EFFICACY OF HIGH-OLEIC CANOLA AND FLAXSEED OILS

FOR CARDIOVASCULAR DISEASE RISK REDUCTION

by

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A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Food and Nutritional Sciences

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ABSTRACT

Considerable interest has focused on the influence of dietary fat quality on cardiovascular disease (CVD) risk. Increasingly, novel dietary oils rich in oleic acid and α-linolenic acid (ALA) are being developed and marketed with an aim to improve fatty acid intakes and reduce CVD risk. The objective of this research was to investigate the efficacy of high-oleic canola oil (HOCO) alone, or blended with flaxseed oil (FXCO), on traditional and emerging clinical biomarkers of CVD risk. An additional aim was to study the influence of dietary and genetic factors on metabolism of ^{13}C-ALA to long-chain PUFA. Using a diet-controlled randomized crossover design, thirty-six hypercholesterolaemic subjects consumed three isoenergetic diets for 28 days each containing ~36% energy from fat, of which 70% was provided by HOCO, FXCO, or a Western dietary fat blend (WD; control). Endpoint measures revealed reductions ($P<0.001$) in serum lipid concentrations, including a 7.4% and 15.1% decrease in LDL-cholesterol after HOCO and FXCO diets, respectively, as compared with the WD control. Moreover, a reduction ($P=0.023$) in plasma E-selectin concentration was found after the FXCO diet compared with the WD control. Consumption of the dietary oils failed to alter whole-body fat oxidation or energy expenditure, nor lead to alterations in body composition. FXCO diet increased ($P<0.001$) plasma ALA ~5-fold, EPA ~3-fold, and DPA ~1.5-fold, but did not modulate DHA levels compared with the WD control. At 24 and 48 hours the amount of administered ^{13}C-ALA recovered as plasma ^{13}C-EPA and ^{13}C-DPA was lower ($P<0.001$) after FXCO diet compared with HOCO and WD diets, suggesting decreased ALA conversion efficiency with very high intakes of dietary ALA. No difference in plasma ^{13}C-DHA enrichment was observed across diets. Moreover, minor alleles of selected single nucleotide

polymorphisms in the FADS1/FADS2 gene cluster were associated with reduced ($P<0.05$) plasma fatty acid compositions and apparent conversion of ^{13}C-ALA. However, increased consumption of ALA in the FXCO diet compensated for lower levels of EPA in minor allele homozygotes. Taken together, substitution of dietary fats common to WD with both HOCO and FXCO represents an effective strategy to target several biomarkers for CVD risk reduction.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

AA   Arachidonic acid (20:4n-6)

AI   Adequate intake

AHA   American Heart Association

ALA   Alpha-linolenic acid (18:3n-3)

AMDR   Acceptable macronutrient distribution range

ANOVA   Analysis of variance

AP   Atom percent

APE   Atom percent excess

Apo   Apolipoprotein

ATP III   Adult treatment program III

AUC   Area under curve

BMI   Body mass index

CE   Cholesterol ester

CAD   Coronary artery disease

CHD   Coronary heart disease

CHO   Carbohydrate

CRP   C-reactive protein

CVD   Cardiovascular disease

DEXA   Dual-energy x-ray absorptiometry

DGLA   Dihomo-gamma-linolenic acid (20:3n-6)

DHA   Docosahexaenoic acid (22:6n-3)

DM   Diabetes mellitus

DPA	Docospentaenoic acid (22:5n-3)
ELOVL	Elongation of very long chain fatty acids
EPA	Eicosapentaenoic acid (20:5n-3)
FADS	Fatty acid desaturase
FDA	Food and Drug Administration
FXCO	Flaxseed/high-oleic canola oil blend
GC	Gas chromatography
GLA	Gamma-linolenic acid (18:3n-6)
HDL	High-density lipoprotein
HOCO	High-oleic canola oil
IL	Interleukin
IMT	Intima-media thickness
IRMS	Isotope ratio mass spectrometry
IOM	Institute of medicine
LA	Linoleic acid (18:2n-6)
LCPUFA	Long-chain polyunsaturated fatty acid
LDL	Low-density lipoprotein
LF	Lower fat
LT	Leukotriene
MetS	Metabolic syndrome
MF	Moderate fat
MI	Myocardial infarction
MUFA	Monounsaturated fatty acids

n-3	Omega-3 (ω-3)
n-6	Omega-6 (ω-6)
n-9	Omega-9 (ω-9)
N	Nitrogen
NCEP	National Cholesterol Education Program
NEFA	Non-esterified fatty acid
npRQ	Non-protein respiratory quotient
OA	Oleic acid (18:1n-9)
PC	Phosphatidylcholine
PDB	Pee Dee Belemnite
PDR	Percent dose recovered
PE	Phosphatidylethanolamine
PG	Prostaglandin
PI	Phosphatidylinositol
PUFA	Polyunsaturated fatty acids
RCFFN	Richardson Centre for Functional Foods and Nutraceuticals
RMR	Resting metabolic rate
SCD	Stearoyl-CoA desaturase
SDA	Stearidonic acid (18:4n-6)
SFA	Saturated fatty acids
sICAM-1	soluble Intercellular adhesion molecule-1
SNP	Single nucleotid polymorphism
STA	Stearic acid (18:0)

sVCAM-1	soluble Vascular cell adhesion molecule-1
TAG	Triglyceride
TC	Total cholesterol
TEF	Thermic effect of food
TFA	Trans fatty acids
TNF	Tumor necrosis factor
TX	Thromboxane
US	United States
VCO2	Volume per time carbon dioxide production
VO2	Volume per time oxygen consumption
WD	Western diet

CHAPTER I

OVERALL INTRODUCTION

1.1 INTRODUCTION

The role of dietary fat quality in the propensity for cardiovascular disease (CVD) risk has been the focus of considerable interest and dietary intervention strategies. Cardiovascular disease, encompassing coronary heart disease (CHD), stroke, and other vascular diseases, remains a major cause of death in Canada and the United States (US) accounting for 29% and 31% of all deaths in 2008, respectively (1,2). Accordingly, professional health organizations emphasize dietary fat quality, recommending the reduction of saturated fatty acids (SFA), trans fatty acids (TFA) and cholesterol intake, while increasing monounsaturated fatty acids (MUFA) and omega-3 polyunsaturated fatty acids (n-3 PUFA) intake as part of first-line dietary intervention strategies targeting CVD risk reduction (3-5). Recently, the US Food and Drug Administration (FDA) has authorized qualified health claims for conventional foods stating canola oil (6) or n-3 PUFA (7) may reduce the risk of CHD. Consequently, consumer awareness regarding dietary fat has become increasingly sophisticated, recognizing the health detriment of SFA and TFA consumption, while shifting interest towards the health attributes of unsaturated fats and oils, namely n-3 PUFA enriched functional foods (8,9). In response to growing consumer demand, as well as dietary recommendations and food labelling regulations (10), the edible oilseed industry has developed novel modified oils with nutritionally superior fatty acid profiles, containing little or no TFA, reduced SFA, while high in MUFA and n-3 PUFA (11-13). High-oleic canola oil represents a novel dietary oil with enhanced

stability, and thus can be used as a substitute for SFA- and TFA-rich dietary oils for numerous food applications, including frying, baking, and blending with other fats (11). Furthermore, flaxseed oil, containing the highest dietary source of alpha-linolenic acid (ALA), has generated substantial interest for use in n-3 PUFA functional food applications targeting cardiovascular health (9,12). As Canada is currently the world's largest crop producer of canola and flaxseed (14), enhanced demand for dietary oils produced from these crops may significantly benefit the Canadian economy (15,16). For these reasons, substantiating the cardioprotective effects of consumption of dietary oils rich in MUFA and ALA provides crucial knowledge to the edible oil and food industry, dietary regulatory organizations, and ultimately enhances population health and wellness.

The marine derived omega-3 fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) have received substantial scientific and public interest for the prevention of CVD risk, however, the specific function of ALA remains a matter of debate. While dyslipidemia is a primary risk factor in predicting CVD events and a major target of dietary intervention (5), the lipid-lowering potential of ALA has been recently challenged (17). In spite of this, approximately half of all cardiovascular events occur in people with normal cholesterol levels, and 20% of all events occur in people with no major risk factors (18). It has been demonstrated that C-reactive protein (CRP) levels, when added to the traditional ways of measuring risk, provide a better assessment than serum lipids alone of detecting who is a high-risk patient (19). Thus, increasing interest in the unique health attributes of ALA, especially related to the vascular endothelium, has stimulated research into examining the effects of ALA on

novel biomarkers of inflammation and endothelial cell function (20-23). However, results from intervention studies investigating the effects of flaxseed oil on inflammatory biomarkers and adhesion molecules are inconsistent and suggest that high intakes of ALA from flaxseed oil may be most effective (24). Taken together, additional human intervention studies are needed to further substantiate the cardioprotective effects of ALA and elucidate potential mechanisms of action.

The low prevalence of chronic disease in populations consuming MUFA-rich Mediterranean diets (25) has stimulated research into the specific health attributes of MUFA (26). With the aim of reducing SFA intakes in Western diets, questions remain as to the optimal dietary replacement, comparing MUFA to PUFA. Evidence from human intervention studies suggests that MUFA have slightly less or similar hypolipidemic effects compared with PUFA, while preventing reductions in HDL-cholesterol levels (26-28). However, it has also been suggested that not all MUFA-rich oils have the same lipid-lowering effect, as canola oil and high-oleic sunflower oil are demonstrated to be more effective than olive oil (29). With respect to emerging biomarkers of CVD risk, few human intervention studies have investigated the effect of MUFA-rich oils on CRP, inflammatory biomarkers and adhesion molecule concentrations. Therefore, the independent effects of MUFA-rich diets as a substitute for dietary SFA on traditional and emerging risk factors for CVD deserve further investigation.

In addition to modulation of blood lipids and systemic inflammation, abdominal obesity remains a critical topic in public health agendas as an underlying risk factor for metabolic

syndrome and CVD risk. Recent evidence suggests that dietary fat quality influences whether it will be channelled towards fat oxidation or storage, contributing to weight balance and obesity risk (30-33). Unsaturated fatty acids compared to SFA have an increased contribution of fat oxidation to the thermic effect of food suggesting that dietary oils with a higher PUFA and MUFA to SFA content are associated with increased levels of whole-body fat oxidation (31-36). However, the selective oxidation of 18-carbon fatty acids is less clear. ALA seems to be highly oxidized, at similar rates as oleic (OA), whereas linoleic acid (LA) and stearic acid are less oxidized and appear to be conserved (37,38). Thus, plant oils rich in MUFA and ALA, particularly high-oleic canola and flaxseed oil, respectively, may be oxidized more rapidly versus stored than conventional oils rich in SFA or LA, ultimately affecting fat deposition and body composition, factors underlying cardiovascular health.

Recently, low blood levels of EPA, DPA, and DHA have been recognized as independent and modifiable risk factors for primary and secondary CVD risk (39-41). Therefore, it has been suggested that the primary biological role of ALA is as a substrate for the synthesis of EPA, DPA, and DHA, but the efficiency of conversion to these long-chain (LC) PUFA is low (42,43). Furthermore, ALA conversion may be influenced by dietary factors, including absolute amount of ALA in the diet, as well as dietary fatty acid composition (44,45). The desaturation of ALA to LCPUFA is mediated through two key enzymes, delta (Δ)5-desaturase and Δ6-desaturase (42). Recent studies suggest that plasma and tissue concentrations of n-6 and n-3 PUFA are strongly associated with several common single nucleotide polymorphisms (SNP) in the fatty acid desaturase

(FADS)1 and FADS2 gene cluster, encoding for Δ5-desaturase and Δ6-desaturase, respectively (46-48). Therefore, investigating dietary strategies and FADS genetic variants that augment ALA conversion will provide insight into the mechanisms behind the cardioprotective effects of ALA and identify those individuals for which ALA consumption would provide the most benefit.

1.2 RATIONALE

Canola and flaxseed oils are considered 'heart-healthy' providing a nutritionally superior fatty acid profile, rich in MUFA and ALA, respectively, while low in SFA. Given the imbalance of dietary fat intakes and the rising prevalence of CVD morbidity and mortality in Western populations, dietary recommendations emphasize the replacement of SFA in the diet with unsaturated fatty acids to target traditional and emerging risk factors for CVD. Although n-3 PUFA have been shown to be cardioprotective, there remains confusion surrounding the specific health attributes of ALA and the optimal daily dose required to favourably modulate circulating lipids, inflammatory biomarkers and endothelial function. Therefore, examining the independent health benefits of dietary ALA, as well as the genetic and dietary factors that modulate ALA conversion is paramount to strengthening the role of ALA in CVD prevention. Furthermore, to date no human intervention studies have specifically investigated the effects of high-oleic canola oil on risk factors for CVD, including blood lipids, inflammatory biomarkers, and body composition. As high-oleic canola oil is being incorporated into the food supply, it is imperative that the efficacy and safety of this novel oil is assessed in the context of human health. Similarly, considering ALA-rich flaxseed oil is not a commonly consumed

oil, blending flaxseed oil with high-oleic canola oil is of interest to enhance ALA intakes in the Western diet.

Using a rigorous diet-controlled human intervention trial design, the primary focus of this research is to delineate the efficacy of the unique fatty acid profiles of both high-oleic canola oil and a flaxseed/high-oleic canola oil blend on serum lipid levels, markers of endothelial inflammation and atherogenesis, whole-body energy expenditure and substrate utilization, as well as body composition. These experimental dietary oils will be specifically compared with a typical Western dietary fat control arm. Furthermore, a secondary focus is to elucidate the dietary and genetic factors that regulate ALA metabolism. This research will investigate ALA conversion efficiency to EPA, DPA and DHA, changes in plasma fatty acid composition and the association with common genetic variants in the FADS1 and FADS2 gene cluster in response to compositional changes to dietary fat provided by the experimental oils. The output of this research is fundamental in advancing our knowledge of the specific cardiovascular benefits of dietary ALA and MUFA provided by flaxseed and high-oleic canola oils.

1.3 OBJECTIVES

The present research has 4 specific objectives:

1. Investigate the efficacy of high-oleic canola oil and a flaxseed/high-oleic canola oil blend on traditional and emerging risk factors for CVD, including circulating lipid and glucose concentrations, inflammatory biomarkers, as well as intima-media thickness.

2. Determine whether high-oleic canola oil and a flaxseed/high-oleic canola oil blend modulate resting metabolic rate, postprandial energy expenditure, thermic effect of food, substrate oxidation, and body composition.

3. Evaluate the apparent conversion of ALA to LCPUFA and beta-oxidation of ALA using stable isotope tracers, as well as quantify changes in plasma fatty acid composition in response to enhanced ALA consumption from high-oleic canola oil and a flaxseed/high-oleic canola oil blend.

4. Examine the associations between SNP in FADS1, FADS2, and ELOVL2 with serum lipids, inflammatory biomarkers, plasma fatty acid profiles, and ALA conversion efficiency in response to enhanced ALA consumption from high-oleic canola oil and a flaxseed/high-oleic canola oil blend.

1.4 HYPOTHESES

The hypotheses to be tested include:

1. Substitution of fats commonly consumed in the Western diet with high-oleic canola oil and the flaxseed/high-oleic canola oil blend will reduce serum lipid concentrations, biomarkers of inflammation, and other endpoint measures of CVD risk.

2. Due to the increased unsaturated fatty acid content of high-oleic canola oil and the flaxseed/high-oleic canola oil blend, consumption of these oils will channel fatty acids towards oxidation versus storage, enhancing energy expenditure and promoting weight maintenance.

3. Increase dietary ALA intake from consumption of high-oleic canola oil and the flaxseed/high-oleic canola oil blend will increase plasma total n-3 PUFA composition, as well as enhance apparent conversion of ALA to LCPUFA.

4. Dietary ALA intake from consumption of high-oleic canola oil and the flaxseed/high-oleic canola oil blend will interact with FADS1 and FADS2 polymorphisms to affect serum lipids, inflammatory biomarkers, plasma fatty acid profiles, and apparent conversion of ALA to LCPUFA.

The following literature review will critically assess the existing body of evidence surrounding the efficacy of dietary ALA and MUFA for the reduction of CVD risk. The first manuscript examines ALA in the current diet, the metabolic fate of dietary ALA, and the effects of ALA on CVD risk factors from human studies. The second manuscript presents a detailed investigation of the effects of dietary MUFA on metabolic disorders culminating in CVD morbidity and mortality. Thereafter, the research addressing each specific objective will be presented providing insight into the efficacy of high-oleic canola oil and the flaxseed/high-oleic canola oil blend for CVD risk reduction.

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CHAPTER II

MANUSCRIPT 1: LITERATURE REVIEW

Subsection 2.2 and 2.3 are excerpts from the book chapter "Evolution of Omega-3 Fatty

Acids in the Human Diet" in press for publication by

Nova Science Publishers, Inc. (Appendix V)

DIETARY ALPHA-LINOLENIC ACID; METABOLISM AND

CARDIOVASCULAR HEALTH

2.1 INTRODUCTION

In recent years, considerable scientific and public interest has centred on the

cardiovascular benefits of omega-3 polyunsaturated fatty acids (n-3 PUFA). While

consumption of marine derived eicosapentaenoic acid (EPA) and docosahexaenoic acid

(DHA) have been substantiated in the prevention of cardiovascular disease (CVD) risk,

the specific function of plant derived alpha-linolenic acid (ALA) remains a matter of

debate. Research proposes that ALA may target CVD risk reduction by reducing blood

lipids, inflammation, arrythmias, as well as improving platelet function, arterial

compliance and endothelial cell function (1). However, it has been suggested that the

main cardioprotective role of ALA remains as a precursor for EPA and DHA. While

DHA represents the primary n-3 PUFA in tissue membranes and plays a critical role in

human health and development (2), ALA is the primary n-3 PUFA in modern day diets

and is classified as the essential n-3 PUFA. Therefore, the purpose of the following

review is to examine ALA in the current diet, the metabolic fate of dietary ALA, genetic variants that regulate ALA metabolism, and the effects of ALA on CVD risk factors from human studies, namely blood lipids and inflammatory biomarkers.

2.2 DIETARY ALPHA-LINOLEIC ACID; CURRENT AND RECOMMENDED INTAKES

In recent years, much interest has focused on population intakes of n-3 PUFA in relation to dietary recommendations. Based on data from the National Health and Nutrition Examination Survey (NHANES) 2007-2008 in the United States (US), PUFA intakes are ~7% of energy (3). More specifically, mean PUFA intakes of men are 19.8 g/day and of women are 14.8 g/day. Alpha-linolenic acid (18:3n-3) accounts for ~9% of total PUFA energy, with an average intake of 1.7 g/day by men and 1.3 g/day by women (~0.6–0.7% of energy intakes). Conversely, linoleic acid (LA; 18:2n-6) is the predominate PUFA in the diet contributing ~88% of total PUFA energy or 17.5 g/day by men and 13.1 g/day by women (~6–7% of energy intakes). Long-chain (LC) PUFA contribute minimally to dietary fat intake. Less than 1% of energy is derived from EPA (20:5n-3), docosapentaenoic acid (DPA; 22:5n-3), and DHA (22:6n-3) combined intakes, with an average intake of 0.17 g/day by men and 0.11 g/day by women. Consequently, the ratio of n-6/n-3 PUFA in the current US diet is approximately 9.2:1, however, this may be as high as 20–25:1 in some individuals (4,5).

In 2002, the US Institute of Medicine's (IOM) Food and Nutrition Board, together with Health Canada, established an Adequate Intake (AI, an intake level necessary to achieve

nutritional adequacy and prevent deficiency symptoms) for ALA as 1.1 g/day for adult

(aged 19-50 y) women and 1.6 g/day for adult men (6). Furthermore, an Acceptable

Macronutrient Distribution Range (AMDR, a range of intakes for a particular energy

source that is associated with reduced risk of chronic disease while providing adequate

intakes of essential nutrients) was set for ALA as 0.6–1.2% of energy. Additionally, up to

10% of the AI for ALA can be provided by EPA and/or DHA. Similar to ALA, an AI was

established for LA as 12 g/day for adult women and 17 g/day for adult men and an

AMDR set at 5–10% of energy for LA. With respect to guidelines for pregnancy and/or

lactation, an AI has been established as 1.4 g/day and 1.3 g/day of ALA during pregnancy

and lactation, respectively. More specifically, due to the importance of DHA in brain,

retinal and cognitive development, several professional organizations recommend

maternal intake of at least 200 mg/day of DHA (7). Recent interest has centred on dietary

guidelines for EPA and DHA specifically. Current recommendations target 250 to 1000

mg/day of EPA+DHA (8,9). Few government and health organizations have outlined

recommendations for an optimal n-6/n-3 PUFA ratio. In 1995, the World Health

Organization/Food and Agriculture Organization (WHO/FAO) joint committee

recommended a ratio between 5:1 and 10:1 (10), recommendations supported by Health

Canada and the IOM (6,11).

Considering dietary sources, the American Heart Association (AHA), as well as the 2010

Dietary Guidelines for American state that the general population should consume at least

two servings (~8 oz) of fish/seafood per week, emphasizing a variety of fatty fish,

including mackerel, salmon, and herring (8,9). These recommendations target a daily

intake of 250 mg/day of EPA+DHA for coronary heart disease (CHD) risk reduction. Furthermore, the AHA recommends the inclusion of ALA-rich vegetable oils and foods, including flaxseed oil, soybean oil and canola oil, as well as flaxseed and walnuts. For patients with documented CHD, the AHA recommends ~1 g/day of EPA+DHA, while patients with hypertriglyceridemia should consume 2–4 g/day of EPA+DHA. Beyond dietary sources, provision of fish oil supplements, under the guidance of a physician, may be necessary to meet these recommendations. The AHA Dietary Guidelines support the US Food and Drug Administration (FDA) ruling that dietary intakes of up to 3 g/day of EPA+DHA are generally recognized as safe (GRAS) for the population, including patients with diabetes, bleeding tendencies, and elevated LDL-cholesterol (12). Of relevance, the IOM and other professional organizations suggest that intakes of ALA above the recommended AI (i.e. > 1.5 g/day) may result in additional health attributes, specifically cardiovascular benefits (6,13).

2.3 DIETARY SOURCES OF ALPHA-LINOLENIC ACID

Contrary to the abundance of n-6 PUFA in the current food supply, n-3 PUFA are rich in only a limited amount of available foods. ALA is found in plant foods, specifically seeds, nuts, and legumes and concentrated in the chloroplasts of green leafy vegetables. ALA is particularly rich in flaxseed, walnuts, soybean and their oils, as well as canola (rapeseed) oil, butternuts, and chia seeds. Although flaxseed oil represents the richest source of ALA (7.258 g/tbsp), it is not commonly consumed (5) compared with soybean oil (0.923 g/tbsp) and canola oil (1.279 g/tbsp) (14). Purslane, a wild leafy vegetable common in the Eastern Mediterranean diet, contains 300–400mg/100g serving (15). The ALA content of

plants can also be influenced by growing region, variety, season and climate. For

example, considering seasonal variations the average ALA content of flaxseed oil ranged

from 52.6% in 2007 to 58.8% in 2010 (16). Moreover, the ALA content of Western

Canadian flaxseed oil can vary substantially based on region, as harvest reports from

2010 indicate that the ALA content of flaxseed oil from Manitoba, Saskatchewan, and

Alberta were 57.1%, 59.3%, and 63.4%, respectively. **Table 2.1** outlines the ALA

content of commonly consumed foods and amounts needed to meet current

recommendations.

Table 2.1: Alpha-linolenic acid composition of selected oils, nuts, and seeds and amounts needed to meet adequate intakes of adult women and men.

Dietary source	ALA	Amount needed to meet AI for women (1.1 g ALA/day)	Amount needed to meet AI for men (1.6 g ALA/day)
	g/tbsp	*tbsp*	*tbsp*
Oil			
Flaxseed (linseed) oil	7.258	0.15	0.22
Walnut oil	1.414	0.78	1.13
Canola (rapeseed) oil	1.279	0.86	1.25
Soybean oil	0.923	1.19	1.73
High-oleic canola oil	0.308	3.57	5.19
Rice bran oil	0.218	5.05	7.34
Olive oil	0.103	10.68	15.53
Palm oil	0.027	40.74	59.26
Nuts			
Walnuts, English	2.574	0.43	0.62
Butternuts	2.472	0.44	0.65
Walnuts, black	0.156	7.05	10.26
Seeds			
Flaxseeds, whole	2.350	0.47	0.68
Chia seeds	1.819	0.60	0.88
Flaxseeds, ground (7 g)*	1.597	0.69	1.00
Pumpkin seeds	0.034	32.25	47.06

Estimated data obtained from US Department of Agriculture National Nutrient Database for Standard Reference, Release 24; 1 tbsp oil = ~13.6 g; 1 tbsp nuts = ~28.35 g; 1 tbsp seeds = ~10.2 g (unless otherwise noted*); ALA, alpha-linolenic acid; AI, adequate intake.

2.4 POLYUNSATURATED FATTY ACID BIOCHEMISTRY, ESSENTIALITY AND ENDOGENOUS STATUS

Polyunsaturated fatty acids, containing two or more double bonds along the length of the hydrocarbon chain, are classified as either n-3 or n-6 PUFA by the location of the first double bond relative to the terminal methyl end of the carbon chain. ALA is the parent n-3 PUFA containing 3 double bonds with the first double bond located at the third carbon relative to the methyl end of the 18-carbon chain. On the other hand, LA is the parent n-6 PUFA. Both ALA and LA are termed essential fatty acids because humans lack the delta (Δ)15- and Δ12-desaturase enzymes required for insertion of a double bond at the n-3 or n-6 position, respectively. In 1929, Burr and Burr first identified the nutritional essentiality of LA, and later ALA, as clinical symptoms of impaired growth and reproduction, scaly skin, tail necrosis and increased mortality in weanling rats were reversed by the addition of either LA or ALA to the diet (17,18). It was not until 1982 that Holman and colleagues specifically recognized ALA deficiency in a 6-year old girl, associated with low serum ALA concentrations and severe neurological abnormalities (19,20).

As parent essential fatty acids, ALA and LA can be metabolized to their LCPUFA derivatives through a series of desaturation and elongation steps; EPA and DHA can be synthesized from ALA, while arachidonic acid (AA; 20:4n-6) can be synthesized from LA (21). Although the LCPUFA are not considered essential, much debate exists as to whether endogenous synthesis of EPA and DHA from ALA is adequate to support growth, physiological needs, and disease risk reduction (22,23). Despite similar intestinal

absorption efficiency for n-6 and n-3 PUFA exceeding 96% (24), plasma and cell membrane concentration of n-3 PUFA is low as compared with n-6 PUFA, reflecting an abundance of n-6 PUFA in the diet compared with n-3 PUFA (25,26). Moreover, with respect to n-3 PUFA, phospholipids of human mononuclear cells, plasma and erythrocyte, as well as brain, heart, and liver tissue predominately contain DHA, whereas cell and tissue concentration of EPA is limited and ALA is negligible. For example, plasma phospholipids contain ~0.1% of total fatty acids of ALA, whereas n-3 LCPUFA concentrations comprise ~0.8% EPA and ~3% DHA. On the other hand, the n-6 PUFA concentrations in plasma phospholipids are substantially higher with ~22% LA, and ~11% AA (26). Structurally, PUFA are incorporated into triglycerides (TAG), phospholipids, and cholesteryl esters of cell and tissue membranes (27). More specifically, PUFAs are incorporated into the *sn*-2 position of the major membrane phospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). PUFAs play a vital role in cellular membranes, maintaining fluidity, protein and cellular functions, as well as influencing gene expression and cell signalling (28). However, functionally, some of the most potent effects of PUFAs are associated with the biosynthesis of eicosanoids from AA and EPA and docosanoids from DHA, regulating and resolving the systemic inflammatory response (28,29). Considering low plasma and tissue levels of ALA, yet the link between dietary ALA and reduced risk for CVD, the metabolic fate of dietary ALA has been extensively investigated. The major metabolic fates of ALA include: 1) biosynthesis of n-3 LCPUFA, 2) hepatic beta (β)-oxidation, 3) carbon recycling for *de novo* lipogenesis, and 4) tissue storage.

2.5 METABOLISM OF POLYUNSATURATED FATTY ACIDS

2.5.1 The Desaturation and Elongation Pathway

The conversion of ALA to n-3 LCPUFA is the metabolic pathway that has received much attention and clinical investigation. The predominate site of ALA desaturation and elongation occurs in the liver, however, also occurs to a lesser extent in other tissues, including the brain and heart (30-32). The majority of the LCPUFA biosynthesis pathway takes place in the endoplasmic reticulum with ALA and LA utilizing, and thus, competing for the same desaturation and elongation enzymes (**Figure 2.1**). Similarly, conversion of oleic acid (OA; 18:1n-9) to mead acid (20:3n-9) shares the same desaturation and elongation enzymes as ALA and LA (33) (**Figure 2.1**). However, OA is not considered essential as it can be synthesized endogenously from the Δ9-desaturation (stearoyl-CoA desaturase) of stearic acid (STA; 18:0). The desaturation and elongation steps primarily alternate, with desaturation being a slower reaction than chain elongation (34,35). Furthermore, the desaturase enzymes have an affinity order of n-3 PUFA > n-6 PUFA > n-9 PUFA. Albeit a higher affinity for n-3 PUFA, the abundance of LA in the Western diet (e.g. corn, safflower, soybean, and sunflower oils) compared with limited dietary sources of ALA (e.g. flaxseed, canola, and soybean oil, and nuts) significantly impedes the metabolism of ALA. Of importance, accumulation of mead acid is a sign of ALA or LA deficiency due to the preferential affinity of the desaturase enzymes for ALA and LA (36).

The first reaction in the conversion pathway is the desaturation of ALA to stearidonic acid (SDA; 18:4n-3) or LA to gamma-linolenic acid (GLA; 18:3n-6) via the rate-limiting

enzyme $\Delta 6$-desaturase (37,38) (**Figure 2.1**). Next, elongation and $\Delta 5$-desaturation

converts SDA to EPA and GLA to AA. Alternatively, LA can undergo chain elongation

to eicosadienoic acid (EDA; 20:2 n-6), $\Delta 8$-desaturation (FADS2) to dihomo-gamma-

linolenic acid (DGLA; 20:3n-6), and $\Delta 5$-desaturation to form AA. In parallel, ALA can

be chain elongated to eicosatrienoic acid (ETE; 20:3n-3), $\Delta 8$-desaturation to

eicosatetraenoic acid (ETA; 20:4n-3), and $\Delta 5$-desaturated to form EPA. Ultimately, AA

is the major n-6 PUFA end product of LA that gets incorporated into the *sn-2* position of

cell membrane phospholipids. Conversely, EPA is quantitatively a minor fatty acid in

tissue membranes and undergoes further elongation to DPA. Controversy has surrounded

the conversion of DPA to DHA. It has been suggested that two chain elongation steps

separated by a $\Delta 4$-desaturation was the mechanism of DHA synthesis (39). However,

studies by Voss et al. (1991) failed to observe $\Delta 4$-desaturase activity in rat liver

microsomes and have identified the Sprecher pathway as the primary metabolic route

producing DHA (40). In this pathway, DPA is elongated to 24:5n-3 and then utilizes the

$\Delta 6$-desaturase enzyme to form 24:6n-3. Next, 24:6n-3 is translocated to the peroxisome

and partially β-oxidized to DHA. Structually, DHA is the predominate n-3 PUFA that is

esterified into tissue membrane phospholipids. It has been hypothesized that multiple use

of the rate-limiting $\Delta 6$-desaturase enzyme for the conversion of ALA to SDA and 24:5n-

3 to 24:6n-3 may lead to a "bottle-neck" in the metabolic pathway and an associated

decreased in the synthesis of DHA (41,42). Another possible rate-limiting step may be

related to the compartmental translocation of 24:6n-3 from the endoplasmic reticulum to

the peroxisome. Both hypotheses demand further investigation.
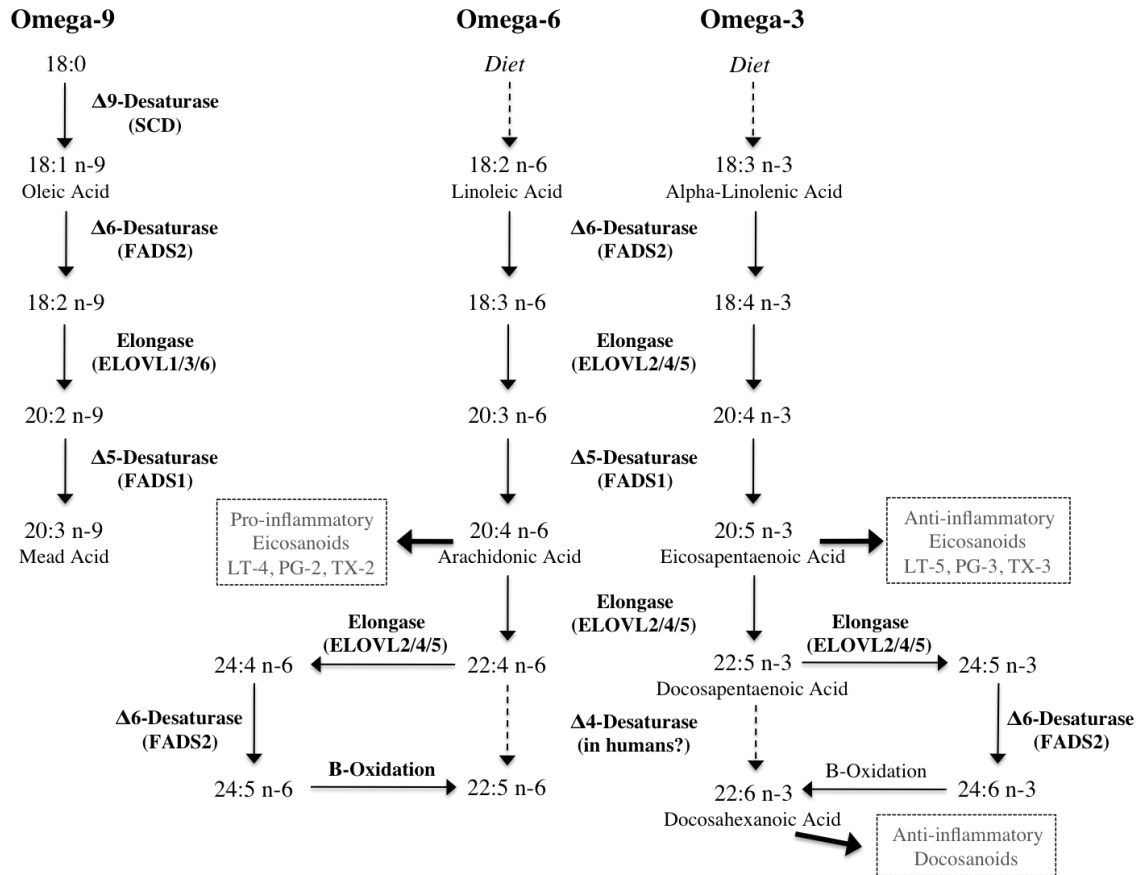
Figure 2.1: The omega-9, omega-6, and omega-3 fatty acid metabolic pathways. ELOVL, elongation of very long chain fatty acids enzyme; FADS, fatty acid desaturase; LT, leukotriene; PG, prostaglandin; SCD, stearoyl-CoA desaturase; TX, thromboxane.

2.5.2 Alpha-Linolenic Acid Conversion to Long-Chain Polyunsaturated Fatty Acids; Results from Dietary Supplementation Studies in Humans

While DHA remains the primary n-3 PUFA in plasma and tissue membranes, ALA is the primary n-3 PUFA in the Western diet. Therefore, several human intervention studies have investigated the extent to which dietary ALA can modulate plasma and tissue levels of LCPUFA (35,43). Flaxseed oil, as well as conventional canola oil, contain high levels of ALA and are typically used in supplementation trials to increased daily intakes of ALA (up to 40 g/day) for an extended duration of time (up to 42 weeks). The consensus

of supplementation trials reveals a direct linear increase in plasma and tissue levels of

EPA with increasing ALA intakes (**Figure 2.2**). More specifically, daily intake of ALA

exceeding 4.5 g for a minimum of 4 weeks resulted in an elevated phospholipid

concentration of EPA ranging from 33–250% (44-48). Studies have also observed an

increase in plasma and tissue levels of DPA, although to a lesser extent than elevations in

EPA levels, after a range of dietary supplementation with ALA. However, the majority of

dietary ALA intervention studies fail to effectively modulate plasma and tissue levels of

DHA (35,43) (**Figure 2.2**).
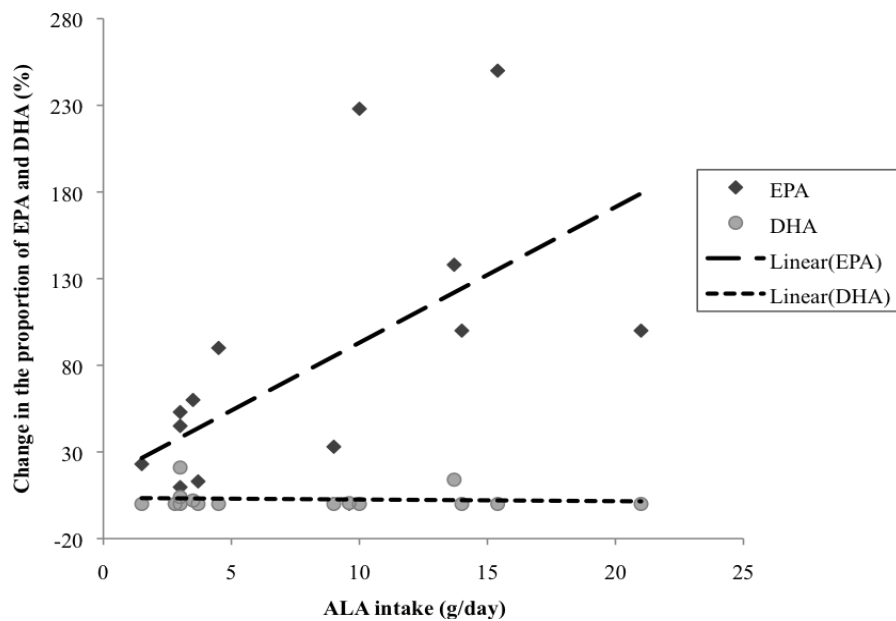
Figure 2.2: The effect of dietary alpha-linolenic acid (ALA) supplementation on the
percent change in the proportion of eicosapentaenoic acid (EPA) and docosahexaenoic
acid (DHA) in plasma total or phospholipids from baseline. Each data point represents
the mean value for an individual study. Data adapted from Brenna et al. (2009) (43).

Differences in background diet and subject characteristics have been reported to

influence the metabolism of ALA to LCPUFA. One of the most studied dietary factors

influencing synthesis and membrane incorporation of n-3 LCPUFA is the absolute

amount of dietary LA, as well as the dietary LA/ALA ratio. Conclusions from a report

published by the International Society for the Study of Fatty Acids and Lipids (ISSFAL)

working group emphasize that a reduction in LA intake in combination with an increase

in n-3 LCPUFA intake is the most effective way to improve n-3 LCPUFA tissue

concentrations (43).

Sex-specific difference may affect biosynthesis of LCPUFA, as DHA composition of

plasma phospholipids have been shown to be higher in women than in men (49). Giltay et

al. (2004) observed a 15% increase in DHA status in women compared with men (50).

Furthermore, administration of oral estradiol increased DHA status by 42%, while

testosterone decreased DHA status by 22%. It is proposed that estrogen may upregulate

ALA metabolism to DHA, and thus, increase maternal DHA status particularly during

pregnancy due to the greater demand of DHA for fetal neurological development (50-52).

Furthermore, age may be a factor in metabolic efficiency as infants exhibit increased

conversion of ALA to LCPUFA, including DHA (53,54). Clark et al. (1992) observed a

105% increase in EPA and 38% increase in DHA composition of erythrocytes in infants

fed formulas with a 3.4% versus 0.7% of total fatty acids of ALA (55). However,

differences in adult age (18–29 versus 45–69 years) may not influence metabolism of

ALA to EPA or DHA (56).

2.5.3 Alpha-Linolenic Acid Conversion to Long-Chain Polyunsaturated Fatty Acids;
Results from Stable Isotope Tracer Studies in Humans

Stable isotope tracer study results support findings from dietary n-3 PUFA

supplementation studies. Although both methods indirectly estimate hepatic ALA conversion to n-3 LCPUFA in humans, the use of stable isotopes, either deuterium- or ^{13}C-labeled, more accurately traces the metabolic fate of ALA and incorporation of the isotope into different pools of interest (57,58). Consensus of stable isotope studies using uniformly labelled ^{13}C-ALA report enzymatic conversion ranging from 0.2–8% to EPA and less than 0.05–4% to DHA (57-64) (**Table 2.2**). However, one study observed 21% conversion to EPA and 9% conversion to DHA in healthy young women (65). Using deuterated-ALA ethyl ester and a physiological compartmental model design, Pawlosky et al. (2001) reported the conversion of plasma ALA to EPA was only about 0.2%, while conversion of EPA to DPA was 63%, and DPA to DHA was 37% (57). Moreover, the conversion efficiency of ALA to DHA was only 0.05%, and EPA to DHA was about 23%. These results suggest that the rate-limiting step of conversion is from ALA to EPA. Finally, stable isotope studies also support enhanced biosynthesis of DHA in women compared with men (61,65).

Recently, Goyens et al. (2006) demonstrated that conversion of ^{13}C-ALA in humans is influenced by the absolute amounts of ALA and LA in the diet, rather than the dietary ratio of LA/ALA (66). At a constant dietary LA/ALA ratio of 7:1, decreasing LA consumption resulted in an increase of ^{13}C-ALA converted to ^{13}C-EPA, however, did not modulate proportions of ^{13}C-DHA. Furthermore, some studies (60,67), but not all (62) have suggested that ALA conversion to LCPUFA is downregulated with increased intake of dietary ALA. Moreover, Burdge et al. (2003) observed a decrease in ALA conversion to EPA by 2-fold and to DPA by 4-fold with a high EPA+DHA diet as compared with

Table 2.2: Estimated conversion and beta-oxidation of alpha-linolenic acid using stable isotope tracers in humans.

Reference	Subjects	ALA Isotope Dose	Diet	Blood Fraction	Conversion to			Oxidation $^{13}CO_2$ (duration)
					EPA	DPA	DHA	
Emken et al. (1994) (59)	Healthy males (n=7)	[^{2}H] 3500mg	15g LA + 2g ALA / 30g LA + 1g ALA	TL / TL	8% / 3.4%	4.2% / 2.6%	4.0% / 3.6%	-- / --
Vermunt et al. (2000) (60)	Healthy subjects (n=13)	[U-^{13}C] 45mg	4.1g LA + 2 g ALA / 4.5g LA + 4.3g ALA	TL	0.12 mg / 0.04 mg	0.05 mg / 0.02 mg	0.01 mg / 0.005 mg	16% (12 h) / 20% (12 h)
Pawlosky et al. (2001) (57)	Healthy males (n=8)	[^{2}H] 1000mg	5g LA + 0.7g ALA	TL	0.2%	0.13	0.05	--
Burdge et al. (2002) (61)	Healthy males (n=6)	[U-^{13}C] 700mg	8g LA + 1g ALA	TAG + NEFA + PC	7.9%	8.1%	ND	33% (24 h)
Burdge & Wootton (2002) (65)	Healthy females (n=6)	[U-^{13}C] 700mg	8g LA + 1g ALA	TAG + PC + NEFA + CE	21.1%	5.9%	9.2%	22% (24 h)
Burdge et al. (2003) (62)	Healthy males (n=14)	[U-^{13}C] 700 mg	17g LA + 2g ALA	TAG + NEFA + PC	2.8%	1.2%	0.04%	34% (24 h)
McCloy et al. (2004) (58)	Healthy females (n=6)	[U-^{13}C] 47mg	0.8g LA + 0.2g ALA (test meal)	PL + CE + TG + NEFA	1.5%	0.6%	0.3%	19% (9 h) / 71% (168 h)
Goyens et al. (2005) (63)	Healthy subjects (n=29)	[U-^{13}C] 30 + 20mg	7% en LA + 0.4% ALA (1 g ALA)	PL	6.9%	--	0.07%	--
Hussein et al. (2005) (64)	Hyperlipidemic subjects (n=38)	[U-^{13}C] 400mg	9g LA + 19g ALA	TL	0.03%	0.02%	<0.01	--

ALA, alpha-linolenic acid; CE, cholesterol esters; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; ND, not detected; NEFA, non-esterified fatty acids; PC, phosphatidylcholine; PL, phospholipids; TAG, triglyceride; TL, total lipids; U-, uniformly labeled; --, not reported.

baseline conversion rates, however, conversion to DHA was not affected (62). Animal studies demonstrate that liver synthesis of DHA is upregulated when dietary n-3 PUFA content is reduced (68). These findings further support the hypothesis of a cyclical pathway utilizing the Δ6-desaturase twice in the biosynthesis of DHA from ALA (42,69). With ALA and 24:5n-3, as well as LA, competing for the Δ6-desaturase enzyme high dietary intakes of ALA and LA may inhibit 24:5n-3 as a substrate for Δ6-desaturation and ultimately, DHA synthesis. Questions remain as to the ideal intake of ALA and LA to maximize conversion efficiency of ALA to LCPUFA in humans.

2.5.4 Beta-Oxidation and Other Metabolic Fates of Alpha-Linolenic Acid

Although conversion of ALA to n-3 LCPUFA receives substantial interest, β-oxidation has been determined as the major metabolic fate of dietary ALA (58) (**Figure 2.3**). Stable isotope tracer studies estimate hepatic β-oxidation of dietary ALA by measuring the proportion of ^{13}C-ALA recovered in breath samples as ^{13}CO$_2$. Results report a range from 16–34% of ^{13}C-ALA is partitioned for β-oxidation over sampling durations of 9–48 hours (**Table 2.2**) (58,60-62,65). However, it has been proposed that results of ^{13}C-ALA β-oxidized may be underestimated by about 30% due to ^{13}CO$_2$ trapping in bicarbonate pools (24,70). Using a longer sampling period, McCloy et al. (2004) confirmed that β-oxidation was the primary metabolic route of dietary ALA observing ~71% of administered ^{13}C-ALA was recovered in breath as ^{13}CO$_2$ over 168 hours (58). Similar to variations in conversion rates, gender may contribute to difference in metabolism, as higher ^{13}C-ALA β-oxidation rates have been observed in males (33% of administered dose) than in females (22% of administered dose) (61,65). A decrease in ALA β-

oxidation in females could therefore result in more substrate available for conversion to LCPUFA (24). As compared with other 18-carbon fatty acids, ALA undergoes increased oxidation (58,71). Over 9 hours, cumulative ^{13}C recovery in breath revealed an oxidation order of ALA (18:3) > elaidate (*trans*18:1) > OA (*cis*18:1) > LA (18:2) > STA (18:0) (71). The preferential oxidation of ALA may be associated with increased affinity for carnitine palmitoyltransferase-1 (72), mediating the transport of LCPUFA across the mitochondrial membrane in the liver and regulating fatty acid oxidation. Of interest, varying the amount of dietary ALA or LCPUFA fails to alter the amount of ^{13}C-ALA recovered as $^{13}CO_2$ (60,62), suggesting that β-oxidation is relatively stable in response to changes in dietary ALA. In addition, studies using indirect calorimetry and the respiratory quotient to examine dietary lipid metabolism have reported an increase in whole-body fat oxidation, as well as increased thermic effect of food, after consumption of diets with a higher PUFA/SFA ratio (73,74).

During β-oxidation of ALA in the mitochondria, carbon units generated in the form of acetyl-CoA can be recycled and used to synthesize fatty acids *de novo* (**Figure 2.3**). Using stable isotope tracers, Burdge et al. (2003) observed recycling of carbon liberated from β-oxidation of ^{13}C-ALA in humans over a 21-day period (75). ^{13}C-labelled SFA and MUFA were identified in plasma PC and TAG at 24 hours post-dose through 21 days of measure. Of interest, ^{13}C-labelled SFA and MUFA was 20% higher in men than in women, coinciding with increased ^{13}C-ALA β-oxidation rates in men than in women (61,65). These findings of reduced ALA β-oxidation and carbon recycling in women further support the hypothesis of an increased availability of ALA for conversion to

LCPUFA in women than in men (24).
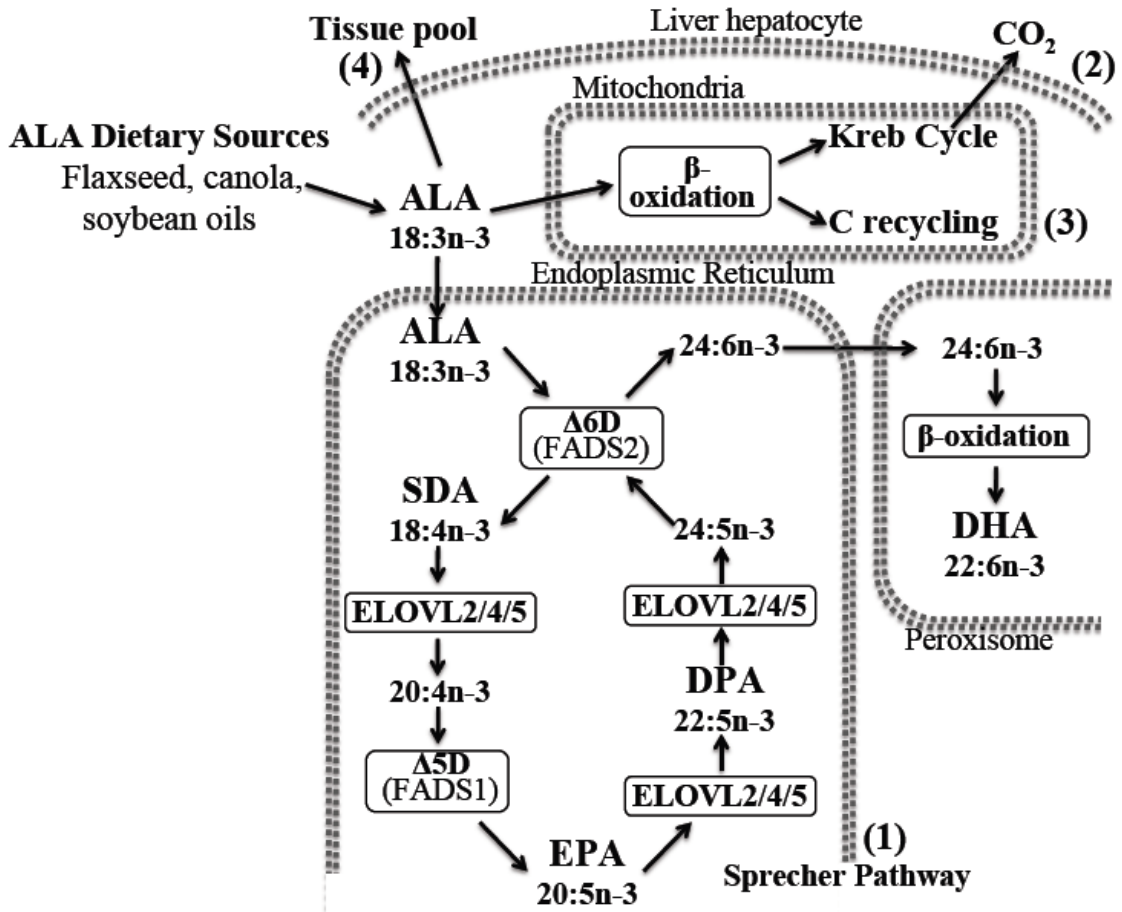
Figure 2.3: Main metabolic fates of alpha-linolenic acid: (1) Conversion to long-chain polyunsaturated fatty acids in the endoplasmic reticulum and peroxisome via Sprecher pathway, (2) β-oxidation in the mitochondria, (3) Carbon recycling for *de novo* fatty acid synthesis, (4) Storage in tissue membrane. ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; Δ5D, delta 5-desaturase; Δ6D, delta 6-desaturase; DPA, docosapentaenoic acid; ELOVL, elongation of very long chain fatty acids enzyme; EPA, eicosapentaenoic acid; FADS, fatty acid desaturase; SDA, stearidonic acid.

Second to β-oxidation, storage of ALA in adipose tissues accounts for a main metabolic disposal route of dietary ALA (58) (**Figure 2.3**). McCloy et al. (2004) reported between 2–11% of ^{13}C-ALA accumulated in abdominal fat 6 hours post-dose, which decreased to 0.6–8% by 168 hours post-dose (58). Extrapolating these measures to the fat content of

the whole-body, the authors estimated that between 4–57% of ^{13}C-ALA was incorporated into whole-body adipose tissue over 168 hours. The main function of adipose tissue is the storage of lipids and mobilization of fatty acids during increased energy demands of the body. Therefore, adipose tissue storage of ALA may be an important metabolic fate, as ALA is the primary n-3 PUFA in adipose tissue, comprising approximately 0.62% weight (76). On the contrary EPA, DPA and DHA only account for approximately 0.01, 0.17, and 0.10% weight of adipose tissue, respectively. Accumulation of ALA in skin may also contribute to the disposal of ALA. Almost half of administered ^{14}C-ALA was found in the skin and fur of guinea pigs 48 hours post-dose (77), however, whether these findings extrapolate to humans has yet to be determined.

2.6 THE EFFECTS OF FADS1 AND FADS2 POLYMORPHISMS ON ALPHA-LINOLENIC ACID METABOLISM AND LONG-CHAIN POLYUNSATURATED STATUS IN HUMANS

The cloning of human desaturase cDNA in 1999 has provided great insight into the molecular regulation of fatty acid desaturase 1 (FADS1) and fatty acid desaturase 2 (FADS2) encoding Δ5-desaturase and Δ6-desaturase, respectively (30,31,78). FADS1 and FADS2 form a gene cluster with FADS3 on human chromosome 11 (11q12-13.1), however, the function of FADS3 remains unknown (38). All three desaturase genes consist of 12 exons and 11 introns, with FADS1 and FADS2 found in a head-to-head orientation, whereas FADS2 and FADS3 are oriented tail-to-tail. Both Δ5-desaturase and Δ6-desaturase consist of 444 amino acids, have 61% amino acid identity and 75% similarity. The gene cluster covers a 91.9 kb region of the human chromosome. Due to

the marked similarities, it has been proposed that the three genes evolved from gene duplication (38,78). These desaturase enzymes are located in the endoplasmic reticulum as membrane-bound proteins, with the highest activity occurring in the liver, followed by the heart, brain, lung and adipose tissue, and to a lesser extent in the skeletal muscle, kidney, pancreas, placenta, and uterus (30-32).

In addition to diet, recent evidence demonstrates that common single nucleotide polymorphisms (SNP) in the FADS1/FADS2 gene cluster modulate ALA, as well as LA, metabolism leading to differences in plasma and tissue PUFA concentrations (33). In 2006, Schaeffer and colleagues published the first study reporting common polymorphisms of the FADS1/FADS2 gene cluster and their haplotypes were associated with differences in serum phospholipid fatty acid concentration in a cohort of 727 European adults (79). More specifically, of the 18 SNPs in the FADS1/FADS2 gene cluster analyzed, minor allele carriers of 11 SNPs had higher concentrations of n-6 LA, EDA, DGLA, as well as n-3 ALA, and lower concentrations of n-6 GLA, AA, and n-3 EPA and DPA in serum phospholipids. Moreover, SNPs genetically explained 28.5% of the variability in serum AA concentrations in the free-living cohort. Of interest, no association was observed between the measured SNPs and DHA concentrations, supporting the hypothesis of limited DHA biosynthesis and diet as the predominant source regulating DHA status.

Follow-up studies in European and North American cohorts of adults, adolescents and children confirm that SNPs in the FADS gene cluster are associated with differences in

fatty acid concentrations in serum phospholipids, as well as plasma, adipose tissue, erythrocytes, and breast milk (33). Martinelli et al. (2008) investigated the influence of 13 SNPs in the FADS1/FADS2 gene cluster in 876 Italian subjects with or without coronary artery disease (CAD) (80). The researchers reported that carriers of FADS haplotypes from 4 SNPs were associated with increased ratio of erythrocyte AA/LA, an estimate of desaturase activity, as well as increased high sensitivity-C-reactive protein (hs-CRP) concentration, and greater risk of CAD. Using two cohorts of children, Rzehak et al. (2010) observed significant associations between several SNPs and plasma fatty acids (81). Moreover, all SNPs were significantly associated with eczema reported within the first 2-years of life in children from Germany, but not children from the Netherlands. The effects of FADS polymorphisms interacting with dietary fatty acids to affect inflammatory responses warrants further investigation to help explain interindividual differences in response to dietary n-3 PUFA.

The significance of FADS polymorphisms in the region of chromosome 11 and plasma PUFA concentrations was recently substantiated in a genome-wide association study by Tanaka and colleagues (82). In the InCHIANTI cohort of 1,075 Italian subjects, results demonstrated that rs174537 was the SNP near FADS1 with the most significant association with plasma AA concentrations. As compared with major allele homozygotes, minor allele homozygotes had lower AA concentrations, accounting for 18.6% of the variability in serum AA concentrations. Furthermore, carriers of the allele associated with increased AA, as well as EDA and EPA, also exhibited an increase in LDL-cholesterol and total cholesterol levels. These effects were also confirmed in the GOLDN study

cohort of 1,076 American subjects (82). Of interest, the researchers also observed a strong association of SNPs in ELOVL2 (rs953413), the gene encoding for the elongase enzyme in the region of chromosome 6, with EPA in the InCHIANTI study cohort, with DPA in the GOLDN study cohort, and DHA in both study cohorts. Taken together, all studies observed significant associations between FADS gene polymorphisms and fatty acid concentrations, substantiating the importance of genetic modulation of fatty acid metabolism and status in humans. Future studies are needed to determine the association between FADS genetic variants and clinical endpoints for CVD and other chronic disorders. Furthermore, whether enhancing the intake of dietary n-3 PUFA can compensate for plasma and tissue levels of PUFA in minor allele carriers warrant further investigation.

2.7 THE EFFECT OF ALPHA-LINOLENIC ACID ON PRIMARY CARDIOVASCULAR ENDPOINTS

2.7.1 Epidemiologic Studies

Substantial evidence supports the inverse relationship between EPA and DHA intake and CVD risk, however, the evidence in support of ALA intake is less clear (83,84). Several observational studies, but not all, have demonstrated that higher ALA intake is associated with a lower prevalence of CVD (1,85-87). In a cross-sectional analysis, Djousse et al. (2001) used food-frequency questionnaires to determine ALA intake in 4,584 subjects participating in The National Heart, Lung, and Blood Institute Family Heart Study. In men and women in the highest quintile of ALA intake, averaging 1.4 and 0.96 g/day, the authors reported a 40 and 58% decrease in prevalence of CHD, respectively (88).

Conversely, Lemaitre et al. (2009) reported an increase in risk of sudden cardiac arrest associated with increased erythrocyte membrane ALA levels (89). As adipose contains the highest tissue proportion of ALA and is a marker of long-term ALA intake (90,91), an inverse association between adipose tissue levels of ALA and CHD risk has been documented in some retrospective case-control studies (92), however, not in others (93,94) potentially due to confounding factors including TFA intakes (93). A recent large case-control study of 3,638 participants from Costa Rica observed a strong inverse relationship between nonfatal myocardial infarction (MI) events and adipose tissue levels ranging from 0.36–1.04% of total fatty acids, corresponding with ALA intakes ranging from 1.1–2.4 g/day as assessed by food-frequency questionnaire (95). The relationship between ALA and MI was nonlinear, with no change in risk reduction with intakes exceeding 1.8 g/day. Moreover, the authors concluded that the cardioprotective effects were directly associated with ALA intakes and independent of EPA and DHA intake or status, suggesting independent anti-inflammatory mechanistic effects of ALA. Indeed, epidemiological evidence supports an inverse association between dietary ALA intake and plasma concentrations of inflammatory biomarkers, including CRP, interleukin (IL)-6, vascular cell adhesion molecule (VCAM)-1, and E-selectin (95,96). Furthermore, increased serum and erythrocyte ALA concentrations have been associated with decreased intima-media thickness (IMT) (97,98) and atherosclerotic plaque progression (99,100).

Large prospective cohort studies offer the strongest epidemiological evidence, as retrospective case-control studies may have limitations with study design, including

inaccuracy of food-frequency questionnaires, as well as subject selection and survival

bias. The majority of earlier prospective cohort studies have demonstrated a reduction in

major CHD events (101-103) or mortality (104-107), however, recent prospective cohort

studies have failed to observe this association (108-110). In an 18-year follow-up of

76,783 women participating in the Nurses' Health Study, higher intake of ALA at 1.4

g/day was associated with a 40% reduced risk of sudden cardiac death (RR=0.60; 95%

CI, 0.37–0.96) (107). Similarly, in a 14-year follow-up of 45,722 men participating in the

Health Professionals Study, ALA intake greater than 1.1 g/day was associated with an

11–12% decrease in total CHD risk with simultaneous low (HR=0.88; 95% CI, 0.78–

0.99) or high (HR=0.89; 95% CI, 0.79–0.99) n-6 PUFA intakes (102). Of particular

interest, in men consuming low daily intakes of LCPUFA (<100 mg/day of EPA+DHA),

each 1 g/day increase in ALA intake was associated with an approximate 50% decrease

in CHD risk, highlighting the importance of ALA in low fish/seafood-based diets.

Recently, a large systematic review investigating the evidence linking dietary factors and

CHD risk failed to observed an association between ALA intakes and CHD events in

145,497 participants among 5 cohort studies (RR=1.01; 95% CI, 0.84–1.18) and

concluded that evidence from randomized controlled trials is inconclusive (84).

Ultimately, results of randomized controlled trials are essential in substantiating

conclusions from epidemiological evidence.

2.7.2 Human Intervention Studies

Few randomized controlled trials have investigated the cardioprotective effects of ALA,

with some observing benefits (111-115), while others have not (116,117). Previous

intervention studies failing to observe an effect of ALA from flaxseed oil (5.5 g/day

ALA) (116) or mustard oil (2.9 g/day ALA) (117) on clinical cardiovascular endpoint

measures may have been masked by their short study design of 1 year or large amounts of

EPA+DHA in the background diet. Conversely, the Indo-Mediterranean Diet Heart Study

reporting a 52% decrease in sudden cardiac death or non-fatal MI after 2 years

consumption of 1.8 g/day ALA from mustard and soybean oil in 1,000 subjects (113) has

been challenged and discounted due to multiple methodical issues, as well as

discrepancies and validity of the data (118). The Lyon Diet Heart Study randomized 605

patients with recent MI to consume either an ALA-rich Mediterranean diet supplemented

with canola oil and canola oil-based margarines (0.81% energy from ALA) or a control

diet (0.27% energy from ALA) (114). After 27 months, a 73% reduction in cardiac death

and non-fatal acute MI (RR=0.27; 95% CI, 0.12–0.59) was demonstrated in the

experimental group as compared with the control group. However, as ALA was only one

component of the Mediterranean dietary intervention, also rich in MUFA, fruits, and

legumes, the independent effect of ALA on the reported reduction of CVD risk has been

challenged (119). Due to discrepancies in previous intervention studies, the Alpha Omega

Study was conducted to add clarity surrounding the efficacy of ALA for CHD risk

reduction (120). The study randomized 4,837 patients who had a MI to consumed one of

four margarines supplemented with EPA+DHA (400 mg/day), ALA (2 g/day),

EPA+DHA+ALA (3.4 g/day), or placebo daily. After 40 months, no effect of EPA+DHA

or ALA supplementation on fatal and nonfatal cardiovascular events was observed.

However, in a subgroup of women consuming ALA a trend ($P = 0.07$) towards a 27%

reduction in major cardiovascular events was noted (HR=0.73; 95% CI, 0.51−1.03) as

compared with consumption of EPA+DHA or placebo. Nevertheless, it has been proposed that the results of the Alpha Omega Trial may have been confounded by the increased content of n-6 PUFA in the margarine as compared with the margarine supplemented in the Lyon Diet Heart Study (121). Taken together, clinical evidence favours the efficacy of ALA in lowering CVD morbidity and mortality, however, a demand for additional randomized controlled trials substantiating the cardioprotective effects of ALA and mechanisms of action remains.

2.8 THE EFFECT OF ALPHA-LINOLENIC ACID ON CARDIOVASCULAR DISEASE RISK BIOMARKERS; HUMAN INTERVENTION STUDIES

The cardioprotective results of ALA demonstrated in epidemiological and intervention studies have stimulated increasing interest in the unique mechanistic attributes of ALA and effects on established and emerging CVD risk biomarkers. As compared with EPA and DHA, ALA has been shown to have similar, yet much more mild, effects on CVD risk biomarkers (1). Whether the observed effects are directly attributed to physiologic effects of ALA or through conversion to n-3 LCPUFA, namely EPA, remains to be elucidated. In any case, human intervention studies suggest that dietary ALA may target a reduction in blood lipids, inflammatory biomarkers and adhesion molecules, as well as other CVD risk factors.

2.8.1 Alpha-Linolenic Acid Effects on Blood Lipids

Clinically, serum levels of blood lipids are an established risk factor for CVD and a primary target of dietary intervention (1,122). Numerous randomized controlled trials

have investigated the influence of increased intake of ALA on blood cholesterol and TAG concentrations. Recent meta-analyses have reported neutral effects on total and LDL-cholesterol and modest effects on HDL-cholesterol and TAG concentrations after dietary ALA intervention (27,123,124). Pan et al. (2009) suggested that the lack of effect of ALA observed in human intervention studies may be related to the use of MUFA or n-6 PUFA as a control arm, with comparable hypolipidemic effects observed between these dietary fatty acids (123). Indeed, using a 6-week randomized crossover design in 23 hypercholesterolemic subjects, Zhao et al. (2004) observed a 10.8, 11.0, and 18.4% reduction in serum total cholesterol, LDL-cholesterol, and TAG concentrations, respectively, with consumption of ALA (6.5% of energy; 18 g/day) as compared with the average American dietary control (125). However, comparable reductions were also observed when subjects consumed the LA-rich diet. Similarly, other studies have failed to observe a change in blood lipids in hyperlipidemic subjects when dietary ALA was compared with n-6 PUFA (126,127) or MUFA (45,128). Conversely, some studies have demonstrated that dietary ALA reduces HDL-cholesterol (27,115,129) or is not as effective in lowering LDL-cholesterol as compared with dietary LA (27,130).

With respect to TAG modulation, variable effects have been reported after dietary ALA intervention as some studies have reported no effect (126,127,129), an increase (115), or a decrease (125,131). Moreover, studies reporting a substantial decrease (>15%) in TAG levels from baseline generally supplied high ALA intakes exceeding 18 g/day (125,131). The magnitude of dietary ALA effect on TAG concentrations have been reported to be influenced by factors including fatty acid content in the background diet and control

diets, as well as subject characteristics such as the degree of triglyceridemia (27). Of interest, recent studies suggest that FADS polymorphisms interact with dietary ALA to modulate serum lipid concentrations, namely total cholesterol and non-HDL-cholesterol (132,133). Taken together, future studies are needed to substantiate the effects of dietary ALA on blood lipids as compared with dietary n-6 PUFA and MUFA, as well as typical Western diets both rich in n-6 PUFA and SFA.

2.8.2 Alpha-Linolenic Acid Effects on Inflammatory Biomarkers and Adhesion Molecules

Activation of the vascular endothelium and a chronic inflammatory response are critical events involved in the initiation and progression of atherogenesis (134). C-reactive protein, serum amyloid A (SAA) and pro-inflammatory cytokines, such as IL-1, IL-6, and tumor necrosis factor (TNF)-α contribute to the development of atherosclerosis by up-regulating endothelial expression of adhesion molecules, including VCAM-1, intercellular adhesion molecule-1 (ICAM-1) and E-selectin (134). More specifically, adhesion molecules mediate monocyte attachment to the endothelium and transmigration into the subendothelial space (135,136). Recent evidence demonstrates that elevated concentrations of circulating inflammatory biomarkers are associated with cardiovascular events (137-139).

Increasing interest in the unique health attributes of ALA especially related to vascular endothelial activation has stimulated research into novel biomarkers of inflammation and endothelial cell function. Dietary intervention studies administering between 8–18 g/day

of ALA from flaxseed oil have observed significant reductions in concentrations of CRP, SAA, IL-6, VCAM-1, ICAM-1 and E-selectin (125,129,140-142). Zhao et al. (2004) observed a 75% decrease in hs-CRP concentrations, as well as a decrease in VCAM-1, ICAM-1, and E-selectin levels after 6 weeks consumption of ~18 g/day (6.5% energy) of ALA as compared with an average American diet (125). Upon further analysis the authors reported that changes in VCAM-1 and hs-CRP were inversely associated with changes in serum EPA concentrations after the ALA-rich diet. Furthermore, low-dose administration of 2 g/day of ALA for 12 weeks has been shown to reduce VCAM-1 by 16% and E-selectin by 23%, yet failed to affect TNF-α, IL-6 or ICAM-1 levels (143). However, recent studies have not observed an effect of ALA intervention on CRP, IL-6, TNF-α, or E-selectin and VCAM-1 (66,144-147). Moreover, Bemelmans et al. (2004) failed to observe a change in IMT progression after consumption of 4.5 g/day of ALA in hypercholesterolemic subjects (148). Nevertheless, the magnitude of effect of ALA on inflammatory biomarkers may be influenced by the background diet (142) and the baseline health status of the subject (144,145).

It is hypothesized that ALA actions on inflammatory biomarkers and adhesion molecules are due to a reduction in the formation of AA derived eicosanoids. As a precursor for EPA, increased incorporation of ALA into cell membrane phospholipids interferes with the conversion of LA to AA and reduces the synthesis of proinflammatory eicosanoids, including 4-series leukotrienes (LT) via the lipoxygenase (LOX) enzyme and 2-series prostaglandins (PG) and thromboxanes (TX) via the cylooxygenase (COX) enzyme (25) (**Figure 2.1**). Conversely, more EPA is available for the synthesis of less inflammatory

eicosanoids, including 5-series LT and 3-series PG and TX (29) (**Figure 2.1**). Indeed, Caughey et al. (1996) observed a 29 and 30% reduction in the production of TXB_2 and PGE_2, respectively, in mononuclear cells after subjects consumed ~14 g/day of ALA from flaxseed for 4 weeks (25). Furthermore, dietary ALA intervention decreased mononuclear cell AA concentrations, and lead to an increase in ALA concentrations by 3-fold and EPA concentrations by 2.3-fold. Nevertheless, independent of changes in 20-carbon fatty acid concentrations, ALA may exhibit direct effects on the modulation of the inflammatory response by regulating transcription factors, such as activation of peroxisome proliferator-activated receptors (PPAR) (149) and inhibition of nuclear factor kappa B (NFκB) (149,150). Therefore, future studies are warranted to substantiate the effects of ALA in inflammatory biomarkers and adhesion molecules, as well as elucidate the underlying mechanisms of action.

2.8.3 Other Cardiovascular Effects of Dietary Alpha-Linolenic Acid

Effects of ALA on other CVD risk biomarkers have been inconsistent. Although some studies have observed a reduction in systolic and diastolic blood pressure (151-154), others studies have not (127,155). Similarly, dietary ALA has been shown to be effective in reducing plasma glucose levels (124,156), however, effects on other markers of insulin resistance have been variable (86,157). Recent reviews have reported that dietary ALA reduces fibrinogen levels (124) and has anticoagulation effects (1,158), while improving arterial compliance (1,151). With respect to the anti-arrhythmic effects of ALA, a recent study reported that dietary ALA was inversely associated with ventricular premature beats (159), however, other studies have failed to observe improvements in arrhythmias

(110,160). As a result, the effects of ALA on these cardiovascular measures are variable and demand further investigation.

Unlike marine derived n-3 PUFA, the efficacy of ALA has not been extensively studied in human clinical trials. Therefore, the following research will try to ascertain and substantiate cardioprotective attributes from consumption of ALA-rich flaxseed oil.

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CHAPTER III

MANUSCRIPT 2: LITERATURE REVIEW

DIETARY MONOUNSATURATED FATTY ACIDS ARE PROTECTIVE AGAINST METABOLIC SYNDROME AND CARDIOVASCULAR DISEASE RISK FACTORS

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3.1 ABSTRACT

Over 50 years of research has sought to define the role dietary fat plays in cardiovascular disease (CVD) risk. Although optimal dietary fat quantity has been keenly pursued over past decades, attention has recently centered on the value of dietary fat quality. The purpose of the present review is to provide a critical assessment of the current body of evidence surrounding efficacy of dietary monounsaturated fatty acids (MUFA) for reduction of traditional risk factors defining metabolic syndrome (MetS) and CVD. Due to existing and emerging research on health attributes of MUFA-rich diets, and to the low prevalence of chronic disease in populations consuming MUFA-rich Mediterranean diets, national dietary guidelines are increasingly recommending dietary MUFA, primarily at the expense of saturated fatty acids (SFA). Consumption of dietary MUFA promotes healthy blood lipid profiles, mediates blood pressure, improves insulin sensitivity and regulates glucose levels. Moreover, provocative newer data suggest a role for preferential oxidation and metabolism of dietary MUFA, influencing body composition and ameliorating the risk of obesity. Mounting epidemiological and human clinical trial data continue to demonstrate the cardioprotective activity of the MUFA content of dietary fat. As the debate on the optimal fatty acid composition of the diet continues, the benefit of increasing MUFA intakes, particularly as a substitute for dietary SFA, deserves considerable attention.

3.2 INTRODUCTION

Considerable scientific interest has focused on the impact of dietary fat in the development of metabolic disorders, leading to cardiovascular disease (CVD) (1,2). The complications associated with metabolic syndrome (MetS) are the primary foundation of CVD morbidity and mortality. Dyslipidemia, hypertension, hyperglycemia, insulin resistance and obesity, namely abdominal obesity, are critical factors contributing to MetS. As MetS is a combination of modifiable risk factors, dietary intervention is targeted in primary prevention and secondary treatment therapies. Cumulative scientific evidence suggests that dietary monounsaturated fatty acids (MUFA) effect reductions in key risk factors for MetS (3-5). Dietary MUFA promote a healthy blood lipid profile, mediate blood pressure, and favourably modulate insulin sensitivity and glycemic control. Conversely, the detrimental effects of diets rich in saturated fatty acids (SFA) have been widely recognized (6,7). Thus, national dietary guidelines with a primary focus on cardiovascular health have emphasized the need to reduce consumption of SFA as compared to a decrease in total dietary fat. With emerging research on the health attributes of MUFA-rich diets, and the low prevalence of chronic disease in populations consuming MUFA-rich Mediterranean diets (8), recommendations have been made to replace SFA intakes with unsaturated fats (9). However, questions still remain as to the optimal dietary replacement for SFA, comparing MUFA intakes to those of polyunsaturated fatty acids (PUFA) and carbohydrates (CHO). Despite PUFA numerous cardiovascular benefits, intakes have been limited to ≤10% due to potential adverse effects, including reduction of HDL-cholesterol levels and increased susceptibility of LDL-cholesterol to oxidation (4,10). Furthermore, the replacement of dietary SFA with

CHO may result in challenges in glucose metabolism and insulin resistance, as well as blood triglyceride (TAG) and HDL-cholesterol levels (11,12). Thus, potential health attributes of increasing MUFA intakes, particularly at the expense of dietary SFA, deserve careful attention. In light of the recent attention challenging the cardioprotective benefits of MUFA (13,14), professional organizations continue to recommend dietary MUFA for the prevention of CVD (15,16). The purpose of the present review, therefore, is to critically assess the current evidence from human clinical trials surrounding the efficacy of dietary MUFA in the reduction of risk factors for MetS, ultimately targeting a reduction in CVD.

3.3 METABOLIC SYNDROME; DEFINITION, PREVALENCE, AND INTERVENTION

The rising prevalence of chronic disease is related to unhealthy lifestyle choices, including atherogenic diets and lack of physical activity. Metabolic syndrome is defined by a collection of metabolic disorders occurring in an individual and associates with an increased risk of developing type 2 diabetes mellitus (DM-II) and CVD (17-19). The primary clinical endpoint of MetS is CVD morbidity and mortality. Since the term was first classified by Reaven in 1988, the definition has evolved to include specific diagnostic criteria by several professional organizations (20). Recently, the National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATP III) defines MetS as an individual possessing any 3 or more of the following 5 risk factors; elevated TAG ($\geq$150 mg/dL (1.7 mmol/L)), reduced HDL-cholesterol (<40 mg/dL (1.03 mmol/L) in men or <50 mg/dL (1.29 mmol/L) in women), elevated fasting glucose ($\geq$100 mg/dL

(5.6 mmol/L)), hypertension (≥130/85 mmHg or drug treatment), or obesity (waist circumference ≥102 cm (40 in) in men or ≥88 cm (35 in) in women) (20), with ethnicity specific values for waist circumference outlined by the International Diabetes Federation (21). Furthermore, emerging risk factors for MetS include a proinflammatory and prothrombotic state (20). Initially it was hypothesized that insulin resistance was the main risk factor for MetS (22), however, recent definitions propose abdominal obesity to be the predominant risk factor underlying MetS (20,21,23,24). The prevalence of MetS ranges worldwide (25), impacted by cultural differences associated with population dietary and lifestyle patterns. For example, the prevalence of MetS in the United States (US) (34.5%) is approximately 3-fold that of Mediterranean countries (25-27); predominated by the epidemic growth of obesity in the United States (28). Currently, approximately 66% of the US population are classified as overweight (BMI >25 kg/m^2) and 33% obese (BMI >30 kg/m^2) (29). The components of the Mediterranean diet are fundamental to the lower prevalence of MetS (30). Although the Mediterranean diet is complex in nature, rich in fruits, vegetables, and whole-grains, the MUFA content of Mediterranean diets accounts for 16–29% of energy (4), with olive oil providing 15–30% of energy (8). Therefore, incorporating MUFA into Western dietary patterns, particularly at the expense of SFA, may target a reduction in risk for MetS and CVD.

3.4 MONOUNSATURATED FAT; STRUCTURE AND SOURCES

Monounsaturated fatty acids are classified as fatty acid chains containing one double bond. Monounsaturated fatty acids possess higher melting points than PUFA, which contain two or more double bonds. Both MUFA and PUFA are liquid at room

Table 3.1: Fatty acid composition of oils, nuts, seeds and fruit high in monounsaturated fat.

	Energy (kcal)	Total Fat (g)	SFA (g)	MUFA (g)	PUFA (g)	n-6 PUFA (g)	n-3 PUFA (g)
Vegetable Oil							
Almond oil	884	100	8.2	69.9	17.4	17.4	0.0
Apricot oil	884	100	6.3	60.0	29.3	29.3	0.0
Avocado oil	884	100	11.6	70.6	13.5	12.5	1.0
Canola oil	884	100	7.4	63.3	28.1	19.0	9.1
Hazelnut oil	884	100	7.4	78.0	10.2	10.1	0.0
Olive oil	884	100	13.8	73.0	10.5	9.8	0.7
High-oleic canola oil	884	100	6.5	72.0	17.1	14.5	2.6
High-oleic safflower oil	884	100	6.2	74.6	14.4	14.4	0.0
High-oleic sunflower oil	884	100	9.7	83.6	3.8	3.6	0.2
Mid-oleic sunflower oil	884	100	9.0	57.3	29.0	29.0	0.0
Nuts and seeds[1]							
Almonds	597	52.8	4.0	33.7	12.6	12.6	0.0
Cashews	574	46.4	9.2	27.3	7.8	7.7	0.2
Hazelnuts	646	62.4	4.5	46.6	8.4	8.4	0.1
Macadamia nuts	718	76.1	11.9	59.3	1.5	1.3	0.2
Mixed nuts	594	51.5	6.9	31.4	10.8	10.5	0.2
Peanuts	585	49.7	6.9	24.6	15.7	15.7	0.0
Peanut butter (smooth)	588	50.4	10.3	23.7	13.9	13.8	0.1
Pistachios	571	46.0	5.6	24.2	13.9	13.6	0.3
Pecans	710	74.3	6.3	44.0	20.6	19.6	1.0
Sesame seeds	565	48.0	6.7	18.1	21.0	20.7	0.4
Tahini (sesame butter)	595	53.8	7.5	20.3	23.6	23.1	0.4
Walnuts (black)	618	59.0	3.4	15.0	35.1	33.1	2.0
Walnuts (English)	654	65.2	6.1	8.9	47.2	38.1	9.1
Fruit							
Avacado, raw	160	14.7	2.1	9.8	1.8	1.7	0.1
Olives, ripe	481	10.7	1.4	7.9	0.9	0.8	0.1
Selected Animal Products							
Ground beef, regular 100g	259	16.3	5.7	7.5	0.6	0.5	0.1
Chicken breast, boneless skinless 100g	690	3.57	1.0	1.2	0.8	0.7	0.1
Egg, large whole 50 g	324	5.3	1.6	2.0	0.7	0.6	0.1
Fried bacon, 3 slices	529	9.6	3.2	4.3	1.1	1.0	0.1

[1]All nuts and seeds are dry roasted, without salted added; Adapted from USDA National Nutrient Database for Standard Reference (http://www.nal.usda.gov/fnic/foodcomp/search/). Accessed: August 18, 2009; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

temperature, whereas MUFA exist as semi-solids or solids when refrigerated. Conversely, SFA contain no double bonds and are solid at room temperature. Structurally, the common MUFA, palmitoleic acid (16:1n–7) and oleic acid (OA; 18:1n–9), are both *cis* isomers of MUFA. The major dietary *trans* isomer of MUFA is elaidic acid (*trans*18:1n–9). Oleic acid is the predominate MUFA in the diet, representing ~92% of *cis*MUFA (4). **Table 3.1** outlines the fatty acid content of food rich in MUFA. Of the MUFA-rich dietary oils, the most commonly consumed are olive and canola oil. Furthermore, over the last decade an increase has occurred in commercial production of high-OA modified dietary oils with increased stability for the use in food processing, as a replacement to dietary oils rich in SFA and trans fatty acids (TFA) (31).

3.5 CURRENT AND RECOMMENDED INTAKES OF DIETARY FATTY ACIDS

The total fat intake from Western diets is similar to that of the Mediterranean diet (**Table 3.2**), however, the type of dietary fat, specifically MUFA, differs vastly. In the US, MUFA intakes are 13–14% of energy, SFA intakes are in excess at 11–12% of energy, and PUFA intakes are ≤7% of energy, of which 85–89% of PUFA intakes are n-6 PUFA, principally linoleic acid (LA) (4,32,33). Conversely, the majority of total fat intake (33–40% of energy) in the Mediterranean diet is represented by MUFA, ranging from 16–29% of energy, with olive oil as the principal fat (4,34,35). The high-MUFA intake of the Mediterranean diet is at the expense of SFA, with intakes of SFA <8% of energy. Thus, an inverse relationship between the Mediterranean diet and coronary heart disease (CHD) risk has been substantiated in both epidemiological studies and randomized clinical trails (1).

Table 3.2: Current nutrient intakes in the Mediterranean and United States as compared to the recommended intakes outlined by health professional organizations.

	Current Intakes			Recommended Intakes					
	Mediterranean[1]	United States[1,2]	United States[3]	Dietary Guidelines[1]	ADA & DC[1]	NCEP ATP III[1]	USDA's MyPyramid[3]	NHLBI's DASH Eating Plan[3]	Harvard Health Eating Pyramid[3]
Total Fat	33–40%	33%	83–87 g	20–35%	20–35%	25–35%	64.8 g	41.1 g	69.0 g
SFA	< 8%	11–12%	28–30 g	< 10%	< 10%	< 7%	17.3 g	10.0 g	12.8 g
MUFA	16–29%	13–14%	32–33 g	--	≤ 25%	≤ 20%	23.5 g	15.0 g	24.8 g
PUFA	< 7%	< 7%	17–18 g	--	≤ 10%	≤ 10%	19.6 g	12.6 g	25.7 g

[1]Percent of daily energy

[2]Means of United States male and females (ages 20–59) from the NHANES,1999–2000

[3]Based on a ~2000 kcal/day

-- not specified, however supports recommendations by other expert organizations

ADA, American Dietetic Association; DASH, Dietary Approaches to Stop Hypertension; DC, Dietitians of Canada; MUFA, monounsaturated fatty acid; NCEP ATPIII, National Cholesterol Education Program Adult Treatment Panel III; NHLBI ,National Heart, Lung, and Blood Institutes; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; USDA, United States Department of Agriculture.

Cardiovascular disease, the clinical outcome of MetS, remains the leading cause of mortality in the Western population (29) and therefore, several professional health organizations have outlined target fatty acid intakes to reduce MetS, DM and CVD risk (**Table 3.2**) (9,36-41). Recently, the recommendations focus on dietary fat quality versus fat quantity with less emphasis on high-CHO diets. The American Diabetes Association (ADA) have modified their previous dietary recommendations for individuals with diabetes, which consisted of high-CHO intakes and restricted total fat to ≤ 30% of energy, with SFA, MUFA, and PUFA at ≤10% of energy (42). The ADA currently recommends that 60–70% of total calories in diets of those affected with DM-I and -II should be obtained from MUFA and CHO, emphasizing individualization of macronutrients by healthcare professionals (43,44). Moreover, the most recent position statement on dietary fatty acids from the ADA and Dietitians of Canada allows for total fat between 20–35% of energy, enhancing MUFA intakes up to 25% of energy (36). The upper limit of total fat at 35% of energy is to minimize intakes of SFA, as well as an upper limit of PUFA intake at 10% of energy due to inconclusive scientific evidence supporting higher intakes of LA for individuals with DM. Furthermore, the NCEP ATP III, endorsed by the American Heart Association (AHA), has recommended dietary guidelines for primary and secondary prevention of CHD with emphasis on monitoring total dietary fat and targeting a reduction in SFA. Similar to the ADA, earlier recommendations by the AHA, NCEP Step I and II diets, limited total fat intake to ≤30% and MUFA intake to ≤15% of energy (45). However, in 2001 the NCEP released revisions to the ATP III guidelines (9) increasing total fat to 25–35% of energy, allowing

a specific increase in MUFA intakes of up to 20% of energy, with a recommendation for

replacing CHO with unsaturated fats for individuals with DM or MetS. Of interest, the

current NCEP ATP III recommendations mirror the dietary fat profile of the

Mediterranean diet (**Table 3.2**) (4,34,35). Recently, the Joint FAO/WHO Expert

Consultation on Fats and Fatty Acids in Human Nutrition recommended that MUFA

intakes be 15–20% of energy, according to total fat intakes (46). Unlike other fatty acids

with a recommended limit, MUFA intakes should be determined by calculating the

difference, i.e. MUFA (% energy) = Total Fat (% of energy) – SFA (% of energy) –

PUFA (% of energy) – TFA (% of energy). Thus, MUFA intakes will range with respect

to the total fat and fatty acid composition of the diet.

As mentioned, olive oil is the predominant fat in the Mediterranean diet, and although

olive oil use is not as common in Western diets, MUFA-rich canola oil use in the US has

increased 5.5–fold from 1985 to 1994 (32). Canola oil, originally naturally bred from

rapeseed oil and low in erucic acid, has grown to become the third largest consumed

vegetable oil in the world and, next to soybean oil, canola oil is the second most

consumed vegetable oil in the US. Canola oil can be regarded as one of the most healthy

consumed vegetable oils with an attractive fatty acid profile distinctively low in SFA, and

rich in MUFA and n-3 PUFA α-linolenic acid (ALA) (**Table 3.1**). Consequently, in 2006

the United States Food and Drug Administration (FDA) authorized a qualified health

claim stating that canola oil (~19 grams daily) may reduce the risk of CHD due its

unsaturated fat content, recommending direct caloric replacement of dietary SFA with

canola oil (47). A recent dietary modeling study revealed that replacing common dietary

fats in the US with canola oil and canola-based spreads would increase the percentage of Americans complying with current dietary intake recommendations for fatty acids, namely SFA, MUFA, and ALA, but not for LA (48). More specifically, a 50% substitution of fats with canola oil would decrease SFA intakes by 4.7%, whereas a 100% substitution would decrease SFA and LA intakes by 9.4% and 44.9%, respectively, while increasing MUFA and ALA intakes by 27.6% and 73.0%, respectively. Based on the emphasis of increasing the intakes of MUFA in the diet, particularly at the expense of SFA, it is timely and appropriate to explore the efficacy of MUFA-rich diets in the prevention of MetS and CVD.

3.6 MONOUNSATURATED FAT AND BLOOD LIPIDS

Numerous randomized controlled trials have investigated the impact of dietary intervention on changes in circulating lipids (49-52). The NCEP ATP III guidelines have outlined risk factors that increase CHD risk over a 10-year period. Traditionally, elevated LDL-cholesterol (>100 mg/dL (2.6 mmol/L)) remains the strongest primary factor in predicting CHD and therefore is a primary target of therapy (53). However, as circulating TAG and HDL-cholesterol concentrations are critical risk factors in MetS, the TC:HDL-cholesterol ratio has been considered a more valuable marker in determining CHD risk (52). Although the hypolipidemic effect of reducing dietary SFA is well-known and remains the primary target of dietary intervention (54), the debate as to whether MUFA, PUFA or CHO should replace SFA in the diet continues.

3.6.1 Effects of Monounsaturated Fat Compared with Saturated Fat

Evidence from randomized controlled trials has substantiated the deleterious effects of dietary SFA on circulating lipids and lipoproteins (49-51). When MUFA isocalorically replace SFA in the diet there are improvements in TC:HDL-cholesterol ratio, namely associated with a decrease in serum LDL-cholesterol levels and preservation of HDL-cholesterol levels. Recently, attention has focused on the lipidemic effects of individual SFA, as stearic acid (STA, 18:0) is considered to have neutral or hypolipidemic effects on circulating lipids compared with other SFA, namely lauric (12:0), myristic (14:0) and palmitic (16:0) acids (52,55). Although only a few studies have directly compared OA to STA intakes, Hunter et al. (2010) collectively showed that when OA replaced STA, LDL-cholesterol levels decreased by 5–13% in 3 of 8 studies, however, had no effect in 5 other studies (55). HDL-cholesterol levels increased in one study between 5–7%, with no effect in 7 of 8 studies. Triglycerides decreased 20–37% in 2 studies; with no effect in 6 other studies. Finally, an estimated directional decrease in TC:HDL-cholesterol ratio was observed in 6 of the 8 studies when OA replaced STA. Overall compared to OA, STA tended to increase LDL-cholesterol and TAG levels, lower HDL-cholesterol levels, and resulted in an increase in the TC:HDL-cholesterol ratio. Thus, novel modified dietary oils with a high-OA content have been developed by agricultural and food industries to replace partially hydrogenated oils rich in TFA and SFA for use in food preparation, including frying, baking, and blending with other fats (31). However, as there are specific food applications that require a solid fat (i.e. shortenings and baked goods), a high-STA fat may provide an alternative to fat-containing TFA (55).

3.6.2 Dietary Monounsaturated Fat versus Carbohydrate for Replacement of Saturated Fat

The effects on CHD risk with substitution of SFA by other macronutrients continue to be a primary focus of public health agendas (14,52,56). Diets rich in CHO, PUFA and MUFA have been compared to those rich in SFA in assessing the ability of each dietary strategy to favourably alter plasma lipids. In studies conducted with healthy subjects comparing high-MUFA diets to high-CHO diets, those on high-MUFA diets showed significant reductions in TAG levels (57-59). Likewise, overweight and obese subjects (60), those with DM-II (5,61,62), and MetS (63) also benefited from the substitution of MUFA-rich diets, as compared to CHO-rich diets, in improving plasma TAG levels. One of the main cardioprotective activities of high-MUFA diets is the ability of MUFA to either preserve or increase HDL-cholesterol levels when compared to CHO-rich diets which mostly produce decreases in HDL-cholesterol levels (5,57,61,63,64). As compared to high-CHO diets, high-MUFA diets more favourably affect the TC:HDL-cholesterol ratio, emphasized by a reduction in LDL-cholesterol and TAG levels, while increasing HDL-cholesterol levels (52). Recently, Cao et al. (2009) conducted a meta-analysis of 30 controlled-feeding studies in subjects with and without diabetes, comparing moderate fat (MF) (30.2–50% of energy; mean MUFA intake 23.6% of energy) versus lower fat (higher CHO) diets (LF) (18.3–30.2 % of energy; mean MUFA intake 11.4% of energy) (65). In all subjects, reductions in LDL-cholesterol levels were similar between the MF and LF diets. However, the MF diet increased HDL-cholesterol levels (2.28 mg/dL; 95% CI, 1.66 to 2.90 mg/dL) and decreased TAG levels (–9.36 mg/dL; 95% CI, −12.16 to −6.08 mg/dL) versus the LF diet. Moreover, in subjects with

diabetes, a further decrease in TAG levels (–24.79 mg/dL) was observed after the MF diet, as well as a decrease in the TC:HDL-cholesterol ratio (–0.62) and non-HDL-cholesterol (–5.39%) versus the LF diet. The authors concluded that MF diets reduced predicted CHD risk by 6.37% in men and 9.34% in women, including subjects with diabetes, compared with the LF diet. Therefore, MUFA versus CHO replacement for SFA may be more beneficial for individuals predisposed to MetS or with DM-II (5,53).

3.6.3 Dietary Monounsaturated Fat versus Polyunsaturated Fat for Replacement of Saturated Fat

Comparison studies and reviews have also examined the action of PUFA-rich versus MUFA-rich diets on plasma lipid modulation (4,52,66-68). Evidence supports the notion that MUFA-rich diets have slightly less or comparable TC and LDL-cholesterol lowering effects to those of PUFA-rich diets. Whereas n-3 PUFA-rich diets may additionally reduce serum TAG (69), MUFA-rich diets have more favourable effects on HDL-cholesterol concentrations. The ability to effectively target an increase in plasma HDL-cholesterol is critical in patients with MetS, DM-II and the prevention of CVD (70,71). When PUFA-rich and MUFA-rich diets were compared for replacement of dietary SFA in healthy adult subjects, those consuming MUFA-rich diets demonstrated a preservation of HDL-cholesterol levels to a greater extent with only a 4% decrease in HDL-cholesterol levels compared to those consuming PUFA-rich diets, which decreased HDL-cholesterol levels by 14% (72). Thus, due to the preservation of HDL-cholesterol with MUFA-rich versus PUFA-rich diets, effects on the TC:HDL-cholesterol ratio where comparable when either MUFA or PUFA replaced dietary SFA (52,72).

3.7 DIETARY MONOUNSATURATED FAT AND BLOOD PRESSURE

Evidence from human clinical studies have shown that dietary MUFA either have neutral or hypotensive effects when compared to diets rich in CHO, n-6 or n-3 PUFA, notably reporting consistent reductions in blood pressure when MUFA are compared to SFA-rich diets (**Table 3.3**). A study comparing hypertensive subjects consuming MUFA-rich and PUFA-rich diets revealed that virgin olive oil high in OA resulted in significant decreases in total blood pressure (73). The hypotensive effect of MUFA also alleviated the need of anti-hypertensive drug therapy by 48%, whereas all subjects on a PUFA-rich diet required further drug therapy. In contrast, a study conducted by Mutanen et al. (1992) failed to observe hypotensive effects of either MUFA or PUFA-rich diets in normotensive subjects (74). Among the studies comparing MUFA and PUFA-rich diets, hypotensive benefits of MUFA-rich diets are observed in individuals predisposed to MetS in 2 clinical trials, whereas 4 of 5 clinical trials observed no difference between MUFA and PUFA diets in healthy individuals (**Table 3.3**).

The effects of MUFA versus CHO-rich diets on blood pressure were compared in a meta-analysis by Shah et al. (2007) (75). Of the 10 intervention trials assessed, MUFA-rich diets were associated with a slight reduction in blood pressure, specifically systolic blood pressure, compared to the CHO-rich diets. Similarly, in this review, 3 of 6 clinical trials observed hypotensive benefits with MUFA-rich diets compared to CHO-rich diets in individuals predisposed to MetS (**Table 3.3**). Muzio et al. (2007) compared consumption

Table 3.3: Human clinical trials investigating the effects of monounsaturated fat and hypertension.

Reference	Subject Characteristics	Study design/duration	Diets	Outcome
Individuals Predisposed to Metabolic Syndrome				

Gulseth et al. (2010) (126)	MetS subjects (n=486)	Randomized, parallel 12 weeks	MUFA
			  39% fat; 10% SFA, 19% MUFA, 7% PUFA
			SFA
			  40% fat; 18% SFA, 13% MUFA, 6% PUFA
			H-CHO
			  30% fat; 9% SFA, 12% MUFA, 6% PUFA
			H-CHO + n-3 PUFA
			  29% fat; 9% SFA, 11% MUFA, 6% PUFA, 1.6 g/d EPA+DHA
				No difference in systolic BP or diastolic BP between diets
				↓ pulse pressure with MUFA vs. SFA in men

Brehm et al. (2009) (95)	Overweight or obese with DM-II subjects (n=124)	Randomized, parallel 12 months	MUFA
			  38% fat; 14% MUFA
			H-CHO
			  28% fat; 8% MUFA
				No difference in diastolic BP between diets

Muzio et al. (2007) (76)	Hypercholesterolemic obese subjects with MetS (n=100)	Randomized 5 months	H-CHO
			  22% fat; 5% SFA, 14% MUFA, 3% PUFA
			MUFA
			  33% fat; 9% SFA, 21% MUFA, 4% PUFA
				↓ systolic BP and HR with MUFA vs. H-CHO

Appel et al. (2005) (77)	Pre-HT or HT (stage 1) subjects (n=164)	Randomized, crossover 6 weeks	H-CHO
			  27% fat; 6% SFA, 13% MUFA, 8% PUFA
			Protein
			  27% fat; 6% SFA, 13% MUFA, 8% PUFA
			MUFA
			  37% fat; 6% SFA, 21% MUFA, 10% PUFA
				↓ systolic & diastolic BP with MUFA & Protein vs. CHO in all subjects

Shah et al. (2005) (127)	DM-II subjects (n=41)	Randomized, crossover 6 weeks, then 14 weeks	H-CHO
			  30% fat; 10% SFA, 10% MUFA, 10% PUFA
			MUFA
			  45% fat; 10% SFA, 25% MUFA, 10% PUFA
				No difference in BP between diets at 6 weeks
				↑ diastolic BP and heart rate at 14 weeks with H-CHO vs MUFA

Piers et al. (2003) (107)	Overweight or obese men (n=8)	Randomized, crossover 4 weeks	SFA
			  40% fat; 24% SFA, 13% MUFA, 3% PUFA
			MUFA
			  40% fat; 11% SFA, 22% MUFA, 7% PUFA
				↓ mean arterial pressure & diastolic BP with MUFA vs. SFA

(Table 3.3 continued on the following page)

Ferrara et al. (2000) (73)	HT subjects (n=23)	Randomized, crossover 6 months	MUFA
			  27% fat; 6% SFA, 17% MUFA, 4% PUFA
			PUFA
			  27% fat; 6% SFA, 11% MUFA, 11% PUFA
				↓ systolic & diastolic BP with MUFA vs. PUFA
				↓ HT drug treatment with MUFA but not PUFA

Thomsen et al. (1995) (128)	DM-II subjects (n=16)	Randomized, crossover 3 weeks	MUFA
			  49% fat; 10% SFA, 30% MUFA, 7% PUFA
			PUFA
			  49% fat; 9% SFA, 10% MUFA, 27% PUFA
				↓ arterial BP with MUFA vs. PUFA

Walker et al. (1995) (129)	DM-II subjects (n=24)	Randomized, crossover 12 weeks	H-CHO
			  23% fat; 9% SFA, 10% MUFA, 4% PUFA
			MUFA
			  36% fat; 11% SFA, 20% MUFA, 5% PUFA
				No differences in BP between diets

Healthy Individuals				

Rasmussen et al. (2006) (130)	Healthy subjects (n=162)	Randomized, parallel 3 months	SFA
			  37% fat; 17% SFA, 14% MUFA, 6% PUFA
			MUFA
			  37% fat; 8% SFA, 23% MUFA, 6% PUFA
			Further randomization with n-3 PUFA (fish oil): 3.6 g/d
				↓ systolic & diastolic BP with MUFA from baseline
				↔ BP with SFA from baseline
				↓ diastolic BP with MUFA vs. SFA
				↔ BP with addition of fish oil supplementation

Aro et al. (1998) (131)	Healthy subjects (n=87)	Randomized, parallel 8 weeks	Control
			  20% fat; 8% SFA, 8% MUFA, 3% PUFA
			MUFA
			  26% fat; 7% SFA, 14% MUFA, 3% PUFA
			PUFA
			  26% fat; 8% SFA, 8% MUFA, 8% PUFA
				No differences in BP between diets

Lahoz et al. (1997) (132)	Healthy subjects (n=42)	4 consecutive diet phases 5 weeks	SFA
			  35% fat; 17% SFA, 14% MUFA, 4% PUFA
			MUFA
			  35% fat; 9% SFA, 21% MUFA, 4% PUFA
			n-6 PUFA
			  35% fat; 10% SFA, 12% MUFA, 13% PUFA
			n-3 PUFA
			  35% fat; 9%SFA, 12% MUFA, 13% PUFA
			(1.6% n-3 PUFA)
				↓ systolic BP with MUFA vs. SFA & n-6 PUFA

(Table 3.3 continued)

Uusitupa et al. (1994) (133)	Healthy subjects (n=159)	Randomized, parallel 6 months	SFA
			  35% fat; 14:19:4 SFA:MUFA:PUFA
			AHA diet
			  32% fat; 10:8:8 SFA:MUFA:PUFA
			MUFA
			  34% fat; 11:11:5 SFA:MUFA:PUFA
			Low-fat
			  30% fat; 12:8:3 SFA:MUFA:PUFA
				↓ systolic BP with AHA only
				↑ BP with SFA in men only

Mutanen et al. (1992) (74)	Healthy subjects (n=59)	Randomized, crossover 3.5 weeks	MUFA
			  38% fat; 13% PUFA
			PUFA
			  38% fat; 16% MUFA
				No differences in BP between diets

Mensink et al. (1990) (134)	Healthy subjects (n=58)	Randomized, parallel 5 weeks	MUFA
			  36% fat; 13% SFA, 15% MUFA, 8% PUFA
			PUFA
			  36% fat; 13% SFA, 11% MUFA, 13% PUFA
				No differences in BP between diets

Mensink et al. (1988) (135)	Healthy subjects (n=47)	Randomized, parallel 5 weeks	H-CHO
			  22% fat; 7% SFA, 9% MUFA, 5% PUFA
			MUFA
			  41% fat; 10% SFA, 24% MUFA, 5% PUFA
				No differences in BP between diets

Direction of effect on biomarkers of hypertension (↑ increased; ↓ decreased; ↔ no effect).

AHA, American Heart Association; BP, blood pressure; CHO, carbohydrate; DM-II, Diabetes Mellitus-II; H-CHO, high-carbohydrate; HT, hypertensive; HR, heart rate; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; vs, versus.

of high-MUFA diets to high-CHO diets in 100 obese subjects with MetS over 5 months

(76). At study cessation, while both groups showed significant reductions in all

components of MetS, only the diet high in MUFA produced a significantly lower systolic

blood pressure, as well as lowered heart rate. In the large randomized, crossover Omni

Heart Trial, 164 subjects with prehypertension or stage-1 hypertension consumed diets

varying in dietary fats for 6 weeks to determine their subsequent risk of hypertension

(77). Compared to a high-CHO diet, consumption of high protein and MUFA diets

produced significant reductions in systolic blood pressure and additional benefits in TAG

and HDL-cholesterol levels.

Considering prospective cohort studies, the SUN (Seguimiento Universidad de Navarra)

study of nearly seven thousand subjects reported that high intake of olive oil for an

average of 28.5 months was associated with a decrease in the incidence of hypertension

in men, but not women (78). Similarly, in the Greek EPIC (European Prospective

Investigation into Cancer and Nutrition) study, olive oil consumption was a primary

dietary factor in the Mediterranean diet preventing hypertension (79). More specifically,

a reduction in both systolic and diastolic blood pressure was noted with olive oil

consumption, even after controlling for vegetable intake. Alongside, there was an inverse

relationship between blood MUFA/SFA ratio and arterial blood pressure. Indeed, olive

oil in Mediterranean diets has potent hypotensive effects (80). However, the OA content

of olive oil, independent of its other components, has been shown to be directly

associated with a reduction in blood pressure (81). As such, strong support can be

obtained from clinical trials of the blood pressure lowering effects of MUFA-rich diets in

both normotensive and hypertensive individuals.

3.8 MONOUNSATURATED FATS, INSULIN RESISTANCE AND DIABETES MELLITUS-II

With the rising prevalence of DM worldwide (82), MUFA have gained attention for their ability to regulate glycemic response and improve insulin sensitivity. Similar to the detrimental effects on circulating lipids, SFA have been shown to impair glycemic control and insulin sensitivity (12), specifically in skeletal muscle cells (83). Therefore, clinical trials replacing dietary SFA with MUFA have noted improvements in insulin sensitivity and glycemic response in individuals predisposed to insulin resistance (84-87), as well as healthy people (88-92) (**Table 3.4**). The KANWU (Kuopio, Aarhus, Naples, Wollongong and Uppsala) Study of 162 healthy subjects reported a reduction in insulin sensitivity following consumption of a SFA-rich diet for 3 months, and that replacement of SFA with a MUFA-rich diet improved insulin sensitivity (90). More specifically, when total daily fat intake was < 37% of energy, an 8.8% increase in insulin sensitivity was observed with the MUFA-rich diet, whereas the SFA-rich diet decreased insulin sensitivity by 12.5%. However, these effects were not observed when total daily fat intakes exceeded 37% of energy. In the development of DM-II, pancreatic β-cells that secrete insulin to counteract postprandial rises in blood glucose become overwhelmed and as a result, fail to effectively provide the necessary insulin to regulate glucose levels (93). Recently, MUFA was shown to have a direct action on β-cell function and lower insulin resistance in a study of 14 healthy men using a randomized, crossover design (88). Data revealed that MUFA improved insulin sensitivity and β-cell function when

compared with SFA. With the incremental substitution of MUFA for SFA, direct linear decreases in insulin resistance were observed.

As a replacement for dietary SFA, high-MUFA diets have been compared to high-CHO diets for preventing insulin resistance and DM-II risk (3,5). An earlier meta-analysis of 10 randomized controlled trials by Garg (1998), assessing the effect of high-MUFA diets in patients with either DM-I or DM-II, reported improvements in glycemic control, as well as lipoprotein profiles, as compared to high-CHO diets (5). Ros (2003) reviewed the evidence on dietary MUFA and metabolic control in DM-II following the comprehensive meta-analysis by Garg (1998) and observed similar beneficial metabolic effects of MUFA-rich diets (3). Following these analyses, Paniagua et al. (2007) demonstrated that compared to SFA or CHO-rich diets, insulin resistant subjects consuming a MUFA-rich diet exhibited improvements in insulin sensitivity, as well as other hormonal and metabolic parameters (85,94). Similarly, when compared to high-CHO and high-SFA diets, diets high in MUFA have been shown to significantly decrease fasting glucose by 3% and insulin by 9.4%, and improve insulin sensitivity by 12.1% (84). In contrast, clinical trials with healthy subjects have observed no difference between MUFA-rich and CHO-rich diets in markers of glucose-insulin homeostasis (89,95,96). However, due to other metabolic abnormalities associated with high-CHO diets, such as the deleterious effects on plasma TAG and HDL-cholesterol levels, (11) high-MUFA diets may be more beneficial for ameliorating the risk of DM-II. Taken together, evidence from prospective cohort studies have reported that dietary MUFA are not associated with increased risk of DM-II in men (97) or women (98) after adjustment for other dietary fats, age and BMI.

Table 3.4: Human clinical trials investigating the effects of monounsaturated fat on glucose and insulin responses.

Reference	Subject Characteristics	Study design/duration	Diets	Outcome
Individuals Predisposed to Metabolic Syndrome				
Brehm et al. (2009) (95)	Obese & overweight subjects with DM-II (n=124)	Randomized 1 year	H-CHO 28% fat; 7-9% MUFA MUFA 38% fat; 14-15% MUFA	No differences in glucose and insulin sensitivity between groups
Due et al. (2008) (84)	Nondiabetic obese subjects (n=46)	Randomized, parallel 6 months	SFA 32% fat; 15% SFA, 10% MUFA, 4% PUFA MUFA 39% fat; 7% SFA, 20% MUFA, 8% PUFA Low-fat 23% fat; 8% SFA, 8% MUFA, 5% PUFA	↓ fasting glucose, insulin, and insulin resistance score with MUFA vs. other diets ↓ HOMA-IR wtih MUFA vs. other diets
Paniagua et al. (2007) (85)	Obese DM-II subjects (n=11)	Randomized, crossover 28 days	SFA 38% fat; 23% SFA, 9% MUFA, 6% PUFA MUFA 38% fat; 9% SFA, 23% MUFA, 6% PUFA H-CHO 20% fat; 6% SFA, 8% MUFA, 6% PUFA	↓ fasting glucose with MUFA and H-CHO vs. SFA ↑ insulin sensitivity (↓ HOMA-IR) with MUFA vs. other diets ↑ postprandial GLP-1 with MUFA vs. H-CHO
Shah et al. (2007) (86)	DM-II subjects (n=11)	Randomized, crossover 15 days	SFA 50% fat; 26% SFA, 20% MUFA, 5% PUFA MUFA 50% fat; 7% SFA, 39% MUFA, 5% PUFA n-6 PUFA 50% fat; 4% SFA, 8% MUFA, 39% PUFA n-3 PUFA 50% fat; 9% SFA, 15% MUFA, 44% PUFA	↓ postprandial insulin response with MUFA and n-3 PUFA vs. SFA and n-6 PUFA ↔ postprandial glucose response between diets
Vega-Lopez et al. (2006) (136)	Hyperlipidemic subjects (n=15)	Randomized, crossover 5 weeks	TFA 30%fat;9% SFA,10%MUFA,8%PUFA,4% TFA SFA 30% fat;15%SFA,11%MUFA,4%PUFA MUFA 32%fat;6%SFA,15%MUFA, 9%PUFA PUFA 28%fat;7%SFA,8%MUFA,12%PUFA	No difference in fasting insulin, fasting glucose, or HOMA between diets

(Table 3.4 continued on the following page)

(Table 3.4 continued)

Gerhard et al. (2004) (137)	DM-II subjects (n=11)	Randomized, crossover 6 weeks	Low-fat
			  20% fat; 4% SFA, 8% MUFA, 6% PUFA
			MUFA
			  40% fat; 6% SFA, 25% MUFA, 6% PUFA
				No difference in fasting glucose, glycemic control or insulin sensitivity between diets

Thomsen et al. (2003) (138)	Overweight subjects with DM-II (n=12)	Randomized, crossover ≥ 1 week	SFA MUFA	↔ glucose or insulin responses between diets ↑ GLP-1 responses with MUFA vs. SFA

Lovejoy et al. (2002) (139)	Healthy, normal and overweight subjects (n=25)	Randomized, crossover 4 weeks	SFA
			  28% fat; 9% SFA
			MUFA
			  28% fat; 9% MUFA
			TFA
			  28% fat; 9% TFA
				↔ insulin sensitivity between diets
				↓ insulin sensitivity with SFA vs. MUFA for overweight subjects

Lauszus et al. (2001) (140)	Pregnant women with gestational DM-II (n=27)	Randomized From 33rd gestational week for 5 weeks	H-CHO
			  30% fat; 13% SFA, 11% MUFA, 6% PUFA
			MUFA
			  37% fat; 10% SFA, 22% MUFA, 5% PUFA
				No difference in fasting insulin and glucose, insulin sensitivity between diets

Rodriguez-Villar et al. (2000) (141)	DM-II subjects (n=12)	Randomized, crossover 12 weeks	CHO
			  29% fat; 6% SFA, 12% MUFA, 5% PUFA
			MUFA
			  40% fat; 8% MUFA, 25% MUFA, 5% PUFA
				No differences in fasting or postprandial glucose and insulin between diets

Luscombe et al. (1999) (142)	DM-II subjects (n=21)	Randomized, crossover 4 weeks	CHO (high-GI diet) 21% fat; 8% SFA, 7% MUFA, 4% PUFA CHO (low-GI diet) 23% fat; 8% SFA, 7% MUFA, 4% PUFA MUFA (high-GI diet) 35% fat; 8% SFA, 18% MUFA, 7% PUFA	No difference in fasting insulin and glucose between diets

Christiansen et al. (1997) (87)	DM-II and obese subjects (n=16)	Randomized, crossover 6 weeks	SFA
			  30% fat; 20% SFA, 5% MUFA, 5% PUFA
			MUFA
			  30% fat; 5% SFA, 20% MUFA, 5% PUFA
			TFA
			  30% fat; 5% SFA, 20% TFA, 5% PUFA
				↔ glycemic control or postprandial glycemic response between diets
				↓ postprandial insulinemia with MUFA vs. SFA and TFA

(Table 3.4 continued on the following page)

Sarkkinen et al. (1996) (143)	IGM subjects (n=22)	Randomized 8 weeks	SFA
			  37% fat; 18%SFA, 11% MUFA, 5% PUFA
			MUFA
			  40% fat; 11% SFA, 19% MUFA, 8% PUFA
			PUFA
			  34% fat; 11% SFA, 10% MUFA, 10% PUFA
				↓ fasting glucose with MUFA vs. SFA
				↔ fasting glucose with PUFA vs. SFA
				↑ glucose effectiveness with MUFA vs. PUFA

Parillo et al. (1992) (144)	DM-II subjects (n=10)	Randomized 15 days	H-CHO
			  20% fat
			MUFA
			  40% fat
				↓ fasting glucose and insulin with MUFA vs. H-CHO

Bonanome et al. (1991) (145)	DM-II subjects (n=19)	Consecutive diets 2 months	H-CHO
			  25% fat; 10% SFA, 10% MUFA, 5% PUFA
			MUFA
			  40% fat; 10% SFA, 25% MUFA, 5% PUFA
				↔ fasting glucose or insulin response between diets

Garg et al. (1988) (61)	DM-II subjects (n=10)	Randomized 28 days	H-CHO
			  25% fat
			MUFA
			  50% fat; 33% MUFA
				↓ plasma glucose and insulin requirements with MUFA vs. H-CHO

Healthy Individuals				

Lopez et al. (2008) (88)	Healthy men (n=14)	Randomized, crossover Single meal 8 hour	NCEP Step-I diet
			  29% fat
			Butter diet
			  38% fat; 0.48 MUFA:SFA
			High-palmitic sunflower oil diet
			  38% fat; 2.42 MUFA:SFA
			Refined olive oil diet
			  38% fat; 5.43 MUFA:SFA
			Vegetables/fish oil diet
			  38% fat; 7.08 MUFA:SFA
				↑ postprandial β-cell function and insulin sensitivity with an increase in the MUFA to SFA ratio of dietary fats

Perez-Jimenez et al. (2001) (89)	Healthy subjects (n=59)	Randomized, crossover 28 days	SFA
			  20% SFA, 12% MUFA, 6% PUFA
			H-CHO
			  28% fat; 10% SFA, 12% MUFA, 6% PUFA
			MUFA
			  38% fat; 10% SFA, 22% MUFA, 6% PUFA
				↑ fasting insulin and mean glucose for the SFA vs. MUFA and H-CHO
				Improvement in insulin sensitivity with MUFA and H-CHO vs. SFA

(Table 3.4 continued)

Vessby et al. (2001) (90)	Healthy subjects (n=162)	Randomized 3 months	SFA 37% fat; 18% SFA, 13% MUFA, 5% PUFA MUFA 37% fat; 10% SFA, 21% MUFA, 5% PUFA	↓ insulin sensitivity with SFA vs. MUFA ↔ insulin secretion between diets
Salas et al. (1999) (91)	Healthy men (n=41)	Consecutive diets 4 weeks	SFA 38% fat; 20% SFA MUFA 38% fat; 22% MUFA NCEP Step-I 47% CHO, 28% fat	↑ insulin on SFA diet ↓ fasting glucose and insulin with MUFA vs. NCEP Step-I diet
Thomsen et al. (1999) (96)	Healthy subjects (n=16)	Randomized, crossover 4 weeks	H-CHO 28% fat; 9% SFA, 8% MUFA, 7% PUFA MUFA 42% fat; 9% SFA, 24% MUFA, 6% PUFA	↔ insulin sensitivity between diets ↔ fasting blood glucose between diets
Thomsen et al. (1999) (146)	Healthy subjects (n=10)	Randomized Single meal 8 hours	CHO SFA MUFA	↔ postprandial glucose or insulin response between diets ↑ GLP-1 and GIP responses with MUFA vs. SFA
Louheranta et al. (1998) (147)	Healthy women (n=15)	Randomized, crossover 4 weeks	SFA 39%fat; 19% SFA, 12% MUFA, 6% PUFA MUFA 41% fat; 13% SFA, 19% MUFA, 6% PUFA	↔ glucose or insulin responses between diets ↔ insulin sensitivity between diets
Joannic et al. (1997) (148)	Healthy men (n=8)	Randomized, crossover Single meal 3 hour	MUFA 47% fat; 4.3 MUFA:PUFA PUFA 47% fat; 0.4 MUFA:PUFA	↓ postprandial glucose and insulin responses with PUFA vs. MUFA
Uusitupa et al. (1994) (92)	Healthy subjects (n=10)	Randomized, crossover 3 weeks	SFA 39% fat; 20% SFA, 12% MUFA, 4% PUFA MUFA 40% fat; 9% SFA, 19% MUFA, 10% PUFA	↓ glucose AUC with MUFA vs. SFA ↑ glucose disappearance rate with MUFA vs. SFA

Direction of effect on biomarkers of glucose and insulin responses (↑ increased; ↓ decreased; ↔ no effect).

AUC, area under curve; CHO, carbohydrate; DM-II, diabetes mellitus-II; GI, glycemic index; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; H-CHO, high-carbohydrate; HOMA-IR, homeostasis model assessment of insulin resistance; IGM, irregular glucose metabolism; MUFA, monounsaturated fatty acid; NCEP, National Cholesterol Education Program; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TFA, trans fatty acid; vs, versus.

3.9 MONOUNSATURATED FAT IN WEIGHT MAINTENANCE AND OBESITY

There is a perception that fat, rich in calories as compared to CHO or protein, is associated with body weight gain leading to obesity (99). However, a strong argument also exists that dietary fat is not the primary cause of the high prevalence of obesity (100,101). Moreover, fat quality may have a stronger correlation to weight gain than fat quantity (102). Considering fat quality and specific effects of dietary fatty acids for risk of obesity, evidence from prospective cohort studies have reported that MUFA intake is not associated with increases in waist circumference or body weight gain (102, 103). In the Health Professionals Study of 16,587 men over a 9-year period, replacement of 2% energy of PUFA or CHO with MUFA was not associated with any change in waist circumference, whereas replacement with TFA or SFA led to an increase (103). Similarly, in the Nurses' Health Study, consumption of MUFA, as well as PUFA, was not associated with an increase in body weight, while TFA and SFA positively correlated with weight gain after 8 years (102). Large prospective cohort studies in the Mediterranean region have revealed that high intakes of olive oil (104) or nuts (105), both rich sources of MUFA, or adherence to a Mediterranean diet (106) were not associated with an increase in weight or risk of obesity over the longer term (104,105).

With respect to human clinical trials, Paniagua et al. (2007) have demonstrated that compared to CHO-rich diets, insulin resistant subjects consuming a MUFA-rich diet showed significantly increased fat oxidation rates and decreased abdomen-to-leg adipose ratios, thus preventing central body fat distribution (94). This finding has important implications for those at risk for MetS since the increase in central adiposity was

associated with a reduction in adiponectin expression and insulin sensitivity following the CHO-rich diet as compared to the MUFA-rich diet. An inverse relationship has been shown between circulating adiponectin levels and body fat percentage as well as central body fat accumulation, specifically visceral adiposity. Similarly, Piers et al. (2003) substituted a SFA-rich diet with MUFA for 4 weeks in 8 overweight and obese men using a randomized crossover design to determine the effects on body weight and composition (107). Assessment of body composition by dual energy x-ray absorptiometry (DEXA) revealed a significant decrease in body mass (-2.1 ± 0.4 kg; $P = 0.0015$) and fat mass (-2.6 ± 0.6 kg; $P = 0.0034$) following the MUFA compared to the SFA-rich diet, albeit no differences in total energy or fat intake were noted between diets. Furthermore, the changes in body mass and fat mass were accompanied with a decrease in waist-to-hip ratio after the MUFA-rich versus the SFA-rich diets. The favorable modifications in body composition and amelioration of weight gain after consumption of MUFA compared to SFA have also been observed in healthy subjects (108).

Of interest and as extensively reviewed by Bergouignan et al. (2009) (83), MUFA is the primary fat composing adipose tissue, however, there appears to be no direct relation between MUFA intake and MUFA levels in adipose. Rather SFA intake seems to be more closely associated with endogenous MUFA levels (109,110). Bergouignan et al. (2009) hypothesized that *in vivo* desaturation of SFA may be related to an increase in MUFA versus SFA in adipose tissue (83). Furthermore, OA preferentially accumulates in subcutaneous fat versus visceral fat, whereas the reverse exists with palmitate (111,112). Thus, since a direct correlation exists between visceral fat and risk factors for metabolic

syndrome (113), OA concentrating in subcutaneous fat versus visceral fat may be less atherogenic. Moreover, dietary MUFA may be preferentially oxidized as compared to other dietary fatty acids, as the degree of fatty acid chain length and unsaturation may contribute to the partitioning of dietary fat to energy expenditure versus energy storage (108,114-117). Furthermore, the metabolism of dietary fat stimulates behavioral changes in food intake preference (118). Indeed, evidence suggests that different dietary fats may elicit varying effects on satiety and total energy intake (119). Taken together, dietary MUFA consumption is associated with maintenance of body weight and favorable shifts in reducing central body fat adiposity, potentially ameliorating obesity risk.

3.10 MONOUNSATURATED FATS AND CARDIOVASCULAR RISK; EPIDEMIOLOGICAL EVIDENCE

As effects on risk markers may not directly translate into effects on clinical outcomes of disease, it is thus critical to assess effects of dietary MUFA on the primary clinical endpoint of MetS, that is CVD morbidity and mortality. Randomized controlled trials are considered the gold standard for evaluating the causal relationship between dietary intervention and chronic disease endpoints in humans; however, to date no randomized controlled trials have investigated dietary MUFA on CVD morbidity and/or mortality as the clinical endpoint (1). Consequently, Rudel and colleagues have challenged the cardioprotective effects MUFA, observing equal coronary artery atherosclerotic effects between dietary MUFA and SFA in nonhuman primates (120). However, it is acknowledged that results from experimental animal models may not always extrapolate to humans. Considering the substantial evidence presently reviewed supporting the

beneficial effects of dietary MUFA on risk factors for MetS and CVD, additional

evidence is needed to uncover the discrepancy between human epidemiological evidence

and experimental animal models. The following literature discusses the evidence from

ecological and prospective cohort studies on effects of MUFA and CVD risk.

3.10.1 Ecological Studies

In a landmark epidemiological trial of 11,579 men aged 40–59 in the Seven Countries

study, Keys and colleagues presented important data revealing that areas consuming a

Mediterranean diet rich in OA from olive oil, even though higher in total fat (33–40% of

energy), exhibited lower incidence of CHD mortality (8). Indeed, in this 15-year follow-

up trial, data continued to emphasize the strong inverse relationship between dietary

MUFA, as well as the ratio of dietary MUFA to SFA, and incidence of CHD mortality.

Conversely, Hegsted and Ausman (1988) reported a positive correlation between dietary

MUFA and CHD mortality in men aged 35–74 from 18 countries (121). It is important,

however, to note the authors emphasized a rather high correlation between MUFA and

SFA intakes and stated that SFA as a confounding variable compromised conclusions

linking dietary MUFA with increase risk of CHD.

3.10.2 Prospective Cohort Studies

Large prospective cohort studies are considered to be the strongest source of evidence of

the observational studies. Recently, a systematic review of 507 prospective cohort studies

confirmed the relationship between a Mediterranean diet and decreased risk of CHD

(RR=0.66; 95% CI, 0.57–0.75), evidence that was further confirmed as effective through

pooled analysis of 94 randomized control trials (1). Of interest, analysis of the

prospective cohort studies revealed strong evidence of an inverse relationship between

dietary MUFA and CHD risk (RR=0.81; 95% CI, 0.68–0.93). Conversely, Mente et al.

(2009) also identified that consumption of foods high in TFA and glycemic load were

attributed to increased CHD risk (RR=1.32; 95% CI, 1.16–1.48; and RR=1.33; 95% CI,

1.13–1.52, respectively). In a 14-year follow-up of 80,082 women in the Nurses' Health

Study, a 5% increase in energy intake from MUFA was associated with a relative risk of

CHD of 0.81 (95% CI, 0.65–1.00) (122). Furthermore, it was estimated that a 5% or 2%

energy replacement of SFA or TFA with MUFA decreased risk of CHD by

approximately 30% and 50%, respectively, whereas a 5% energy replacement of MUFA

with CHO increased risk of CHD by approximately 25%. Results of the Finnish ATBC

(Alpha-Tocopherol, Beta-Carotene) Cancer Prevention Study revealed that after

adjustment for vitamin E, C, and β-carotene intakes, an inverse association existed

between MUFA intakes and CHD mortality (RR between the extreme quintiles=0.73;

95% CI, 0.56–0.95) (123). Conversely, a pooled analysis of 11 American and European

cohort studies conducted by Jakobsen et al. (2009) failed to identify a causal link between

MUFA intake and decreased CHD risk (14). These authors reported that a 5% energy

substitution of MUFA for SFA resulted in a hazard ratio of 1.19 (95% CI, 1.00–1.42) for

CHD events and 1.01 (95% CI, 0.73–1.41) for CHD deaths. The authors, however,

discussed that the association of MUFA intakes with CHD risk may be confounded by

incomplete adjustments for TFA intakes, as MUFA intakes in Westernized diets are

primarily from meat, dairy and hydrogenated oils (124). Moreover, data from the Nurses'

Health Study reported a strong correlation between MUFA intakes and SFA ($r = 0.81$)

and TFA ($r = 0.55$) (122). Taken together, observational evidence supports dietary MUFA for reduction of CVD risk, however, results from large randomized controlled trials are crucial to substantiate the cardioprotective effects of dietary MUFA.

3.11 CONCLUSION

As dietary intervention remains the primary strategy for the prevention of CVD risk, professional organizations continue to ascertain the optimal fatty acid profile for population intake recommendations. This critical assessment of randomized controlled trials demonstrates that dietary MUFA prevent or ameliorate MetS and CVD risk by favourably modulating blood lipids, blood pressure and insulin sensitivity. Moreover, MUFA preferential oxidation and metabolism influence body composition and potentially ameliorate the risk of obesity (**Figure 3.1**). Considering dietary replacement of SFA, as compared to CHO, MUFA are effective at preserving HDL-cholesterol levels, lowering TAG levels, and improving insulin sensitivity; benefits which are especially important in individuals with MetS and DM. As compared to PUFA, MUFA have slightly less or comparable plasma LDL-cholesterol and TC lowering effects, however, ameliorate reductions in HDL-cholesterol levels, and potentially provide hypotensive effects. The majority of epidemiological data favour the cardioprotective activity of dietary MUFA. More specifically, strong evidence from prospective cohort studies suggests that dietary MUFA are associated with a 20% reduced risk in CHD events (1). It has also been well established that the intake of a Mediterranean diet rich in MUFA contributes to reducing CHD in both healthy adults and those with established chronic disease.
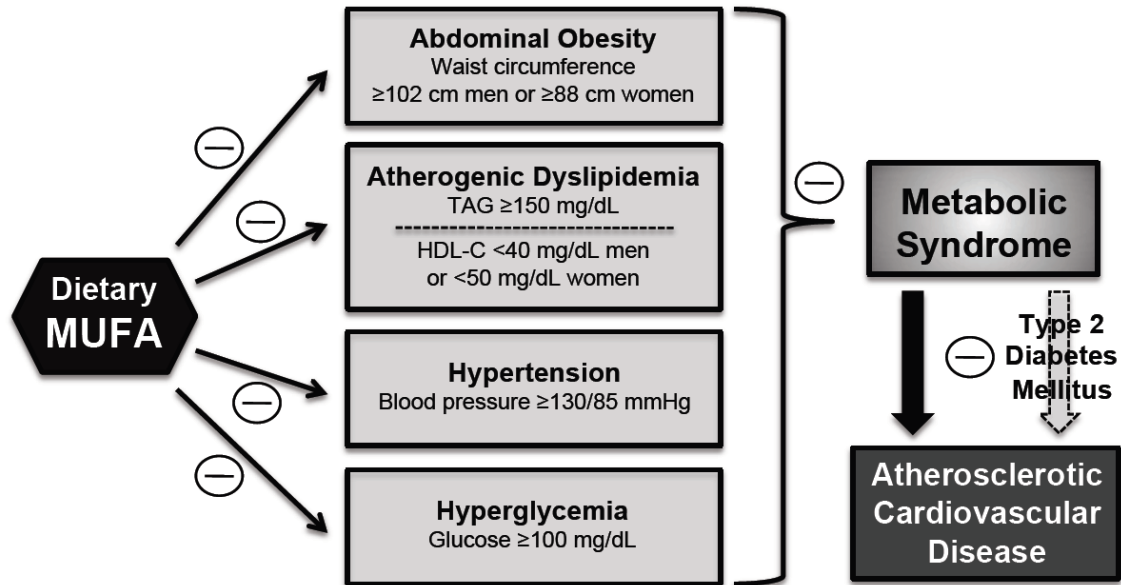
Figure 3.1: Dietary monounsaturated fats for the prevention of metabolic syndrome and atherosclerotic cardiovascular disease risk.

In North America, where consumption of SFA and TFA are in excess, a dietary movement is occurring to reduce the content of these deleterious fats from commercial production of foods. With the escalating use of MUFA-rich canola oil, replacing common dietary fats with canola oil and canola-based spreads would increase the percentage of North Americans complying with current dietary intake recommendations for fatty acids (48). Consumer awareness of the health implication of dietary fats is increasing (125) and there is a demand for modified dietary oils with a high-OA content for the use in cooking and food preparation in replace of partially hydrogenated oils rich in TFA and SFA (31). Novel dietary oils rich in OA with enhanced oxidative stability, such as high-oleic canola oil, provide an attractive healthful alternative to increase dietary MUFA and reduce SFA in commercial food use. With epidemiological and human clinical research substantiating the cardioprotective value of dietary MUFA, increasing population consumption of MUFA, specifically as a substitute for SFA, will embark beneficial implication for MetS, CVD and overall health.

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LGG and SHJ conducted the literature search and paraphrasing of research articles utilized in the manuscript. LGG wrote the manuscript and all authors contributed to revisions of the manuscript and reviewed the final version. The authors declare no conflict of interest.

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CHAPTER IV

MANUSCRIPT 3:

HIGH-OLEIC CANOLA AND FLAXSEED OILS MODULATE SERUM LIPIDS AND INFLAMMATORY BIOMARKERS IN HYPERCHOLESTEROLEMIC SUBJECTS

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4.1 ABSTRACT

Recently, novel dietary oils with modified fatty acid profiles have been manufactured to improve fatty acid intakes and reduce CVD risk. Our objective was to evaluate the efficacy of novel high-oleic canola oil (HOCO), alone or blended with flaxseed oil (FXCO), on circulating lipids and inflammatory biomarkers versus a typical Western diet (WD). Using a randomised, controlled, crossover trial, thirty-six hypercholesterolemic subjects consumed three isoenergetic diets for 28 days each containing ~36% of energy from fat, of which 70% was provided by HOCO, FXCO, or WD. Dietary fat content of SFA, MUFA, PUFA omega-6, and omega-3 was 6, 23, 5, 1% of energy for HOCO; 6, 16, 5, 7.5% of energy for FXCO; and 11.5, 16, 6, 0.5% of energy for WD. After 28 days, compared with WD, LDL-cholesterol was reduced 15.1% ($P < 0.001$) with FXCO and 7.4% ($P < 0.001$) with HOCO. Total cholesterol (TC) was reduced 11% ($P < 0.001$) with FXCO and 3.5% ($P = 0.002$) with HOCO compared with WD. Endpoint TC differed between FXCO and HOCO ($P < 0.05$). FXCO consumption reduced HDL-cholesterol 8.5% ($P < 0.001$) and LDL:HDL ratio by 7.5% ($P = 0.008$) versus WD. FXCO significantly decreased E-selectin concentration compared with WD ($P = 0.02$). No differences were observed in inflammatory markers after HOCO compared with WD. In conclusion, consumption of novel high-oleic canola oil alone or blended with flaxseed oil are cardioprotective through lipid lowering effects. The incorporation of flaxseed oil may also target inflammation by reducing plasma E-selectin.

4.2 INTRODUCTION

Considerable interest has focused on the influence of dietary fatty acids on cardiovascular disease (CVD) risk (1), with attention centered on the value of dietary fat quality (2-4). Evidence from prospective cohort studies and controlled clinical trials support the use of dietary unsaturated fatty acids for the reduction of CVD risk factors (1-3,5). Therefore, dietary guidelines with a focus on cardiovascular health have recommended replacing SFA intakes with unsaturated fats (6). Increased consumption of novel dietary oils rich in MUFA and alpha-linolenic acid (ALA) may improve the fatty acid imbalance typical of modern Western diets, high in SFA and the n-6/n-3 fatty acid ratio (7). Recent advances in the edible oil industry have produced dietary oils with nutritionally superior fatty acid profiles (8). High-oleic canola oil (HOCO) is rich in MUFA, low in SFA, and exhibits a low ratio of n-6/n-3 fatty acids. With enhanced oxidative stability, HOCO is an attractive oil replacement for high SFA-high TFA oil varieties currently used in the food industry. Furthermore, recommendations have been made to increase dietary n-3 fatty acid intake (7). Flaxseed oil is a rich source of ALA, however, as flaxseed oil is less commonly consumed, blending flaxseed oil with other dietary oils provides a viable option to increase ALA intakes in Western diets.

Dyslipidemia, specifically elevated LDL-cholesterol, is a primary risk factor in predicting CVD events and a major target of dietary intervention (9). Recently, elevated concentrations of circulating inflammatory biomarkers have been associated with cardiovascular events (10-12). C-reactive protein (CRP) and proinflammatory cytokines, such as interleukin (IL)-6, initiate the development of atherosclerosis by upregulating

endothelial expression of adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin (13). Therefore, reducing both circulating LDL-cholesterol levels and inflammatory biomarkers are important in ameliorating CVD risk.

To date, the efficacy of HOCO consumption in modulating established biomarkers of CVD risk has not been investigated in a human clinical study. Additionally, although a high dose of flaxseed oil consumption has been reported to reduce inflammatory biomarkers in at risk subjects (14), the effects of flaxseed oil on serum lipids have been inconsistent (14,15). Therefore, the objectives of this human clinical study were to evaluate the efficacy of HOCO and a flaxseed/high-oleic canola oil (FXCO) blend in modulating circulating lipids and inflammatory biomarkers associated with CVD risk as compared with a typical Western diet.

4.3 Experimental Methods

4.3.1 Subjects

Thirty-nine individuals (fourteen males and twenty-five females) were recruited using flyers and media advertisements. Subjects were screened for LDL-cholesterol after 12 hours of fasting, and detailed blood chemistry analyses were performed. Inclusion criteria for the study were serum LDL-cholesterol >3.0 mmol/L, aged 18–65 years, and BMI between 22 and 36 kg/m^2. Before study enrolment, subjects underwent a routine physical examination by the study physician. Exclusion criteria were documented atherosclerotic disease, inflammatory disease, diabetes mellitus, uncontrolled hypertension, kidney

disease, cancer, tobacco smoking, use of lipid lowering medications for at least 3 months prior to starting the study, alcohol consumption >2 servings/day, or excessive exercise expenditure of >16,735 kJ (4000 kcal)/wk. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Bannatyne Campus Research Ethics Board (Protocol no. B2007:071) and the St. Boniface General Hospital Research Review Committee (Ref no. RCC/2007/0862). Written informed consent was obtained from all subjects. The study is registered in the ClinicalTrials.gov registry (Identifier #NCT00927199).

4.3.2 Experimental Design

A randomised, single-blind, crossover, controlled-diet clinical trial was conducted at the Clinical Research Unit at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), University of Manitoba. The study was designed as three phases with 28 days per phase separated by 4 to 8 week washout periods during which subjects consumed their habitual diets. Subjects were randomised to the three experimental diets using a 3 x 3 Latin-square design. Diets were individualized to meet daily energy requirements for weight maintenance for each subject as determined by the Mifflin equation (16), multiplied by a factor of 1.7 for medium physical activity. The study diets were prepared in the metabolic kitchen of the Richardson Centre Clinical Nutrition Research Unit and the food ingredients weighed within 0.5 g. Diets consisted of three isoenergetic meals prepared according to a 3 day cycle menu providing a variety of foods. In order to ensure stability of the flaxseed oil, experimental oils were added to cold foods; provided in milkshakes at breakfast and puddings at lunch and dinner. Subjects consumed one of

three daily meals (breakfast) at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) under supervision, while other meals (lunch and dinner) were prepared and cold-packed for take out. Subjects were instructed to consume only foods and beverages provided by the RCFFN and to refrain from alcoholic and caffeinated beverages during intervention periods. Subjects were advised to maintain their typical physical activity level and asked to report any symptoms or changes in health and medications throughout the study. Subjects' body weights were measured under supervision every morning before breakfast using a medical scale (Detecto, Webb City, MO, USA) to monitor weight stability.

4.3.3 Experimental Diets

Experimental diets were designed as typical Western diets containing 50% of energy as carbohydrate, 15% as protein, and 35% as fat, of which 70% was provided by the experimental oil. Diets were identical in composition throughout each phase, except for the type of experimental oil. Macronutrient profiles of experimental diets (**Table 4.1**) were analyzed using the nutrient composition software FOOD PROCESSOR (Food Processor version 7.81, Salem, OR, USA). Experimental oils tested included 1/ high-oleic canola oil (HOCO) (~70% oleic acid; Canola Harvest HiLo®; Richardson Oilseed Limited, Lethbridge, AB, Canada); 2/ A 1:1 blend of the high-oleic canola oil and flaxseed oil (FXCO) (~55% ALA and no lignans; Bioriginal Food & Science Corp., Saskatoon, SK, Canada); and 3/ A blend of oils typical of a Western diet (WD) including non-salted butter (12%), extra-virgin olive oil (35%), vegetable lard (35%), and sunflower oil (>60% linoleic acid) (18%). Fatty acid profiles of experimental oils are

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reported in **Table 4.2**.

Table 4.1: Macronutrient profile of the three experimental diets[*].

	Western diet		High-oleic canola oil diet		Flaxseed and high-oleic canola oil diet	
	g/day	% energy	g/day	% energy	g/day	% energy
Energy (kJ/d)	2500		2500		2500	
Carbohydrate	305	48.8	305	48.8	304	48.7
Fiber	20	3.3	20	3.2	20	3.2
Protein	90	14.4	90	14.4	90	14.4
Fat	102	36.8	102	36.8	103	36.9
SFA	31.2	11.2	15.7	5.6	17.0	6.1
MUFA	44.8	16.1	63.5	22.9	44.2	15.9
PUFA	18.0	6.5	15.9	5.7	34.1	12.3
18:2n-6	16.5	5.9	13.3	4.8	13.5	4.9
18:3n-3	1.3	0.5	2.4	0.8	20.6	7.4
n-6 to n-3 ratio	12.8		5.5		0.7	
Cholesterol (mg/d)	201.1		169.8		169.4	

[*]The macronutrient profile of the three experimental diets were estimated using FOOD PROCESSOR software (version 7.81; Food Processor, Salem, OR).

Table 4.2: Fatty acid composition of the three experimental dietary oils[*].

Fatty acid	Western diet	High-oleic canola oil	Flaxseed and high-oleic canola oil blend
	g/100 g total fatty acids		
Σ SFA	28.6	6.6	7.5
10:0	0.3	--	--
12:0	0.4	--	--
14:0	1.7	--	--
16:0	18.2	3.9	4.6
18:0	7.6	1.8	2.7
20:0	0.3	0.6	0.3
Σ MUFA	48.3	75.2	44.1
16:1n-7	1.4	0.2	--
18:1n-9	46.5	73.7	43.4
20:1n-9	0.4	1.3	0.7
Σ PUFA	22.6	17.9	48.4
18:2n-6	21.7	16.3	15.9
18:3n-3	0.8	1.7	32.4

[*]Values were determined by gas-liquid chromatography of triplicate samples of the dietary oil blends (-- indicates undetected fatty acid).

4.3.4 Blood Sampling and Serum Lipid Analysis

On days 1, 2, 28 and 29 of each phase, 12 hour fasted serum and EDTA plasma samples were collected. Within 1 hour of blood collection, serum, plasma and red blood cell (RBC) fractions were separated by centrifugation at 3000 rpm for 20 min at 4^oC, aliquoted and immediately stored at -80^oC until further analysis.

Serum TC, HDL-cholesterol, TAG and glucose levels were determined by automated enzymatic methods on a Vitros-350 chemistry analyzer (Ortho-Clinical Diagnostics, Markham, ON, Canada). Serum LDL-cholesterol levels were calculated by the Friedewald equation (17).

4.3.5 Plasma Inflammatory Biomarker and Adhesion Molecule Analysis

Plasma CRP levels were measured using quantitative colorimetric sandwich ELISA according to manufacturer's guidelines (R & D Systems, Minneapolis, MN, USA). IL-6 levels were measured by high-sensitivity ELISA (R & D Systems, Minneapolis, MN, USA). The intra-assay and inter-assay CV values were 2.31 and 4.26%, and 2.51 and 8.04%, for CRP and IL-6, respectively.

Plasma soluble adhesion molecules (VCAM-1, ICAM-1, E-selectin) were measured simultaneously by flow cytometry using multianalyte profiling performed on a Luminex-100 IS system (Luminex Corporation, Austin, TX, USA). Plasma concentrations of sVCAM-1, sICAM-1, sE-selectin were determined using a MILLIPLEX MAP human CVD panel-1 (3-plex) kit according to the manufacturer's guidelines (HCVD1-67AK,

Millipore Corporation, Billerica, MA, USA). Acquired median fluorescent intensity data were analyzed using a weighted 5-parameter logistic curve by the IS 2.3 software (Luminex Corporation, Austin, TX, USA). The sensitivity of the assay reported by the manufacturer had a minimum detectable concentration of 0.016, 0.009, and 0.079 ng/mL for sVCAM-1, sICAM-1, and sE-selectin, respectively. Intra-assay and inter-assay CV values were 7.4 and 10.9%, 8.8 and 11.3%, and 6.0 and 7.4% for sVCAM-1, sICAM-1, and sE-selectin, respectively.

For analyses of inflammatory biomarkers by ELISA and adhesion molecules by LUMINEX, controls (low, medium, high) supplied by the respective assay manufacturer and subject plasma samples were assayed in duplicate by a single laboratory technician with all samples for each subject run in one assay.

4.3.6 Plasma Fatty Acid Profile Analysis

Plasma total lipids were extracted by the Folch method (18) using chloroform:methanol (2:1, v/v) containing 0.01% BHT (Sigma-Aldrich, Oakville, ON, Canada) and heptadecanoic acid (17:0) as an internal standard (Sigma-Aldrich, Oakville, ON, Canada). Extracted fatty acids were methylated with methanolic HCl. Fatty acid methyl esters were separated on a Supelcowax 10 column (30 m x 0.25 mm with 0.25 μm film thickness; Supelco, Bellefonte, PA, USA) using an Agilent 6890N gas chromatograph equipped with a flame-ionization detector (Agilent Technologies, Mississauga, ON, Canada). The oven was programmed from 70°C to 240°C in four temperature steps (70°C for 1 min, rise of 25°C/min, 180°C for 2 min, rise of 3°C/min, 220°C for 10 min, rise of

20°C/min, 240°C for 15 min). Samples were run with a 10:1 split ratio and helium was used as the carrier gas with a column flow rate of 1.0 ml/min. Temperatures for the injector and detector were set at 280 and 300°C, respectively. Individual fatty acids were identified by comparison with known standards (NuChek Prep Inc., Elysian, MN, USA). Individual fatty acids were calculated according to the peak area relative to the total area and expressed as the percentage of total fatty acids.

4.3.7 Intima-Media Thickness Assessment

A subset of study subjects (n=18; randomised selection from study population) underwent clinical endothelial health assessment at onset of the study (phase 1; day 1–3) and at end of each treatment phase (day 24–26) by common carotid arterial ultrasound to assess changes in intima-media thickness (IMT). Common carotid IMT was performed with the use of an annular array ultrasound imaging system (9L probe, GE Vivid 7, Milwaukee, IL, USA). Subjects were examined in the supine position. Ultrasound scans of the right and left common carotid arteries were performed at the bifurcation of the first proximal center of internal carotid arteries as previously described (19). All measurements were made offline of the longitudinal carotid IMT scans using dedicated computer software (GE Echopac BT 08, Milwaukee, IL, USA). Average and maximal IMT values of each segment were measured as previously described (19). All ultrasound scans were performed by two trained sonographers and recorded ultrasound images were analyzed blindly at the Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, Winnipeg, Canada.

4.3.8 Statistical Analyses

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Results are expressed as means ± SEM unless otherwise noted. For variables with non-normal distribution, as determined by Shapiro-Wilk value < 0.05, statistical analyses were conducted after a logarithmic (base 10) transformation. Data on inflammatory biomarkers and adhesion molecules were not normally distributed and are reported as the median and 25th and 75th percentiles. Effects of dietary treatment were examined using a mixed model ANOVA procedure with diet, sequence, and phase as fixed factors and subject as a random factor in the model. Baseline values were inserted into the model as covariates for serum lipid measurements. Significant diet effects were examined with Bonferroni adjustment for multiple comparisons. For serum lipids percent change from baseline for each group was analyzed with a two-tailed paired student t-test. Pearson correlation analyses were conducted to test associations between lipid levels and inflammatory biomarkers. Statistical significance was set at $P < 0.05$ for all analyses. For all data, baseline and endpoint values are reported as averages of days 1 and 2, and days 28 and 29, respectively.

4.4 RESULTS

4.4.1 Subject Characteristics

Baseline characteristics of subjects who completed the study are presented in **Table 4.3**. Thirty-six subjects (thirteen males and twenty-three females; five premenopausal) completed the study. Two subjects withdrew from the study due to relocation of residence and one withdrew due to work-related issues. All subjects showed good

tolerance to experimental diets and reported consuming all meals provided to them. No

side effects were associated with the experimental diets. Subjects reported no change in

physical activity and no significant differences were noted in body weight after

consumption of the three experimental diets.

Table 4.3: Baseline characteristics of the subjects.

Anthropometric and serum lipid measurements (n=36)	Mean ± SD
Age (y)	47.49 ± 11.93
Body weight (kg)	78.80 ± 17.09
Height (cm)	165.50 ± 9.78
Body mass index (kg/m^2)	28.56 ± 4.62
Total cholesterol (mmol/L)	5.94 ± 1.03
LDL-cholesterol (mmol/L)	3.70 ± 0.95
HDL-cholesterol (mmol/L)	1.41 ± 0.35
Triglycerides (mmol/L)	1.84 ± 1.09
Plasma inflammatory biomarkers (n=36)	Median (25th-75th percentile)
C-reactive protein (mg/L)	1.34 (0.66–2.65)
Interleukin-6 (pg/mL)	1.59 (1.02–2.22)
sVCAM-1 (ng/mL)	1073.46 (915.28–1215.78)
sICAM-1 (ng/mL)	148.09 (134.96–159.50)
sE-selectin (ng/mL)	28.74 (19.55–36.25)
Carotid intima-media thickness (n=16)	Mean ± SD
Average (mm)	0.61 ± 0.10
Maximum (mm)	0.70 ± 0.11

Values are means ± SD for anthropometric and serum lipid
measurements (n=36), and carotid intima-media thickness (n=16) or
median (25th–75th percentiles) for plasma inflammatory biomarkers
(n=36); sVCAM-1, soluble vascular cell adhesion molecule-1;
sICAM-1, soluble intercellular adhesion molecule-1; sE-selectin,
soluble E-selectin.

4.4.2 Plasma Fatty Acids

After consumption of the experimental diets, changes in the plasma fatty acid

concentrations (**Table 4.4**) reflected the fatty acid profile of the experimental diets

(**Table 4.1**), verifying subjects' compliance with the experimental diets. As expected, plasma total MUFA, specifically 18:1n-9, was higher after consumption of the HOCO diet compared with both the FXCO diet ($P < 0.001$) and WD control ($P < 0.001$). Plasma

Table 4.4: Plasma fatty acid concentration at the end of each of the three experimental diets.

Total Fatty Acid (%)	Western diet	High-oleic canola oil diet	Flaxseed and high-oleic canola oil diet	P value*
Σ SFA	28.46 ± 0.29^a	26.30 ± 0.31^b	26.80 ± 0.34^b	<0.001
14:0	0.72 ± 0.04^a	0.73 ± 0.04^a	0.69 ± 0.04^a	0.473
16:0	19.77 ± 0.25^a	18.33 ± 0.27^b	18.37 ± 0.29^b	< 0.001
18:0	6.94 ± 0.13^a	6.27 ± 0.12^b	6.82 ± 0.14^a	< 0.001
Σ MUFA	26.18 ± 0.49^a	30.93 ± 0.58^b	26.13 ± 0.49^a	<0.001
16:1n-7	1.65 ± 0.08^a	$1.53 \pm 0.08^{a,b}$	1.51 ± 0.08^b	0.028
18:1n-9	21.94 ± 0.44^a	26.45 ± 0.58^b	21.87 ± 0.45^a	<0.001
18:1n-7	1.68 ± 0.04^a	1.90 ± 0.07^b	1.75 ± 0.04^a	<0.001
Σ PUFA	43.01 ± 0.61^a	40.38 ± 0.62^b	44.55 ± 0.58^c	<0.001
Σ n-6 PUFA	39.68 ± 0.60^a	36.85 ± 0.60^b	36.13 ± 0.58^b	<0.001
18:2n-6	29.95 ± 0.51^a	27.33 ± 0.49^b	28.73 ± 0.50^c	<0.001
18:3n-6	0.44 ± 0.03^a	0.48 ± 0.03^a	0.29 ± 0.02^b	<0.001
20:3n-6	1.67 ± 0.06^a	1.67 ± 0.05^a	1.08 ± 0.04^b	<0.001
20:4n-6	6.90 ± 0.24^a	6.70 ± 0.23^a	5.49 ± 0.18^b	<0.001
Σ n-3 PUFA	3.32 ± 0.07^a	3.54 ± 0.08^a	8.42 ± 0.21^b	<0.001
18:3n-3	0.74 ± 0.03^a	0.84 ± 0.03^a	4.46 ± 0.18^b	<0.001
20:5n-3	0.54 ± 0.03^a	0.62 ± 0.04^a	1.74 ± 0.11^b	<0.001
22:5n-3	0.54 ± 0.02^a	0.54 ± 0.02^a	0.75 ± 0.03^b	<0.001
22:6n-3	$1.50 \pm 0.05^{a,b}$	1.54 ± 0.05^a	1.47 ± 0.04^b	0.030
n-6/n-3 ratio	12.10 ± 0.28^a	10.59 ± 0.28^b	4.43 ± 0.17^c	<0.001

Values are means ± SEM (n=36); [a,b,c] Values within a row with different superscript letters were significantly different between treatment groups ($P < 0.05$). *P values are shown for the treatment effect analyzed by mixed model ANOVA (with Bonferroni adjustment for multiple comparisons).

total PUFA and total n-3 PUFA (including 18:3n-3, 20:5n-3, 22:5n-3) were higher after consumption of the FXCO diet as compared with both the HOCO diet ($P < 0.001$) and WD control ($P < 0.001$). No change in plasma DHA (22:6n-3) content was observed after consumption of the FXCO diet compared with the WD control ($P = 0.683$), however,

there was a slight decrease in plasma DHA content after the FXCO diet compared with the HOCO diet ($P = 0.025$). Plasma total SFA, total n-6 PUFA (specifically 18:2n-6), and n-6/n-3 ratio were lower after both the HOCO and FXCO diets compared with the WD control ($P < 0.001$ for all). Furthermore, plasma n-6/n-3 ratio was lower after the FXCO diet compared with the HOCO diet ($P < 0.001$). No significant differences in baseline fatty acid concentrations across the groups indicated no carryover effect and adequate washout periods between treatment phases (data not shown).

4.4.3 Serum Lipid Concentrations

Concentrations of fasting serum lipids and glucose at the end of each treatment phase are presented in **Table 4.5**. Serum lipid percent change from baseline is presented in **Figure 4.1**. After the 28 day treatment phase, serum TC concentrations were reduced when subjects consumed the HOCO diet (5.27 ± 0.14 mmol/L; $P < 0.001$) and FXCO diet (5.12 ± 0.13 mmol/L; $P < 0.001$) compared with the WD control (5.65 ± 0.16 mmol/L). TC percent change from baseline was reduced by 3.5% ($P = 0.002$) and 11.0% ($P < 0.001$) when subjects consumed the HOCO and FXCO diets, respectively, compared with the WD control. Furthermore, TC endpoint values ($P = 0.025$) and percent change from baseline (7.5%; $P = 0.015$) were lower when subjects consumed the FXCO diet as compared with the HOCO diet.

Table 4.5: Serum lipid and glucose concentrations at the end of each of the three experimental diets.

Serum Lipids	Western diet	High-oleic canola oil diet	Flaxseed and high-oleic canola oil diet	P value[*]
Total cholesterol (mmol/L)	5.65 ± 0.16^a	5.27 ± 0.14^b	5.12 ± 0.13^c	<0.001
LDL-cholesterol (mmol/L)	3.53 ± 0.14^a	3.10 ± 0.12^b	3.08 ± 0.12^b	<0.001
HDL-cholesterol (mmol/L)	1.37 ± 0.06^a	1.33 ± 0.06^a	1.28 ± 0.06^b	<0.001
Triglycerides (mmol/L)	1.63 ± 0.16	1.84 ± 0.18	1.65 ± 0.14	0.060
Total:HDL-cholesterol	4.37 ± 0.23	4.24 ± 0.22	4.32 ± 0.24	0.267
LDL:HDL-cholesterol	2.76 ± 0.17^a	2.49 ± 0.14^b	2.62 ± 0.17^b	<0.001
Non-HDL-cholesterol	4.28 ± 0.17^a	3.94 ± 0.14^b	3.84 ± 0.14^c	<0.001
Glucose (mmol/L)	5.04 ± 0.16	4.99 ± 0.15	4.97 ± 0.13	0.328

Values are means ± SEM (n=36); [a,b,c] Values within a row with different superscript letters were significantly different between treatment groups ($P < 0.05$). *P values are shown for the treatment effect analyzed by mixed model ANOVA (with Bonferroni adjustment for multiple comparisons).

Similarly, endpoint serum LDL-cholesterol concentrations were reduced after the HOCO diet (3.10 ± 0.12 mmol/L; $P < 0.001$) and FXCO diet (3.08 ± 0.12 mmol/L; $P < 0.001$) compared with the WD control (3.53 ± 0.14 mmol/L). LDL-cholesterol percent change from baseline was reduced by 7.4% ($P < 0.001$) and 15.1% ($P < 0.001$) after the HOCO and FXCO diets, respectively, compared to the WD control. However, no differences were observed in endpoint or percent change from baseline in LDL-cholesterol concentrations between the FXCO and HOCO diets.

No differences were observed in endpoint TAG concentrations between the treatment groups ($P = 0.060$; trend). With respect to percent change from baseline, no differences were observed for serum TAG concentrations between the treatment groups.
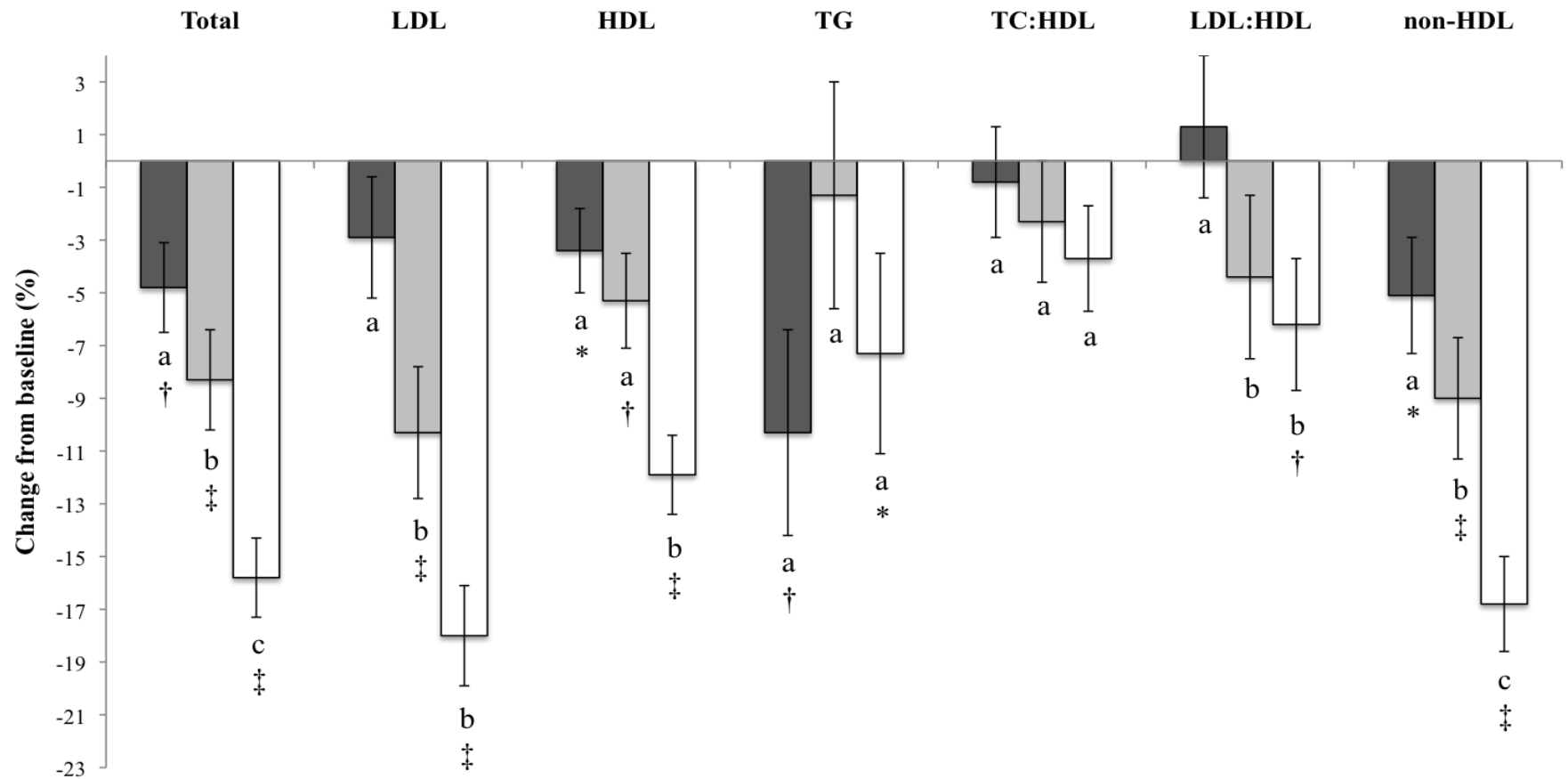
Figure 4.1. Percent changes in serum lipids from baseline in response to the three treatment diets; Western diet ▇ , high-oleic canola oil ▣ , flaxseed/high-oleic canola oil blend ▢ . Values are means ± SEM (n=36). [a,b,c] Mean values with unlike superscript letters between treatment groups are significantly different at $P \leq 0.05$ (mixed model ANOVA followed by Bonferroni adjustment for multiple comparisons). Mean values were significantly difference when compared within treatment group from baseline: *$P \leq 0.05$, †$P \leq 0.01$, ‡$P \leq 0.001$ (two-tailed paired-Student t test). TC, total cholesterol.

Endpoint serum HDL-cholesterol concentrations were reduced after the FXCO diet (1.28 ± 0.06 mmol/L) as compared with the HOCO diet (1.33 ± 0.06 mmol/L; $P = 0.008$) and WD control (1.37 ± 0.06 mmol/L; $P < 0.001$). The FXCO diet reduced HDL-cholesterol concentrations from baseline by 6.6% ($P = 0.006$) and 8.5% ($P < 0.001$) as compared with the HOCO diet and WD control, respectively. No differences were observed in endpoint or percent change from baseline in HDL-cholesterol concentrations between the HOCO diet and WD control.

Endpoint LDL:HDL-cholesterol ratios were reduced after the HOCO diet (2.49 ± 0.14; $P < 0.001$) and FXCO diet (2.62 ± 0.17; $P = 0.018$) compared with the WD control (2.76 ± 0.17). Both the HOCO and FXCO diets reduced LDL:HDL-cholesterol ratio from baseline by 5.7% ($P = 0.002$) and 7.5% ($P = 0.008$), respectively, as compared with the WD control. Endpoint and percent change from baseline in serum TC:HDL-cholesterol ratios did not differ after the treatment periods. Endpoint non-HDL-cholesterol was reduced after the HOCO diet (3.94 ± 0.14; $P = 0.003$) and FXCO diet (3.84 ± 0.14; $P < 0.001$) compared with the WD control (4.28 ± 0.17). Both the HOCO and FXCO diets reduced non-HDL-cholesterol from baseline by 3.9% ($P = 0.004$) and 11.7% ($P < 0.001$), respectively, compared with the WD control. Furthermore, non-HDL-cholesterol endpoint values ($P = 0.031$) and percent change from baseline (7.8%; $P = 0.030$) were lower when subjects consumed the FXCO diet compared with the HOCO diet.

No significant effects were observed in fasting serum glucose endpoint levels between treatment groups, nor were changes from baseline values observed.

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4.4.4 Plasma Inflammatory Biomarkers and Adhesion Molecule Concentrations

Results for measures of inflammatory biomarkers by ELISA and adhesion molecules by LUMINEX were within the detection limits of the assay. No significant differences were observed in endpoint concentrations for CRP or IL-6 between the treatment groups (**Table 4.6**). A decrease in endpoint E-selectin concentrations was observed after consumption of the FXCO diet compared with the WD control ($P = 0.023$), however, not in comparison with the HOCO diet ($P = 0.34$). No significant changes were observed in endpoint concentrations for sVCAM-1 and sICAM-1 between the treatment groups (**Table 4.6**). As compared with the WD control, after the subject consumed the FXCO diet, the change in endpoint E-selectin concentrations was directly associated with changes in TC ($r = 0.413$; $P = 0.012$), LDL-cholesterol ($r = 0.383$; $P = 0.021$) and non-HDL-cholesterol ($r = 0.340$; $P = 0.042$) concentrations (**Table 4.7**). However, changes in E-selectin concentrations following the consumption of the FXCO diet compared with the WD control did not correlate with other lipid parameters or plasma fatty acid concentrations (data not shown). There were no correlations between changes in lipid concentrations after the HOCO diet and changes in inflammatory biomarkers.

4.4.5 Intima-Media Thickness

A subset of sixteen subjects (age, 48.7 ± 11.9 years; BMI, 30.53 ± 4.64; four males and twelve females (four premenopausal)) completed the assessment of common carotid IMT. Two subjects withdrew due to relocation of residence. There were no significant changes detected in right posterior wall or left posterior wall average or maximum IMT between the dietary treatments or from baseline values at study entry (**Table 4.6**).

Table 4.6: Plasma inflammatory biomarker concentrations and carotid intima-media thickness at the end of each of the three experimental diets.

Plasma inflammatory biomarkers (n=36)	Western diet Median (25th–75th percentile)	High-oleic canola oil diet Median (25th–75th percentile)	Flaxseed and high-oleic canola oil diet Median (25th–75th percentile)	P value[*]
CRP (mg/L)	1.10 (0.57–2.31)	1.03 (0.46–2.53)	0.77 (0.52–2.02)	0.219
IL-6 (pg/mL)	1.48 (0.91–1.74)	1.48 (0.93–1.96)	1.32 (0.82–1.90)	0.227
sVCAM-1 (ng/mL)	1104.62 (954.77–1228.41)	1065.56 (938.87–1225.76)	1092.15 (980.12–1165.57)	0.195
sICAM-1 (ng/mL)	139.67 (128.16–149.38)	142.05 (126.16–162.63)	145.08 (131.21–161.43)	0.226
sE-selectin (ng/mL)	23.19 (16.97–30.91)[a]	21.63 (15.90–31.6)[a,b]	21.99 (16.17–29.23)[b]	0.027
Carotid IMT (n=16)	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**P value[*]**
Average (mm)	0.58 ± 0.02	0.59 ± 0.02	0.59 ± 0.03	0.968
Maximum (mm)	0.67 ± 0.03	0.67 ± 0.03	0.68 ± 0.03	0.967

Values are median (25th–75th percentile) for plasma inflammatory biomarkers (n=36) and mean ± SEM for carotid intima-media thickness; [a,b] Values within a row with different superscript letters were significantly different between treatment groups ($P <$ 0.05). *P values are shown for the treatment effect analyzed by mixed model ANOVA (with Bonferroni adjustment for multiple comparisons). CRP, C-reactive protein; IL-6, interleukin-6; sVCAM-1, soluble vascular cell adhesion molecule-1; sICAM-1, soluble intercellular adhesion molecule-1; sE-selectin, soluble E-selectin; IMT, intima-media thickness.

Table 4.7: Correlation coefficients among the change in plasma E-selectin and the changes in serum lipids when subjects consumed the FXCO diet compared with the WD[*].

	Δ E-selectin	
	r	*P* value
Δ Total cholesterol (mmol/L)	0.413	0.012
Δ LDL-cholesterol (mmol/L)	0.383	0.021
Δ HDL-cholesterol (mmol/L)	0.218	0.202
Δ Triglycerides (mmol/L)	0.055	0.751
Δ Total:HDL-cholesterol	0.211	0.216
Δ LDL:HDL-cholesterol	0.246	0.148
Δ Non-HDL-cholesterol	0.340	0.042

FXCO, Flaxseed and high-oleic canola oil diet; WD, Western diet.
[*]Pearson correlation analyses were conducted to test associations.

4.5 DISCUSSION

The present results are the first to demonstrate the lipid-lowering efficacy of low-SFA diets enriched with novel HOCO alone or blended with ALA-rich flaxseed oil. Compared with the WD control, we observed substantial decreases in TC for both the HOCO and the FXCO diets after 28 days, with the FXCO diet further reducing TC beyond that of HOCO (**Table 4.5; Figure 4.1**). The present study observed similar reductions in LDL-cholesterol concentrations after the consumption of the HOCO and FXCO diets compared with the WD control. Reports examining the lipid-lowering action of PUFA-rich versus MUFA-rich diets support the notion that PUFA-rich diets reduce TC and LDL-cholesterol concentrations comparable to MUFA-rich diets, and that PUFA oils elicit a slight TAG lowering effect (2,20-22). Similarly, compared with the HOCO diet, the FXCO diet and WD control both higher in dietary PUFA content, tended to reduce endpoint TAG concentrations; however, due to large individual variation, there was no difference in percent change in TAG levels from baseline between the dietary interventions examined.

The ability of HOCO to reduce TC and LDL-cholesterol, as well as preserve HDL-cholesterol, is of particular interest since to date the efficacy of HOCO in modulating blood lipids has not been assessed. Furthermore, it has previously been reported that not all MUFA-rich oils elicit the same effects on plasma cholesterol concentrations (23), suggesting the importance of other oil-derived fatty acid and non-lipid components. Reports suggest that ALA-rich flaxseed oil interventions fail to modify TC and LDL-cholesterol levels when compared with other dietary interventions (14,15). However, these results could be confounded by the use of MUFA and n-6 PUFA dietary controls. Limited work has directly compared dietary flaxseed oil with MUFA-rich oils. Whereas Singer et al. (1990) observed a reduction in TAG, as well as TC and LDL-cholesterol levels after 2-week supplementation with 60 ml/day of flaxseed oil but not with olive oil (24), Li et al. (1999) failed to find differences in plasma lipids after 4-weeks of a canola oil or flaxseed oil-enriched diet (25). In the present study, substitution of 50% HOCO with flaxseed oil in the FXCO treatment group was effective in further reducing TC compared with the HOCO treatment group.

FXCO reduced HDL-cholesterol from baseline, resulting in lower endpoint HDL-cholesterol levels than the WD control (**Table 4.5; Figure 4.1**). Previous studies administering high doses of flaxseed oil to hypercholesterolemic subjects have observed reductions in HDL-cholesterol levels (26-29). Generally, dietary strategies replacing SFA with PUFA results in a reduction in plasma TC and LDL-cholesterol and a parallel decrease in plasma HDL-cholesterol concentrations. Although concern exists that the

cardioprotection associated with low LDL-cholesterol is diminished with simultaneous reductions in HDL-cholesterol, it has been shown that rates of cholesterol efflux from macrophage cells to serum are not affected (30). Furthermore, endpoint LDL:HDL-cholesterol ratios were reduced in response to the HOCO and FXCO diet as compared with the WD control (**Table 4.5**). The LDL:HDL-cholesterol ratio is valuable in evaluating CVD risk across many populations (31). As well, non-HDL-cholesterol provides a single measure of the atherogenic apo B-containing lipoproteins and can thus provide a tool for cardiovascular risk assessment (6,31). After the FXCO diet, non-HDL-cholesterol levels decreased beyond that of the HOCO diet and the WD control. Therefore, the additive effects of ALA and oleic acid in the FXCO diet may have provided additional hypolipidemic effects that extend beyond those incurred by the HOCO diet alone.

In addition to dyslipidemia, elevated CRP levels associate with clinical manifestations of atherosclerosis and CVD risk (10). The intricate communication between inflammatory stimuli and endothelial cell adhesion molecules regulates inflammatory responses and the progression of atherosclerosis (13). Thus, a direct association may exist between plasma concentrations of VCAM-1, ICAM-1 and E-selectin and the extent of atherosclerosis and incidence of CVD risk (11,12). *In vitro* studies have shown the ability of oleic acid to inhibit cytokine-induced expression of VCAM-1, ICAM-1 and E-selectin in endothelial cells (32,33). Although human clinical trials have yet to specifically investigate effects of HOCO on inflammatory biomarkers, Keogh et al. (2005) failed to observe any effect of a MUFA-rich diet on serum CRP or plasma adhesion molecules in forty healthy adults

(34). Likewise, consumption of the HOCO-rich diet for 28 days did not affect inflammatory biomarker measures. Results of clinical trials investigating effects of flaxseed oil on inflammatory biomarkers and adhesion molecules are inconsistent (14). It has been suggested that the discrepancy may be dose related, as intakes exceeding 14 g/d of ALA from flaxseed oil have been shown to be more effective. In the present study, after 4-week supplementation of 21 g/day (7.5% energy) of ALA in the FXCO diet, a reduction was seen in E-selectin as compared with the WD control, however, no reductions in other inflammatory biomarkers were observed. In a 6-week randomised crossover trial which examined hypercholesterolemic subjects consuming 6.5% ALA from walnuts and flaxseed oil daily, Zhao et al. (2004) observed significant reductions in serum CRP, VCAM-1, ICAM-1 and E-selectin, as compared with an average American diet (28). Similarly, decreases in CRP, VCAM-1, as well as IL-6, have been reported with supplementation of 15 ml/day flaxseed oil (8.1 g ALA/d) for 12 weeks (26,35,36), however, no effects on ICAM-1 or E-selectin were observed (35,36). In contrast, recently Nelson et al. (2007) failed to observe decreases in CRP or IL-6 in healthy abdominally obese subjects consuming 5% of energy from ALA for 8 weeks (37). Similar to the latter study, we observed no change in plasma CRP or IL-6 concentration following the FXCO diet.

Unlike VCAM-1 and ICAM-1, E-selectin activity is specific to the surface of stimulated endothelial cells, mediating the rolling of monocytes along the cell surface (38). Furthermore, the expression of E-selectin directly associates with dyslipidemia. It was previously shown that effective lipid-lowering intervention reduced plasma E-selectin

concentrations in dyslipidemic subjects; however, the lipid-lowering effect was not associated with a reduction in VCAM-1 or ICAM-1 (39). Of interest, in the present study a significant correlation was observed between changes in plasma E-selectin and TC, LDL-cholesterol, and non-HDL-cholesterol concentrations when subjects consumed the FXCO diet compared with the WD control (**Table 4.7**). However, albeit the reduction in serum lipids following the consumption of the FXCO and HOCO diets, there was no change in VCAM-1 or ICAM-1 concentrations. Since the FXCO diet resulted in reductions in TC and non-HDL-cholesterol concentrations beyond that of the HOCO diet (**Table 4.5**), we speculate that the acute effects of FXCO consumption on E-selectin concentrations may be attributed to the magnitude of reductions in circulating lipids.

The discrepancy between the present results and those of previous studies that reported reductions in inflammatory biomarkers may be related to subject baseline levels of those biomarkers. In the present study, subject baseline levels of inflammatory biomarkers CRP and IL-6 were in the healthy range compared with those of subjects examined previously (26,35,40). Similarly, studies that failed to observe an effect of ALA intervention on inflammatory biomarkers have attributed the absence of response to a 'floor effect'; the inability to detect changes due to low baseline levels (37,41). Another consideration may be the duration of the present study. Although a 4-week intervention is typically sufficient to observe significant alterations in blood lipids, previous studies reporting reductions in inflammatory markers were of 6–12 weeks in duration (26,28,35,36). Similarly, the limited study duration may also explain the absence of treatment effects on carotid IMT. Bemelmans et al. (2004), using a parallel-arm design and a 2-year dietary

intervention, found that 4.5 g/day of ALA yielded no significant effect on IMT

progression (40). The present study focused on examining whether a high dose of ALA,

approximately 3.5-fold greater than that used in Bemelmans et al. (2004), utilizing a

crossover design, would have acute effects on IMT progression; however, no positive

action was observed.

The plasma fatty acid concentrations reflected fatty acid profiles of the experimental oils,

indicating compliance to the dietary interventions (42,43). After consumption of the

ALA-rich FXCO diet, an approximate 5-fold increase in plasma ALA (18:3n-3)

concentrations and 3-fold increase in EPA (20:5n-3) concentrations were observed

compared with the HOCO diet and WD control. However, there were no differences in

plasma DHA (20:6n-3) concentrations between the FXCO diet and the WD control.

These results are consistent with previous stable isotope tracer studies demonstrating the

linear relationship between dietary ALA intakes and plasma EPA, with no direct

relationship between ALA intakes and plasma DHA due to limited conversion rates (44).

Nonetheless, the increase in plasma concentration of ALA, EPA and DPA after the

FXCO diet may be cardioprotective as an inverse association has been found between

plasma concentrations of combined EPA and DHA, as well as ALA, and risk of fatal

ischemic heart disease (45). Furthermore, the higher plasma MUFA concentration after

the HOCO diet may provide cardiovascular benefits, as MUFA has been shown to be

resistant to oxidative modifications of LDL-cholesterol (46).

A potential limitation of this study is that the experimental diets were not balanced for

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dietary cholesterol levels, however it has been reported that in humans dietary fatty acids are primary determinants of serum cholesterol, whereas dietary cholesterol has minimal effect on modulating serum cholesterol levels (3,47,48). Furthermore, the average daily intake of cholesterol in each experimental diet was considerably lower than the AHA recommendation of <300 mg/day (9). Moreover, the feasibility of incorporating both HOCO and FXCO into typical diets requires further consideration. In order to maintain total fat energy intake, it is crucial to target fat substitution versus fat supplementation of the diet. The high-stability properties of HOCO make it a practical substitution for TFA-rich partially hydrogenated vegetable oils in food processing, frying and for culinary purposes (8). Increased dietary ALA intake can be achieved by fortifying dressings, spreads and margarines with the FXCO blend as a replacement of traditional products. Currently, the US Food and Drug Administration (FDA) has authorized a qualified health claim stating that canola oil (~19 g/day) may reduce the risk of CHD due to its unsaturated fat content, recommending direct caloric replacement of dietary SFA with canola oil (49). Therefore, increased compliance with dietary recommendations and targeting a reduction in CHD risk would be possible by replacing a proportion of commonly used dietary oils and spreads in the Western diet with high-oleic canola oil alone or blended with flaxseed oil.

In conclusion, the present study is the first human clinical trial to investigate effects of HOCO on serum lipids and other markers of CVD risk. HOCO alone or when blended with flaxseed oil effectively reduced serum TC and LDL-cholesterol compared with a WD control. Moreover, the ALA-rich FXCO may further target inflammation and

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atherogenic pathways by reducing plasma E-selectin. Substitution of dietary fats common to the WD with both HOCO and flaxseed oil is a feasible option to target dietary recommendations and risk factors for CVD.

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BRIDGE TO CHAPTER V

The data presented in Chapter IV substantiate the lipid-lowering efficacy of both high-oleic canola and flaxseed oils when substituted for dietary fats common to the Western diet. Moreover, since the observed reduction in plasma E-selectin was directly associated with lower circulating lipid concentrations, but not changes in plasma n-3 LCPUFA proportions after consumption of the flaxseed/high-oleic canola oil diet, the data emphasize independent health benefits of dietary ALA. Beyond traditional and emerging risk factors for CVD, abdominal obesity underlies health complications culminating CVD morbidity and mortality. Data from animal and human studies argue for a more rapid metabolic disposal of OA and ALA compared particularly with SFA. As such, it can be suggested that plant oils rich in OA and ALA, particularly high-oleic canola oil and flaxseed oil, would be oxidized more rapidly and result in less body fat accumulation than conventional oils such as lard and dairy fats which are richer in SFA. However, no systematic studies have explored these questions, particularly in the face of the current global epidemic of obesity.

Using whole-body indirect calorimetry and dual x-ray absorptiometry methods, the purpose of the following study was to simultaneously investigate changes in energy expenditure and substrate utilization with alterations in body composition after consumption of high-oleic canola oil and the flaxseed/high-oleic canola oil blend, as compared with current North American fatty acid intakes using the Western dietary control.

CHAPTER V

MANUSCRIPT 4

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EFFECT OF HIGH-OLEIC CANOLA AND FLAXSEED OILS ON ENERGY EXPENDITURE AND BODY COMPOSITION IN HYPERCHOLESTEROLEMIC SUBJECTS

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absorptiometry: substrate metabolism

5.1 ABSTRACT

Objective: The fatty acid profile of dietary fats may contribute to its channelling toward oxidation versus storage, influencing energy and weight balance. Our objective was to compare the effects of diets enriched with high-oleic canola oil (HOCO), alone or blended with flaxseed oil (FXCO), on energy expenditure, substrate utilization, and body composition versus a typical Western diet (WD). *Materials/Methods:* Using a randomized crossover design, 34 hypercholesterolemic subjects (n=22 females) consumed 3 controlled diets for 28 days containing ~49% energy from carbohydrate, 14% energy from protein, and 37% energy from fat, of which 70% of fat was provided by HOCO rich in oleic acid, FXCO rich in alpha-linolenic acid, or WD rich in saturated fat. Indirect calorimetry measured energy expenditure and substrate oxidation. Body composition was analyzed by dual-energy x-ray absorptiometry. *Results:* After 28 days, resting and postprandial energy expenditure and substrate oxidation was not different after consumption of the HOCO or FXCO diets compared with a typical Western diet. No significant changes in body composition measures were observed between diets. However, the android-to-gynoid ratio tended to increase ($P = 0.055$) after the FXCO diet compared with the HOCO diet. *Conclusions:* The data suggest that substituting a typical Western dietary fatty acid profile with HOCO or FXCO does not significantly modulate energy expenditure, substrate oxidation or body composition in hypercholesterolemic males and females.

5.2 INTRODUCTION

The epidemic growth of obesity in North America is propelled by unhealthy lifestyle choices (1-3). Recently, much attention has focused on the influence of dietary fat composition in energy and weight balance (4-7). Evidence from stable isotope-labelled fatty acid (8-10) and indirect calorimetry (11-16) studies have shown increased oxidation of long-chain unsaturated fatty acids, namely oleic acid, compared with long-chain SFA. However, controversy remains as to the impact of dietary fat composition on whole-body substrate oxidation or energy expenditure, with recent indirect calorimetry studies failing to observe an effect (17-19), or reporting that subject body composition (13,14,20) and gender (21) may alter the response to dietary fat. Additionally, few human studies have simultaneously investigated changes in body composition associated with shifts in components of daily energy expenditure after dietary fat intervention (12,14,19).

High-oleic canola oil (HOCO) and flaxseed oil are low in SFA and high in unsaturated fatty acids, oleic acid and alpha-linolenic acid (ALA), respectively. However, to date, the efficacy of consuming HOCO, alone or blended with flaxseed oil (FXCO), on energy and weight balance as compared with a typical Western dietary fatty acid profile has not been studied in humans. Therefore, the objective of the present study was to investigate the effects of chronic consumption of HOCO and FXCO on resting and postprandial energy expenditure, substrate oxidation, and body composition.

5.3 EXPERIMENTAL METHODS

5.3.1 Subjects

Thirty-nine individuals between the ages 18 and 65 years (fourteen males and twenty-five females) were recruited using flyers, newspaper and radio advertisements. Fasting blood was sampled to screen for biochemical and haematological parameters. Inclusion criteria were serum LDL-cholesterol >3.0 mmol/L, and BMI between 22-36 kg/m^2. The present study was conducted according to the principles expressed in the Declaration of Helsinki, and all procedures were approved by the University of Manitoba's Biomedical Research Ethics Board (Protocol no. B2007:071). All subjects provided written informed consent prior to starting the study. This study was registered with ClinicaTrials.gov (Identifier #NCT00927199).

5.3.2 Experimental Design

A detailed report of this study design has been published previously, thus methods are only briefly summarized here (22). The study used a randomized, single-blind, crossover, controlled-diet design consisting of three phases with 28 days per phase separated with a 4 to 8-week washout period. Women were studied during the same phase of their menstrual cycle for each treatment phase. A typical high-fat Western diet (WD) was prepared by the metabolic kitchen at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) consisting of three isoenergetic meals using a 3-day meal cycle. Food ingredients were weighed within 0.5 g based on subjects individual daily energy requirements for weight maintenance calculated by the Mifflin equation (23) and multiplied by an activity factor of 1.7. Subject body weights were recorded daily under

supervision before breakfast. If subject body weight fluctuated in the first week of the study, energy intake was adjusted by shifting the activity factor to maintain subject body weight. Subjects' breakfast meals were consumed under supervision at the RCFFN daily, with lunch and dinner meals prepared for take out. Throughout the study, subjects were instructed to maintain their physical activity level and report any changes in health.

5.3.3 Test Meals

The macronutrient profile of the test meals were identical in composition and designed to contain 50% of energy as carbohydrate, 15% as protein and 35% as fat. The experimental oils, providing 70% of fat intake, included 1/ HOCO (approximately 70% oleic acid; Canola Harvest HiLo®; Richardson Oilseed Limited, Lethbridge, AB, Canada); 2/ a 1:1 blend of the HOCO and flaxseed oil (FXCO) (Approximately 55% ALA and no lignans; Bioriginal Food & Science Corporation, Saskatoon, SK, Canada); 3/ a blend of oils typical of a WD including non-salted butter (12%), extra-virgin olive oil (35%), vegetable lard (35%), and sunflower oil (>60% linoleic acid) (18%). Experimental oils were blended in cold foods as milkshakes at breakfast and puddings at lunch and dinner. **Table 5.1** outlines the macronutrient profile of the three test meals consumed during indirect calorimetry analysis. The fatty acid profiles of the experimental oils and macronutrient profile of the experimental diets have been published previously (22). A questionnaire was administered at the end of each phase to assess sensory characteristics of the treatments and any experienced side effects from consumption of the experimental diets (Chapter V Supplement in "Appendix III").

Table 5.1: Energy and macronutrient profile of the three test meals used for indirect calorimetry analysis[*].

	Western diet		High-oleic canola oil diet		Flaxseed and high-oleic canola oil diet	
	g/meal	% of Energy	g/meal	% of Energy	g/meal	% of Energy
Carbohydrate	94.36 ± 15.87	48.37	94.26 ± 15.85	48.31	94.26 ± 15.85	48.31
Fiber	5.60 ± 0.89	2.89	5.60 ± 0.89	2.88	5.60 ± 0.89	2.88
Protein	29.86 ± 6.96	15.20	29.83 ± 6.95	15.18	29.83 ± 6.95	15.18
Fat	32.66 ± 5.17	37.77	32.67 ± 5.17	37.78	32.88 ± 5.21	38.02
SFA	10.57 ± 2.01	12.16	5.73 ± 1.39	6.56	5.88 ± 1.40	6.73
MUFA	11.82 ± 1.90	13.66	18.05 ± 2.88	20.86	11.64 ± 1.87	13.45
PUFA	5.94 ± 1.02	6.90	5.24 ± 0.92	6.09	11.31 ± 1.81	13.11
n-6 PUFA	4.60 ± 0.81	5.30	3.00 ± 0.57	3.45	3.28 ± 0.61	3.77
n-3 PUFA	0.21 ± 0.04	0.25	0.56 ± .09	0.64	6.65 ± 1.06	7.68
PUFA:SFA ratio	0.57		0.95		21.99	
PUFA:MUFA:SFA ratio	0.56:1.12:1.0		0.91:3.15:1.0		1.92:1.98:1.0	
Daily Energy (kcal/d)	2463.58 ± 390.28		2463.58 ± 390.28		2463.58 ± 390.28	
Test Meal Energy (kcal/d)	780.74 ± 133.54		780.79 ± 133.53		789.79 ± 133.53	

[*]The energy and macronutrient profile of the three test meals (average of breakfast ($n=22$) and dinner ($n=12$)) were estimated using Food Processor software (version 7.81; Food Processor, Salem, OR). All values are means ± SD.

5.3.4 Indirect Calorimetry Measurements

On a single day during week 1 of phase 1 (study baseline) and week 4 of each phase

(phase endpoints), energy expenditure was analyzed by indirect calorimetry using an

open-circuit ventilated canopy (Vmax Encore software, Summit Technologies Inc.,

Burlington, ON, Canada) recording the rate (L/min) of oxygen consumption (VO_2) and

carbon dioxide production (VCO_2). Each day prior to respiratory measurements,

calibration of the flow sensor with a syringe was conducted using reference gas standards

(16% O_2, 4% CO_2, 80% N_2; and, 26% O_2, 74% N_2) (Summit Technologies Inc.,

Burlington, ON, Canada). Subjects were measured under standardized conditions in the

fasted state (12 hour fast for breakfast group (n=22), 6 hour fast for dinner group (n=12)).

Upon arriving at the RCFFN, subjects lay supine for 15 minutes before a 30 minute

resting metabolic rate (RMR) measure was recorded prior to the test meal. Subjects were

allowed 40 minutes to consume their controlled test meal under supervision. After the

meal, 6 hour postprandial energy expenditure was measured in 30 minutes intervals and

no additional food or beverages were permitted. All measures were supervised with

subjects resting supine on a bed with their head placed under the transparent ventilated

canopy. Subjects were permitted to watch movies or read, and were asked to refrain from

speaking while under the hood. Washroom breaks were permitted during the 30 minute

intervals when subjects were not being measured, otherwise subjects were advised to

remain in a rested supine state.

The VO_2 and VCO_2 values (L/min) recorded by the Vmax Encore software (Summit

Technologies Inc., Burlington, ON, Canada) were extracted into a spreadsheet. Subjects'

weights were recorded prior to analysis and the assumption of a constant nitrogen (N)

excretion (0.14 gN/kg body weight/day) was used in place of urinary nitrogen

measurements (11). Therefore, non-protein respiratory quotient (npRQ) was calculated

based on the equation described by Westenskow et al. (1988) (24).

$$npRQ = \frac{npVCO_2}{npVO_2} = \frac{VCO_2 - \left(\left(bodyweight \times \frac{0.14}{1440}\right) \times 6.03\right)}{VO_2 - \left(\left(bodyweight \times \frac{0.14}{1440}\right) \times 4.88\right)} \qquad [1]$$

Where 6.03 and 4.88 is the volume (L) of CO_2 and O_2 utilized per gram of N

metabolized. Using the npRQ and $npVO_2$ derived from equation [1], total energy

expenditure (EE_{total}), including RMR and postprandial energy expenditure, was calculated

based on equations described by Lusk (1924) (25) and Schutz (1995) (26).

$$EE_{total}\left(\frac{kcal}{min}\right) = npVO_2 \times \left[4.686 + \left(\left(\frac{npRQ - 0.707}{0.293}\right) \times 0.361\right)\right] \qquad [2]$$

Where 4.686 is the calories per volume (kcal/L) of O_2 consumed and 0.707 is the RQ

corresponding to 100% fat oxidation, 0.293 is the difference between the RQ for

carbohydrate and fat oxidation (i.e. $1.00 - 0.707$), and 0.361 is the difference in the

calories per volume (kcal/L) of O_2 consumed between carbohydrate and fat oxidation (i.e

$5.047 - 4.686$). Using the npRQ and $npVO_2$ derived from equation [1], carbohydrate

oxidation (CHO_{ox}) and fat oxidation (Fat_{ox}) were calculated based on the equations

described by Jequier et al. (1987) (27).

$$CHO_{ox}\left(\frac{g}{min}\right) = npVO_2 \times \left(\frac{npRQ - 0.707}{0.293 \times 0.746}\right) \qquad [3]$$

$$Fat_{ox}\left(\frac{g}{min}\right) = npVO_2 \times \left(\frac{1.00 - npRQ}{0.293 \times 2.019}\right) \qquad [4]$$

Where 0.746 and 2.019 is the volume (L) of O_2 consumed per gram of carbohydrate and

fat oxidized, respectively.

Resting metabolic rate recorded prior to the meal was assumed to remain constant throughout the 6-hour period post meal consumption. Average postprandial energy expenditure, fat oxidation and carbohydrate oxidation were calculated for each time interval over the 6 hours post meal consumption. Thermic effect of food (TEF) was calculated as the area under the 6-hour curve of postprandial energy expenditure plotted against time minus the projected RMR over the 6 hour postprandial period using GraphPad Prism version 4.0c (GraphPad Software, Inc., La Jolla, CA, USA).

5.3.5 Dual-Energy X-Ray Absorptiometry Measurements

Body composition measures were assessed after a 12-hour fast on days 1 and 29 of each phase by dual-energy x-ray absorptiometry (DEXA) scanning after quality assurance calibration of the machine (Lunar Prodigy Advance, GE Healthcare, Madison, WI, USA). Prodigy Encore 2005 software version 9.30.044 (GE Healthcare, Madison, WI, USA) calculated the regions of interest, including android fat mass and gynoid fat mass as a percentage of total fat mass, as well as the ratio of android-to-gynoid fat (percent android fat divided by percent gynoid fat). Android fat mass reflects the abdominal region, whereas gynoid fat mass reflects region of the hips, buttocks and upper thighs. More specifically, the android region was defined inferiorly at the pelvis cut line, superiorly at 96mm above the pelvis cut line, and laterally at the arm cut lines. The gynoid region was defined superiorly below the pelvis cut line, inferiorly at 96mm below the pelvis cut line, and laterally at the outer leg cut lines.

5.3.6 Statistical Analyses

Statistical analysis was performed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA) using linear mixed model analysis of covariance (ANCOVA) with subject as a random factor and treatment as an independent factor. Repeated measures were used to examine the existence of effects of time and time x treatment interaction. For thermogenic data, BMI was tested as a covariate. The effect of dietary treatment, sequence, phase, time of meal (breakfast versus dinner) and gender were included in the model as fixed factors when their effect on the independent variable was significant. Significant treatment effects were examined with Bonferroni post hoc test for multiple comparisons. For body composition, changes from baseline within each treatment group were analyzed with a two-tailed paired student t-test. Pearson correlation analyses were conducted to test associations between body composition and energy expenditure or substrate oxidation variables. Given that the present study was an extension of a clinical trial investigating the lipid-lowering efficacy of HOCO and FXCO (22), a sample size of 34 subjects was utilized. Therefore, using a P-value of 0.05 with a power of 80%, the ratio of effect estimate/variance for this study would be 0.68. Statistical significance was set at $P \leq 0.05$ for all analyses. Results are expressed as means ± SEM.

5.4 RESULTS

5.4.1 Subject Characteristics

Subject baseline characteristics are presented in **Table 5.2**. Thirty-four subjects (12 males and 22 females (5 premenopausal)) completed the study. There were 8 lean subjects (BMI 22.5 ± 1.2 kg/m^2), 13 overweight subjects (BMI 27.0 ± 1.6 kg/m^2), and 13 obese

subjects (BMI 32.6 ± 2.8 kg/m^2). No differences in baseline BMI, fat mass, or android fat was observed between male and females. Baseline percent body fat and gynoid fat were higher ($P < 0.001$), while fat free mass and the android-to-gynoid ratio were lower ($P < 0.001$) in females compared with males.

Table 5.2: Subject baseline characteristics.

Characteristic	All subjects	Males	Females
n	34	12	22
Anthropometric measurements			
Age (y)	48.24 ± 11.88	48.75 ± 11.36	47.95 ± 12.40
Body mass (kg)	77.09 ± 16.02	86.26 ± 10.80	72.08 ± 16.36[†]
Height (m)	1.65 ± 0.10	1.73 ± 0.07	1.61 ± 0.08[‡]
Body mass index (kg/m^2)	28.12 ± 4.49	28.92 ± 3.64	27.68 ± 4.91
Body fat (%)	38.01 ± 7.36	32.16 ± 4.49	41.20 ± 6.66[‡]
Fat mass (kg)	29.52 ± 9.18	28.00 ± 6.55	30.35 ± 10.39
Fat free mass (kg)	47.56 ± 10.47	58.26 ± 5.81	41.73 ± 7.32[‡]
Android fat (%)	45.81 ± 6.07	43.40 ± 4.19	47.12 ± 6.60
Gynoid fat (%)	42.75 ± 8.98	33.20 ± 5.03	47.96 ± 5.74[‡]
Android:Gynoid fat	1.11 ± 0.21	1.33 ± 1.17	0.99 ± 0.11[‡]
RMR (kcal/min)	0.812 ± 0.176	0.937 ± 0.130	0.758 ± 0.167[‡]
Serum Lipid Measurements			
Total cholesterol (mmol/L)	5.94 ± 1.05	6.00 ± 1.05	5.90 ± 1.08
LDL-cholesterol (mmol/L)	3.73 ± 0.96	3.86 ± 0.97	3.65 ± 0.96
HDL-cholesterol (mmol/L)	1.39 ± 0.34	1.22 ± 0.32	1.48 ± 0.32[*]
Triglycerides (mmol/L)	1.81 ± 1.10	2.01 ± 1.51	1.70 ± 0.82
Glucose (mmol/L)	5.39 ± 1.30	5.74 ± 2.01	5.20 ± 0.65

All values are means ± SD. Mean values were significantly different between males and females: [*]$P \leq 0.05$, [†]$P \leq 0.01$, [‡]$P \leq 0.001$ (independent T-test).

Three subjects withdrew from the study due to work-related issues or relocation of residence. Two subjects did not participate due to discomfort with the ventilated canopy for indirect calorimetry analysis. Subjects did not report a change in physical activity during the study protocol. No major side-effects from the treatments were noted. Sensory analysis revealed that treatments formulated with HOCO received more favourable

sensory characteristic ratings ($P < 0.005$) as compared with the WD control formulations (Chapter V Supplement in "Appendix III").

5.4.2 Energy Expenditure and Substrate Oxidation by Indirect Calorimetry

Fasting and postprandial energy expenditure and substrate oxidation rates for each treatment group are presented in **Table 5.3**. After 28 days, no differences were observed in RMR, RQ, and substrate oxidation measured in the fasting state between treatment groups. After consumption of the test meals, no differences were observed in hourly average postprandial energy expenditure between treatment groups (**Figure 5.1**). In addition, total postprandial energy expenditure, RQ, TEF, as well as fat and CHO oxidation rates did not differ between treatment groups. When energy expenditure, as well as fat and CHO oxidation rates, were expressed as a factor of fat-free mass no differences were noted between treatment groups (data not shown).

In subsequent analysis, effects of gender and BMI on resting and postprandial energy expenditure and substrate oxidation were tested after consumption of the test meals. Gender resulted in a significant effect ($P < 0.05$) on RMR, postprandial energy expenditure, and TEF. However, further analysis revealed no gender x treatment

Table 5.3: Fasting and postprandial energy expenditure and substrate oxidation of subjects after consuming the treatment diets.

	Western diet	High-oleic canola oil diet	Flaxseed and high-oleic canola oil diet	*P*-value
Fasting				
RMR (kcal/min)	0.929 ± 0.031	0.946 ± 0.038	0.927 ± 0.034	0.647
Fat oxidation (g/min)	0.101 ± 0.007	0.101 ± 0.007	0.100 ± 0.007	0.992
CHO oxidation (g/min)	-0.007 ± 0.013	-0.003 ± 0.011	-0.005 ± 0.012	0.908
Respiratory quotient	0.78 ± 0.01	0.79 ± 0.01	0.79 ± 0.01	0.832
Postprandial				
PEE (kcal/min)	1.067 ± 0.034	1.070 ± 0.037	1.055 ± 0.035	0.599
Fat oxidation (g/min)	0.088 ± 0.005	0.089 ± 0.006	0.085 ± 0.006	0.895
CHO oxidation (g/min)	0.062 ± 0.011	0.060 ± 0.010	0.066 ± 0.012	0.968
TEF (kcal/meal)	48.468 ± 4.538	45.261 ± 3.310	46.141 ± 4.408	0.588
Respiratory quotient	0.83 ± 0.01	0.83 ± 0.01	0.84 ± 0.01	0.931

Values are means ± SEM; n = 34. CHO, carbohydrate, RMR, resting metabolic rate; PEE, postprandial energy expenditure; TEF, thermic effect of food.
P-values are shown for the treatment effect between groups analyzed by mixed model ANCOVA (with the Bonferroni post hoc test for multiple comparisons).

interaction on these dependent variables. No effect of gender on resting and postprandial substrate oxidation was observed. Conversely, BMI impacted ($P < 0.01$) RMR, postprandial energy expenditure, as well as resting and postprandial substrate oxidation. Further analysis revealed no BMI x treatment interaction on these dependent variables. No effect of BMI on TEF was observed. BMI positively correlated with RMR ($r = 0.569$; $P < 0.001$), postprandial energy expenditure ($r = 0.567$; $P < 0.001$), resting fat oxidation ($r = 0.446$; $P < 0.001$), and postprandial fat oxidation ($r = 0.369$; $P < 0.001$), while negatively correlated with resting CHO oxidation ($r = -0.196$; $P < 0.048$). BMI did not correlate with TEF ($r = 0.014$; $P = 0.887$). No effect of the time of meal (breakfast versus dinner) on resting and postprandial energy expenditure or substrate oxidation was observed.

A.

B.
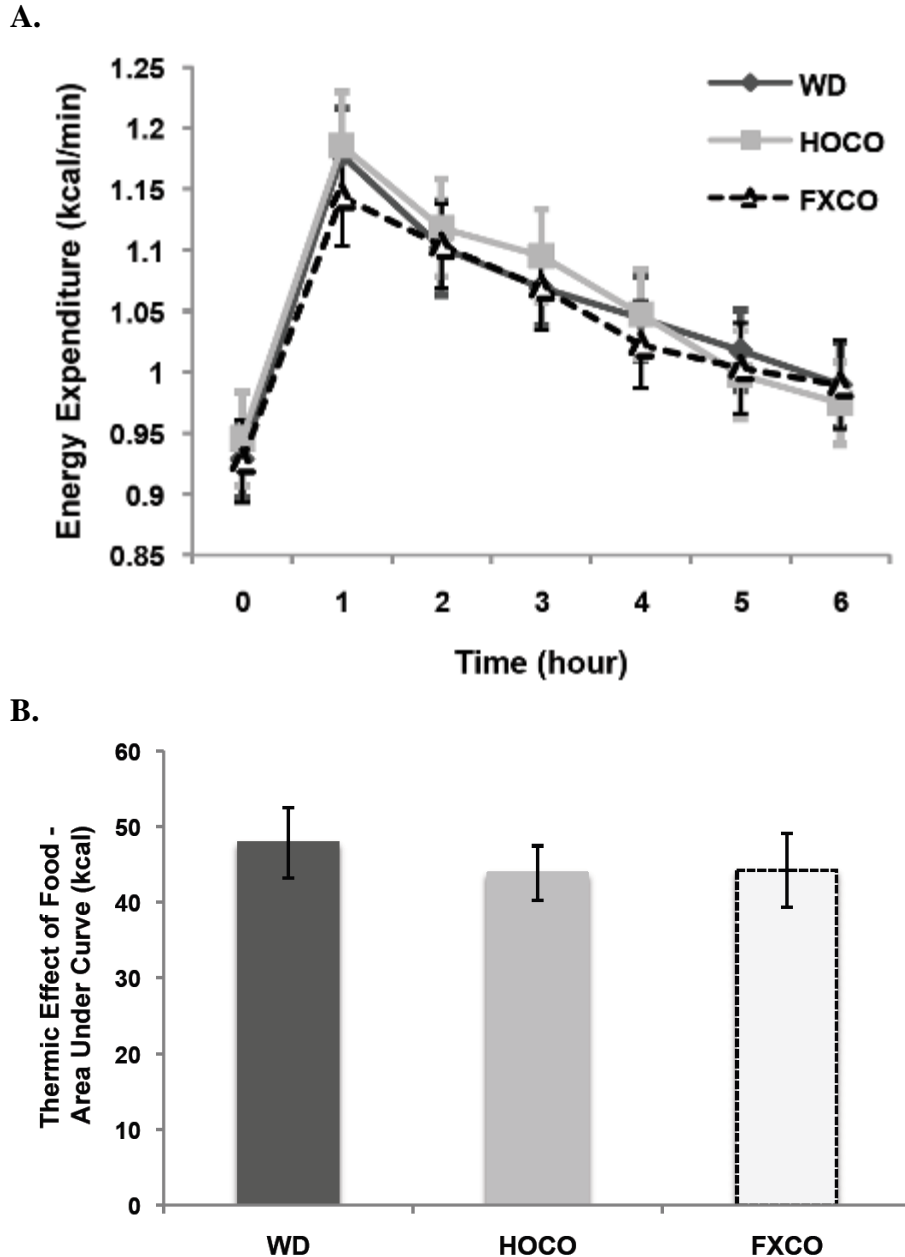
Figure 5.1A: Resting (hour 0) and postprandial (hour 1-6) energy expenditure for subjects (n=34) after consumption of the three experimental diets; Western (WD), high-oleic canola oil diet (HOCO), flaxseed/high-oleic canola oil diet (FXCO). **Figure 5.1B:** Area under the curve for thermic effect of food (postprandial energy expenditure area – resting metabolic rate area) measured for 6 hours after subjects (n=34) consumed the three experimental diets. Values are means ± SEM. Repeated measures mixed model ANCOVA determined no significant treatment effect, time effect, or treatment x time interaction.

5.4.3 Body Composition by Dual-Energy X-Ray Absorptiometry

No differences were observed in baseline body composition measures between treatment groups (**Table 5.4**). After 28 days, BMI, fat mass, and fat free mass were reduced ($P < 0.05$) from baseline within each treatment group (data not shown). However, BMI, fat mass, fat free mass, as well as percent body fat, android fat and gynoid fat did not differ at endpoint between treatment groups. A trend towards a reduction in the android-to-gynoid ratio was observed after consumption of the FXCO diet compared with the HOCO diet, however the difference did not reach statistical significance ($P = 0.055$).

Table 5.4: Body composition at the end of each of the three experimental diets.

	Western diet	High-oleic canola oil diet	Flaxseed and high-oleic canola oil diet	P-value
Body mass (kg)	75.63 ± 2.72	75.50 ± 2.66	75.77 ± 2.71	0.667
Body mass index (kg/m^2)	27.60 ± 0.78	27.55 ± 0.75	27.65 ± 0.78	0.656
Body fat (%)	37.69 ± 1.36	37.78 ± 1.39	37.78 ± 1.35	0.921
Fat mass (kg)	28.82 ± 1.67	28.85 ± 1.68	28.92 ± 1.64	0.958
Fat free mass (kg)	46.81 ± 1.73	46.65 ± 1.69	46.86 ± 1.74	0.525
Android fat (%)	45.67 ± 1.25	45.65 ± 1.28	45.79 ± 1.19	0.954
Gynoid fat (%)	42.16 ± 1.60	42.38 ± 1.61	41.99 ± 1.66	0.205
Android/Gynoid fat ratio	1.12 ± 0.04	1.11 ± 0.04	1.13 ± 0.04	0.055

Values are means ± SEM; n = 34.
P-values are shown for the treatment effect between groups analyzed by mixed model ANOVA (with the Bonferroni post hoc test for multiple comparisons).

Subsequent analysis revealed that gender influenced ($P < 0.001$) baseline and endpoint percent body fat, fat free mass, gynoid fat, and the android-to-gynoid fat ratio. However, further analysis revealed no gender x treatment interaction on these dependent variables. No effect of gender on baseline or endpoint fat mass and android fat was observed.

5.5 DISCUSSION

The present results demonstrate that consumption of the MUFA-rich HOCO diet or ALA-rich FXCO diet for 28-days as compared with a typical Western diet fatty acid profile does not modulate resting or postprandial energy expenditure and substrate oxidation. Data further suggest that consumption of the experimental oils in the context of energy controlled diets does not differentially affect body composition measures. Results of this study have important implications in further substantiating the role of fat quality, versus fat quantity, in the context of a typical Western diet on energy metabolism and the influence on body composition. Labelling fatty acids with stable isotope tracers is an effective means of measuring individual fatty acid oxidation and incorporation into tissues (28). Over 9 hours, cumulative ^{13}C recovery in breath revealed an oxidation order of laurate (12:0) > ALA (18:3) > elaidate (*trans*18:1) > OA (*cis*18:1) > LA (18:2) > palmitate (16:0) > STA (18:0) (9). These results were similar to those of Jones et al. (1985) investigating the oxidation of labeled 18-carbon fatty acid in 6 healthy men and observing an increase oxidation of OA > LA > STA (29). However, few human studies have examined the effect of consumption of dietary fat ranging in fatty acid profile on whole-body fat oxidation, which includes oxidation of both dietary fatty acids and those produced via *de novo* lipogenesis. Because specific fatty acids regulate transcription factors (30), the assessment of total whole-body fat oxidation and energy expenditure using indirect calorimetry may provide more insight into the metabolic role of dietary fat.

Our finding of no effect of dietary fatty acids on resting and postprandial energy expenditure, as well as substrate oxidation, coincides with those of recent studies (17-19,

31). After feeding a high-fat diet rich in either MUFA or SFA to eight healthy men, Cooper et al. (2009) (17) failed to observe a difference in resting or 24-hour energy expenditure measures using a metabolic chamber. However, discrepancies from human trials remain as increasing the ratio of MUFA to SFA (12-14,16) or PUFA to SFA (11,16) in the diet has previously been shown to increase fat oxidation, TEF, or both. Several potential mechanisms have been proposed to suggest larger contribution of dietary unsaturated fatty acid to thermogenesis than SFA, namely faster gastric emptying (32), increased intestinal absorption (29,33), and preferential hepatic oxidation (9,29). More specifically, MUFA and PUFA have been shown to be more effective than SFA in upregulating PPARα expression, stimulating the transcription of genes involved in fat oxidation and thermogenesis while suppressing the genes regulating fatty acid synthesis (30,34). Despite these proposed mechanisms of action, no changes in fat oxidation or thermogenesis were noted in the present study.

Potential explanation for the discrepancy between the present results observing no effect of dietary fat on thermogenesis compared with previous studies may be related to differences the caloric load and fat content of the diet. Previous studies may have magnified the metabolic response to dietary fat intake by administering >50% of energy from total fat (15-18) or >20 of energy from SFA (12,14,16,19). Although the present study provided ~37% energy from total fat and utilized a WD control containing ~11% of energy from SFA, fatty acid intakes more typical of current Western intakes (35), the moderate fat content of the diets may not have mechanistically challenged endogenous lipid trafficking sufficiently to alter energy expenditure and fat oxidation. Another dietary

consideration is the influence plant versus animal derived fat on thermogenesis and substrate oxidation. Previous studies have reported an increase in postprandial fat oxidation rates and/or thermogenesis after unsaturated fats from vegetable origin versus that of animal fats from dairy products (13,14,16). Conversely, the experimental oils investigated in the present study were predominately vegetable fats, with only a small content of the WD control containing fat from animal origin (12% non-salted butter).

Energy expenditure and substrate oxidation play critical roles in fat balance and fat stores (36), and may influence weight gain and obesity in humans. Animal studies have reported a simultaneous increase in diet-induced thermogenesis and decrease in body fat deposition following MUFA and PUFA rich diets as compared with SFA rich diets (37,38). In humans, Kein et al. (2005) (12) demonstrated a simultaneous decrease in fat oxidation and an increase in fat mass with high-SFA versus high-MUFA diets. However, Piers et al. (2003) (19) failed to observe a correlation between changes in fat mass and postprandial fat oxidation after 4-week consumption of high-SFA or high-MUFA diets, attributing the favourable modifications in body composition to reduced energy intake and/or increased physical activity after the high-MUFA diet. In the present study after 4-weeks of dietary intervention, no changes in endpoint values of body composition were observed between diets, consistent with the lack of effect on energy expenditure or substrate oxidation. In spite of this, there was a trend towards an increase in the android-to-gynoid ratio after consumption of the FXCO diet compared with the HOCO diet, however, this did not reach statistical significance. An increase in the android-to-gynoid ratio is considered unfavorable, as shifts of adipose deposition to the android region,

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reflecting adiposity in the abdomen, are generally associated with an increase in CVD risk (39). Previously, we reported a decrease in CVD risk factors, serum lipids and inflammatory biomarkers after consumption of the FXCO diet (22). Further statistical assessment revealed no correlation between the android-to-gynoid ratio with measured CVD risk factors after the FXCO diet. Nevertheless, although not significant, the slight increase in the android-to-gynoid ratio after the ALA-rich FXCO diet cannot be explained and the long-term effects require further investigation.

A strength of the present study includes the use of a 4-week supervised diet-controlled design, thus reducing a confounding effect of the antecedent diet on energy expenditure and substrate oxidation (40,41). Furthermore, TEF accounts for ~10% total daily energy expenditure and is strongly subject to intraindividual variation (42). Genetic polymorphisms of transcription factors (43) and uncoupling proteins (44) regulating energy expenditure and substrate metabolism may have altered subjects' response to dietary interventions regardless of the study duration. Therefore, the use of a crossover design reduces the influence of genetic and other interindividual variations between subjects (45). Potential limitations of the study may include the limited 6-hour period for data collection during indirect calorimetry as previous studies using stable isotopes reveal peak oxidation of fatty acid at approximately 6 hours after substrate administration (9,29). However, the present results coincide with recent results observing no change in 24-hour energy expenditure measured in a metabolic chamber after consumption of MUFA-rich compared with SFA-rich diets (17). Considering the present data, a future study would require approximately 55 subjects to demonstrate a relevant 10% increase in

postprandial energy expenditure after consumption of a diet rich in unsaturated fatty acids versus SFA at a 0.05 significance level with a power of 80%. Therefore, future human intervention studies are still needed before it can be determined with confidence if fatty acid composition affects thermogensis.

In summary, the present study demonstrated that in the context of current Western macronutrient intakes, altering dietary fatty acid composition had no major effect on whole-body energy expenditure, substrate oxidation or body composition during the 4-week controlled dietary intervention. While the long-term effects on obesity prevention require further investigation, results suggest that substitution of dietary fats typical to the Western diet with HOCO alone, or blended with flaxseed oil, does not module energy and weight balance.

5.6 ACKNOWLEDGEMENTS & AUTHORS' CONTRIBUTIONS

LGG was responsible for subject recruitment, management of the clinical trial, data collection, laboratory and statistical analysis, and writing the manuscript. KSR contributed to subject recruitment, was the clinical coordinator for the trial, and assisted in conducting indirect calorimetry and DEXA measures and collecting data. PJHJ was responsible for the conception and design of the project, submission for ethical approval and acquiring financial support. All authors contributed to revisions of the manuscript and review of the final version. The authors declare no conflict of interest.

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BRIDGE TO CHAPTER VI

Using a diet-controlled randomized crossover design, Chapter IV demonstrated that high-oleic canola oil and the flaxseed/high-oleic canola oil blend effectively modulates circulating serum lipid levels. Furthermore, the flaxseed/high-oleic canola oil blend may provide additional cardioprotective benefits by targeting a reduction in E-selectin levels in hypercholesterolemic men and women. However, using a controlled dietary regimen in the context of typical Western macronutrient intakes, no effects on body composition or substrate utilization and energy expenditure were observed in Chapter V after incorporation of the experimental oils. Although indirect calorimetry effectively measures total whole-body fat oxidation, stable isotopes more precisely trace the metabolic fate of individual fatty acids. Therefore, there is specific interest regarding the impact of dietary fat on the metabolism of ALA as assessed by stable isotope tracers.

It is well-established that the conversion of ALA to EPA and DHA is limited, however, the influence of enhanced consumption of ALA conversion to LCPUFA is unclear. While ALA is the primary n-3 PUFA in the diet, DHA is the predominant n-3 PUFA in cell and tissue membranes. Given that the cardioprotective effects of EPA and DHA have been substantiated and considering the sustainability of current wild fish resources are being challenged, there is much interest if ALA can provide a functional source of endogenous EPA and DHA. In addition, recent evidence demonstrates that single nucleotide polymorphisms in the FADS1/FADS2 gene cluster can influence the conversion of ALA to EPA and DHA. Therefore, a defect in the activity of $\Delta 5$- and $\Delta 6$-desaturases may indeed be a risk factor for CVD, downregulating the biosynthesis of EPA and DHA and

influencing the health outcomes associated with increased ALA consumption.

Using stable isotope tracers, the following study evaluates the apparent conversion of ALA to EPA, DPA and DHA, as well as β-oxidation of ALA in response to enhanced ALA consumption from high-oleic canola oil and the flaxseed/high-oleic canola oil blend. In addition, the following chapter will delineate whether genetic variations in the FADS gene cluster interact with increased dietary intakes of ALA to affect serum lipids, inflammatory biomarkers, plasma fatty acid profiles, and ALA conversion efficiency.

CHAPTER VI

MANUSCRIPT 5

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DIETARY OILS AND FADS1-FADS2 GENETIC VARIANTS MODULATE ^{13}C-ALPHA-LINOLENIC ACID METABOLISM AND PLASMA FATTY ACID COMPOSITION

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6.1 ABSTRACT

Objective: Desaturation of dietary ALA to omega-3 (n-3) long-chain polyunsaturated fatty acids (LCPUFA) is mediated through fatty acid desaturases (FADS1-FADS2) and may be influenced by dietary FA composition. The objective was to investigate effects of diets enriched in flaxseed oil (FXCO) or high-oleic canola oil (HOCO) versus a Western Diet (WD) fat blend and FADS1-FADS2 single nucleotide polymorphisms (SNP) on plasma fatty acids, as well as [U-^{13}C]ALA apparent conversion and β-oxidation.

Materials/Methods: Using a randomized crossover design, 36 hyperlipidemic subjects consumed 3 isoenergetic diets for 28 days enriched in FXCO (20.6 g/d ALA), HOCO (2.4 g/d ALA), or WD (1.3 g/d ALA). On day 27, blood was sampled at $t = 0$, 24, and 48 hours after subjects consumed 45 mg of [U-^{13}C]ALA. Subjects were genotyped for rs174537, rs174545, rs174561, and rs174583 in the FADS1-FADS2 gene cluster.

Results: FXCO increased plasma ALA ~5-fold ($P<0.001$), EPA ~3-fold ($P<0.001$), and DPA ~1.5-fold ($P<0.001$), with no change in DHA compared with HOCO or WD diets. At 24 and 48 hours, [U-^{13}C]ALA recovered as plasma ^{13}C-EPA and ^{13}C-DPA was lower ($P<0.001$) after FXCO diet compared with HOCO and WD diets. No change in ^{13}C-DHA was observed between diets. At 48 hours post-dose, [U-^{13}C]ALA cumulative oxidation was similar (~19%; $P=0.788$) between diets. Minor allele homozygotes of selected FADS genotypes had lower ($P<0.05$) plasma composition of EPA, AA, EPA/ALA, AA/LA and lower ($P<0.05$) ^{13}C-EPA at 24 and 48 hours compared with major allele carriers following all diets. *Conclusion:* Very high ALA intake by minor allele homozygotes compensated for lower apparent FADS activity, as determined using stable isotope [U-^{13}C]ALA, resulting in increased plasma composition of cardioprotective EPA.

6.2 INTRODUCTION

Considerable research supports a reduction in cardiovascular disease risk (CVD) with increased consumption and high plasma concentrations of omega-3 (n-3) polyunsaturated fatty acids (PUFA) (1-3). Alpha-linolenic acid (ALA; 18:3n-3), the plant-derived essential n-3 PUFA, is the precursor for the biosynthesis of eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DPA; 22:5n-3), and docosahexanoic acid (DHA; 22:6n-3) (4). The documented cardioprotective benefits of increased EPA and DHA status include anti-inflammatory, anti-thrombotic, anti-hypertensive and anti-arrhythmic effects, as well as positive actions on circulating lipid concentrations (1,5-7). While the specific cardiovascular effects attributed to ALA remain unclear, ALA is the predominate n-3 PUFA in the diet and increased consumption of ALA may be particularly cardioprotective for individuals with low combined EPA and DHA intake and status (2).

Both dietary and metabolic factors are known to affect plasma and tissue PUFA levels. The desaturation of dietary precursor ALA to EPA and DHA, as well as n-6 PUFA linoleic acid (LA; 18:2n-6) to arachidonic acid (AA; 20:4n-6), is mediated through two key enzymes, delta (Δ)6-desaturase and Δ5-desaturase (4). Although increased consumption of ALA has been shown to increase net plasma and tissue EPA concentrations, studies using stable isotope tracers and ALA supplements suggest that ALA is readily oxidized and undergoes limited enzymatic conversion, ranging from 0.2 to 8% for EPA and less than 0.05 to 4% for DHA (4,8). Furthermore, the efficiency of ALA conversion may be dependent on dietary factors, including dietary fatty acid composition (9,10). Few studies have investigated the extent of increased consumption of

ALA on metabolic conversion and oxidation using ^{13}C-labeled ALA. Therefore, investigating dietary strategies that augment ALA conversion and improve n-3 PUFA status furthers our understanding of the cardiovascular effects of individual PUFA, namely ALA and EPA.

In addition to dietary factors, studies suggest that plasma and tissue concentrations of n-6 and n-3 PUFA are strongly associated with several common single nucleotide polymorphisms (SNP) in desaturase genes FADS1 and FADS2, encoding for Δ5- and Δ6-desaturases, respectively (11-13), as well as elongase genes ELOVL2 (14). Recently, Martinelli et al. (2008) and Bokor et al. (2010) reported an association between various FADS polymorphisms and estimated desaturase activity as determined by product-to-precursor ratio (i.e. AA/LA or EPA/ALA) (15,16). However, to our knowledge, studies have not investigated FADS genetic variants and their association with [U-^{13}C]ALA conversion and oxidation in humans, a more precise measure of desaturase activity. Consequently, genetic variants that influence LCPUFA biosynthesis and status may impact traditional and emerging biomarkers of CVD risk.

Therefore, the aim of this study was to investigate the efficiency of uniformly labelled [U-^{13}C]ALA apparent conversion to LCPUFA and β-oxidation in response to enhanced ALA consumption in the form of high-oleic canola oil (HOCO) and a flaxseed/high-oleic canola oil blend (FXCO). A secondary objective was to examine effects of SNPs in FADS1, FADS2, and ELOVL2 on plasma fatty acid composition, [U-^{13}C]ALA apparent conversion and oxidation, serum lipids and plasma inflammatory biomarkers, and if SNP

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associated changes in plasma fatty acid composition could be compensated for by substantially increasing ALA intake.

6.3 EXPERIMENTAL METHODS

6.3.1 Subjects

Thirty-nine individuals (14 males and 25 females) were recruited using flyers, newspaper and radio advertisements. Fasting blood was sampled and screened for biochemical and haematological parameters. Exclusion criteria included history of atherosclerotic disease, inflammatory disease, diabetes, uncontrolled hypertension, kidney disease, cancer, smoking, use of prescription and natural lipid lowering medications, chronic alcohol consumption (>2 servings/day), or excessive exercise expenditure (>4000 kcal/week). The study was conducted according to the principles expressed in the Declaration of Helsinki. Study procedures were approved by the University of Manitoba's Biomedical Research Ethics Board (Protocol no. B2007:071 and B2009:129). All subjects provided written informed consent. This study was registered with ClinicalTrials.gov (Identifier #NCT00927199).

6.3.2 Experimental Design

The study utilized a 3-phase randomized, single-blind, crossover design. Each study phase consisted of a 4-week controlled dietary intervention separated by 4–8 week washout periods during which subjects consumed their habitual diet. Prior to starting the study and during the duration of the study, subjects were instructed to avoid consumption of fish and fish oil supplements. During each study phase, subjects consumed only foods

provided by the metabolic kitchen at the Richardson Centre for Functional Foods and

Nutraceuticals (RCFFN) and were prohibited from consuming alcohol and caffeinated

beverages. The treatment diets consisted of three isoenergetic meals distributed using a 3-

day meal cycle. Subjects consumed breakfast meals under supervision at the RCFFN

daily, with lunch and dinner meals prepared for take out. To avoid fluctuations in body

weight, subject's individual daily energy requirements were determined using the Mifflin

equation (17), then applying an activity factor of 1.7 for medium physical activity. Food

ingredients were prepared within 0.5 g of each subject's individual calculated energy

requirements. Body weights were assessed daily before breakfast to monitor weight

maintenance. If body weight fluctuated during the first week of the study, energy intake

was adjusted accordingly and maintained in each study phase. Throughout the study,

subjects were instructed to maintain their physical activity levels and report changes in

health or medication.

6.3.3 Experimental Diets

Fatty acid profiles of the experimental oils and macronutrient profile of the experimental

diets have been reported previously (18). Experimental diets were comparable in

composition containing approximately 49% of energy as carbohydrate (approximately

3% of energy as fibre), 14% as protein and 37% as fat. The experimental oils provided

70% of fat intake, and therefore, altered the fatty acid composition of the experimental

diets. The experimental oils tested included 1/ HOCO (approximately 70% oleic acid;

Canola Harvest HiLo®; Richardson Oilseed Limited, Lethbridge, AB, Canada); 2/ a 1:1

blend of the HOCO and flaxseed oil (FXCO) (approximately 55% ALA; Bioriginal Food

& Science Corporation, Saskatoon, SK, Canada); 3/ a blend of oils typical of a Western

Diet (WD control) including non-salted butter (12%), extra-virgin olive oil (35%),

vegetable shortening (35%), and sunflower oil (>60% linoleic acid) (18%). Experimental

oils were blended into milkshakes at breakfast and puddings at lunch and dinner. **Table**

6.1 outlines the macronutrient composition of the test meals administered at breakfast for

the stable isotope tracer substudy, as analyzed by Food Processor version 7.81 (ESHA

Research; Salem, OR, USA).

Table 6.1: Energy and macronutrient profile of the three breakfast test meals used in the stable isotope tracer substudy[1].

	Western diet		High-oleic canola oil diet		Flaxseed and high-oleic canola oil diet	
	g/meal	% of Energy	g/meal	% of Energy	g/meal	% of Energy
Energy (kcal/day)	711.0		706.5		708.5	
Carbohydrate	84.6	47.6	84.6	47.9	84.6	47.8
Fiber	4.5	2.5	4.5	2.5	4.5	2.5
Protein	25.6	14.4	25.6	14.5	25.6	14.4
Fat	29.8	37.7	29.8	38.0	30.0	38.1
SFA	8.7	11.0	3.4	4.4	3.7	4.7
MUFA	14.2	18.0	20.1	25.6	13.1	16.6
PUFA	6.8	8.6	5.6	7.1	12.7	16.2
LA (18:2n-6)	6.2	7.9	4.8	6.2	4.8	6.1
ALA (18:3n-3)	0.5	0.6	0.7	0.9	7.9	10.0
n-6/n-3 ratio	12.2		6.8		0.6	

[1]The energy and macronutrient profile of the three breakfast test meals were based on a 2500 kcal/day projected diet and estimated using FOOD PROCESSOR software (version 7.81; Food Processor, Salem, OR, USA). ALA, alpha-linolenic acid; LA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

6.3.4 Administration of [U-^{13}C]Alpha-Linolenic Acid and Sample Collection

Uniformly labelled [U-^{13}C]ALA (Spectra Stable Isotopes, Columbia, MD, isotopic purity

>98% enriched) was mixed in margarine at a ratio of 1:100 (w/w), respectively. On day

27 of each phase, 12 hour fasted blood was sampled to determine background enrichment of ^{13}C-labeled ALA, EPA, DPA and DHA in plasma. Then, breath samples were collected to measure background $^{13}CO_2$ excretion using a breath collection bag fitted to a mouthpiece with a gas collection port (EasySampler^{TM}, Quintron Instrument Co., Milwaukie, WI) where vacuum tubes were inserted to collect expired breath samples. Thereafter, subjects simultaneously consumed their treatment milkshake and their standardized breakfast meal containing 45 mg of [U-^{13}C]ALA dissolved in 4.5 g of margarine and spread on an English muffin with jam, an egg omelette, and juice. The breakfast meal and treatment milkshake was consumed by all participants within 10 minutes after administration of the tracer. Breath samples were collected at 1 hour intervals for the first 8 h after tracer intake. Between hour 4 and 5 after tracer intake, subjects consumed a standardized lunch. Fasting blood and breath were further sampled at 24 and 48 hours post tracer dose. Blood was sampled in EDTA-containing tubes, centrifuged within 1 hour of blood collection. Plasma was separated from red blood cells and the buffy coat layer after centrifugation at 3000 rpm for 20 min at 4^oC, aliquoted and immediately stored at -80^OC until analysis. Breath sampled in vacuum tubes were stored at room temperature until further analysis.

6.3.5 Sample Analysis

Serum lipids and plasma inflammatory biomarkers, including CRP, IL-6, E-Selectin, VCAM-1 and ICAM-1 were measured as previously described (18). Total lipids were prepared from plasma by extraction (19) with chloroform-methanol (2:1 v/v) containing 0.01% BHT (Sigma-Aldrich, Oakville, ON, Canada) using heptadecanoic acid as an

internal standard (Sigma-Aldrich, Oakville, ON, Canada). Fatty acid extracts were

methylated with methanolic HCL. The absolute fatty acid composition in plasma total

lipids was analyzed using an Agilent 6890N gas chromatograph (GC) equipped with a

flame ionization detector (Agilent Technologies, Mississauga, On, Canada) as previously

described in detail (18).

[13]C enrichment of n-3 fatty acid methyl esters from total plasma lipids were analyzed

using an Agilent 6890N GC (Agilent Technologies, Mississauga, On, Canada) coupled to

a Delta V Plus isotope ratio mass spectrometry (IRMS) via a Finnegan GC combustion

III interface (Thermo Fisher Scientific Inc., Bremen, Germany). The GC was equipped

with a split/splitless injector and the column was a fused silica SP-2560 capillary column

(100m X 0.25mm X 0.2μm film thickness; Supelco, Bellefonte, PA, USA). The GC oven

was programmed from 70°C to 250°C in four temperature steps (70°C for 2 min, rise

25°C/min, 190°C for 3 min, rise 2°C/min, 220°C for 15 min, rise 45°C, 250°C for 10

min). Samples were run with a 5.0 split ratio and He was used as the carrier gas with a

column flow rate of 0.8 ml/min. Temperature for the injector was set at 280°C.

Temperature for the combustion reactor was 960°C. Samples were run in duplicate with

all samples from the one subject analyzed in a single run. [13]C enrichment of CO_2 in

breath samples was analyzed by IRMS (ABCA, SerCon Ltd., Cheshire, UK) and

measured using ABCA breath analyzer software version 500.1.12 (SerCon Ltd.,

Cheshire, UK). A reference gas containing 5% CO_2 was used to calibrate the IRMS and

He was used as the carrier gas.

6.3.6 Stable Isotope Calculations

The difference between the $^{13}C/^{12}C$ ratio of both the plasma and breath samples was expressed as the delta ($\delta^{13}C$; in units of ‰ or per mil) and was normalized against the international standard Pee Dee Belemnite limestone (PDB), which has a $^{13}C/^{12}C$ ratio of 0.0112372. ^{13}C enrichment in the plasma and breath samples was calculated as:

$$\delta^{13}C_{PDB} = \frac{(R_S - R_{PDB})}{R_{PDB}} \times 1000 \qquad [1]$$

where R_S is the $^{13}C/^{12}C$ ratio in the sample, and R_{PDB} is the constant $^{13}C/^{12}C$ ratio of PDB = 0.0112372 (20). Atom percent ($AP^{13}C$) was calculated based on the equation described by Slater et al. (2001) (20) as:

$$AP^{13}C = \frac{100}{\dfrac{1}{\left(\dfrac{\delta}{1000} + 1\right) R_{PDB}} + 1} \qquad [2]$$

where δ is measured $\delta^{13}C_{PDB}$. ^{13}C Enrichment of the sample at each time interval of interest above the baseline level (background ^{13}C enrichment) is expressed as atom percent excess (APE) and calculated as:

$$APE = (AP^{13}C)_{tn} - (AP^{13}C)_{t0} \qquad [3]$$

where $AP^{13}C$ is derived from equation [2], therefore $(AP^{13}C)_{tn}$ is the measured abundance of the enriched sample at time n (i.e. 24 hour), while $(AP^{13}C)_{t0}$ is the measured abundance of the baseline sample before tracer administration (20).

6.3.7 Estimation of ^{13}C Fatty Acid Oxidation

Percent dose recovered (PDR) per minute from β-oxidation of [U-^{13}C]ALA in breath was calculated based on the equation described by Freemantle et al. (2008) (21) as:

$$PDR_{ox} = \frac{APE \times VCO_2}{mmol\ {}^{13}C\ \text{administered}} \times 100\% \qquad [4]$$

where APE is derived from equation [3] and VCO_2 is calculated by multiplying the CO_2 production constant (300 mmol/hour) by body surface area (21). The amount of ^{13}C administered (mmol) was calculated based on the equation described by McCloy et al. (2004) (22) as:

$$mmol\ C = \frac{mg\ [U-{}^{13}C]ALA\ \text{administered}}{\text{molecular weight of}\ [U-{}^{13}C]ALA} \times \%\ \text{chemical purity} \times$$
$$[(0.99 \times \#\ {}^{13}C) + (0.01 \times total\ \#C)] \qquad [5]$$

where the chemical purity of administered $[U-{}^{13}C]ALA$ was 98% and the isotopic purity was 0.99 accounting for 99% labelling efficiency of administered $[U-{}^{13}C]ALA$ (isotopic purity), and 0.01 accounting for the 1% of naturally occurring ^{13}C in administered $[U-{}^{13}C]ALA$. Area under the curve of PDR was calculated using GraphPad Prism version 4.0c (GraphPad Software, Inc., La Jolla, CA, USA) to determine cumulative oxidation of administered $[U-{}^{13}C]ALA$.

6.3.8 Calculation of ^{13}C Enrichment in Plasma Fatty Acids

To calculate the absolute amount (mg) of $[U-{}^{13}C]ALA$ dose recovered in plasma n-3 fatty acid pools of interest, the differences in relative tracee enrichments attributed to variation in tracee pool sizes after consumption of the three experimental diets needed to be taken into account (10). Therefore, the absolute amount of ^{13}C-labelled fatty acid was calculated by correcting for the plasma concentration of this specific fatty acid quantified by GC:

$$\text{Dose}\ (mg)\ {}^{13}C\ \text{recovered} = APE \times \text{fatty acid poolsize} \times$$
$$\text{molecular weight of}\ C\ \text{in fatty acid} \qquad [6]$$

where APE is derived from equation [3], fatty acid pool size (mg) is calculated based on plasma volume of 4.5% of body weight (23) multiplied by the fatty acid concentration, and the molecular weight of carbons in the plasma fatty acid pool of interest. Percent dose recovered of administered $[U\text{-}^{13}C]$ALA in plasma n-3 fatty acid pools of interest was then calculated as:

$$PDR_{Plasma} = \frac{\text{dose (mg) }^{13}C \text{ recovered}}{\text{dose (mg) }^{13}C \text{ administered}} \times 100\% \qquad [7]$$

where dose (mg) ^{13}C recovered is derived from equation [6], and dose (mg) ^{13}C administered is 45 mg dose administered multiplied by molecular weight of the carbons in the $[U\text{-}^{13}C]$ALA.

6.3.9 Single Nucleotide Polymorphism Genotyping

To examine the influence of specific desaturation and elongation enzyme genetic variants on $[U\text{-}^{13}C]$ALA conversion and plasma fatty acid composition, study subjects were genotyped for five selected SNP, rs174545, rs174583, rs174561, rs174537, and rs953413, that have been reported to be associated with differences in plasma n-6 and n-3 PUFA composition (11-15). Genomic DNA was extracted from white blood cells using commercial QIAGEN #69504 DNeasy Blood and Tissue Kit according to the manufacturer's instructions (QIAGEN Sciences, Maryland, USA). The concentration and integrity of the genomic DNA was assessed by Thermo Scientific NanoDrop 2000 micro-volume spectrophotometer (Thermo Fisher Scientific, Wilmington Delaware, USA). DNA samples were genotyped using the ABI #4403311 TAQMAN GTXPRESS MASTER MIX on Applied Biosystems StepOnePlus Real-Time PCR System and Applied Biosystems Taqman assays.

6.3.10 Statistical Analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Results are expressed as means ± SEM unless otherwise noted. Effects of dietary treatment on plasma fatty acid composition and [U-^{13}C]ALA recovery in plasma and breath samples were analyzed using linear mixed model ANOVA with subject as a random factor and treatment as an independent factor. Repeated measures were used to examine the existence of effects of time and time x treatment interaction. The effect of dietary treatment, sequence, phase and gender were included in the model as fixed factors. Significant treatment effects were examined using Bonferroni post hoc tests for multiple comparisons. Pearson correlation analyses were conducted to test associations. Deviations from Hardy-Weinberg proportions for the genotypes of each SNP were tested using chi-square tests. Each SNP was analyzed separately and categorized as homozygous for the major allele (coded 11), heterozygous (coded 12), and homozygous for the minor allele (coded 22). The mean serum lipids, plasma inflammatory biomarkers, plasma fatty acid % composition and ^{13}C-labelled fatty acid composition within each treatment group were compared among the major and minor allele homozygotes and heterozygotes using Kruskal-Wallis test. Mann-Whitney U tests were used to determine differences between major allele carriers and minor allele homozygotes for the variables of interest. Statistical significance was set at $P < 0.05$ for all analyses.

6.4 RESULTS

6.4.1 Subject Characteristics

Thirty-six hypercholesterolemic individuals (13 males and 23 females; 5

postmenopausal) completed the 3-phase study design and were genotyped for selected
SNPs. One subject withdrew from the study due to work-related issues and two subjects
withdrew due to relocation of residence. The men and women were on average 46.5 ±
11.6 y and 48.4 ± 12.3 y (mean ± SD), weighed 89.5 ±12.8 kg and 72.7 ±16.4 kg, and had
a body mass index of 29.8 ±3.9 and 27.9 ± 4.9 kg/m^2, respectively. The baseline
characteristics, including lipid and inflammatory biomarker concentrations, of the study
population (n=36) have been previously published (18). The stable isotope study was
conducted in a subset of subjects (n=26).

6.4.2 Plasma Total Fatty Acid Composition

No differences in baseline plasma fatty acid % composition were noted between the
treatment groups, indicating no carryover effect of the dietary intervention and adequate
washout periods. For n-3 PUFA, endpoint plasma composition of ALA was >5-fold
higher ($P < 0.001$) after consumption of the FXCO diet (4.42 ± 0.24% total fatty acids)
than after the HOCO diet (0.86 ± 0.04%) and the WD control (0.70 ± 0.03%). Plasma
composition of EPA was ~3-fold higher ($P < 0.001$) after consumption of the FXCO diet
(1.66 ± 0.13%) than after the HOCO diet (0.60 ± 0.04%) and the WD control (0.49 ±
0.04%). Consequently, the ratio of plasma EPA/ALA was lower ($P < 0.001$) after
consumption of the FXCO diet (0.40 ±0.04) than after the HOCO diet (0.72 ± 0.06) and
the WD control (0.72 ± 0.06). Plasma composition of DPA was ~1.5-fold higher ($P <$
0.001) after consumption FXCO diet (0.76 ± 0.03%) than after the HOCO diet (0.54 ±
0.03%) and the WD control (0.54 ± 0.02%). However, plasma composition of DHA was
similar ($P = 0.205$) between the FXCO diet (1.49 ± 0.05%), the HOCO diet (1.55±

0.06%) and the WD control (1.53 ± 0.06%). For the n-6 PUFA of interest, endpoint

plasma composition of LA was lower ($P < 0.001$) after consumption of the HOCO diet

(27.13 ± 0.57%) than after the FXCO diet (28.83 ± 0.55%) and the WD control (30.55 ±

0.51%). Furthermore, plasma composition of LA differed ($P < 0.001$) between the FXCO

diet and the WD control. Plasma composition of AA was lower ($P < 0.001$) after

consumption of the FXCO diet (5.46 ± 0.22%) than after the HOCO diet (6.54 ± 0.29%)

and the WD control (6.82 ± 0.31%). Plasma composition of AA differed ($P = 0.048$)

between the HOCO diet and the WD control. Consequently, the ratio of plasma AA/LA

was lower ($P < 0.001$) after consumption of the FXCO diet (0.19 ± 0.01) than after the

HOCO diet (0.24 ± 0.01) and the WD control (0.22 ± 0.01). Furthermore, the AA/LA

ratio differed ($P < 0.001$) between the HOCO diet and the WD control. In addition, the

ratio of plasma AA/EPA was lower ($P < 0.001$) after consumption of the FXCO diet

(3.72 ± 0.30) than after the HOCO diet (12.03 ± 0.77) and the WD control (15.04 ± 0.71),

and also differed ($P < 0.001$) between the HOCO diet and the WD control.

6.4.3 Enrichment of Stable Isotope in Plasma as ^{13}C-Labelled Fatty Acids

[13]C enrichment of plasma ALA, EPA, DPA and DHA were observed at both 24 and 48

hours post-dose. Differences in the tracee pool sizes after consumption of the treatment

diets for 4-weeks resulted in differential dilution of tracer between diets. Therefore, a

lower ^{13}C enrichment (APE) of ALA, EPA and DPA was observed after the FXCO diet

compared with the WD and HOCO diets ($P < 0.001$) at both 24 and 48 hours post-dose.

There was no difference in ^{13}C enrichment of DHA between the diets.

Following adjustments for dietary influences on plasma fatty acid pool size (Formula 6), percent dose of administered ^{13}C recovered in plasma ALA, EPA, DPA, and DHA at 24 and 48 hours post-dose is shown in **Table 6.2**. Furthermore, the mean absolute amount of plasma ^{13}C-labelled n-3 fatty acids is shown in **Figure 6.1**. Measured ^{13}C-ALA was highest in plasma total lipids 24 hours post-dose (**Figure 6.1A**). The mean plasma ^{13}C-ALA was higher after the FXCO diet (6.42 ± 0.53 mg; ~18% dose recovered) compared with the WD control (4.17 ± 0.52 mg; ~12% dose recovered; $P < 0.001$) and HOCO diet (4.38 ± 0.54 mg; ~13% dose recovered; $P < 0.001$). At 48 hours, plasma ^{13}C-ALA approached baseline levels, however, remained higher after the FXCO diet compared with the WD control ($P = 0.015$) and the HOCO diet ($P = 0.033$). Measured ^{13}C-EPA was highest in plasma total lipids 24 hours post-dose (**Figure 6.1B**). The mean plasma ^{13}C-EPA was highest after the HOCO diet (1.38 ± 0.17 mg: ~4.0% dose recovered) compared with the FXCO diet (0.83 ± 0.08 mg; ~2.4% dose recovered; $P < 0.001$) and WD control (1.09 ± 0.13 mg; ~ 3.1% dose recovered; $P = 0.017$). Furthermore, mean plasma ^{13}C-EPA at 24 hours post-dose was higher ($P = 0.043$) after the WD control compared with the FXCO diet. At 48 hours post-dose, mean plasma ^{13}C-EPA slightly decreased, however, remained higher after the HOCO diet compared with the WD control ($P = 0.013$) and FXCO diets ($P < 0.001$). At 48 hours post-dose, plasma ^{13}C-DPA was higher after the WD control (0.27 ± 0.04 mg; ~0.8% dose recovered; $P = 0.004$) and the HOCO diet (0.25 ± 0.04 mg; ~0.7% dose recovered; $P = 0.004$) compared with the FXCO diet (0.14 ± 0.02 mg; ~0.4% dose recovered; $P = 0.004$) (**Figure 6.1C**). At 24 hours post-dose, mean plasma ^{13}C-DHA was similar between diets (**Figure 6.1D**). However, at 48 hours post-dose mean plasma ^{13}C-DHA was higher after the WD control (0.09 ± 0.02 mg; ~0.3%

dose recovered) and the HOCO diet (0.08 ± 0.02 mg; ~0.2% dose recovered) compared

with the FXCO diet (0.03 ± 0.02 mg; ~0.1% dose recovered), although not significantly

so (P=0.146), and may be attributed to wide variation particularly after the FXCO diet.

Statistical analysis revealed no effect of gender on plasma ^{13}C-labelled fatty acid

composition.

Table 6.2: Percent dose of administered ^{13}C recovered in plasma ALA, EPA, DPA, and DHA at 24 and 48 hours after intake of a single dose of [U-^{13}C]ALA in experimental diets.

^{13}C-Fatty Acid	Western diet	High-oleic canola oil diet	Flaxseed and high-oleic canola oil diet	P-value
^{13}C-ALA				
24 h	11.97 ± 1.50[a]	12.56 ± 1.55[a]	18.41 ± 1.52[b]	<0.001
48 h	4.48 ± 0.52[a]	4.60 ± 0.45[a]	5.85 ± 0.64[b]	0.009
^{13}C-EPA				
24 h	3.13 ± 0.38[a]	3.95 ± 0.49[b]	2.39 ± 0.24[c]	<0.001
48 h	2.73 ± 0.29[a]	3.54 ± 0.37[b]	2.29 ± 0.21[a]	<0.001
^{13}C-DPA				
24 h	0.68 ± 0.11[a]	0.74 ± 0.09[a]	0.34 ± 0.04[b]	<0.001
48 h	0.77 ± 0.12[a]	0.73 ± 0.11[a]	0.41 ± 0.05[b]	0.001
^{13}C-DHA				
24 h	0.18 ± 0.04[a]	0.17 ± 0.05[a]	0.14 ± 0.04[a]	0.812
48 h	0.25 ± 0.06[a]	0.22 ± 0.06[a]	0.10 ± 0.05[a]	0.146

Values are means ± SEM; n=26. [a,b,c]Mean values within a row with unlike superscript letters were significantly different between treatment groups (P<0.05). P-values are shown for the treatment effect analyzed by mixed model ANOVA (Bonferroni post hoc test for multiple comparisons). ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; h, hour.

A/
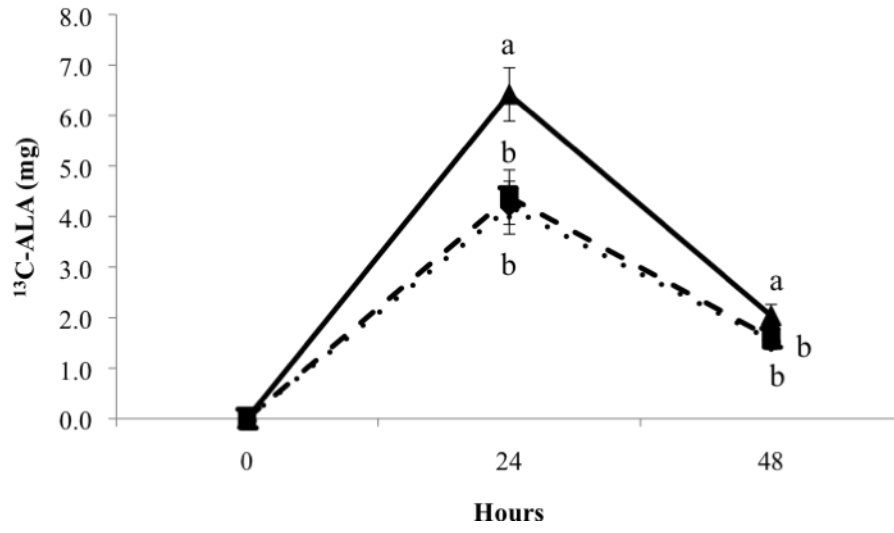
B/
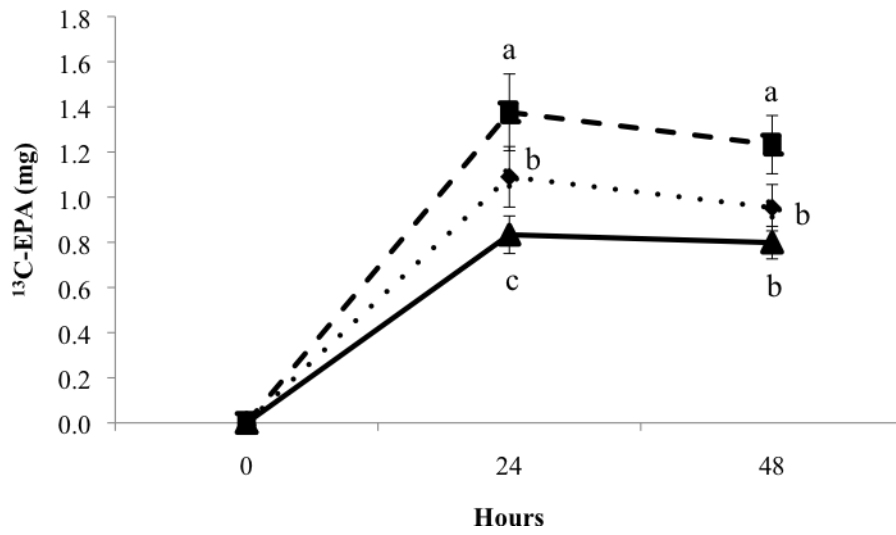
C/
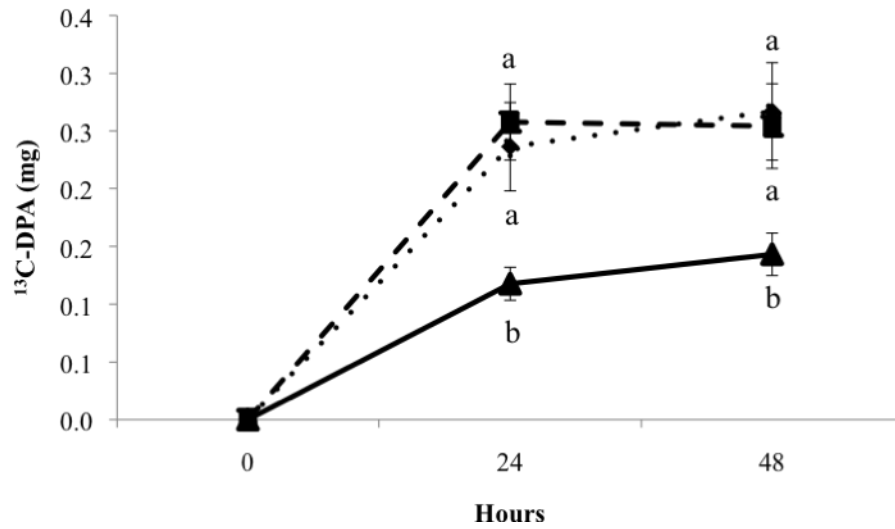
D/
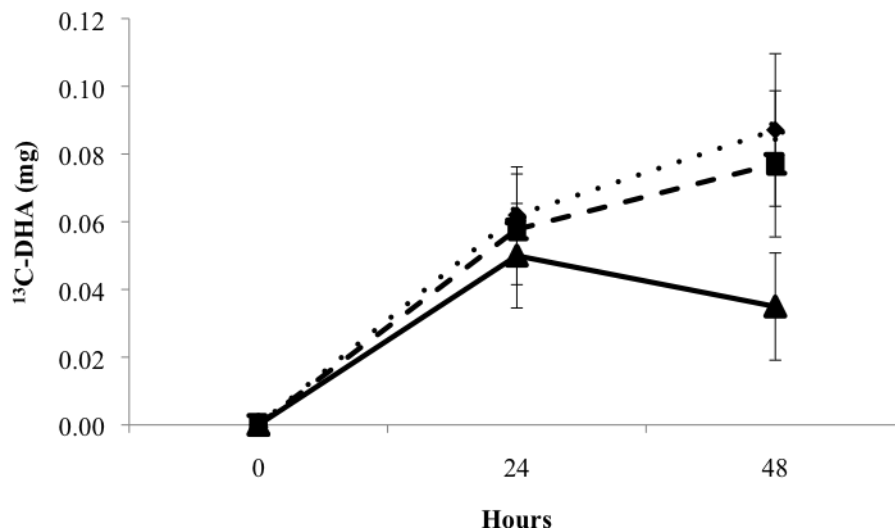
Figure 6.1: Absolute amount (mg) of administered ^{13}C recovered as plasma A/ ^{13}C-ALA; B/ ^{13}C-EPA; C/ ^{13}C-DPA; and D/ ^{13}C-DHA at 24 and 48 hours after intake of a single dose of [U-^{13}C]ALA in experimental diets: Western diet (dotted line), high-oleic canola oil diet (dashed line), flaxseed/high-oleic canola oil diet (solid line). Values are means ± SEM; n=26. Mean values with unlike superscript letters are significantly different between treatment groups at $P<0.05$ (mixed model ANOVA followed by Bonferroni post hoc test for multiple comparisons). ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.

At 48 hours post-dose Pearson correlation coefficients revealed a negative correlation between plasma ^{13}C-ALA and plasma EPA/ALA ratio ($r = -0.373$, $P = 0.001$), as well as AA/LA ratio ($r = -0.360$, $P = 0.001$). Conversely, a positive correlation was observed between plasma ^{13}C-EPA and plasma EPA/ALA ratio ($r = 0.504$, $P < 0.001$), as well as AA/LA ratio ($r = 0.488$, $P < 0.001$). No correlation was observed between plasma ^{13}C-DPA or ^{13}C-DHA and plasma EPA/ALA or AA/LA ratios. The Pearson correlation coefficients and P-values were similar at 24 hours post-dose to results at 48 hours post-dose (data not shown).

6.4.4 Beta-Oxidation of [U-^{13}C]Alpha-Linolenic Acid

The peak rate of [U-^{13}C]ALA β-oxidation recovered in breath was $3.4 \pm 0.1\%$ dose/hour after the WD control and $3.2 \pm 0.1\%$ dose/hour after the HOCO diet, and was reached at approximately 5 hours post-dose. In contrast, peak rate of oxidation was $3.3 \pm 0.2\%$ dose/hour after the FXCO diet, and was reached at approximately 6 hours post-dose. No differences existed in hourly oxidation rates between treatment groups (**Figure 6.2A**). Cumulative oxidation of ^{13}C recovered in breath reached 19.1 ± 0.6, 18.8 ± 0.6, and $18.7 \pm 0.7\%$ at 48 hours post-dose after consumption of the WD control, HOCO, and FXCO diets and was not significantly different between diets (**Figure 6.2B**).

A/

B/
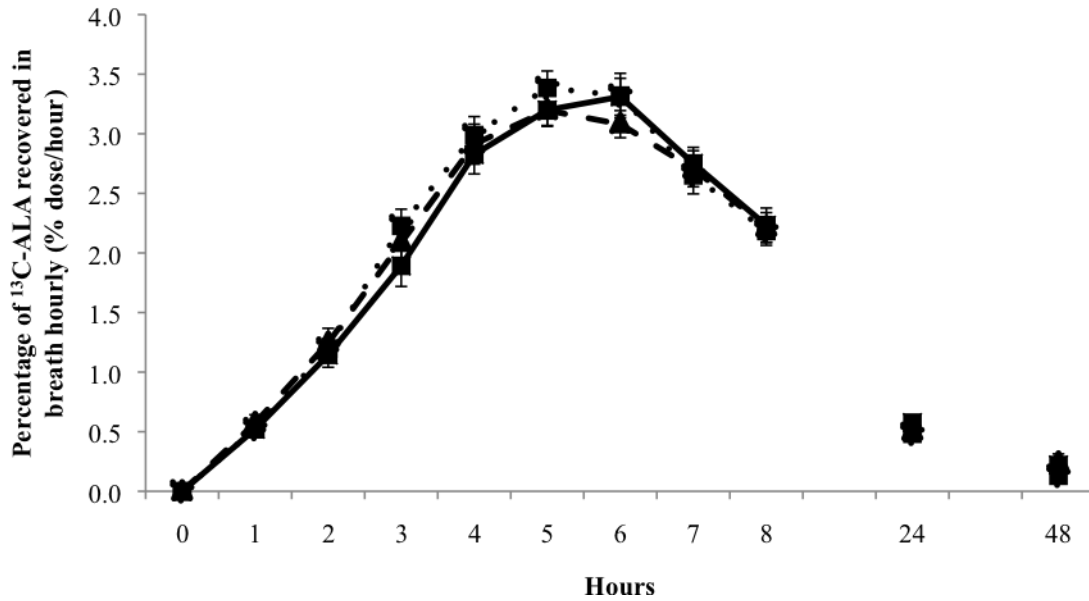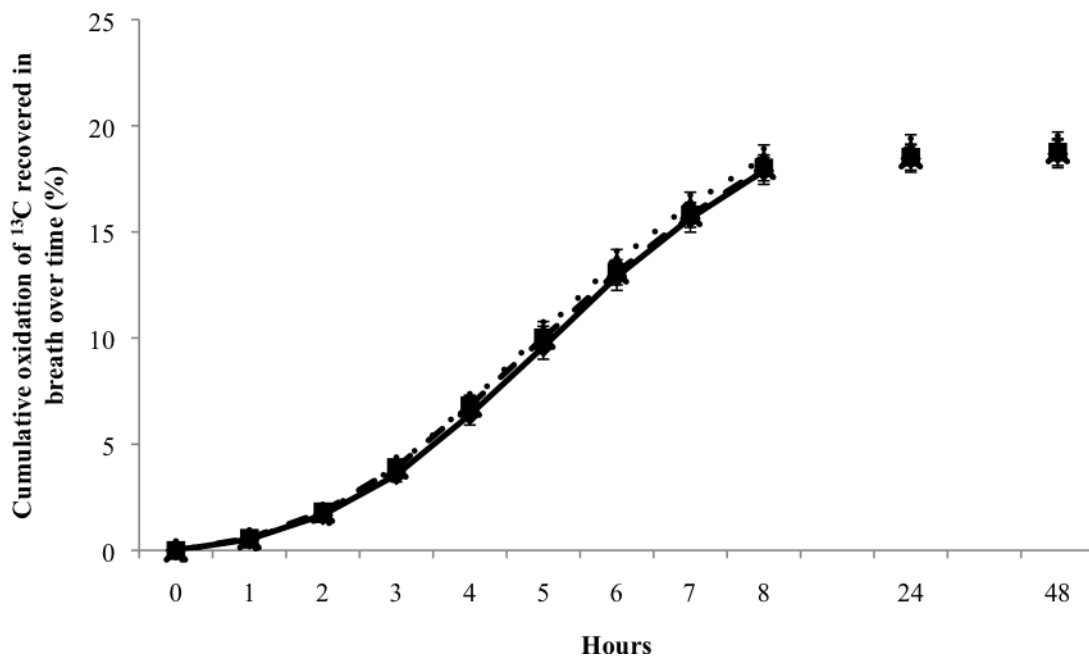
Figure 6.2: β-oxidation of administered ^{13}C shown as A/ percentage of dose recovered in breath as $^{13}CO_2$ hourly and, B/ cumulative recovery in breath as $^{13}CO_2$ over time after intake of a single dose of [U-^{13}C]ALA in experimental diets: Western diet (dotted line), high-oleic canola oil diet (dashed line), flaxseed/high-oleic canola oil diet (solid line). Values are means ± SEM; n=26.

Pearson correlation coefficients were calculated between cumulative oxidation of ^{13}C recovered in breath (AUC for 48 hours) and the percent dose recovered as plasma ^{13}C-labeled fatty acids at 48 hours. The cumulative recovery of $^{13}CO_2$ in breath was negatively correlated with plasma ^{13}C-ALA ($r = -0.260$, $P = 0.022$) and ^{13}C-DPA ($r = -0.347$, $P = 0.002$), but not with ^{13}C-EPA and ^{13}C-DHA.

6.4.5 Single Nucleotide Polymorphism Characteristics and Association with Plasma Fatty Acids

Minor allele frequencies in our study ranged between 34.7% and 41.7%. Genotype distribution for each SNP, in the whole group and the subset of subjects in the stable isotope analysis, did not deviate from Hardy-Weinberg equilibrium (**Table 6.3**). With respect to association of SNPs with plasma fatty acids at the end of each experimental diet, ELOVL2 (rs953413) was not associated with changes in plasma fatty acid % composition (Table III.9 – III.15 in "Appendix III"). For the n-6 PUFA, subjects homozygous for the minor allele for rs174537, rs174545, rs174561 and rs174583 had lower ($P < 0.001$) plasma composition of AA compared with major allele carriers after consumption of each experimental diet (**Table 6.4** and Table III.9 – III.10 in "Appendix III"). Whereas, only after consumption of the WD control did subjects homozygous for the minor allele for each of the 4 SNPs have lower ($P = 0.007$) plasma levels of gamma-linolenic acid (GLA; 18:3n-6) compared major allele carriers. Although no significant associations for the studied SNPs were observed for plasma LA, subjects homozygous for the minor allele for each of the 4 SNPs had lower ($P < 0.001$) plasma composition of AA/LA ratio compared with major allele carriers. Considering n-3 PUFA, the results for

Table 6.3: Characteristics of the selected single nucleotide polymorphisms associated with desaturation and elongation of fatty acids.

SNP	Gene	Chromosome Position (bp)[1]	Alleles (major/minor)	Genotype[2]			MAF %	HWE
rs174537	FADS1 intron	61309256	G/T	GG	GT	TT		
				14 (38.9)	18 (50.0)	4 (11.1)	36.1	0.881
rs174545	FADS1 UTR-3	61325882	C/G	CC	CG	GG		
				14 (38.9)	18 (50.0)	4 (11.1)	36.1	0.881
rs174561	FADS1 intron	61339284	T/C	TT	TC	CC		
				15 (41.7)	17 (47.2)	4 (11.1)	34.7	0.972
rs174583	FADS2 intron	61366326	C/T	CC	CT	TT		
				13 (36.1)	19 (52.8)	4 (11.1)	37.5	0.746
rs953413	ELOVL2 intron	11120845	G/A	GG	GA	AA		
				11 (30.6)	20 (55.6)	5 (13.9)	41.7	0.682

[1]Position in basepairs (bp) was derived from the National Center for Biotechnology (NCBI) dbSNP Build 136, based on NCBI Human Genome Build 36.3 (April 2012) of chromosome 11.

[2]Number of subjects for each genotype; percentage in parentheses

ELOVL, elongation of very long-chain fatty acids; FADS, fatty acid desaturase; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

plasma composition of EPA were similar with its n-6 PUFA counterpart AA with respect to the direction of the differences by genotype. Subjects homozygous for the minor allele for each of the 4 SNPs had lower ($P < 0.05$) plasma composition of EPA compared with major allele carriers (**Table 6.5** and Table III.11 – III.13 in "Appendix III"). Whereas, only after consumption of the FXCO diet did subjects homozygous for the minor allele for each of the 4 SNPs have lower ($P = 0.002$) levels of DPA compared with major allele carriers. Although no significant associations for the studied SNPs were observed for plasma ALA, subjects homozygous for the minor allele for each of the 4 SNPs had lower ($P < 0.005$) plasma composition of EPA/ALA ratio compared with major allele carriers. Of interest, although composition of EPA was lower in the subjects homozygous for the minor allele (n=4) for each of the 4 SNPs, plasma composition of EPA increased after consumption of the ALA-rich FXCO diet compared with the HOCO diet ($P = 0.048$) and WD control ($P = 0.036$) (**Table 6.5; Figure 6.3**). No significant associations for the studied SNPs were observed for other plasma fatty acid compositions, including dihomo-gamma-linolenic acid (DGLA; 20:3n-6), stearidonic acid (SDA; 18:4n-3), or DHA after consumption of the different experimental diets. Associations of SNPs in rs174561 (FADS1) and rs174583 (FADS2) with selected plasma n-6 and n-3 fatty acids at the end of each experimental diet are shown in **Tables 6.4** and **6.5**, respectively, and Table III.9 – III.14 in "Appendix III".

Table 6.4: Selected plasma n-6 polyunsaturated fatty acid concentrations (% of total) at the end of each experimental diet classified by rs174561 (FADS1) and rs174583 (FADS2) genotype.

Fatty Acid	Allele	rs174561 (FADS1)			rs174583 (FADS2)		
		Western Diet (Control)	High-Oleic Canola Oil Diet	Flax/High-Oleic Canola Oil Diet	Western Diet (Control)	High-Oleic Canola Oil Diet	Flax/High-Oleic Canola Oil Diet
18:2n–6 (LA)	MM	29.26 ± 0.85	26.76 ± 0.87	28.12 ± 0.95	28.63 ± 0.84	26.36 ± 0.96	27.40 ± 0.93
	Mm	30.31 ± 0.76	27.71 ± 0.67	29.46 ± 0.57	30.63 ± 0.71	27.88 ± 0.61	29.81 ± 0.58
	mm	30.99 ± 0.77	27.87 ± 0.85	27.96 ± 1.20	30.99 ± 0.77	27.87 ± 0.85	27.96 ± 1.20
	P	0.469	0.750	0.465	0.112	0.392	0.099
18:3n–6 (GLA)	MM	0.54 ± 0.04	0.54 ± 0.05	0.32 ± 0.03	0.55 ± 0.05	0.54 ± 0.06	0.33 ± 0.04
	Mm	0.41 ± 0.03	0.46 ± 0.04	0.29 ± 0.03	0.42 ± 0.03	0.47 ± 0.04	0.29 ± 0.02
	mm	0.21 ± 0.09[*]	0.29 ± 0.10	0.15 ± 0.06	0.21 ± 0.09[*]	0.29 ± 0.10	0.15 ± 0.06
	P	0.004	0.093	0.062	0.007	0.128	0.062
20:3n–6 (DGLA)	MM	1.66 ± 0.10	1.69 ± 0.07	1.01 ± 0.06	1.67 ± 0.11	1.57 ± 0.16	1.02 ± 0.06
	Mm	1.66 ± 0.07	1.69 ± 0.07	1.11 ± 0.05	1.65 ± 0.07	1.41 ± 0.11	1.09 ± 0.05
	mm	1.72 ± 0.19	1.79 ± 0.15	1.25 ± 0.17	1.72 ± 0.19	1.97 ± 0.07	1.25 ± 0.17
	P	0.858	0.399	0.204	0.881	0.377	0.362
20:4n–6 (AA)	MM	7.89 ± 0.35	7.67 ± 0.29	6.07 ±0.26	7.88 ± 0.40	7.63 ± 0.32	6.00 ± 0.29
	Mm	6.47 ± 0.25	6.30 ± 0.25	5.34 ± 0.18	6.63 ± 0.25	6.47 ± 0.26	5.47 ± 0.19
	mm	5.00 ± 0.04[*]	4.75 ± 0.14[*]	3.92 ± 0.17[*]	5.00 ± 0.04[*]	4.75 ± 0.14[*]	3.92 ± 0.17[*]
	P	<0.001	<0.001	0.003	0.001	<0.001	0.006
AA/LA ratio	MM	0.27 ± 0.01	0.29 ± 0.01	0.22 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	0.22 ± 0.01
	Mm	0.21 ± 0.01	0.23 ± 0.01	0.18 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.18 ± 0.01
	mm	0.16 ± 0.00[*]	0.17 ± 0.01[*]	0.14 ± 0.01[*]	0.16 ± 0.00[*]	0.17 ± 0.01[*]	0.14 ± 0.01[*]
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are means ± SEM; n = 36. MM represents homozygotes for the major allele, Mm represents heterozygotes, and mm represents homozygotes for the minor allele. P-values are analyzed by Kruskal-Wallis test. [*]Indicates mean values within column for minor allele homozygotes significantly differ from major allele carriers within SNP genotype analyzed by Mann-Whitney U test ($P < 0.05$). AA, arachidonic acid; DGLA, dihomo-gamma-linolenic acid; FADS, fatty acid desaturase; GLA, gamma-linolenic acid; LA, linoleic acid.

Table 6.5: Selected plasma n-3 polyunsaturated fatty acid concentrations (% of total) at the end of each experimental diet classified by rs174561 (FADS1) and rs174583 (FADS2) genotype.

		rs174561 (FADS1)			rs174583 (FADS2)		
Fatty Acid	Allele	Western Diet (Control)	High-Oleic Canola Oil Diet	Flax/High-Oleic Canola Oil Diet	Western Diet (Control)	High-Oleic Canola Oil Diet	Flax/High-Oleic Canola Oil Diet
18:3n–3 (ALA)	MM	0.74 ± 0.05	0.80 ± 0.05	4.48 ± 0.22	0.75 ± 0.05	0.79 ± 0.05	4.40 ± 0.24
	Mm	0.72 ± 0.04	0.89 ± 0.05	4.30 ± 0.32	0.72 ± 0.03	0.88 ± 0.05	4.38 ± 0.30
	mm	0.81 ± 0.06	0.82 ± 0.11	5.04 ± 0.31	0.81 ± 0.06	0.82 ± 0.11	5.04 ± 0.31
	P	0.568	0.442	0.317	0.564	0.394	0.367
20:5n–3 (EPA)	MM	0.64 ± 0.05	0.71 ± 0.05	2.17 ± 0.16	0.66 ± 0.05	0.71 ± 0.06	2.23 ± 0.18
	Mm	0.50 ± 0.04	0.61 ± 0.05	1.55 ± 0.10	0.50 ± 0.03	0.61 ± 0.04	1.58 ± 0.09
	mm	$0.32 \pm 0.07^{*}$	$0.36 \pm 0.11^{*}$	$0.91 \pm 0.19^{*}$	$0.32 \pm 0.07^{*}$	$0.36 \pm 0.11^{*}$	$0.91 \pm 0.19^{*}$
	P	0.009	0.045	<0.001	0.006	0.049	<0.001
22:5n–3 (DPA)	MM	0.57 ± 0.03	0.56 ± 0.04	0.81 ± 0.04	0.57 ± 0.04	0.56 ± 0.04	0.83 ± 0.04
	Mm	0.55 ± 0.02	0.55 ± 0.02	0.75 ± 0.03	0.55 ± 0.02	0.55 ± 0.02	0.75 ± 0.03
	mm	0.42 ± 0.08	0.39 ± 0.10	$0.53 \pm 0.07^{*}$	0.42 ± 0.08	0.39 ± 0.10	$0.53 \pm 0.07^{*}$
	P	0.222	0.214	0.009	0.216	0.210	0.007
22:6n–3 (DHA)	MM	1.54 ± 0.08	1.56 ± 0.08	1.52 ± 0.08	1.57 ± 0.09	1.59 ± 0.09	1.56 ± 0.08
	Mm	1.47 ± 0.07	1.54 ± 0.09	1.46 ± 0.06	1.46 ± 0.07	1.52 ± 0.08	1.44 ± 0.06
	mm	1.47 ± 0.06	1.44 ± 0.09	1.35 ± 0.08	1.47 ± 0.06	1.44 ± 0.09	1.35 ± 0.08
	P	0.836	0.825	0.520	0.677	0.694	0.327
EPA/ALA ratio	MM	0.90 ± 0.07	0.92 ± 0.08	0.51 ± 0.05	0.92 ± 0.08	0.94 ± 0.10	0.53 ± 0.06
	Mm	0.71 ± 0.05	0.72 ± 0.06	0.38 ± 0.03	0.72 ± 0.05	0.73 ± 0.06	0.38 ± 0.02
	mm	$0.39 \pm 0.06^{*}$	$0.41 \pm 0.07^{*}$	$0.18 \pm 0.04^{*}$	$0.39 \pm 0.06^{*}$	$0.41 \pm 0.07^{*}$	$0.18 \pm 0.04^{*}$
	P	0.004	0.005	0.004	0.002	0.003	0.003

Values are means $\pm$ SEM; n = 36. MM represents homozygotes for the major allele, Mm represents heterozygotes, and mm represents homozygotes for the minor allele. P-values are analyzed by Kruskal-Wallis test. *Indicates mean values within column for minor allele homozygotes significantly differ from major allele carriers within SNP genotype analyzed by Mann-Whitney U test ($P < 0.05$). ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FADS, fatty acid desaturase.
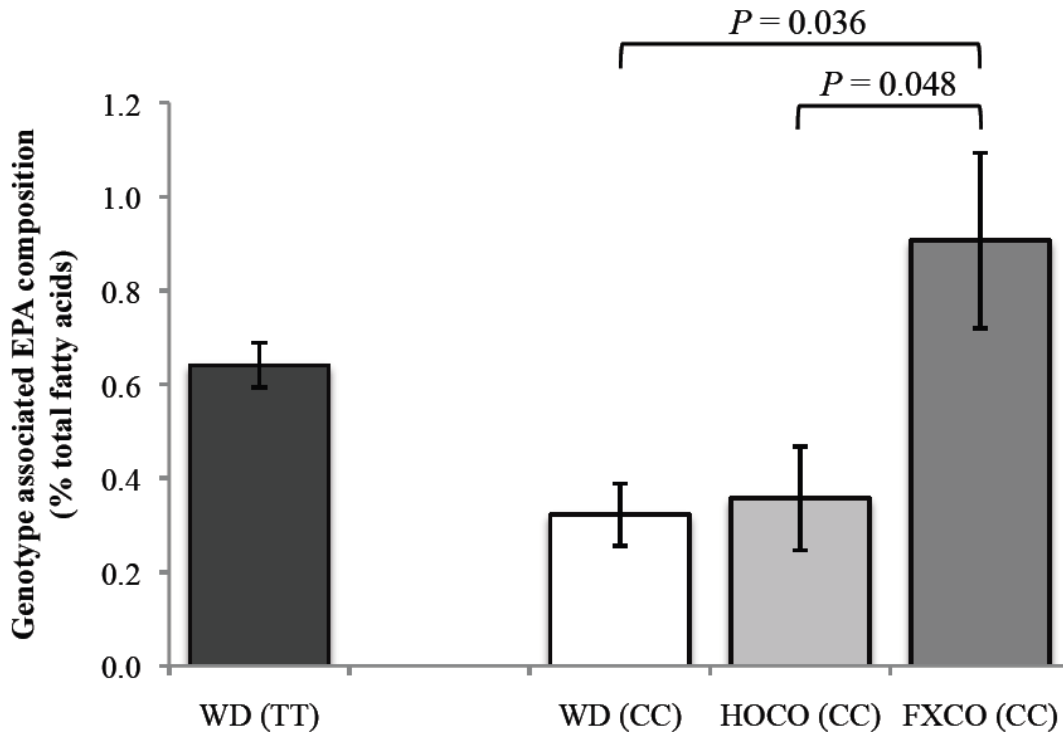
Figure 6.3: Associations of the rs174561 (FADS1) polymorphism with plasma eicosapentaenoic acid (EPA) composition in subjects homozygous for the major allele (TT; n = 15) after consumption of the Western dietary (WD) control and subjects homozygous for the minor allele (CC; n = 4) after consumption of the treatment diets; Western diet (WD), high-oleic canola oil diet (HOCO), flaxseed/high-oleic canola oil diet (FXCO). Values are means ± SEM. P-values are analyzed by ANOVA (Bonferroni post hoc test for multiple comparisons).

6.4.6 Single Nucleotide Polymorphism Association with Plasma ^{13}C-Labelled Fatty Acids

After consumption of each of the experimental diets subjects homozygous for the minor allele for rs174545, rs174583, rs174561 and rs174537 had lower ($P < 0.001$) levels (mg and % dose recovered) of plasma ^{13}C-EPA at both 24 and 48 hours compared with major allele carriers (**Table 6.6** and Table III.16 – III.19 in "Appendix III"). After consumption of the WD control and HOCO diet subjects homozygous for the minor allele for each of

Table 6.6: Percent dose of administered ^{13}C recovered as plasma labelled-fatty acids 48 hours after intake of a single dose of [U-^{13}C]ALA in experimental diets classified by rs174561 (FADS1) and rs174583 (FADS2) genotype.

| Fatty Acid | Allele | rs174561 (FADS1) | | | rs174583 (FADS2) | | |
		Western Diet (Control)	High-Oleic Canola Oil	Flax/High-Oleic Canola Oil	Western Diet (Control)	High-Oleic Canola Oil	Flax/High-Oleic Canola Oil
^{13}C-ALA	MM	4.21 ± 1.19	4.82 ± 0.82	6.62 ± 1.61	4.36 ± 1.36	4.84 ± 0.94	6.62 ± 1.86
	Mm	4.12 ± 0.53	4.64 ± 0.69	5.66 ± 0.64	4.05 ± 0.49	4.64 ± 0.65	5.73 ± 0.60
	mm	6.26 ± 1.67	3.99 ± 0.66	4.95 ± 1.82	6.26 ± 1.67	3.99 ± 0.66	4.95 ± 1.82
	P	0.356	0.951	0.912	0.363	0.922	0.913
^{13}C-EPA	MM	3.77 ± 0.47	4.37 ± 0.63	2.68 ± 0.22	3.76 ± 0.54	4.40 ± 0.73	2.76 ± 0.24
	Mm	2.67 ± 0.34	3.69 ± 0.47	2.51 ± 0.27	2.75 ± 0.32	3.72 ± 0.44	2.48 ± 0.25
	mm	0.89 ± 0.28[*]	1.35 ± 0.37[*]	0.75 ± 0.25[*]	0.89 ± 0.28[*]	1.35 ± 0.37[*]	0.75 ± 0.25[*]
	P	0.005	0.006	0.014	0.005	0.007	0.014
^{13}C-DPA	MM	1.06 ± 0.25	1.04 ± 0.23	0.44 ± 0.10	1.10 ± 0.28	1.10 ± 0.26	0.43 ± 0.12
	Mm	0.76 ± 0.15	0.66 ± 0.12	0.45 ± 0.07	0.76 ± 0.14	0.65 ± 0.11	0.46 ± 0.07
	mm	0.20 ± 0.14[*]	0.36 ± 0.10	0.19 ± 0.06	0.20 ± 0.14[*]	0.36 ± 0.10	0.19 ± 0.06
	P	0.044	0.095	0.153	0.043	0.084	0.145
^{13}C-DHA	MM	0.32 ± 0.12	0.26 ± 0.16	0.14 ± 0.06	0.33 ± 0.14	0.25 ± 0.19	0.16 ± 0.06
	Mm	0.29 ± 0.09	0.21 ± 0.07	0.12 ± 0.06	0.29 ± 0.08	0.22 ± 0.07	0.11 ± 0.06
	mm	0.04 ± 0.08	0.19 ± 0.09	0.05 ± 0.18	0.04 ± 0.08	0.19 ± 0.09	0.05 ± 0.18
	P	0.114	0.769	0.659	0.111	0.931	0.521

Values are means ± SEM; n = 26. MM represents homozygotes for the major allele, Mm represents heterozygotes, and mm represents homozygotes for the minor allele. P-values are analyzed by Kruskal-Wallis test. [*]Indicates mean values within column for minor allele homozygotes significantly differ from major allele carriers within SNP genotype analyzed by Mann-Whitney U test ($P < 0.05$). ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FADS, fatty acid desaturase.

the 4 SNPs had lower ($P < 0.05$) levels (mg and % dose recovered) of ^{13}C-DPA (22:5n-3) at 24 hours compared with major allele carriers. At 48 hours, the lower ($P < 0.05$) levels of ^{13}C-DPA was maintained for the minor allele homozygous subjects after consumption of the WD control, but not after the HOCO or FXCO diets. No significant associations for the studied SNPs were observed for plasma levels of ^{13}C-ALA or ^{13}C-DHA after consumption of each experimental diet (Table III.16 and III.19 in "Appendix III"). No significant associations for the studied SNPs were observed with hourly or cumulative oxidation of ^{13}C recovered in breath. Associations of SNPs in rs174561 (FADS1) and rs174583 (FADS2) with percent dose of administered ^{13}C recovered in plasma n-3 fatty acids at 48 hours post-dose are shown in **Table 6.6**.

6.4.7 Single Nucleotide Polymorphism Association with Plasma Inflammatory Biomarkers and Serum Lipids

With respect to association of SNPs with plasma inflammatory biomarkers, after consumption of the HOCO diet subjects homozygous for the minor allele for rs174537, rs174545, rs174561, and rs174583 had lower ($P < 0.05$) plasma concentrations of VCAM-1 (883.45 ± 98.44 ng/ml) compared with major allele carriers (1033.75 ± 44.78 to 1171.41± 60.69 ng/ml; Table III.6 – III.8 in "Appendix III"). However, no significant associations for the studied SNPs were observed for plasma levels of CRP, IL-6, ICAM-1, or E-selectin after consumption of each experimental diet (Table III.6 – 8 in "Appendix III"). Furthermore, no significant associations for the studied SNPs were observed for serum lipid levels after consumption of each experimental diet (Table III.2 – 5 in "Appendix III").

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6.5 DISCUSSION

The main finding of the present study was that a high intake (~20 g/day) of dietary ALA decreased apparent conversion of $[U\text{-}^{13}C]$ALA to EPA and DPA. More specifically, maximal absolute amounts of plasma ^{13}C-EPA and ^{13}C-DPA were approximately 24% and 47% lower, respectively, after consumption of the FXCO diet compared with the WD control. Furthermore, using stable isotope tracers, the present study substantiates that dietary ALA fails to modulate plasma DHA levels (8,9,24,25).

An additional finding was that FADS1 and FADS2 genetic variants in the genes encoding for Δ5- and Δ6-desaturases, respectively, were associated with differences in $[U\text{-}^{13}C]$ALA apparent conversion to LCPUFA, as well as plasma n-3 and n-6 PUFA composition after consumption of the treatment diets. In particular, plasma ^{13}C-EPA levels, as well as plasma composition of EPA, EPA/ALA and AA/LA, were lower in subjects homozygous for the minor allele compared with carriers of the major allele. Of interest, the present study demonstrated that in minor allele homozygotes, increased consumption of ALA in the FXCO diet resulted in higher plasma EPA levels, beyond that of major allele homozygotes consuming a typical Western Diet (WD control).

Metabolism of ALA to EPA is influenced by dietary factors, including the absolute amount of dietary ALA, LA, the n-6/n-3 PUFA ratio, as well as dietary EPA and/or DHA (8,9,24,26,27). Vermunt et al. (2000) were the first to report a reduction in plasma ^{13}C-labeled EPA after consumption of a diet rich in ALA (8.3 g/day of ALA) compared with an oleic acid-rich diet (9). Similarly, in the present study a decrease in plasma ^{13}C-EPA,

as well as ^{13}C-DPA, was observed with very high intakes of ALA in the FXCO diet as compared with the HOCO diet and WD control. Of interest, the slight 1 g increase in daily ALA intake, and simultaneous decrease in the LA/ALA ratio, with the HOCO diet resulted in a 26% increase in maximal absolute amount of plasma ^{13}C-EPA levels at 24 hours post-dose beyond that of the WD control, however, failed to modulate plasma total n-3 LCPUFA status. Conversely, the 15-fold increase in daily ALA intake with the FXCO diet may have compromised apparent conversion efficiency resulting in a decrease in plasma ^{13}C-labeled LCPUFA compositions. While this finding may be of theoretical interest it may not have clinical significance as a 3-fold increase in plasma EPA and 1.5-fold increase in DPA proportions were found after the FXCO diet. These results are in agreement with previous literature showing a linear relationship between dietary ALA and plasma levels of EPA (8,24). An increase in plasma and tissue EPA composition, despite no change in DHA composition, has independent cardioprotective benefits (1). Mechanistically, EPA competes with AA as substrates for cyclooxygenase and lipoxygenase enzymes in the synthesis of eicosanoids (28). Therefore, we speculate that the 4-fold decrease in plasma AA/EPA composition after consumption of the FXCO diet may have clinical implications associated with a decrease in the synthesis of proinflammatory eicosanoids that warrant further investigation.

The availability of substrate, namely dietary fatty acids, is a major contributor in the regulation of its β-oxidation (29,30). Although plasma ^{13}C-ALA was higher after the ALA-rich FXCO diet, peak oxidation rate (~3% dose/hour) and cumulative oxidation (~19% at 48 hours) of [U-^{13}C]ALA was similar between the treatment diets, and thus, not

influenced by the absolute amount of ALA in the diet. Evidence indicates that the primary metabolic fate of ALA is β-oxidation, reporting between 16–34% β-oxidation of ^{13}C-ALA over a sampling duration of 9–48 hours (9,22,31-34). Storage in adipose tissue accounts for a second major disposal route of dietary ALA (22). While we speculate that excess ALA from the FXCO diet may have been incorporated into adipose triglycerides, our study failed to measure changes in adipose tissue fatty acid composition in response to the treatment diets. Nevertheless, our results substantiate that conversion to LCPUFA is a minor metabolic pathway of dietary ALA. Present data demonstrated maximum recovery of administered [U-^{13}C]ALA as 3–4% ^{13}C-EPA, 0.7–0.8% ^{13}C-DPA, and 0.2–0.3% ^{13}C-DHA, results in accordance with previous studies (8,24).

Recent evidence reveals that SNPs in the FADS1-FADS2 gene cluster are associated with differences in the fatty acid composition of plasma or serum phospholipids (11-16,35-39), adipose tissue (35), erythrocyte membrane (11,13-15,36,40), and breast milk (36,39,41). More specifically, minor alleles of analyzed SNPs from previous studies results in increased proportions of desaturation precursors (i.e. ALA, LA, EDA, DGLA) and decreased proportions of desaturation products (i.e. EPA, DPA, GLA, AA). To our knowledge, the present study is the first to report a lower apparent conversion of [U-^{13}C]ALA to EPA, as measured by lower plasma ^{13}C-EPA, a surrogate marker of conversion efficiency, in subjects homozygous for the minor allele of rs174537, rs174545, rs174561, and rs174583. As both FADS2 and FADS1 are sequentially utilized in the conversion of ALA to EPA, polymorphisms in either of these enzymes were associated with decreased levels of ^{13}C-EPA. Moreover, data revealed that major allele

homozygotes in the measured SNPs exhibited approximately a 3 to 4-fold increase in ^{13}C-EPA as compared with minor allele homozygotes, irrespective of dietary treatment group. Other studies have reported an association between FADS polymorphisms and desaturase transcription or activity as estimated by product-to-precursor ratio (15,16,36,40). Similarly, our results demonstrated a decrease in plasma levels of both AA/LA and EPA/ALA ratio in subjects homozygous for the minor allele within each treatment group. Indeed, plasma EPA/ALA and AA/LA composition positively correlated with plasma ^{13}C-EPA levels. These results are of importance as previous evidence suggests a lack of agreement between product-to-precusor ratio and actual desaturase enzyme activity, particularly in disease states (42,43).

FADS2 is essential in the biosynthesis of DHA from 24:6n-3, however, our results failed to demonstrate an association between any of the SNPs measured and ^{13}C-DHA levels. Furthermore, polymorphisms in the FADS1-FADS2 gene cluster did not result in changes in plasma DHA levels, in agreement with earlier studies (11-14,36). These findings further substantiate the hypothesis of limited DHA biosynthesis and that direct consumption of DHA is the primary way to increase DHA status (12,44,45).

Recent cross-sectional studies report that dietary PUFA modulate the association between FADS polymorphisms and serum lipid levels (37,38). However, the present intervention study failed to observe an association between FADS polymorphisms and serum lipid levels or plasma inflammatory biomarkers within treatment groups, results that may be attributed to a lack of statistical power. Nevertheless, elevated plasma and tissue levels of

EPA plus DHA are associated with decreased CVD morbidity and mortality (1,3,46,47). Therefore, a valuable implication of the present research is that hypercholesterolemic subjects homozygous for the minor allele had ~50% lower plasma EPA proportions and may be predisposed to increased CVD risk. However, results demonstrated that in minor allele homozygotes, substantial intakes of ALA in the FXCO diet compensated for the lower apparent FADS activity and these individuals obtained higher plasma levels of EPA, beyond that of major allele carriers consuming a typical WD control (**Figure 6.3**). Indeed, plasma EPA composition similarly increased after the FXCO diet for all 4 SNPs measured, while AA composition decreased. Therefore, despite having lower apparent conversion efficiency of $[U\text{-}^{13}C]$ALA to plasma ^{13}C-EPA, minor allele homozygotes may obtain cardiovascular benefit from high-ALA intakes by increasing plasma EPA composition, albeit no changes in DHA status.

In summary, our results demonstrate using stable isotope tracer $[U\text{-}^{13}C]$ALA that plasma level of essential fatty acids ALA and LA, their biologically active LC-PUFA derivatives, as well as their apparent conversion efficiency are influenced not only by dietary fatty acid composition but also by genetic variants in the FADS1-FADS2 gene cluster. Moreover, the results confirm that a large range in dietary ALA intake does not increase conversion of ALA to DHA, substantiating the importance of direct intake of DHA-rich dietary sources. Albeit no change in DHA composition, increased plasma EPA composition is associated with decreased CVD endpoints (1). Therefore, consumption of ALA-rich FXCO resulting in increased plasma EPA composition may be cardioprotective, especially in individuals with unfavourable FADS genetic variants.

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6.6 ACKNOWLEDGEMENTS & AUTHORS' CONTRIBUTIONS

The authors' responsibilities were as follows – LGG: subject recruitment, management of the clinical trial, data collection, stable isotope analyses and interpretation of the data, laboratory and statistical analyses, and writing the manuscript; SVH: Stable isotope and GC-IRMS training and technical assistance, and stable isotope analyses and interpretation of the data; SCC: Analysis of $^{13}CO_2$ in breath samples and guidance on the interpretation of the data; NY and PKE: genotyping and assistance in genetic data analysis; TCR and PJHJ: conception and design of the project, submission for ethical approval and acquiring financial support. All authors contributed to revisions of the manuscript and review of the final version. The authors declare no conflict of interest.

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CHAPTER VII

OVERALL CONCLUSION

7.1 SUMMARY AND IMPLICATIONS

The results of the present research have important implications for dietary mangement of cardiovascular health, particularly related to dyslipidemia. Currently, ~40% of Canadians have high blood cholesterol (1). The National Cholesterol Education Program Adult Treatment Program III (NCEP ATP III) guidelines outline traditional risk factors which increase coronary heart disease (CHD) risk over a 10-year period (2). Elevated LDL-cholesterol (>2.60 mmol/L) remains the strongest primary factor in predicting CHD and a primary target of dietary intervention. Evidence from human clinical trials reveals an approximate 1% decrease in CHD events with every 1% decrease in serum LDL-cholesterol levels (3). Data from the present research demonstrates that as compared with the Western dietary (WD) control, high-oleic canola oil (HOCO) reduced LDL-cholesterol by ~7%. Moreover, blending HOCO with flaxseed oil (FXCO) resulted in a further reduction in LDL-cholesterol by ~15%. Therefore, results suggest that substituting fats common to the Western diet with HOCO and FXCO is efficacious as a first-line dietary intervention strategy targeting dyslipidemia and may reduce CHD risk by 7 and 15%, respectively. With respect to absolute serum cholesterol levels, at study baseline subjects were clinically classified as borderline-high hyperlipidemic for serum total cholesterol (TC), LDL-cholesterol and triglyerceride (TAG) levels (4). However, after the short-term dietary intervention of 4 weeks, consumption of HOCO and FXCO reduced subjects LDL-cholesterol levels to within the range classified as near optimal

(2.60–3.35 mmol/L). Moreover, albeit the reduction in HDL-cholesterol levels after consumption of the FXCO diet, endpoint HDL-cholesterol levels were still classified as moderate, while TC and TAG fell to desirable and normal levels, respectively. Finally, both the FXCO and HOCO diet reduced non-HDL-cholesterol levels to meet the goals outlined by the NCEP ATP III panel.

Systemic inflammation is recognized as an emerging risk factor for cardiovascular disease (CVD) (4,5). Therefore elevated circulating inflammatory biomarkers, such as C-reactive protein (CRP) (>3 mg/dL), provide additional clinical information beyond serum lipid levels for detecting at-risk patients (6). After consumption of the FXCO diet, a reduction was observed in CRP levels, however, this decrease failed to reach statistical significance, which may be related to the short 4-week dietary intervention design, as discussed in Chapter IV. Nevertheless, the FXCO blend may still be targeting inflammation and atherogenic pathways via a reduction in E-selectin levels, an independent risk factor for CVD (7,8). Of interest, data shown in Chapter IV suggest that the anti-inflammatory effects of alpha-linolenic acid (ALA)-rich FXCO may be independent of increases in levels of long-chain polyunsaturated fatty acids (LCPUFA), as changes in plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) failed to correlate with changes in E-selectin levels after consumption of the FXCO diet. These results are of significance, further supporting independent anti-inflammatory and cardioprotective effects of ALA-rich flaxseed oil.

The impact of specific fatty acids on energy expenditure, whole-body substrate

utilization, and consequently body weight balance are crucial considering the rising obesity epidemic in Western populations. Given the available literature demonstrating that unsaturated fatty acids are more rapidly diverted for energy use and fat oxidation as compared with saturated fatty acids (SFA) (9-14), the data presented in Chapter V observing no effect of HOCO or FXCO on energy expenditure or substrate oxidation were unexpected. As discussed in Chapter V, previous studies may have magnified the metabolic response to dietary fat intake by administering substantial total fat (>50% of energy) or SFA (>20% of energy) intakes not typical of the current average American diet. Therefore, the failure to enhance thermogenesis, fat oxidation or modulate body composition after consumption of HOCO and FXCO described in Chapter V may be secondary to the moderate SFA, monounsaturated fatty acid (MUFA), and n-6 PUFA content provided by the WD control in the context of the weight maintaining controlled dietary design. Nevertheless, observing no change in energetic or body composition measures after consumption of HOCO and FXCO still provides valuable insight surrounding the maintenance of both energy and weight balance.

Elevated plasma and tissue levels of EPA, docosapentaenoic acid (DPA), and DHA are associated with decreased CVD morbidity and mortality (15-17). Recently, the 'Omega-3 Index' or 'OmegaScore™', a diagnostic test comparing blood n-3 LCPUFA concentrations to established cut-offs, has been increasingly recognized as an independent and modifiable CVD risk factor that is gaining momentum in clinical practice to identify at-risk patients (18,19). Data from Chapter IV and VI demonstrate that the ALA-rich FXCO diet resulted in a 3-fold increase in plasma EPA composition

and a 1.5-fold increase in plasma DPA composition, however, did not alter plasma DHA status compared with the Western dietary control. Although combined EPA plus DHA have both shared and complementary cardiovascular benefits (20), elevated plasma composition of EPA is independently associated with decreased non-fatal cardiovascular endpoints (20,21). Therefore, the present research suggests that consumption of ALA-rich FXCO resulting in increased plasma EPA composition provides additional cardioprotective effects.

The present research enhances our understanding of dietary and genetic factors that regulate ALA metabolism. In accordance with previous findings (22,23), data from Chapter VI demonstrate that substantial intake of dietary ALA decreases biosynthesis of LCPUFA as measured in plasma using stable isotope techniques. Moreover, results from Chapter IV reveal that subjects have slightly lower plasma DHA levels after consumption of the FXCO diet compared with the HOCO diet, supporting the hypothesis of a potential curvilinear relationship between ALA intakes and DHA status (24,25). In addition, the research finding that fatty acid desaturase (FADS)1 and FADS2 polymorphisms are associated with decreased conversion of ALA to LCPUFA and lower EPA status has significant implications in the initiation and progression of atherosclerosis and CVD risk (26). Of importance, increased consumption of ALA in the FXCO diet may be especially cardioprotective in individuals with unfavourable alleles by compensating for their lower EPA status. Nevertheless, the present research further substantiates the hypothesis of limited DHA biosynthesis and the need for direct consumption of DHA for optimal cardiovascular health (27-30).

Dietary recommendations for n-3 PUFA have progressed significantly in the last 10 years, particularly for EPA and DHA (31). Whereas traditional recommendations have focused on nutritional essentiality and adequacy to prevent nutrient deficiency, current recommendations consider the amount required to reduce chronic disease risk (31,32). Thus, governmental and professional health organizations with a focus on primary and secondary prevention of CVD target intakes of 250–1000 mg/day of EPA+DHA (20,31,32). However, challenges in meeting these recommended intakes of EPA and DHA via enhanced fish consumption in Western diets exist, including the sustainability of fish resources (33-35), as well as consumer concerns regarding environmental toxins (36,37), taste preferences, preparation or availability of fish (32). Therefore, given that flaxseed provides an abundant and sustainable resource for dietary ALA, there is much interest if ALA can provide a functional source of endogenous EPA and DHA. Hypothetically, considering the apparent conversion of ALA to EPA of ~2.5% shown in Chapter VI with the use of stable isotope tracers, consumption of ~20 g/day of ALA provided by the FXCO diet would generate ~500 mg/day of EPA, an amount falling within the targeted recommended range for EPA+DHA indicated above. However, dietary ALA failed to modulate DHA status. This finding has important implications in support of the ongoing vigorous debate whether DHA is conditionally essential as professional organizations strive to reconsider a Dietary Recommended Intake (DRI) value for LCPUFA (30,38). Nevertheless, substitution of commonly used dietary oils in the Western diet with HOCO and flaxseed oil will increase compliance with dietary recommendations for fatty acid, namely SFA, MUFA, and n-3 PUFA and target a

reduction in CVD risk.

The present research supports the current United States Food and Drug Administration (US FDA) qualified health claims for canola oil (39) and n-3 PUFA (40) for CVD risk reduction. In addition, the present findings provide valuable information for novel health claims in Canada. Scientifically verified nutrient content claims, nutrient function claims, and disease risk reduction claims used on food labels and advertising are important tools for the enhancement of consumer awareness and knowledge as to the nutritional value and health attributes of conventional and functional foods. In addition to the approved health claim in Canada stating: "*A healthy diet low in saturated and trans fat may reduced risk of heart disease*" (41), in February 2012 a new health claim was approved in Canada stating: "*Replacing saturated fat with unsaturated fat from vegetable oil lowers cholesterol – High cholesterol is a risk factor for heart disease*" (42). The new health claim supports the vegetable oil industry in its movement to eliminate trans fatty acids (TFA) and reduce SFA from the diet while communicating with Canadians the health attributes of dietary PUFA and MUFA. In addition, approved nutrient content claims include "*low in saturated fatty acids*", "*free of trans fatty acids*", and "*source of omega-3 polyunsaturated fatty acids*" (43). The versatility and stability properties of HOCO make it a practical replacement option for TFA/SFA-rich partially hydrogenated vegetable oils in food processing, frying, and culinary purposes (44). Furthermore, blending flaxseed oil into traditional products such as dressings, spreads and margarines will enhance dietary ALA intake. Therefore, foods incorporating HOCO and flaxseed oil will permit the use of some of the aforementioned health claims. Ultimately, these trends in use will

stimulate the opportunity for more 'heart-healthy' canola and flaxseed oil containing functional food opportunities to meet the demands and interest of today's health conscious consumer.

An increase in the demand for flaxseed oil, HOCO, and functional foods enriched with these products have important economical implications with respect to both edible oilseed production and health care costs in Canada. Within 13 years of market introduction (1986), canola oil consumption increased 167-fold (45). Currently, canola oil is the #1 consumed vegetable oil in Canada, #2 in the US, and #3 worldwide (46). Next to wheat, canola is the second largest crop harvested in Canada, contributing ~19% to total crop production with ~58% exported internationally (47). Thus, canola contributes $15.4 billion dollars to the Canadian economy annually (46). Flaxseed is the eighth largest crop produced in Canada, accounting for ~1.5% of total crop production with ~83% exported internationally (47), and contributes ~$1 billion dollars to the Canadian economy annually (48). Taken together, the results of the present research substantiate the 'heart-healthy' position of both canola and flaxseed oil for health care professionals and consumers, enhancing the demand for crop production and adding to the economic value of canola and flaxseed crops within Canada.

The total economic burden of heart disease and stroke in Canada accounts for more than $20.9 billion annually in physical services, hospital cost, lost wages and reduced productivity (49). Health Canada estimates that chronic diseases attributed to poor eating patterns, including SFA-rich diets, accounts for approximately $6.3 billion annually and

that a substantial component of this cost is preventable (50). Therefore, replacing SFA with MUFA and PUFA dietary options, such as HOCO and FXCO, may aid in reducing the economic burden of diet-related disease in Canada. Ultimately, consumption of canola and flaxseed oils will help improve the health of the Canadian population via prevention of CVD morbidity and mortality.

7.2 LIMITATIONS AND FUTURE DIRECTIONS

Results from the present body of research provide valuable insight into the cardioprotective effects of HOCO, alone or blended with flaxseed oil, as well as dietary and genetic influences on the metabolism of ALA to LCPUFA. However, considering limitations in the present work, the following suggested future research directions would provide additional information regarding molecular mechanisms and clinical efficacy of dietary MUFA and ALA in cardiovascular health.

The present study provided a large gap in ALA intakes, ranging from typical intakes (1.3 g/day of ALA), to recommended intakes (2.4 g/day of ALA), to substantial intakes (20 g/day of ALA). Therefore, a dose-response study within the range of more achievable daily intakes of ALA (i.e. 1–10 g/d of ALA) is suggested to determine optimal levels needed to target specific clinical endpoints. In addition, the use of an EPA/DHA treatment arm as a positive control would contribute further knowledge regarding the comparative effects of plant and marine derived n-3 PUFA on CVD risk factors.

The present research assessed traditional serum lipid endpoint measures and estimated

atherogenic apolipoprotein (apo)-B containing lipoproteins by calculating non-HDL-cholesterol. Future studies including the analysis of alternative lipoprotein measurements, such as lipoprotein subfraction size and concentration, apo-B and apo-AI, lipoprotein(a), as well as lipoprotein-associated phospholipase A_2 may provide additional information related to atherogenesis (51) and the beneficial health effects of MUFA-rich and ALA-rich diets.

Although the present 4-week intervention design was sufficient in length to observe alterations in serum lipids, previous studies demonstrating a reduction in inflammatory biomarkers after consumption of ALA-rich diets were at least 6-weeks in duration (52,52-54). Therefore, future studies investigating effects of dietary ALA and MUFA on inflammatory biomarkers should employ a minimum 6-week dietary intervention. In addition, subjects should be screened for baseline CRP levels to target an at-risk population (i.e. CRP >3 mg/dl) and avoid a potential 'floor effect', as discussed in Chapter IV. Moreover, while carotid ultrasounds measuring intima-media thickness (IMT) is a reliable clinical marker of vascular health (55), this method may not be the ideal measure of endothelial function in short-term dietary intervention studies. Future studies should consider alternative measures of endothelial function and vascular health that are sensitive to a short-term study design, such as reactive hyperemia peripheral arterial tonometry or pulse wave velocity analysis (56,57).

The present study demonstrated that consumption of ALA increased plasma EPA status while decreasing arachidonic acid (AA) status. Given that EPA and AA are precursors

for eicosanoids modulating an inflammatory response (58), future investigations should directly measure changes in eicosanoid levels, such as thromboxane and prostaglandin concentrations, after consumption of ALA and MUFA-rich diets. In addition, the interaction between dietary ALA intake with FADS genetic variants in modulating inflammatory eicosanoids demands further investigation to elucidate mechanisms regulating CVD risk reduction. Furthermore, as fatty acids are ligands for transcription factors regulating transcription and expression of genes involved in fatty acid oxidation and systemic inflammation (59,60), future studies should measure the effects of treatment oils on transcription factors, such as peroxisome proliferator-activated receptors (PPARs) expression, to further elucidate molecular mechanisms of action.

Results from Chapter VI revealed that FADS1/FADS2 genetic variants are associated with plasma fatty acid concentrations. Therefore, unfavourable alleles may be a confounding variable to the cardioprotective benefits of dietary ALA. This observation has important implications as single nucleotide polymorphisms (SNP) in subject populations may account for discrepancies between results of epidemiological studies and intervention trials surrounding the effects of ALA on CVD endpoint measures. Therefore, future studies should incorporate measures of FADS genetic variants into the study design to determine if SNPs may exist as confounding variables explaining the heterogeneity of efficacy of dietary ALA intervention. Furthermore, the interactive effects of FADS genetic variants and dietary ALA on CVD endpoint measures require further substantiation.

The major strength of the present research is the use of a diet-controlled randomized crossover design, considered the 'gold standard' for evaluating nutritional treatment interventions (61). Precise control of the diet isolates the effects of the specific actions of treatment oils on endpoint measures and reduces confounding influences of the antecedent diet. Moreover, the crossover design limits the influence of genetic, metabolic and other interindividual variations. A suggestion is for future studies to consider the present study design at several research centres simultaneously. Multicentre trials allow for a larger, more diverse subject population, including a wider range of baseline and genetic characteristics. Furthermore, multicentre trials assist in increasing the generalizability of the study. Finally, as stable isotope tracers remain a more precise surrogate measure of desaturase activity, future studies should consider their use when investigating ALA metabolism to LCPUFA, however, considering the high cost of stable isotopes the use of a subset study population is recommended.

7.3 FINAL CONCLUSION

The prevalence of CVD in North America may be modifiable through changes in dietary fat quality. Today's health conscious consumers are savvy to dietary recommendations emphasizing cardiovascular benefits from replacement of SFA with unsaturated fatty acids and enhancing n-3 PUFA intakes. In parallel, the edible oilseed industry has responded by increasing production of traditional and novel 'heart-healthy' dietary oils for use in food production, namely flaxseed oil and high-oleic canola oil. The totality of the present research is the first to demonstrate the efficacy of high-oleic canola oil for CVD risk reduction in humans. More specifically, high-oleic canola oil exhibits

cardioprotective benefits via lipid-lowering effects and favourable changes in plasma fatty acid profiles, while maintaining energy and weight balance. In addition, the present research advances our understanding of the metabolic mechanisms underlying the health benefits associated with consumption of ALA-rich flaxseed oil. Incorporation of flaxseed oil into the diet effectively targets traditional and emerging biomarkers of CVD risk by reducing serum lipid concentrations, decreasing E-selectin levels, and increasing plasma n-3 PUFA status. Furthermore, the present research findings demonstrate that ALA conversion to LCPUFA is influenced not only by dietary fatty acid composition but also genetic variants in the FADS1/FADS2 gene cluster. Accordingly, these results have important implications for individuals with unfavourable alleles for FADS1 and FADS2, revealing that increased consumption of ALA will provide cardiovascular benefits associated with an increase in EPA status. Taken together, substitution of dietary fats common to the Western diet with both high-oleic canola and flaxseed oils represents a feasible option to target dietary recommendations, biomarkers for CVD risk reduction, and ultimately, improve population health and wellness.

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APPENDICES

APPENDIX I

ETHICS APPROVAL FOR STUDIES CORRESPONDING TO CHAPTERS IV, V,

AND VI

BANNATYNE CAMPUS
Research Ethics Boards

UNIVERSITY OF MANITOBA

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Winnipeg, Manitoba
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APPROVAL FORM

JUN 2 2 2007

Principal Investigator: Dr. P. Jones
Sponsor: Flax Canada 2015

Protocol Reference Number: B2007:071
Date of REB Meeting: April 30, 2007
Date of Approval: June 19, 2007
Date of Expiry: April 30, 2008

Protocol Title: "Efficacy of Consumption of Canola and Flax Oils in Management of Hypercholesterolemia and Other Disease Risk Factors"

The following is/are approved for use:

- Protocol dated June 7, 2007
- Research Participant Information and Consent Form, Version dated June 7, 2007
- Advertisement dated April 16, 2007

The above was approved by Dr. Nicholas Anthonisen, Chair, Biomedical Research Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated June 7, 2007. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations*.

This approval is valid for one year from the date of the meeting at which it was reviewed. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Nicholas Anthonisen, MD, Ph.D
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary
Telephone: (204) 789-3255/ Fax: (204) 789-3414

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AUG 0 3 2007

APPROVAL FORM

Principal Investigator: Dr. P. Jones
Sponsor: Flax Canada 2015

Protocol Reference Number: B2007:071
Date of Approval: July 31, 2007

Protocol Title: "Efficacy of Consumption of Canola and Flax Oils in Management of Hypercholesterolemia and Other Disease Risk Factors"

The following is/are approved for use:

- Protocol Amendment dated July 20, 2007
- Research Participant Information and Consent Form, Version dated July 20, 2007
- Advertisement dated July 20, 2007

The above was approved by Dr. Ian Maclean, Acting Chair, Biomedical Research Board, Bannatyne Campus, University of Manitoba on behalf of the committee as per your letter dated July 20, 2007. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations.*

A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Ian Maclean, Ph.D
Acting Chair,
Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary
Telephone: (204) 789-3255/ **Fax:** (204) 789-3414

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Mendet: Please copy to heah and file *Pete*

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APPROVAL FORM

OCT 2 2 2007

Principal Investigator: Dr. P. Jones
Sponsor: Flax Canada 2015

Protocol Reference Number: B2007:071
Date of Approval: October 9, 2007

Protocol Title: "Efficacy of Consumption of Canola and Flax Oils in Management of Hypercholesterolemia and Other Disease Risk Factors"

The following is/are approved for use:

* Protocol dated October 2, 2007
* Research Participant Information and Consent Form, Version dated July 20, 2007

The above was approved by Dr. Nicholas Anthonisen, Chair, Biomedical Research Board, Bannatyne Campus, University of Manitoba on behalf of the committee as per your letter dated October 2, 2007. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba.   The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations*.

A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Nicholas Anthonisen, MD, Ph.D
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
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APPROVAL FORM

Principal Investigator: Dr. P. Jones
Sponsor: Flax Canada 2015

Protocol Reference Number: B2007:071
Date of Approval: December 5, 2007

Protocol Title: "Efficacy of Consumption of Canola and Flax Oils in Management of Hypercholesterolemia and Other Disease Risk Factors"

The following is/are approved for use:

- Amendment dated November 15, 2007
- Research Participant Information and Consent Form, Version dated November 19, 2007

The above was approved by Dr. Nicholas Anthonisen, Chair, Biomedical Research Board, Bannatyne Campus, University of Manitoba on behalf of the committee as per your letters dated November 15, 2007 and December 5, 2007. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations*.

A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Nicholas Anthonisen, MD, Ph.D
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
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MAY 06 '09

Principal Investigator: Dr. P. Jones
Sponsor: Flax Canada 2015

Ethics Reference Number: B2007:071
Date of Approval: April 30, 2009
Date of Expiry: April 30, 2010

Protocol Title: Efficacy of Consumption of Canola and Flax Oils in Management of Hypercholesterolemia and Other Disease Risk Factors

The following is/are approved for use:

- **Annual Approval**

The above was approved by Dr. Nicholas Anthonisen, Chair, Biomedical Research Board, Bannatyne Campus, and University of Manitoba on behalf of the committee per submission dated April 6, 2009. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada*.

This approval is valid until the expiry date only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Nicholas Anthonisen, MD, Ph.D
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Ethics Reference Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone:** (204) 789-3255/ **Fax:** (204) 789-3414

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APPROVAL FORM

Principal Investigator: Dr. P. Jones
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Ethics Reference Number: B2007:071
Date of Approval: April 30, 2010
Date of Expiry: April 30, 2011

Protocol Title: Efficacy of Consumption of Canola and Flax Oils in Management of Hypercholesterolemia and Other Disease Risk Factors

The following is/are approved for use:

- **Annual Approval**

The above was approved by Dr. Nicholas Anthonisen, Chair, Biomedical Research Board, Bannatyne Campus, and University of Manitoba on behalf of the committee per your letter dated April 1, 2010. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba.   The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada.*

This approval is valid until the expiry date only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes.   The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Nicholas Anthonisen, MD, Ph.D
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

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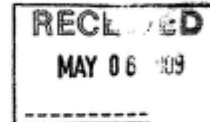
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APPROVAL FORM

Principal Investigator: Dr. P. Jones
Sponsor: Flax Canada 2015

Ethics Reference Number: B2007:071
Date of Approval: April 30, 2011
Date of Expiry: April 30, 2012

Protocol Title: Efficacy of Consumption of Canola and Flax Oils in Management of Hypercholesterolemia and Other Disease Risk Factors

The following is/are approved for use:

- **Annual Approval**

The above was approved by Dr. Nicholas Anthonisen, Chair, Biomedical Research Board, Bannatyne Campus, and University of Manitoba on behalf of the committee per your letter dated April 20, 2011. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada*.

This approval is valid until the expiry date only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Nicholas Anthonisen, MD, Ph.D
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

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DEC 2 0 2007

Research Review Committee
Approval Form

Principal Investigator: Peter Jones, PhD

RRC Reference Number: RRC/2007/0862

Date: December 14, 2007

Protocol Title: Efficacy of Consumption of Canola and Flax Oils in Management of Hypercholesterolemia and Other Disease Risk Factors

The following is/are approved for use:

- **Protocol dated October 2, 2007**
- **Research Participant and Information Consent Form dated November 19, 2007**
- **Advertisement dated July 20, 2007**

The above was approved by Dr. B. Light, Chairperson, Research Review Committee, St. Boniface General Hospital, on behalf of the Committee. As the recommendations by the Research Review Committee have been met, final approval is now granted.

Any significant changes to the study Protocol and Informed Consent Form, must be reported to the Research Review Committee along with any other documents required as per Standard Operating Procedures for Clinical Investigators.

Sincerely yours,

Dr. B. Light
Chairperson, Research Review Committee
St. Boniface General Hospital

Please quote the above reference number on all correspondence.
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Affiliated with the University of Manitoba/Affilié à l'Université du Manitoba

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BANNATYNE CAMPUS
Research Ethics Boards

UNIVERSITY
OF MANITOBA

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. P. Jones
Sponsor: CIHR

Ethics Reference Number: B2009:129
Date of Approval: October 20, 2009
Date of Expiry: October 20, 2010

Protocol Title: Genetic Basis for Heterogeneity in Response of Plasma Lipids to Plant Sterols Supplementation and Fatty Acid Dietary Modification: Freezer Study

The following is/are approved for use:

- Protocol submitted September 25, 2009
- Research Subject Information and Consent Form, Version dated 10/9/2009
- Cover letter to participants submitted October 8, 2009

The above underwent expedited review and was approved as submitted on October 20, 2009 by Dr. Nicholas Anthonisen, Chair, Biomedical Research Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated October 8, 2009. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada.*

This approval is valid for one year only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Nicholas Anthonisen, MD, Ph.D
Chair,
Biomedical Research Ethics Board
Bannatyne Campus

Please quote the above Ethics Reference Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone:** (204) 789-3255/ **Fax:** (204) 789-3414

www.umanitoba.ca/faculties/medicine/research/ethics

APPENDIX II

FORMS CORRESPONDING TO STUDIES DESCRIBED IN CHAPTERS IV, V,

AND VI

STUDY ADVERTISEMENTS – POSTER 1

POSTER 2

Worried about your CHOLESTEROL?
Want to improve your HEALTH?

We are conducting a study to investigate
the health benefits associated with
CANOLA OIL and **FLAX OIL**
consumption.

Are you eligible?

- Open to men and women, 18-65 years
- Not taking medication to lower blood fats
- Non-smoker
- Dedicated to learning about nutrition and health

How will you benefit?

- All meals provided for duration of study
- Lower your cholesterol and improve your health
- Receive personalized health information
- Receive compensation for your participation

Please call: (204) 272-1551
Ask for Leah
Dr. Peter Jones, Principal Investigator

Richardson Centre
for Functional Foods
and Nutraceuticals

July 20, 2007

RCFFN WEBSITE AD

EFFICACY OF CONSUMPTION OF CANOLA AND FLAX OILS IN MANAGEMENT OF HYPERCHOLESTEROLEMIA AND OTHER DISEASE RISK FACTORS

The Richardson Centre for Functional Foods and Nutraceuticals at the University of Manitoba is conducting a study to learn more about the efficacy of consuming canola and flax oils in managing hypercholesterolemia and other disease risk factors.

The study is open to men and postmenopausal women who meet the following criteria:

- Between 20-60 years
- Borderline hypercholesterolemic (low density lipoprotein cholesterol (LDL-C) > 3.0 mmol/L)
- Are *not* taking medication to lower blood lipid levels
- Do *not* have hypertension

We will provide volunteers with 3 meals a day for 3 one month test periods, each test month will be followed by a one month period during which volunteers may consumer their normal diets.

For more information about the study, please see the additional information sheet.

Volunteers will be compensated for their participation.

If Interested, Please Call: (204) 474-9787

Dr. Peter Jones, Principal Investigator

CTV NEWS CLIP

U OF M LOOKS FOR HEART-SMART OILS

A research team at the University of Manitoba is studying the effects of canola and flaxseed oils on cholesterol levels. Every day, 21 people eat breakfast together, and then get to take home a kit filled with a pre-made lunch and dinner. One group is eating meals with canola and flax oils, while another group is eating meals with no oils. The study will determine whether canola lowers cholesterol and whether it should be deemed a heart-smart oil. Results from the study should be known within the next year.

Watch at Noon, 6 p.m., and 11:30 p.m.

November 16, 2007

GENERAL INFORMATION FORM

Canola and Flax oils Nutrition Study*
Additional Information
August 2007

The Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) is located in the SmartPark at the Fort Garry Campus of University of Manitoba.

General information
Canola oil and flax oil both contain an essential omega-3 fatty acid, alpha-linolenic acid (ALA). "Essential" means that the human body does not make this fatty acid, so to be beneficial it must be consumed in the diet. ALA can reduce cardiovascular disease risk by several means. First, it is a starting point to make other helpful fatty acids including those commonly consumed from fish oil supplements. Second, it helps maintain healthy outer layers of the cells that make up our body. Third, it may reduce inflammation which has been implicated in cardiovascular disease.

The Study
The feeding portion of the study is planned to commence in mid September 2007, however subjects who are unable to start at this time but still interested should also inquire further. Subjects enrolled will participate for a total of approximately five months, consisting of 3 one month treatment phases separated by 4 weeks washout or BREAK phases. During treatment phases the subjects must only consume food provided by the Centre. During washout or BREAK phases subjects may resume their normal diets. Subjects will receive financial compensation for participation in the study.

| Treatment 1 | → | BREAK | → | Treatment 2 | → | BREAK | → | Treatment 3 |

The treatment consists of different oils:
1. Control oil (normal cooking oil such as safflower oil)
2. Canola oil
3. Canola/flax oil blend

The different treatment oils are incorporated into the diet. During treatment phases all meals are provided by the Centre. Subjects will come to the Centre every morning, between 6:30-9:30am on weekdays and 8-10am on weekends, and consume breakfast here. They will leave with a cooler containing their food for the rest of the day.

On days 1, 2, 27, 28 and 29 of each treatment phase subjects will have a 12 hour fasting **blood sample** taken. Blood will be analyzed for total lipid profile, glucose levels, as well as other parameters. Once during each treatment phase subjects will consume ALA that is labeled to allow us to measure the conversion of ALA to other beneficial fatty acids. Additionally, at the beginning and end of each treatment phase subjects will undergo 2 procedures to measure the body composition and energy expenditure. The first procedure is called **dual energy x-ray absorptiometry (DEXA)**. It takes approximately 7 minutes and measures the body fat percentage and distribution.

The second procedure is called **energy expenditure** and requires that the subject remain lying down for approximately 6 hours while the air that they breathe out is measured. Subjects may read or watch movies during this time.

August 30, 2007

Subjects have the option to undergo **flow mediated dilation** at a local hospital (to be announced). This procedure uses an (non-invasive) ultrasound to assess artery function, looking at both blood vessel diameter and blood flow. Subjects are only eligible for this procedure if they do not have high blood pressure and are not on any blood pressure lowering medications. Eligible subjects are highly encouraged to participate in this section. Additional compensation for this section is provided.

If you are interested in participating in this study contact the Centre **474-9787 or 272-1551**. Interested subjects may come to the Richardson Centre for an information session for a detailed explanation of the study and the centre. To be eligible for the study, subjects must come to the centre for blood tests to make sure your cholesterol level meets those required for our study. You must have fasted for 12 h and had no alcohol for 24 h before this blood test.

Frequently asked questions:
Which kind of meals will be served?
A standard North American diet including foods such as spaghetti, chicken, etc. The treatment oils will be used to cook the different foods. During treatment phases the subjects must only consume food provided by the Centre. During washout or BREAK phases subjects may resume their normal diets.

Is the labeled ALA dangerous?
No. The labeled ALA is non-radioactive and non-toxic.

How much is the radiation dose that I will receive for doing the DEXA test?
The amount of radiation that you will receive for the DEXA test per scan is 1% of the radiation dose that you would be exposed to if you were taking a flight across Canada.

If I already know my cholesterol level, can I avoid the 1ˢᵗ blood draw?
No. In order to keep our study controlled, all volunteers have to have their blood tested at the same place, since different laboratories may produce different results.

*EFFICACY OF CONSUMPTION OF CANOLA AND FLAX OILS IN MANAGEMENT OF HYPERCHOLESTEROLEMIA AND OTHER DISEASE RISK FACTORS

August 30, 2007

SUBJECT CONSENT FORM

UNIVERSITY OF MANITOBA

St-Boniface General Hospital

RESEARCH SUBJECT INFORMATION AND CONSENT FORM

Title of Study: Efficacy of Consumption of Canola and Flax Oils in Management of Hypercholesterolemia and Other Disease Risk Factors

Investigator: Peter Jones, PhD

Richardson Centre for Functional Foods and Nutraceuticals

University of Manitoba

196 Innovation Drive, Smartpark

Winnipeg, Manitoba R3T 6C5

Phone: 204 474 9787

You are being asked to participate in a research study. Please take your time to review this Information and Consent Form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family. This consent form may contain words that you do not understand. Please ask the study doctor or study staff to explain any words or information that you do not clearly understand.

Purpose of Study

The purpose of the study is to examine how alpha-linolenic acid (ALA), as well as ALA in combination with oleic acid, which are naturally found in canola oil and flax oil, will affect your body weight and fat content, blood fat levels as well as cardiovascular diseases (CVD) biomarkers. The canola and flax oils will be supplemented in an oil form and will be added to diets as provided by the metabolic kitchen at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN).

Study procedures

If you agree to take part in this study, as part of a pre-screening visit, you will be asked to have a fasting (nothing to eat or drink 12 hours before the test) blood sample of approximately two teaspoons taken to measure your blood fat levels. In addition, your blood pressure will be measured. If you meet eligibility requirements, you will be invited back for further screening where a fasting blood sample of four teaspoons will be taken to do a complete blood count, and biochemistry profile. All baseline values must be normal

Efficacy of consumption of Canola and Flax Oils in Management of
Hypercholesterolemia and Other Disease Risk Factors

as verified by the study physician prior to enrollment in the study and any abnormality in
tests performed at screening will result in exclusion. An electrocardiogram (EKG) may
be performed at the discretion of the physician in charge. Prior to beginning the study,
you will undergo a physical examination by a physician to ensure that you are in good
health. During the physical examination, the physician will measure your vital signs
examine the normality of body systems and ask you some questions regarding your
medical history. The study physician and or study staff will review medical history and
ask questions to determine whether you are eligible to participate.

If you are female and are not post-menopausal you will be asked to take a pregnancy test
prior to beginning the study and subsequently before each DEXA scan.

Any change in your health status at any point during the study needs to be reported to the
study investigators.

The study will consist of 3 phases of 30 days each during which you will consume a fixed
composition precisely controlled weight-maintaining diet. At the end of each phase, a
washout period of 4 weeks will be followed during which you will consume your habitual
diets. The 3 phases of treatments will include:

1. Control phase: Dietary fat will represent higher saturated fat not
   atypical of current North American intakes. Fat will comprise 35% of
   total energy and be largely saturated fat with substantial levels of
   omega-6 linoleic acid provided

2. Canola oil phase: Dietary fat consumed will provide 35% of total
   energy and will be comprised of up to 70% canola oil.

3. Canola/Flax oil blend phase: Dietary fat consumed will provide 35%
   of total energy and will be comprised of up to 70% canola and flax oil
   blend

This study is with double-blind design which means that neither you nor the study staff
will know which variation of the treatments that you will be receiving. You will receive
all 3 treatments, however, it will be unknown the order you will be given in. In an
emergency, this information will be made available.

Study diets will be prepared in the metabolic kitchen of the RCFFN. You will consume at
least 1 of 3 daily meals at the RCFFN under supervision. The other meals will be
prepared and packed to be taken out. The treatment oils will be provided as a part of the
meals given as appropriate for each phase. You will be asked to consume only the
prepared meals and not to consume alcohol or caffeinated beverages.

We will measure the amount of fat in your body using a procedure called dual energy x-
ray absorptiometry (DEXA). These analyses will be performed 6 times in total during the
study, once at the beginning of each phase and once at the end of each phase. For this

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November 19, 2007

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Efficacy of consumption of Canola and Flax Oils in Management of
Hypercholesterolemia and Other Disease Risk Factors

procedure, you will need to lie in a horizontal position for about 5-10 minutes while the
scan arm passes from your head to your feet. The radiation from this test is very low
dosage (equivalent to approximately 1 day of natural background radiation). The dosage
is 1000 times less than the limit for trivial exposure. You will be asked not to wear
anything metal (metal may affect bone density values which will affect body composition
calculations). In addition, you will need to ensure that you will not undergo barium
tests/exams, or a nuclear medicine scan or injection with an x-ray dye within two weeks
prior to your DEXA scan.

During the first and fourth week of each dietary phase, you will have your energy
expenditure measured using a canopy hood ventilation system. You will have a plexiglass
canopy placed over your head for 30 minutes before breakfast as the first measurement,
and 5.5 hours after breakfast as the second measurement. You will be asked to consume
breakfast within 30 minutes in between the first and second measurements. Since the air
in the room is directed through the hood, you can therefore breathe normally during the
test. The rate at which your body is burning calories will be determined by measuring the
rate of oxygen that you consume and the carbon dioxide that you produce while the
ventilation system is in operation.

During the first and fourth week of each dietary phase, you will undergo endothelial
health assessment using the ultrasound approach which will measure arterial function and
vascular reactivity. The ultrasound sessions will be conducted at the local clinical
facilities in Winnipeg area.

During days 1 and 28 of each four-week test phase, you will undergo pulse wave analysis
after 10 minutes of rest in supine position. Pulse wave analysis consists of a noninvasive
pressure sensor lightly applied to the radial artery held by a wrist band for 90 seconds.
Pulse wave analysis can noninvasively evaluate cardiac information including pulse,
systolic pressure, diastolic pressure and other cardiovascular parameters.

During days 1, 2, 28, 29 and 30 of each four-week test diet phase, fasting blood samples
(approximately 6 teaspoons) will be obtained for assessment of blood fat, fatty acid
profile and other CVD biomarkers including insulin glucose concentrations and
inflammatory markers, oxidative stress markers and markers of adiposity. On day 28, you
will be required to consume a small quantity of carbon-labeled fatty-acid. The labeled
fatty acid is almost identical to regular fatty acid, except that a small amount of carbon in
the fatty acid is being replaced with a heavier form of carbon. The conversion of certain
fatty acids naturally found in the body will be analyzed using the rate of the tagged fatty
acid being converted. This labeled fatty acid is non-radioactive and not toxic

Each blood test will take approximately 5 minutes. The total amount of blood drawn
during each phase of the study will be approximately 10 tablespoons. The total blood
volume required for this trial will be approximately 2 cups.

Initials of Subject:_____

Efficacy of consumption of Canola and Flax Oils in Management of
Hypercholesterolemia and Other Disease Risk Factors

Risks and Discomforts

As with any clinical trial, there may be as yet unknown or unforeseen risks of taking part.

The canola and flax oils contained within the meals at the proposed level has been shown
to have no known direct negative side effects on health in several dozen existing animal
and human experiments. Some known risks, although rare, are associated with placing a
needle into a vein. These include the possibility of infection, perforation or penetration of
the needle through the vein, and bleeding, pain, or bruising at the site. In case you feel
any discomfort during the experimental trial a physician, Dr. Kesselman, will be
available to contact at any time. Dr. Kesselman can be reached at 204 954 4486.

Benefits

You may not benefit from participation in this research; however, the study should
contribute to a better understanding of the effects of canola and flax oils on body weight
and blood fat levels as well as cardiovascular diseases (CVD) biomarkers. You will also
receive access to your test results when they become available.

Costs

All clinic and professional fees, diagnostic and laboratory tests that will be performed as
part of this study are provided at no cost to you. There will be no cost for the study
treatment that you will receive.

Payment for participation

You will receive up to a maximum of $1000 at completion of this study for your time and
inconvenience of the study schedule. This amount will be divided into 3 equal portions
and 1 portion given after each phase. If you withdraw early from the study, you will
receive an appropriate pro-rated fraction of this amount.

Alternatives

You do not have to participate in this study. The study coordinators, physician and
principal investigator will answer any questions you have about the experimental group
of this study. You should be aware that lipid lowering medications exist as an alternative
to lowering blood cholesterol levels.

Confidentiality

Medical records that contain your identity will be treated as confidential in accordance
with the Personal Health Information Act of Manitoba. The RCFFN staff involved with
your care may review/copy medical information that may reveal your identity. With your
permission, the study doctor will also write to your Family Doctor to tell him/her that you
are taking part in a study or to obtain further medical information. The Biomedical

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> Efficacy of consumption of Canola and Flax Oils in Management of
> Hypercholesterolemia and Other Disease Risk Factors

Research Ethics Board at the University of Manitoba may also review your research-related records for quality assurance purposes. If you are a research subject from St. Boniface General Hospital, your research related records may be reviewed by St. Boniface General Hospital for quality assurance purposes. If the results of the trial are published, your identity will remain confidential. Personal information such as your name, address, telephone number and/or any other identifying information will not leave the Richardson Centre for Functional Foods and Nutraceuticals.

Study samples will be stored in the freezer at the RCFFN. Only the study coordinators and the principal investigator will have access to the samples. Your samples will not be used for any additional analyses, nor stored for any longer than 2 years, nor shared with any other group, other than is indicated in the protocol, without your specific consent.

Voluntary Participation/Withdrawal From the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision to not participate or to withdraw from the study will not affect your other medical care.

Your participation in this study may be terminated without your consent by the study coordinators, physician or principal investigator. The study staff will withdraw you if he/she feels that participation is no longer in your best interest, or if you fail to follow the directions of the study staff.

If you decide to participate, you will agree to cooperate fully with the study visit schedule, and will follow the study staff's instructions.

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study.

Should you wish to withdraw your participation from the study, you must inform the study coordinators so that your file can be officially close.

Medical Care for Injury Related to the Study

In the event of an injury that occurs to you as a direct result of participating in this study, or undergoing study procedures you should immediately notify the study physician, Dr. Kesselman at 204 954 4486 or go to your nearest emergency room to receive necessary medical treatment. You are not waiving any of your legal rights by signing this consent form nor releasing the investigator or the sponsor from their legal and professional responsibilities. If any health abnormalities are identified in the clinical tests conducted during this experiment, Dr. Kesselman will be contacted, who will inform you of the results.

Efficacy of consumption of Canola and Flax Oils in Management of
Hypercholesterolemia and Other Disease Risk Factors

Questions

You are free to ask any questions that you may have about your treatment and your rights
as a research subject. If any questions come up during or after the study or if you have a
research-related injury, contact the study doctor and the study staff.

Investigator:	Dr. Peter Jones	Tel No.	204 474 9787
Coordinator:	Leah Gillingham	Tel No.	204 272 1551 or 204 474 8383
Study Physician:	Dr. Edward Kesselman	Tel No.	2049544486

For questions about your rights as a research subject, you may contact:

The Biomedical Research Ethics Board, University of Manitoba at 789-3389

Do not sign this consent form unless you have a chance to ask questions and have
received satisfactory answers to all of your questions.

Consent

I agree to allow the study doctor to inform my family doctor that I am participating in this
study or to obtain information regarding my medical history.

Yes No

1. I have read and understood this Information and Consent Form, and I freely
   and voluntarily agree to take part in the clinical trial (research study)
   described above.

2. I understand that I will be given a copy of the signed and dated Information
   and Consent Form. I have received an explanation of the purpose and duration
   of the trial, and the potential risks and benefits that I might expect. I was given
   sufficient time and opportunity to ask questions and to reflect back my
   understanding of the study to study personnel. My questions were answered to
   my satisfaction.

3. I agree to cooperate fully with the study doctor and will tell him if I
   experience any side effects, symptoms or changes in my health.

4. I am free to withdraw from the study at any time, for any reason, and without
   prejudice to my future medical treatment.

Initials of Subject:_____

> Efficacy of consumption of Canola and Flax Oils in Management of
> Hypercholesterolemia and Other Disease Risk Factors

5. I have been assured that my name, address and telephone number will be kept confidential to the extent permitted by applicable laws and/or regulations.

6. By signing and dating this document, I am aware that none of my legal rights are being waived.

Signature: Date/Time:

Printed name of above:

I confirm that I have explained the purpose, duration etc of this clinical trial, as well as any potential risks and benefits, to the subject whose name and signature appears above. I confirm that I believe that the subject has understood and has knowingly given their consent to participate by his/her personally dated signature.

Signature: Date/Time:

Printed name of above: Study role:

ALL SIGNATORIES MUST DATE THEIR OWN SIGNATURE

Efficacy of Consumption of Canola and Flax Oils in Management of
Hypercholesterolemia and Other Disease Risk Factors

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19/08/2007 Initials of Subject: _____

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November 19, 2007

Initials of Subject:_____

SUBJECT CONSENT FORM CORRESPONDING TO GENETICS STUDY

DESCRIBED IN CHAPTERS VI

October 8, 2009

UNIVERSITY OF **MANITOBA**

RESEARCH SUBJECT INFORMATION AND CONSENT FORM

RESEARCH SUBJECT INFORMATION AND CONSENT FORM FOR GENETICS ANALYSIS

Title of Study: Genetic Basis for Heterogeneity in Response of Plasma Lipids to Plant Sterols Supplementation and Fatty Acid Dietary Modification: Freezer Study

Investigator: Peter Jones, PhD
Richardson Centre Functional Foods and Nutraceuticals
University of Manitoba
196 Innovation Drive, Smartpark
Winnipeg, Manitoba R3T 6C5
Phone: (204) 474-9787

You are being asked to participate in a research study using samples collected in previous clinical trials. Participation is voluntary and you do not need to consent to the use of your biological samples in this study. Please take your time to review this Information and Consent Form. You may take your time to make your decision about participating in this research study and you may discuss it with your regular doctor, friends and family. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand.

NATURE AND DURATION OF PROCEDURE

We would like to investigate genetic variability in the responses of individuals to certain trial interventions by combining data collected from numerous previously completed clinical trials. From the blood drawn during one of the following clinical studies:

1) Evaluation of Plant Sterol and Cholesterol Absorption in Overweight Hypercholesterolemic Men with or without Coronary Heart Disease, REB #J2005:144, University of McGill
2) Relative Efficacy of Plant Sterols Given One or Three Times per Day in Management of Hypercholesterolemia, REB #J2005:148, University of McGill
3) Efficacy of Sterol Fortified Low Fat Soy Beverage on Cholesterol Metabolism, Inflammation and Oxidative Status in Humans – Study I, REB# 2007:110, University of Manitoba
4) Efficacy of Sterol Fortified Low Fat Soy Beverage on Cholesterol Metabolism, Inflammation and Oxidative Status in Humans – Study II, REB# 2007:110A, University of Manitoba
5) Efficacy of Consumption of Canola and Flax oils in the Management of Hypercholesterolemia and Other Disease Risk Factors, REB# 2007:071, University of Manitoba

Research subject ICF for genetic analysis   **October 8, 2009**

We would like to extract DNA and perform genetic analyses using a laboratory technique that recognizes specific genes to determine why some people decrease their cholesterol levels better than others in response to different diets. DNA is a molecule found in the cells of your body that is organized into genes that contain all of the information needed to make the proteins that perform specific biological functions in your body. As this analysis will be carried out on previously collected biological samples no additional visits to the research facility or from study staff will be required for this study.

CONFIDENTIALITY AND SAFEKEEPING OF DNA SAMPLES

All of the information obtained about you and the results of the research will be treated confidentially. We will protect your confidentiality by assigning your DNA sample a specific code. This code will link you to your DNA sample and can only be decoded by the principal researcher or an individual authorized by the latter. Samples of your DNA will be kept at the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, under the supervision of Dr. Peter Jones for a 2-year period following the end of the research project. After this time, all samples will be destroyed. Your DNA samples will only be used for the purpose of this research project.

Your participation and the results of the research will not appear in your medical record. Although the results of this study may be published or communicated in other ways, it will be impossible to identify you. Unless you have provided specific authorization or where the law permits or a court order has been obtained, your personal results will not be made available to third parties such as employers, government organizations, insurance companies, or educational institutions. This also applies to your spouse, other members of your family and your physician. However, for the purposes of ensuring the proper management of research, it is possible that a member of an ethics committee, or a representative from the Richardson Center for Functional Foods and Nutraceuticals, may consult your research data and record. You can communicate with the research team to obtain information on the general progress or the results of the research project. Project updates will be mailed at the end of the project. However, we will not communicate any individual results to you.

POTENTIAL RISKS AND/OR BENEFITS

As the DNA will be extracted from blood samples that have already been taken, there is no additional invasive procedure to undergo and no physical risk to you. However, when you donate blood or tissue for genetic testing or research, you are sharing genetic information, not only about yourself, but also about biological (blood) relatives who share your genes or DNA. There is a potential risk that information gained from genetic research could eventually be linked to you. You should be aware that genetic information cannot be protected from disclosure by court order. This potential re- identification of the information (e.g., to an employer or insurer) could lead to loss of privacy and to possible future discrimination in employment or insurance, against you or your biological relatives. Due to the rapid pace of technological advances, the potential future use of genetic information is unknown and therefore the potential future risks are also unknown.

Initials of Subject: _____

Research subject ICF for genetic analysis   **October 8, 2009**

While there may be no direct benefits to you for taking part in these additional analyses, we hope that these results will provide us with the information on genetic characteristics of people in which dietary strategies results in an enhanced or inferior cholesterol-lowering capacity.

QUESTIONS

If you have any questions or concerns regarding this study (REB# B2009:129) please do not hesitate to contact:

Dr. Peter Jones,
Richardson Center for Functional Foods and Nutraceuticals
Phone # 1-204 474-9787
or

The Bannatyne Research Ethics Board.
Bannatyne Campus, University of Manitoba
Phone # 1-204-789-3389

SIGNATURE OF PARTICIPANT

The procedures associated with this research study have been outlined to me in this consent form. I have had the opportunity to contact study staff and ask questions concerning any and all aspects of the project and procedures involved, and may continue in the future to ask further questions at any time, as it is my right to do so. I am aware that I may refuse to participate as well as withdraw my consent at any time. I acknowledge that no guarantee or assurance has been given by anyone as to the results to be obtained and that my participation in this study is completely voluntary. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. Samples will not be utilized for any additional analyses, nor stored for any prolonged period, nor shared with any other group, other than is indicated in the protocol, without my specific consent.

I, _____, have read the above description. I have been made aware of all the procedures, advantages and disadvantages of the study, which have been explained to me.

_____   _____
Signature of Subject   Date
By signing this consent form, you have not waived any of the legal rights that you have as a participant in a research study.

_____   _____
Signature of clinical coordinator   Date

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11/13/2009   Initials of Subject: _____

SUBJECT SCREENING FORM

CANOLA / FLAX SCREENING

NOTES:

Name:	DOC

How did you hear about the study?

(H): (C/W): E-mail:	Leave a message? Y…N

Sex: M…F Age (18-65): Weight: lbs Kg Height: ' '' [''] BMI (kg/m2):	Postmenopausal: Y…N DOB: Quick BMI:
Cholesterol lowering medication? Medication that affects lipid metabolism? *(in the last 3 months)*	Y…N Lipitor Cholestyramine Colestipol Fish oil capsules Plant sterols Other
Do you have high blood pressure?	Y (controlled, uncontrolled)…N Type of medication? What is your BP?

Smoker	Y….N	
Alcohol	Y….N	/day, /wk
Diabetes mellitus	Y….N	
Thyroid disease	Y….N	Stable doses of medication? Y…N
Kidney disease	Y….N	
Liver disease	Y….N	
Heart disease	Y….N	

Other medications	Y….N	Specify
Vitamin, Mineral supplement	Y….N	Specify
Herbal, food supplement	Y….N	Specify
Laxatives, Stool Softners	Y….N	
Fiber	Y….N	
Allergies (food)	Y….N	Specify
Lactose Intolerant	Y….N	
Vegetarian	Y….N	
Any metallic bone components	Y….N	
Exercise	Y….N	hrs/wk types
Other		

- 1/2 -

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First blood screening:

Date:
Time:

Second blood screening:

Date:
Time:

Medical exam:

Date:
Time:

ELIGIBLE: Y...N

Start date:
Subject code:

**Please refrain from fish oil capsules, flax oil, omega-3 supplements and fish 2 month before the start of the study and 2 weeks before screening

**Please fast for 12 hours from food/beverage, 24 hours from alcohol before screening

- 2/2 -

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MEDICAL SCREENING FORM

Flax/Canola Study 2007
Screening Medical Examination Form

Phase screening	Study Physician Dr. Edward Kesselman	Subject Code
Date of Visit _____/_____/_____ MM DD YR	Investigator Dr. Peter Jones	

COMPLETE PHYSICAL EXAMINATION

A. Vital Signs

Body Weight:_____ lbs _____ kg   **Height:** _____cm

Respiration:_____

Blood Pressure (seated): _____/_____ mmHg   **Heart Rate:**_____bpm
               systolic diastolic

Race/Ethnic Origin:
☐ **Caucasian**   ☐ **African-American/Canadian**   ☐ **Asian**   ☐ **Other:**_____

B. Body Systems (Check the appropriate box if organ system was examined. If not done, write N/D in the box)

	Normal	Abnormal	*Details of abnormal finding
1) Ears, Nose, Throat			
2) Eyes			
3) Dermatological			
4) Musculoskeletal			
5) Lymph Nodes			
6) Neurological			
7) Cardiovascular			
8) Respiratory			
9) Endocrine			
10) Urogenital			
11) Gastrointestinal (complete section C)			

C. Gastrointestinal Cont…

Bowel Habits: Frequency_____/Day   **Urination:** Frequency_____/Day

             Consistency_____   Nocturia_____/Night

Medications:

Flax/Canola Study 2007
Screening Medical Examination Form

Hospitalizations:

Family History:

D. Medical History **Exclusion** Criteria Screening Questionnaire	YES	NO
Have you taken a medication affecting lipid metabolism (cholestyramine, colestipol, niacin, colfibrate, gemfibrozil, probucol, HMG-CoA reductase inhibitors, and high-dose dietary supplements, plant sterols or fish oil capsules) within the past 3 months?		
Do you take any natural or pharmaceutical weight loss supplements or products?		
Do you smoke?		
Do you consume large amounts of alcohol? (more than 2 drinks per day or 12 drinks per week)		
Do you have diabetes mellitus?		
Do you have kidney disease?		
Do you have liver disease?		
Do you have heart disease?		
Do you have uncontrolled thyroid disease or hypertension? (Subject will be accepted if she is on a stable dose of a thyroid or blood pressure medication that has no known effects on blood lipid metabolism.)		
Are you pregnant or do you intend to become pregnant?		

Flax/Canola Study 2007
Screening Medical Examination Form

E. Additional Physician Notes
Based on the inclusion and exclusion criteria above, and the medical exam is the subject eligible to participate in the study protocol (circle one): YES   NO
Physician's Signature:_____
Date:_____

SUBJECT STUDY PROGRESS FORM

Patient Study Progress

Study ID: _____

PHASE 1

1. Day 1 (mm/dd/yyyy): ___/___/___ ☐
- DEXA
- Blood 2 EDTA, 2 Serum, 2 Heparin

2. Day 2 (mm/dd/yyyy): ___/___/___ ☐
- Blood 2 EDTA, 2 Serum, 2 Heparin

3. Week 1 (mm/dd/yyyy): ___/___/___ ☐
- Energy Expenditure
- Breath Collection during EE

4. Week 1 Side Effects Questionnaire: ___/___/___ ☐

---

5. Day 27 (mm/dd/yyyy): ___/___/___ ☐
- Flow Mediated Dilation @ Health Sciences Centre ☐
- Blood 2 EDTA, 2 Serum, 2 Heparin
- ^{13}C-ALA administration (before blood)   **Time: ___: ___**
- Give Breath Sampling Kits to Subjects

6. Day 28 (mm/dd/yyyy): ___/___/___ ☐
- Flow Mediated Dilation @ Health Sciences Centre ☐
- Blood 2 EDTA, 2 Serum, 2 Heparin (24hr ^{13}C- ALA) **Time: ___: ___**
- Collect Breath Sampling Kits from Subjects
- Side Effects Questionnaire

7. Day 29 (mm/dd/yyyy): ___/___/___ ☐
- DEXA before breakfast
- Blood 2 EDTA, 2 Serum, 2 Heparin (48hr ^{13}C- ALA) **Time: ___: ___**
- Side Effects Questionnaire

8. Week 4 (mm/dd/yyyy): ___/___/___ ☐
- Energy Expenditure

9. Week 4 Side Effects Questionnaire: ___/___/___ ☐

PHASE 2

10. Day 56 (mm/dd/yyyy): ___/___/___ ☐
  - DEXA
  - Blood 2 EDTA, 2 Serum, 2 Heparin

11. Day 57 (mm/dd/yyyy): ___/___/___ ☐
  - Blood 2 EDTA, 2 Serum, 2 Heparin

12. Week 1 (mm/dd/yyyy): ___/___/___ ☐
  - Energy Expenditure
  - Breath Collection during EE

13. Week 1 Side Effects Questionnaire: ___/___/___ ☐

---

14. Day 83 (mm/dd/yyyy): ___/___/___ ☐
  - Flow Mediated Dilation @ Health Sciences Centre ☐
  - Blood 2 EDTA, 2 Serum, 2 Heparin
  - ^{13}C-ALA administration (before blood)   **Time: ___ : ___**
  - Give Breath Sampling Kits to Subjects

15. Day 84 (mm/dd/yyyy): ___/___/___ ☐
  - Flow Mediated Dilation @ Health Sciences Centre ☐
  - Blood 2 EDTA, 2 Serum, 2 Heparin (24hr ^{13}C- ALA) **Time: ___ : ___**
  - Collect Breath Sampling Kits from Subjects
  - Side Effects Questionnaire

16. Day 85 (mm/dd/yyyy): ___/___/___ ☐
  - DEXA before breakfast
  - Blood 2 EDTA, 2 Serum, 2 Heparin (48hr ^{13}C- ALA) **Time: ___ : ___**
  - Side Effects Questionnaire

17. Week 4 (mm/dd/yyyy): ___/___/___ ☐
  - Energy Expenditure

18. Week 4 Side Effects Questionnaire: ___/___/___ ☐

PHASE 3

19. Day 112 (mm/dd/yyyy): ___/___/___ ☐
- DEXA
- Blood 2 EDTA, 2 Serum, 2 Heparin

20. Day 113 (mm/dd/yyyy): ___/___/___ ☐
- Blood 2 EDTA, 2 Serum, 2 Heparin

21. Week 1 (mm/dd/yyyy): ___/___/___ ☐
- Energy Expenditure
- Breath Collection during EE

22. Week 1 Side Effects Questionnaire: ___/___/___ ☐

---

23. Day 139 (mm/dd/yyyy): ___/___/___ ☐
- Flow Mediated Dilation @ Health Sciences Centre ☐
- Blood 2 EDTA, 2 Serum, 2 Heparin
- ^{13}C-ALA administration (before blood)   **Time: ___:___**
- Give Breath Sampling Kits to Subjects

24. Day 140 (mm/dd/yyyy): ___/___/___ ☐
- Flow Mediated Dilation @ Health Sciences Centre ☐
- Blood 2 EDTA, 2 Serum, 2 Heparin (24hr ^{13}C- ALA) **Time: ___:___**
- Collect Breath Sampling Kits from Subjects
- Side Effects Questionnaire

25. Day 141 (mm/dd/yyyy): ___/___/___ ☐
- DEXA before breakfast
- Blood 2 EDTA, 2 Serum, 2 Heparin (48hr ^{13}C- ALA) **Time: ___:___**
- Side Effects Questionnaire

26. Week 4 (mm/dd/yyyy): ___/___/___ ☐
- Energy Expenditure

27. Week 4 Side Effects Questionnaire: ___/___/___ ☐

SUBJECT MENSTRUAL CYCLE CHECKLIST

Canola Flax Study 2007/2008
MENSTRUAL CYCLE CHECKLIST

PHASE 1

Start (Day 1)
-Date (dd/mm/yyyy)   ___/___/___

-Day 1 of last cycle (dd/mm/yyyy)
   ___/___/___

-Calculated Day 14 of cycle (dd/mm/yyyy)
   ___/___/___

-Pregnancy Test (pre-DEXA) __ **Yes** __ **No**

-Results __ **Positive** __ **Negative**

End (Day 28)
-Date (dd/mm/yyyy)   ___/___/___

-Day 1 of last cycle (dd/mm/yyyy)
   ___/___/___

-Calculated Day 14 of cycle (dd/mm/yyyy)
   ___/___/___

-Pregnancy Test (pre-DEXA) __ **Yes** __ **No**

-Results __ **Positive** __ **Negative**

PHASE 2

Start (Day 1)
-Date (dd/mm/yyyy)   ___/___/___

-Day 1 of last cycle (dd/mm/yyyy)
   ___/___/___

-Calculated Day 14 of cycle (dd/mm/yyyy)
   ___/___/___

- Pregnancy Test (pre-DEXA) __ **Yes** __ **No**

-Results __ **Positive** __ **Negative**

End (Day 28)
-Date (dd/mm/yyyy)   ___/___/___

-Day 1 of last cycle (dd/mm/yyyy)
   ___/___/___

-Calculated Day 14 of cycle (dd/mm/yyyy)
   ___/___/___

- Pregnancy Test (pre-DEXA) __ **Yes** __ **No**

-Results __ **Positive** __ **Negative**

PHASE 3

Start (Day 1)
-Date (dd/mm/yyyy)   ___/___/___

-Day 1 of last cycle (dd/mm/yyyy)
   ___/___/___

-Calculated Day 14 of cycle (dd/mm/yyyy)
   ___/___/___

- Pregnancy Test (pre-DEXA) __ **Yes** __ **No**

-Results __ **Positive** __ **Negative**

End (Day 28)
-Date (dd/mm/yyyy)   ___/___/___

-Day 1 of last cycle (dd/mm/yyyy)
   ___/___/___

-Calculated Day 14 of cycle (dd/mm/yyyy)
   ___/___/___

- Pregnancy Test (pre-DEXA) __ **Yes** __ **No**

-Results __ **Positive** __ **Negative**

Study Co-ordinator Signature:_____

ENERGY EXPENDITURE TRACKING FORM

Phase: Week: Day: Meal: Date:

ENERGY EXPENDITURE TRACKING FORM

Patient: _____		Patient: _____	
Time	**Status**	**Time**	**Status**
___ : ___	ON	___ : ___	On
___ : ___	Meal Bf / Din	___ : ___	Meal Bf / Din
___ : ___	First Bite	___ : ___	First Bite
___ : ___	ON	___ : ___	ON
___ : ___	OFF	___ : ___	OFF
___ : ___	ON	___ : ___	ON
___ : ___	OFF	___ : ___	OFF
___ : ___	ON	___ : ___	ON
___ : ___	OFF	___ : ___	OFF
___ : ___	ON	___ : ___	ON
___ : ___	OFF	___ : ___	OFF
___ : ___	ON	___ : ___	ON
___ : ___	OFF	___ : ___	OFF
___ : ___	ON	___ : ___	ON
___ : ___	OFF	___ : ___	OFF

BREATH SAMPLING FORM

FLAX-CANOLA STUDY 2007-2008

SUBJECT CODE: **DATE:**

PHASE: **DAY:**

HEIGHT: **WEIGHT:**

*TRACER (name/amount): U-^{13}C$_{18}$-Alpha-Linolenic Acid (omega-3) / 45mg

THEORETICAL TIME	REAL TIME	TUBE #	Comments
		0	
*BREAKFAST		--	
		1	
		2	
		3	
		4	
LUNCH (first bite)		--	
		5	
		6	
		7	
		8	
DINNER		--	
		24	
		48	

COMMENTS:

***REMEMBER TO FAST 12hrs BEFORE DAY 28 BLOOD DRAW AND DEXA

COORDINATOR'S NOTES FORM

Coordinator's Notes
FLAX/CANOLA Study 2007

Subject ID:_____ Phase:_____ Date:_____

Subject ID:_____ Phase:_____ Date:_____

Subject ID:_____ Phase:_____ Date:_____

Subject ID:_____ Phase:_____ Date:_____

Subject ID:_____ Phase:_____ Date:_____

STUDY CALENDAR

February 08   [AM479]

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Jan-27 – Feb-2	Jan-27 *** GROUP 6 ***	28 ***Day 1*** Blood Draw DEXA + PWA c/u 11:40am (ST, B)	29 ***Day 2*** Blood Draw EE 5:30pm.	30	31	Feb-1	2 Breakfast 8-10am
Feb-3 – 9	3 Breakfast 9-10am Physical w/ Dr. K.	4	5	6	7	8	9 Breakfast 8-10am
Feb-10 – 16	10 Breakfast 9-10am	11	12	13	14	15	16 Breakfast 8-10am
Feb-17 – 23	17 Breakfast 9-10am	18	19	20	21 Energy Expenditure (6.5 hours) - Tues Feb 19 to Sun Feb 24 - Book EE date with Jen or Leah St.Boniface - Carotid Ultrasound (11:30am-12:15pm) - Book w/ Jen or Leah	22	23 ***Day 27*** Blood Draw Isotope+Breath Samp
Feb-24 – Mar-1	24 ***Day 28*** Blood Draw DEXA + PWA	25 ***Day 29*** Blood Draw END OF PHASE 1 PH 2 starts Mar 17	26	27	28	29	Mar-1

February 08

March 08

Leah Gillingham

21/01/2008 4:19 PM

SMOOTHIE INSTRUCTIONS

How to Make the Flax Study Smoothies:

Treatment **A** and **C** Smoothies:

Equipment:

- tall plastic measuring cup
- PC hand-held blender
- spatula
- paper cup with lid
- scale

Ingredients: frozen fruit, sherbet, FAT-FREE SKIM milk, treatment oil in brown glass bottle

How to make:

1. Place tall plastic measuring cup on scale, then ZERO/TARE
2. Add frozen fruit to the nearest 1g, then ZERO/TARE
3. Add sherbet to the nearest 1g, then ZERO/TARE
4. Add FAT FREE SKIM MILK to the nearest 1g, then ZERO/TARE
5. Add treatment oil SLOWLY to measure to the nearest 1g
6. Using hand-held blender, blend all the ingredient until COMPLETELY blended
7. Detach blender arm and scrape blade and head with spatula as much as possible
8. Scrape the smoothie into the paper cup
9. Put lid on TIGHT and label with subject code, "F" (for FLAX), breakfast
10. Rinse all pieces before next smoothie

***Please ask manager if you have any questions about equipment, ingredients or preparation
***If a mistake happens, don't be shy, let manager know

SMOOTHIE INSTRUCTIONS

How to Make the Flax Study Smoothies:

Treatment B Smoothies:

Equipment:

- tall plastic measuring cup
- PC hand-held blender
- spatula
- paper cup with lid
- scale

Ingredients: frozen fruit, sherbet, FAT-FREE SKIM milk, unsalted butter, lard, extra virgin olive oil, sunflower oil

Prepare Oil:

- Heat a bowl of butter in the microwave until it just turns to liquid
- Heat a bowl of lard in the microwave until its just turns liquid
  *MAKE SURE BUTTER AND LARD ARE MELTED COMPLETELY BUT DO NOT OVER HEAT

How to make:

1. Place tall plastic measuring cup on scale, then ZERO/TARE
2. Add frozen fruit to the nearest 1g, then ZERO/TARE
3. Add sherbet to the nearest 1g, then ZERO/TARE
4. Add FAT FREE SKIM MILK to the nearest 1g, then ZERO/TARE
5. Add olive oil SLOWLY to measure to the nearest 1g, then ZERO/TARE
6. Add sunflower oil SLOWLY to measure to the nearest 1g, then ZERO/TARE
7. Use a spoon to add liquid lard to the nearest 1g, then ZERO/TARE
8. Use a spoon to add liquid butter to the nearest 1g, then ZERO/TARE
9. ******Microwave all ingredients in the plastic measuring cup for 30 seconds******
10. Using hand-held blender, blend all the ingredient until COMPLETELY blended (make sure all the butter and lard is blended and there are no large chunks!!!)
11. Detach blender arm and scrape blade and head with spatula as much as possible
12. Scrape the smoothie into the paper cup
13. Put lid on TIGHT and label with subject code, "F" (for FLAX), breakfast
14. Rinse all pieces before next smoothie

   ***Please ask manager if you have any questions about equipment, ingredients or preparation
   ***If a mistake happens, don't be shy, let manager know

PUDDING INSTRUCTIONS

How to Make the Flax Study PUDDINGS:

SEPARATE DIET CARDS INTO GROUPS AND DO ONE TREATMENT AT A TIME

Equipment:

- Small round plastic container with lid
- Large Spoon and Large Fork (for Treatment B Puddings)

Treatment B PUDDINGS:

Ingredients: prepared pudding (following exact instruction on the pudding box), unsalted butter, lard, extra virgin olive oil, sunflower oil

Prepare Oil:

- Heat a bowl of butter in the microwave until it just turns to liquid
- Heat a bowl of lard in the microwave until it just turns to liquid
  *MAKE SURE BUTTER AND LARD ARE MELTED COMPLETELY BUT DO NOT OVER HEAT

How to make Treatment B puddings:

1. Place small round plastic container with lid on scale, then ZERO/TARE
2. **Add prepared pudding FIRST!,** then ZERO/TARE
3. 1st: Add olive oil SLOWLY to measure to the nearest 1g, then ZERO/TARE
4. 2nd: Add sunflower oil SLOWLY to measure to the nearest 1g, then ZERO/TARE
5. 3rd: Use a spoon to add liquid lard to the nearest 1g, then ZERO/TARE
6. 4th: Use a spoon to add liquid butter to the nearest 1g
7. **Use a fork to mix the pudding immediately after adding butter and lard. If butter and lard gets hard, put in microwave for 30 seconds before mixing.**
8. Put lid on TIGHT and label with subject code, "F" (for FLAX), breakfast

Treatment A and C PUDDINGS:

Ingredients: prepared pudding (following exact instruction on the pudding box), treatment oil in brown glass bottle

How to make Treatment A and C puddings:

1. Place small round plastic container with lid on scale, then ZERO/TARE
2. **Add pudding FIRST!,** then ZERO/TARE
3. Add treatment oil SLOWLY to the nearest 1g
4. DO NOT mix treatment A or C pudding with a fork, they will get mixed in the morning instead
5. Put lid on TIGHT and label with subject code, "F" (for FLAX), breakfast

SUBJECT DAILY ENERGY EXPENDITURE CALCULATOR

Patient Total Energy Expenditure Calculator

Mifflin Equation

Patient: **XX400**   **PHASE:** 2

Date (mm/dd/yyyy): 10-Mar-08

Men   RMR = (9.99 x Wt) + (6.25 x Ht) - (4.92 x age) + 5
Wt(kg)	0.0
Ht(cm)	0
Age	0

Resting Metabolic Rate   5 kcal
Activity Factor   1.7

Total energy expenditure   **8.5 Kcal**

Women   RMR = (9.99 x Wt) + (6.25 x Ht)- (4.92 x age) - 161
Wt(kg)	81.8
Ht(cm)	162.5
Age	58

Resting Metabolic Rate   1386.447
Activity factor   1.7

Total energy expenditure   **2356.96 Kcal**

SUBJECT DIET CARDS

PATIENT _____XX400_____

DAY 1   **PHASE:** **2**

Total Cal	3000	kcal /day	
BREAKFAST	Grams		Grams

	Grams		Grams
Tropicana Orange Juice BKC	223.9		
		Treatment	
Scrambled Eggs Dish		Treatment Oil: A	27.9
Large Whole FreshEgg-Raw-Each	11.9	Nonfat Skim Milk-No Added Vit /	99.5
Raw Egg White-Fresh-Cup Measure	179.1	Strawberries-Frozen,Unsweetd,	99.5
Tomatoes-Chopped/Sliced,Red,Raw,Ripe-Cup	64.7	Orange Sherbet	99.5
Becel - Original	7.0		
Bagel and Jam			
Whole Wheat Bagel (4 1/2" diameter)	44.8	**TREATMENT**	_____
Kraft Strawberry Jam KFT	19.9		

PATIENT _____XX400_____

DAY 1   **PHASE:** **2**

Total Cal	3000	kcal /day	
LUNCH	Grams		Grams

	Grams		Grams
Dole OrangeStrawberryBananaJuice-RTD TRO	223.9	**Cucumber and Tomato Salad**	
		Cucumber-Peeled, Chopped	99.5
Pita Pizza		Tomatoes-Chopped	99.5
Whole Wheat Pita Pocket Bread 6 1/2"	109.5	S&W White Distilled Vinegar-Tb	4.0
Contadina Pizza Sauce-Original DLM	64.7	Canola Oil	5.0
Chicken Breast-w/o Skin-Boneless-Roasted	32.8		
Mushrooms-White-Raw MUC	44.8	**Treatment**	
Sweet Green Bell Peppers-Raw-Ring	36.8	Treatment Oil: A	27.9
Baby Zucchini Squash-Raw	39.8	Prepared Pudding	156.7
White Onions-Raw-Chopped-Cup	24.9		
Mozzarella Cheese-Part Skim-Shredded	29.9		
		TREATMENT	_____

PATIENT _____XX400_____

DAY 1   **PHASE:** **2**

Total Cal	3000	kcal /day	
DINNER	Grams		Grams

	Grams		Grams
Apple Juice + Vit C-Cnd/Bottled,Unsw	238.9	**Treatment**	
		Treatment Oil: A	27.9
Chicken Dish		Prepared Pudding	156.7
Chicken Breast-w/o Skin-Boneless-Roasted	114.5		
Cranberry Sauce-Canned,Sweetened-Cup	54.7		
Butter-Salted LOL	11.9		
Peeled Potato-Boiled w/o Skin-Each	149.3	**TREATMENT**	_____
Whole Carrots (7.5" Long)-Raw-Each	99.5		

PATIENT ____XX400____

DAY 2 PHASE: **2**

Total Cal	3000	kcal /day

BREAKFAST	Grams		Grams
Fruit Dish		**Treatment**	
Dried Apricots DFA	20.9	Treatment Oil: A	27.9
Seedless Raisins-Cup-Unpacked	20.9	Nonfat Skim Milk-No Added	99.6
		Raspberries-Frozen,Unswee	99.6
French Toast Dish		Raspberry Sherbet	99.6
Maple Syrup	30.9		
Canola Oil	5.0		
RCFFN French Toast Mix	210.3		
Whole Wheat Bread	79.7		

TREATMENT	_____

PATIENT ____XX400____

DAY 2 PHASE: **2**

Total Cal	3000	kcal /day

LUNCH	Grams		Grams
Tropicana Orange Juice BKC	224.2	**Treatment**	
		Treatment Oil: A	27.9
Ham Sandwhich		Prepared Pudding	156.9
Whole Wheat Bread	79.7		
Yellow Mustard-Prep	14.95		
Ham, sliced, extra lean, (5% fat)-Slice	69.8		
Cheese, Low Fat, Cheddar-Slice	24.9		
Leaf Lettuce-Raw, Shredded FDA	24.9		
Large Sweet Green Bell Peppers-Raw-Each	19.9	TREATMENT	_____
Tomatoes-Chopped/Sliced,Red,Raw,Ripe-Cup	24.9		
RCFFN Cream of Broccoli Soup	328.8		

PATIENT ____XX400____

DAY 2 PHASE: **2**

Total Cal	3000	kcal /day

DINNER	Grams		Grams
Spaghetti Dish		**Treatment**	
Spaghetti Noodles-Enr-Ckd	224.2	Treatment Oil: A	27.9
RCFFN Spaghetti Sauce	237.2	Prepared Pudding	156.9
Parmesan Cheese-Grated	14.0		
1% Fat Cottage Cheese	44.8		

TREATMENT	_____

	PATIENT	XX400

DAY 3

PHASE: 2

Total Cal	3000	kcal /day

BREAKFAST	Grams		Grams
Fruit Dish		**English Muffin Dish**	
Peaches in Juice-Cnd-Halves/Slices-Cup	65.7	Whole Wheat English Muffin-Toaste	59.7
Florida Orange-Cup	65.7	Kraft Strawberry Jam KFT	29.9
Banana Slices-Cup	49.8		
1% Fat Cottage Cheese	99.5	**Treatment**	
		Treatment Oil: A	27.9
		Nonfat Skim Milk-No Added Vit A	99.5
Scrambed Egg Dish with CHEESE		Mango -Frozen,Unsweetd,Thawed-	99.5
Large Whole FreshEgg-Raw-Each	19.9	Mango Sherbet	99.5
Large Raw Egg White-Fresh-Each	134.4		
Butter-Salted LOL	8.0		
Cheese, Low Fat, Cheddar-Shredded-Cup	19.9		

TREATMENT _____

	PATIENT	XX400

DAY 3

PHASE: 2

Total Cal	3000	kcal /day

LUNCH	Grams		Grams
Tropics Orange Kiwi PassionJuice-RTD TRO	248.8	**Treatment**	
		Treatment Oil: A	27.9
Chicken Fajitas Dish		Prepared Pudding	156.7
RCFFN Chicken for Fajitas	84.6		
RCFFN Vegetables for Fajitas	124.4		
Flour Tortilla-10 inch	60.7		
Dessert			
Tropical Fruit Salad-LightSyrup-Cnd DLM	89.57	**TREATMENT** _____	

	PATIENT	XX400

DAY 3

PHASE: 2

Total Cal	3000	kcal /day

DINNER	Grams		Grams
Apple Juice-Canned/Bottled,Unsweetened	209.0		
		Treatment	
Soup Dish		Treatment Oil: A	27.9
Canola Oil	5.0	Prepared Pudding	156.7
RCFFN Soup Tomato Macaroni	288.6		
Beef Stirfry			
Long Grain White Rice-Enr-Ckd	174.2		
Carrots+Celery for StirFry	58.7	**TREATMENT** _____	
Beef for StirFry	110.5		

STUDY END QUESTIONNAIRE

CANOLA FLAX STUDY 2007/08 EXIT QUESTIONNAIRE

STUDY ID: _____   DATE: _____

***Please answer the following questions honestly and to the best of your ability.

Treatment Sensory Questionnaire

PHASE 1

1. In Phase 1, considering the FLAVOURS of the OILS added to the **PUDDINGS** (not the pudding flavours, i.e. pistachio versus chocolate), rank your acceptance of the puddings on a scale of 1 to 9.
   (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely") - Please circle a number.

   1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

2. In Phase 1, considering the TEXTURE of the OILS added to the **PUDDINGS**, rank your acceptance of the puddings on a scale of 1 to 9.
   (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely") - Please circle a number.

   1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

3. With the **PUDDINGS** in Phase 1, did you detect the presence of off tastes/odour on a scale of 1 to 7?
   (1 = no off-taste/odour; 7 = strong off-taste/odour). Off-taste/odour is defined as "the presence of something agreeable or disagreeable differing from the typical pudding due to the oils added"

   1 - 2 - 3 - 4 - 5 - 6 - 7

4. In Phase 1, considering the FLAVOURS of the OILS added to the **SMOOTHIES** (not the smoothie flavours, i.e. raspberry versus mango), rank your acceptance of the smoothies on a scale of 1 to 9.
   (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely")

   1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

5. In Phase 1, considering the TEXTURE of the OILS added to the **SMOOTHIES**, rank your acceptance of the smoothies on a scale of 1 to 9.
   (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely")

   1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

6. With the SMOOTHIES in Phase 1, did you detect the presence of off tastes/odour on a scale of 1 to 7?
   (1 = no off-taste/odour; 7 = strong off-taste/odour). Off-taste/odour is defined as "the presence of something agreeable or disagreeable differing from the typical pudding due to the oils added"

   1 - 2 - 3 - 4 - 5 - 6 - 7

Other Comments:

1

PHASE 2

1. In Phase 2, considering the FLAVOURS of the OILS added to the **PUDDINGS**, rank your acceptance of the puddings on a scale of 1 to 9.
    (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely")

    1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

2. In Phase 2, considering the TEXTURE of the OILS added to the **PUDDINGS**, rank your acceptance of the puddings on a scale of 1 to 9.
    (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely")

    1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

3. With the **PUDDINGS** in Phase 2, did you detect the presence of off tastes/odour on a scale of 1 to 7?
    (1 = no off-taste/odour; 7 = strong off-taste/odour). Off-taste/odour is defined as "the presence of something agreeable or disagreeable differing from the typical pudding due to the oils added"

    1 - 2 - 3 - 4 - 5 - 6 - 7

4. In Phase 2, considering the FLAVOURS of the OILS added to the **SMOOTHIES** (not the smoothie flavours, i.e. raspberry versus mango), rank your acceptance of the pudding on a scale of 1 to 9.
    (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely")

    1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

5. In Phase 2, considering the TEXTURE of the OILS added to the **SMOOTHIES**, rank your acceptance of the smoothies on a scale of 1 to 9.
    (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely")

    1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

6. With the **SMOOTHIES** In Phase 2, did you detect the presence of off tastes/odour on a scale of 1 to 7?
    (1 = no off-taste/odour; 7 = strong off-taste/odour). Off-taste/odour is defined as "the presence of something agreeable or disagreeable differing from the typical pudding due to the oils added"

    1 - 2 - 3 - 4 - 5 - 6 - 7

Other Comments:

PHASE 3

1. In Phase 3, considering the FLAVOURS of the OILS added to the **PUDDINGS**, rank your acceptance of the pudding on a scale of 1 to 9.
    (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely")

    1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

2. In Phase 3, considering the TEXTURE of the OILS added to the **PUDDINGS**, rank your acceptance of the pudding on a scale of 1 to 9.
(9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely")

1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

3. With the **PUDDINGS** in Phase 3, did you detect the presence of off tastes/odour on a scale of 1 to 7?
(1 = no off-taste/odour; 7 = strong off-taste/odour). Off-taste/odour is defined as "the presence of something agreeable or disagreeable differing from the typical pudding due to the oils added"

1 - 2 - 3 - 4 - 5 - 6 - 7

4. In Phase 3, considering the FLAVOURS of the OILS added to the **SMOOTHIES** (not the smoothie flavours, i.e. raspberry versus mango), rank your acceptance of the pudding on a scale of 1 to 9.
(9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely")

1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

5. In Phase 3, considering the TEXTURE of the OILS added to the **SMOOTHIES**, rank your acceptance of the smoothies on a scale of 1 to 9.
(9 = "like very much"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely")

1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9

6. With the **SMOOTHIES** in Phase 3, did you detect the presence of off tastes/odour on a scale of 1 to 7?
(1 = no off-taste/odour; 7 = strong off-taste/odour). Off-taste/odour is defined as "the presence of something agreeable or disagreeable differing from the typical pudding due to the oils added"

1 - 2 - 3 - 4 - 5 - 6 - 7

Other Comments:

Can you guess what treatment oils you were on during each phase? Please write the appropriate letter associated with the oil beside each of the 3 phases:

X – Canola Oil   **Y** – Canola/Flax Oil Blend   **Z** – Average American Oil Blend

PHASE 1 _____   PHASE 2 _____   PHASE 3 _____

Any General Comments on the Treatment Smoothies and Puddings:

3

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Treatment Side Effects:

1   Did you experience any gastrointestinal side-effect(s) during any of the phases such as diarrhea, constipation, increased flatulence, cramping, nausea?

    a.   YES

    b.   NO

    c.   If YES, please indicate which phase, what side-effect(s), duration of side effect (i.e. 1-2 days, 3-5 days, 1 weeks, >1 week), and did you take any pharmacological treatments to resolve the side-effect

PHASE	SIDE EFFECT(S) i.e. diarrhea, constipation, increased flatulence, cramping, nausea, other	DURATION i.e. 1-2 days, 3-5 days, 1 week, >1 week	Medication to treat
PHASE 1			
PHASE 2			
PHASE 3			

Other Comments: _____

_____

2   Did you experience any other side effects, positive or negative, such as softer skin, more energy, etc?

    a.   YES

    b.   NO

    c.   If YES, please give details and indicate the phase: _____

_____

_____

Supplements, Medications and Foods:

1.   Please list the natural health products or supplements that you were taking during the study:

_____

_____

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2. Did you consistently take these supplement(s) throughout each of the 3 phases?

    a. YES

    b. NO

    c. If NO, please indicate which phase (i.e. 1, 2, or 3) where there was a change in supplements and what the change was.

_____

_____

3. Please list the prescription medication(s) that you were taking during the study (not including short term medications like Tylenol for 1 day):

_____

_____

4. Did you consistently take these medication(s) throughout each of the 3 phases?

    a. YES

    b. NO

    c. If NO, please indicate which phase (i.e. 1, 2, or 3) where there was a change in medication(s) and what the change was.

_____

_____

5. Did you consume any omega-3 rich foods during the washout periods, such as ground flaxseed, flaxseed oil, omega-3 supplements or oils (including fish oil pills), fish (more than 1 time per week), large amounts of canola oil (i.e. more than 2 tablespoons per day)?

    a. YES

    b. NO

    c. If YES, please indicate what foods and approximate quantity per day or week.

_____

_____

Any General Comments on Side-effects, supplements, medications:

5

Physical Activity:

Light Physical Activity

1. When you are at work/school, which of the following best describes what you do?
   a. Mostly sitting or standing
   b. Mostly walking
   c. Mostly heavy labour or physically demanding work

2. Was this activity level consistent throughout each of the 3 phases?
   a. YES
   b. NO
   c. If NO, please indicate which phase your activity level changed and if it was an increase or decrease in activity? (Circle the appropriate PHASE and ARROW).
      i. Phase 1 - ↓ or ↑
      ii. Phase 2 - ↓ or ↑
      iii. Phase 3 - ↓ or ↑

Moderate Physical Activity

3. Do you do moderate activities for at least 10 minutes at a time, such as brisk walking, bicycling, vacuuming, gardening, or anything else that causes a small increase in breathing or heart rate?
   a. YES
   b. NO

4. How often a week do you do these activities for at least 10 minutes at a time?
   a. _____ hours and/or minutes per day
   b. _____ days per week

5. Was this activity level consistent throughout each of the 3 phases?
   a. YES
   b. NO
   c. If NO, please indicate which phase your activity level changed and if it was an increase or decrease in activity? (Circle the appropriate PHASE and ARROW).
      i. Phase 1 - ↓ or ↑
      ii. Phase 2 - ↓ or ↑
      iii. Phase 3 - ↓ or ↑

Vigorous Physical Activity

6. Do you do vigorous activities for at least 10 minutes at a time, such as running, aerobics, heavy yard work, or anything else that causes large increases in breathing or heart rate?
   a. YES
   b. NO

7. How often do you do these activities for at least 10 minutes at a time?
   a. _____ hours and/or minutes per day
   b. _____ days per week

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8. Was this activity level consistent throughout each of the 3 phases?
   a. YES
   b. NO
   c. If NO, please indicate which phase your activity level changed and if it was an increase or decrease in activity? (circle the appropriate PHASE and ARROW)
      i. Phase 1 - ↓ or ↑
      ii. Phase 2 - ↓ or ↑
      iii. Phase 3 - ↓ or ↑

Please give us any other general comments, feedback or suggestions:

Thank you so much for taking the time to complete this questionnaire! Please return to Leah ☺

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ADDITIONAL RESULTS AND TABLES CORRESPONDING TO STUDIES

DESCRIBED IN CHAPTERS V AND VI

CHAPTER V SUPPLEMENT

Assessment of Sensory Characteristics and Side effects

Methods

For each treatment, milkshakes and puddings containing the experimental oils, subjects

completed a short sensory questionnaire at the end of each study phase. Flavour and

texture was assessed using a 9-point hedonic scale (9 = "like extremely"; 8 = "like very

much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither like nor dislike"; 4 =

"dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike

extremely"), as described by Peryam and Girardot (1). Furthermore, the presence of off-

taste/odour for each treatment was evaluated using a 7-point intensity scale (1= no off-

taste/odour; 7 = strong off-taste/odour), as described by Lawless and Heymann (2).

Subjects were asked to report if they experienced side effects (1 = yes; 2 = no) from

consumption of the experimental diets containing the treatment milkshakes and puddings

at the end of each study phase. Scores after each phase were compiled for statistical

analysis using linear mixed model analysis of covariance (ANCOVA) and presented as

mean ± SD. Statistical significance was set at $P \leq .05$ for all analyses.

Results

The supplemental table (**Table III.1**) outlines the sensory characteristic ratings after

consumption of the treatments at the end of each phase. The majority of subjects reported

no side effects from consumption of the experimental diets. The reported side effects

included gastro-intestinal irregularities, namely constipation, followed by diarrhea and

flatulence for an average duration of 3–5 days. Finally, side effects did not differ between

treatment groups, and therefore, may have been a result of the controlled background

experimental diet.

Table III.1: Comparison of sensory characteristics and side effect ratings after consumption of pudding and milkshake treatments.

	Western diet	High-oleic canola oil diet	Flaxseed and high-oleic canola oil diet	P-value
Pudding flavour	5.8 ± 1.9^a	7.0 ± 1.5^b	5.9 ± 2.4^a	0.002
Pudding texture	4.2 ± 2.3^a	7.2 ± 1.6^b	6.7 ± 1.6^b	< 0.001
Pudding off-taste/odour	2.6 ± 2.0	2.0 ± 1.6	2.8 ± 2.1	0.057
Milkshake flavour	6.3 ± 2.0^a	7.3 ± 1.9^b	$6.9 \pm 1.8^{a,b}$	0.005
Milkshake texture	4.9 ± 2.6^a	7.6 ± 1.4^b	7.3 ± 1.4^b	<0.001
Milkshake off-taste/odour	2.4 ± 1.9^a	1.4 ± 0.8^b	$2.0 \pm 1.4^{a,b}$	0.001
Side effects	1.7 ± 0.5	1.7 ± 0.4	1.8 ± 0.4	0.568

Values are means $\pm$ SD; n = 34. [a,b,c] Mean values with unlike superscript were significantly different between treatment groups ($P < 0.05$). P-values are shown for the treatment effect between groups analyzed by mixed model ANOVA (with the Bonferroni post hoc test for multiple comparisons).

References

(1) Peryam DR, Girardot NF. Advanced taste-test method. Food Eng 1952;194:58.

(2) Lawless HT, Heymann H. Sensory evaluation of food. New York: Chapman & Hall; 1998.

GC-IRMS CHROMATOGRAM
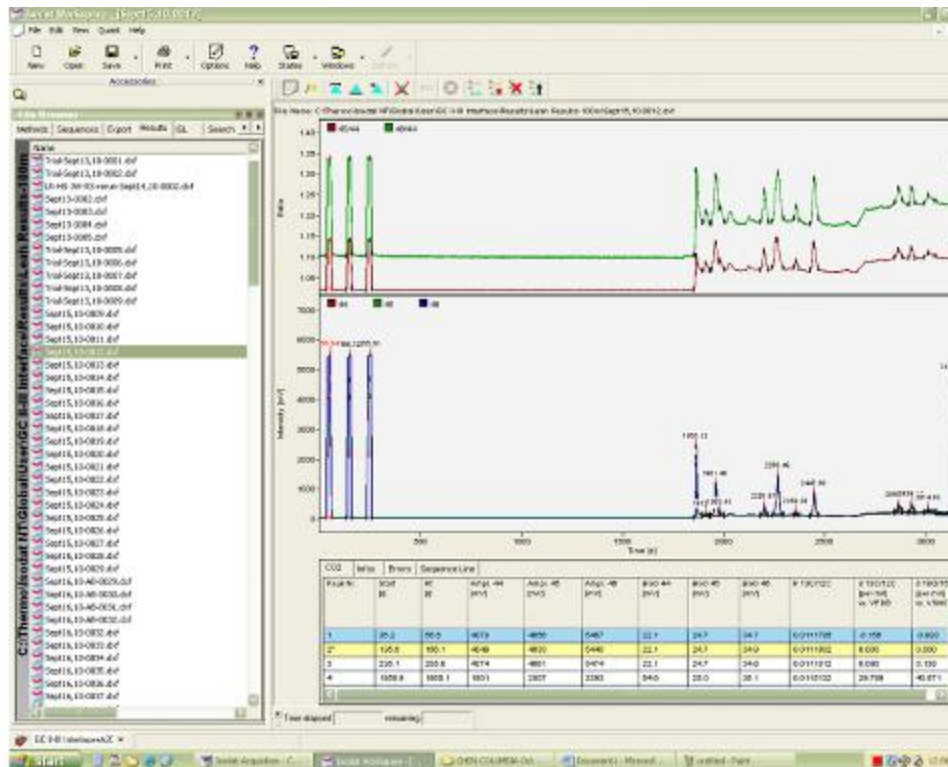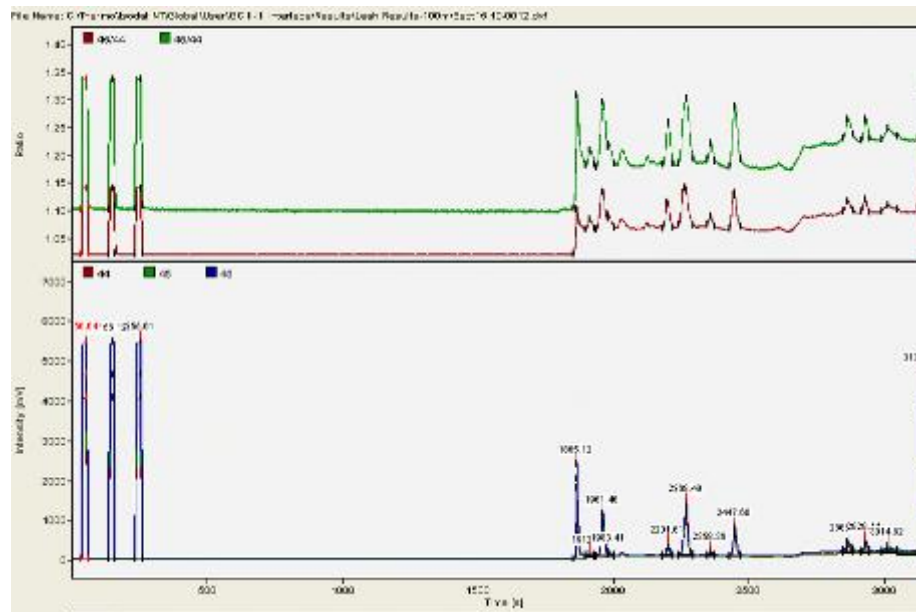
SINGLE NUCLEOTIDE POLYMORPHISM (SNP) TABLES

Table III.2: Serum total cholesterol and LDL-cholesterol at the end (Day 29) of each experimental diet classified by SNP genotype.

| | | Total Cholesterol | | | | | | LDL cholesterol | | | | | |
| | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | |
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	5.95	0.26	5.54	0.23	5.30	0.23	3.66	0.25	3.20	0.20	3.09	0.23
Heterozygous G/T	18	5.48	0.22	5.08	0.18	4.95	0.18	3.46	0.19	3.01	0.18	3.04	0.16
Homozygous T/T	4	5.36	0.45	5.19	0.40	5.25	0.27	3.45	0.36	3.15	0.23	3.22	0.17
P-Value		0.483		0.408		0.456		0.935		0.726		0.424	
rs174545 (FADS1)													
Homozygous C/C	14	5.95	0.26	5.54	0.23	5.30	0.23	3.66	0.25	3.20	0.20	3.09	0.23
Heterozygous C/G	18	5.48	0.22	5.08	0.18	4.95	0.18	3.46	0.19	3.01	0.18	3.04	0.16
Homozygous G/G	4	5.36	0.45	5.19	0.40	5.25	0.27	3.45	0.36	3.15	0.23	3.22	0.17
P-Value		0.483		0.408		0.456		0.935		0.726		0.424	
rs174561 (FADS1)													
Homozygous T/T	15	5.92	0.24	5.53	0.22	5.34	0.22	3.63	0.23	3.18	0.18	3.13	0.22
Heterozygous C/T	17	5.48	0.24	5.06	0.19	4.89	0.18	3.47	0.20	3.01	0.19	3.00	0.17
Homozygous C/C	4	5.36	0.45	5.19	0.40	5.25	0.27	3.45	0.36	3.15	0.23	3.22	0.17
P-Value		0.519		0.402		0.267		0.970		0.731		0.393	
rs174583 (FADS2)													
Homozygous C/C	13	6.03	0.26	5.58	0.25	5.38	0.24	3.73	0.26	3.26	0.20	3.16	0.24
Heterozygous C/T	19	5.46	0.21	5.07	0.17	4.92	0.17	3.41	0.18	2.98	0.17	2.99	0.16
Homozygous T/T	4	5.36	0.45	5.19	0.40	5.25	0.27	3.45	0.36	3.15	0.23	3.22	0.17
P-Value		0.294		0.366		0.256		0.708		0.511		0.372	
rs953413 (ELOVL2)													
Homozygous A/A	11	5.39	0.38	5.00	0.26	4.98	0.28	3.39	0.32	2.95	0.22	3.05	0.25
Heterozygous A/G	20	5.77	0.18	5.43	0.18	5.18	0.16	3.65	0.15	3.24	0.14	3.12	0.14
Homozygous G/G	5	5.76	0.32	5.25	0.31	5.18	0.36	3.37	0.40	2.86	0.45	2.98	0.43
P-Value		0.671		0.468		0.732		0.306		0.320		0.775	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.3: Serum triglyceride and HDL-cholesterol at the end (Day 29) of each experimental diet classified by SNP genotype.

| | | Triglycerides | | | | | | HDL | | | | | |
| | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | |
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	1.79	0.26	1.96	0.31	1.80	0.30	1.48	0.11	1.45	0.11	1.38	0.11
Heterozygous G/T	18	1.55	0.25	1.74	0.26	1.45	0.14	1.32	0.06	1.27	0.07	1.24	0.07
Homozygous T/T	4	1.48	0.23	1.84	0.19	2.02	0.29	1.24	0.35	1.16	0.17	1.10	0.37
P-Value		0.467		0.541		0.242		0.422		0.253		0.447	
rs174545 (FADS1)													
Homozygous C/C	14	1.79	0.26	1.96	0.31	1.80	0.30	1.48	0.11	1.45	0.11	1.38	0.11
Heterozygous C/G	18	1.55	0.25	1.74	0.26	1.45	0.14	1.32	0.06	1.27	0.07	1.24	0.07
Homozygous G/G	4	1.48	0.23	1.84	0.19	2.02	0.29	1.24	0.17	1.16	0.17	1.10	0.18
P-Value		0.467		0.541		0.242		0.422		0.253		0.447	
rs174561 (FADS1)													
Homozygous T/T	15	1.76	0.24	1.93	0.29	1.77	0.28	1.49	0.10	1.46	0.10	1.40	0.10
Heterozygous C/T	17	1.56	0.26	1.75	0.28	1.46	0.15	1.30	0.06	1.25	0.07	1.21	0.07
Homozygous C/C	4	1.48	0.23	1.84	0.19	2.02	0.29	1.24	0.17	1.16	0.17	1.10	0.18
P-Value		0.520		0.543		0.246		0.252		0.165		0.285	
rs174583 (FADS2)													
Homozygous C/C	13	1.86	0.27	2.03	0.33	1.88	0.32	1.45	0.11	1.39	0.10	1.35	0.11
Heterozygous C/T	19	1.51	0.24	1.70	0.25	1.42	0.14	1.35	0.06	1.32	0.08	1.27	0.07
Homozygous T/T	4	1.48	0.23	1.84	0.19	2.02	0.29	1.24	0.17	1.16	0.17	1.10	0.18
P-Value		0.207		0.332		0.146		0.631		0.432		0.615	
rs953413 (ELOVL2)													
Homozygous A/A	11	1.49	0.17	1.76	0.16	1.57	0.12	1.34	0.10	1.24	0.08	1.21	0.09
Heterozygous A/G	20	1.62	0.19	1.79	0.23	1.70	0.23	1.37	0.07	1.37	0.08	1.27	0.07
Homozygous G/G	5	1.99	0.86	2.22	0.92	1.64	0.41	1.49	0.21	1.37	0.21	1.44	0.25
P-Value		0.842		0.773		0.817		0.601		0.381		0.488	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.4: Serum total:HDL-cholesterol and LDL:HDL-cholesterol at the end (Day 29) of each experimental diet classified by SNP genotype.

		Total:HDL-Cholesterol Ratio						LDL:HDL-Cholesterol Ratio					
		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	4.40	0.44	4.19	0.41	4.24	0.45	2.77	0.36	2.46	0.29	2.54	0.35
Heterozygous G/T	18	4.29	0.27	4.18	0.29	4.19	0.26	2.68	0.17	2.42	0.14	2.56	0.17
Homozygous T/T	4	4.64	0.84	4.68	0.60	5.21	0.97	3.03	0.66	2.89	0.49	3.21	0.63
P-Value		0.750		0.567		0.432		0.759		0.600		0.391	
rs174545 (FADS1)													
Homozygous C/C	14	4.40	0.44	4.19	0.41	4.24	0.45	2.77	0.36	2.46	0.29	2.54	0.35
Heterozygous C/G	18	4.29	0.27	4.18	0.29	4.19	0.26	2.68	0.17	2.42	0.14	2.56	0.17
Homozygous G/G	4	4.64	0.84	4.68	0.60	5.21	0.97	3.03	0.66	2.89	0.49	3.21	0.63
P-Value		0.750		0.567		0.432		0.759		0.600		0.391	
rs174561 (FADS1)													
Homozygous T/T	15	4.33	0.42	4.13	0.39	4.20	0.42	2.71	0.34	2.42	0.28	2.52	0.33
Heterozygous C/T	17	4.35	0.28	4.23	0.30	4.22	0.27	2.73	0.17	2.45	0.15	2.58	0.18
Homozygous C/C	4	4.64	0.84	4.68	0.60	5.21	0.97	3.03	0.66	2.89	0.49	3.21	0.63
P-Value		0.638		0.500		0.408		0.604		0.531		0.379	
rs174583 (FADS2)													
Homozygous C/C	13	4.54	0.45	4.34	0.41	4.39	0.46	2.88	0.38	2.57	0.30	2.64	0.36
Heterozygous C/T	19	4.20	0.27	4.08	0.29	4.09	0.26	2.62	0.17	2.35	0.15	2.49	0.18
Homozygous T/T	4	4.64	0.84	4.68	0.60	5.21	0.97	3.03	0.66	2.89	0.49	3.21	0.63
P-Value		0.797		0.587		0.463		0.828		0.614		0.453	
rs953413 (ELOVL2)													
Homozygous A/A	11	4.24	0.46	4.21	0.36	4.31	0.41	2.72	0.40	2.51	0.29	2.68	0.35
Heterozygous A/G	20	4.44	0.26	4.21	0.27	4.37	0.32	2.84	0.20	2.54	0.19	2.66	0.23
Homozygous G/G	5	4.37	0.95	4.42	0.98	4.16	0.85	2.51	0.49	2.22	0.33	2.37	0.48
P-Value		0.653		0.978		0.869		0.613		0.816		0.826	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.5: Serum glucose at the end (Day 29) of each experimental diet classified by SNP genotype.

		Glucose					
		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet	
	n	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)							
Homozygous G/G	14	4.88	0.10	4.85	0.13	4.79	0.09
Heterozygous G/T	18	5.19	0.29	5.12	0.28	5.15	0.23
Homozygous T/T	4	4.91	0.40	4.94	0.31	4.76	0.34
P-Value		0.909		0.979		0.780	
rs174545 (FADS1)							
Homozygous C/C	14	4.88	0.10	4.85	0.13	4.79	0.09
Heterozygous C/G	18	5.19	0.29	5.12	0.28	5.15	0.23
Homozygous G/G	4	4.91	0.40	4.94	0.31	4.76	0.34
P-Value		0.909		0.979		0.780	
rs174561 (FADS1)							
Homozygous T/T	15	4.86	0.10	4.82	0.12	4.78	0.09
Heterozygous C/T	17	5.23	0.31	5.16	0.30	5.19	0.24
Homozygous C/C	4	4.91	0.40	4.94	0.31	4.76	0.34
P-Value		0.922		0.974		0.638	
rs174583 (FADS2)							
Homozygous C/C	13	4.88	0.11	4.80	0.12	4.79	0.10
Heterozygous C/T	19	5.18	0.28	5.14	0.27	5.13	0.22
Homozygous T/T	4	4.91	0.40	4.94	0.31	4.76	0.34
P-Value		0.914		0.957		0.809	
rs953413 (ELOVL2)							
Homozygous A/A	11	5.09	0.21	5.05	0.23	5.04	0.22
Heterozygous A/G	20	4.87	0.12	4.85	0.12	4.87	0.14
Homozygous G/G	5	5.63	0.95	5.43	0.91	5.21	0.59
P-Value		0.756		0.685		0.697	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.6: Plasma CRP and IL-6 at the end (Day 29) of each experimental diet classified by SNP genotype.

		CRP						IL-6				
		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	2.08	0.49	1.99	0.43	1.69	0.43	1.47	0.18	1.73	0.28	1.32	0.19
Heterozygous G/T	18	1.26	0.26	1.39	0.31	1.18	0.27	1.37	0.09	1.51	0.14	1.43	0.15
Homozygous T/T	4	1.42	0.32	1.11	0.24	1.28	0.54	1.41	0.25	1.85	0.69	1.74	0.42
P-Value		0.446		0.336		0.633		0.905		0.991		0.550	
rs174545 (FADS1)													
Homozygous C/C	14	2.08	0.49	1.99	0.43	1.69	0.43	1.47	0.18	1.73	0.28	1.32	0.19
Heterozygous C/G	18	1.26	0.26	1.39	0.31	1.18	0.27	1.37	0.09	1.51	0.14	1.43	0.15
Homozygous G/G	4	1.42	0.32	1.11	0.24	1.28	0.54	1.41	0.25	1.85	0.69	1.74	0.42
P-Value		0.446		0.336		0.633		0.905		0.991		0.550	
rs174561 (FADS1)													
Homozygous T/T	15	1.94	0.48	1.87	0.42	1.58	0.42	1.42	0.17	1.70	0.26	1.27	0.18
Heterozygous C/T	17	1.33	0.27	1.47	0.32	1.25	0.27	1.41	0.09	1.52	0.14	1.47	0.15
Homozygous C/C	4	1.42	0.32	1.11	0.24	1.28	0.54	1.41	0.25	1.85	0.69	1.74	0.42
P-Value		0.733		0.676		0.934		0.995		0.999		0.344	
rs174583 (FADS2)													
Homozygous C/C	13	2.16	0.52	2.06	0.46	1.75	0.47	1.45	0.19	1.54	0.23	1.31	0.20
Heterozygous C/T	19	1.24	0.25	1.38	0.30	1.17	0.25	1.38	0.09	1.65	0.19	1.42	0.14
Homozygous T/T	4	1.42	0.32	1.11	0.24	1.28	0.54	1.41	0.25	1.85	0.69	1.74	0.42
P-Value		0.438		0.352		0.697		0.979		0.899		0.523	
rs953413 (ELOVL2)													
Homozygous A/A	11	1.37	0.37	1.61	0.48	1.25	0.44	1.23	0.12	1.71	0.32	1.29	0.17
Heterozygous A/G	20	1.94	0.36	1.75	0.32	1.65	0.30	1.62	0.12	1.72	0.18	1.59	0.17
Homozygous G/G	5	0.72	0.15	0.92	0.33	0.64	0.09	0.99	0.13	1.10	0.17	1.02	0.17
P-Value		0.263		0.542		0.245		0.020		0.227		0.282	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.7: Plasma VCAM-1 and ICAM-1 at the end (Day 29) of each experimental diet classified by SNP genotype.

		VCAM-1						ICAM-1					
		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	1071.10	41.30	1033.75	44.78	1072.07	33.03	136.79	6.58	142.76	8.16	143.72	6.36
Heterozygous G/T	18	1189.95	64.25	1171.41	60.69	1146.77	56.73	143.72	7.35	148.02	8.26	146.22	8.54
Homozygous T/T	4	946.21	116.61	883.45	98.44	949.25	124.06	137.52	20.33	131.13	2.37	142.40	8.48
P-Value		0.145		0.024		0.303		0.487		0.396		0.988	
rs174545 (FADS1)													
Homozygous C/C	14	1071.10	41.30	1033.75	44.78	1072.07	33.03	136.79	6.58	142.76	8.16	143.72	6.36
Heterozygous C/G	18	1189.95	64.25	1171.41	60.69	1146.77	56.73	143.72	7.35	148.02	8.26	146.22	8.54
Homozygous G/G	4	946.21	116.61	883.45	98.44	949.25	124.06	137.52	10.16	131.13	2.37	142.40	8.48
P-Value		0.145		0.024		0.303		0.487		0.396		0.988	
rs174561 (FADS1)													
Homozygous T/T	15	1078.13	39.08	1048.08	44.09	1091.39	36.31	137.68	6.19	143.95	7.69	145.32	6.14
Heterozygous C/T	17	1190.74	68.14	1166.86	64.19	1134.12	58.66	143.34	7.79	147.28	8.72	144.95	8.95
Homozygous C/C	4	946.21	116.61	883.45	98.44	949.25	124.06	137.52	10.16	131.13	2.37	142.40	8.48
P-Value		0.163		0.041		0.433		0.619		0.446		0.897	
rs174583 (FADS2)													
Homozygous C/C	13	1083.06	42.70	1039.17	48.01	1081.21	34.28	136.99	7.11	142.01	8.78	143.79	6.87
Heterozygous C/T	19	1175.51	62.46	1160.45	58.44	1136.58	54.62	143.22	6.97	148.26	7.81	146.04	8.08
Homozygous T/T	4	946.21	116.61	883.45	98.44	949.25	124.06	137.52	10.16	131.13	2.37	142.40	8.48
P-Value		0.224		0.041		0.413		0.573		0.361		0.982	
rs953413 (ELOVL2)													
Homozygous A/A	11	1008.10	54.21	996.38	68.69	1008.13	65.49	130.88	7.51	131.13	7.82	131.88	7.97
Heterozygous A/G	20	1184.41	54.45	1144.66	51.88	1152.33	43.25	149.10	5.86	153.96	6.92	154.39	6.49
Homozygous G/G	5	1084.38	122.92	1047.65	108.51	1062.37	92.70	126.06	12.84	133.18	15.33	135.05	13.14
P-Value		0.108		0.241		0.193		0.180		0.174		0.114	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.8: Plasma E-selectin at the end (Day 29) of each experimental diet classified by SNP genotype.

		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet	
	n	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
rs174537 (FADS1)							
Homozygous G/G	14	24.29	2.60	23.80	2.98	23.43	2.58
Heterozygous G/T	18	26.40	2.72	26.13	2.45	23.89	2.38
Homozygous T/T	4	20.12	1.52	17.54	2.06	19.35	2.10
P-Value		0.541		0.343		0.721	
rs174545 (FADS1)							
Homozygous C/C	14	24.29	2.60	23.80	2.98	23.43	2.58
Heterozygous C/G	18	26.40	2.72	26.13	2.45	23.89	2.38
Homozygous G/G	4	20.12	1.52	17.54	2.06	19.35	2.10
P-Value		0.541		0.343		0.721	
rs174561 (FADS1)							
Homozygous T/T	15	23.60	2.52	23.27	2.82	22.91	2.46
Heterozygous C/T	17	27.13	2.78	26.73	2.52	24.37	2.47
Homozygous C/C	4	20.12	1.52	17.54	2.06	19.35	2.10
P-Value		0.388		0.258		0.637	
rs174583 (FADS2)							
Homozygous C/C	13	24.10	2.80	23.31	3.17	23.06	2.76
Heterozygous C/T	19	26.42	2.57	26.34	2.33	24.12	2.26
Homozygous T/T	4	20.12	1.52	17.54	2.06	19.35	2.10
P-Value		0.492		0.286		0.682	
rs953413 (ELOVL2)							
Homozygous A/A	11	21.48	2.56	20.17	2.40	19.72	1.94
Heterozygous A/G	20	24.95	2.13	24.86	2.15	23.92	2.05
Homozygous G/G	5	32.10	6.44	30.91	6.85	27.99	6.27
P-Value		0.266		0.237		0.254	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.9: Plasma linoleic acid and gamma-linoleic acid at the end (Day 29) of each experimental diet classified by SNP genotype.

| | | LA | | | | | | | GLA | | | | |
| | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | | Western Diet (Control) | | High-Oleic Canola Oil | | Flaxseed/High-Oleic Canola Oil Diet | |
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	28.98	0.86	26.65	0.93	27.89	0.99	0.55	0.05	0.55	0.05	0.32	0.04
Heterozygous G/T	18	30.46	0.73	27.74	0.63	29.56	0.55	0.42	0.03	0.46	0.04	0.29	0.03
Homozygous T/T	4	30.99	0.77	27.87	0.85	27.96	1.20	0.21	0.09	0.29	0.10	0.15	0.06
P-Value		0.265		0.622		0.286		0.005		0.082		0.072	
rs174545 (FADS1)													
Homozygous C/C	14	28.98	0.86	26.65	0.93	27.89	0.99	0.55	0.05	0.55	0.05	0.32	0.04
Heterozygous C/G	18	30.46	0.73	27.74	0.63	29.56	0.55	0.42	0.03	0.46	0.04	0.29	0.03
Homozygous G/G	4	30.99	0.77	27.87	0.85	27.96	1.20	0.21	0.09	0.29	0.10	0.15	0.06
P-Value		0.265		0.622		0.286		0.005		0.082		0.072	
rs174561 (FADS1)													
Homozygous T/T	15	29.26	0.85	26.76	0.87	28.12	0.95	0.54	0.04	0.54	0.05	0.32	0.03
Heterozygous C/T	17	30.31	0.76	27.71	0.67	29.46	0.57	0.41	0.03	0.46	0.04	0.29	0.03
Homozygous C/C	4	30.99	0.77	27.87	0.85	27.96	1.20	0.21	0.09	0.29	0.10	0.15	0.06
P-Value		0.469		0.750		0.465		0.004		0.093		0.062	
rs174583 (FADS2)													
Homozygous C/C	13	28.63	0.84	26.36	0.96	27.40	0.93	0.55	0.05	0.54	0.06	0.33	0.04
Heterozygous C/T	19	30.63	0.71	27.88	0.61	29.81	0.58	0.42	0.03	0.47	0.04	0.29	0.02
Homozygous T/T	4	30.99	0.77	27.87	0.85	27.96	1.20	0.21	0.09	0.29	0.10	0.15	0.06
P-Value		0.112		0.392		0.099		0.007		0.128		0.062	
rs953413 (ELOVL2)													
Homozygous A/A	11	30.38	0.63	27.17	0.43	29.10	0.46	0.37	0.06	0.44	0.06	0.24	0.03
Heterozygous A/G	20	29.37	0.76	27.35	0.74	28.49	0.78	0.49	0.03	0.51	0.04	0.32	0.03
Homozygous G/G	5	31.31	1.51	27.61	1.90	28.88	1.64	0.43	0.10	0.45	0.10	0.26	0.05
P-Value		0.516		0.849		0.939		0.146		0.608		0.326	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.10: Plasma arachidonic acid and arachidonic-to-linoleic acid ratio at the end (Day 29) of each experimental diet classified by SNP genotype.

| | | AA | | | | | | AA/LA Ratio | | | | |
| | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | | Western Diet (Control) | | High-Oleic Canola Oil | | Flaxseed/High-Oleic Canola Oil Diet | |
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	7.92	0.37	7.70	0.31	6.08	0.28	0.27	0.01	0.29	0.01	0.22	0.01
Heterozygous G/T	18	6.53	0.24	6.35	0.24	5.38	0.18	0.22	0.01	0.23	0.01	0.18	0.01
Homozygous T/T	4	5.00*	0.04	4.75*	0.14	3.92*	0.17	0.16*	0.00	0.17*	0.01	0.14*	0.01
P-Value		<0.001		<0.001		0.003		<0.001		<0.001		<0.001	
rs174545 (FADS1)													
Homozygous C/C	14	7.92	0.37	7.70	0.31	6.08	0.28	0.27	0.01	0.29	0.01	0.22	0.01
Heterozygous C/G	18	6.53	0.24	6.35	0.24	5.38	0.18	0.22	0.01	0.23	0.01	0.18	0.01
Homozygous G/G	4	5.00	0.04	4.75	0.14	3.92	0.17	0.16	0.00	0.17	0.01	0.14	0.01
P-Value		<0.001		<0.001		0.003		<0.001		<0.001		<0.001	
rs174561 (FADS1)													
Homozygous T/T	15	7.89	0.35	7.67	0.29	6.07	0.26	0.27	0.01	0.29	0.01	0.22	0.01
Heterozygous C/T	17	6.47	0.25	6.30	0.25	5.34	0.18	0.21	0.01	0.23	0.01	0.18	0.01
Homozygous C/C	4	5.00	0.04	4.75	0.14	3.92	0.17	0.16	0.00	0.17	0.01	0.14	0.01
P-Value		<0.001		<0.001		0.003		<0.001		<0.001		<0.001	
rs174583 (FADS2)													
Homozygous C/C	13	7.88	0.40	7.63	0.32	6.00	0.29	0.28	0.01	0.29	0.01	0.22	0.01
Heterozygous C/T	19	6.63	0.25	6.47	0.26	5.47	0.19	0.22	0.01	0.23	0.01	0.18	0.01
Homozygous T/T	4	5.00	0.04	4.75	0.14	3.92	0.17	0.16	0.00	0.17	0.01	0.14	0.01
P-Value		0.001		<0.001		0.006		<0.001		<0.001		<0.001	
rs953413 (ELOVL2)													
Homozygous A/A	11	6.74	0.31	6.48	0.30	5.40	0.22	0.22	0.01	0.24	0.01	0.19	0.01
Heterozygous A/G	20	7.21	0.34	7.05	0.29	5.65	0.25	0.25	0.01	0.26	0.01	0.20	0.01
Homozygous G/G	5	6.03	0.81	5.79	0.94	5.03	0.67	0.19	0.02	0.21	0.03	0.17	0.02
P-Value		0.437		0.367		0.757		0.174		0.080		0.373	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.11: Plasma alpha-linolenic acid and eicosapentaenoic acid at the end (Day 29) of each experimental diet classified by SNP genotype.

		ALA						EPA					
		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet		Western Diet (Control)		High-Oleic Canola Oil		Flaxseed/High-Oleic Canola Oil Diet	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	0.74	0.05	0.79	0.05	4.40	0.22	0.66	0.05	0.71	0.06	2.20	0.17
Heterozygous G/T	18	0.72	0.04	0.89	0.05	4.37	0.31	0.50	0.03	0.61	0.04	1.56	0.10
Homozygous T/T	4	0.81	0.06	0.82	0.11	5.04	0.31	0.32*	0.07	0.36*	0.11	0.91*	0.19
P-Value		0.567		0.291		0.369		0.004		0.035		<0.001	
rs174545 (FADS1)													
Homozygous C/C	14	0.74	0.05	0.79	0.05	4.40	0.22	0.66	0.05	0.71	0.06	2.20	0.17
Heterozygous C/G	18	0.72	0.04	0.89	0.05	4.37	0.31	0.50	0.03	0.61	0.04	1.56	0.10
Homozygous G/G	4	0.81	0.06	0.82	0.11	5.04	0.31	0.32	0.07	0.36	0.11	0.91	0.19
P-Value		0.567		0.291		0.369		0.004		0.035		<0.001	
rs174561 (FADS1)													
Homozygous T/T	15	0.74	0.05	0.80	0.05	4.48	0.22	0.64	0.05	0.71	0.05	2.17	0.16
Heterozygous C/T	17	0.72	0.04	0.89	0.05	4.30	0.32	0.50	0.04	0.61	0.05	1.55	0.10
Homozygous C/C	4	0.81	0.06	0.82	0.11	5.04	0.31	0.32	0.07	0.36	0.11	0.91	0.19
P-Value		0.568		0.442		0.317		0.009		0.045		<0.001	
rs174583 (FADS2)													
Homozygous C/C	13	0.75	0.05	0.79	0.05	4.40	0.24	0.66	0.05	0.71	0.06	2.23	0.18
Heterozygous C/T	19	0.72	0.03	0.88	0.05	4.38	0.30	0.50	0.03	0.61	0.04	1.58	0.09
Homozygous T/T	4	0.81	0.06	0.82	0.11	5.04	0.31	0.32	0.07	0.36	0.11	0.91	0.19
P-Value		0.564		0.394		0.367		0.006		0.049		<0.001	
rs953413 (ELOVL2)													
Homozygous A/A	11	0.80	0.06	0.88	0.06	4.31	0.42	0.50	0.04	0.58	0.05	1.47	0.15
Heterozygous A/G	20	0.72	0.03	0.79	0.03	4.41	0.17	0.59	0.05	0.67	0.06	1.92	0.16
Homozygous G/G	5	0.69	0.08	0.97	0.16	4.98	0.73	0.42	0.09	0.51	0.11	1.57	0.24
P-Value		0.238		0.303		0.836		0.206		0.698		0.235	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.12: Plasma docosapentaenoic acid and docosahexaenoic acid at the end (Day 29) of each experimental diet classified by SNP genotype.

| | | DPA | | | | | | DHA | | | | |
| | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | | Western Diet (Control) | | High-Oleic Canola Oil | | Flaxseed/High-Oleic Canola Oil Diet | |
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	0.57	0.03	0.56	0.04	0.81	0.04	1.57	0.08	1.59	0.08	1.55	0.07
Heterozygous G/T	18	0.55	0.02	0.55	0.02	0.75	0.03	1.45	0.07	1.52	0.09	1.43	0.06
Homozygous T/T	4	0.42	0.08	0.39	0.10	0.53	0.07	1.47	0.06	1.44	0.09	1.35	0.08
P-Value		0.218		0.220		0.012		0.531		0.614		0.320	
rs174545 (FADS1)													
Homozygous C/C	14	0.57	0.03	0.56	0.04	0.81	0.04	1.57	0.08	1.59	0.08	1.55	0.07
Heterozygous C/G	18	0.55	0.02	0.55	0.02	0.75	0.03	1.45	0.07	1.52	0.09	1.43	0.06
Homozygous G/G	4	0.42	0.08	0.39	0.10	0.53	0.07	1.47	0.06	1.44	0.09	1.35	0.08
P-Value		0.218		0.220		0.012		0.531		0.614		0.320	
rs174561 (FADS1)													
Homozygous T/T	15	0.57	0.03	0.56	0.04	0.81	0.04	1.54	0.08	1.56	0.08	1.52	0.08
Heterozygous C/T	17	0.55	0.02	0.55	0.02	0.75	0.03	1.47	0.07	1.54	0.09	1.46	0.06
Homozygous C/C	4	0.42	0.08	0.39	0.10	0.53	0.07	1.47	0.06	1.44	0.09	1.35	0.08
P-Value		0.222		0.214		0.009		0.836		0.825		0.520	
rs174583 (FADS2)													
Homozygous C/C	13	0.57	0.04	0.56	0.04	0.83	0.04	1.57	0.09	1.59	0.09	1.56	0.08
Heterozygous C/T	19	0.55	0.02	0.55	0.02	0.75	0.03	1.46	0.07	1.52	0.08	1.44	0.06
Homozygous T/T	4	0.42	0.08	0.39	0.10	0.53	0.07	1.47	0.06	1.44	0.09	1.35	0.08
P-Value		0.216		0.210		0.007		0.677		0.694		0.327	
rs953413 (ELOVL2)													
Homozygous A/A	11	0.54	0.05	0.53	0.05	0.72	0.05	1.50	0.08	1.51	0.09	1.45	0.07
Heterozygous A/G	20	0.57	0.02	0.56	0.02	0.79	0.03	1.45	0.05	1.52	0.06	1.46	0.05
Homozygous G/G	5	0.46	0.06	0.44	0.08	0.68	0.07	1.69	0.22	1.66	0.25	1.58	0.17
P-Value		0.330		0.377		0.442		0.251		0.699		0.452	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.13: Plasma eicosapentaenoic-to-alpha-linolenic acid ratio and total polyunsaturated fatty acids at the end (Day 29) of each experimental diet classified by SNP genotype.

		EPA/ALA Ratio						PUFA					
		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet		Western Diet (Control)		High-Oleic Canola Oil		Flaxseed/High-Oleic Canola Oil Diet	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	0.92	0.07	0.94	0.09	0.53	0.05	43.45	1.11	40.92	1.08	44.84	1.15
Heterozygous G/T	18	0.71	0.05	0.72	0.06	0.38	0.03	42.97	0.88	40.43	0.88	44.97	0.64
Homozygous T/T	4	0.39	0.06	0.41	0.07	0.18	0.04	41.62	0.64	38.31	0.78	41.68	1.30
P-Value		0.002		0.003		0.003		0.444		0.233		0.189	
rs174545 (FADS1)													
Homozygous C/C	14	0.92	0.07	0.94	0.09	0.53	0.05	43.45	1.11	40.92	1.08	44.84	1.15
Heterozygous C/G	18	0.71	0.05	0.72	0.06	0.38	0.03	42.97	0.88	40.43	0.88	44.97	0.64
Homozygous G/G	4	0.39	0.06	0.41	0.07	0.18	0.04	41.62	0.64	38.31	0.78	41.68	1.30
P-Value		0.002		0.003		0.003		0.444		0.233		0.189	
rs174561 (FADS1)													
Homozygous T/T	15	0.90	0.07	0.92	0.08	0.51	0.05	43.60	1.05	40.94	1.01	45.07	1.09
Heterozygous C/T	17	0.71	0.05	0.72	0.06	0.38	0.03	42.80	0.91	40.38	0.93	44.77	0.65
Homozygous C/C	4	0.39	0.06	0.41	0.07	0.18	0.04	41.62	0.64	38.31	0.78	41.68	1.30
P-Value		0.004		0.005		0.004		0.386		0.221		0.190	
rs174583 (FADS2)													
Homozygous C/C	13	0.92	0.08	0.94	0.10	0.53	0.06	43.03	1.11	40.51	1.08	44.32	1.11
Heterozygous C/T	19	0.72	0.05	0.73	0.06	0.38	0.02	43.28	0.89	40.73	0.89	45.32	0.70
Homozygous T/T	4	0.39	0.06	0.41	0.07	0.18	0.04	41.62	0.64	38.31	0.78	41.68	1.30
P-Value		0.002		0.003		0.003		0.429		0.248		0.142	
rs953413 (ELOVL2)													
Homozygous A/A	11	0.66	0.06	0.66	0.05	0.37	0.04	43.20	0.51	39.92	0.39	44.36	0.74
Heterozygous A/G	20	0.83	0.06	0.87	0.08	0.45	0.05	42.83	0.92	40.83	0.82	44.65	0.85
Homozygous G/G	5	0.66	0.16	0.60	0.16	0.36	0.08	43.30	2.46	39.61	3.13	44.57	2.11
P-Value		0.191		0.215		0.590		0.910		0.831		0.897	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.14: Plasma total n-6 and n-3 polyunsaturated fatty acids at the end (Day 29) of each experimental diet classified by SNP genotype.

		N-6 PUFA							**N-3 PUFA**				
		Western Diet (Control)		**High-Oleic Canola Oil Diet**		**Flaxseed/High-Oleic Canola Oil Diet**		**Western Diet (Control)**		**High-Oleic Canola Oil**		**Flaxseed/High-Oleic Canola Oil Diet**	
	n	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
rs174537 (FADS1)													
Homozygous G/G	14	39.91	1.09	37.27	1.10	35.88	1.16	3.54	0.10	3.65	0.11	8.96	0.22
Heterozygous G/T	18	39.75	0.86	36.86	0.83	36.85	0.67	3.22	0.09	3.56	0.10	8.13	0.35
Homozygous T/T	4	38.59	0.55	35.30	0.66	33.85	1.15	3.03	0.21	3.00	0.34	7.83	0.53
P-Value		0.542		0.393		0.189		0.047		0.103		0.123	
rs174545 (FADS1)													
Homozygous C/C	14	39.91	1.09	37.27	1.10	35.88	1.16	3.54	0.10	3.65	0.11	8.96	0.22
Heterozygous C/G	18	39.75	0.86	36.86	0.83	36.85	0.67	3.22	0.09	3.56	0.10	8.13	0.35
Homozygous G/G	4	38.59	0.55	35.30	0.66	33.85	1.15	3.03	0.21	3.00	0.34	7.83	0.53
P-Value		0.542		0.393		0.189		0.047		0.103		0.123	
rs174561 (FADS1)													
Homozygous T/T	15	40.12	1.03	37.31	1.02	36.08	1.10	3.49	0.11	3.63	0.10	8.99	0.21
Heterozygous C/T	17	39.56	0.89	36.80	0.88	36.72	0.70	3.24	0.09	3.58	0.10	8.05	0.36
Homozygous C/C	4	38.59	0.55	35.30	0.66	33.85	1.15	3.03	0.21	3.00	0.34	7.83	0.53
P-Value		0.505		0.372		0.232		0.110		0.113		0.068	
rs174583 (FADS2)													
Homozygous C/C	13	39.49	1.08	36.85	1.09	35.31	1.09	3.54	0.11	3.66	0.12	9.01	0.23
Heterozygous C/T	19	40.05	0.86	37.17	0.85	37.18	0.71	3.23	0.09	3.57	0.09	8.14	0.33
Homozygous T/T	4	38.59	0.55	35.30	0.66	33.85	1.15	3.03	0.21	3.00	0.34	7.83	0.53
P-Value		0.471		0.368		0.104		0.061		0.101		0.092	
rs953413 (ELOVL2)													
Homozygous A/A	11	39.85	0.52	36.41	0.34	36.42	0.45	3.35	0.13	3.51	0.14	7.95	0.51
Heterozygous A/G	20	39.51	0.92	37.29	0.83	36.07	0.89	3.32	0.08	3.54	0.10	8.58	0.23
Homozygous G/G	5	40.04	2.19	36.03	2.86	35.76	2.26	3.26	0.30	3.58	0.33	8.81	0.48
P-Value		0.940		0.865		0.973		0.998		0.907		0.704	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.15: Plasma total saturated and monounsaturated fatty acids at the end (Day 29) of each experimental diet classified by SNP genotype.

| | | SFA | | | | | | MUFA | | | | |
| | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | | Western Diet (Control) | | High-Oleic Canola Oil | | Flaxseed/High-Oleic Canola Oil Diet | |
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	14	28.98	0.55	26.99	0.51	26.96	0.47	25.14	0.75	29.76	0.82	25.49	0.91
Heterozygous G/T	18	28.19	0.31	25.71	0.34	26.50	0.34	26.54	0.70	31.42	0.92	26.20	0.51
Homozygous T/T	4	27.89	1.18	26.53	1.50	27.59	2.33	28.20	1.03	32.79	1.00	28.06	1.99
P-Value		0.425		0.160		0.789		0.132		0.190		0.503	
rs174545 (FADS1)													
Homozygous C/C	14	28.98	0.55	26.99	0.51	26.96	0.47	25.14	0.75	29.76	0.82	25.49	0.91
Heterozygous C/G	18	28.19	0.31	25.71	0.34	26.50	0.34	26.54	0.70	31.42	0.92	26.20	0.51
Homozygous G/G	4	27.89	1.18	26.53	1.50	27.59	2.33	28.20	1.03	32.79	1.00	28.06	1.99
P-Value		0.425		0.160		0.789		0.132		0.190		0.503	
rs174561 (FADS1)													
Homozygous T/T	15	28.78	0.55	26.78	0.52	26.76	0.49	25.18	0.70	29.93	0.78	25.48	0.85
Heterozygous C/T	17	28.31	0.30	25.82	0.34	26.65	0.32	26.59	0.74	31.37	0.98	26.26	0.54
Homozygous C/C	4	27.89	1.18	26.53	1.50	27.59	2.33	28.20	1.03	32.79	1.00	28.06	1.99
P-Value		0.562		0.422		0.921		0.131		0.249		0.483	
rs174583 (FADS2)													
Homozygous C/C	13	29.10	0.58	27.11	0.53	27.07	0.50	25.45	0.74	30.11	0.80	25.96	0.84
Heterozygous C/T	19	28.14	0.30	25.70	0.32	26.45	0.32	26.25	0.72	31.10	0.93	25.84	0.60
Homozygous T/T	4	27.89	1.18	26.53	1.50	27.59	2.33	28.20	1.03	32.79	1.00	28.06	1.99
P-Value		0.303		0.130		0.636		0.191		0.298		0.532	
rs953413 (ELOVL2)													
Homozygous A/A	11	28.20	0.51	26.56	0.49	26.41	0.55	26.31	0.40	31.19	0.54	26.90	0.65
Heterozygous A/G	20	28.53	0.40	26.26	0.41	26.82	0.32	26.26	0.77	30.57	0.73	25.97	0.72
Homozygous G/G	5	28.75	0.95	25.90	1.25	27.58	1.83	25.56	1.66	31.80	3.09	25.11	1.45
P-Value		0.946		0.368		0.864		0.793		0.695		0.317	

Values are means ± SEM; n = 36; *P*-values are analyzed by Kruskal-Wallis test.

Table III.16: Percent dose of administered ^{13}C recovered in plasma alpha-linolenic acid acid 24 and 48 hours after intake of a single dose of ^{13}C-ALA in experimental diets classified by SNP genotype.

| | | 24hr - ALA PDR | | | | | | 48hr - ALA PDR | | | | | |
| | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | | Western Diet (Control) | | High-Oleic Canola Oil | | Flaxseed/High-Oleic Canola Oil Diet | |
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	7	9.93	2.45	12.72	2.88	16.78	2.15	4.36	1.36	4.84	0.94	6.62	1.86
Heterozygous G/T	15	11.58	1.66	13.98	2.19	17.95	2.21	4.05	0.49	4.64	0.65	5.73	0.60
Homozygous T/T	4	16.99	6.44	7.00	1.94	22.99	3.78	6.26	1.67	3.99	0.66	4.95	1.82
P-Value		0.607		0.177		0.468		0.363		0.922		0.913	
rs174545 (FADS1)													
Homozygous C/C	7	9.93	2.45	12.72	2.88	16.78	2.15	4.36	1.36	4.84	0.94	6.62	1.86
Heterozygous C/G	15	11.58	1.66	13.98	2.19	17.95	2.21	4.05	0.49	4.64	0.65	5.73	0.60
Homozygous G/G	4	16.99	6.44	7.00	1.94	22.99	3.78	6.26	1.67	3.99	0.66	4.95	1.82
P-Value		0.607		0.177		0.468		0.363		0.922		0.913	
rs174561 (FADS1)													
Homozygous T/T	8	9.78	2.12	13.98	2.79	17.13	1.89	4.21	1.19	4.82	0.82	6.62	1.61
Heterozygous C/T	14	11.79	1.77	13.35	2.26	17.84	2.37	4.12	0.53	4.64	0.69	5.66	0.64
Homozygous C/C	4	16.99	6.44	7.00	1.94	22.99	3.78	6.26	1.67	3.99	0.66	4.95	1.82
P-Value		0.532		0.172		0.481		0.356		0.951		0.912	
rs174583 (FADS2)													
Homozygous C/C	7	9.93	2.45	12.72	2.88	16.78	2.15	4.36	1.36	4.84	0.94	6.62	1.86
Heterozygous C/T	15	11.58	1.66	13.98	2.19	17.95	2.21	4.05	0.49	4.64	0.65	5.73	0.60
Homozygous T/T	4	16.99	6.44	7.00	1.94	22.99	3.78	6.26	1.67	3.99	0.66	4.95	1.82
P-Value		0.607		0.177		0.468		0.363		0.922		0.913	
rs953413 (ELOVL2)													
Homozygous A/A	9	10.64	3.29	12.62	2.42	16.98	2.64	4.93	1.16	3.90	0.85	5.04	1.08
Heterozygous A/G	12	11.79	1.68	10.87	1.71	18.06	1.70	3.91	0.69	4.60	0.40	6.78	1.01
Homozygous G/G	5	14.80	3.57	16.52	5.63	21.83	5.17	5.01	0.75	5.82	1.53	5.05	1.23
P-Value		0.273		0.730		0.925		0.652		0.400		0.525	

Values are means ± SEM; n = 26; *P*-values are analyzed by Kruskal-Wallis test.

Table III.17: Percent dose of administered ^{13}C recovered in plasma eicosapentaenoic acid 24 and 48 hours after intake of a single dose of ^{13}C-ALA in experimental diets classified by SNP genotype.

| | | 24hr - EPA PDR | | | | | | 48hr - EPA PDR | | | | | |
| | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | | Western Diet (Control) | | High-Oleic Canola Oil | | Flaxseed/High-Oleic Canola Oil Diet | |
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	7	3.74	0.70	4.77	0.80	2.65	0.36	3.76	0.54	4.40	0.73	2.76	0.24
Heterozygous G/T	15	3.42	0.50	4.36	0.63	2.71	0.30	2.75	0.32	3.72	0.44	2.48	0.25
Homozygous T/T	4	0.96	0.11	0.95	0.36	0.74	0.16	0.89	0.28	1.35	0.37	0.75	0.25
P-Value		0.006		0.006		0.014		0.005		0.007		0.014	
rs174545 (FADS1)													
Homozygous C/C	7	3.74	0.70	4.77	0.80	2.65	0.36	3.76	0.54	4.40	0.73	2.76	0.24
Heterozygous C/G	15	3.42	0.50	4.36	0.63	2.71	0.30	2.75	0.32	3.72	0.44	2.48	0.25
Homozygous G/G	4	0.96	0.11	0.95	0.36	0.74	0.16	0.89	0.28	1.35	0.37	0.75	0.25
P-Value		0.006		0.006		0.014		0.005		0.007		0.014	
rs174561 (FADS1)													
Homozygous T/T	8	3.83	0.61	4.73	0.70	2.57	0.32	3.77	0.47	4.37	0.63	2.68	0.22
Heterozygous C/T	14	3.34	0.53	4.35	0.67	2.76	0.31	2.67	0.34	3.69	0.47	2.51	0.27
Homozygous C/C	4	0.96	0.11	0.95	0.36	0.74	0.16	0.89	0.28	1.35	0.37	0.75	0.25
P-Value		0.006		0.006		0.014		0.005		0.006		0.014	
rs174583 (FADS2)													
Homozygous C/C	7	3.74	0.70	4.77	0.80	2.65	0.36	3.76	0.54	4.40	0.73	2.76	0.24
Heterozygous C/T	15	3.42	0.50	4.36	0.63	2.71	0.30	2.75	0.32	3.72	0.44	2.48	0.25
Homozygous T/T	4	0.96	0.11	0.95	0.36	0.74	0.16	0.89	0.28	1.35	0.37	0.75	0.25
P-Value		0.006		0.006		0.014		0.005		0.007		0.014	
rs953413 (ELOVL2)													
Homozygous A/A	9	2.79	0.62	3.62	0.80	2.20	0.43	2.77	0.58	3.39	0.51	1.99	0.41
Heterozygous A/G	12	3.63	0.65	4.42	0.79	2.44	0.37	2.88	0.46	3.90	0.64	2.38	0.27
Homozygous G/G	5	2.53	0.61	3.40	1.03	2.64	0.52	2.33	0.40	2.93	0.80	2.64	0.47
P-Value		0.527		0.510		0.894		0.912		0.534		0.584	

Values are means ± SEM; n = 26; *P*-values are analyzed by Kruskal-Wallis test.

Table III.18: Percent dose of administered ^{13}C recovered in plasma docosapentaenoic acid 24 and 48 hours after intake of a single dose of ^{13}C-ALA in experimental diets classified by SNP genotype.

| | | 24hr - DPA PDR | | | | | | 48hr - DPA PDR | | | | | |
| | | Western Diet (Control) | | High-Oleic Canola Oil Diet | | Flaxseed/High-Oleic Canola Oil Diet | | Western Diet (Control) | | High-Oleic Canola Oil | | Flaxseed/High-Oleic Canola Oil Diet | |
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	7	0.99	0.20	1.00	0.19	0.36	0.09	1.10	0.28	1.10	0.26	0.43	0.12
Heterozygous G/T	15	0.66	0.14	0.74	0.12	0.36	0.06	0.76	0.14	0.65	0.11	0.46	0.07
Homozygous T/T	4	0.20	0.15	0.29	0.10	0.21	0.03	0.20	0.14	0.36	0.10	0.19	0.06
P-Value		0.049		0.023		0.400		0.043		0.084		0.145	
rs174545 (FADS1)													
Homozygous C/C	7	0.99	0.20	1.00	0.19	0.36	0.09	1.10	0.28	1.10	0.26	0.43	0.12
Heterozygous C/G	15	0.66	0.14	0.74	0.12	0.36	0.06	0.76	0.14	0.65	0.11	0.46	0.07
Homozygous G/G	4	0.20	0.15	0.29	0.10	0.21	0.03	0.20	0.14	0.36	0.10	0.19	0.06
P-Value		0.049		0.023		0.400		0.043		0.084		0.145	
rs174561 (FADS1)													
Homozygous T/T	8	0.92	0.19	0.95	0.17	0.36	0.08	1.06	0.25	1.04	0.23	0.44	0.10
Heterozygous C/T	14	0.67	0.15	0.75	0.13	0.36	0.06	0.76	0.15	0.66	0.12	0.45	0.07
Homozygous C/C	4	0.20	0.15	0.29	0.10	0.21	0.03	0.20	0.14	0.36	0.10	0.19	0.06
P-Value		0.063		0.030		0.385		0.044		0.095		0.153	
rs174583 (FADS2)													
Homozygous C/C	7	0.99	0.20	1.00	0.19	0.36	0.09	1.10	0.28	1.10	0.26	0.43	0.12
Heterozygous C/T	15	0.66	0.14	0.74	0.12	0.36	0.06	0.76	0.14	0.65	0.11	0.46	0.07
Homozygous T/T	4	0.20	0.15	0.29	0.10	0.21	0.03	0.20	0.14	0.36	0.10	0.19	0.06
P-Value		0.049		0.023		0.400		0.043		0.084		0.145	
rs953413 (ELOVL2)													
Homozygous A/A	9	0.48	0.15	0.68	0.20	0.35	0.09	0.72	0.23	0.68	0.23	0.45	0.10
Heterozygous A/G	12	0.70	0.16	0.82	0.11	0.31	0.05	0.71	0.15	0.82	0.13	0.38	0.08
Homozygous G/G	5	0.98	0.33	0.66	0.26	0.39	0.08	1.00	0.36	0.60	0.23	0.42	0.10
P-Value		0.324		0.398		0.718		0.677		0.320		0.847	

Values are means ± SEM; n = 26; *P*-values are analyzed by Kruskal-Wallis test.

Table III.19: Percent dose of administered ^{13}C recovered in plasma docosahexaenoic acid 24 and 48 hours after intake of a single dose of ^{13}C-ALA in experimental diets classified by SNP genotype.

	n	24hr - DHA PDR						48hr - DHA PDR					
		Western Diet (Control)		High-Oleic Canola Oil Diet		Flaxseed/High-Oleic Canola Oil Diet		Western Diet (Control)		High-Oleic Canola Oil		Flaxseed/High-Oleic Canola Oil Diet	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
rs174537 (FADS1)													
Homozygous G/G	7	0.24	0.10	0.25	0.11	0.20	0.07	0.33	0.14	0.25	0.19	0.16	0.06
Heterozygous G/T	15	0.19	0.05	0.16	0.06	0.09	0.06	0.29	0.08	0.22	0.07	0.11	0.06
Homozygous T/T	4	0.02	0.08	0.07	0.22	0.24	0.12	0.04	0.08	0.19	0.09	0.05	0.18
P-Value		0.273		0.447		0.257		0.111		0.931		0.521	
rs174545 (FADS1)													
Homozygous C/C	7	0.24	0.10	0.25	0.11	0.20	0.07	0.33	0.14	0.25	0.19	0.16	0.06
Heterozygous C/G	15	0.19	0.05	0.16	0.06	0.09	0.06	0.29	0.08	0.22	0.07	0.11	0.06
Homozygous G/G	4	0.02	0.08	0.07	0.11	0.24	0.12	0.04	0.08	0.19	0.09	0.05	0.18
P-Value		0.237		0.447		0.257		0.111		0.931		0.521	
rs174561 (FADS1)													
Homozygous T/T	8	0.22	0.09	0.24	0.10	0.17	0.07	0.32	0.12	0.26	0.16	0.14	0.06
Heterozygous C/T	14	0.20	0.05	0.15	0.06	0.10	0.07	0.29	0.09	0.21	0.07	0.12	0.06
Homozygous C/C	4	0.02	0.08	0.07	0.11	0.24	0.12	0.04	0.08	0.19	0.09	0.05	0.18
P-Value		0.295		0.455		0.437		0.114		0.769		0.659	
rs174583 (FADS2)													
Homozygous C/C	7	0.24	0.10	0.25	0.11	0.20	0.07	0.33	0.14	0.25	0.19	0.16	0.06
Heterozygous C/T	15	0.19	0.05	0.16	0.06	0.09	0.06	0.29	0.08	0.22	0.07	0.11	0.06
Homozygous T/T	4	0.02	0.08	0.07	0.11	0.24	0.12	0.04	0.08	0.19	0.09	0.05	0.18
P-Value		0.237		0.447		0.257		0.111		0.931		0.521	
rs953413 (ELOVL2)													
Homozygous A/A	9	0.16	0.06	0.08	0.08	0.08	0.07	0.22	0.13	0.14	0.04	0.02	0.10
Heterozygous A/G	12	0.16	0.07	0.19	0.07	0.15	0.07	0.23	0.09	0.22	0.12	0.10	0.05
Homozygous G/G	5	0.25	0.09	0.26	0.11	0.24	0.09	0.34	0.13	0.37	0.12	0.25	0.09
P-Value		0.622		0.269		0.378		0.655		0.276		0.324	

Values are means ± SEM; n = 26; *P*-values are analyzed by Kruskal-Wallis test.

APPENDIX IV

BOOK CHAPTER PUBLICATION OF THESIS RELEVANCE

The following book chapter is in press for publication in:

'The Omega‑3 Fatty Acid Deficiency Syndrome: Opportunity for Disease Prevention'

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THE EVOLUTION OF OMEGA-3 FATTY ACIDS IN THE HUMAN DIET

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ABSTRACT
        The evolution of the human diet over the past 10,000 years has lead to considerable changes in dietary fatty acid composition, predominately omega-3 polyunsaturated fatty acids (n-3 PUFA). The diets of our Paleolithic ancestors consisted of wild plant and animal foods abundant in alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). With the domestication of animals and plants during the Agricultural Revolution, shifts in the macro and micro-nutrient composition of formerly wild foods initiated the elimination of n-3 PUFA from the diet. Over the past 100 years the Industrial Revolution resulted in a manufactured diet that the human genome was not adapted to, abundant in refined grains, fats and oils rich in n-6 PUFA while deficient in n-3 PUFA. The current fatty acid imbalance of Western diets hinders the conversion of ALA to n-3 LCPUFA, an already inefficient pathway utilizing non-evolved enzymatic machinery. Recently, several professional health organizations have outlined recommendations for n-3 PUFA, with the latest dietary guidelines targeting a minimum intake of 250 mg/day of EPA+DHA for adults. Despite these recommendations, current intakes of n-3 PUFA in Western diets are low and challenges regarding availability, safety and sustainability of fish resources exist. Taken together, a new era of n-3 PUFA enriched functional foods and dietary supplements is emerging to enhance n-3 PUFA intakes. This review explores the evolution of n-3 PUFA in the human diet and emphasizes recent recommendations and novel dietary options to realign our modern fatty acid intake with our Paleolithic genome.

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