDL/IDL (1.006-1.019 g/mL), LDL (1.019-1.055 g/mL) and HDL (1.055-1.21 g/mL). For each fraction, total cholesterol, triglycerides, free cholesterol, phospholipids and protein were determined. Cholesterol esters were computed as a difference in total and free cholesterol.

In the VLDL fraction, there were no significant differences observed in the composition of the particle between the diet groups. In the LDL fraction, triglycerides were higher in the Hi-LM and Hi-PO diet groups compared with Hi-LO and Hi-LO Low-LN diet groups ($p \leq 0.05$). In the HDL fraction, cholesterol esters were lower in the Hi-LO group compared to Hi-LM, Hi-LO Low-LN and Hi-PO diet groups ($p \leq 0.05$). Also, Hi-LM contained the lowest triglycerides compared with all other diet groups ($p \leq 0.05$) (Table 3-4).

Small LDL particles are considered a high risk for atherosclerosis compared to larger LDL particles [29]. On the other hand, small HDL particles are considered more cardio-protective compared with larger HDL particles [78]. Analyses of particle diameters revealed, LDL particle diameter for Hi-LO Low-LN was significantly smaller compared to Hi-PO diet group ($p \leq 0.05$). Also, Hi-LM and Hi-LO showed significantly smaller HDL particle diameter compared to Hi-LO Low-LN, Hi-PO and Hi-OL diet groups ($p \leq 0.05$) (Figure 3-13). CETP transfers cholesterol esters from HDL to VLDL and LDL in exchange for triglycerides. Hamsters show CETP activity similar in humans and

therefore present a good model to understand the changes seen in the LDL and HDL fractions in the triglyceride and cholesterol esters.

Liver lipids Analyses

Experiment 1- Pilot Gerbil Study

Analyses of the liver revealed a no significant differences between the diet groups for total cholesterol and free cholesterol. Cholesterol esters were calculated as a difference between total cholesterol and free cholesterol. No significant differences were seen for cholesterol esters between the diet groups (Table 3-5).

Experiment 2- Gerbil Study

Analyses of the liver revealed no significant differences between the diet groups for total cholesterol, free cholesterol and cholesterol esters except total cholesterol between Hi-LM and Hi-LO Low-LN diet groups (Table 3-6).

Experiment 3- Hamster Study

Analyses of the liver revealed no significant differences between the diet groups for total cholesterol, free cholesterol and cholesterol (Table 3-7).

Expression of the Reverse Cholesterol Transport genes

RNA was isolated from the hamster liver and cDNA was synthesized using random primers. Real time PCR was performed for reverse cholesterol transport genes, namely, ABCA1, ApoA1, CETP and SR-B1 using specific primers. GAPDH was used as an endogenous control. Relative gene expression was computed to determine fold change compared to Hi-LM (positive control).

Relative to Hi-LM diet all diets showed 2 fold decreased gene expression for **ABCA1** (Figure 3-14).

Relative to Hi-LM diet all diet groups showed increased gene expression for **ApoA1** with Hi-OL showing 2.2 fold increase compared to the Hi-LM group and 1.5 fold increase compared to other diet groups (Figure 3-14).

Relative to Hi-LM diet group Hi-LO Low-LN showed similar gene expression, Hi-LO and Hi-PO showed almost 2.5 fold decrease in gene expression compared to Hi-LM diet group. Hi-OL on the other hand showed a 2.6 fold increase in expression for **CETP** relative to Hi-LM diet group (Figure 3-14).

The gene expression for **SR-B1** decreased almost 2.5 fold for Hi-LO Low-LN, Hi-LO and Hi-PO diet groups whereas Hi-OL showed similar gene expression for SR-B1 compared with Hi-LM (Figure 3-14).

To summarize, the results of feeding dietary fats with higher saturation in gerbils revealed that gerbils showed changes in TC ranging from 99 to 306mg/dL (184 ± 45) suggesting the effectiveness of the model to fatty acid sensitivity. Compared with Hi-LM, all diets showed decrease in TC and HDL-C. All test diets with higher dietary fat saturation had similar plasma and liver lipid profiles.

The results of feeding study in hamsters revealed changes in TC in Hi-LO Low-LN, Hi-PO and Hi-OL were lower compared with Hi-LM and Hi-LO and HDL-C was lower in Hi-PO and Hi-OL compared with Hi-LM and Hi-LO. There were no significant differences in TC: HDL-C ratio and TG between the diet groups. Lipoprotein TG composition was significantly different between diet groups for LDL and HDL particles. In the HDL particles, CE was significantly lower in case of Hi-LO

compared with Hi-LM, Hi-LO Low-LN and Hi-PO. There were no significant differences observed in liver lipids between the diet groups. Relative fold differences were observed in the expression of the RCT genes between the diet groups compared to Hi-LM.

Specific Aim 2

The **second objective** of the study was to examine the extent to which the amount and type of Saturated Fatty acids affect lipoproteins. Since hamsters are shown to depict human lipoprotein metabolism, they were chosen as the model of evaluation for the study.

Diet Given and Consumed

In order to see the effects in response to type and amount of the saturated fatty acids, pelleted diet was fed to the hamsters. Food intake was monitored every two to three days. After a week of acclimatization, the hamsters were randomly assigned to one of the feeding groups - Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=15), 45%LM (n=15), 30%LM (n=15); 21%CON (n=15); and Palmitic Acid supplemented group with varying percentages of fat - 30%PA (n=15), 45%PA (n=15). Over the 7 week feeding period, there were no significant differences in amount of food provided between the diet groups. Analyses of diet consumed revealed a significant higher consumption of food intake for 45%LM diet group compared with 60%LM and 45%PA. Food consumption in other diet groups was not significantly different (Figure 3-15).

For the study, diets were formulated such that percentage of calories from protein, monounsaturated fatty acids and polyunsaturated fatty acids were kept constant while calories from fat were replaced with calories from carbohydrate. 60%LM provided the highest and 21% CON the least kcalories among the diet groups. Kcalories from diets containing 45%fat were significantly higher than 30% fat (Figure 3-16). Analyses of kcalories consumed revealed kcalories consumed for 60% and 45% LM diet groups was significantly higher compared to all other diet groups. 21% CON group consumed lesser kcalories compared to 45%PA, but there were no significant differences in the kcalories consumed between 21%CON and 30% fat diet groups (Figure 3-16).

Body Weight and growth patterns were similar between all diet groups.

There was a progressive increase in body weight with age for all diet groups over the 7 week feeding period indicating the adequacy of the diets with the trend indicating the highest increase in body weight for 45% fat diet groups compared with other diet groups. Even though there was a trend observed, the changes did not show significant differences (Figure 3-17).

There were no significant differences in the initial body weight, but the final body weight for 21%CON diet was significantly lower than the 45%LM diet group reflected as a change in body weight (Figure 3-18). The change in body weight was not significantly different between the other diet groups. Plotting the kcalories consumed against change in body weight revealed that with an increase in the consumption of kcalories, there was a linear increase in the change in body weight (Figure 3-19).

Liver weight (n=48) and Liver weight to body weight (n=48) ratios did not differ significantly between the six diet groups (Table 3-8).

Plasma Total Cholesterol, n-HDL-Cholesterol and Triglycerides

To fully elucidate the effects of the amount and type of saturated fatty acid, plasma and liver lipid profiles for 48 hamsters were examined. The 48 hamsters were randomly chosen based on their final body weights. Analyses of plasma revealed that the mean plasma TC (Figure 3-34) was significantly higher in the 60%LM compared with all diet groups except 45%LM ($p \leq 0.05$). Also the hypercholesterolemic effect was higher in 45%LM compared with 21%CON, and PA supplemented groups. Mean plasma HDL-C (Figure 3-35) was significantly lower in 21%CON compared with 60%LM, 45%LM and 45%PA diet groups ($p \leq 0.05$). Plasma nHDL-C (Figure 3-36) was significantly higher in the 60%LM compared with all diet groups except 45%LM ($p \leq 0.05$). Also, nHDL-C was higher in 45%LM compared with 21%CON, and PA supplemented groups. TC:HDL-C and nHDL-C:HDL-C ratios did not differ significantly between the diets (Table 3-9).

Mean plasma triglycerides (Figure 3-34) were significantly different between the 60%LM and 21%CON diet groups ($p \leq 0.05$). Triglycerides were higher in all diets compared with 21%CON, but they did not reach significance.

Lipoprotein Fraction Analyses

CETP transfers cholesterol esters from HDL to VLDL and LDL in exchange for triglycerides. Hamsters show CETP activity similar in humans and therefore can help in

better understanding of the changes for the LDL and HDL fractions in TG and CE. To fully elucidate the changes, plasma lipoproteins were separated using sequential ultracentrifugation. Three lipoprotein fractions were isolated using density cuts of lipoproteins for humans, namely, VLDL/IDL (1.006-1.019 g/mL), LDL (1.019-1.055 g/mL) and HDL (1.055-1.21 g/mL). For each fraction, total cholesterol, triglycerides, free cholesterol, phospholipids and protein were determined. Cholesterol esters were computed as a difference in total and free cholesterol.

In the VLDL fraction, free cholesterol was higher for 60%LM compared with PA supplemented diets. Also, compared to 30%PA, 45%LM showed a significant increase in free cholesterol ($p \leq 0.05$). Cholesterol esters were lowest in the 45%PA group compared with all other diet groups ($p \leq 0.05$). Protein showed highest increases for 30%PA compared with all other diet groups ($p \leq 0.05$) (Table 3-10). Total cholesterol in the VLDL fraction was significantly higher for 60%LM compared to 30% fat and 45% PA diet groups (Table 3-11).

In the LDL fraction, triglycerides were lowest in the 60%LM diet group compared with all other diet groups ($p \leq 0.05$). Also, 60%LM showed highest increases in cholesterol esters, but did not show a significant difference (Table 3-10). Total cholesterol in the LDL fraction was significantly higher for 60%LM compared to all diet groups (Table 3-11).

In the HDL fraction, 60%LM showed highest triglycerides and protein compared with all other diet groups, but significant lower phospholipids level compared with all diets except 45%LM ($p \leq 0.05$) (Table 3-10). Total cholesterol in the HDL fraction was significantly higher for 60%LM compared to 30% LM diet group (Table 3-11).

Particle diameters

Small LDL particles are considered a high risk for atherosclerosis compared to larger LDL particles [29]. On the other hand, small HDL particles are considered more cardio-protective compared with larger HDL particles [78]. Analyses of particle diameters revealed no significant differences between the diet groups (Figure 3-22).

Liver lipids Analyses

Analyses of the liver revealed no significant differences between the diet groups for total cholesterol, free cholesterol between the diet groups. Cholesterol esters were computed as the difference between TC and FC. 30%PA showed significantly higher CE compared with 45%LM (Table 3-12).

Expression of the Reverse Cholesterol Transport genes

RNA was isolated from the hamster liver and cDNA was synthesized using random primers. Real time PCR was performed for reverse cholesterol transport genes, namely, ABCA1, ApoA1, CETP and SR-B1 using specific primers. GAPDH was used as an endogenous control. Relative gene expression was computed to determine fold change compared to 21%CON (positive control).

Relative to 21% CON, 60%LM, 30% LM, 30% PA and 45% PA showed similar gene expression for **ABCA1** whereas 45% LM showed 2 fold decreased gene expression for ABCA1, relative to 21% CON (Figure 3-23).

For **ApoA1**, 45% LM showed 2.8 fold increase in gene expression whereas 60%LM, 30%LM, 30%PA and 45% PA showed decreased gene expression with 30% PA and 45% PA showing 6 and 4.3 fold decrease in expression relative to 21% CON. 30% PA and 45% PA showed 2 to 4 fold higher decrease in expression compared to 60% and 30% LM (Figure 3-23).

60%LM, 30% LM, 30% PA and 45%PA showed decreased gene expression for **CETP**, relative to 21% CON. 60% LM and 45% PA showed a 3 fold decrease in the expression for CETP compared to 21% CON and a 2 fold decrease compared with 30% PA. 45% LM on the other hand showed a 2 fold increase in the expression of CETP relative to 21%CON (Figure 3-23).

60% LM, 30% LM, and 30% PA showed similar gene expression for **SR-B1** relative to 21% CON whereas 45% LM and 45% PA showed 2 fold decreased gene expression relative to 21%CON. (Figure 3-23).

To summarize, the results from feeding diets with different type and amounts of saturated fatty acids in hamsters revealed that kcalorie consumption was linearly related to change in Body Weight. Plasma TC and n-HDL-C was lower in all diet groups compared with 60%LM and 45% LM supplemented groups. No significant difference in HDL-C and TC:HDL-C, nHDL: HDL ratio were observed between the diet groups. 60% LM lipoprotein profile showed highest CE and lowest TG in the LDL particle and low FC in the HDL particle compared with other groups. VLDL and LDL fractions for 60% LM supplemented group carried the highest amounts of TC. Particle sizes were not significantly different between the diet groups. Relative fold

differences were observed in the expression of RCT genes between the diet groups relative to 21%CON.

The gerbil and hamster studies (Specific Aim 1) showed no significant differences in the lipid and liver profiles after feeding blended, genetically modified or interesterified fat compared with naturally occurring coconut and soybean oil. Also, feeding palmitic acid showed no adverse effects at 45% and 30% fat intake (Specific Aim 2). These results led us to see the effects of these oils in human postprandial state using an oral fat challenge (Specific Aim 3).

Specific Aim 3

The postprandial state is a reflection of the metabolic state in humans due to regular diet intake. The **third objective** of the study was to evaluate postprandial changes in lipids following an oral challenge to test oils - Linoleic acid rich soybean oil (Hi-LO), Palm-Olein rich Palm oil (Hi-PO) and Oleic acid rich veggie fruit oil (Hi-OL) . 9 subjects started the study, but only 6 subjects completed the study with all the 3 diets. The data presented is for n=6 who completed all the 3 oral fat challenges. The 3 subjects that are not included in the study were dropped out due to not completing all the 3 diets. The human study was planned to be completed within the 4 months of the semester, therefore, we could not have the subjects come in at a later time.

To elucidate the postprandial effects of the test oils, plasma lipids and lipoproteins were analyzed. Triglycerides peaked at 2 hours after the ingestion of the test oil diets. No significant differences were seen in the response of the test oil diets between the diet groups at 0, 2, 4 and 8 hours. Mean plasma total cholesterol showed no significant

differences between the three diets. There was a small non-significant increase in total cholesterol from baseline at 2 hours, and then returned to baseline at 4 and 8 hours. Mean plasma HDL-C showed no significant difference between the diets at 0, 2, 4 and 8 hours after the ingestion of the test fats (Table 3-13).

To evaluate the postprandial effects of dietary fatty acids on lipoproteins, triglyceride rich fractions were isolated from plasma samples by sequential discontinuous ultracentrifugation for each time point namely Sf > 400, Sf 60-400 and Sf 20-60. Analyses of the lipoprotein subfractions revealed no significant differences in the triglyceride content between the test diet groups at 0, 2, 4 and 8 hours (Table 3-14).

CETP is active during triglyceridemic state. CETP activity was measured in plasma at 0, 2, 4 and 8 hours. Analyses of the CETP activity in plasma revealed no significant differences in activity between the diet groups at 0, 2, 4 and 8 hours.

To summarize, an oral fat challenge with Palm oil, Blended oil and Soybean oil showed no significant difference in plasma lipids, chylomicron fractions and CETP activity at 0, 2, 4 and 8 hours.

In Summary,

- Specific Aim 1: In the animal models : no adverse effects were seen in the lipid and lipoprotein profiles for the naturally occurring soybean oil, blended veggie oil, genetically modified soybean oil and palm olein oil compared with coconut supplemented diet- positive control
- Specific Aim 2: Palmitic acid supplemented diets showed improvement in TC and n-HDL-C compared with the lauric and myristic supplemented groups.

- Specific Aim 3: In the human study: No significant differences were seen in plasma lipids with palm oil or blended veggie oil diets compared with soybean diet.

Table 3-1: Specific Aim 1: Experiment 1- Diet Consumed, Change in Body Weight (BW) and Liver Weight for gerbils in the pilot study over 4 weeks feeding period

	CHOW (n=3)	Hi-LM (n=6)	Hi-OL (n=6)	Hi-LO (n=6)
Diet Consumed (g)	6.7±0.6	5.2±0.4	5.3±0.5	5.2±0.4
Change in BW(g)	15±3.0	9±3.6	7±1.3	9±6.4
Liver Weight (g)	2.1±0.3	1.9±0.2	1.9±0.1	1.7±0.2

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=6), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=6) or Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=6) for 4 weeks. Values are the Mean ± SD. For comparative purposes, the values from 3 chow-fed gerbils are also shown (these animals were not included in the statistical analyses). Data was analyzed using a one way analysis of variance (ANOVA).

- Gerbils were fed every 2-3 days. Diet consumed was estimated as the difference between diet provided and spilled diet collected from the cage. There was no significant difference in the diet consumed between the diet groups.
- Gerbils were weighed weekly. Change in body weight was taken as the difference in initial weight and weight at the end of the 4 week feeding period. There were no significant differences in body weight between the diet groups.
- Animals were sacrificed after 4 week feeding period. Livers were harvested and weighed. There were no significant differences observed between the diet groups following the 4 week feeding period.

Table 3-2: Specific Aim 1: Experiment 2- Diet Consumed, Change in Body Weight and Liver weight for gerbils after 4 weeks feeding period

	CHOW	Hi-LM	Hi-SLO	Hi-LO	Hi-PO	Hi-OL
	(n=4)	(n=13)	(n=15)	Low-LN (n=13)	(n=15)	(n=15)
Diet Consumed (g)	6.5±0.1	4.7±0.4	5.2±0.2	4.8±0.1	4.6±0.2	5.0±0.3
Change in BW (g)	8±7.2	10±4.4	11±4.1	9±4.0	8±4.1	8±4.7
Liver Weight (g)	2.1±0.5	1.8±0.2	1.6±0.2	1.6±0.1	1.6±0.1	1.8±0.3
LW: BW ratio (%)	2.9±0.6	2.7±0.2 ^a	2.4±0.1 ^b	2.4±0.2 ^b	2.6±0.2 ^{ab}	2.7±0.3 ^a

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=13), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=13), Stearic acid and Linoleic Acid supplemented diet using Interesterified Soybean Oil (Hi-SLO, n=15) for 4 weeks. Values are the Mean ± SD. For comparative purposes, the values from 3 chow-fed gerbils are also shown (these animals were not included in the statistical analyses). Data was analyzed using a one way analysis of variance (ANOVA).

- Gerbils were fed every 2-3 days. Diet consumed was estimated as the difference between diet provided and spilled diet collected from the cage. There was no significant difference in the diet consumed between the diet groups.
- Gerbils were weighed weekly. Change in body weight was taken as the difference in initial weight and weight at the end of the 4 week feeding period. There were no significant differences in body weight between the diet groups.
- Animals were sacrificed after 4 week feeding period. Livers were harvested and weighed. There were no significant differences observed between the diet groups following the 4 week feeding period.
- Liver weight to body weight ratio was significantly higher in Hi-LM and Hi-OL diet groups compared to Hi-Lo Low-LN and Hi-PO diet groups. Diet groups in the same row sharing different superscripts were significantly different from each other ($p \leq 0.05$).

Table 3-3: Specific Aim 1: Experiment 3- Diet Consumed, Change in Body Weight and Liver weight for hamsters after 4 weeks of feeding period

	Hi-LM (n=15)	Hi-LO Low-LN (n=15)	Hi-PO (n=15)	Hi-LO (n=15)	Hi-OL (n=15)
Diet Consumed (g)	5.7±0.6	6.2±0.4	5.9±0.4	6.1±0.6	6.2±0.5
Change in BW (g)	28±6.4	33±5.6	31±5.9	29±8.7	31±8.6
Liver Weight (g)	4.9±0.7	5.1±0.7	4.8±0.7	4.8±0.7	4.5±0.5
LW: BW ratio (%)	4.2±0.5	4.2±0.6	4.0±0.4	4.1±0.4	3.9±0.3

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15) for 4 weeks. Values are the Mean ± SD. Data was analyzed using a one way analysis of variance (ANOVA).

- Hamsters were fed every 2-3 days. Diet consumed was estimated as the difference between diet provided and spilled diet collected from the cage. There was no significant difference in the diet consumed between the diet groups.
- The hamsters were weighed weekly. Change in body weight was taken as the difference in initial weight and weight at the end of the 4 week feeding period. There were no significant differences in body weight between the diet groups.
- Animals were sacrificed after 4 week feeding period. Livers were harvested and weighed. There were no significant differences observed between the diet groups following the 4 week feeding period.
- Liver weight to body weight ratio was not significantly different between the diet groups.

Table 3-4: Specific Aim 1: Experiment 3 - Lipoprotein particle composition for hamsters after 4 week feeding period

	Hi-LM	Hi-LO Low-LN	Hi-PO	Hi-LO	Hi-OL
VLDL					
% FC	6.4±0.8	6.6±0.9	5.8±0.6	5.5±0.8	5.8±0.5
% CE	13.6±1.8	12.8±1.4	11.2±2.1	10.8±2.2	13.2±1.8
% TG	53.8±4.0	54.8±3.2	57.6±4.8	57.4±5.0	54.6±4.3
% PL	15.9±0.5	16.5±0.8	15.9±1.2	15.5±1.1	15.7±0.9
% Pro	10.2±1.6	9.3±1.0	9.4±1.5	10.8±2.6	10.7±1.5
LDL					
% FC	5.8±1.8	5.9±1.9	5.1±1.2	7.3±0.7	5.5±1.1
% CE	27.1±4.5	27.0±3.5	29.5±1.4	29.5±0.9	29.1±2.7
% TG	10.6±1.8 ^a	7.1±1.7 ^b	10.2±1.2 ^a	8.2±1.2 ^b	7.6±1.3 ^b
% PL	22.4±3.0	23.4±2.4	22.5±0.5	23.2±1.5	22.7±2.3
% Pro	34.1±3.2	36.5±7.4	32.6±2.2	31.8±1.7	35.1±2.1
HDL					
% FC	3.6±1.0	2.9±1.0	3.3±0.6	3.9±0.8	2.6±0.9
% CE	19.1±0.4 ^a	19.6±1.6 ^a	19.6±1.0 ^a	16.9±0.6 ^b	18.0±2.2 ^{ab}
% TG	0.7±0.1 ^a	3.8±1.0 ^b	3.5±1.9 ^b	2.3±0.8 ^b	5.3±1.4 ^b
% PL	33.7±1.9 ^a	31.4±1.4 ^b	33.3±1.2 ^a	31.9±1.0 ^{ab}	30.3±1.7 ^b
% Pro	42.9±2.5 ^{ab}	42.3±2.1 ^{ab}	40.3±1.7 ^a	45.1±1.7 ^b	43.8±3.1 ^b

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15) for 4 weeks. Plasma was isolated by centrifugation. Pooled plasma samples (3 - 4 per each diet group) were fractionated by sequential ultracentrifugation using the density cuts for humans - V+IDL fraction (1.006<d<1.019g/ml), LDL fraction (1.019<d<1.055) and HDL fraction (1.063<d<1.21g/ml). Fractions were Values are the Mean ± SD. Data was analyzed using a one way analysis of variance

(ANOVA). There was no significant difference in the composition of the V+IDL fraction between the diet groups. For LDL and HDL fractions, diet groups in the same row sharing different superscripts were significantly different from each other ($p \leq 0.05$).

Table 3-5: Specific Aim 1: Experiment 1- Pilot Gerbil Study Liver Lipids (mg/g liver) after 4 week feeding period

Liver lipids (mg/g liver)	CHOW (n=3)	Hi-LM (n=6)	Hi-OL (n=6)	Hi-LO (n=6)
Total Cholesterol	4.5±1.0	5.8±0.3	5.9±0.8	6.9±1.5
Free Cholesterol	1.8±0.5	2.5±0.3	2.3±0.3	2.7±0.6
Cholesterol Esters	2.7±0.6	3.3±0.3	3.5±0.7	4.3±1.2

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=6), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=6) or Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=6) for 4 weeks. Liver lipids were extracted using Folch's method. Total Cholesterol and Free cholesterol in the extracts were measured enzymatically. Cholesterol esters were calculated by subtracting Free Cholesterol from Total Cholesterol. Values are the Mean ± SD. For comparative purposes, the values from 3 chow-fed gerbils are also shown (these animals were not included in the statistical analyses). Data was analyzed using a one way analysis of variance (ANOVA). Liver lipids were not significantly different between the diet groups.

Table 3-6: Specific Aim 1: Experiment 2- Gerbil Study Liver Lipids (mg/g liver) for gerbils after 4 weeks feeding period

Liver lipids (mg/g liver)	CHOW (n=4)	Hi-LM (n=13)	Hi-SLO (n=15)	Hi-LO Low-LN (n=13)	Hi-PO (n=15)	Hi-OL (n=15)
Total Cholesterol	2.4±0.3	3.2±0.4 ^a	3.4±0.4 ^{ab}	3.7±0.4 ^b	3.6±0.4 ^{ab}	3.4±0.5 ^{ab}
Free Cholesterol	1.9±0.1	2.3±0.3	2.6±0.3	2.6±0.4	2.6±0.3	2.5±0.3
Cholesterol Esters	0.5±0.2	0.9±0.5	0.9±0.4	1.1±0.4	1.0±0.5	0.9±0.5

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=13), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=13), Stearic acid and Linoleic Acid supplemented diet using Interesterified Soybean Oil (Hi-SLO, n=15) for 4 weeks. Liver lipids were extracted using Folch's method. Total Cholesterol and Free cholesterol in the extracts were measured enzymatically. Cholesterol esters were calculated by subtracting Free Cholesterol from Total Cholesterol. Values are the Mean ± SD. For comparative purposes, the values from 3 chow-fed gerbils are also shown (these animals were not included in the statistical analyses). Data was analyzed using a one way analysis of variance (ANOVA). No significant differences were seen in the liver lipids between the diet groups.

Table 3-7: Specific Aim 1: Experiment 3- Liver Lipids (mg/g liver) for hamsters after 4 week feeding period

Liver lipids (mg/g liver)	Hi-LM (n=15)	Hi-LO Low-LN (n=15)	Hi-PO (n=15)	Hi-LO (n=15)	Hi-OL (n=15)
Total Cholesterol	3.7±0.5	3.9±0.4	3.8±0.5	4.0±0.6	3.9±0.5
Free Cholesterol	1.6±0.5	1.8±0.4	1.6±0.5	1.7±0.9	1.7 ±0.4
Cholesterol Esters	2.2±0.5	2.1±0.6	2.2±0.6	2.2±0.9	2.2±0.5

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15) for 4 weeks. Liver lipids were extracted using Folch's method. Total Cholesterol and Free cholesterol in the extracts were measured enzymatically. Cholesterol esters were calculated by subtracting Free Cholesterol from Total Cholesterol. Values are the Mean ± SD. Data was analyzed using a one way analysis of variance (ANOVA). No significant differences were seen in the liver lipids between the diet groups following the 4 week feeding period.

Table 3-8: Specific Aim 2: Body Weight and Liver Weight for hamster after 7 weeks feeding period

	60%LM (n=8)	45%LM (n=8)	30%LM (n=8)	21%CON (n=8)	30%PA (n=8)	45%PA (n=8)
Liver Weight (g)	5.7 ± 0.7	6.3 ± 0.9	5.5 ± 1.0	5.7 ± 1.0	5.4 ± 1.0	5.7 ± 1.1
LW/BW (%)	4.1± 0.3	4.3 ± 0.4	4.0 ± 0.5	4.3 ± 0.6	3.9 ± 0.5	4.1 ± 0.5

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=15); 45%LM (n=15), 30%LM (n=15) ; 21%CON (n=15); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=15); 45%PA (n=15) for 7 weeks. Values are the Mean ± SD. Data was analyzed using a one way analysis of variance (ANOVA).

- Animals were sacrificed after 4 week feeding period. Livers were harvested and weighed. There were no significant differences observed between the diet groups following the 7 week feeding period.
- Liver weight to body weight ratio was not significantly different between the diet groups.

Table 3-9: Specific Aim 2: Plasma lipoprotein ratios in hamsters after 7 weeks feeding period

	60%LM (n=8)	45%LM (n=8)	30%LM (n=8)	21%CON (n=8)	30%PA (n=8)	45%PA (n=8)
TC:HDL-C	7.7 ± 1.5	7.2 ± 0.8	7.2 ± 0.8	8.4 ± 0.9	7.0 ± 1.7	7.3 ± 0.9
nHDL: HDL-C	6.7 ± 1.5	6.2 ± 0.8	6.2 ± 0.8	7.4 ± 0.9	6.0 ± 1.7	6.3 ± 0.9

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=8); 45%LM (n=8), 30%LM (n=8) ; 21%CON (n=8); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=8); 45%PA (n=8) for 7 weeks. Values are the Mean ± SD. Data was analyzed using a one way analysis of variance (ANOVA).

Table 3-10: Specific Aim 2: Lipoprotein particle composition in hamsters after 7 weeks feeding period

	60%LM	45%LM	30%LM	21%CON	30%PA	45%PA
VLDL						
% FC	7.0±0.5 ^a	6.7±0.5 ^a	6.5±0.3 ^{ab}	5.9±0.5 ^{ab}	5.4±0.6 ^b	5.7±1.0 ^b
% CE	14.4±2.7 ^a	12.4±1.8 ^a	14.2±1.2 ^a	14.2±2.7 ^a	11.3±1.0 ^a	7.6±3.3 ^b
% TG	53.0±4.6 ^a	55.7±2.9 ^a	53.0±2.2 ^a	51.8±6.7 ^a	48.8±5.3 ^b	56.2±6.9 ^a
% PL	14.2±0.8	14.5±0.9	14.3±0.4	13.3±0.9	12.9±1.4	13.4±1.8
% Pro	11.4±2.4	10.6±1.7	12.0±0.9	14.8±3.3	21.6±6.0	17.1±5.4
LDL						
% FC	10.1±0.9	8.9±2.8	7.2±3.1	7.0±3.3	7.8±1.7	11.3±1.7
% CE	30.1±1.0	25.3±4.4	24.1±4.6	25.8±2.1	23.9±1.3	20.5±1.4
% TG	8.6±0.5 ^a	9.8±1.2 ^b	10.8±0.7 ^b	13.8±0.2 ^b	12.9±0.6 ^b	12.8±2.3 ^b
% PL	22.3±2.1	21.7±4.5	20.3±5.4	21.8±6.9	23.7±2.8	24.5±2.5
% Pro	28.8±1.0	34.5±11.1	37.7±12.8	31.6±11.7	31.7±5.2	30.8±2.6
HDL						
% FC	4.3±0.5 ^a	5.1±0.5 ^b	5.6±0.5 ^{ab}	5.4±0.3 ^{ab}	5.3±0.4 ^{ab}	5.2±0.6 ^{ab}
% CE	20.1±3.6	21.8±5.2	18.1±0.3	19.1±0.2	19.4±0.8	19.1±1.6
% TG	2.5±0.4 ^a	1.4±0.2 ^b	1.3±0.2 ^b	1.4±0.2 ^b	1.4±0.2 ^b	1.3±0.1 ^b
% PL	25.1±8.4 ^a	33.2±3.9 ^{ab}	36.0±1.3 ^b	35.2±1.4 ^b	35.6±1.2 ^b	36.6±1.0 ^b
% Pro	48.0±4.9 ^a	38.6±6.1 ^a	37.9±1.9 ^a	38.8±1.5 ^a	38.3±2.2 ^a	37.8±1.2 ^b

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=8); 45%LM (n=8), 30%LM (n=8) ; 21%CON (n=8); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=8); 45%PA (n=8) for 7 weeks. Plasma was isolated by centrifugation. Pooled plasma samples (4per each diet group) were fractionated by sequential ultracentrifugation using the density cuts for humans - V+IDL fraction (1.006<d<1.019g/ml), LDL fraction (1.019<d<1.055) and HDL fraction (1.063<d<1.21g/ml) Fractions values are the Mean ± SD. Data was analyzed using a one way analysis of variance (ANOVA). For the lipoprotein fractions, diet groups in the same row sharing different superscripts were significantly different from each other (p ≤ 0.05).

Table 3-11: Specific Aim 2: Total Cholesterol (mg/dL) in lipoproteins in hamsters after 7 weeks feeding period

	60%LM	45%LM	30%LM	21%CON	30%PA	45%PA
(mg/dL)						
VLDL	29 ± 5.6 ^a	19 ± 4.7 ^{ab}	18 ± 1.8 ^b	19 ± 6.3 ^{ab}	16 ± 3.7 ^b	18 ± 3.9 ^b
LDL	34 ± 5.2 ^a	20 ± 3.8 ^b	14 ± 3.8 ^b	13 ± 3.0 ^b	14 ± 3.8 ^b	15 ± 1.4 ^b
HDL	41 ± 8.1 ^a	55 ± 11.6 ^{ab}	59 ± 7.2 ^b	48 ± 7.7 ^{ab}	55 ± 7.1 ^{ab}	58 ± 1.5 ^{ab}

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=8); 45%LM (n=8), 30%LM (n=8) ; 21%CON (n=8); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=8); 45%PA (n=8) for 7 weeks. Plasma was isolated by centrifugation. Pooled plasma samples (4per each diet group) were fractionated by sequential ultracentrifugation using the density cuts for humans - V+IDL fraction (1.006<d<1.019g/ml), LDL fraction (1.019<d<1.055) and HDL fraction (1.063<d<1.21g/ml). Total cholesterol in the fractions was measured enzymatically. Values are the Mean ± SD. Data was analyzed using a one way analysis of variance (ANOVA). For the lipoprotein fractions, diet groups in the same row sharing different superscripts were significantly different from each other (p ≤ 0.05).

Table 3-12: Specific Aim 2: Liver Lipids (mg/g liver) for hamsters after 7 weeks feeding period

Liver lipids (mg/g liver)	60%LM (n=8)	45%LM (n=8)	30%LM (n=8)	21%CON (n=8)	30%PA (n=8)	45%PA (n=8)
Total Cholesterol	5.2 ± 0.9	4.9 ± 1.3	4.5 ± 0.5	5.2 ± 1.2	5.5 ± 1.1	5.3 ± 1.6
Free Cholesterol	3.9 ± 0.8	3.8 ± 1.2	3.0 ± 0.8	3.8 ± 0.8	3.8 ± 1.2	3.8 ± 1.7
Cholesterol Esters	1.3±0.5 ^{ab}	1.1 ± 0.5 ^a	1.5±0.4 ^{ab}	1.5±0.5 ^{ab}	1.7 ± 0.6 ^b	1.5±0.3 ^{ab}

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=8); 45%LM (n=8), 30%LM (n=8) ; 21%CON (n=8); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=8); 45%PA (n=8) for 7 weeks. Liver lipids were extracted using Folch's method. Total Cholesterol and Free cholesterol in the extracts were measured enzymatically. Cholesterol esters were calculated by subtracting Free Cholesterol from Total Cholesterol. Values are the Mean ± SD. Data was analyzed using a one way analysis of variance (ANOVA). No significant differences were seen in the liver lipids between the diet groups for total cholesterol and free cholesterol. For Cholesterol esters, diet groups in the same row sharing different superscripts were significantly different from each other ($p \leq 0.05$).

Table 3-13: Specific Aim 3: Plasma Lipids at 0, 2, 4 and 8 hours following the oral fat challenge

Plasma Lipids (mg/dL plasma)	Hi-PO (n=6)	Hi-LO (n=6)	Hi-OL (n=6)
hours			
Triglycerides			
0	88 ± 21.0	82 ± 39.9	80 ± 28.7
2	173 ± 44.0	146 ± 73.8	187 ± 81.4
4	123 ± 17.9	137 ± 44.8	128 ± 55.9
8	54 ± 11.2	59 ± 22.0	54 ± 18.3
Total Cholesterol			
0	183 ± 36.6	189 ± 16.9	190 ± 31.0
2	191 ± 38.5	195 ± 23.1	200 ± 43.7
4	190 ± 36.3	187 ± 23.4	191 ± 35.5
8	181 ± 28.6	188 ± 24.3	188 ± 32.8
HDL-C			
0	54 ± 16.1	56 ± 14.0	55 ± 17.8
2	52 ± 13.2	56 ± 15.3	60 ± 15.3
4	52 ± 13.4	55 ± 13.3	58 ± 15.0
8	56 ± 16.3	58 ± 15.0	60 ± 16.6

Subjects were given an oral fat challenge with either Linoleic Acid supplemented fat using soybean oil - Hi-LO (n=6), Palmitic acid supplemented fat using Palm Olein - Hi-PO (n=6) or Oleic acid supplemented fat using Veggie fruit oil - Hi-OL (n=6) with a 2 week wash out period between each challenge. Blood was collected at 0, 2, 4 and 8 hours. Plasma was isolated by centrifugation. Triglycerides and Total Cholesterol were measured enzymatically. Following precipitation of apoB lipoproteins, HDL-C was measured enzymatically. Values are the Mean ± SD. Data was analyzed using a one way analysis of variance (ANOVA). There were no significant differences in the plasma lipids at 0, 2, 4 and 8 hours between the diets.

Table 3-14: Specific Aim 3: Plasma Lipoprotein Subfractions for Postprandial Human Study

Plasma Lipids (mg/dL plasma)	Hi-PO (n=6)	Hi-LO (n=6)	Hi-OL (n=6)
hours			
Sf > 400			
0	1.2 ± 0.7	1.8 ± 1.2	1.2 ± 0.5
2	7.7 ± 7.1	6.7 ± 5.5	8.4 ± 4.1
4	4.3 ± 1.6	7.8 ± 3.8	5.3 ± 4.1
8	1.4 ± 0.7	1.8 ± 0.8	1.7 ± 1.5
Sf 60- 400			
0	8.5 ± 3.1	6.5 ± 3.5	9.1 ± 4.5
2	14.7 ± 13.8	15.7 ± 14.0	19.6 ± 10.6
4	9.0 ± 4.7	13.4 ± 6.0	12.0 ± 3.4
8	3.0 ± 0.8	3.9 ± 2.2	4.8 ± 4.2
Sf 20- 60			
0	9.6 ± 3.8	7.5 ± 4.8	9.0 ± 2.5
2	12.9 ± 10.9	10.7 ± 7.2	17.5 ± 7.6
4	13.5 ± 4.1	14.4 ± 10.2	10.9 ± 2.8
8	4.1 ± 1.4	5.5 ± 3.3	4.7 ± 3.3

Subjects were given an oral fat challenge with either Linoleic Acid supplemented fat using soybean oil - Hi-LO (n=6), Palmitic acid supplemented fat using Palm Olein oil - Hi-PO (n=6) or Oleic acid supplemented fat using Veggie fruit oil - Hi-OL (n=6) with a 2 week wash out period between each challenge. Blood was collected at 0, 2, 4 and 8 hours. Plasma was isolated by centrifugation and lipid concentrations were measured enzymatically. To fully characterize the postprandial effects of dietary blends on lipoproteins, TAG rich fractions (Sf > 400, Sf 60-400 and Sf 20-60) were isolated from plasma samples by sequential discontinuous ultracentrifugation for each time point. Triglycerides in the fractions were measured enzymatically. Values are the Mean ± SD. Data was analyzed using a one way analysis of variance (ANOVA). There were no significant differences in the lipoprotein subfractions at 0, 2, 4 and 8 hours between the diet groups.

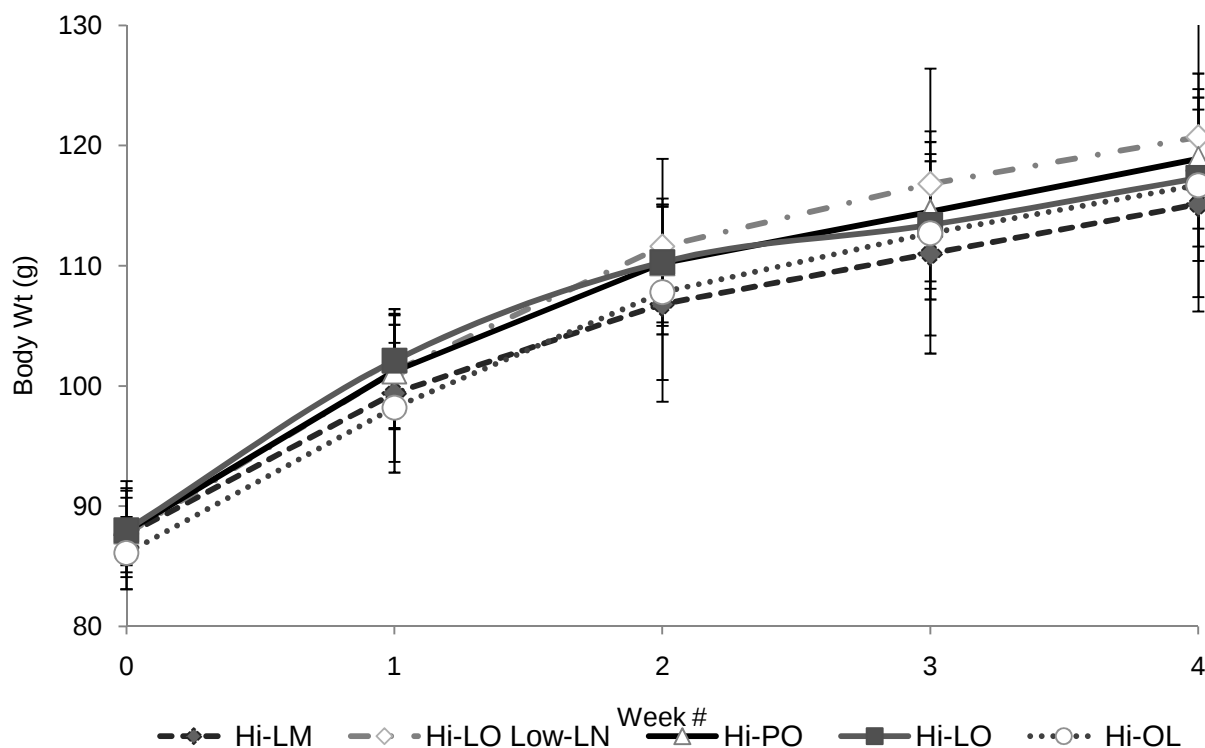


Figure 3-1. Specific Aim 1: Experiment 3: Growth in hamsters over 4 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein Oil (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15) for 4 weeks. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). There was no significant difference in the growth of hamsters between the diet groups over the 4 week feeding period.

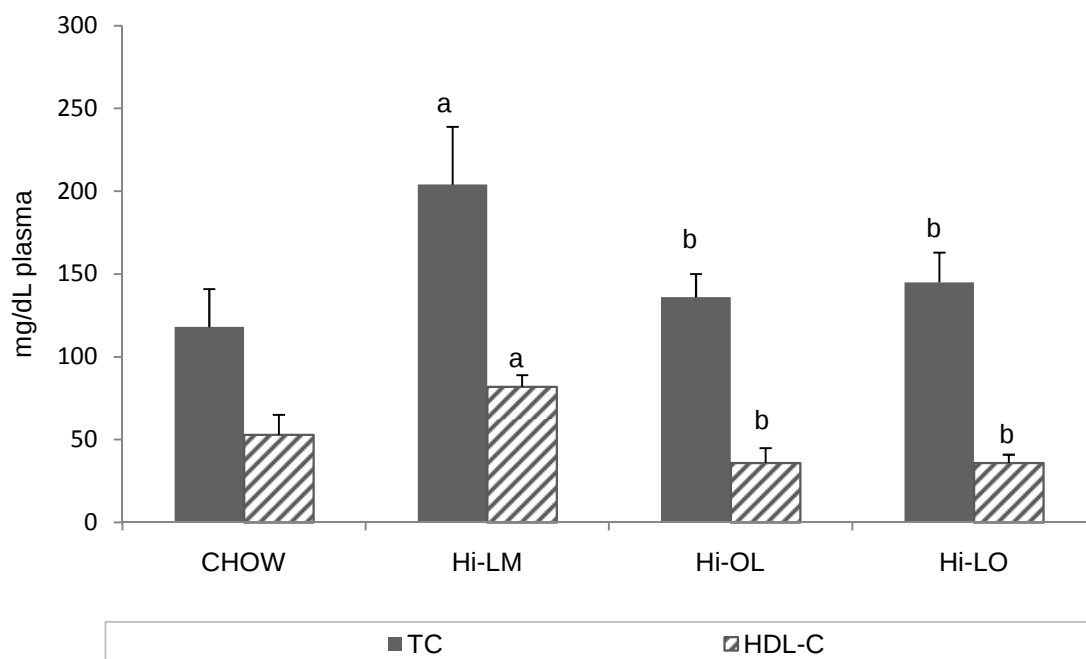


Figure 3-2. Specific Aim 1: Experiment 1: Plasma Total Cholesterol and HDL-C after 4 weeks feeding period

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=6), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=6) or Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=6) for 4 weeks. Plasma was isolated by centrifugation. Total Cholesterol was measured enzymatically. Following precipitation of apoB containing lipoproteins, HDL-C was measured enzymatically. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$). For comparative purposes, the values from 3 chow-fed gerbils are also shown (these animals were not included in the statistical analyses).

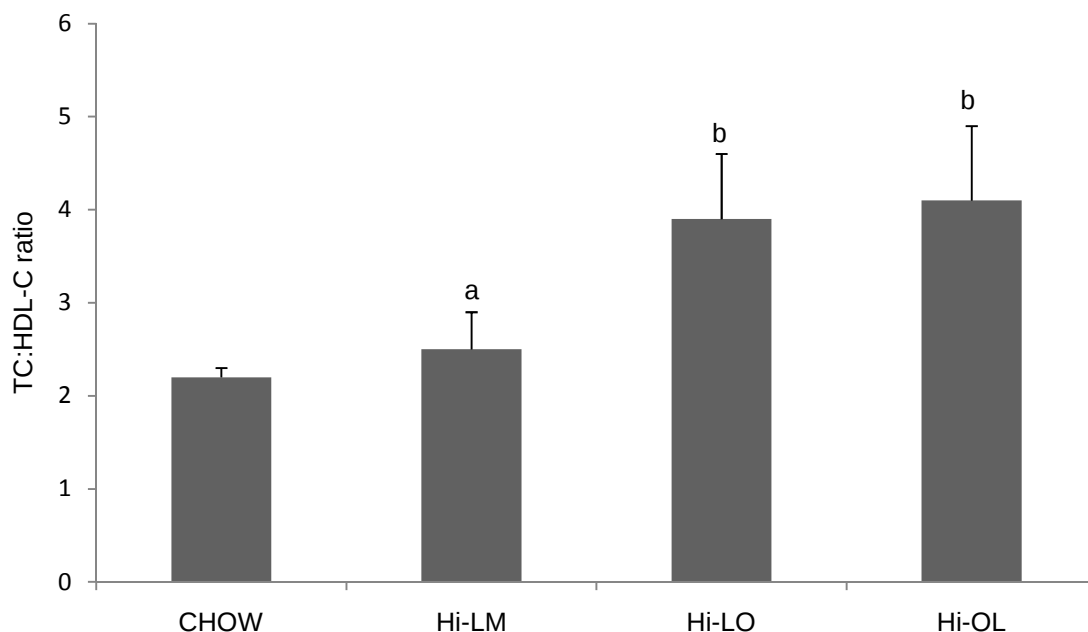


Figure 3-3. Specific Aim 1: Experiment 1: TC to HDL-C ratio after 4 weeks feeding period

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=6), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=6) or Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=6) for 4 weeks. TC: HDL-C ratio is a more sensitive risk marker for dyslipidemia. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$). For comparative purposes, the values from 3 chow-fed gerbils are also shown (these animals were not included in the statistical analyses).

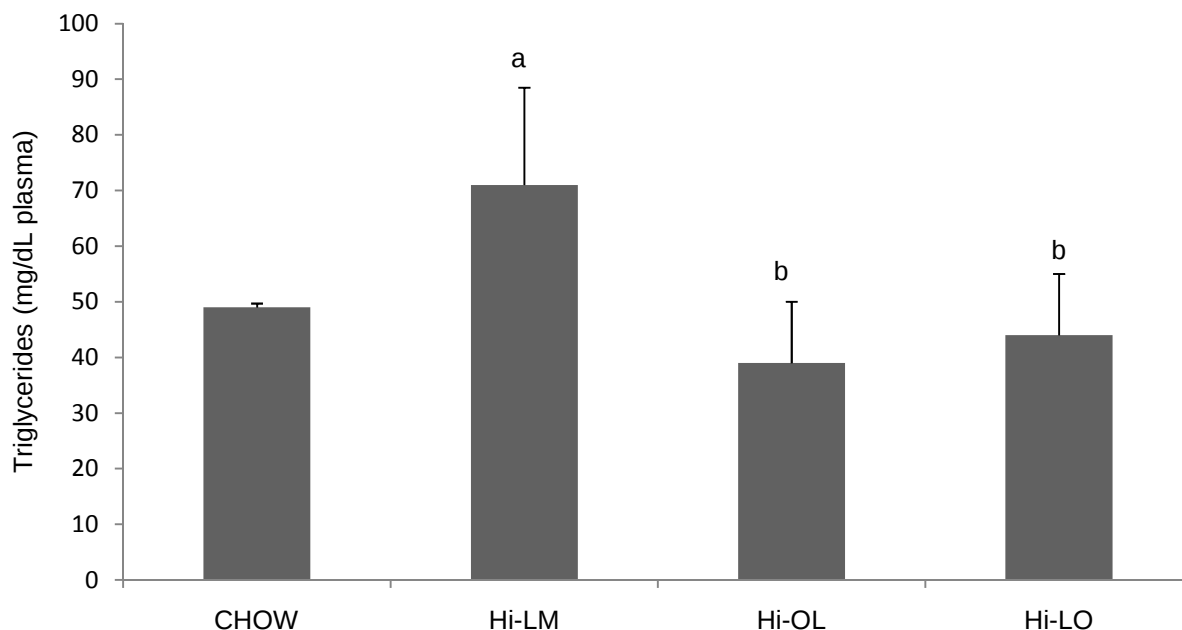


Figure 3-4. Specific Aim 1: Experiment 1: Plasma Triglycerides in gerbils after 4 weeks feeding period for the pilot study

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=6), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=6) or Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=6) for 4 weeks. Plasma was isolated by centrifugation and triglycerides were measured enzymatically. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$). For comparative purposes, the values from 3 chow-fed gerbils are also shown (these animals were not included in the statistical analyses).

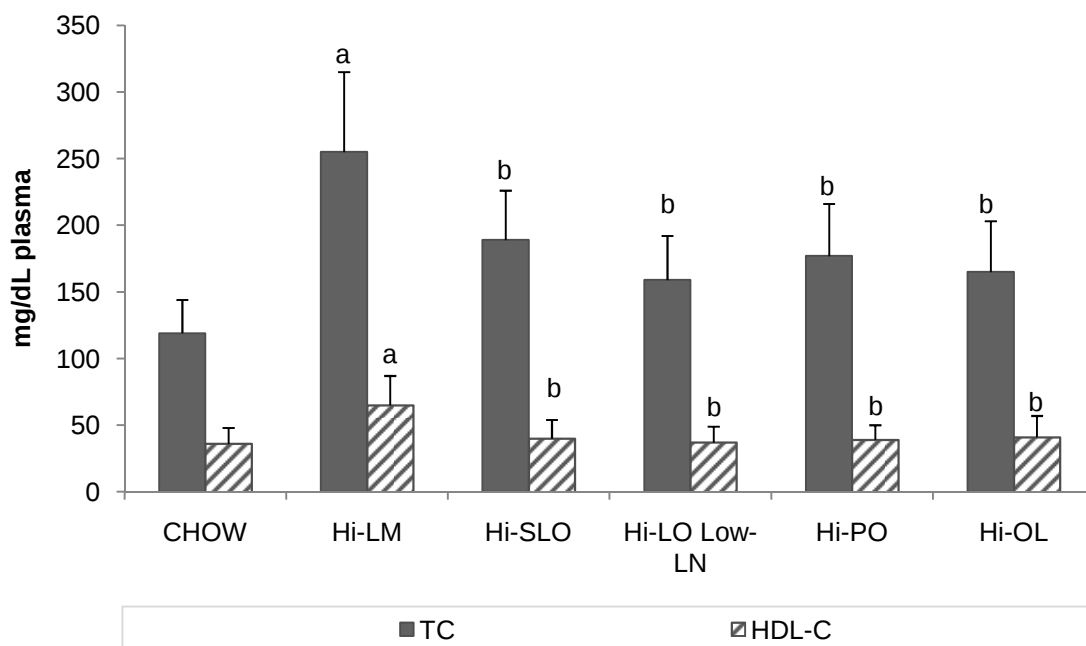


Figure 3-5. Specific Aim 1: Experiment 2: Plasma Total Cholesterol and HDL-C in gerbils after 4 weeks feeding period

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=13), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein Oil (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=13), Stearic acid and Linoleic Acid supplemented diet using Interesterified Soybean Oil (Hi-SLO, n=15) for 4 weeks. Plasma was isolated by centrifugation. Total Cholesterol was measured enzymatically. Following precipitation of apoB containing lipoproteins, HDL-C was measured enzymatically. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$). For comparative purposes, the values from 4 chow-fed gerbils are also shown (these animals were not included in the statistical analyses).

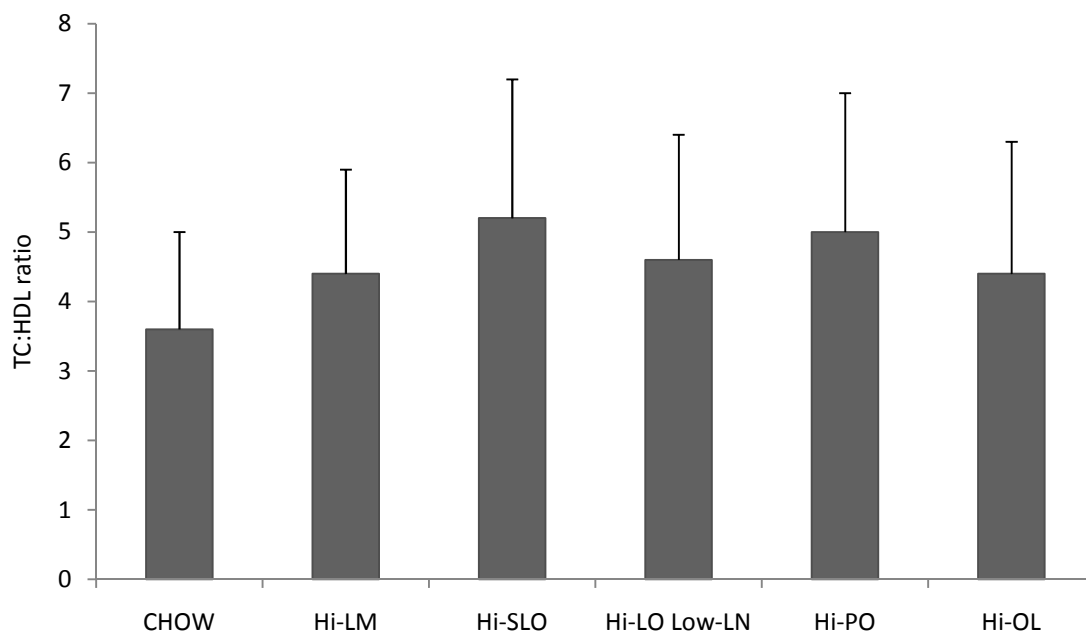


Figure 3-6. Specific Aim 1: Experiment 2: TC to HDL-C ratio in gerbils after 4 weeks feeding period

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=13), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein Oil (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=13), Stearic acid and Linoleic Acid supplemented diet using Interesterified Soybean Oil (Hi-SLO, n=15) for 4 weeks. TC: HDL-C ratio is a more sensitive risk marker for dyslipidemia. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing common letters were significantly different from each other ($p \leq 0.05$). For comparative purposes, the values from 3 chow-fed gerbils are also shown (these animals were not included in the statistical analyses). There were no significant differences observed in the TC:HDL-C ratio between the diet groups.

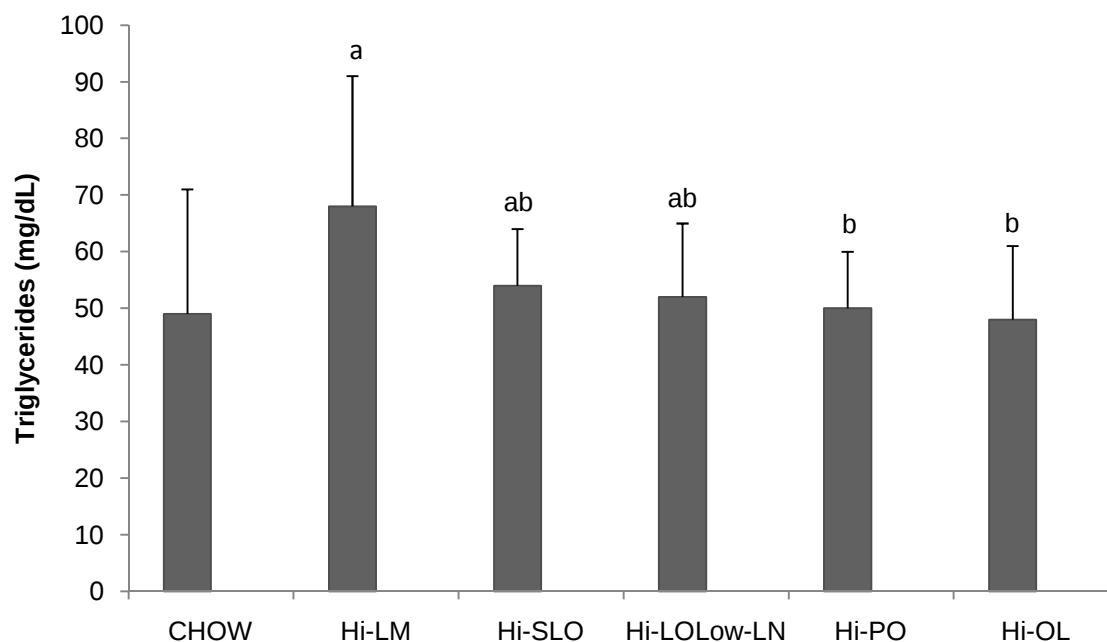


Figure 3-7. Specific Aim 1: Experiment 2: Plasma Triglycerides in gerbils after 4 weeks feeding period

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=13), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein Oil (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=13), Stearic acid and Linoleic Acid supplemented diet using Interesterified Soybean Oil (Hi-SLO, n=15) for 4 weeks. Plasma was isolated by centrifugation and triglycerides were measured enzymatically. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$). For comparative purposes, the values from 3 chow-fed gerbils are also shown (these animals were not included in the statistical analyses).

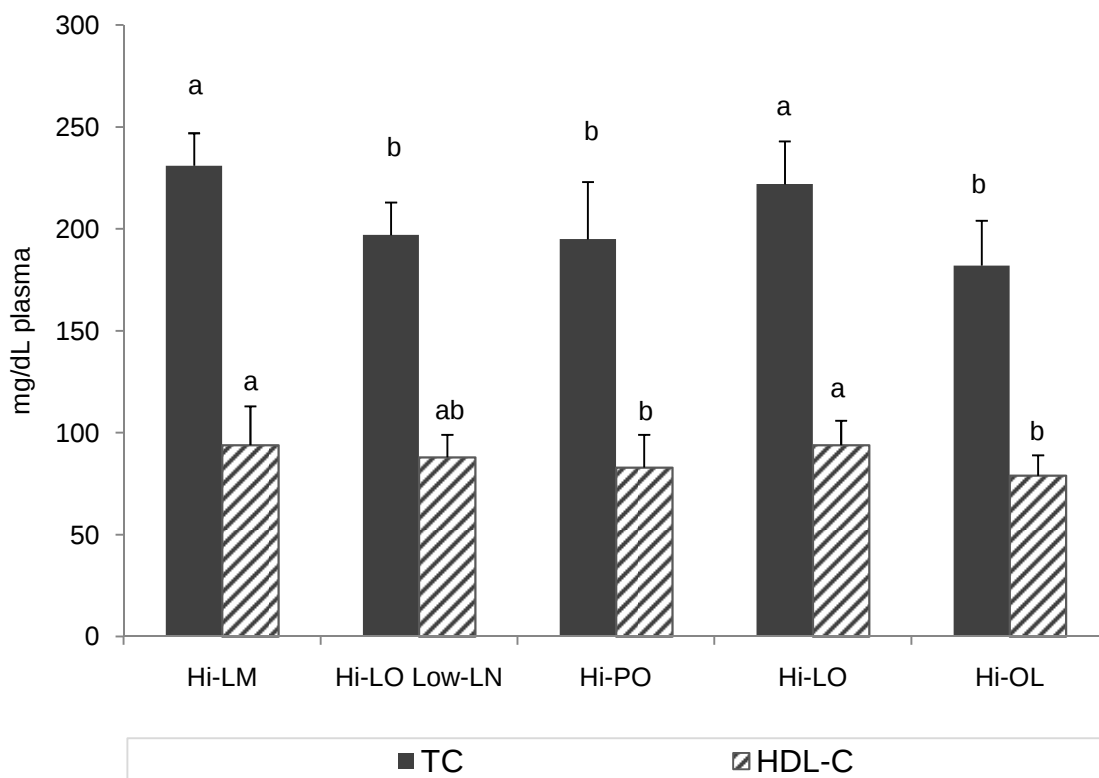


Figure 3-8. Specific Aim 1: Experiment 3: Plasma Total Cholesterol and HDL-C in gerbils after 4 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15) for 4 weeks. Plasma was isolated by centrifugation. Total Cholesterol was measured enzymatically. Following precipitation of apoB containing lipoproteins, HDL-C was measured enzymatically. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$).

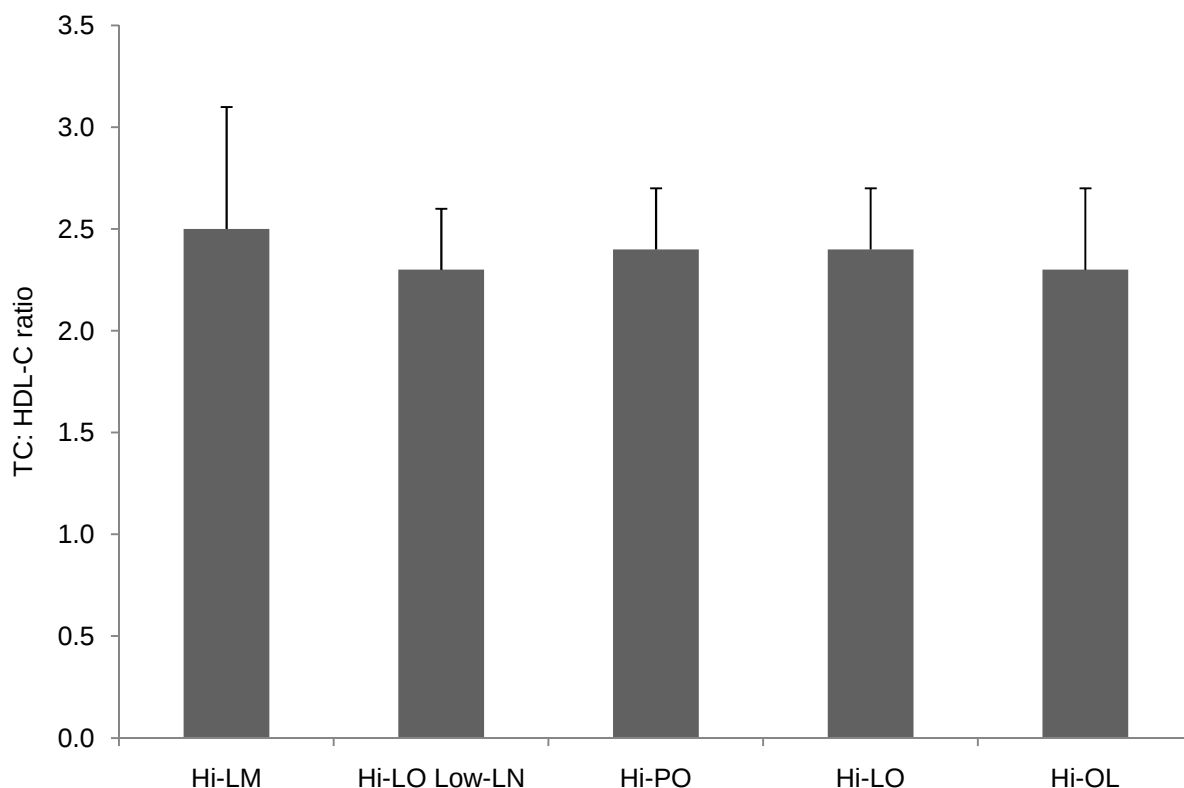


Figure 3-9. Specific Aim 1: Experiment 3: Total Cholesterol to HDL-C ratio in hamsters after 4 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15) for 4 weeks. TC: HDL-C ratio is a more sensitive risk marker for dyslipidemia. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). There were no significant differences in the TC:HDL-C ratio between the diet groups.

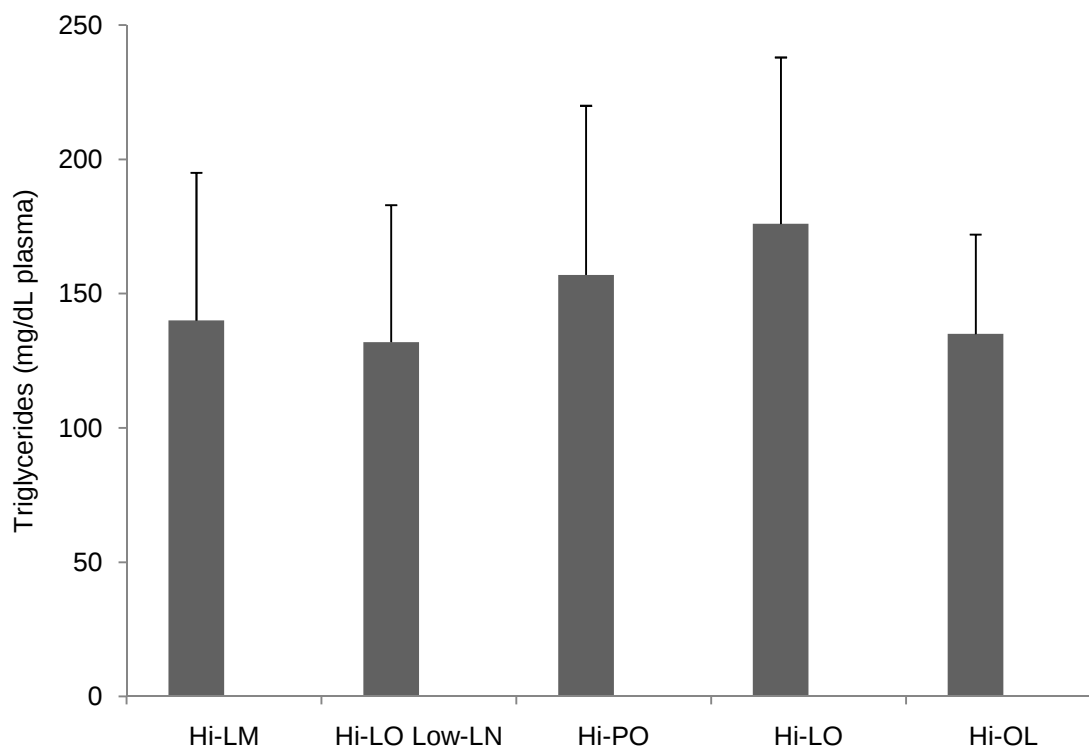


Figure 3-10. Specific Aim 1: Experiment 3: Plasma Triglycerides in hamsters after 4 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15) for 4 weeks. Plasma was isolated by centrifugation and Triglycerides were measured enzymatically. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). There were no significant differences in plasma triglycerides following a 4 week feeding period between the diet groups.

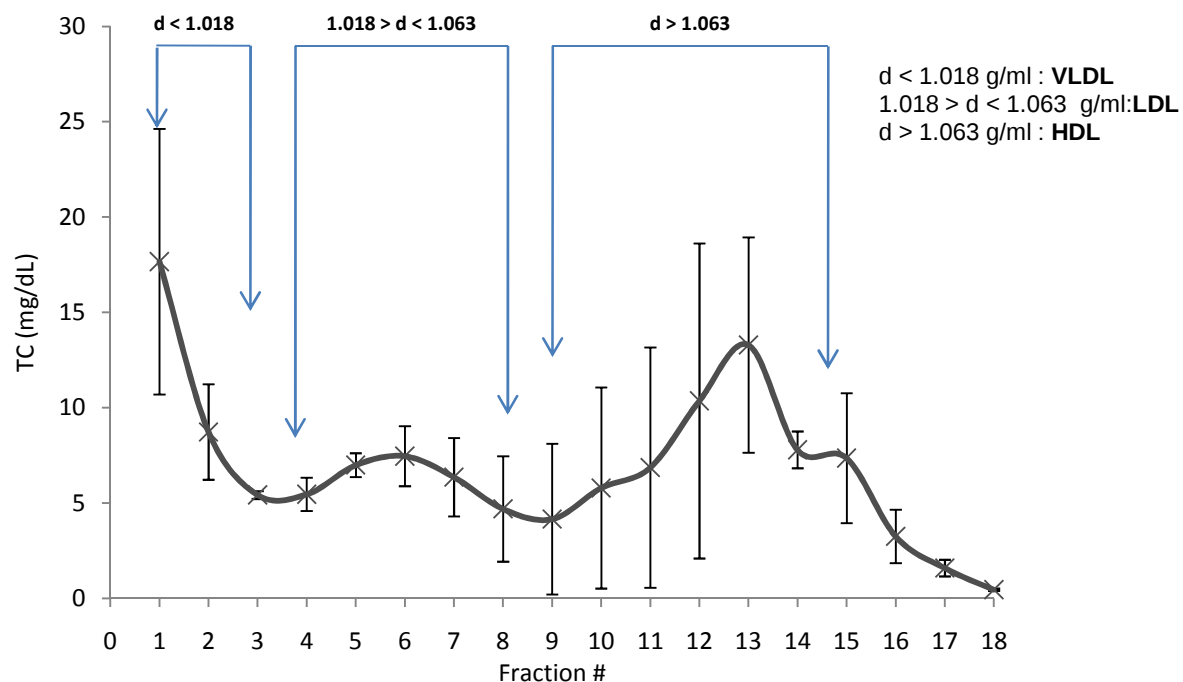
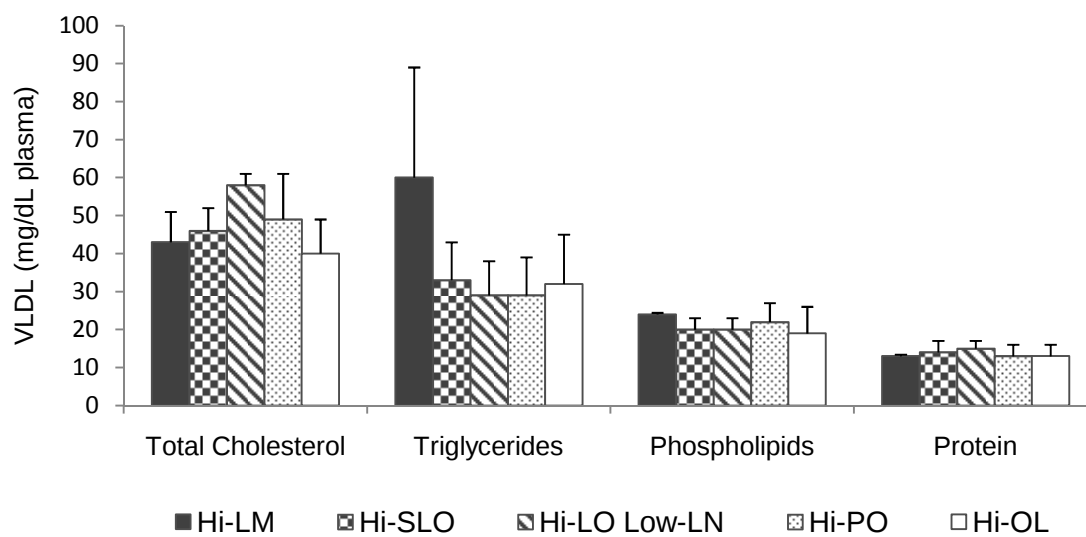
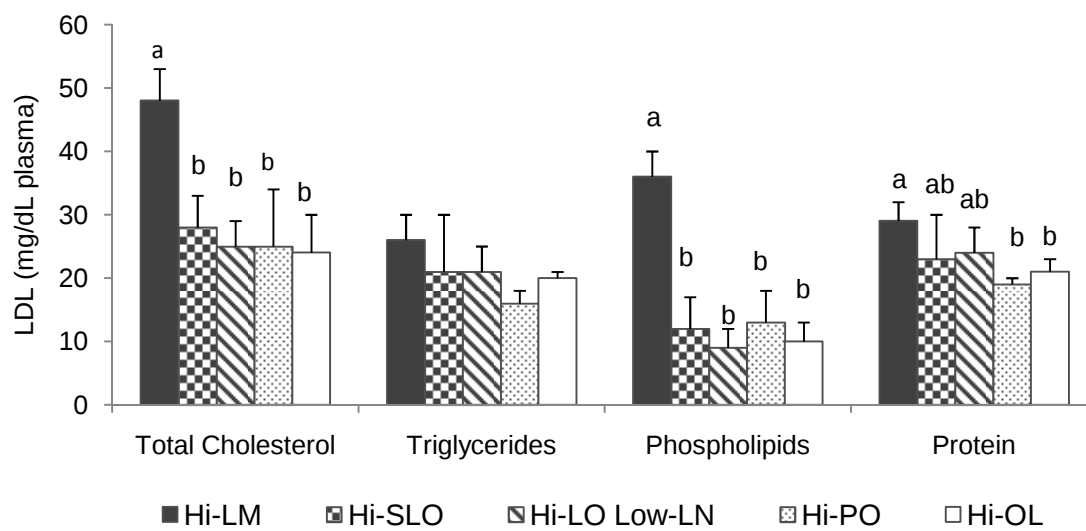


Figure 3-11. Lipoprotein fractions designated based on density cuts.

Plasma was isolated by centrifugation and lipid concentrations were measured enzymatically. Pooled plasma samples (3-4 per each diet group) were fractionated by discontinuous ultracentrifugation. Fractions were designated based on plasma density cuts $d < 1.018$ g/ml, $1.018 < d < 1.063$ g/ml and $d > 1.063$ g/ml and denoted V+IDL, LDL and HDL respectively.



(A)



(B)

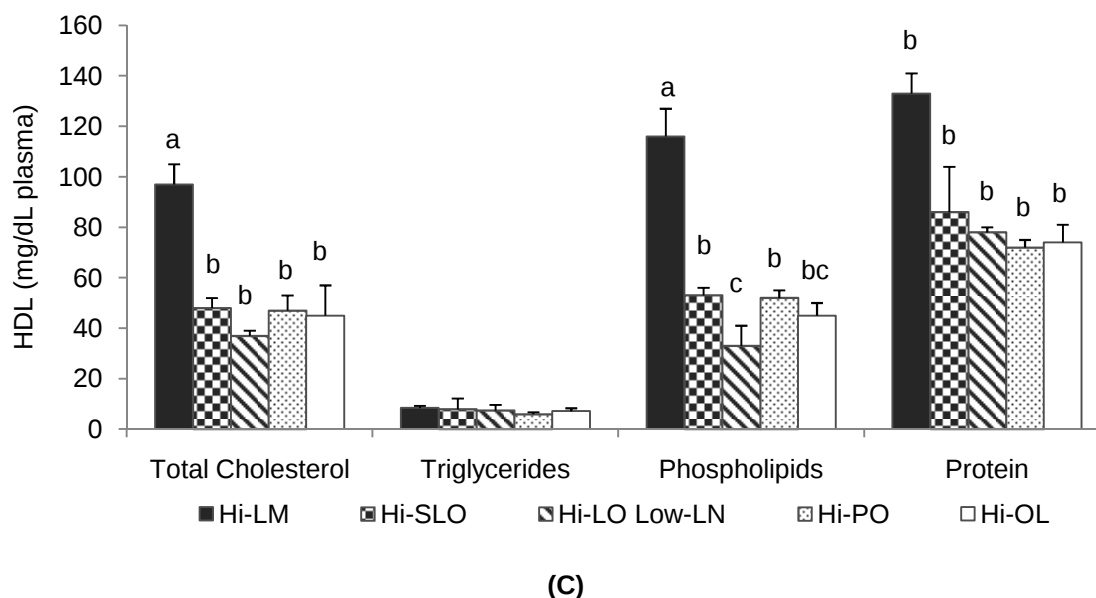


Figure 3-12. Specific Aim 1: Experiment 2: Plasma Lipoproteins Analyses for gerbils after 4 weeks feeding period

Male Mongolian gerbils (8wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=13), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein Oil (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=13), Stearic acid and Linoleic Acid supplemented diet using Interesterified Soybean Oil (Hi-SLO, n=15) for 4 weeks. Plasma was isolated by centrifugation. Pooled plasma samples (3-4per each diet group) were fractionated by discontinuous ultracentrifugation. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA).

- (A) Composition of V+IDL fraction ($d < 1.018 \text{g/ml}$): No significant differences were observed in the plasma lipoproteins in the VLDL fraction.
- (B) Composition of LDL fraction ($1.018 < d < 1.063$): Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$).
- (C) Composition of HDL fraction ($d > 1.063 \text{g/ml}$): Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$).

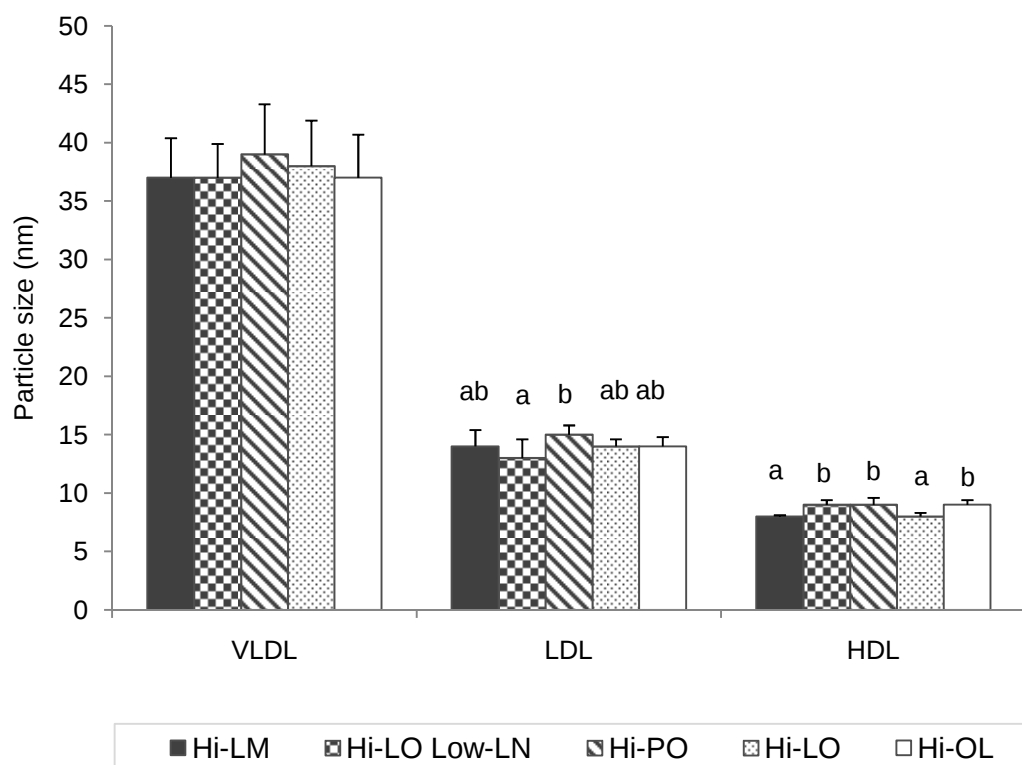


Figure 3-13. Specific Aim 1: Experiment 3: Lipoprotein Particle Diameters for hamsters after 4 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15) for 4 weeks. Plasma was isolated by centrifugation. Pooled plasma samples (3-4per each diet group) were fractionated by sequential ultracentrifugation. Particle diameters were calculated from core to surface ratio (Van Heek and Zilversmit). Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$).

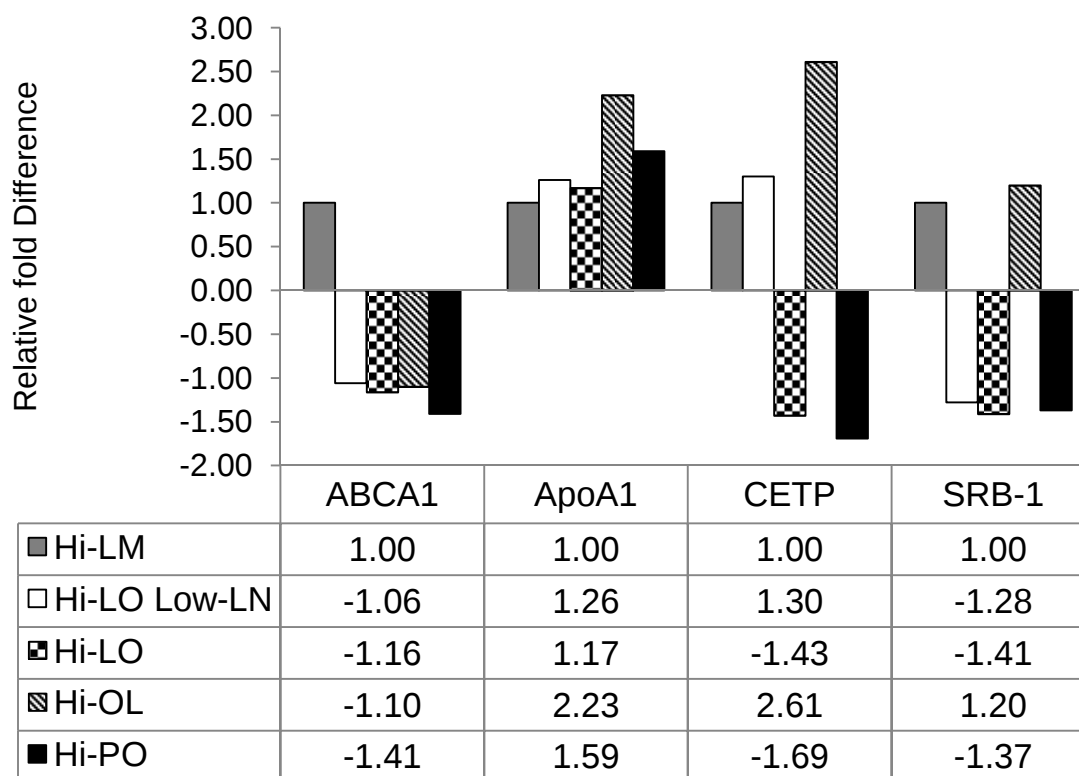


Figure 3-14. Specific Aim 1: Experiment 3: Expression of Reverse Cholesterol transport genes in hamsters following 4 week feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet using Coconut oil (Hi-LM, n=15), Oleic Acid supplemented diet using Veggie Fruit Oil (Hi-OL, n=15), Palmitic Acid supplemented diet using Palm Olein (Hi-PO, n=15), Linoleic Acid supplemented diet using Low Linolenic Soybean Oil (Hi-LO Low-LN, n=15), Linoleic Acid supplemented diet using Soybean Oil (Hi-LO, n=15) for 4 weeks. RNA was isolated from the liver and cDNA was synthesized using random primers. Real time PCR was performed using specific primers (4-5 samples per diet group). Ct values were normalized using GAPDH as an endogenous control. Relative gene expression was calculated [$2^{-(\text{avg. (Ct}_{\text{gene of interest}}) - \text{avg. (Ct}_{\text{Housekeeping gene}})})$]. The relative expression values were used to determine fold changes in the test diet compared to Hi-LM (positive control). The figure shows the relative fold difference for ABCA1, ApoA1, CETP and SR-B1 for all diet groups relative to Hi-LM.

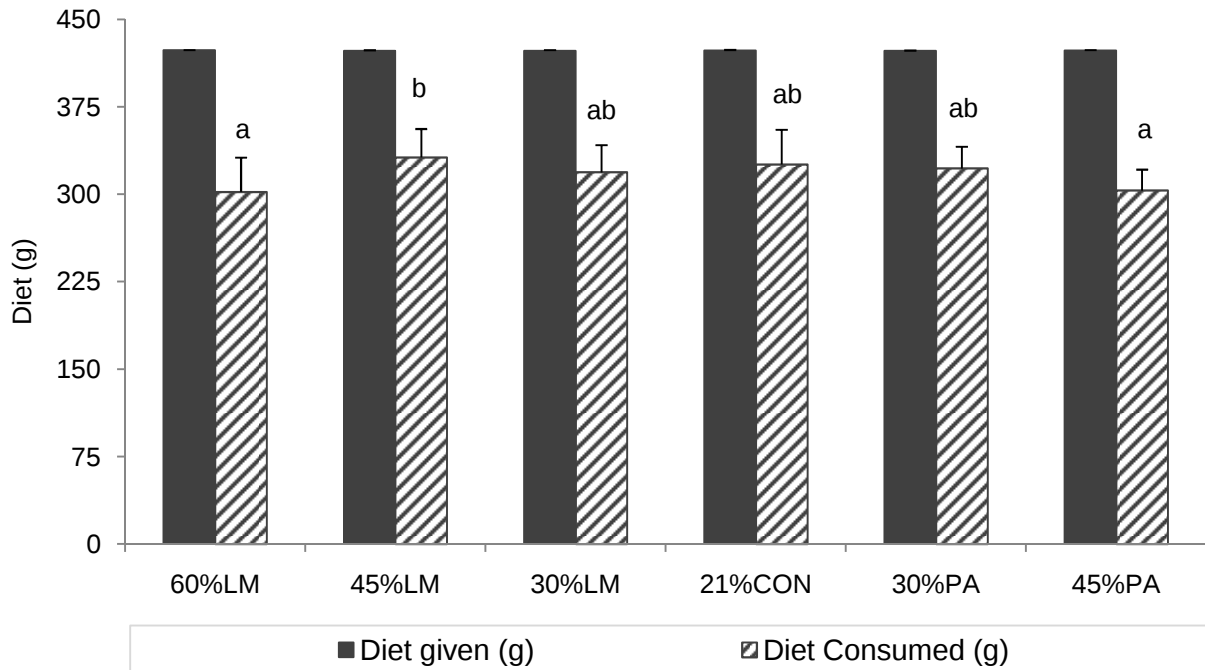


Figure 3-15. Specific Aim 2: Diet Given and Consumed by hamsters over 7 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=15); 45%LM (n=15), 30%LM (n=15); 21%CON (n=15); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=15); 45%PA (n=15) for 7 weeks. Diet consumed was estimated as the difference between diet provided and spilled diet collected from the cage. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). There was no significant difference in the diet given (g) between the diet groups over the 7 week feeding period. For diet consumed, diet groups sharing different letters were significantly different from each other ($p \leq 0.05$).

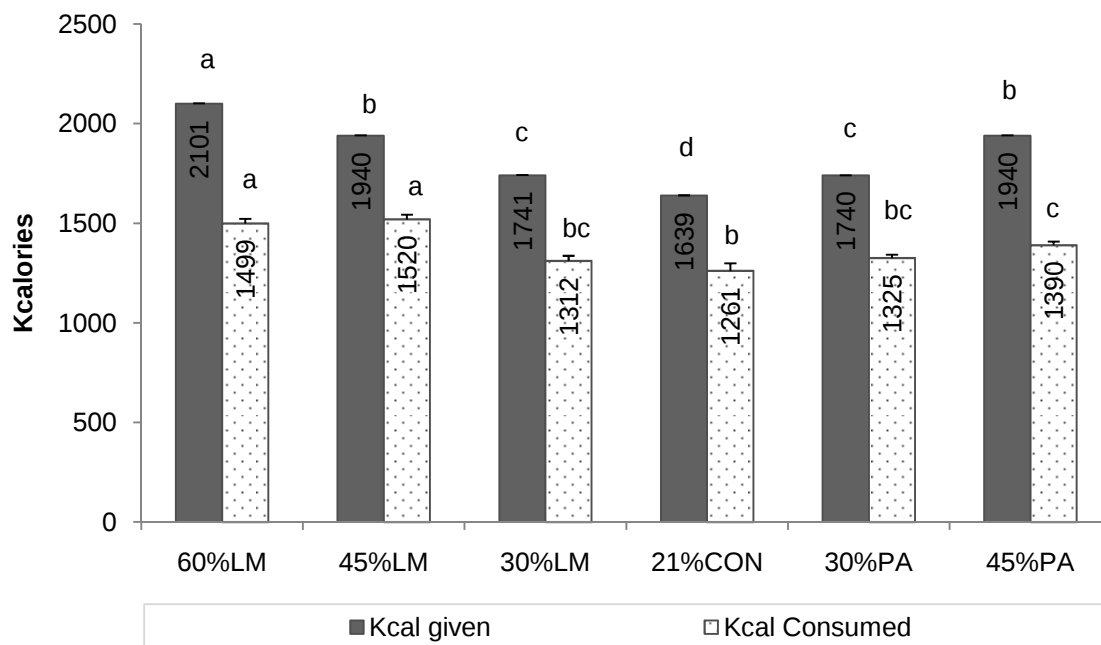


Figure 3-16. Specific Aim 2: Kcalories Given and Consumed by hamsters over 7 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=15); 45%LM (n=15); 30%LM (n=15); 21%CON (n=15); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=15); 45%PA (n=15) for 7 weeks. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$).

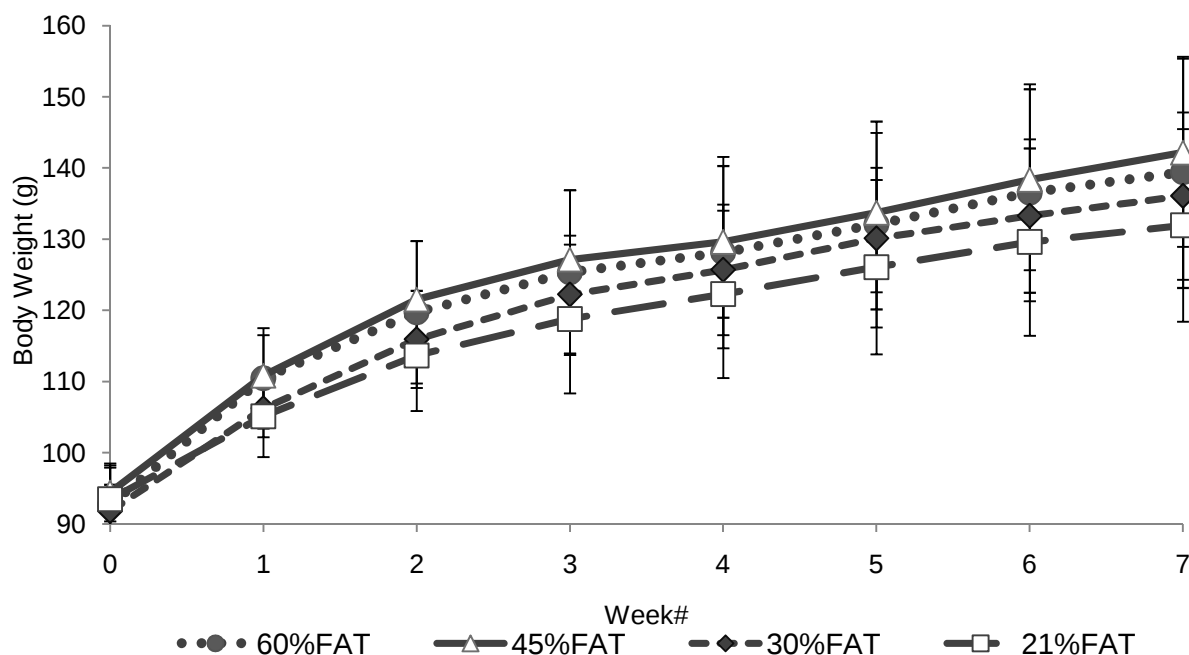


Figure 3-17. Specific Aim 2: Growth in hamsters over 7 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=15); 45%LM (n=15), 30%LM (n=15) ; 21%CON (n=15); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=15); 45%PA (n=15) for 7 weeks. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). There was no significant difference in the growth of hamsters between the diet groups over the 4 week feeding period.

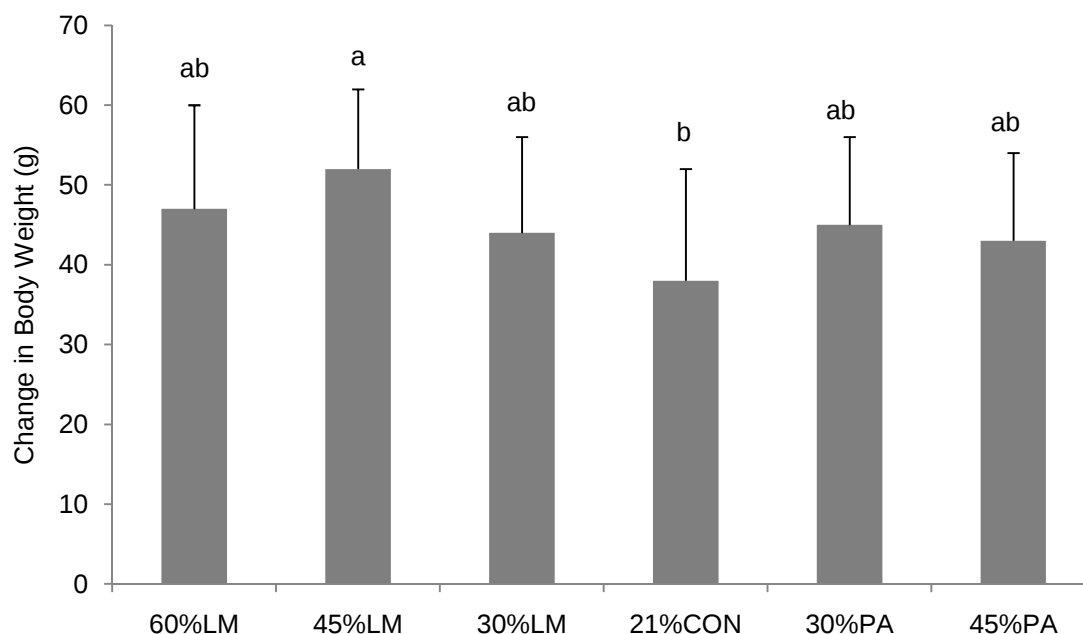


Figure 3-18. Specific Aim 2: Change in Body weight in hamsters over 7 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=15); 45%LM (n=15), 30%LM (n=15) ; 21%CON (n=15); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=15); 45%PA (n=15) for 7 weeks. The hamsters were weighed weekly. Change in body weight was taken as the difference in initial weight and weight at the end of the 7 week feeding period. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$).

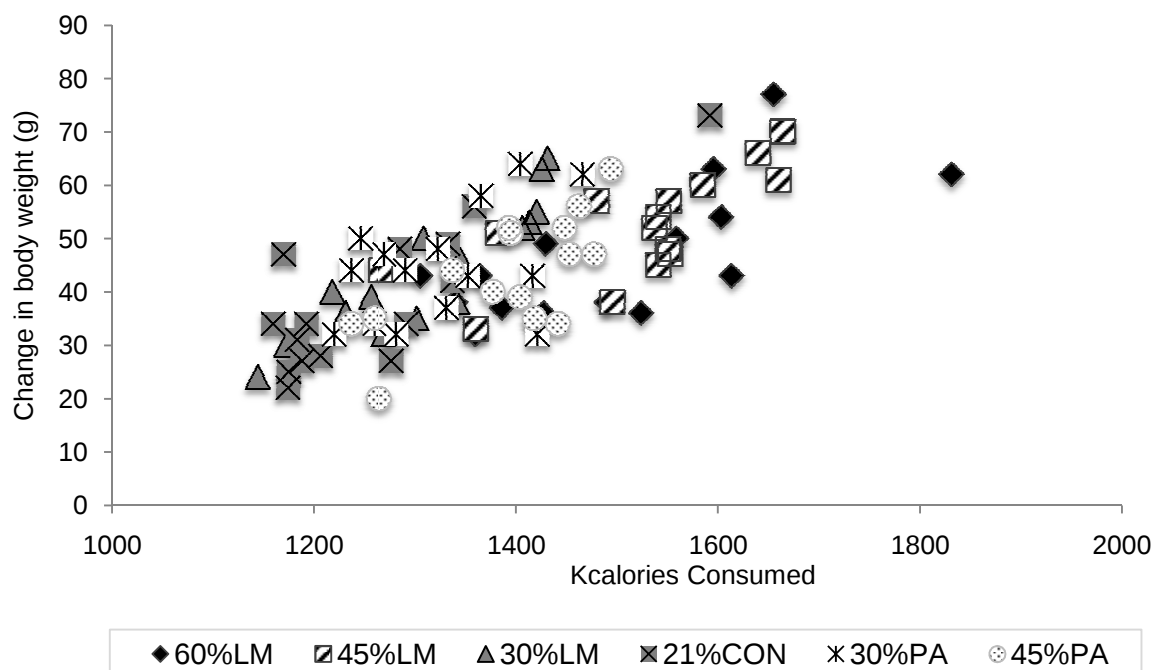


Figure 3-19. Specific Aim 2: Change in Body Weight in hamsters with respect to Kcalories consumed over 7 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=15); 45%LM (n=15), 30%LM (n=15); 21%CON (n=15); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=15); 45%PA (n=15) for 7 weeks. The hamsters were weighed weekly. Change in body weight was taken as the difference in initial weight and weight at the end of the 7 week feeding period. Kcalories consumed were calculated by multiplying kcal/g diet consumed.

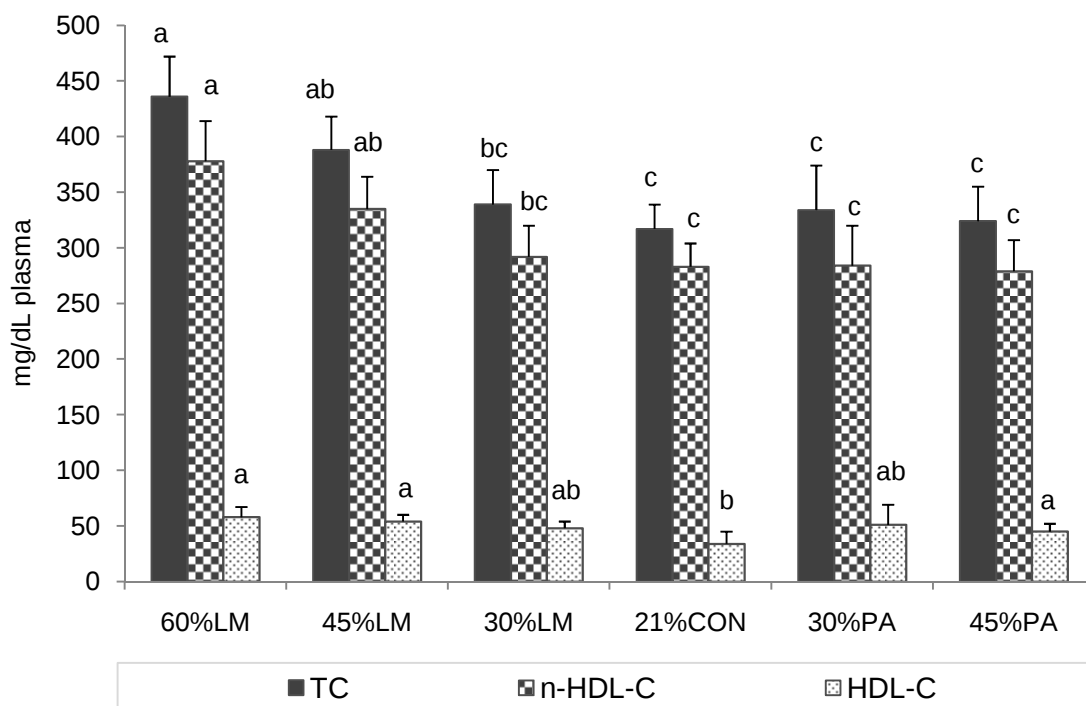


Figure 3-20. Specific Aim 2: Plasma Lipids in hamsters after 7 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=8); 45%LM (n=8), 30%LM (n=8); 21%CON (n=8); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=8); 45%PA (n=8) for 7 weeks. Plasma was isolated by centrifugation and Total Cholesterol was measured enzymatically. Following precipitation of apoB containing lipoproteins, HDL-C was measured enzymatically. nHDL was calculated as a difference of Total Cholesterol and HDL-C. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$).

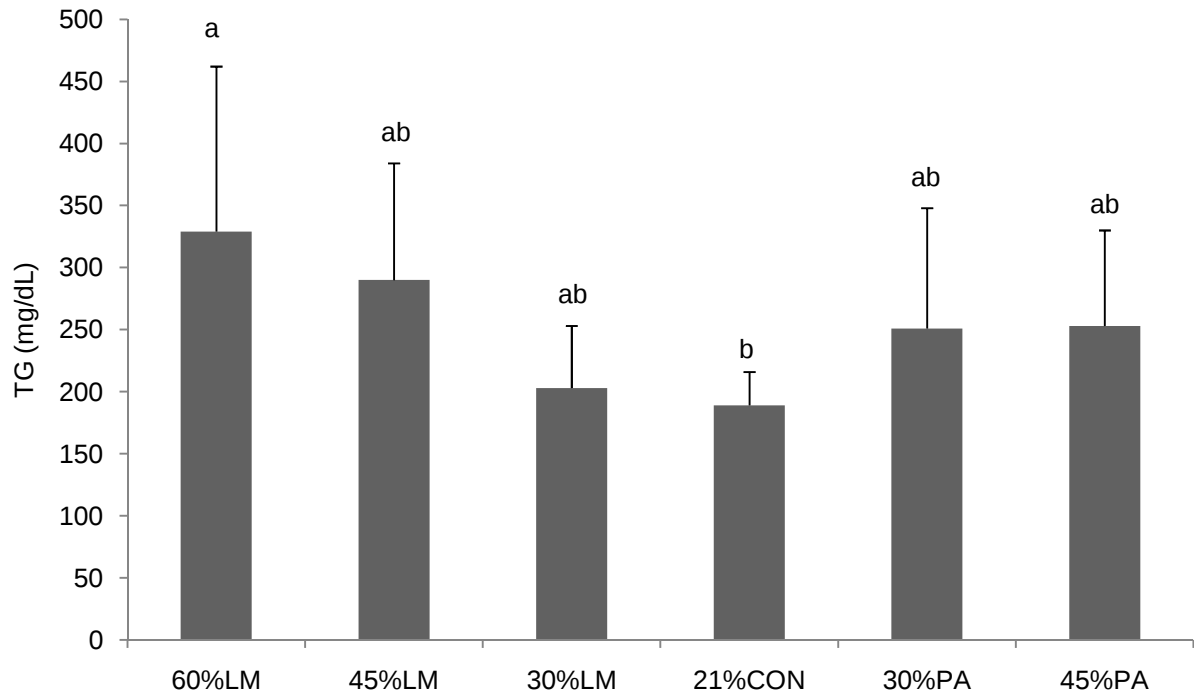


Figure 3-21. Specific Aim 2: Plasma Triglycerides for hamsters after 7 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=8); 45%LM (n=8), 30%LM (n=8) ; 21%CON (n=8); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=8); 45%PA (n=8) for 7 weeks. Plasma was isolated by centrifugation and Triglycerides were measured enzymatically. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing different letters were significantly different from each other ($p \leq 0.05$).

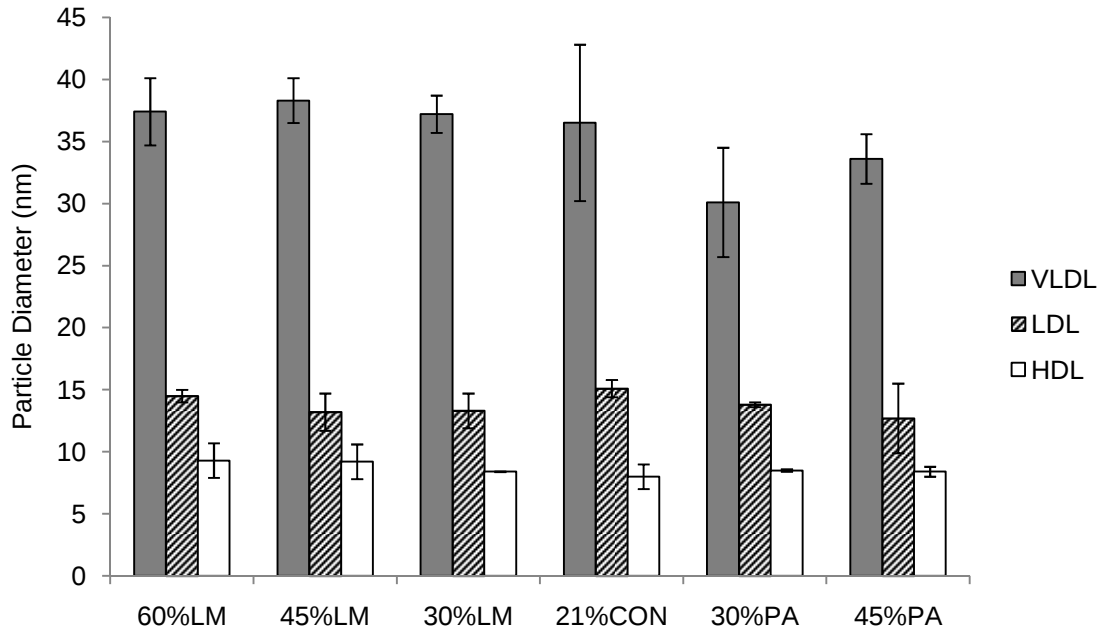


Figure 3-22. Specific Aim 2: Lipoprotein Particle Diameters in hamsters after 7 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=8); 45%LM (n=8), 30%LM (n=8) ; 21%CON (n=8); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=8); 45%PA (n=8) for 7 weeks. Plasma was isolated by centrifugation. Pooled plasma samples (4per each diet group) were fractionated by sequential ultracentrifugation. Particle diameters were calculated from core to surface ratio (Van Heek and Zilversmit). Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). Diet groups sharing common letters were significantly different from each other ($p \leq 0.05$).

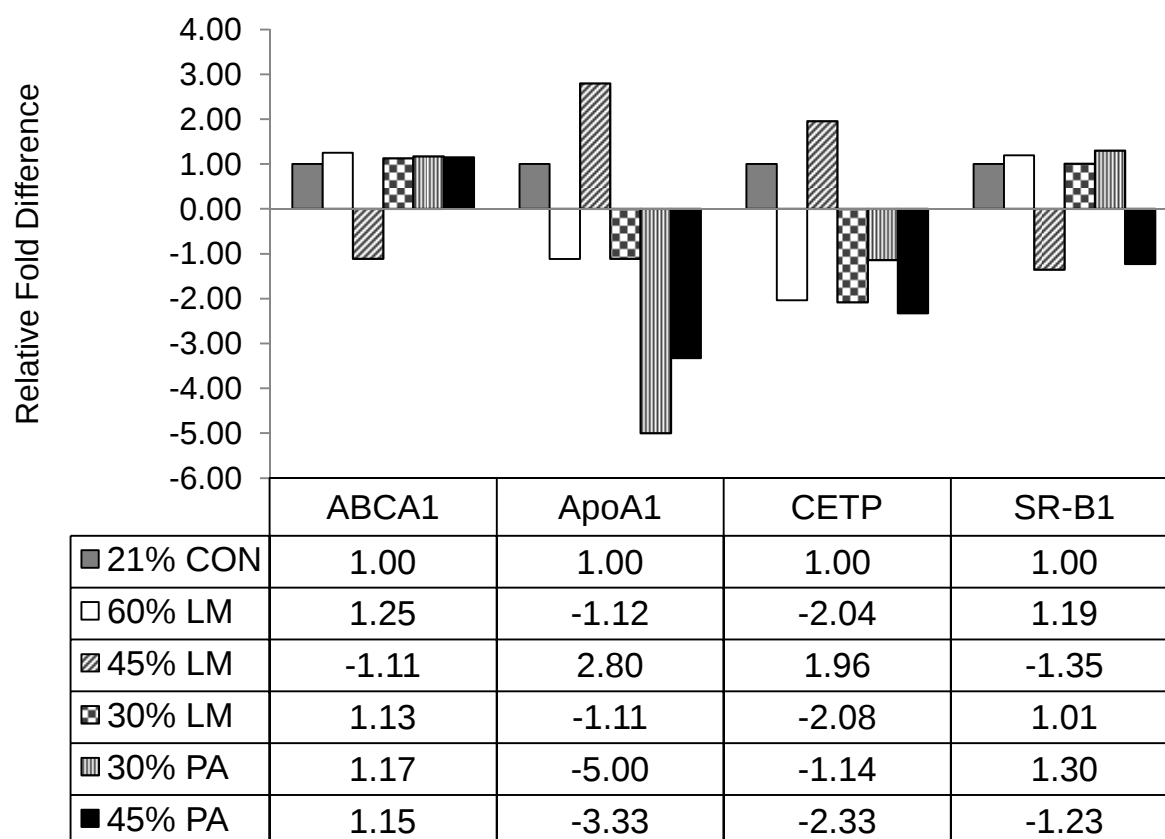


Figure 3-23. Specific Aim 2: Expression of Reverse Cholesterol transport genes in hamsters after 7 weeks feeding period

Male Golden Syrian hamsters (6wks of age) were fed Lauric and Myristic Acid supplemented diet with varying percentage of fat - 60%LM (n=8); 45%LM (n=8), 30%LM (n=8) ; 21%CON (n=8); and Palmitic Acid supplemented group with varying percentages of fat; 30%PA (n=8); 45%PA (n=8) for 7 weeks. RNA was isolated from the liver and cDNA was synthesized using random primers. Real time PCR was performed using specific primers (4-5 samples per diet group). Ct values were normalized using GAPDH as an endogenous control. Relative gene expression was calculated [$2^{-(\text{avg. (Ct}_{\text{gene of interest}}) - \text{avg. (Ct}_{\text{Housekeeping gene}})})$]. The relative expression values were used to determine fold changes in the test diet compared to 21% CON (positive control). The figure shows the relative fold difference for ABCA1, ApoA1, CETP and SR-B1 for all diet groups relative to 21% CON.

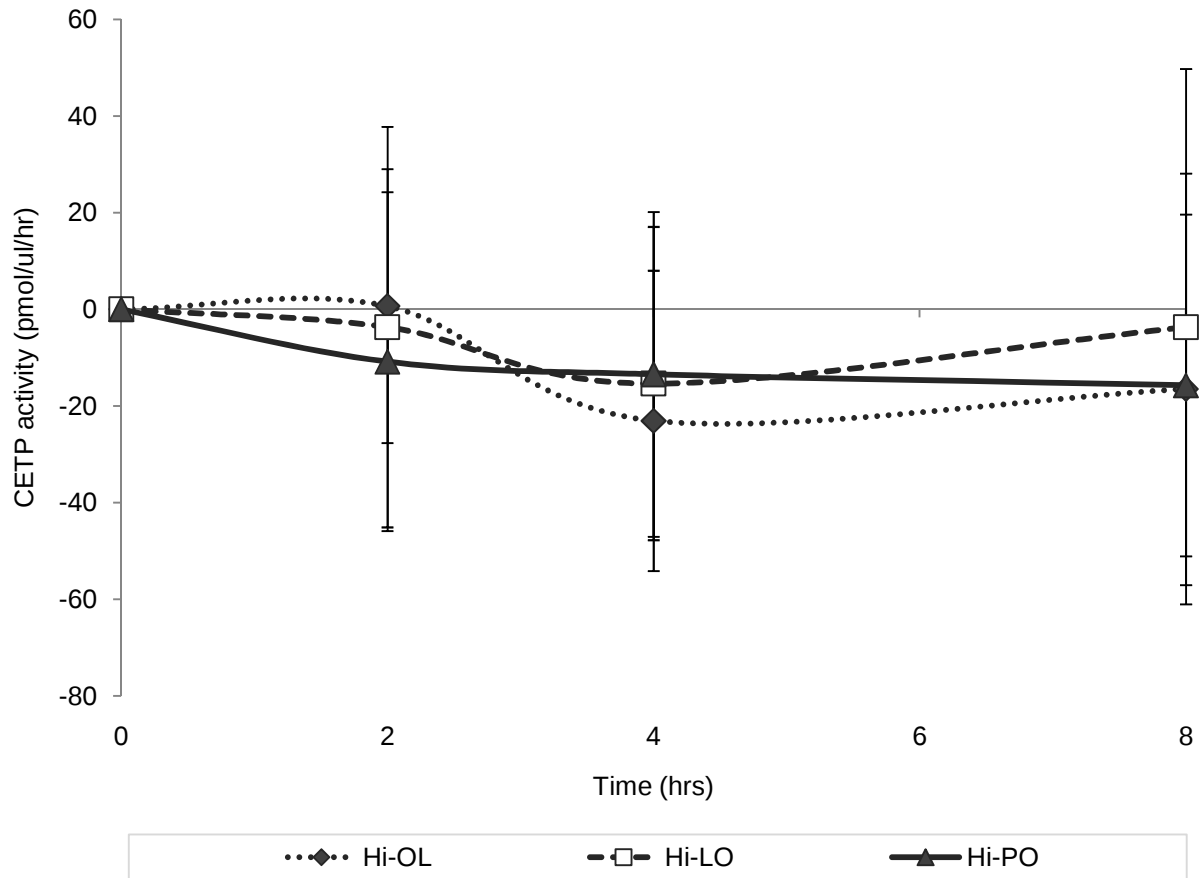


Figure 3-24. Specific Aim 3: CETP activity at 0, 2, 4 and 8 hours following the oral fat challenge

Subjects were given an oral fat challenge with either Linoleic Acid supplemented fat using soybean oil - Hi-LO (n=6), Palmitic acid supplemented fat using Palm Olein - Hi-PO (n=6) or Oleic acid supplemented fat using Veggie fruit oil - Hi-OL (n=6) with a 2 week wash out period between each challenge. Blood was collected at 0, 2, 4 and 8 hours. Plasma samples were used to analyze CETP activity. Values are the Mean $\pm$ SD. Data was analyzed using a one way analysis of variance (ANOVA). No significant differences were observed in CETP activity at 0, 2, 4 and 8 hours between the diets.

Chapter 4: Discussion

Consumption of partially hydrogenated vegetable oils (PHVO) containing trans-fatty acids (tFA) and saturated fatty acids (SFA) have been associated with higher incidence of coronary heart disease. An attempt to eliminate PHVO from the food supply has led to finding alternatives with higher dietary fat saturation compared to naturally occurring vegetable oils. In the present study we evaluated the effects of dietary fat saturation on lipoprotein metabolism using – High Linoleic soybean oil (Hi-LO), High Linoleic Low Linolenic Soybean Oil (Hi-LO Low-LN), High Stearic and Linoleic Soybean oil (Hi-SLO), High Oleic Oil Blend (palm, corn & sunflower) (Hi-OL) and a palm oil fraction with an increased content of oleic acid (Hi-PO). Experimental and observational data have established that consumption of PHVO containing tFA and SFA lead to similar increases in total cholesterol and LDL-cholesterol (LDL-C). Also, among individual SFA, lauric and myristic acids show the most increases in TC and LDL-C compared to palmitic and stearic acids. Therefore, we used a diet high in Lauric and Myristic acid (Hi-LM) as a positive control for our study. The effects of the diets were evaluated in gerbil and hamster model since gerbils are highly sensitive to fatty acid manipulations and hamsters are widely accepted as appropriate models to depict human lipoprotein metabolism.

In gerbils and hamsters, amount of diet consumed was not significantly different between the diet groups suggesting equal acceptance of the oils contained in the pellets. In the 4 weeks of the study period, body weight increased for all gerbils and hamsters in all the diet groups with no signs of illness or deficiency. The growth through the span of the study is indicative of adequacy of the diet. Body weights did not differ

significantly between the diet groups suggesting that isocaloric consumption of diets, irrespective of the type of fat consumed may not have effects on body weight.

Liver weights were not significantly different between the diet groups for gerbils or for hamsters. In the gerbil model, when body weight and liver weight were taken as a ratio, Hi-SLO and Hi-LO Low-LN were significantly lower to Hi-LM and Hi-OL groups. The differences seen seem to be a statistical artifact. The body weight reported was taken during the feeding period of the study, whereas the liver weights are representative of the fasting state. Also, a hind side of the study, we did not take the absolute adipose weight for the gerbils. Therefore, it would be difficult to conclude why these differences were observed.

Plasma TC in gerbils indicated changes ranging from 99 mg/dL to 306 mg/dL (184 ± 45) establishing the effectiveness of the model to fatty acid sensitivity. Hi-LM showed highest increase in TC compared with all other diet groups. Also, Hi-LM showed maximum rise in HDL-C compared with other diet groups. In the LDL and HDL fractions of plasma, Hi-LM contained the most amount of cholesterol compared with other diets. Lauric acid has the greatest TC and HDL-C raising effect compared with other SFA, MUFA and PUFA. This effect is even more pronounced in gerbils since almost 70% of TC is carried in the HDL representing the major system for transport for cholesterol [71]. Plasma TC in hamsters indicated changes ranging from 123 mg/dL to 261 mg/dL (205 ± 27) establishing the effectiveness of the model to fatty acid sensitivity. Analyses of plasma revealed that TC and HDL-C were significantly higher for Hi-LM and Hi-LO compared with other diet groups. Increased TC and HDL-C in the case of Hi-LO group is an unexpected result. In a study to evaluate the effects of different fatty acids by

Lichtenstein et al, they also observed no significant differences between LM rich Coconut oil and LO rich Soybean oil. At the same time, there were differences between butter diet and soybean oil enriched diet. It is highly plausible that there is a differential effect of dietary fatty acid and their specific combinations on lipoprotein metabolism. Based on these results however, it might not be correct to conclude that consuming linoleic acid supplemented oils, as in case of soybean oil raises total and HDL-Cholesterol.

Another aspect that warrants attention is that in the diets for gerbils and hamsters compared to Hi-LM diet, PUFA to SFA (P:S) ratio was higher in all other diets. It could be speculated, as shown earlier by Hayes et al, level of PUFA in the diet could be a determinant of how the SFA affect lipoproteins [79].

In the gerbil model, plasma TG was also significantly higher in the Hi-LM diet compared with all other diet groups. Amongst the lipoproteins, VLDL is the major carrier for TG. In the gerbil model, Hi-LM diet contained the highest amount of TG in the VLDL particle compared with all other diet groups. This is in line with documented results from gerbil model studies where SFA raises TG relative to MUFA and PUFA diets [71]. In the hamster model, plasma TG was not significantly different between the diet groups. This observation was again an unexpected result based on data available on hamsters, but it is also known that hamsters have mouth pouches where there is a possibility food could remain. The standard deviation for TG ($148 \text{ mg/dL} \pm 56$) suggests that this difference might be due to variation in fasting status of the hamster.

In the hamster model, analyses of lipoprotein revealed no significant differences in the composition of the VLDL particle. In the LDL particle, TG increased in Hi-LM and

Hi-PO compared with Hi-LO and Hi-LO Low-LN. Also, in the HDL particle TG was lowest in Hi-LM compared to all diet groups and CE was lowest in Hi-LO compared with other diet groups.

CETP transfers cholesterol esters from HDL to VLDL and LDL in exchange for triglycerides. Hamsters show CETP activity similar in humans and therefore present a good model to elucidate mechanisms associated with Reverse Cholesterol Transport (RCT). In an attempt to understand the differences we saw in the LDL and HDL lipoproteins, we analyzed the expression of RCT genes. Based on the results, in the LDL particle, TG was highest in Hi-LM diet group along with being lowest in the HDL particle along with higher CE. These analyses led to the speculation that CETP expression might be altered with Hi-LM consumption. ABCA1, ApoA1, CETP and SR-B1 in the liver tissue were explored and analyses were made using fold differences between the diet groups relative to Hi-LM. The trend showed, compared to Hi-LM, expression of CETP was almost 2.5 fold lower in soybean supplemented Hi-LO and tropical oil fraction Hi-PO and 2.6 fold increased expression in blended oil Hi-OL. Increased expression of CETP would suggest increased TG in the HDL and decreased expression would indicate decreased TG in the HDL particles. The trend did not show these differences in the TG and CE in the LDL and HDL lipoproteins. Based on available literature, not much has been done to interpret our analyses to previous data. Dorfman et al analyzed CETP activity in hamsters fed different dietary fatty acids, and the results showed no difference in the activity [64]. More research is warranted to explain the differences that we see in the plasma and lipoproteins in this study. Other

pathways that might influence these differences need to be studied, especially LDL-C pathway.

Mechanisms associated with increase in Plasma TC and LDL-C has been studied in the gerbil and the hamster models. Gerbils are HDL models with almost 70% of the cholesterol transported in the HDL particle as opposes to hamsters, which like humans, exhibit the effects of dietary fatty acids in plasma through changes in LDL-C and moderately through HDL-C. It is speculated that the changes seen in the plasma lipids is due to either the alteration in the rate of LDL production or/and receptor dependent LDL uptake by the liver [80]. SFA is associated with depressing the activity and expression of LDL receptors, thereby increasing the concentration of circulating plasma cholesterol. On the other hand, unsaturated FA tends to accelerate the rate of receptor dependent LDL uptake resulting in lowering of circulating LDL concentrations [81]. Also, the effect is more pronounced in the presence of dietary cholesterol. Dietary cholesterol suppresses LDL receptors and SFA along with dietary cholesterol has a combined effect on LDL receptor uptake, and PUFA tends to partially restore the suppression due to dietary cholesterol [80]. Horton et al demonstrated that the changes induced due to receptor dependent LDL transport paralleled changes in LDL receptor protein and mRNA levels.

Along with LDL receptors, activity of ACAT is also known to increase with unsaturated FA consumption. In contrast, SFA are speculated to inhibit ACAT activity resulting in decreased conversion of FC to CE and thereby, increasing the FC pool. An increase in FC pool mediated feedback repression of the LDL receptor pathway, further intensifying the effects. There is little data available on the effects of blended,

genetically modified and interesterified fats on these pathways relative to naturally occurring oils. This would be an interesting venue for future studies to understand the underlying mechanisms that control the changes in plasma cholesterol level at the level of expression and how they in sync with the HDL regulation genes.

Small LDL particles are considered atherogenic compared to the larger LDL particles and also there is emerging data that small HDL particles might have a pro atherogenic effect. Based on Heek and Zilversmit formula for particle size measurement, LDL and HDL particles showed significant differences between diet groups in hamsters. To fully elucidate the effects of the diets on the particle size and compositions, subfractions of the lipoproteins need to be evaluated. For the current study we did not analyze the subfractions of LDL and HDL particles and the differences we see are very small to conclude their effects.

Analyses of the liver lipids revealed no significant differences between the diet groups in the gerbil and the hamster models. Studies comparing the effects of SFA and unsaturated FA show decrease in liver lipids with SFA consumption [82, 83]. In the hamster study, Hi-LM and Hi-LO diets induced similar plasma concentrations and therefore, it may be speculated that liver lipids for both would be similar, although the mechanisms may not be the same. Little is known about the effects of blended and genetically modified oils on liver metabolism. More research is warranted to explain and understand if these effects are reproducible and to what degree they apply to humans.

Saturated fatty acids (SFA) have been associated with increased CVD risk and have been a subject of debate for establishing the recommendations of Dietary Guidelines for the last 50 years. In terms of replacing SFA, Carbohydrates (CHO) are

generally considered as a safe substitution. There is emerging data that SFA may not pose adverse effects and there may not be any additional effects with SFA being replaced by CHO. In the present study we examined the extent to which the amount and type of SFA affect plasma lipids and lipoproteins. Diets were formulated such that percentage of calories from protein, MUFA and PUFA were kept constant while calories from Lauric and Myristic (60%LM, 45%LM, 30%LM) or Palmitic Acid (45%PA, 30%PA) were replaced with calories from carbohydrate and compared to 21% CON diet. To elucidate the effects, we used a hamster model, evaluated plasma and liver lipids and analyzed the RCT gene expression for holistic understanding of the diets.

In the present study, diet consumed and body weights showed interesting results. Diet consumed was highest for 45% LM group and interestingly, 45%PA and 60% LM diet groups consumed significantly lesser diet compared to 45%LM. One of the plausible explanations could be that 45% LM had a higher acceptance for the diet compared to 45%PA and 60%LM. Also, there could be a higher satiety effect of 45%PA and 60%LM compared to 45%LM. Whether these effects are due to taste, palatability, satiety or digestibility needs further investigation.

Based on the grams consumed, kcalories were affected likewise and analyses of the results show that kcalories consumed were directly associated with increase in body weight. Kcalorie consumption for 45%LM was higher compared to all diets, and the trend revealed that 45%LM had the most weight gain, but change in weight was significantly different only between 45% PA and 21% CON.

Results of plasma analyses showed that 60%LM and 45%LM raised plasma TC and nHDL-C more than all other diets. But the most striking result was that 45%LM

showed increases in TC and nHDL-C more than 45%PA without any significant differences in HDL-C. Comparing the P:S ratio for these diets suggest plausible explanations. It is speculated that P:S ratio close to 1 diminishes the effects of SFA on plasma cholesterol. 21%CON and 30% fat diets had P:S ratios of 4.64 and 0.9 respectively, and consequently there were no significant differences in their plasma lipid profile. 45%fat and 60%LM had P:S ratios of 0.4 and 0.23 respectively and it would be expected that all three would show similar increases, but 45%PA showed significantly lower plasma TC and nHDL-C compared with 45%LM and 60%LM, establishing a higher cholesterolemic effect of Lauric and Myristic acid compared with Palmitic acid.

Also, as previously shown in studies, SFA has a higher potential to raise HDL-C compared to unsaturated FA was verified in the present study too. 60% LM, 45%LM and 45%PA revealed significantly higher HDL-C levels compared with the 21%CON diet. Similar trend was seen with 30% LM and 30%PA compared to 21%CON, but the differences did not reach significance.

Another aspect of understanding these results are in the light of the fact that these diets represented replacement of SFA with CHO. Compared to a high CHO diet (21%CON), all SFA diets showed increase in TC, nHDL-C as well as HDL-C with no effect on TC:HDL-C ratio. This is in agreement with current literature suggesting that due to the magnitude of changes between the lipoproteins in a high fat versus a high CHO diet TC:HDL-C ratio is unaffected [30, 53]. Therefore, in this respect CHO replacement of SFA, there seems to be no additional beneficial effect.

Predictive models and studies conducted in humans suggest replacement of SFA with CHO suggest that a high CHO diet is likely to have higher TG compared with high

SFA diet [52]. The present study revealed the opposite results. High CHO or 21% CON diet showed lowest TG compared to all other diets in trend, although the differences were only significant between 60%LM and 21%CON. In the present study, 21%CON contained 21%, 54% and 25% energy from fat, CHO and protein respectively. In study models used to assess differential effects of CHO on lipoprotein metabolism have used CHO higher than 55% and fat higher than 30% [84]. Our study's design even though represents substitution of CHO for fat, was primarily set up to see the differential effects of individual FA substitutions of lauric and myristic versus palmitic acid with respect to CHO. Further research is warranted to understand the differential effects in TG metabolism in high fat versus a high CHO diet.

Lipoprotein analysis revealed that in case of 60% LM, TG was lowest in the LDL and highest in the HDL particles compared to all diets. The results suggest that there might be a potential inhibitive role of CETP involved which we investigated as a part of studying the RCT genes. Also, TC in VLDL and LDL were significantly higher in 60%LM compared to all other diets. The results from the lipoprotein TC analyses need further investigation to understand underlying mechanisms. A plausible explanation could be that there is an increase in the secretion of the VLDL/LDL particles. Apo B is the specific marker for number of VLDL/LDL particles or it could be that the number of particles is the same but the lipoproteins have an increased concentration of TC. This could be due to the suppressive effect of SFA on the LDL receptor and hence clearance of LDL-C. Whether the effect of higher VLDL/LDL-C in 60%LM is due to increased synthesis or decreased clearance needs further investigation.

Liver weights were not significantly different between diets. Also analyses of liver lipids revealed no significant differences in TC, FC and CE between the diet groups. It would be interesting to investigate why no differences were observed. A plausible thought could be that there is a suppressive effect of SFA on LDL receptors and an increased clearance of FC due to up regulation of ACAT and 7-alpha hydroxylase in the 21%CON diet compared to other diet groups. Therefore, even if there is an increased uptake of nHDL-C from the circulation, its clearance is also up regulated. The effects could be explored further by investigating the expression of genes involved in LDL metabolism.

To understand some of the differences seen in the lipoprotein composition and to investigate the changes with replacing CHO for SFA, we looked at the expression of the RCT genes in the liver tissue. The most striking differences were seen in the expression of ABCA1, ApoA1, CETP and SR-B1 for the 45% LM diet group relative to 21%CON, and compared to all other diet groups. The trend seemed to be working in the opposite direction compared to all other diets. Plasma lipids and lipoproteins for 45% LM behaved differently compared to 45% PA and similar to 60% LM. Further investigation is warranted to understand the trends in the differences seen and if there are other pathways that might be involved in the regulation.

The postprandial state represents the phase between food ingestion and the post-absorptive state, and is the habitual metabolic state for most duration of the day. Characteristic to this phase is the rise in TG and TG-rich lipoproteins, Chylomicrons and VLDL of intestinal and hepatic origin respectively. For the present study we presented an oral fat challenge in 6 healthy subjects to investigate the effects of an oleic and

linoleic acid supplemented blended oil (Hi-OL), palm oil with a higher fraction of oleic acid supplemented oil (Hi-PO) and high linoleic acid supplemented oil (Hi-LO) on postprandial lipids and lipoproteins.

Analyses of the results show a rise in TG at 2 hours and slowly declining through 4 and 8 hours subsequently. No significant differences were observed between the diet groups for TG, TC and HDL-C at 0, 2, 4 and 8 hours. A tropical oil based Hi-PO and blended oil Hi-OL represent alternatives that can be used in place of PHVO and contain higher dietary SFA compared to soybean oil, Hi-LO. Based on the results on plasma lipids in this pilot study, it may be speculated that these alternatives can be used interchangeably.

To further investigate the effects of these oils, we observed their effects on CM/VLDL subfractions. Sf>400 is produced by the intestine and carries TG derived from ingested fat. Analyses of the results reveal that Sf>400 increased substantially from 0 to 2 hours indicating the incorporation of TG in the chylomicron particle from fasting to fed state. In sync with the plasma TG results, Sf>400 declined at 4 and 8 hours subsequently. During the fed state, CM gets cleaved of TG and CM remnants are taken up by the liver.

Sf 60-400 and Sf 20-60 represent chylomicron remnants in addition to hepatically synthesized VLDL particles. Their hepatic origin can be justified by the fact that the level of TG in these particles is higher in the fasting state compared with the CM Sf>400 particles. At 2 hours, Sf 60-400 increased in TG concentration and declined at 4 and 8 hours. For Sf 20-60, TG increased at 2 and 4 hours and then decreased at 8 hours. Plausible explanation to these changes could be the shift in metabolic state of fed to

early fasting. No significant differences were observed between the diet groups at any time point. This further strengthens the idea that consumption of Hi-Po and Hi-OL will not pose any adverse effects compared with Hi-LO.

CETP is active in the postprandial state. An increase in CETP activity is considered to be atherogenic due to its potential to transfer cholesterol esters to VLDL/LDL particles and thereby increasing the concentration of LDL-C. In the present study, no significant changes in the CETP activity were observed between the diet groups at any time point. The baseline CETP activity for individual subjects varied on each study day. Therefore, these results from CETP activity need to be further investigated in a larger population to derive conclusive results.

The results from the study suggest no adverse effects of the diets compared with Hi-LO. But these results also need to be interpreted with caution. The number of subjects in this pilot study are only suggestive of the direction, but cannot be used as conclusive. There are some limitations to the study that need to be addressed that could potentially impact the results of the study. The oral fat challenge was given as a “smoothie” and the test oil was added to it. There was no way of measuring the level of incorporation of the oil in the smoothie the left overs. The oil had the tendency to stick to the sides of the blender and therefore complete ingestion of the amount is a concern. Another limitation was the control on the subjects regarding the fasting period before the study day. Even though the subjects were asked to maintain similar diets before the study day and fast for 12 hours, there was no way of verifying these concerns.

In light of the above observations and limitations, it is evident that a larger study is needed to evaluate the full potential of these alternative oils. The study needs to

include a much larger population and should be a feeding study to see the complete effects of these oils with increased dietary SFA on lipids and lipoproteins.

To Summarize

In the first study we evaluated the effects of naturally occurring oils (Coconut and Soybean oils), interesterified fat, blended oil, palm oil fraction and a genetically modified fat in a gerbil and hamster model. The results from the study suggest that compared to the Hi-LM coconut oil diet, all diet formulations presented decrease in plasma lipids irrespective of SFA formulations. No adverse effects were seen in terms of lipoprotein composition or liver lipids. The present study agrees with the hypothesis that oil formulations with specific SFA will not cause any adverse effects in lipids and lipoproteins.

The second study was an attempt to investigate the differential effects of replacing specific SFA with CHO on lipoprotein metabolism. The analysis of the study shows higher cholestrolemic effect of lauric and myristic acid at 45% and 60% intake compared with palmitic acid at 45%. Also for diets that maintain P:S ratio close to 1 did not show any adverse effects on plasma lipoproteins. It would therefore be interesting to speculate that if P:S ratio in a diet is maintained, SFA intake up to 30% would not have any adverse effects. Also, replacement of SFA with CHO will induce no additional benefit.

In the final study we evaluated the effects of a tropical oil fraction (Hi-PO) and a blended oil (Hi-OL) in comparison to the most widely used oil source in the US, Soybean oil (Hi-LO). The analyses of the study suggest no adverse postprandial effects

of these oils on plasma lipids and lipoproteins. More research is warranted to explore the potential effects of these oils in a larger population.

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ABSTRACT**EFFECTS OF DIETARY FAT SATURATION ON LIPOPROTEIN METABOLISM IN
RODENTS AND HUMANS****by****DEEPINDER KAUR****MAY 2011****Advisor:** Dr. Pramod Khosla**Major:** Nutrition and Food Science**Degree:** Doctor of Philosophy

Consumption of trans-fatty acids (tFA) and saturated fatty acids (SFA) have been associated with higher incidence of coronary heart disease. Experimental and observational data suggests that consumption of PHVO containing tFA, like SFA leads to increased total cholesterol and LDL-cholesterol (LDL-C) but compared to tFA, SFA raises HDL-cholesterol (HDL-C). An attempt to eliminate PHVO from the food supply has resulted in the need to find alternatives which despite their higher dietary fat saturation as compared to naturally occurring vegetable oils, may be preferable to PHVO. However, amongst SFA, the effects on plasma lipoproteins are variable. Thus, we hypothesized that specific combinations of dietary SFA will not contribute to an adverse lipid profile.

We used gerbils and hamsters as our study models to determine the effects of Lauric and Myristic Acid (Hi-LM), Oleic Acid (Hi-OL), Palmitic Acid (Hi-PO), Linoleic Acid

(Hi-LO Low-LN), Stearic acid and Linoleic Acid (Hi-SLO) supplemented diets on plasma and liver lipids and lipoproteins. The results revealed that compared to the Hi-LM diet, all diets had lower TC, LDL-C and HDL-C. Liver lipids were similar for all the diet groups. Also, we analyzed the gene expression for the reverse Cholesterol transport genes. No significant differences in ABCA1, ApoA1, CETP or SR-B1 were observed between the diet groups compared with Hi-LM diet. The data suggested no additional adverse effects on CHD risk factors compared with Hi-LM diet.

Carbohydrates are generally considered a safe replacement for SFA. We used a hamster model to evaluate the extent to which the amount and type of SFA affect lipoproteins. Diets were formulated such that percentage of calories from protein, MUFA and PUFA were kept constant while calories from Lauric and Myristic (60%LM, 45%LM, 30% LM) or Palmitic Acid (45%PA, 30% PA) were replaced with calories from carbohydrate and compared to 21% CON diet. Plasma TC and n-HDL-C was lower in all diet groups compared with 60%LM and 45% LM supplemented groups. Liver lipids were similar in all diet groups. Analysis of Reverse Cholesterol Transport genes revealed no significant differences in expression between the diet groups compared with 21% CON. Data suggests no adverse effect of PA at any level of consumption and no net effect of replacing CHO for at any level of SFA.

The postprandial state is a reflection of the metabolic state in humans due to regular diet intake. We used an oral challenge to test oils with various fatty acid compositions (Hi-PO, Hi-LO and Hi-OL) to evaluate postprandial changes in lipids at 0, 2, 4 and 8 hours. No significant differences were seen in TC, HDL-C, TC:HDL-C and TG between the test oils. We analyzed the chylomicron fractions following the oral fat

challenge and data revealed no significant differences in the chylomicron fractions between the test oils. Additionally, we measured CETP activity in plasma following the challenge and no significant differences were observed. The data suggests in agreement with the hypothesis, oil formulations with specific SFA will not cause any adverse effects in lipids and lipoproteins.

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