MECHANISMS OF ACTION OF DIETARY FATTY ACIDS IN A SYRIAN HAMSTER MODEL: THE ROLE OF FATTY ACID ETHANOLAMIDES ON FEEDING INTAKE, BODY COMPOSITION AND ENERGY EXPENDITURE

By

Lyyn (Lin) Lin

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This Thesis is dedicated to my parents

Qixin Lin and Ping Ren

For Giving Me a Strong Mind and a Kindhearted Soul

"When you are lonely, when you feel yourself an alien in the world, play Chess.

This will raise your spirits and be your counsellor in war" (Aristotle)

Abstract

Replacement of saturated fatty acids (SFA) with monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) impacts risk of atherosclerosis and cardiovascular disease (CVD). However, although dietary fatty acids (DFA) have been established as an important factor related to CVD, their exact mechanisms of action have not been clearly established. One of the possible mechanisms is that DFA convert to fatty acid ethanolamides (FAEs), such as oleoylethanolamide (OEA), palmitoylethanolamide (PEA) and arachidonoylethanolamide (AEA), which are thought to associate with lipid signalling, fat oxidation and appetite control. Hence, the objectives of this thesis were to identify the impact of diets containing corn oil, canola oil, DHA + canola oil and fish oil on plasma and organ levels of FAEs as well as energy metabolism and lipid profiles in Syrian Golden hamsters. Forty-eight hamsters were provided diets containing 6% treatment oil for 30 d before sacrifice. Across all diets, in proximal small intestine and liver, animals fed canola oil showed higher ($p<0.05$) levels of OEA than corn oil and fish oil fed groups, but no difference compared to those fed DHA +canola oil. In plasma, fish oil fed animals showed higher ($p<0.05$) OEA and PEA levels and lower ($p<0.05$) AEA levels compared to all other groups. Feed intakes (g/d), oxygen consumption (ml/g) and body composition of total fat (%) and mass (g) did not differ across groups. However, energy expenditure associated with fat oxidation (%) was higher ($p<0.01$) in canola oil and DHA + canola oil fed hamsters compared to those consuming corn oil and fish oil. Also, body composition of fish oil fed animals showed a lower ($p<0.01$) total lean mass (g) compared to other three groups and a lower ($p<0.01$) total mass (g) compared to DHA + canola oil diets, but no difference compared to animals fed the canola oil diet. None of the treatments had any effect on triglyceride (TG) or C-reactive protein (CRP) levels. The

fish oil group showed a higher ($p<0.01$) plasma total cholesterol (TC) levels than all other three groups. No differences existed between DHA + canola oil and fish oil groups in HDL or Non-HDL levels, but these levels were different ($p<0.01$) compared to corn oil group and canola oil groups. To conclude, different DFA affect whole body energetics and plasma lipid profiles. Also DFA produced marked shifts in plasma and organ levels of OEA, PEA and AEA. These dietary induced shifts in FAEs may translate into discernable changes in energy expenditure and lipid levels which in turn influence CVD risk.

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CHAPTER 1: LITERATURE REVIEW

1. Introduction

Cardiovascular disease (CVD) is currently one of the leading causes of death in North America. Hypercholesterolemia is a well-established risk factor for the development of atherosclerosis, a component of CVD. Food choice profoundly influences health outcomes such as heart health and its related risk factors such as obesity. Replacement of saturated fatty acids (SFA) with monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) impacts risk of atherosclerosis and CVD. In North America, dietary sources of oils vary substantially in fatty acid composition, such as canola oil high in MUFA; corn oil high in omega-6 and fish oil high in omega-3. Although dietary fatty acids (DFA) have been established as important factors related to CVD, their exact mechanisms of action are not clearly understood. One of the possible mechanisms is that DFA convert to fatty acid ethanolamides (FAEs), such as oleoylethanolamide (OEA), plamitoylethanolamide (PEA) and arachidonoylethanolamide (AEA), which associate with lipid signalling, fat oxidation and appetite control. Hence, the purpose of this research was to identify the impact of diets containing corn oil, canola oil, canola + DHA oil and fish oil on plasma and organ levels of FAE as well as energy metabolism and lipid profile in Syrian Golden hamsters. Accordingly, we have investigated the effects of corn oil, high-oleic canola oil, high-oleic canola +DHA oil and fish oil on food intake, body weight, plasma lipid profile, inflammatory marker (C-reactive protein), body composition, energy expenditure, tissue fatty acid composition, and the levels of FAEs.

1.2 Cardiovascular Disease

Currently, CVD is the leading cause of death in North America (Arnett et al., 2005). In 2006, 29.2% of deaths were from CVD; and mortality data showed that 1 of every 2.9 deaths was from CVD in the United States (Lloyd-Jones et al., 2010). According to the Public Health Agency of Canada (Government of Canada, 2010), the definition of CVD refers to more than one disease of the circulatory system, including heart failure, stroke, hypertension, coronary heart diseases (CHD), congenital cardiovascular defects and atherosclerosis. The vast majority of coronary heart diseases are caused by atherosclerosis from the narrowing of the coronary arteries due to build up of plaque. Scientists and researchers continue to search for optimal approaches to reduce the morbidities associated with this disease especially CHD and suppress potential risk factors.

1.3 Hypercholesterolemia and Diet

Hypercholesterolemia, or dyslipidemia, is a well-established risk factor for the development of CVD, including CHD and atherosclerosis. The fundamental relation between dyslipidemia and atherosclerosis is related to the elevated level of low density lipoprotein cholesterol (LDL-C) in blood. Over 33% of American adults exhibit elevated circulating LDL-C values and 16% possess low high density lipoprotein cholesterol (HDL-C) levels. The linkages between hypercholesterolemia and cardiovascular challenges are well established in that for every 1% decrease in serum LDL-C levels there is an associated 1% decline in CHD events (NCEP, 2002). Current treatments using lipid-lowering drugs have been shown to reduce LDL-C levels, which slows the progression of atherosclerosis and other coronary conditions (Fodor et al, 2000). In the 2009 Heart Disease and Stroke Statistics update report, it is indicated that serum lipid levels are risk factors for CHD when elevated total cholesterol (TC) > 200mg/dl (> 5.17mmol/L), elevated

LDL-C >100 mg/dl (> 2.6mmol/L); reduced HDL-C <40 mg/dl (< 1.03mmol/L) and elevated triglycerides (TG) >150 mg/dl (> 1.69mmol/L) (Lloyd-Jones et al, 2010). Patients identified as having LDL-C concentrations above 130mg/dl (> 3.36mmol/L), or with mild to moderate hypercholesterolemia, are thus routinely counselled to modify their diet with respect to high dietary intake of SFA, total fat and cholesterol (Moreau et al., 2002). Also, Conway et al (2006) indicate that the treatment of hypercholesterolemia using food choice approaches should focus on decreasing caloric consumption from SFA and trans-fat intakes. In fact, ample evidence stems from epidemiological, clinical and experimental research clarifying that food choice is a profound influencing factor on health outcomes, especially for heart health.

Although measurement of individual serum lipid parameters including TC, LDL-C and HDL-C is an important component of most cardiovascular screening algorithms, current guidelines for cardiovascular risk detection have more recently emphasized the importance of non-HDL-C, specific lipid ratios (TC to HDL-C, LDL-C to HDL-C), apolipoproteins (ApoB100, ApoA-1) and C-reactive protein (CRP) as predictive markers for CVD risk (Ridker et al, 2005; . Sinning et al (2011). For example, regulation of the synthesis of apolipoprotein (apo) A1, a major constituent of HDL-C, can in turn affect levels of circulatory HDL-C. Similarly, in humans, the expression of apoB100 correlates with levels of hepatic production of very low density liproprotein cholesterol (VLDL-C) (Sundaram & Yao, 2010). Not only do lipid profiles impact cardiovascular health, extensive evidence now suggests that inflammation is a key mechanism of pathogenicity associated with the development and progression of atherosclerosis and CVD (Lloyd-Jones et al., 2006). Circulatory CRP levels exist as an inflammatory biomarker for prediction of CVD risk, vascular inflammation, vessel damage and also serve as an indicator of lipid-lowering drug efficacy (Ridker et al., 2005). Lloyd-Jones et al (2006) indicate that, in humans, elevation of CRP

levels is associated with CVD based on CRP levels at low < 1.0mg/L, intermediate 1.0 to 3.0mg/L and high > 3.0 mg/L concentrations. For example, women with higher predicted CVD risk, and possessing intermediate or high CRP levels, are at greater risk of CVD than women with high predicted CVD risk but low levels of CRP. The American Heart Association (2011) also indicate that the higher CRP levels (upper third of the CRP levels) relate to a higher risk of having a heart attack when compare to the lower third of the CRP levels in various population sub-categories including men, women and elderly. To conclude, food choice is important for human health, especially for the cardiovascular system. Replacing trans-fatty acids and SFA with unsaturated fatty acids (UNSFA) can decrease multiple cardiovascular risk factors and inflammation.(Mozaffarian et al, 2010). It is therefore important for us to understand the function and mechanisms of action of different dietary fatty acids (DFA).

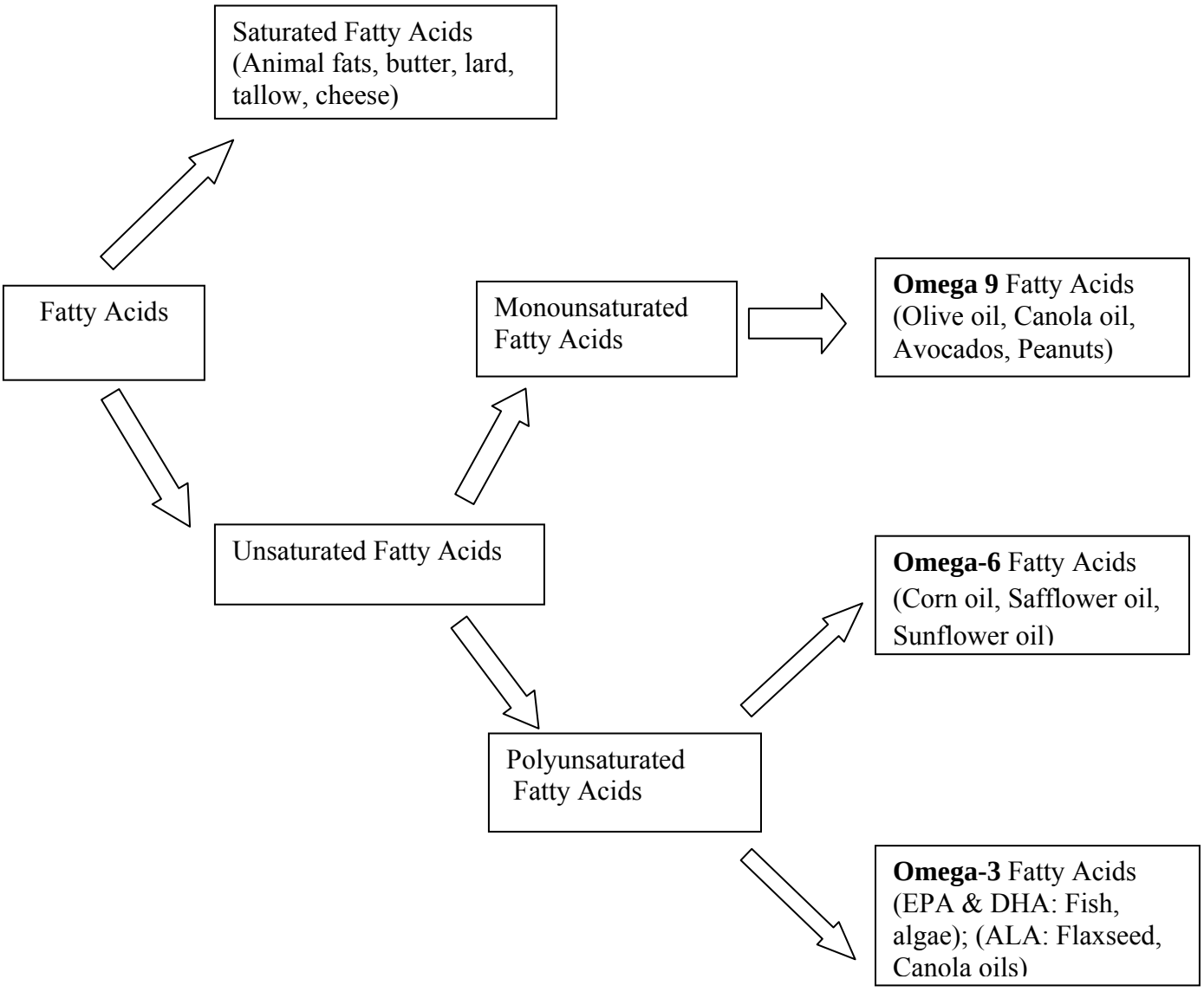
1.4 The Impact of Dietary Fatty Acids on Cardiovascular Health

Recent studies have found that dietary fat quality, as well as fat quantity, impacts CVD risk. Dietary fatty acids, depending on their chain length, chemical structure and number of double bonds, have the ability to regulate several aspects of metabolism, including fat oxidation, and cholesterol and lipoprotein metabolism (Salter & Tarling, 2007). The high SFA to USFA ratio of Western diets tend to impair insulin sensitivity, promote obesity and increase risk of coronary disease (McCarty, 2010). Also, SFA are associated with elevation of LDL-C, but replacing SFA with MUFA can reduce circulatory cholesterol levels, especially LDL-C levels, and in turn atherosclerosis and CVD (Salter & Tarling, 2007; Kris-Etherton, 1999). For instance, high-MUFA diets are better at reducing LDL-C levels, inflammation and lipoprotein oxidation compared to a high carbohydrate diet (López-Miranda et al., 2010). Mente, de Koning, Shannon, & Anand,

(2009) provide a systematic review of 507 prospective cohort studies to confirm that high MUFA intake can decrease CHD events by 20%. Some studies have also replaced SFA with PUFA and found significant effects on reducing the risk of CVD. For example, (Hu et al., 1997) show that MUFA and PUFA are more effective in reducing the risk of CVD, compared to a high-carbohydrate mixed with low-fat diet. Also, high-carbohydrate diets can reduce LDL-C levels, but not the ratio of LDL-C to HDL-C, due to the reduction of HDL-C being the same as that of LDL-C levels. However, when USFA replace SFA, these fats reduce LDL-C and also the ratio of LDL-C to HDL-C, as the proportion of LDL-C is much more significantly reduced than HDL-C (Sacks & Katan, 2002).

The major food sources of different types of DFA are listed in Figure 1. Current Western diets contain high SFA (~12%) based food products obtained mainly from animal fats (meat and dairy), partially hydrogenated fats (trans-fat), 200-400 mg of cholesterol from eggs, dairy and meat per day and low monounsaturated fatty acid (MUFA) (~14% of energy) based food products derived mainly from olives oil and canola oil; a diet which is linked to a higher risk of CVD and other chronic diseases (Lichtenstein et al., 2006). In contrast, the Mediterranean diet is represented by an abundant consumption of virgin olive oil, with a high MUFA (16% to 29% of calories) and low SFA (7% to 8% of calories), which differs in many respects from the Western diet and protects against CVD due to its low concentration of SFA (Kris-Etherton, 1999).

Figure 1. Food Source for Different Types of Dietary Fatty Acids



Source is based on: Lichtenstein et al., 2006; Gillingham, et al., 2011; Svensson, et al., 2011; Wen et al., 2003

In 1994, Willett (1994) summarized National Research Council (NRC, 1989) recommendation for dietary intake, which suggested reduction of total fat intake to less than 30% of calories, a reduction in SFA intake to less than 10% of calories and a lowering of the intake of cholesterol to less than 300 mg daily. Kris-Etherton (1999) introduced that for healthy Americans, the recommended energy consumption be increased to 15% energy from MUFA, up to 10% from PUFA and less than 10% obtained from SFA, in order to prevent obesity and reduce related diseases. The American Heart Association (Lichtenstein et al., 2006) suggests a strategy for CVD risk reduction in the general population, which includes limiting intake of SFA to < 7% of energy, trans fat to < 1% of energy and cholesterol to < 300 mg/d. According to the Dietary Guidelines Advisory Committee (DGAC, 2010), the recommendation for dietary SFA intake is being reduced from the current level of 12% of the diet to 7% with the substitution of SFA by USFA, but not by carbohydrates. In this context, the optimal dietary replacement of SFA by increasing MUFA and PUFA intakes deserves significant attention and warrants additional scientific evidence (Hite et al., 2010).

1.4.1 Monounsaturated Fatty Acids

Monounsaturated fatty acids (MUFA) are classified as fatty acid chains containing one double bond. The predominant MUFA in the diet is oleic acid (C18:1, cis-9), representing over 92% of total MUFAs, which is colloquially referred to as an omega-9 fatty acid (Kris-Etherton, 1999). In North America, the total intake of oleic acid in the diet is in the range of 12% to 18% of total energy consumption (Lopez-Huertas, 2010). The most commonly consumed MUFA are found in olive and canola oil, which contain 73% and 60% of total fat as oleic acid, respectively. Moreover, high-oleic modified canola oil (70% oleic acid in total fat content) production has been increasing

over the last decade due to better oil stability which is critically important for the food processing industry (Canola Council of Canada, 2011).

Lopez-Miranda et al (2010) report that the Mediterranean diet has been distinguished as the dietary pattern which best fulfills the need of nourishment, health and a lower incidence of chronic diseases, especially CHD. The difference between Mediterranean diet and other healthy dietary models is the customary high intake of olive oil. MUFA show many health beneficial effects in terms of CVD risk including improvement of blood lipid profile by decreasing TC and LDL-C levels, increasing HDL-C levels, reducing oxidative stress, improving endothelial function, as well as improving blood pressure and inflammatory status (Lopez-Miranda et al, 2010). Indeed, Lopez-Huertas (2010) show that replacing 5% of SFA with oleic acid can reduce CHD risk by as much as 20-40% through LDL-cholesterol reduction. An increase of oleic acid intake is therefore beneficial for a number of risk factors associated with CVD.

As part of a Mediterranean diet, olive oil is not common in Western diets and the cost of olive oil is much higher than other local oils. In order to obtain a similar amount of oleic acid in Western diets, canola oil has become a popular alternative in North America. Canola, a type of rapeseed plant (*Brassica napus* or *Brassica Campestris*) contains more than 40% oil and is the world's third leading source of vegetable oil. Canola oil can be regarded as one of the healthiest consumed vegetable oils with a desirable fatty acid profile containing the lowest SFA amount, compared to other vegetable oils commonly consumed in the United States (Canola Oil Petition, 2006). Moreover, classical canola oil is rich in oleic acid, α-linolenic acid and possesses zero trans-fat and cholesterol concentrations (Canola council, 2011). The Food and Drug Administration (2006) certified a qualified health claim stating that 19 grams of canola oil daily may reduce the risk of CHD and recommended the direct caloric replacement of dietary SFA with

canola oil. This claim suggested that replacing SFA with USFA from canola oil should not increase caloric intake, so it corresponds to about 19 g of canola oil (11.2 g of MUFA) (FDA, 2006). In another words, replacing 5% of SFA with MUFA can reduce the risk of CVD.

Due to the fact that oils with high levels of MUFA are more stable than those with higher levels of PUFA, high-oleic canola oil offers advantages of being more heat-resistant and possessing a longer shelf-life than classic canola oil, olive oil or fish oil. Thus, high-oleic canola oil can be regarded as favourable oil in food industries and food services. Clinical studies have shown that consumption of high-oleic canola oil or canola blended with flaxseed oil exerts cardio-protective benefits through reduction of circulating LDL-C levels. Also, the combination of flaxseed oil (high in omega-3) and high oleic canola oil showed a favourable lowering of plasma E-selectin concentrations, which improves inflammatory status in human studies (Gillingham, et al, 2011). More studies are required to determine the function of high-oleic canola oil and the combination of canola oil combined with omega-3 fatty acid rich oils in terms of cardiovascular health benefits.

1.4.2 Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFA) include two types of fatty acids, omega-3 and omega-6. Humans cannot synthesize PUFA since they lack the □12- and □15-desaturase enzymes required in PUFA formation (Adkins & Kelley, 2010). As such, humans need to obtain PUFA from diet. Omega-3 and omega-6 fats differ from each other in their chemical structure. From the methyl end of the fatty acid, the first double bond of the omega-3 is between the third and fourth carbon atoms, while with omega-6 fatty acids the first double bond is after the sixth carbon atom (Adkins & Kelley, 2010). A strong correlation exists between omega-6 and omega-3 fatty acids

because they both respond differently to inflammation in atherogenesis. It is important to understand the fundamental metabolic differences between omega-6 and omega-3 fatty acids.

1.4.2.1 Omega-6 Fatty Acids

Linoleic acid (LA) exists as a precursor of arachidonic acid (AA). The conversation from LA to AA shares the same enzymes as convert omega-3 ALA to EPA, including the □6- and □5-desaturases. Generally, omega-6 can be converted to proinflammatory eicosanoids such as the two series prostaglandins (PGE_2) and thromboxanes (TXA_2). TXA_2 can continue to be converted to TXB_2 (Adkins & Kelley, 2009). Also AA produces series-2 PGs and TXBs and series-4 LTs, while EPA produces series-3 PGs and TXBs, and series-5 LTs. The AA-derived eicosanoids are pro-inflammatory factors, which can increase vascular permeability and activate the production of proinflammatory cytokines (SanGiovanni et al., 2005). However, some studies have found that LA is not restricted to increasing plasma LDL but also increases HDL cholesterol levels.

1.4.2.2 Omega-3 Fatty Acids

From the standpoint of vascular disease prevention, omega-3 fatty acids are the most extensively studied PUFA. Major types of omega-3 fatty acids include plant derived ALA, (C18:3n-3) and animal derived eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). These long chain omega-3 fatty acids have been shown to effectively improve multiple cardiovascular risk factors in epidemiological (Adkins & Kelley, 2010), cell culture (Nowak et al., 2011), animal model (Kitamura et al., 2011), and human clinical investigations (Svensson et al, 2011).

Although ALA is the precursor of EPA, the conversion of ALA in humans is extremely variable ranging from 0.2 to 21% for EPA and 0 to 9% for DHA. Unfortunately, ALA does not possess the same health benefits as EPA and DHA on cardiovascular diseases and insulin resistance (Vermunt et al, 2000), creating vigorous debate as to the relative importance of these two categories of omega-3 fatty acids in the diet.

Eicosapentaenoic acid (EPA) is the precursor of DHA and can be converted to other eicosanoids such as thromboxane-3 (TXB3), leukotriene-5 (LTB5) and prostaglandin-3 (PG3) (SanGiovanni et al. 2005). The eicosanoids produced from EPA serve to reduce the inflammatory response and to counteract the actions of other eicosanoids produced from AA (SanGiovanni et al., 2010). However, EPA may also directly compete with AA for storage and metabolism in the cell membrane lipids thereby increasing the production of PG_3, TXB_3 and LTB5 that have less inflammatory effects as well as reduce levels of PG_2, TXB_2 and LTB4 (SanGiovanni et al., 2010).

Docosahexaenoic acid (DHA) is the only omega-3 fatty acid stored inside human tissues and plays very important rules on regulation of brain development, heart health and prevention of inflammation. DHA decreases inflammatory marker cytokines, TNF-α and IL-1β in blood mononuclear cells and inhibits COX-2 *in vitro*, decreasing the amount of PGE2 and LTB4 production in healthy men (Adkins & Kelley, 2010). EPA and DHA act as anti-inflammation factors which can modulate inflammation, reduce LTB4 and other cytokines which in turn decrease the risk of asthma, arthritis, CAD and some types of cancer. For example, studies have shown that DHA decreases the inflammatory marker IL-6 and TGs in hypertriglyceridemic men (Kelley et al., 2009). The impaired secretion of apo B in cells treated with DHA showed an improvement of oxidation, aggregation and autophagocytic degradation of apo B (Sundaram & Yao, 2010). However, Meyer et al (2007) have indicated that fish oil raises the level of cholesterol

and apo B in LDL particles. As such, the health benefits of different dietary fatty acid intake are controversial and it is imperative to investigate this area further.

To conclude, omega-6 and omega-3 fatty acids form different structural and functional components including eicosanoids which appear be antagonistic in their effects. Since long-chain EPA and DHA, as well as AA, compete for the same enzymes including COX-2 and LOX-5, a high omega-3 intake will increase the formation of three-series eicosanoids and be expected to decrease inflammation. Therefore, it is important to balance the uptake of moega-6 and omega-3 FA in order to prevent eicosanoid dysfunctions and may be useful for the treatment of different illnesses and metabolic disorders (Wen & Chen 2003).

1.5 Regulation of Gene Expression by Fatty Acids

With the intention of transportation and utilization of lipids by various organs and tissues as an energy source, mammals have converted insoluble lipids into soluble lipoprotein forms primarily produced in the liver and intestine (Sundaram & Yao, 2010). The liver is the major organ regulating the synthesis, uptake and secretion of lipoproteins, as well as the major site of formation of cholesterol and TG. The formation of lipoproteins is associated with specific gene transcription and translation processes.

The relationship between gene and protein expression is regulated by extracellular signalling. For living cells to adapt to their environment, to manage their homeostasis, and to grow, they must modify their transcription pathways depending on factors resulting in altered expression of specific genes (Sagduyu, 2002). The majority of genes are expressed in two steps. The first one is termed transcription, occurring in the nucleus and involving the production of RNA by copying a DNA template using the enzyme RNA polymerase. The second step is termed

translation which occurs in cytoplasm and translates messenger RNA (mRNA) into a classified sequence of amino acids resulting in the production of a protein (Lorkowski & Cullen, 2003).

Salter & Tarling (2007) have indicated that DFA and also fatty acid derivatives have direct effects on the regulation of enzymes, apolipoproteins and receptors involved in lipid metabolism by modulating gene expression. Also, Vallim & Salter (2010) point out that dietary PUFA intake can down-regulate specific genes involved in fatty acid synthesis. Very long chain PUFA, including EPA and DHA, appear to be able to inhibit lipogenesis by reducing the expression of stearoyl CoA desaturase 1, fatty acid synthase and acetyl coA carboxylase. As well, EPA and DHA can promote lipolysis and fatty acid oxidation by increasing the activity of acyl CoA oxidase, acyl CoA transferase-1, ketoacyl-CoA thiolase and hormone-sensitive lipase (Tai & Ding, 2010). Sterol regulatory binding proteins (SREBPs), peroxisome proliferator activated receptors (PPARs) and liver x-receptor (LXR) exist as important transcription factors in the fatty acid-induced regulation of gene transcription. More specifically, SREBPs belong to the basic helix-loop-helix and leucine zipper family of membrane-bound transcription factors. PPARs and LXR belong to the class of nuclear hormone receptors that form obligate heterodimers with retinoid X receptors (RXR) (Vallim & Salter, 2010). The difference between LXR and PPARs can be distinguished based on the fact that LXR is known to regulate bile acid synthesis and lipogenesis, whereas PPARs are associated with regulation of fatty acid metabolism and inflammation (Goldwasser et al., 2010) (Varga et al, 2011).

1.5.1 Peroxisome Proliferator Activated Receptors

Peroxisome proliferator activated receptors (PPARs) are the most well studied and representative nuclear receptors that are regulated by fatty acids. Peroxisome proliferator activated

receptors (PPARs) function by dimerizing with RXR and binding to DNA sequences termed the PPAR response element**.** The three major PPAR isoforms alpha, beta and gamma are all ligand-activated transcription factors. Each isoform has specific and interrelated functions in certain organs and tissues (Bocher et al. 2002).

PPAR-γ is mainly found in adipose tissue and macrophages. Two different types of adipose tissues exist, white adipose tissue (WAT) and brown adipose tissue (BAT). White adipose tissue (WAT) is used for energy storage, supplying energy when other tissues are in need and producing adipokines supporting whole body metabolism. The factor PPAR-γ is important in regulating WAT adipocytes, such that a dominant-negative PPAR-γ mutation can lead to the development of progressive lipodystrophy in humans and mice (Goldwasser et al., 2010). Compared to WAT, BAT is particularly used for energy expenditure, so BAT is important in temporary storage of fats and thus is protective against obesity and related metabolic problems in human. PPAR-γ is highly expressed in BAT and is necessary for BAT formation (Wang 2010). PPAR-β can be found in most tissues and is weakly activated by fatty acids. Also, PPAR-β does not significantly correlate with fatty acid metabolism and does not affect cholesterol metabolism in mouse macrophage tissues (Barish, 2006).

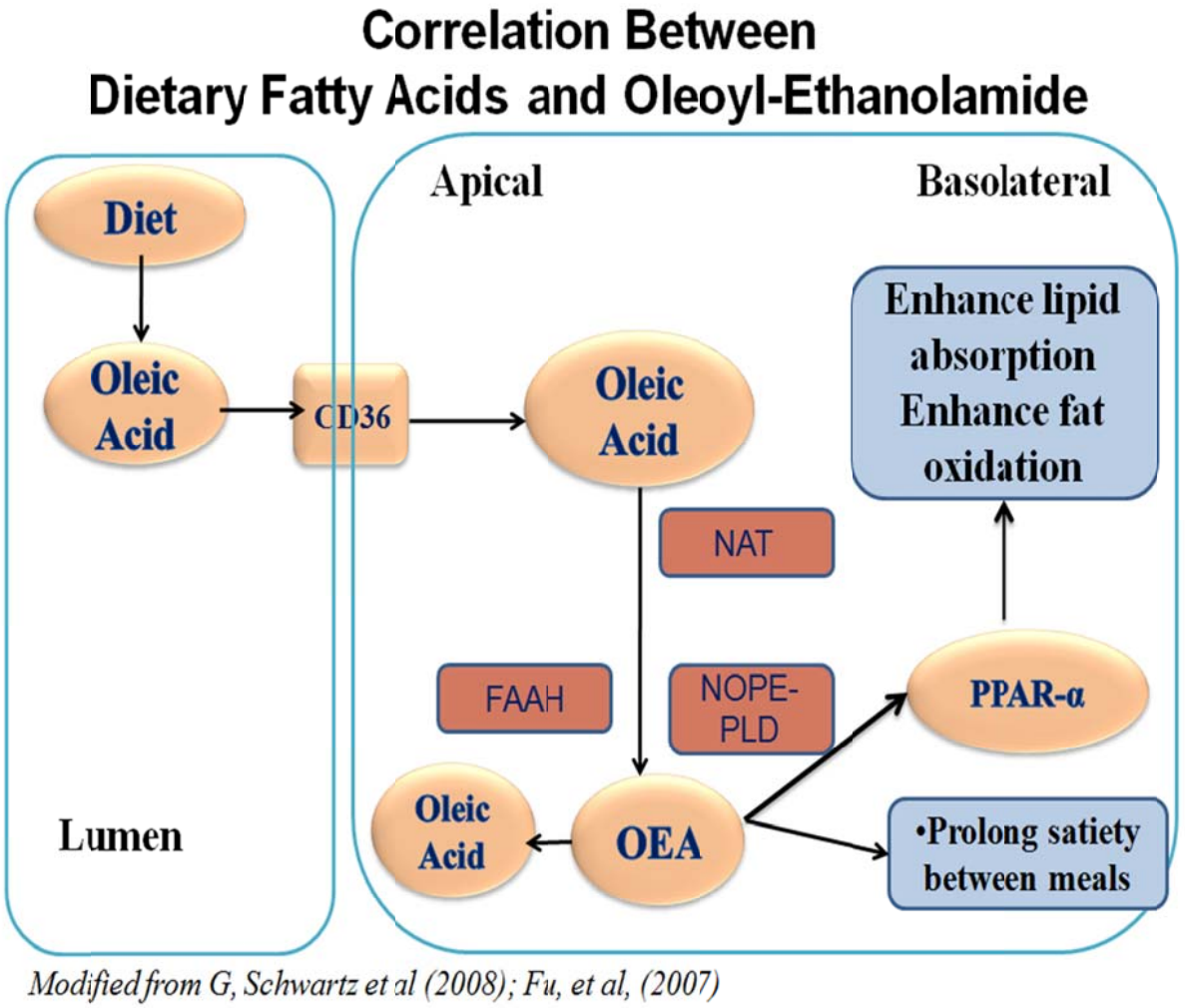
The most important isoform of PPAR on fatty acid regulation is PPAR-α. PPAR-α is expressed in the liver, heart, muscle and kidney, where it regulates fatty acid catabolism (Zavvar Reza et al., 2009). PPAR-α can be activated by fatty acids, eicosanoids, oxidized fatty acids and cholesterol reducing drugs, such as fibrates. This factor is also involved in elevating levels of apoA-1 and thus HDL-C concentrations. Moreover, PPAR-α is important in the up-regulation of triglyceride synthesis, promoting ketone body synthesis and glycogenesis in the liver (Ribet,et al.,

2010). In addition, PPAR-α can be expressed in BAT where combined with PGC1α to increase thermogenesis.

Diet impacts PPAR activity. For instance, a high omega-3 fatty acid diet reduces PPAR-α expression in adipose tissue, but does not affect PPAR-γ expression. In addition, omega-3 fatty acids can bind with PPAR-α and PPAR-γ resulting in the promotion of β-oxidation and adipogenesis. Docosahexaenoic acid (DHA) can bind with RXRs and affect RXR dependent transcription in brain, but EPA cannot activate RXR. Compared to PUFA, SFA cannot directly bind to PPAR-α (Seo et al., 2005).

There is limited evidence on how MUFA regulates PPAR-α directly, but a few studies have showed that oleoylethanolamide (OEA), a derivative of oleic acid, functions as a PPAR-α agonist to enhance lipolysis in mouse adipocytes (Fu et al., 2003). The function of OEA may associate with the participation in the satiety-induce effects by delaying meal initiation and prolonging the interval between meals and inhibiting food intake by activating PPAR-α (Fu et al., 2003). It is important to understand how MUFA affects PPAR-α through OEA. As such, many potential possibilities exist describing how oleic acid can regulate PPAR-α activity as well as affect satiety after the consumption of oleic acid. Also, it is helpful to understand the modulation of OEA through dietary MUFA by understanding the mechanism of actions between OEA, PPAR-α, fat oxidation and energy expenditure (Figure 2).

Figure 2 Correlation Between Dietary Fatty Acids and Oleoylethanolamide



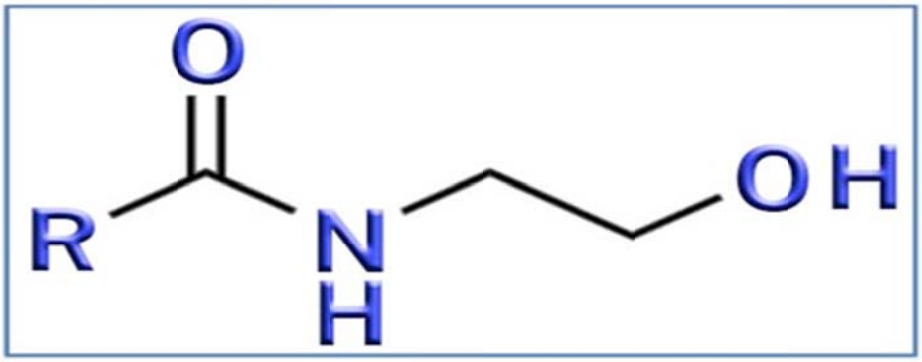
NAT, N-acyltransferase; NAPE, N- acylphosphatidylethanolamine; NAPE-PLD, NAPE-hydrolyzing phospholipase D; FAAH, fatty acid amide hydrolase; OEA, oleoylethanolamide; PPAR-α, peroxisome proliferator activated receptor

1.5 Fatty Acid Ethanolamides

The gastrointestinal tract participates critically in the regulation of absorption and digestion, controlling multiple aspects of this process through both neuronal and humoral mechanisms in vertebrates (Astarita, 2005). Humoral signals, such as leptin, cholecystokinin, glucagon-like peptide-1, and ghrelin have attracted attention in this field through regulation of neural inputs, but lipid mediators are also involved and have received considerable attention due to recent advances in science (Broberger, 2005).

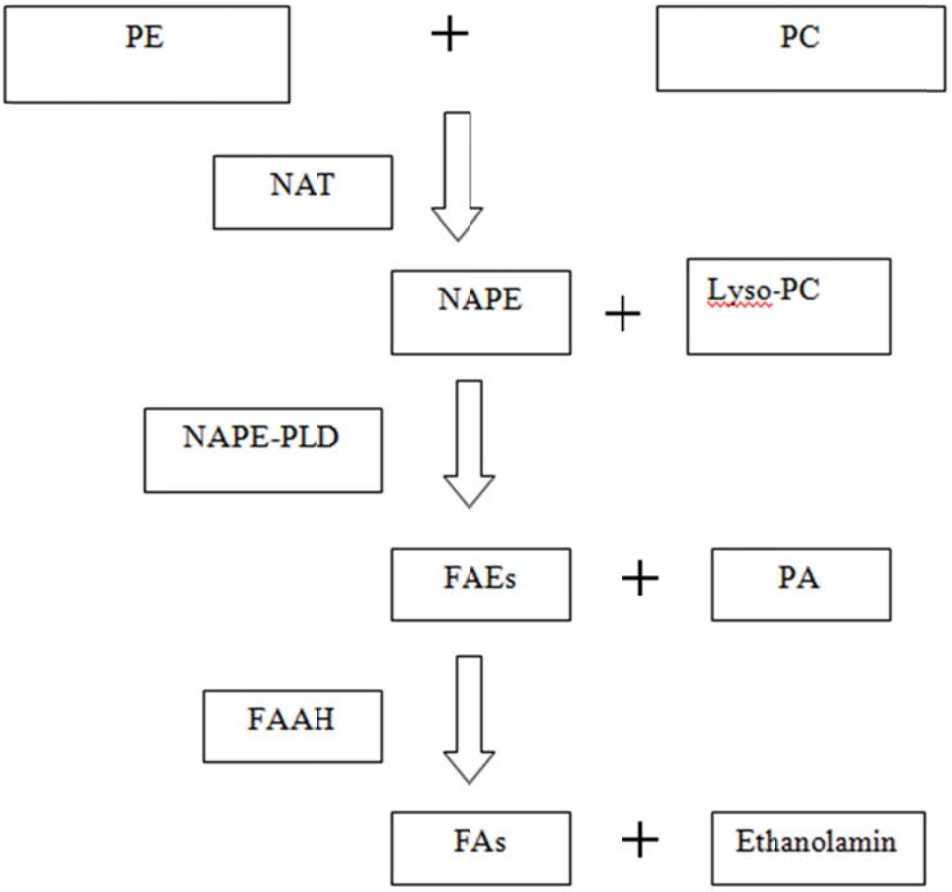
Fatty acid ethanolamides (also called N-acylethanolamines, FAEs) are a group of lipid mediators which have been shown to play a role as lipid signalling molecules distributed in plants and animal tissues (Artmann et al., 2008a)(Ozalp & Barroso, 2009). The general structure of FAEs contains several types of acyl groups linking to the nitrogen atom of ethanolamine (Figure 3) The synthesis of FAEs involves serial enzymes and an N-acylphosphatidylethanolamine (NAPE) intermediate. In the first step, NAPE is synthesised by a calcium-stimulated N-acyltransferase (NAT) from a donor phospholipid to the amino group of an ethanolamine phospholipid. In the second step, NAPE undergoes catabolism by a NAPE-hydropyzing phospholipase D (NAPE-D) to form FAEs. FAEs can then be degraded by fatty acid amide hydrolase (FAAH) and break down into FAs and ethanolamine (Figure 4). Although, OEA, PEA, and AEA are formed by amide linkage of ethanolamine to FA including oleic acid, palmitic acid and arachidonic acid, respectively, their functions are very different (Lo Verme et al, 2005). For example, OEA is involved in peripheral appetite regulation and may have some potential benefits as an anti-obesity pharmaceutical for regulating proximal small intestine satiety signalling (Fu et al, 2008). However, AEA is believed to bind with cannabinoid receptors (CRs) and increase food intake. PEA has been shown anti-inflammatory activities and anorexic properties (Lambert et al., 2002).

Figure 3. General Structures of Fatty Acid Ethanolamides



(Modified from Edgar (2009) from source: http://en.wikipedia.org/wiki/File:N-acylethanolamine.png)

Figure 4. Proposed Route of Fatty Acid Ethanolamides in Vertebrate Tissues



PC, phosphatidylcholine; PE, phosphatidylethanolamine, NAT, N-acyltransferase; NAPE, N-acylphosphatidylethanolamine; NAPE-PLD, NAPE-hydrolyzing phospholipase D; FAEs, Fatty acids ethanolamides; PA, phosphatidic acid; FAAH, fatty acid amide hydrolase, FAs, Fatty acids

Modified source from Jin Fu et al., (2007)**;** Artmann et al., (2008)**;** Petersen et al., (2006)

1.6.3 Oleoylethanolamide (OEA)

Oleoylethanolamide (OEA) is an endogenous lipid mediator primarily in the small intestine (Lo Verme, et al, 2005). Studies have shown that oral administration or intraperitoneal injection of OEA stimulates OEA mobilization in the mucosal layer of the duodenum and jejunum in rat models, but not in other sections such as the ileum, stomach, colon, or in the internal organs and tissues such as the liver, brain, heart and in plasma (Fu et al. 2007).

The biosynthesis of OEA requires two steps including two different enzymes called NAT and NAPE-PLD. In the first step, an oleic acid residue is catalyzed by NAT. Then, NAPE can be formed by N-acylation from PE, PC, lyso-PC or cardiolipin (Thabuis et al. 2007). In the second step, NAPE is split by NAPE-PLD and forms OEA with a phosphatidic acid (PA) molecule. OEA deactivation is related to intracellular hydrolysis, so OEA can be broken down into oleic acid and ethanolamine by means of FAAH (Fu et al., 2007).

In normal physiological conditions, dietary oleic acid is transported into enterocytes using an enzyme called fatty acid translocase, at which point some of the oleic acid is converted to OEA (Schwartz, et al. 2008). In food products, the natural concentration of OEA is very low, about 2 ug/g in cocoa powder, oatmeal or nuts (Thabuis et al, 2008). It is important to know how dietary fat intake is linked to OEA mobilization and the subsequent stimulation of PPARs. Based on Schwartz's hypothetical model (2008), most of the oleic acid entering via CD36 is converted to TG and phosphatidylcholine (PC). A small amount of oleic acid is converted into OEA through the biosynthesis pathway, which activates PPAR-α and then prolongs the satiety between meals (Figure 3).

The action of OEA determines the level of activation of PPAR-α (Guzmán et al., 2004). Guzman et al. (2004) have suggested that stimulation of OEA enhances fat utilization through

activation of PPAR-α and this effect may be able to contribute to anti-obesity functions of oleic acid. In 2003, Fu et al studied the correlation between OEA and PPARs. This study design included a mouse model with one group of wild-type mice and one group of PPAR-α knockout mice that were administered OEA. Results show that food intake and body weight were decreased in wild-type mice only. Thus, OEA can activate PPAR-α with a half-maximal concentration (EC50) value of 50 nM, but OEA failed to activate PPAR-γ and PPAR-β. In addition to mice, OEA can activate PPAR-α in human and rodents as well as cause a reduction of food intake (Thabuis et al., 2007; Schwartz et al., 2007). To conclude, OEA is a natural PPAR-α ligand, which can regulate PPAR-α target gene expression.

Many studies have shown that OEA exerts strong effects on the regulation of lipid metabolism. Yang et al (2006) studied the mechanism of OEA on fatty acid uptake in the proximal small intestine and suggested that OEA can impact appetite regulation, body weight, and enhance fatty acid uptake by enterocytes. Likewise, Schwartz et al (2008) showed that OEA has beneficial effects in inhibiting food intake, reducing blood lipid levels and enhancing peripheral fatty acid catabolism. Also, Thabuis et al (2008) have indicated that OEA has the ability to increase β-oxidation, weight loss and evoke analgesic effects. Moreover, the efficiency of OEA on activation of PPAR-α is 500-900 times higher than seen with fibrates, a PPAR-α agonist drug used for reducing LDL-cholesterol level (Guzmán et al. 2004).

Moise et al (2008) for the first time determined an endocanabinoid signalling system in a hamster model. This study used neuroanatomical, biochemical and behavioural pharmacological approaches and concluded that FAAH inhibitor (URB597) did not change unconditioned or conditioned social defeat behaviour, but demonstrated that FAAH does suppress anxiety-like behaviour by engaging functional CB1 receptors. More studies are required in the future to

analyze the pharmacological effects of OEA in regulating food intake, fatty acid metabolism and energy expenditure in animal models and humans.

1.6.1 Arachidonoylethanolamide

Arachidonoylethanolamide (also called anandamide, AEA) is an endogenous ligand for canabinoid receptors (CRs), belonging to the FAE family which was first identified in brain (Hansen & Diep, 2009). The amount of AEA in total FAEs is less than 5%, presenting in the order of pmol/g of tissue, which is due to the low levels of NAPE causing the low prevalence of AA in sn-1 position of phospholipids. Arachidonoylethanolamide is a ligand for CR1 and CR2 and also G protein-coupled receptor 119 (GPR119), so it is involved in modulating food intake either through acting in the brain or through locally stimulating signalling molecules from the gastrointestinal system (Hansen & Diep, 2009). As opposed to OEA, AEA increases food intake by reducing gastric and intestinal mobility through activation of CRs in tissues. Other functions of AEA include causing hypothermia, antinociception, vasodilation and participating in anti-inflammatory responses, such that AEA has been implicated in several physiological conditions including vascular tone, obesity, and cancer (Marczylo et al., 2009). Virodhamine is an isomer of AEA, which is formed from AA combined with ethanolamine through an ester linkage. Due to the function of virodhamine acting as a partial agonist on CB1 and CB2 receptors, it is important to distinguish virodhamine from AEA during chromatography separation to avoid the interference of virodhamine while measuring AEA levels (Ozalp & Barroso, 2009).

1.6.2 Palmitoylethanolamide

Palmitoylethanolamide (PEA) is a palmitate fatty acid derivative, synthesized by refluxing ethanolamine with palmitic acid. Detectable levels of PEA can be found in rat brain, liver and skeletal muscle and other tissues (Lambert et al., 2002). In rat blood plasma, the level of PEA was found to be 16.7 + 2.7 pmol/ml (Lambert et al., 2002). The regulatory functions of PEA include inflammation and nociception, mediated through a variety of molecular mechanism which involves PPAR-α. Other studies investigating PEA show that this metabolite can inhibit the secretion of TNF-α in human adipose tissue (Ozalp & Barroso, 2009), have cell-protective properties and illicit similar physiological effects to AEA without affinity for CRs (Lam et al., 2010).

1.7 Conclusion

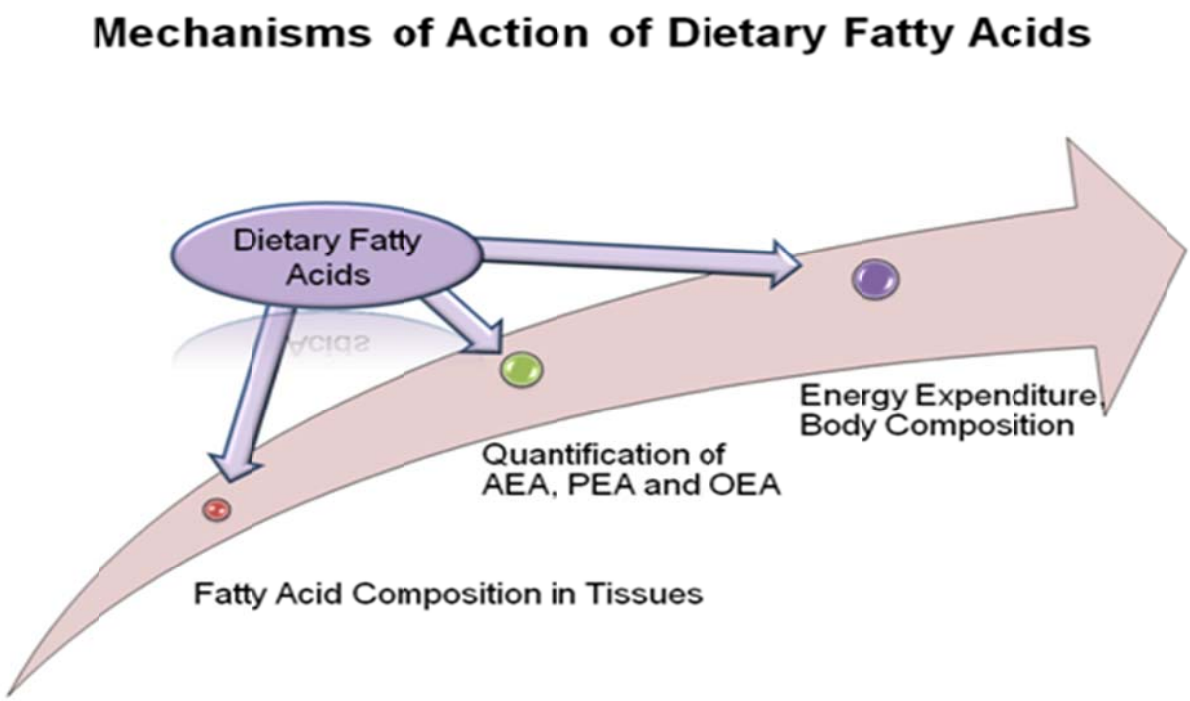
A series of FAEs regulate molecular mechanism, with their functions strongly associating with many physiological processes. Due to the fact that FAEs are formed from fatty acids, different DFAs may affect the quantity of FAEs in tissues. Thus consumption of high MUFA, high PUFA or high SFA diets can be predicted to substantially affect the distribution and levels of tissue FAEs. Moreover, FAEs are agonists of PPAR-α, so it is important to explore correlations between FAEs and fat oxidation, energetics, feed intake and body composition. As such, this present thesis focuses on the above two perspectives and seeks to explain associations between dietary fatty acids, FAEs and metabolic response including food intake, body weight, plasma lipid profile, inflammatory marker (C-reactive protein), body composition, energy expenditure, tissue fatty acid composition, and the levels of FAEs.

1.8 Study Rationale

As discussed above, substantial evidence indicates high-oleic acid oil is thought to reduce LDL-cholesterol levels, to protect against oxidative stress, inflammation and lipid peroxidation. Moreover, the efficacy of dietary oils and a novel formulation for dispersible high-oleic oil has not been tested, especially in mechanistic studies. In light of the popularity of MUFA and PUFA rich food products and the established efficacy of olive oil in cholesterol lowering, more work ought to be carried out exploring the efficacy of high-oleic canola oil with or without enriched DHA versus other oils.

This study aims at using a Golden Syrian male atherogenic hamster model fed controlled diets to determine the association between dietary fatty acids, FAEs and downstream metabolic response including food intake, body weight, plasma lipid profile, inflammatory status, body composition, energy expenditure, tissue fatty acid composition, and the levels of FAE (Figure 5). The proposed study aims to contribute to the evaluation of health benefits of using different dietary oils, especially the novel high-oleic canola oil with and without enrichment with DHA oil and define how these benefits may play a role in improving public health and decreasing CVD risk.

Figure 5 Mechanism of Action of Dietary Fatty Acid through the conversion of Fatty Acid Ethanolamides



1.9 Objectives and Null Hypothesis

1.9.1 Objectives

The purpose of this study is to investigate the effects of different types of dietary oils, including corn oil, high-oleic canola oil, DHA + canola oil and fish oil, on fatty acid metabolism via cellular regulatory pathways mediated by OEA, PEA and AEA.

Specific objectives include:

1) To determine the effects of corn oil, high-oleic canola oil, DHA + high-oleic canola oil (DHA + canola oil) and fish oil on liver and RBC FA composition in a hypercholesterolemia hamster model.
2) To determine the effects of corn oil, high-oleic canola oil, DHA + canola oil and fish oil on OEA, PEA and AEA levels in the proximal small intestine, liver and plasma tissues in a hypercholesterolemia hamster model.
3) To assess the effects of corn oil, high-oleic canola oil, DHA + canola oil and fish oil, and levels of OEA, AEA and PEA associated with these diets, on body composition, energy expenditure, food intake and weight change in a hypercholesterolemia hamster model.
4) To assess the effects of corn oil, high-oleic canola oil, DHA + canola oil and fish oil on plasma lipid levels (TC, TG, HDL, and non-HDL cholesterol), and inflammatory status in a hypercholesterolemia hamster model.

1.9.2 Null Hypotheses:

1). Corn oil, high-oleic canola oil, DHA + canola oil and fish oil will have no effect on red blood cell or liver tissue fatty acid composition in a hypercholesterolemia hamster model.
2) Corn oil, high-oleic canola oil, DHA + canola oil and fish oil have no effect on proximal

small intestine, liver or plasma fatty acid ethanolamide (OEA, PEA and AEA) levels in a hypercholesterolemia hamster model.

3) The levels of OEA, AEA and PEA generated through dietary interventions will not associate with changes in body composition, energy expenditure, food intake or weight change in a hypercholesterolemia hamster model.

4) Corn oil, high-oleic canola oil, DHA + canola oil and fish oil have no effect on plasma lipid levels (TC, TG, HDL, and non-HDL cholesterol) or inflammatory status in a hypercholesterolemia hamster model.

CHAPTER 2:

2 Materials and Methods:

2.1 Experimental Animals and Study Design

A 4-week hamster trial, approved by the Animal Ethics Board of the University of Manitoba, Richardson Centre for Functional Foods and Nutraceuticals Pre-Clinical Animal Facility (Winnipeg, MB, Canada) was conducted according to the Canadian Animal Care guidelines. After arrival, forty-eight, male Golden Syrian hamsters (Charles River Laboratories, Montreal, Quebec) weighing 80-100g were acclimatized for one week individually in plastic cages and were housed in a temperature-controlled room with a 12 h light/dark cycle. During acclimatization, hamsters were fed *ad libitum,* a pelleted rodent standard laboratory chow diet (Prolab RMH 3000 Diet), and were given free access to water.

Following the acclimatization period, hamsters were randomly assigned into one of four different experimental treatments (n=12), then were fed for 4 weeks semi-purified hypercholesterolemic diets containing 10% fat (wt/wt) and 0.25% cholesterol, prepared at the beginning of the experimental period and stored at 4°C. Dietary fatty acid composition of the different experimental diets are shown in Table 1.

Table 1 Composition of Experimental Diets for Hamsters

Diet (% wt/wt)	**Corn oil**	**High-oleic canola oil**	**DHA + High-oleic canola oil**	**Fish oil**
Casein	20	20	20	20
Cornstarch	26	26	26	26
Sucrose	33	33	33	33
DL-methionine	0.5	0.5	0.5	0.5
Cellulose	5	5	5	5
Mineral mixture [a]	4	4	4	4
Vitamin mixture [b]	1	1	1	1
Choline bitartrate	0.2	0.2	0.2	0.2
Butylated hydroxytoluene	0.002	0.002	0.002	0.002
Cholesterol	0.25	0.25	0.25	0.25
Fish oil	0	0	0	6
Corn oil	6	0	0	0
Beef tallow (98%) + safflower (2%) mix	4	4	4	4
High-oleic canola oil	0	6	0	0
High-oleic canola oil enriched with DHA	0	0	6	0

-a,b: based on AIN-93 recommendations

Dietary ingredients were purchased from Dyets Inc. (Bethlehem, PA) except cornstarch. High-oleic canola oil and DHA + canola oil (10% DHA oil + 90% high oleic canola oil) were specially obtained from Dow Agro-Sciences, while fish oil and beef tallow were purchased from Dyets, Inc (Bethlehem, PA). Corn and safflower oils were purchased from local stores. From the literature review, we understood that replacing more than 5% of SFA with USFA can reduce the risk of CVD, but the specific efficacy between replacing SFA with MUFA, omega-3 or omega-6 is still not clear. Due to this purpose, the four experimental treatments in this study contained (i) 6% corn oil and 4% beef tallow/safflower oil, (ii) 6% high-oleic canola oil and 4% beef tallow/safflower oil, (iii) 6% DHA + canola oiland 4% beef tallow/safflower oil, and (iv) 6% fish oil and 4% beef tallow/safflower oil. All treatment groups contained a total of 10 wt% added fat.

Mineral and vitamin mixtures were purchased independently from Dyets Inc. (Bethlehem, PA), which followed AIN-93G growth purified diet for rodents recommended by the American Institute of Nutrition (Reeves, et al., 1993). Other nutrient contents in the diet were calculated based on the AIN-93G diet recommendation for the growth of rodents. For example, 2.5g/kg of choline bitartrate was recommended for growth of rodents (Reeves, et al., 1993), so we used 0.2g of choline bitartrate for our hamsters since the average weight is around 100 g.

The experiment was conducted using a completely randomized block design with four blocks according to the following model

$$\gamma_{ij} = \mu + t_i + \rho_j + \varepsilon_{ij}$$

Where μ is the general mean, t_i is the treatment effect, ρ_j is the block effect, and ε_{ij} is the experimental error. Each experimental period (block) continued for 2929d with the specific experiment treatment and the time frame during the feeding period as listed in Appendix 2.

2.2 Data Collection

2.2.1 Food Intake and Body Weight

Food intake and body weight were measured every three days during the 4 week feeding period at identical times each day (10:00-11:30am). Food intake was obtained based on the amount of food given and leftover amounts within individual cages. If body weight loss was more than 30%, the hamster would have to be euthanized, however in this study, no hamsters were excluded due to weight loss.

2.2.2 Energy Expenditure Assessment

On days 21 and 22, two hamsters from each treatment in one block were selected for energy expenditure assessment using the MM100-metabolic monitor system (CWE, Inc, Ardmore, USA). This system performs highly accurate measurements of oxygen consumption (VO_2) [average O_2 consumption in ml/hr x 10], carbon dioxide production (VCO_2) [average CO_2 production in ml/hour x 10], and respiratory gas exchange ratio (RER) [VCO_2/VO_2] in small animals. Hamsters were housed in individual air chambers for 500mins (~~80-100 min/cycle) to obtain acquisition of 5 full cycles of data per hamster. The percentage of total O_2 consumed by carbohydrate and fat was calculated using the formula of Lusk (Lusk, 1923):

1) carbohydrate (%) = 100 (R-0.707)/0.293 R=RER
2) fat (%) = 100 (1.00-R)/0.293 R=RER

The values used for respiratory quotients varied from 1.00, for pure carbohydrate, to 0.707 for pure fat (Lusk, 1923).

2.2.3 Blood Sample Collection

At the end of the 4 week feeding trial, hamsters were euthanized after 12h fasting by isofluorane (2.5%) with O_2 inhalation (gas flow rate at 2.5 L/min) followed by phlebotomy. Blood samples were collected by decapitation into pre-coated heparin tubes to prevent blood coagulation. Plasma and RBC samples were separated by centrifugation at 3500 rpm for 20 min at 4°C, and then stored at -80°C. Plasma samples were retaining for lipid profile analysis while RBC samples were stored in discrete tubes for fatty acid composition measurements.

2.2.4 Tissue Collection and Processing

Following a midline laparotomy, the liver and small intestine divided into proximal (PROX) and distal (DIOS) sections were collected. Small intestinal sections were excised, rinsed in chilled saline (154 mM; pH 7.4) containing a protease inhibitor (0.1 mM phenylmethylsulfonyl fluoride), wrapped with aluminum foil, flash frozen in liquid nitrogen, and then stored at -80ºC. All hamsters' liver and proximal small intestine samples were individually fractured into small pieces under liquid nitrogen. Mixtures of liver or proximal small intestine were then collected into several separated storage tubes for different analyses.

2.2.5 Body Composition of Hamsters

Immediately after collecting samples of blood and tissues, the body composition of all animals was measured by dual energy X-ray absorptiometry (DEXA) (Lunar Prodigy Advance, GE Healthcare, Madison, WI, USA). The whole-body DEXA scans included the percentage of total fat mass (%TFM), total fat mass (TFM) and total lean mass (TLM) assessed using small animal analysis operational parameters. The DEXA data were determined using Encore 2005 software version 9.30.044 (GE Healthcare, Madison, WI, USA).

2.3 Methodologies for Endpoint Measurements

2.3.1 Plasma Lipid Profile and Inflammatory Marker Analyses

Plasma TC , HDL-C, TG and CRP concentrations were measured using an Abbott Spectrum CCX Analyzer, Vitro Chemistry System 350 (Ortho-Clinical Diagnostics, Inc. Rochester, NY, USA) utilizing enzymatic reagents (Abbott A-GENT) in conjunction with commercial enzymatic kits, standardized reagents, and appropriate standards (Abbott Diagnostics, Montréal, Quebec). For the hamster model, non-HDL-C [VLDL + intermediate density lipoprotein (IDL) + LDL-C] instead of LDL-C values were calculated by subtracting HDL-C from TC (Wang, et al, 2003), due to the Friedewald equation (Friedewald, et, al., 1972) not being applicable to this species due the large amount of cholesterol carried in HDL particles (Matas et al., 1994).

2.3.2 Lipid Extraction of Tissues

Lipid extraction of RBC and liver were carried out according to the Folch method (1957). The percentage of lipid of total tissue weight was calculated for individual hamsters. External fatty acid standards were labelled based on the manufacture's description. Heptadecanoic acid (C17:0) was used as the internal standard. Exactly 0.4 mg of heptadecanoic acid was diluted with chloroform to obtain the final concentration of 0.4mg/ml hepatdecanoic acid in the homogenate. Samples were stored at 4°C for further analyses.

2.3.3 Red Blood Cell Lipid Extraction

First of all, 250 ul of RBC was mixed with methanol and chloroform together with 0.02% butylated hydroxytoluene (BHT) (Sigma-Aldrich, Oakville, ON, Canada). Secondly, 100 ul of heptadecanoic acid as an internal standard (Sigma-Aldrich, Oakville, ON, Canada) was added into

the RBC mixture following by vortexing and centrifugation for 15 min at 2000 rpm and 4°C to extract the lipid. The solvent layer was then transferred into a new tube and chloroform and NaCl added. Samples were vortexed, then centrifuged for 10 min at 2000 rpm and 4°C. The top solvent phase was discarded, then TUP (chloroform: methanol: water=3:48:47) added to the tube and mixed with the lower phase. The final mixture was dried down under nitrogen; then toluene added and the tube contents dried down again for methylation procedures.

2.3.4 Liver Tissue Lipid Extraction

Liver tissues were homogenized in chloroform-methanol (2:1, v/v) with 100 ul of heptadecanoic acid (0.4mg/ml). Methanol was used to rinse the homogenization rotor, then samples were vortexed and centrifuged 15min at 2000 rpm at 4°C. The solvent layer was transferred to a new tube and mixed with chloroform and 0.73% NaCl. Samples were again vortexed and centrifuged. The top solvent phase was discarded and 2ml of TUP (chloroform: methanol: water=3:48:47) rinsed down into the tube and mixed with the lower phase. The final mixture was dried down under nitrogen; after which toluene was added and the mixture dried down again prior to methylation.

2.3.5 Red Blood Cell and Liver Fatty Acid Sample Methylation

The identical method of methylation was used for both RBC and liver tissues. Methanolic HCl was added to the sample mixture and placed into a preheated oven for 1 h at 80°C. Subsequently, deionized water was added to samples, which were vortexed and centrifuged for 5 min at 2000 rpm at 4°C. The top layer of the solvent phase was transferred into a new tube. Petroleum ether was added to the lower phase, then the sample vortexed and centrifuged. The top

phase (2nd run) was then combined with the first run top phase, and these combined top phases combined with deionized water, vortexed and centrifuged for 5 min at 2000 rpm at 4°C. Finally, the top layer of the solvent phase (3rd run) was transferred into a clean tube and dried down under nitrogen. Hexane was added and the resulting FA methyl esters collected into GC vials for FA analysis.

2.3.6 Fatty Acid Analysis by Gas Liquid Chromatography

Extracted FA methyl esters were separated on a Supelcowax-10 30m x 0.25mm internal diameter x 0.25um film thickness column (Supelco, Bellefonte, PA, USA), which were connected to an Agilent 6890N gas chromatograph equipped with a flame ionisation detector (Agilent Technologies, Mississauga, ON, Canada). The oven temperature schedule was initial temperature, 70°C for 1 min; ascended to 180°C at 25°C/min, held for 2 mins; ascended to 220°C at 3°C/min, held for 10 mins; and ascended to 240°C at 20°C/min, held for 15 min (Gillingham et al. 2010). Samples were run with a 10:1 split ratio. Helium was the carrier gas with a column flow rate of 1.0 ml/min. Injector and detector temperatures were set at 280°C and 300°C, respectively. The percentage of each FA was calculated according to each fatty acid peak area relative to the total FAs of interest area.

2.3.7 Standard Preparation of Oleoylethanolamide, Palmitoylethanolamide, and Arachidonoylethanolamide

Individual OEA, OEA-d4, PEA and PEA-d4 (Cayman Chemicals, Ann Arbor, MI) stock solutions were prepared by adding acetonitrile (5mg/ml, 100ug/ml, 0.5mg/ml, 100ug/ml, respectively). Individual AEA, AEA-d8, and virodhamine (O-AEA) (Cayman Chemicals, Ann

Arbor, MI, USA) stock solutions were prepared by drying the supplied solution under nitrogen gas and reconstituting them in acetonitrile (5mg/ml, 100ug/ml and 5mg/ml, respectively). Stock solutions were stored at -20°C for further dilutions, which were performed in acetonitrile on ice. A combination of APO-EA-dx (AEA-d8, PEA-d4 and OEA-d4) was used as the internal standard in this study. The APO-EA-dx was prepared by diluting the stock solutions and mixing OEA-d4 (2.5ng/ml), AEA-d8 (5ng/ml) and PEA-d4 (2.5ng/ml) together. Internal standards were stored at -20°C for further analyses which were performed on ice. Plasma, liver and proximal small intestinal tissue samples were used for FAEs analysis. Marczylo et Al. (2008) indicate that the liquid-liquid extraction method for plasma samples took an overly lengthy processing time (approximately 2h/sample), so suggested use of a solid-liquid extraction method. Thus, this study selected a solid-liquid extraction method for plasma samples and a liquid-liquid extraction method for liver and proximal small intestinal tissues.

2.3.8 Plasma Fatty Acid Ethanolamides Solid-Liquid Extraction

Plasma samples were mixed with 70ul of mixed OEA-d_4, AEA-d_8 and PEA- d_4, and then 825ul of deionized water was added to make up the total volume of 1 ml. Samples were then vortexed and centrifuged at 1,200 x g for 10 min at 4 °C. Methanol and deionized water were added into an Oasis HLB 1 cc, 30 mg cartridge and drained down using a syringe. Subsequently, samples were introduced into the cartridge; drawing them under gentle vacuum at a low flow rate using a syringe; and discarding the flow through the cartridge. Methanol (40%) was washed into the cartridge and discarded in the flow through the cartridge. Acetonitrile was then added into the cartridge and the flow collected into to a clean tube to elute OEA, AEA and PEA. The eluants

were dried under nitrogen; with 150 ul of acetonitrile added before transfer into GC vials for UPLC-MS/MS analysis.

2.3.9 Tissue Fatty Acid Ethanolamide Liquid-Liquid Extraction Procedures

Samples of liver and proximal small intestine tissues were mixed with 70ul of mixed OEA-d_4, AEA-d_8 and PEA-d_4. Ice-cold acetone was added to the sample; homogenized and rinsed with an additional amount of acetone. Samples were vortexed and centrifuged at 2000 g for 15 min at 4 °C. Supernatants were transferred into clean tubes and dried under nitrogen gas. Chloroform-methanol (2:1, v/v) and deionized water were then added into the dried samples, which were vortexed and centrifuged at 2000 g for 15 min at 4 °C. The upper phases of each mixture were discarded and the lower phases transferred into another clean tube; dried under nitrogen gas and combined with 150ul of acetonitrile. Finally, samples were transferred into GC vials for UPLC-MS/MS analysis.

2. 3.10 Ultra-performance Liquid Chromatography/Mass Spectrometry

Chromatographic separation was carried out in an Acquity UPLC system (Waters, US) equipped with a C18 column (Acquity UPLC BEH, 1.0 x 100mm length, 1.7 um). The mobile phase A consisted of milliQ-water with 0.1% formic acid. Mobile phase B was acetonitrile (optima-grade) with 0.1% formic acid. The LC gradient conditions were as follows: 0.0 to 6.0 min, 30% A; 6.0-6.1 min, 24%A, and then equilibrated at 30% A until 7.0 min. The flow rate employed was 0.2 ml/min. The column temperature was kept constant at 40°C. Samples were maintained at 4°C throughout.

Analyses were quantified using tandem electro-spray mass spectrometry in positive-ion mode (ES^+). Source parameters included capillary (kV) 3.00, source temperature of 120°C, desolvation temperature of 500 °C, cone gas flow of 50 L/h, desolvation gas flow 600 L/h. Subsequently, MS/MS conditions for monitoring each precursor $[M+H]^+$ ion comprised entry, collision, and exit energies of 1, 17, 1v, respectively. Product ions were monitored in multiple reaction monitoring (MRM) mode. Injection volumes for samples and standards were 10 ul with needle overfill. The key MRM parameters are summarized in Table 2. Limits of quantification (LOQ) were 0.2 fg on column and the limits of detection (LOD) were 0.5 fg on column.

Table 2. Mass Spectrometric Parameters for the Quantitative Analysis of the Different N-acylethanolamides

N-acylethanolamide	Retention Time (min)	Precursor Ion (m/z)	Product Ion (m/z)	Cone Voltage (V)	Collision Energy (V)
Virodhamine	3.23	348.4	61.9	18	17
AEA	2.92	348.4	61.9	18	17
AEA-d8	2.84	356.5	62.5	18	17
PEA	3.84	300.0	62	28	17
PEA-d4	3.81	304.3	65.8	28	17
OEA	4.32	326.5	61.9	28	17
OEA-d4	4.27	330.0	65.8	28	17

Ten-point calibration curves were performed using different concentration arrangements. The AEA, PEA and OEA peaks were integrated using Masslynx software version 4.1 (Waters Corp., Milford, MA). Microsoft Excel software 2007 was used to calculate the concentration of AEA, PEA and OEA using calibration curves of concentration against relative response calculated as:

Relative response (y) = Peak area /(IS area/[APO-EA-dx])

where IS = the peak area of the AEA-d8, OEA-d4 or PEA-d4 internal standard and [APO-EA-dx] = the concentration of the AEA-d8, OEA-d4 or PEA-d4 internal standard. Peak area = the peak area of AEA, OEA or PEA (Ozalp & Barroso, 2009).

2.4 Statistical Analysis and Power Calculation

This was an "intention to treat" design; thus data from all hamsters were analyzed. Results are presented as mean +/- standard error and differences between treatments at endpoint were compared by using a one-way analysis of variance (ANOVA) and LSD test for the determination of treatment effects using SPSS (PASW statistics 18). The multiple comparisons were focused on each treatment comparing to each other treatment and also each treatment compared to the control treatment. Results were considered significant at $p<0.05$.

CHAPTER 3

3 RESULTS

3.1 Food Intake

Food intake results of hamsters are summarized in Table 3. Data demonstrated no significant differences between treatment groups in average weekly food intake at weeks 2, 3, or 4 of the study. However, during week 1 hamsters consuming canola oil showed 14%, 20% and 9% higher (p=0.004) food intake compared to hamsters consuming corn oil, fish oil or DHA + canola oil, respectively.

Table 3 Effect of Dietary Intervention on Feed Intake of Hamsters[1]

	Feed Intake over Feeding Period across Different Treatments			
	Corn oil	Canola oil	DHA + canola oil	Fish oil
Feeding Period	n[2] = 12	n = 12	n= 12	n= 12
Week 1	16.47 ± 0.75[a]	19.24 ± 0.88[b]	17.35 ± 0.84[a]	15.21 ± 0.62[a]
Week 2	17.69 ± 0.80	18.48 ± 0.71	17.78 ± 0.83	15.85 ± 0.63
Week 3	16.24 ± 0.47	16.75 ± 0.59	17.04 ± 0.52	15.49 ± 0.51
Week 4	15.74 ± 0.65	16.49 ± 0.56	17.76 ± 0.78	16.02 ± 0.59

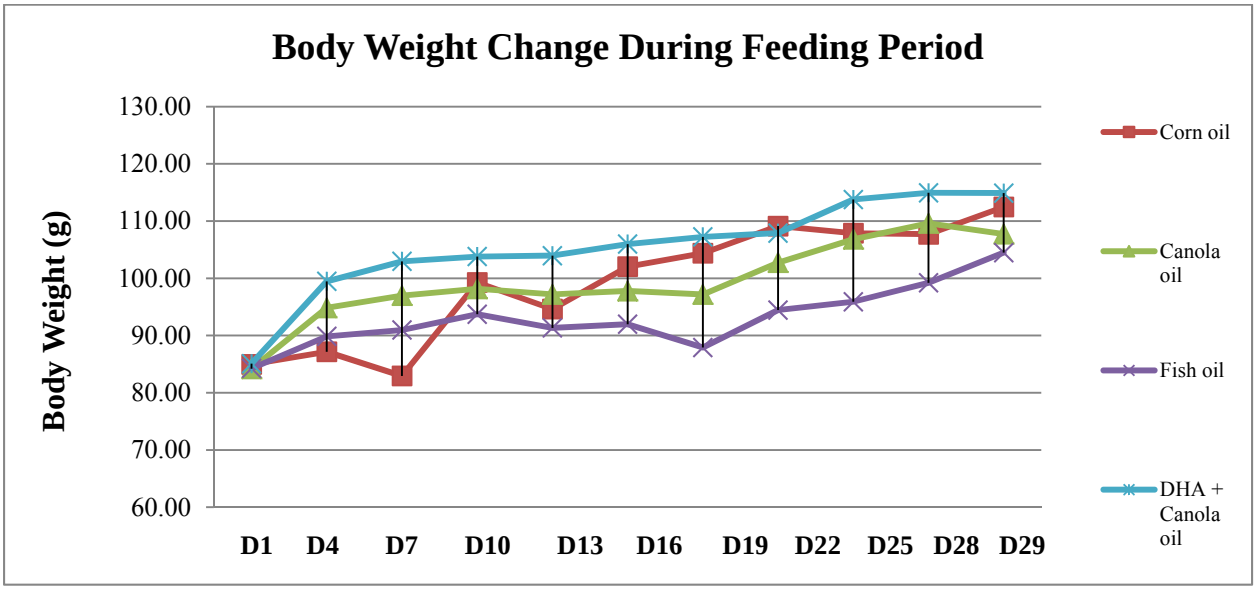
[1] Values are expressed as means (g) ± SEM. Values with different letter superscripts are significantly different from each other $p<0.05$

[2]number of hamsters

3.2 Body Weight

Body weight data are provided in Figure 1 and Table 2. A consistent increase in body weight was observed across all treatment groups throughout the feeding period, which indicated good tolerance of the experimental diets (Figure 6). Mean weekly body weights did not significantly differ between the groups at initial arrival and week 1 (Table 4). However, at week 2 and week 3, the DHA + canola oil group showed a higher ($p<0.01$) body weight compared to canola and fish oil fed groups, but not corn oil fed group. At week 4, the DHA + canola oil fed group showed a higher ($p=0.013$) body weight compared to the fish oil fed group.

Figure 6 Effect of Dietary Intervention on Body Weight of Hamsters[1]



[1] Values are expressed as means (g) ± SEM. Values with different letter superscripts are significantly different from each other $p<0.05$

Table 4 Effect of Dietary Intervention on Body Weight of Hamsters[1]

	Weekly Body Weight over the Feeding Period across Different Treatments			
	Corn oil	Canola oil	DHA + canola oil	Fish oil
Feeding Period	n[2]=12	n=12	n=12	n=12
Initial (g)	84.97± 1.02	84.18 ± 1.04	85.08 ± 0.97	84.26 ± 1.04
Week 1	101.22 ± 2.61	101.33 ± 2.58	103.52 ± 1.98	97.14 ± 2.57
Week 2	100.28 ± 2.89^{ac}	96.43 ±2.55^{a}	104.1 ±2.26^{c}	90.18 ±2.00^{b}
Week 3	106.63 ±3.22^{ac}	101.54 ±2.82^{a}	108.62 ±2.30^{c}	93.31 ±2.60^{b}
Week 4	112.47 ± 3.69^{a}	108.57 ±2.96^{ab}	114.57 ±2.63^{a}	100.07 ±3.36^{b}

[1] Values are expressed as means (g) ± SEM. Values with different letter superscripts are significantly different from each other $p<0.05$

[2] n=number of hamsters

3.3 Plasma Lipid Profile and C-Reactive Protein Levels

Plasma TC, TG, DHDL-C and Non-D-HDL and CRP endpoint values and their percentage differences are listed in Table 5. None of the treatments had any effect on TC or CRP levels. The fish oil group showed a higher ($p<0.01$) plasma total cholesterol level than all other three groups. There was no difference between DHA + canola oil group and fish oil group in D-HDL level or Non-HDL level, but these levels were different ($p<0.01$) compared to the corn oil and canola oil fed groups.

Table 5 Plasma Lipid Profile and CRP Levels of Hamsters[1]

	Lipid Profile and CRP in Different Treatments				
Measurement	Corn oil (n^2=12)	Canola oil (n=12)	DHA + canola oil (n=12)	Fish oil (n=12)	p Value
Total Cholesterol (mmol/L)	8.19 ± 0.26^a	7.74 ± 0.37^a	8.61 ± 0.47^a	11.15 ± 0.52^b	<0.01
Triglyceride (mmol/L)	2.69 ± 0.30	2.75 ± 0.27	2.61 ± 0.25	2.05 ± 0.24	0.239
D-HDL[3] (mmol/L)	4.67 ± 0.16^a	4.47 ± 0.18^a	3.28 ± 0.13^b	3.13 ± 0.18^b	<0.01
Non-HDL (mmol/L)[4]	3.31 ± 0.25^a	3.28 ± 0.22^a	5.32 ± 0.48^c	8.02 ± 0.54^b	<0.01
CRP[5] (mg/L)	4.33 ± 0.14	4.67 ± 0.19	4.58 ± 0.15	4.78 ± 0.22*	0.336

[1] Values are expressed as means (g) ± SEM. Values with different letter superscripts are significantly different from each other $p<0.05$

[2]number of hamsters

[3]Direct-High Density Lipoprotein

[4]Non-HDL: calculation: Non-HDL = Total Cholesterol – D-HDL

[5] C –Reactive Protein

*number of hamsters: n=10

3.4 Energy Expenditure and Body Composition

Energy expenditure and body composition data are reported in Table 6. The energy expenditure data demonstrated no significant difference between all treatment groups in total oxygen consumption. However, the total oxygen consumed which was attributable to fat oxidation, expressed as percent of total energy expenditure, was higher ($p<0.01$) in canola oil and DHA+ canola oil fed groups compared to corn oil and fish oil fed groups (Table 6). Consequently, the total oxygen consumed as a function of carbohydrate oxidation was significantly lower ($p<0.01$) in canola oil and DHA + canola oil fed groups compared to corn oil and fish oil fed groups.

Body composition data indicate that hamsters fed with different diets did not manifest any significant differences in percentage of total fat (%) and total fat mass (g) (Table 6). However, total lean mass (g) was significantly lower ($p<0.05$) in the fish oil fed group compared to the other three groups.

Table 6 Energy Expenditure and Body Composition[1]

Plasma Lipid Profile Across Different Treatments					
Animal trial endpoint	Corn oil (n^2=12)	Canola oil (n=12)	DHA + canola oil (n=12)	Fish oil (n=12)	p value
Energy expenditure					
Total oxygen consumed by fat (%)	56.75 ± 2.27[a]	68.89 ± 2.71[b]	72.7 ± 2.54[b]	64.18 ± 2.58[c]	<0.01
Total oxygen consumed by carbohydrate (%)	42.72 ± 2.26[a]	30.96 ±2.70[b]	36.04 ± 2.64[b]	27.42 ± 2.61[b]	<0.01
Oxygen consumption (ml/g body wt)	0.76 ± 0.23	0.86 ±0.03	0.77 ± 0.31	0.84 ± 0.03	0.072
Body composition					
Total fat (%)	49.95 ± 2.15	51.45± 1.81	52.38± 2.23	52.6± 1.91	0.788
Total fat mass (g)	46.83 ± 3.30	45.33 ± 1.95	49.58 ± 2.89	42.17 ± 2.39	0.274
Total lean mass (g)	46 ± 1.66[a]	43.17 ± 2.22[a]	44.58 ± 2.11[a]	37.83 ± 2.18[b]	<0.05
Total mass (g)	95 ± 3.29[a]	90.92± 0.89[ab]	96.67 ± 2.54[a]	82.25 ± 3.45[b]	<0.01

[1] Values are expressed as means (g) ± SEM. Values with different letter superscripts are significantly different from each other $p<0.05$

[2]number of hamsters

3.5 Fatty Acid Composition of Different Dietary Treatments

The FA composition of different dietary treatments is outlined in Table 7. The fatty acid composition in dietary treatments was generally representative of the related treatment oils as it reflected the different composition of omega-3, omega-6 and omega-9 FA rich oils used in the dietary preparation process (corn oil 62.22%, canola oil 8.84%, DHA + canola oil 1.33% and fish oil 0.65%). Levels of LA and total PUFAs were higher in corn oil fed animals compared with other groups. The levels of OA, ALA and total MUFA were higher in the canola oil fed group compared with other groups. The fish oil fed group contained the highest SFA compared with other groups and was the only group where EPA and DPA were found in appreciable amounts. DHA was detected in both fish oil and DHA + canola oil fed groups, but not in other groups.

Table 7 Fatty Acid Composition of Different Dietary Treatments[1]

Fatty Acids of Interest (%, w/w)	Treatments			
	Corn oil (n^2=12)	Canola oil (n=12)	DHA + Canola oil (n=12)	Fish oil (n=12)
c14:0	0.27	0.30	1.04	5.75
c14:1	ND[3]	ND	ND	ND
c16:0 (PA)	14.28	9.87	11.80	23.26
c16:1n7	ND	ND	ND	ND
c18:0 (SA)	14.96	17.74	17.59	28.35
c18:1n9 (OA)	21.97	57.03	47.33	7.32
c18:2n6 (LA)	46.21	11.56	9.47	2.57
c18:3n6	ND	ND	ND	ND
c18:3n3 (ALA)	0.74	1.31	0.97	0.98
c20:0	0.60	0.84	0.80	0.74
c20:1n9	0.21	1.04	0.82	0.76
c20:2n6	ND	ND	ND	ND
c20:3n6	ND	ND	ND	ND
c20:4n6 (AA)	ND	ND	ND	0.76
c20:5n3 (EPA)	ND	ND	ND	8.63
c22:0	0.16	0.31	0.30	ND
c22:4n6	ND	ND	ND	ND
c22:5n3 (DPA)	ND	ND	ND	1.77
c22:6n3 (DHA)	ND	ND	6.17	6.97
c24:0	0.14	ND	0.17	ND
c24:1n9	ND	ND	ND	ND
Total SFA	30.40	29.06	31.71	58.10
Total MUFA	22.18	58.07	48.15	15.54
Total PUFA	46.96	12.86	16.60	21.67
Total Fatty Acids	99.54	100.00	96.46	95.31
n-6:n-3	62.22	8.84	1.33	0.65

[1] Values are expressed as means (g) $\pm$ SEM. [2]number of hamsters

[3]ND=Not detected

3.6 Fatty Acid Composition of Liver and Red Blood Cells

Fatty acid composition data of liver and RBC are found in Table 8. In liver tissues, ALA, EPA, DPA and DHA were found in all treatment groups, due to the biological conversion of ALA to EPA and DHA in tissues (Table 8). Levels of LA and total PUFA were significantly higher ($p<0.05$) in corn oil fed animals compared to other three treatment group. Levels of OA and total MUFA in liver were significantly higher ($p<0.05$) in the canola oil fed group compared to all other three treatment groups. Furthermore, in liver, levels of palmitic acid (PA), EPA and DPA were significantly higher ($p<0.05$) in the fish oil fed group compared to other three treatment groups. Also, the levels of DHA in liver were significantly higher ($p<0.05$) in the fish oil and DHA + canola oil fed groups compared to canola oil and corn oil fed groups (Table 8). The ratio of omega-6/ omega-3 was significantly different between each group in liver tissues (Table 8).

Table 8 Fatty Acid Composition of Liver Tissues of Hamsters[1]

Fatty Acids of Interest (%, w/w)	Treatments Corn oil (n[2]=12)	Canola oil (n=12)	DHA + Canola oil (n=12)	Fish oil (n=12)
c14:0	0.15 ±0.01[a]	0.17 ±0.03[ab]	0.12 ±0.00[b]	0.20 ±0.01[c]
c14:1	ND[3]	ND	ND	ND
c16:0 (PA)	13.48± 0.34[a]	13.87 ±0.64[ac]	15.44 ±0.40[c]	20.55 ± 0.75[b]
c16:1n7	0.96 ±0.05[a]	1.06 ±0.07[a]	0.94 ±0.09[a]	2.29± 0.16[b]
c18:0 (SA)	27.22±2.43[a]	25.74 ±2.49[a]	27.92 ±0.63[a]	18.23 ±2.42[b]
c18:1n9 (OA)	20.70 ±0.81[a]	25.96 ±2.47[b]	19.29 ±0.66[a]	20.50 ±2.24[a]
c18:2n6 (LA)	17.16 ±1.50[a]	12.85 ±0.33[b]	10.47 ±0.32[c]	7.82 ±0.52[d]
c18:3n6	0.02 ±0.02[a]	ND	ND	0.08 ±0.02[b]
c18:3n3 (ALA)	0.17 ±0.01[ac]	0.22 ±0.02[b]	0.17 ±0.01[ac]	0.12 ±0.03[c]
c20:0	0.18 ±0.02	0.18 ±0.01	0.16 ±0.02	0.17 ±0.02
c20:1n9	0.28 ±0.02[a]	0.35 ±0.01[a]	0.21 ±0.01[b]	0.12 ±0.01[c]
c20:2n6	0.60 ±0.02[a]	0.68 ±0.03[a]	0.20 ±0.01[b]	0.17 ±0.01[b]
c20:3n6	1.52 ±0.06[a]	1.54 ±0.07[a]	0.53 ±0.05[b]	0.68 ±0.04[b]
c20:4n6 (AA)	8.72 ±0.28[a]	7.53 ±0.26[b]	7.25 ±0.28[b]	4.84 ±0.23[c]
c20:5n3 (EPA)	0.01 ±0.01[a]	0.09 ±0.01[a]	0.90 ±0.05[b]	5.43 ±0.27[c]
c22:0	0.22 ±0.04[abc]	0.29 ±0.01[a]	0.22 ±0.03[ac]	0.16 ±0.03[bc]
c22:4n6	0.46 ±0.08[a]	0.39 ±0.01[ab]	0.30 ±0.01[b]	0.31 ±0.03[b]
c22:5n3 (DPA)	0.02 ±0.01[a]	0.23 ±0.01[b]	0.38 ±0.02[b]	1.89 ±0.10[c]
c22:6n3 (DHA)	4.39 ±0.25[a]	5.43 ±0.26[a]	11.62 ±0.40[b]	12.07 ±1.18[b]
c24:0	0.28 ±0.01	0.23 ±0.01	0.23 ±0.01	1.13 ±0.91
c24:1n9	0.18 ±0.04	0.17 ±0.02	0.17 ±0.04	0.27 ±0.03
Total SFA	41.50 ±2.44	40.48 ±2.60	44.06 ±0.55	35.63 ±3.95
Total MUFA	22.13 ±0.87[a]	27.55 ±2.47[b]	20.61 ±0.73[c]	24.63 ±2.32[d]
Total PUFA	32.82 ±1.50[a]	28.97 ±0.66[b]	31.81 ±0.75[a]	33.36 ±1.38[a]
Total FAs	96.45±3.61	97.00 ±0.33	96.49 ±0.37	96.98 ±0.15
n-6:n-3	6.31 ±0.43[a]	3.91 ±0.16[b]	1.45 ±0.05[c]	0.80 ±0.12[d]

[1] Values are expressed as means (g) ± SEM. Values with different letter superscripts are significantly different from each other $p<0.05$ [2]number of hamsters; [3]ND=Not detected

The fatty acid composition of RBC is shown in Table 9. Fatty acid composition of RBC was very similar as in liver tissues except that levels of ALA were not detectable. Levels of LA and total PUFA were significantly higher ($p<0.05$) in corn oil fed animals compared to other three treatment group. Likewise, levels of OA and total MUFA in RBC were significantly higher ($p<0.05$) in the canola oil fed group compared to all other three treatment groups. Furthermore, in RBC, levels of PA, EPA and DPA were significantly elevated ($p<0.05$) in the fish oil fed group compared to other three treatment groups. Levels of DHA in RBC were significantly higher ($p<0.05$) in the fish oil and DHA + canola oil fed groups compared to canola oil and corn oil fed groups (Table 9). The ratio of omega-6/omega-3 was significantly different between each group in RBC.

Table 9 Fatty Acid Composition of Red Blood Cells of Hamsters[1]

	Treatments			
Fatty Acids of Interest (%, w/w)	Corn oil (n^2=12)	Canola oil (n=12)	DHA + Canola oil (n=12)	Fish oil (n=12)
c14:0	0.02 ± 0.01[a]	ND	0.04 ±0.03[b]	0.33 ±0.06[a]
c14:1	ND[3]	ND	ND	ND
c16:0 (PA)	21.16 ±0.49[a]	20.12 ±0.30[a]	24.06 ±0.57[a]	26.00 ±0.49[b]
c16:1n7	0.61 ±0.07[a]	0.64 ±0.04[a]	0.62 ±0.06[a]	2.00± 0.16[b]
c18:0 (SA)	15.83 ±0.38[a]	15.06 ±0.28[ab]	15.29 ±0.28[a]	14.34 ±0.13[b]
c18:1n9 (OA)	19.92 ±0.57[a]	24.38 ±0.41[b]	22.49 ±0.57[c]	19.79 ±0.52[a]
c18:2n6 (LA)	19.56 ±0.65[a]	13.86 ±0.42[b]	11.69 ±0.42[c]	8.33 ±0.48[d]
c18:3n6	ND	ND	ND	ND
c18:3n3 (ALA)	ND	ND	ND	ND
c20:0	ND	ND	ND	ND
c20:1n9	0.13 ±0.06[a]	0.39 ±0.05[b]	0.13 ±0.05[a]	0.03 ±0.03[a]
c20:2n6	0.19 ±0.07[a]	0.35 ±0.10[a]	ND[b]	ND[b]
c20:3n6	1.29 ±0.03[a]	1.36 ±0.04[a]	0.66 ±0.07[b]	0.66 ±0.02[b]
c20:4n6 (AA)	11.74 ±0.78[a]	12.16 ±0.40[a]	10.88 ±0.67[a]	7.78± 0.33[b]
c20:5n3 (EPA)	ND	ND	0.80 ±0.09[a]	6.40 ±0.61[b]
c22:0	0.08 ±0.06	0.06 ±0.04	0.03 ±0.03	ND
c22:4n6	2.07 ±0.26[a]	1.98 ±0.12[a]	1.15 ±0.12[b]	0.87 ±0.07[b]
c22:5n3 (DPA)	0.43 ±0.15[a]	1.00 ±0.07[ab]	0.75 ±0.15[b]	2.31± 0.18[c]
c22:6n3 (DHA)	2.47 ±0.21[a]	3.28 ±0.11[a]	6.81 ±0.44[b]	6.19 ±0.34[b]
c24:0	0.13 ±0.10	0.09 ±0.06	0.04± 0.04	ND
c24:1n9	1.57 ±0.27	1.89 ±0.21	1.29 ±0.29	1.22 ±0.24
Total SFA	37.22 ±0.89[a]	35.34 ±0.43[a]	39.47 ±0.66[b]	40.67 ±0.67[b]
Total MUFA	22.23 ±0.63[a]	27.30 ±0.44[b]	24.53 ±0.75[a]	23.04± 0.59[a]
Total PUFA	37.74 ±1.35[a]	33.96 ±0.61[b]	32.75 ±0.99[b]	32.48 ±0.76[b]
Total Fatty Acids	97.19 ±0.48	96.59± 0.43	96.74 ±0.27	96.24 ±0.43
n-6:n-3	13.41 ±1.40[a]	7.02 ±0.23[b]	3.01±0.14[c]	1.34± 0.22[d]

[1] Values are expressed as means (g) ± SEM. Values with different letter superscripts are significantly different from each other $p<0.05$

[2]number of hamsters

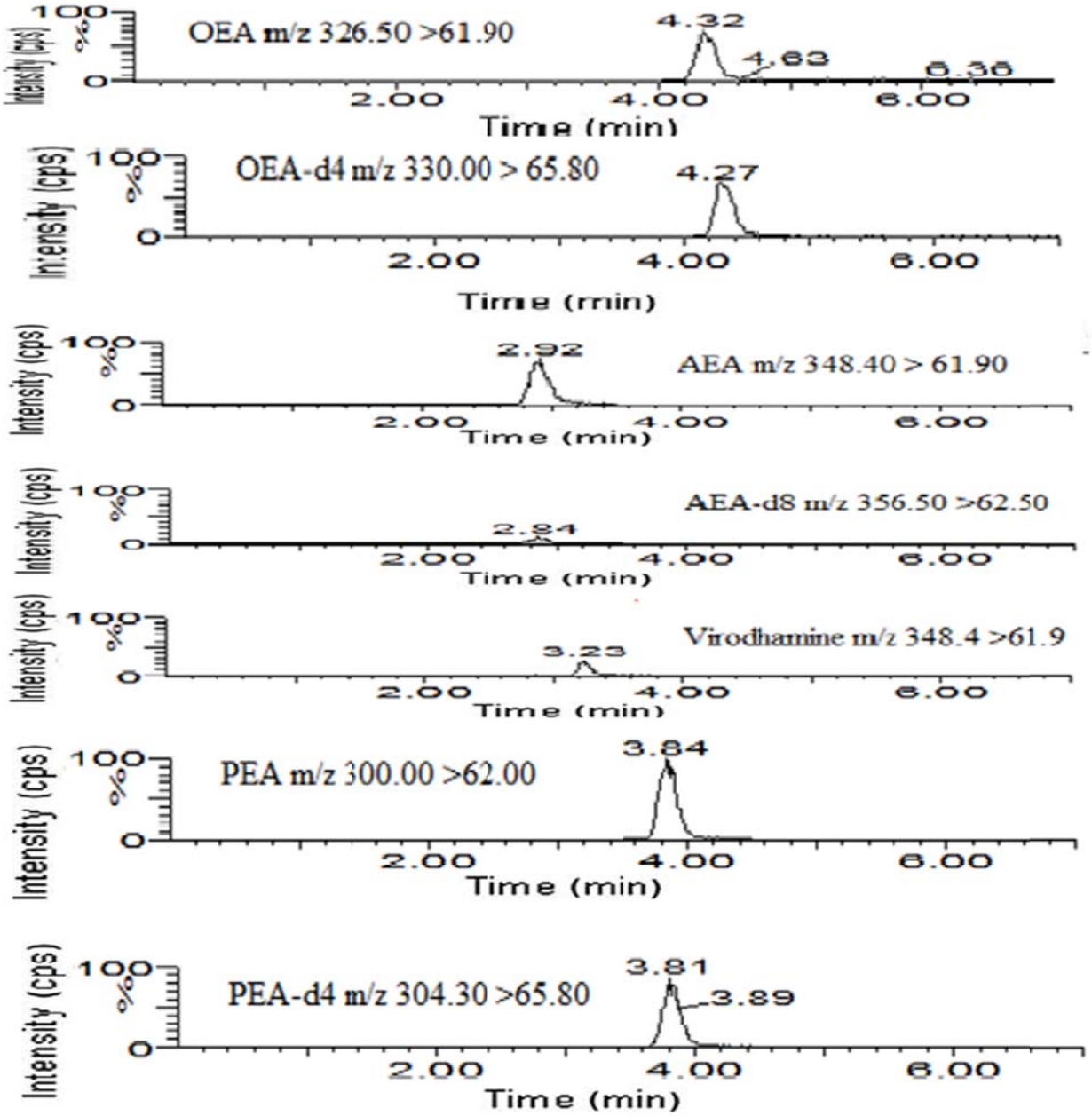
[3]ND=Not detected

3.7 Separation of Fatty Acid Ethanolamide Compounds by UPLC-MS/MSs

The determination of FAEs includes two sections, including chromatographic separation of all compounds; and chromatographic measurement of AEA, PEA and OEA in hamster plasma and tissue samples, so as to quantify their variations after administration of the different dietary fatty acid treatments.

Chromatographic separation of all compounds is described in Figure 7. The Figure shows the total ion current chromatogram obtained after injection of individual extract of one standard samples including OEA, OEA-d4, AEA, AEA-d8, virodhamine, PEA, PEA-d4. As observed, after optimization of fragmentation conditions, all of the peaks were assessed as departure of the detector from baseline. The MRM transitions selected were m/z 326.50 >61.90 for OEA, m/z 330.0 >65.80 for OEA-d4, m/z 348.40 >61.90 for AEA and virodhamine, m/z 356.50 >62.50 for AEA-d8, m/z 300.00 > 62.00 for PEA and m/z 304.30 >65.80 for PEA-d4, which provided lower background noise. This chromatography result was optimized to avoid interference of virodhamine with the analysis of AEA due to the relative close proximity of retention times (3.23 min versus 2.92 min). The second determination was to focus on the analysis of AEA, OEA and PEA.

Figure 7 Separation of Fatty Acid Ethanolamide Compounds



OEA: Oleoylethanolamide

AEA: Arachidonoylethanolamide

PEA: Palmitoylethanolamide

Calibration curves of OEA, AEA and PEA are listed in Figure 8, 9, and 10, respectively. The calibration curves were used for measuring the quantity of AEA, PEA and OEA in hamster samples. Concentrations of OEA, AEA and PEA differed across proximal small intestine, liver and plasma, so different concentration ranges were selected for each FAE species. For OEA, linearity was confirmed in the range from 0.1 ng/ml to 20 ng/ml, which was used for plasma sample calculations, while a range from 0.1 ng/ml to 100 ng/ml was selected for proximal small intestine and liver sample calculations (Figure 8). For AEA, linearity was confirmed in the range from 0.1 ng/ml to 100 ng/ml for plasma calculations and in the range from 0.1 ng/ml to 20 ng/ml for proximal small intestine and liver calculations (Figure 9). For PEA, linearity was confirmed in the range from 0.1 ng/ml to 20 ng/ml for plasma calculation, while a range from 0.1 ng/ml to 250 ng/ml was selected for proximal small intestine and liver calculations. (Figure 10). To be specific, calibration curves were prepared using a blank sample (acetonitrile) and a ten-point concentration span covering the whole calibration range for AEA and OEA. These concentrations were 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 ng/ml. For PEA, the selected concentrations were 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 ng/ml for the lower range and 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 150.0, 200.0 and 250.0 ng/ml for the higher range. The peak area ratios of each compounds of relative to the internal standard were used for calculations. The coefficient of determination (R^2) of the calibration curve was $\geqslant$ 0.996 for all FAE species.

Figure 8-a The Calibration Curves of Oleoylethanolamide used in Assessment of Intestine, Liver of Hamsters

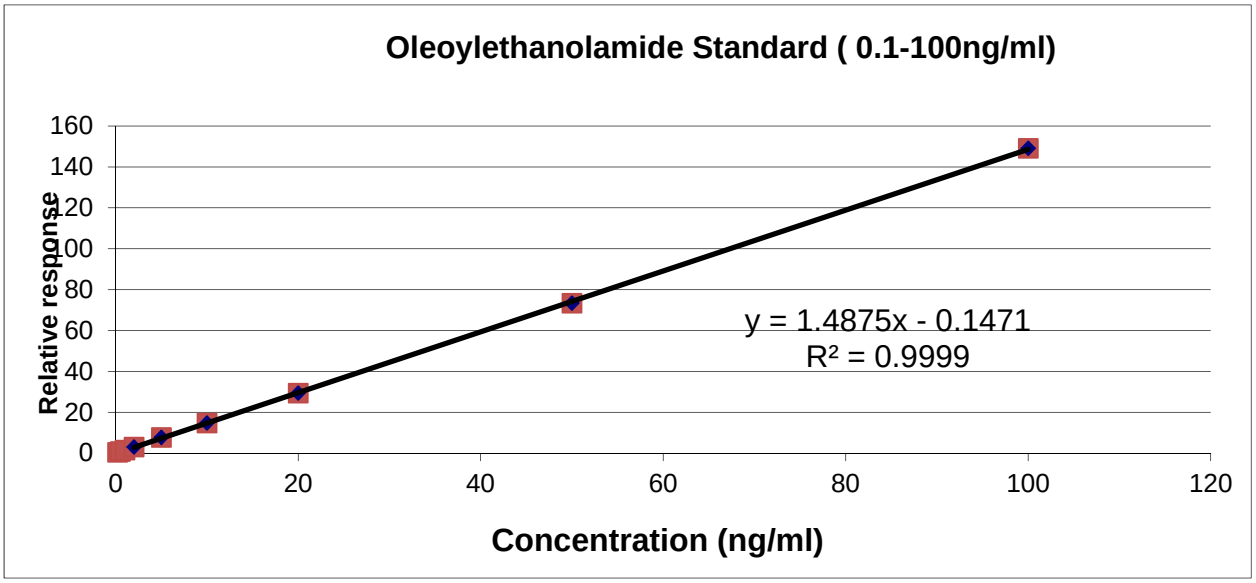


Figure 8-b The Calibration Curves of Oleoylethanolamide used in Assessment of Plasma of Hamsters

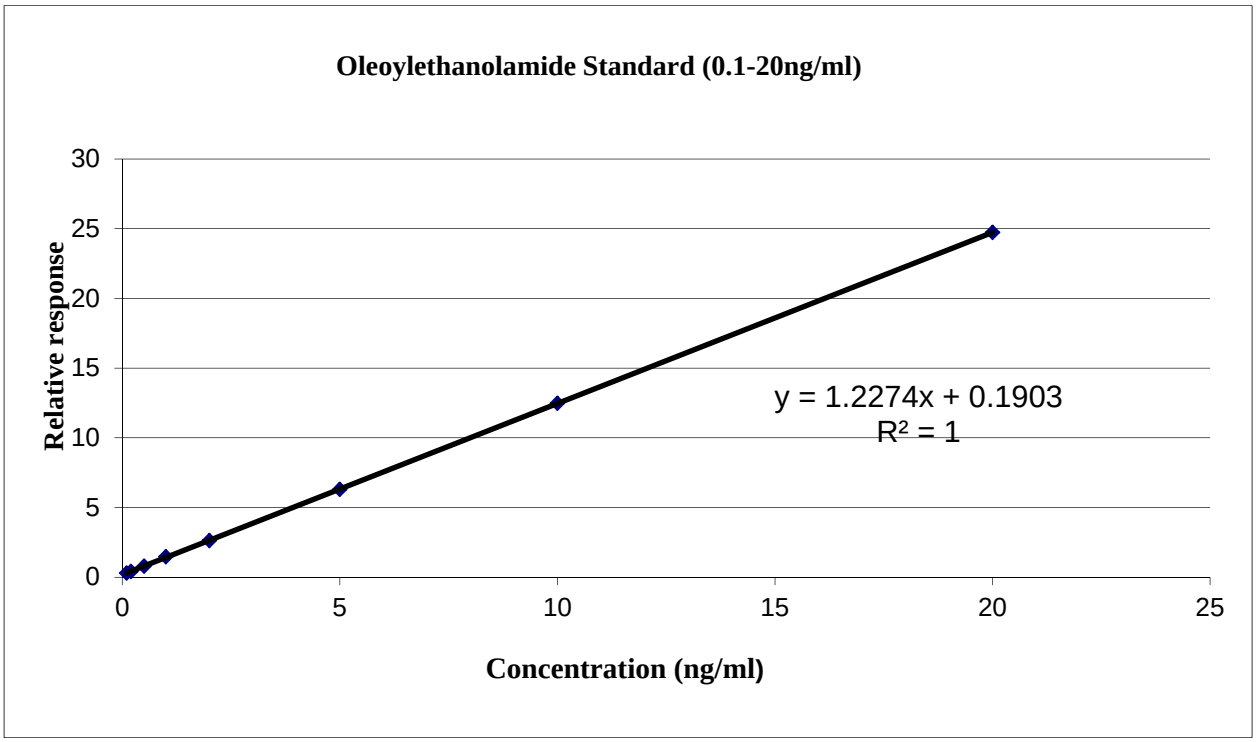


Figure 9-a The Calibration Curves of Arachidonoylethanolamide used in Assessment of Intestine and Liver of Hamsters

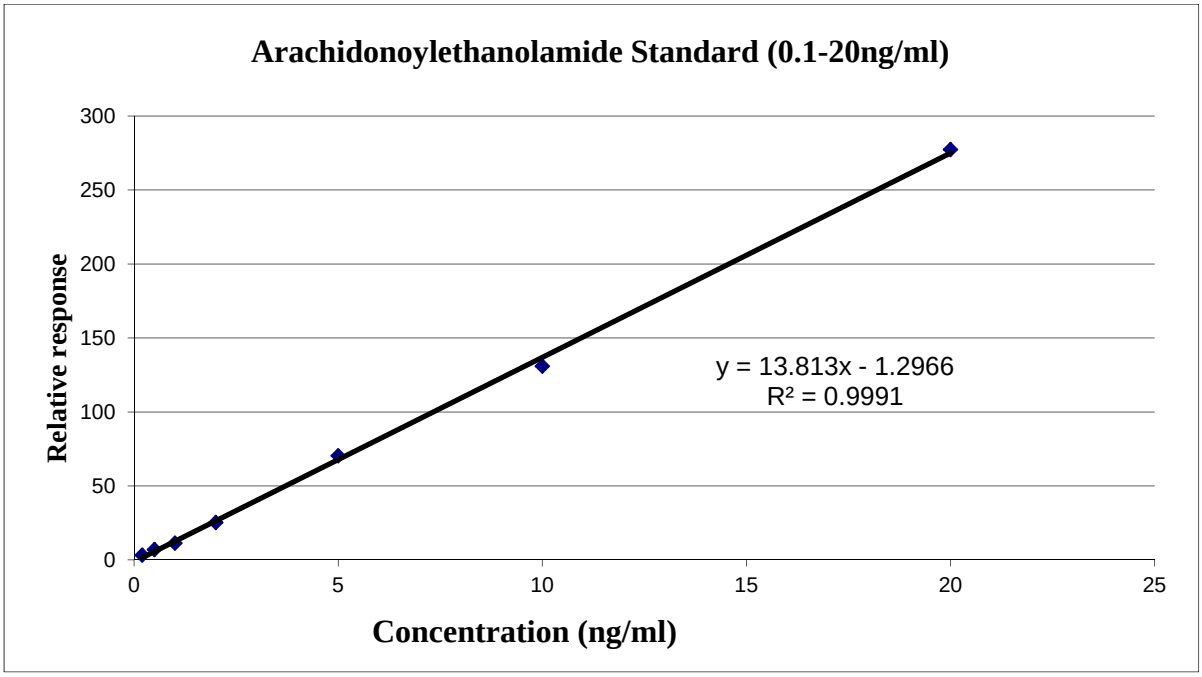


Figure 9-b. The Calibration Curves of Arachidonoylethanolamide used in Assessment of Plasma of Hamsters

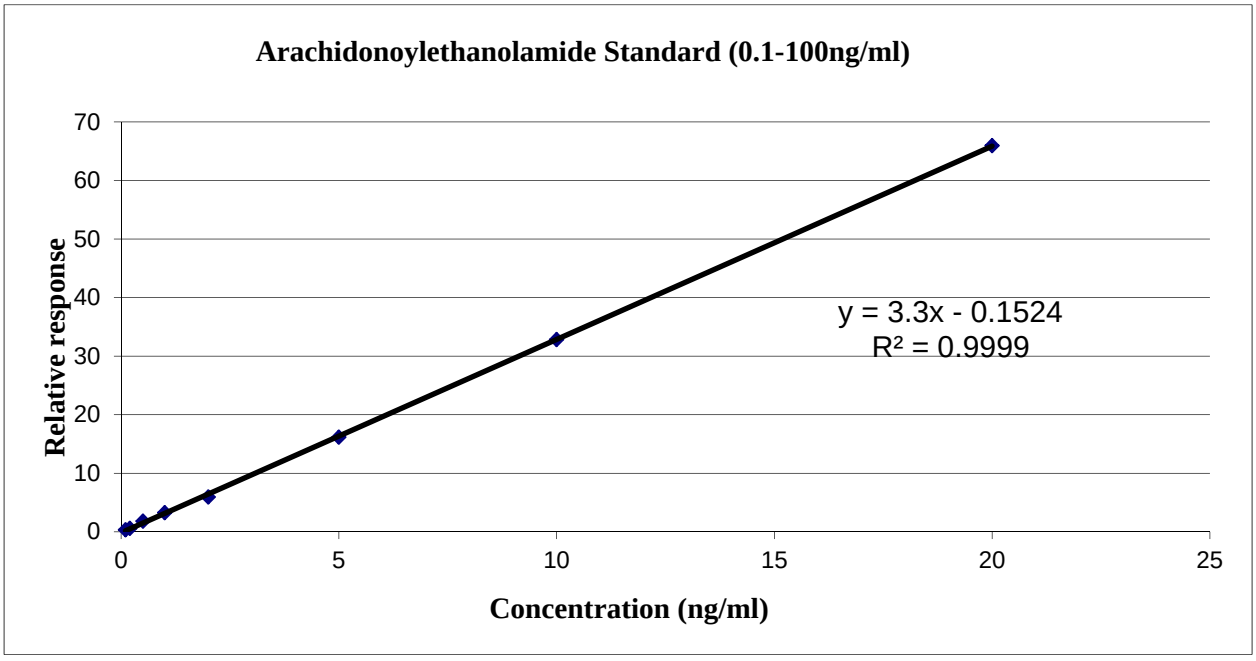


Figure 10-a The Calibration Curves of Palmitoylethanolamide used in Assessment of Intestine and Liver of Hamsters

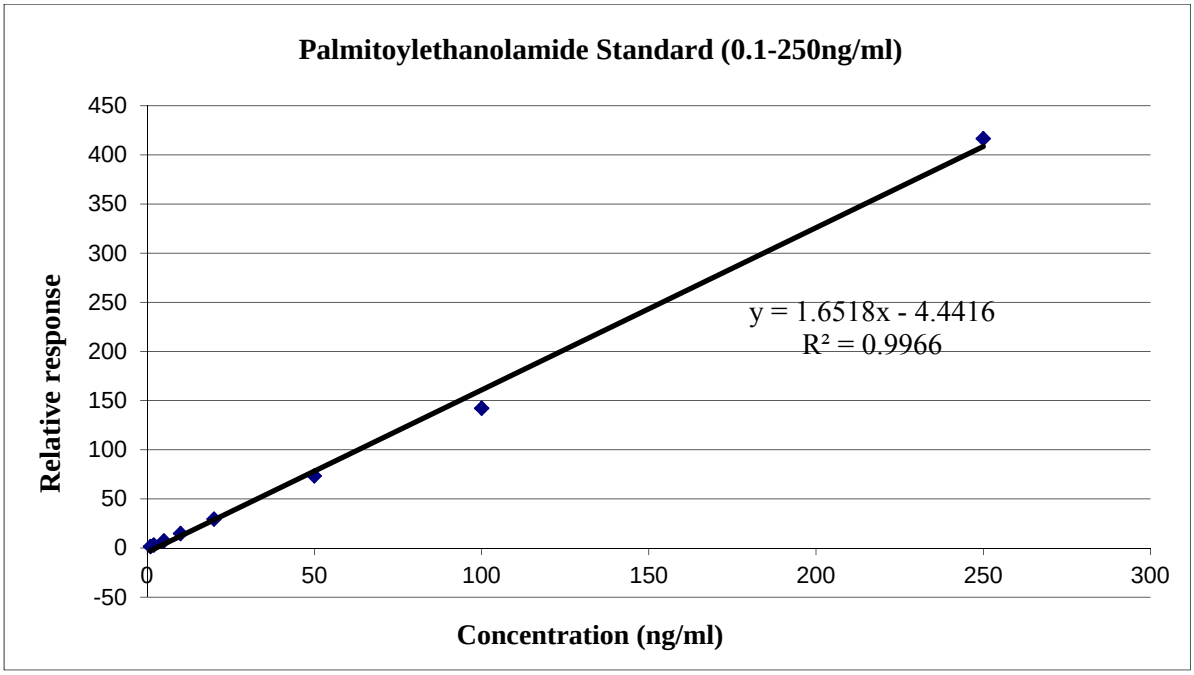
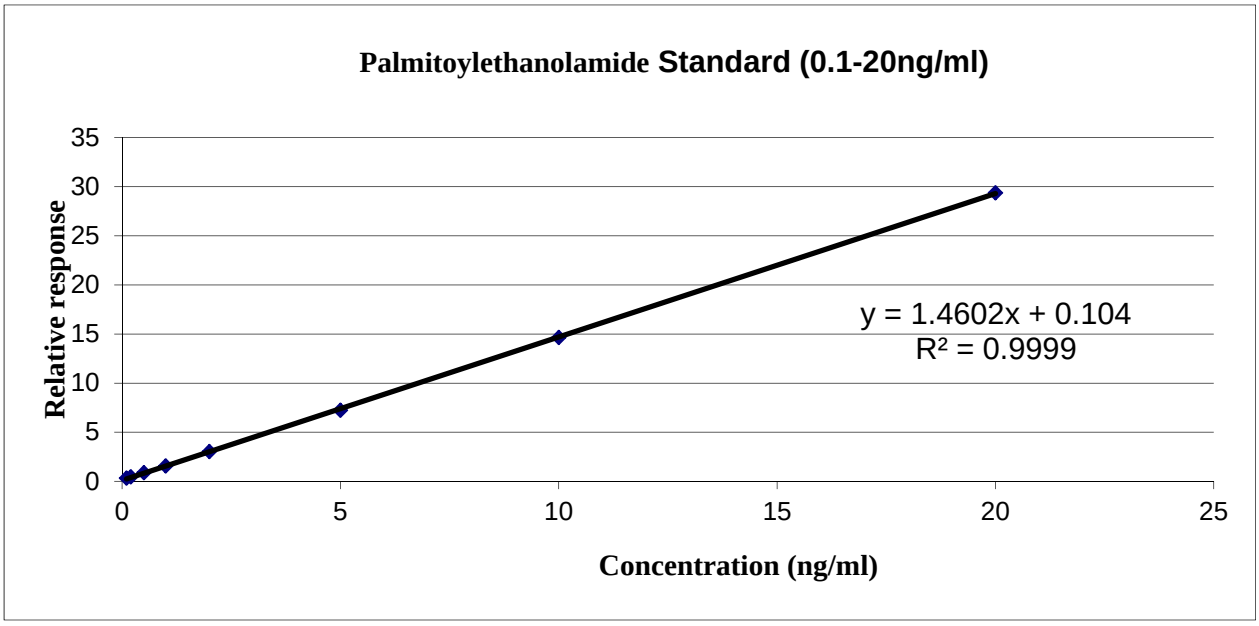


Figure 10-b The Calibration Curves of Palmitoylethanolamide used in Assessment of Plasma of Hamsters



3.8 Accuracy and Precision

The accuracy and precision values for FAEs assessments are given in Table 9. Accuracy and precision of the method of separation of OEA, AEA and PEA were assessed in eight replicates of quality control samples consisting of internal standard concentration levels: OEA-d4 at 2.5 ng/ml, PEA-d4 at 2.5 ng/ml and AEA-d8 at 5 ng/ml. These results indicate the high degree of reproducability of the UPLC/MS system. All compounds were reproduced with equal to or less than 5% precision error between runs.

Table 10 Accuracy and Precision Results for OEA, AEA and PEA Standard Measurements

Analytical Comparison	Number of Measurements (n)	Ratio of Peak Area	Relative Response	Measure Value[1] (ng/ml)	True Value[2] (ng/ml)	Accuracy (%)	Between-Run Precision (%)
OEA[3]/ OEA-d4	7.00	56.53	141.31	96.19	100.00	96.19	2.74
AEA[4] / AEA-d8	7.00	12.60	63.02	4.91	5.00	98.19	3.68
PEA[5] / PEA-d4	7.00	32.82	82.05	96.58	100.00	96.58	3.12

[1]Measure Value: the concentration after running the control in UPLC/MS

[2]True Value: the prepared concentration inside the control before running UPLC/MS

[3]OEA: Oleoylethanolamide

[4]AEA: Arachidonoylethanolamide

[5]PEA: Palmitoylethanolamide

3.9 Determination of Fatty Acid Ethanolamide Levels in Proximal Small Intestine, Liver and Plasma

The concentrations of OEA, AEA and PEA in proximal small intestine, liver and plasma are shown in Table 10, 11, and 12, respectively. In proximal small intestine, the levels of OEA were significantly higher ($p=0.012$) in canola oil and DHA + canola oil groups compared to animals fed corn or fish oil. There were no differences between all treatment groups in AEA and PEA levels (Table 10). In liver tissues, the levels of OEA were significantly higher ($p=0.029$) in canola oil and DHA + canola oil treatment groups compared to corn or fish oil treatment groups (Table 11). The levels of AEA were significantly lower ($p=0.001$) in the fish oil fed group compared to all other three treatment groups. No significant differences in levels of PEA were seen across any of the four treatment groups. In plasma, the fish oil fed group contained significantly higher ($p<0.001$) levels of OEA and PEA, but lower ($p=0.017$) levels of AEA, compared to other three groups (Table 12).

Table 11 Determination of Fatty Acid Ethanolamide Levels in Proximal Small Intestine of Hamsters[1]

Intestine					
FAEs[2]	corn oil (ng/g)	canola oil (ng/g)	DHA canola oil (ng/g)	Fish oil (ng/g)	ANOVA: p value
OEA[3]	101.45 ± 8.38[a]	136.38 ± 8.39[b]	127 ± 8.49[b]	118.51 ±4.35[a]	P=0.012
AEA[4]	8.10 ± 1.31	7.16 ± 0.39	9.59 ±1.83	5.54 ±1.02	P=0.173
PEA[5]	206.69 ± 17.74	196.92± 10.56	206.36 ±13.05	208.41 ±15.00	P=0.025

[1] Values are expressed as means (g) ± SEM. Values with different letter superscripts are significantly different from each other $p<0.05$

[2] FAEs**:** Fatty Acid Ethanolamide

[3]OEA: Oleoylethanolamide

[4]AEA: Arachidonoylethanolamide

[5]PEA: Palmitoylethanolamide

Table 12 Determination of Fatty Acid Ethanolamide Levels in Livers of Hamsters[1]

Liver

FAEs[2]	corn oil (ng/g)	canola oil (ng/g)	DHA + canola oil (ng/g)	Fish oil (ng/g)	ANOVA: p value
OEA[3]	83.17 ±3.91[a]	116.86 ±12.71[b]	108.49 ±13.03[b]	81.02 ±5.95[a]	p=0.029
AEA[4]	2.41 ±0.12[a]	2.25 ±0.23[a]	2.05 ±0.28[a]	1.31 ±0.13[b]	p=0.001
PEA[5]	138±15.73	132.89 ±16.30	131.72 ±13.93	113.06 ±10.19	p=0.604

[1] Values are expressed as means (g) ± SEM. Values with different letter superscripts are significantly different from each other $p<0.05$

[2] FAEs**:** Fatty Acid Ethanolamide

[3]OEA: Oleoylethanolamide

[4]AEA: Arachidonoylethanolamide

[5]PEA: Palmitoylethanolamide

Table 13 Determination of Fatty Acid Ethanolamide Levels in Plasma of Hamsters[1]

Plasma					
FAEs[2]	corn oil (ng/ml)	canola oil (ng/ml)	DHA + canola oil (ng/ml)	Fish oil (ng/ml)	ANOVA: P value
OEA[3]	5.39 $\pm$0.25^{a}	6.21 $\pm$0.41^{a}	5.46 $\pm$0.24^{a}	7.55 $\pm$0.45^{b}	$p<0.01$
AEA[4]	0.69 $\pm$0.05^{a}	0.67 $\pm$0.50^{a}	0.60 $\pm$0.04^{ab}	0.47 $\pm$0.58^{b}	$p=0.017$
PEA[5]	5.48 +0.19^{a}	5.19 +0.30^{ab}	6.03 +0.22^{ac}	7.38 +0.38^{d}	$p<0.01$

[1] Values are expressed as means (g) $\pm$ SEM. Values with different letter superscripts are significantly different from each other $p<0.05$

[2] FAEs**:** Fatty Acid Ethanolamide

[3]OEA: Oleoylethanolamide

[4]AEA: Arachidonoylethanolamide

[5]PEA: Palmitoylethanolamide

CHAPTER 4

4. DISCUSSION SECTION

4.1 Primary Findings

4.1.1 The Impact of Dietary Fatty Acid on Levels of Fatty Acid Ethanolamides

Short-term feeding of Male Golden Syrian hamsters with semi-purified hypercholesterolemic diets containing 10% fat (wt/wt) and 0.25% cholesterol was found to influence levels of OEA, AEA and PEA in proximal small intestine, liver and plasma after 12 hrs food deprivation, in a manner that depended on dietary fatty acid composition. In general, levels of OEA, AEA and PEA were higher in the proximal small intestine compared with liver and plasma. Especially the canola oil diet, with its high oleic acid content, increased levels of OEA in the proximal small intestine relative to animals fed diets rich in corn or fish oil, however, not compared with the diet containing in DHA + canola oil. Our results are interesting because several studies report that dietary oleic acid converts to OEA mainly in the small intestine where the OEA then exerts important functions including activation of PPAR-α, regulating satiety, as well as modifying feeding behaviour and fat utilization (Fu et al., 2007; Artmann et al., 2008).

Canola oil fed animals also showed higher levels of OEA in liver compared to hamsters fed corn or fish oil, but not compared to those consuming DHA + canola oil, while levels of OEA overall were lower compared to the levels of OEA in proximal small intestine. The fish oil fed group showed reduced levels of hepatocyte AEA compared to all other three oil groups. It is reasonable to suggest that a fish oil diet supplemented with high levels of EPA and DHA exerts a minimal impact on levels of AEA (Artmann et al., 2008). However, in the corn oil diet supplemented with high levels of LA, conversion to AA in tissues becomes substantial. The

elevated AA concentrations may have then have led to high production of AEA in the corn oil fed animals (Berger et al., 2001).

In plasma, the levels of AEA, OEA and PEA were tenfold lower compared to the levels observed in intestine and liver tissues. Here, the highest levels of PEA, but lowest levels of AEA were identified with feeding fish oil compared to other three oil diets. As discussed for liver tissue, fish oil may have imposed minor effects on levels of AEA, so low levels of AEA in fish oil could be expected (Artmann et al, 2008). For levels of PEA, based on the literature review, we understand that PA and ethanolamine can combine to form PEA, so a higher PA composition could correlate with a higher formation of PEA in the tissues. Artmann et al (2008) pointed out that diets containing higher PA levels cause elevations in levels of PEA in rat livers, but that levels of PEA in rat brain were not affected by DFA composition between all treatments in that study. In such cases, our fish oil group contained higher levels of dietary PA; also the levels of PA as a fraction of total FA in liver and RBC were higher compared to other three oil groups, so we could expect PEA values to be higher in fish oil-fed groups. However, it is not clear why the significant difference was manifest only in plasma, but not in intestine or liver tissues. In plasma, the most remarkable result was the higher levels of OEA found in fish oil-fed groups rather than in either the canola oil or DHA + canola oil fed group where the supply of oleic acid was considerably greater.

Due to the substantial distinction in levels of OEA, PEA and AEA in proximal small intestine, liver and plasma, our results suggest specific mechanisms of control of FAE levels across various body tissues. The direct activation of PPARs by oleic acid or other fatty acids cannot be ruled out. Indeed, previous work has shown that substantial differences in OEA, AEA and PEA exist across the numerous tissue types in rats and human studies (Fu, et al, 2007; Artmann, et al, 2008;

Marczylo, et al, 2009). Also, the quantified levels of OEA, AEA and PEA in the present study showed similar quantitative results as have other studies conducted in rats, mice and humans (Fu, et al, 2007; Artmann, et al., 2008). Lo Verme et al., (2005) pointed out that OEA levels changed in response to nutrient status and NAT activity in duodenum and jejunum of rats or mice, but the synthesis of OEA in rat brain was stimulated only by pharmacological, and not dietary, treatments.

Although the values for FAEs in hamsters from the present study appear to be close to those found previously in rats, mice and humans, there exists a continuing need for more complete characterization of FAEs levels across different organ systems in the hamster. Also, it would be interesting in future studies to examine the activities of key modulatory enzymes including NAT, NAPE-PLD and FAAH, used in the formation and degradation of OEA.

4.1.2 The Levels of Fatty Acid Ethanolamides Modulating Fat Oxidation, Food Intake and Body Composition

The observed shifts in FAEs levels across the various dietary treatment groups in the present experiment were associated with changes in energy and lipid levels in the groups examined. Most notable was the enhanced proportion of energy expenditure attributable to fat oxidation in the canola oil fed group, compared with corn and fish oil fed animals. The canola oil fed group enhanced fat oxidation concurrent with highest levels of intestine and liver OEA, suggesting that OEA may play an important role in energetics in this sub-species. Although this is the first study examining the correlation between OEA and energetics in hamsters, many studies have previously reported that OEA activates PPAR-α which in turn regulates several aspects of lipid metabolism, fat oxidation and lipid absorption (J Lo Verme et al., 2005). It can be speculated that the OEA is a metabolic regulator that enhances fat oxidation via such a PPAR-α mechanism.

If that is the case, then it can be predicted that dietary oils rich in oleic acid such as canola and olive oil may play a protective role in obesity by stimulating fat oxidation, effectively tilting energy partitioning for oxidation versus storage in fat depots. More work investigating the linkage between OEA and other FAEs and PPAR-α expression and activity is clearly warranted.

4.1.3 Food Intake and Body Composition

Fu et al (2003) and Rodríguez et al (2001) have indicated that OEA can decrease food intake in animals from 40-70% using intra-peritoneal injection or oral feeding after long-term food deprivation. In such cases, OEA may serve as a therapeutic target for regulation of food intake through activation of PPAR-α and possess anorectic effects through its actions in reducing body weight (Lithander et al., 2008). In our study, during the feeding period food intake was the same across all treatment oil groups, except for the first week when hamsters were adjusting to the treatment diet from chow diet. Our results suggest that the conversion of oleic acid to OEA through DFA is not as significant as using therapeutic OEA directly in terms of reducing food intake, however, present results do reveal that OEA is sensitive to qualitative dietary fat intake in hamsters. These results resemble those emerging from a rat study from Artamann et al (2008). In addition, OEA and AEA have been shown to have opposite effects in the small intestine in terms of their actions on food deprivation and re-feeding in rodents. Although the present study did not examine food intake and levels of FAEs during a re-feeding condition, other similar studies showed the correlation of OEA and AEA levels between free-feeding and re-feeding in rats. For example, in the starvation stage, OEA levels were significantly decreased, but AEA levels were increased. After 24hr food deprivation, during the re-feeding stage, OEA levels were significantly

increased, but AEA levels were decreased which indicated the opposite effects between OEA and AEA (Gómez et al., 2002).

Another notable physiological response to dietary fat pattern seen in the present experiment was the observation of a diminished body weight and fat mass in the fish oil fed group of hamsters. This reduction in fat occurred independent of food intake, as food intakes were not different across treatment groups over the majority of the experimental period. Since the energy intakes appeared not to vary substantially across groups, the only explanation to account for a reduced total and fat mass in the fish oil fed group is either that energy absorption or expenditure were altered in the fish oil diet fed group. It is unlikely that the absorption of calories from fish oil is any less than that from other dietary oils.

Another feasible explanation is that energy expenditure was elevated in fish oil fed animals relative to other groups. Indeed, several other studies have suggested that long chain omega-3 fatty acids undergo preferred oxidation compared with other dietary fatty acids (Sethi et al., 2002). However, the excite mechanisms have not been clearly established. PPAR-α activation has been suggested as a mechanism (Tai & Ding, 2010), but it is interesting to note that the level of OEA was highest, and AEA lowest, in plasma of fish oil fed hamsters. Rather than analyzing individual OEA, AEA or PEA levels in different tissues, it may be helpful to look for FAEs ratios and levels in plasma and how such ratios associate with fat oxidation and subsequent body fat mass levels. Clearly, how FAEs are affected by fish oil feeding requires further study. It is curious, however, that despite the lower total and fat masses in fish oil fed animals, no observation of increased fat oxidation by respiratory gas exchange was seen in the present study.

4.2 Secondary Findings

4.2.1 Lipid Profile and Inflammatory Markers

Plasma TG-lowering properties were not significant different between treatment groups in our study. As well, the fish oil-fed group showed significantly higher levels of TC and non-HDL-C compared to other three treatment groups. In general, fish oil is used as a dietary strategy for lowering TG, especially for severely hypertriglyceridemic patients with TG >1000mg/dl or for rats (Stone, 1996). However, the effect on TG levels was not significant in hamsters in our experiment (Harris, 1996). Also, Stone (1996) indicated that fish oil is able to elevate LDL-C in humans with lower plasma levels of TG. A trend to lower circulating TG levels was seen in the present study, however, the hamster model is likely not an optimal model in replicating the TG lowering action of fish oil seen in humans.

Although CRP has been reported as anti-inflammation marker and a predictive factor for CVD, insufficient data are available to evaluate potential effects of DFA consumption on non-lipid risk factors such as inflammation, insulin resistance or weight change. Two randomized dietary trials were accomplished exploring dietary actions on CRP and were reviewed by Lichtenstein et al (2003). One of these studies failed to show any significant effect on CRP (Lichtenstein et al., 2003), but another showed a higher CRP in trans-fatty acid (TFA) group compared to MUFA (Mozaffarian & Clarke, 2009). Mozaffarian (2009) has pointed out that depending on the percentage of TFA replaced with SFA, MUFA or PUFA, the effects of CRP levels were different. It is possible that replacing 6% of beef tallow to other DFA may not have been sufficient to expose a difference in CRP levels across dietary treatments in the present study.

4.2.2 Tissue Fatty Acid Composition Effects of Dietary Fatty Acid

The role of DFA composition in modulating risk markers for disease has been established for several decades. Consumption of oils which are high in specific in modulating DFA generally produce such actions through mechanisms that involve changes in the fatty acid profiles across several tissues (Batres-Cerezo et al., 1991). In the present work substantial alterations in the fatty acid profiles of liver and RBC were observed when the fat composition of the diet was altered. Particularly, tissue levels of MUFA, as well as omega-6 and omega-3 fatty acids reflected the presence of those fatty acids in the diets provided. Numerous studies have demonstrated the functional changes of cellular systems that occur as a consequence of alterations in membrane and other organelle fatty acid composition. For instance, DFA alter blood and organ lipid metabolism in hamsters (Jones et al, 1990). As such, it can be speculated that in the present study, feeding hamsters different fats resulted in substantial shifts in FA composition within multiple body tissues which in turn might be expected to modulate both energy and lipid pathways resulting in shifts in risk markers for disease.

4.2.3 Strength, Limitations and Future Work

A major strength of this present work was development of a rigorous system for analysis of FAEs. Not only is the systematic detection of FAEs, but also the methodology behind the determination, very important in this study. Our solid-liquid-phase extraction method for plasma samples and liquid-liquid-phase extraction method for tissues samples showed faster, more accurate and higher reproducibility results compared to other results using GC/MS or HPLC systems previously utilized to assay FAEs (Marczylo et al., 2009). Also, the data acquired from the UPLC/MS system provided a high accuracy, reproducibility and precision which resembled the results of Ozalp & Barroso, (2009). Without development of the current methodology, it

would have been a challenge for us to make this experiment possible. Furthermore, developing FAE extraction and determination methodologies has enabled this approach to be available for future studies in both animals and humans. We would like to search the correlation between DFA, FAEs levels and energetic effects in humans, which could lead to new discoveries in the context regulation of energy balance, prevention of obesity and reduction of CVD risk.

Several limitations existed in the present study. First, the correlation between FAEs and energetics, fat oxidation and body composition could have been shown to be linked with the PPAR activity and regulation of PPAR gene expression. It would have been desirable to have conducted PPAR- α gene expression work; however, methodological challenges prevented such analyses from being conducted. In future studies, the correlation between the effects of DFA and FAEs on the modulation of PPAR-α regulated genes (eg. CD36, L-FABP) should be measured using quantitative real-time PCR (RC-qPCR). Secondly, the size of the animal model did not allow for sufficient sample collection; thus we were unable to assess all blood lipids and FAEs at every desired timepoint. It would have been useful to more fully understand the satiety effects of FAEs by looking at animals after variable periods of food deprivation. Thirdly, the atherogenic diet and the restrictive activity conditions imposed in the present animal model do not match for Health Canada recommendations for healthy living. As such, it is difficult to interpret the results of this trial in the context of health human lifestyles. Fourth, although a hamster model more closely reflects human lipoprotein metabolism compared to other animal models such as the mouse or rat, (Briand, 2010), hamsters do not show significant effect on TG levels compared to rats or humans (Harris, 1996). Also, hamster studies showed a consistent drop in HDL-C and increase in LDL-C in fish oil treatment, which indicates that fish oil feeding does not affect hamster lipoprotein metabolism as it does in humans (Harris, 1997). However, other animals may

not be a better option, such as rats are a poor model for human lipoprotein metabolism as most of their serum cholesterol transporting to HDL not in LDL fraction as humans (Harris, 1997). Due to this limitation, our plasma lipid profile results may not be able to optimally reflect the human plasma lipid profile in the same types of conditions. It would be necessary to examine the effect of different DFA treatment on plasma lipid profile in clinical studies.

CONCLUSION

Two primary findings arise from the present research project. First, this study determined that levels of FAEs in hamster tissues and plasma reflect the fatty acid composition of the diet and organs, in a manner that was tissue specific. Second, the pattern of FAEs, particularly OEA, associated with fat oxidation, food intake, and changes in body composition in hamsters. In conclusion, the present results demonstrate for the first time in a hamster model that diet fat selection indeed exerts a profound influence on the pattern of the three FAEs across tissues and that this pattern is dependent on the tissue type involved. In summary, it appears that FAEs may possess a functional role in mediating the beneficial effects of qualitative dietary fatty acid composition on risk markers tied to energy balance and circulating lipid levels. Although considerably more information is required to fully elucidate this area, the present study has suggested that each of these three FAEs appears to possess a specific function in cellular regulation and that these act individually in a reciprocal manner to control energy pathways with OEA inducing and AEA suppressing cellular fatty acid oxidation.

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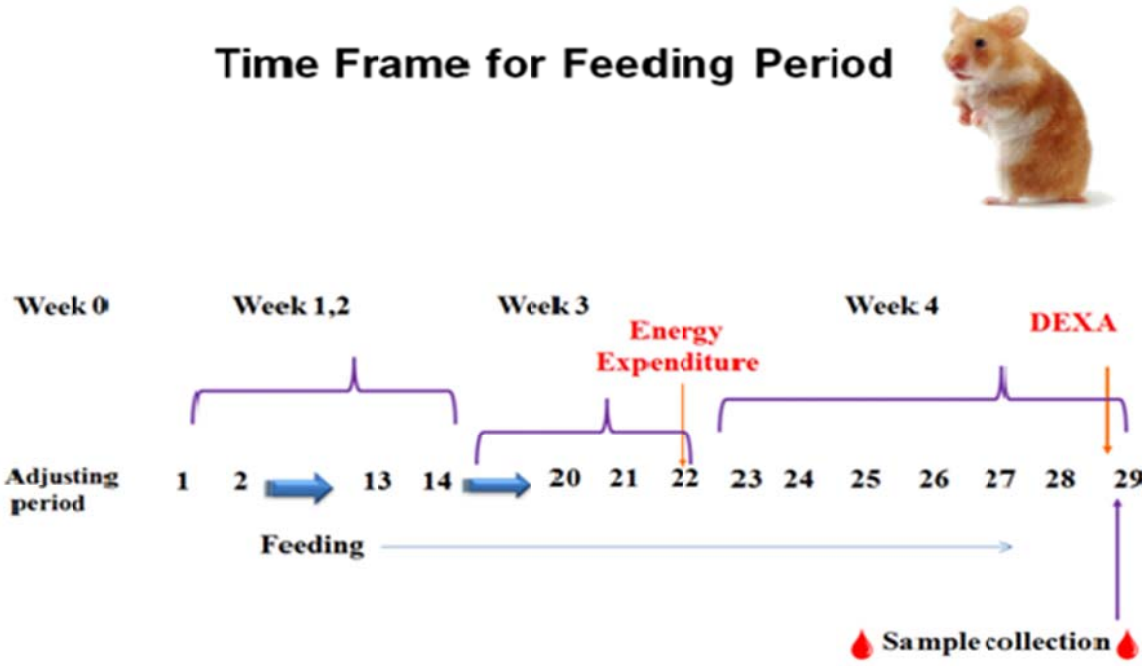
APPENDIX


Time Frame for Feeding Period
Week 0
Week 1,2
Week 3
Week 4
Energy Expenditure
DEXA
Adjusting period
1 2 13 14 20 21 22 23 24 25 26 27 28 29
Feeding
Sample collection