Evaluating effects of foods containing high oleic canola oil, DHA, and fibre on body composition and fatty acid metabolism: The CONFIDENCE (canola oil and fibre with DHA enhanced) study

by

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Abstract

Metabolic syndrome (MetS), a spectrum of chronic disease risk factors, has been identified as a major contributor to the development of cardiovascular disease (CVD). Current recommendations for dietary management of people with MetS involve modifications in food intake including higher consumption of long chain n-3 fatty acids and dietary fibre. Preliminary results from two independent human trials conducted recently in our laboratory revealed the potential of high oleic canola oil and docosahexaenoic acid (HOCO)(DHA) and barley derived high molecular weight β-glucan in managing multiple MetS risk factors. No intervention has combined HOCO, DHA, and β-glucan to examine the health benefit with a portfolio of these bioactives. The hypothesis of the study was combining highly bioactive components such as high oleic canola oil, barley β-glucan and DHA would impart favorable changes in plasma and RBC fatty acid profiles, compared to the individual bioactives listed above. Thirty-five volunteers were randomized and twenty-nine completed the study with a dropout rate of 17.1%. Mean plasma and red blood cell (RBC) total DHA concentrations, which were analyzed among all participants as a measure of adherence, increased significantly in the DHA-enriched treatment ($P<0.0001$ and $P<0.0001$ for HOCODHA control flour and HOCODHA barley flour) compared to control oil-control flour. The plasma and RBC n-6: n-3 ratio was reduced after consumption of HOCODHA-control flour compared to control oil- control flour ($P<0.0001$ and $P<0.0001$ for plasma and RBC n-6: n-3 ratio, respectively). The omega-3 index increased as well after the consumption of HOCODHA –barley flour diet compared to the control oil- barley flour ($P<0.0001$). Similar elevation was

observed for omega-3 index following HOCODHA-control flour diet compared to control oil-control flour diet ($P<0.0001$). The present study failed to see differences in body composition with the HOCODHA-barley flour treatment versus control oil-control flour treatment. In conclusion, significant increases in plasma EPA and DHA levels, as well as the omega-3 index, provide evidence supporting the cardioprotective effects of HOCODHA. The present study demonstrated that in the context of current Western macronutrient intakes, altering the dietary fatty acid composition and adding β-glucan had no major effect on body composition during the 28 days controlled dietary intervention.

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Abbreviations

ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
BMI	Body mass index
CVD	Cardiovascular disease
CONFIDENCE	Canola oil and fibre with DHA enhanced
D_2O	Deuterium oxide
DBP	Diastolic blood pressure
DEXA	Dual-energy x-ray absorptiometry
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FDA	Food and Drug Administration
FSR	Fractional synthesis rate
HOCO	High oleic canola oil
HOCODHA	High oleic canola oil with docosahexaenoic acid
GC-FID	Gas chromatography equipped with flame ionization detection
GC-IRMS	Gas chromatography with combustion isotope ratio mass spectrometry
HDL-C	High-density lipoprotein cholesterol

LA	Linoleic acid
LDL-C	Low-density lipoprotein cholesterol
MetS	Metabolic syndrome
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
TG	Triglycerides
RBC	Red blood cell
SBP	Systolic blood pressure
SFA	Saturated fatty acid

Chapter I

Overall introduction

1.1 Introduction

Cardiovascular disease (CVD), as a major public health problem currently, has caused 17.5 million deaths across the world. Estimations show that in 2030, about 23.6 million deaths will be caused by CVD (Cardozo et al., 2014). Metabolic syndrome (MetS), with central obesity (waist circumference: men>102 cm; women>88cm), diminished high-density lipoprotein (HDL) cholesterol (men<1.0 mmol/L; women<1.3 mmol/L), systemic hypertension (≥130/≥85 mm Hg), elevated triglycerides (≥1.69 mmol/L) and elevated fasting plasma glucose (≥6.1 mmol/L), has become a leading health issue due to its association to CVD (Mottillo et al., 2010). In addition, elevated concentrations of inflammatory biomarkers have been associated with cardiovascular events (Gillingham et al., 2011). Furthermore, higher fatty acid oxidation rates have been found to be associated with lower risk of CVD (Kein et al., 2005). Total body fat and body fat distribution are also reported to be strongly related to CVD risk (Cui et al., 2013).

An association between dietary fats and cardiovascular health has been widely accepted for decades. The quality of fat is generally specified by the relative content of SFA, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), including the proportion or amount of essential fatty acids, including, linoleic acid (LA) and α-linolenic acid (ALA; 18:3n3), as well as the proportion or amount of long-chain n-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Schwab et al., 2014). A large body of evidence

supports the notion that replacing SFA with n-9 MUFA, PUFA, and lower n-6/n-3 ratio diets provides health benefits (Senanayake et al., 2014; Rasmussen et al., 2006; Mensink et al., 1992; Rivellese et al., 2003 & Bjermo et al., 2012). In addition, recent recommendations have been made that EPA and DHA consumption have protective effects on CVD risk (Salter, 2013).

Canola oil as a source of MUFA and ALA may help reduce the risk of MetS and CVD (Gillingham et al., 2011; Liu et al., 2016). Also, canola oil has been claimed to possess the lowest content of SFA among vegetable oils in the US (Shimizu et al., 2008). Oil industries have recently produced a novel type of high-oleic canola oil which is high in MUFA, low in SFA and exhibits a low ratio of n-6: n-3 (Tarrago-Trani, Philips, & Lemar, 2006; Gillingham et al., 2011).

Various modifications in food intake may contribute to dietary management in people at high risk for CVD, such as higher consumption of vegetables, fruits and whole grain food components with their bioactive composition. β -glucan is classified as part of the glucan family and its polysaccharides that have been extensively studied for several health benefits including CVD (Slavin, 2005). The properties against CVD risk of β-glucan by reducing serum lipids were first investigated by Keys and Grande (1960) more than 50 years ago (Rondanelli et al., 2011). β-glucan has received interest by researchers due to its effects on insulin sensitivity, serum lipid reduction, and macronutrient absorption reduction which all exert a positive action on CVD risk factors (Behall, Scholfield & Hallfrisch, 2004). Health Canada has recently announced a health claim regarding an intake of 3 g barley β-glucan per day is the effective dosage for CVD risk reduction (Health Canada, 2012).

1.2 Rationale

Dietary recommendations for people with high risk of CVD involve quantitative and qualitative modifications in food intake. Dietary fatty acids and soluble fibres have recently received considerable attention in the area of nutrition therapy research as ways to manage various pathologies related to CVD. The effects of HOCO, MUFA and ALA enriched diets have been well reported. DHA as an n-3 fatty acid found in marine sources has been shown to possess effective benefits against CVD risk. Furthermore, HOCO with DHA has been shown to have stronger beneficial effects on CVD risk factors than individual components, or regular canola oil alone (Jones et al., 2014). β-glucan intake has also been shown to have effective benefits in risk reduction of CVD by improving various risk factors including blood TC, LDL, and HDL cholesterol levels along with body fat. Portfolios of different bioactives have proven evidence in CVD risk reduction (Ramprasath et al., 2013; Jenkins et al., 2015). However, no intervention has combined HOCO, DHA and β-glucan to examine the health benefit using a portfolio of these particular bioactives. We expect to maximize the health benefits with the consumption of a combination of HOCODHA and β-glucan in CVD risk reduction compared to these individual bioactives provided separately. The current study, therefore, provided an opportunity to examine the synergetic effects that the above bioactives may deliver when consumed in combination.

1.3 Objectives

1. To investigate the potential of functional food products containing combinations of HOCO, DHA, and β-glucan compared to control diets in the management of plasma and red blood cell (RBC) fatty acid profiles and de novo fatty acid synthesis of palmitic acid in participants with metabolic syndrome.

2. To investigate the long term effects of these novel food products on body fat distribution in participants with metabolic syndrome.

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Bridge to chapter II

The following chapter comprises a literature review, which provides a broad overview of the current knowledge of associations between dietary fatty acids and CVD risk. In particular, the review is focused on health benefits of replacing saturated fats with unsaturated fats and the argument on the optimal amounts of different fat types in a healthy diet. In addition, this chapter also reviews the current findings on topics including effects of β-glucan on body fat distribution. This chapter is an introduction to the background of the CONFIDENCE (canola oil and fibre with DHA enhanced) study.

Chapter II

Literature review

2.1 Effect of dietary fatty acids on CVD risk factors

The Mediterranean diet is one of many healthful diet recommendations across the world. High MUFA content is one of the major reasons that Mediterranean diets have been widely recommended. Dietary MUFA may reduce CVD risk by helping reduce blood total cholesterol, LDL cholesterol and also inhibiting LDL oxidation. MUFA-rich diets have been reported to reduce CVD risk by reducing various risk factors based on many studies (Kris-Etherton et al., 1999; Gillingham et al., 2011). A randomized crossover study that examined the effects of the diets differing in fatty acid profile on serum lipids and lipoproteins concluded that high-MUFA diets resulted in a 13% lowering in triglyceride concentration compared to a Western diet (Kris-Etherton et al., 1999). The study also estimated that MUFA consumption decreased CVD risk by 25% with the MUFA substitution for SFA in the diets (Kris-Etherton et al., 1999). A review examining the effects of modifying dietary LA and ALA intakes on long chain omega-3 PUFA status in human adults reviewed over 20 different studies dating from 1994 to 2014 and concluded that it is possible to increase n-3 long chain fatty acid status by reducing LA and/or increasing ALA intake in humans, although decreasing LA intake to below 2.5% of energy may be required to specifically increase levels of DHA (Wood et al., 2015). Another review conducted based on 25 epidemiologic studies that involved a total population of 280,000 participants showed that morbidity and mortality decreased with the increase of

fish consumption (Caterina, 2011). MUFAs have also been shown to reduce CVD risk factors by reducing plasma low density lipoprotein-cholesterol (LDL-C) concentrations and oxidation status (Lopez-Huertas, 2009). A review by Harland in 2009 reviewed 10 intervention studies on canola oil replacing SFAs that were published in England and conducted during the two decades from January 1988 to December 2008. Results showed that with each g/day increase in MUFA intake, reductions in TC by 0.05 and LDL-C by 0.04 mmol/L, respectively, were observed (Harland, 2009). It was also calculated that each 10 g/day increase in MUFA intake was associated with a reduction in TC of 9.8% (Harland, 2009). The literature above established the potential of MUFAs to prevent and manage CVD risk factors in humans.

2.1.1 Dietary fatty acids and circulating fatty acid profiles

Circulating fatty acid profile not only can reflect the quality of the fatty acids consumed but can also serve as a biomarker for certain metabolic diseases like hyperlipidemia and atherosclerosis (Rise et al, 2007). Plasma and RBC fatty acids analyses are the main approaches in assessing circulating fatty acid profiles (Ramprasath et al., 2013). A clinical study, involving 30 subjects aged 45-65 years, with the treatment of DHA and EPA enriched milk, showed an increase in plasma EPA concentration of 33% and DHA of 20% after consuming the treatments for 4 weeks (Lopez-Huertas, 2014). A randomized controlled study which involved young healthy adults examined effects on blood lipid profiles after consumption of meals enriched with different oils, including 40 g/day of butter, olive oil, or sunflower oil, and showed that both olive and sunflower oils consumption lowered postprandial

triglyceride lipoprotein levels significantly (Mekki et al., 2002). Omega-3 fatty acids intake may increase the plasma omega-3 fatty acid profile as well as the omega-3 index without changing body weight. Long chain omega-3 PUFAs have been extensively studied for their beneficial effects against CVD, such as modifying lipid profile, reducing high blood pressure as well as mediating inflammatory responses (Cottin et al., 2011). A meta-analysis done by Hartweg et al. (2008) concluded that omega-3 supplements lowered triglycerides, but raised LDL cholesterol levels. Senanayake et al. (2013) showed that when 15% of the total oil was consumed as DHA oil, plasma n-3 PUFA significantly increased compared to other non-DHA treatments without changing body weight. Another randomized controlled crossover study showed increases in the omega-3 index and plasma n-3 PUFA levels and a reduction in n-6/n-3 ratio without changing body mass index (BMI) after consumption of krill oil or fish oil, which are rich sources of n-3 dietary fatty acids (Ramprasath et al., 2013). The effects of long-term dietary fatty acids on human circulating fatty acid profile and CVD risk markers are critical for research on CVD risk prevention.

2.1.2 Canola oil consumption and CVD risk factors

Canola oil, as a source of MUFA, ALA and vitamin E, has been reported to possess clinical health benefits (Shimizu et al., 2008). Health Canada approved a health claim stating that replacement of 10 mL/d of saturated fat with canola oil may help reduce cholesterol levels (Health Canada, 2012). A review by Lin et al. in 2013 revealed that there was an average of 15.8% reduction in circulating triglyceride concentrations with the consumption of a canola oil based diet, compared with the high SFA-based

diet (Lin et al., 2013). In addition, a clinical study examined the effect of canola oil on cardiovascular risk factors in a population with MetS showed significant reduction of -0.45 mmol/L in triglycerides level after consumption of high MUFA canola oil enriched with ALA (Baxheinrich et al., 2012). Also, about 42% of the study patients no longer suffered from the MetS after the treatment (Baxheinrich et al., 2012).

The oil industry has recently produced a novel type of high-oleic canola oil (HOCO) which is high in MUFA (70%), low in SFA and exhibits a low ratio of n-6: n-3 (Tarrago-Trani, et al., 2006; Gillingham et al., 2011). A randomized controlled study examined the effects of HOCO and flaxseed oils on serum lipids and inflammatory biomarkers in hypercholesterolemic subjects. Results showed significantly higher plasma MUFA and slightly higher DHA concentrations with the consumption of HOCO compared to flaxseed oil (Gillingham et al., 2011). Another randomized controlled trial examined the effect of DHA-enriched HOCO on CVD risk factors and showed significantly higher plasma total MUFA and low blood triglyceride concentration compared to the placebo after consumption of 60 g/day HOCO for 4 weeks (Jones et al., 2014). Baxheinrich et al. (2012) demonstrated a 7.8 kg weight reduction, 2.7 BMI reduction, 9.9 cm waist circumference reduction, 2.9% reduction in body fat and 3% increase in lean mass after consumption of 38% energy as oleic acid enriched rapeseed oil compared to a conventional diet for 26 weeks.

2.2 Effect of dietary fatty acids on fatty acid synthesis and fatty acid oxidation

Excess intakes of dietary fatty acids for human daily activity may not necessarily result in obesity. Obesity results from higher lipogenesis rates than β -oxidation or lipolysis rates (Delany et al, 2002). The effects of different dietary fats on fatty acid synthesis depend on the types of the fatty acids consumed. For example, higher dietary unsaturated fatty acid consumptions could lead to inhibition of fatty acid synthesis rate (Delany et al, 2002). The chain length and degree of saturation of the fats consumed determine the extent of the inhibition of fatty acid oxidation and synthesis. Both animal and human studies showed a pattern of selective oxidation of long chain PUFA over SFA (Jones & Schoeller, 1988; Clandinin et al., 1995; Takeuchi et al., 1995). Moreover, some studies showed greater oxidation rate of oleic acid compared with linoleic or linolenic acids (Jones, et al., 1985; Forsgren, 1969).

Previous studies reviewed the ability of dietary fatty acid to modulate fatty acid synthesis and oxidation in the human body (Jones et al., 2008; Couet et al., 1997, Wood et al., 2015). Wood and his colleagues reviewed 20 clinical trials estimating the dietary intervention on n-3 long chain PUFA status and concluded that both increases in ALA and decreases in LA intake resulted in significant increases in EPA and DHA status (Wood et al., 2015). Studies pointed out that dietary oleic acid and LA had higher absorption and oxidation rates than did saturated fatty acids (Jones et al., 1985). Jones et al. (2008) conducted a randomized controlled crossover study which involved 15 participants consuming 3 diets high in oleic acid, LA or ALA breakfasts and showed potential increases in fatty acid oxidation rates and body

energy expenditure with oleic acid treatment. Fish oils have also been proven to stimulate fat oxidation in the human body; Couet et al. (1997) reported a significant increase in fat oxidation rates with fish oil consumption compared to a control diet after a 12-week intervention without any change in body weight.

Stable isotope tracers have been using to determine human whole body de novo fatty acid synthesis rate. Incorporating hydrogen atoms into fatty acids during fatty acid de novo synthesis and elongation since hydrogen atoms could not replace the ones in the preformed fatty acids (Leitch & Jones, 1992). It is important to better understand human fat metabolism, body fat composition and related metabolic risk factors by introducing stable isotope tracers.

2.3 Effect of β-glucan on CVD risk factors

β-glucan is a type of dietary fibre that may deliver cardioprotective effects such as lowering cholesterol levels, reducing blood pressure, lowering blood glucose level, and reducing body weight (El Khoury et al., 2012). A recent controlled crossover clinical trial which involved 29 hypercholesterolemic subjects with treatments of different dosages and molecular weights of barley β-glucan demonstrated reductions in serum TC with 3 g/day high molecular weight barley β-glucan intake when compared to low molecular weight barley β-glucan intake (Wang et al., 2016). The mechanism of β-glucan's effect on cholesterol is similar to that dietary fibre. β-glucan consumption increases bile acid excretion and increased activity of cholesterol 7 α-hydrolase that leads to the elimination of cholesterol in the body (Zhang et al., 1992). β-glucan may also increase the hepatic synthesis of bile acid and promote the movement to the large intestine (Zhang et al., 1992).

Physiochemical properties of β-glucan such as molecular weight, solubility, and viscosity are related to the bioactive property of β-glucan (Urkus & Temelli, 1998; El Khoury et al., 2012). The mechanisms explaining the satiating property of β-glucan can be various, such as gastric emptying, macronutrients absorption inhibiting, and short chain fatty acid fermentation (El Khoury et al., 2012).

High molecular weight β-glucan possesses high viscosity and is effective in altering total cholesterol (Wang et al. 2016; Wolever et al., 2010). A meta-analysis that included eleven clinical trials examine the lipid lowering effect of β-glucan from barley showed barley and β-glucan isolated from barley lowered both total cholesterol and LDL cholesterol (AbuMweis et al., 2010).

Studies have shown dietary fibre may reduce body weight and body fat through increasing satiety, decreasing absorption of macronutrients and altering secretion of gut hormones (Slavin, 2005). A review by Slavin (2005) showed the ability of dietary fibre on body weight reduction based on human epidemiologic studies. An animal study showed that consumption of oat β-glucan for 6 weeks resulted in improved oxidative stress, which may result in fat oxidation change (Wilczak et al., 2015). Dong and his colleagues conducted a randomized controlled pre-clinical animal study using high, medium and low dose (2000 mg/kg body weight; 1200 mg/kg body weight; 800 mg/kg body weight) oat β-glucan and control as treatments to diabetic mice for 6 weeks. Results showed a higher body weight and food intake reduction in the high and medium dosage group compared to the control group (Dong et al., 2011). A randomized controlled trial showed significant weight, BMI and visceral fat reduction after consuming 7 g/day of β -glucan for 12 weeks (Shimizu et al., 2008).

Another randomized controlled study showed that body weight and BMI were significantly reduced with the consumption of 1.5 g/day oat β-glucan for 12 weeks; the body fat percentage also decreased significantly (Chang et al., 2013).

2.4 Combination of different functional ingredients on CVD marker levels

Researchers have tried to combine different functional ingredients to maximize the health effects of individual components by combining different bioactives into a single functional food. Recently, Natale et al. (2013) conducted a double blind, randomized, crossover study gave n-3 fatty acids, folates, β-glucans, and tocopherol enriched diet to subjects with mild mixed hyperlipidemia for 4 weeks. Results showed significant reduction in fasting plasma triglycerides with consumption of food with the combination of n-3 fatty acids, folates, β-glucans, and tocopherol. Another randomized controlled trial using low fat, viscous fibres, soy protein as a portfolio diet showed significant LDL reduction without changing HDL after 6-week treatment, also improved endothelial function and inflammatory markers (Keith et al., 2015). Furthermore, a randomized controlled study that involved 241 participants with hyperlipidemia across Canada conducted by Jenkins et al. (2015) compared a portfolio diet including soy protein, viscous fibre and nuts to a DASH diet for 24 weeks, showed a significant reduction in cholesterol and blood pressure. Another clinical study investigated the effect of combining MUFA, viscous fibre and plant sterol on cholesterol after consumption for 4 weeks, results showed a significant increase in HDL compared to the control; also there were reductions in the ratio of total to HDL cholesterol and C-reactive protein (Jenkins et al., 2010). Many other studies demonstrated that portfolio diets with different bioactives

might deliver synergistic health benefits for CVD risk reduction (Jenkins et al, 2005; Jenkins et al., 2007; Kendall & Jenkins, 2004).

2.5 Summary

As stated in the above literature review, the individual effects of HOCO, MUFA and ALA enriched diets have been well reported. DHA as an n-3 fatty acid found in marine sources has shown effective benefits against CVD risk. Furthermore, HOCO with DHA has been shown to have stronger beneficial effects on CVD risk factors than individual components, or regular canola oil alone (Senanayake et al., 2013). β-glucan intake has also been shown to have effective benefits in risk reduction of CVD by improving various risk factors such as blood TC, LDL, HDL cholesterol along with body fat (Queenan et al., 2007; AbuMweis, Jew & Ames, 2010; Ripsin et al., 1992). Portfolios of different bioactives have proven evidence in CVD risk reduction (Ramprasath et al., 2013; Jenkins et al., 2015; Jenkins et al, 2005; Jenkins et al., 2007; Kendall & Jenkins, 2004). No intervention has combined HOCO, DHA and β -glucan to examine the health benefit with a portfolio of these bioactives. As discussed previously, there is a rationale for adding these bioactives together in that it could be expected that the combination would have delivered a synergistic effect. One would expect to maximize the health benefits with the consumption of a combination of HOCODHA and β-glucan in CVD risk reduction compared to individual bioactives provided separately.

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Bridge to chapter III

In order to explain the current knowledge gaps discussed in chapters I and II, The CONFIDENCE (canola oil and fibre with DHA enhanced) study was conducted. The following chapter comprises a manuscript, which provides comprehensive information of the study design. This chapter also introduces the study objectives, primary and secondary outcomes and various analytical methods within the entire intervention study. In addition, as the preliminary results of analyses, the post-treatment fatty acid profiles of all complete participants are presented to reflect the high compliance of the entire study across all dietary phases.

Chapter III

Plasma and red blood cell fatty acid changes following consumption of food consisting high-oleic canola oil, DHA and β-glucan

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3.1 Introduction

Cardiovascular disease (CVD) as a major public health problem has caused 17.5 million deaths of people in the world. Estimations show that in 2030, about 23.6 million deaths will be caused by CVD (Cardozo et al., 2014). Metabolic syndrome (MetS), with central obesity (waist circumference: men>102 cm; women>88cm), diminished high-density lipoprotein (HDL) cholesterol (men<1.0 mmol/L; women<1.3 mmol/L), systemic hypertension (≥130/≥85 mm Hg), elevated triglycerides (≥1.69 mmol/L) and elevated fasting plasma glucose (≥6.1 mmol/L), has become a leading health issue due to its association to CVD (Mottillo et al., 2010). An association between dietary fats and cardiovascular health has been widely accepted for decades. A large body of evidence supports that replacing saturated fatty acids (SFA) with n-9 monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and lower n-6/n-3 ratio diets provide health benefits (Senanayake et al., 2014; Rasmussen et al., 2006; Mensink et al., 1992; Rivellese et al., 2003 & Bjermo et al., 2012). In addition, recent recommendations have been made that consumption of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) has protective effects on CVD (Salter, 2013; Kris-Etherton et al., 2002; Rasmussen et al., 2006). RBC fatty acids reflect tissue fatty acid profiles indicating long-term effects of dietary fatty acids. More specifically, the omega-3 index, which is the sum of EPA+DHA levels in the erythrocyte membranes, has been considered as a biomarker of cardiovascular health (Flock et al., 2013).

Canola oil exists as a source of MUFA and α-linolenic acid (ALA). ALA may help reduce the risk of MetS and CVD (Gillingham et al., 2011). Oil industries have

recently produced a novel type of high-oleic canola oil (HOCO) which is high in MUFA, low in SFA and exhibits a low ratio of n-6: n-3 (Gillingham et al., 2011). Previous studies showed a significant reduction in blood triglyceride concentrations after HOCO consumption (Gillingham et al., 2011; Esposito et al., 2004; Sirtori et al., 1986). In addition, studies have shown that MUFA- enriched diets reduce abdominal fat and central obesity (Liu et al., 2016). Furthermore, recent work suggests that EPA and DHA consumption has a protective effect on CVD (Salter, 2013). β-glucan has many bioactive properties including serum lipid reduction, macronutrient absorption reduction and satiety increase which all exert a positive action on CVD risk factors (Behall et al., 2004; Keogh et al., 2003; AbuMweis, Jew & Ames, 2010; Wolever et al., 2010). Health Canada has recently announced a health claim regarding an intake of 3 g barley β-glucan per day to reduce blood cholesterol (Health Canada, 2012).

Although HOCO, DHA, and β-glucan have been found to be effective in CVD risk reduction, to date, no studies have combined these ingredients together targeting the MetS and CVD risk factors. Hence the current study aims to investigate the effect of consuming a combination of HOCO, DHA and β-glucan on various MetS and CVD biomarkers including abdominal fat, body fat distribution and plasma and RBC fatty acid profiles.

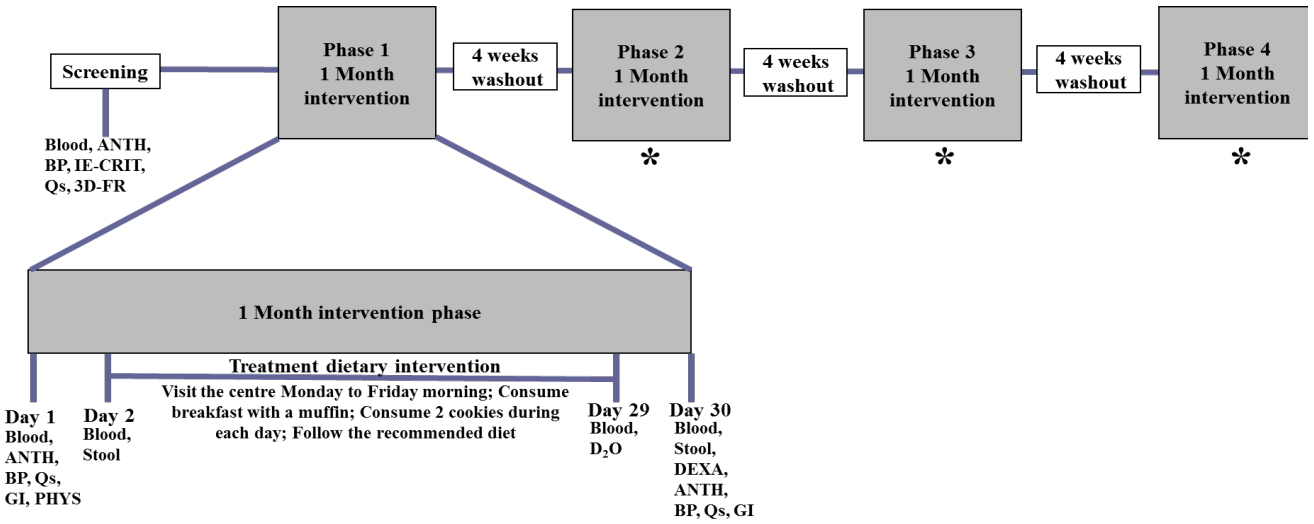
3.2 Experimental methods

3.2.1 Human interventional trial

A randomized, single-blind, crossover trial was conducted at the Clinical Nutrition Research Unit at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), University of Manitoba. The study design consisted of 4 dietary intervention phases of 28 days per phase, with each phase separated by a 4-week washout period (Figure 1). Participants consumed breakfast meals under supervision along with the treatment foods during weekdays from d 2 to 29 of each phase. Treatment muffins and cookies for the weekend were packed and provided to participants to take out on Fridays. Participants were strongly recommended to maintain consistency in their physical activities during the experimental period. Compliance with the treatment products was determined from checklists and by measuring the amount of left-over food if any in the containers returned by the participants the following day. Participants were given recommendations by a registered dietician to follow a typical Western diet that meets the Canadian Recommended Nutrient Intake. Treatments were isocalorically incorporated into muffins and cookies and consumed at breakfast evening snacks and supper. Participants were required to consume one muffin along with their breakfast and two cookies during the rest of the day.

The study protocols (with ethical considerations) were reviewed and approved by research ethics boards at each participating institution, including the Biomedical Ethics Board at the University of Manitoba (B2014:029). The trial was registered at clinical trials.gov under the registration number NCT02091583.

Figure 3.1. Schematic representation of the experimental protocol



* - Procedures are same as Phase 1; Blood - 12 h fasted blood collection; ANTH - Anthropometric measurements; BP – Blood pressure; IE-CRIT - Inclusion and Exclusion criteria; Qs - Questionnaires (Fish and seafood consumption, concomitant medications, adverse events, physical activity); DEXA - Dual energy X-ray absorptiometry

3.2.2 Test foods

Muffin and cookie recipes were developed by Agriculture and Agri-Food Canada laboratories at the RCFFN metabolic kitchen. Control oil (55% Ghee: 25% safflower oil: 20% sunflower oil), HOCODHA (85%: 15%) (Richardson Oilseed Limited; DSM Nutritional Products, Inc.), all-purpose wheat flour and barley flour (cultivar CDC Rattan; Alberta Barley Commission) was incorporated into cookies and muffins (Table 3.2.1 & Table 3.2.2). Macronutrients, including protein, carbohydrates and fibre contents of the foods were analyzed using laboratory methods at the Agriculture and Agri-Food Canada laboratories and results were incorporated into FOOD PROCESSOR (ESHA Research, Salem, Oregon, United States). Total fat and

fatty acid profile of the foods were measured at the RCFFN laboratories (Winnipeg, MB, Canada). Flavors of muffins including vanilla and spice while cookies were made with lemon, ginger and chocolate flavors. Flavors of muffins and cookies were provided in rotation with an equal number of days per flavor during each treatment period. Macronutrient contents of treatment foods were similar between different treatments and also there were no differences in the nutrition profiles between different flavors of muffins or cookies.

Table 3.2.1 Nutritional composition of study muffins

Per muffin	Control flour + HOCODHA	Barley flour + control oil	Barley flour + HOCODHA	Control flour + control oil
Weight (g)	113	108	109	112
Energy (kcal)*	379	383	383	379
Total carbohydrates (g)*	32.7	36.6	36.6	32.7
Total protein (g)*	8.34	8.79	8.79	8.34
Total fibre (g)*	0.36	5.77	5.77	0.36
β glucan (g)*	0	2.18	2.18	0
Total fat (%Wt)	23.5	22.9	22.8	22.5
SFA (%)	13.2	34.2	14.8	34.1
MUFA (%)	56.7	27.2	52.4	27.9
PUFA (%)	30.1	38.6	32.8	38.0
n-3 PUFA (%)	5.59	1.01	5.64	0.86
n-6 PUFA (%)	24.5	37.6	27.2	37.1

*indicates that the values have been calculated based on values of the ingredients.

Muffins were made with two different flavors including vanilla and spice. Values

presented here are the average of both the flavors and there were no major differences between flavors for the above measures.

Table 3.2.2 Nutritional composition of study cookies

Per cookie	Control flour + HOCODHA	Barley flour + control oil	Barley flour + HOCODHA	Control flour + control oil
Weight (g)	52.0	50.0	52.0	53.0
Energy (kcal)*	213	221	221	213
Total carbohydrates (g)*	23.0	25.0	25.0	23.0
Total protein (g)*	4.00	4.00	4.00	4.00
Total fibre (g)*	0.46	3.17	3.17	0.46
β glucan (g)*	0	1.09	1.09	0
Total fat (%Wt)	24.0	24.0	23.0	24.0
SFA (%)	12.5	35.4	12.6	33.8
MUFA (%)	63.4	29.0	64.2	31.1
PUFA (%)	24.1	35.7	23.2	35.2
n-3 PUFA (%)	6.44	0.61	6.28	0.48
n-6 PUFA (%)	17.5	34.9	16.9	34.7

*indicates that the values have been calculated based on values of the ingredients.

Wt: Weight Muffins were made with two different flavors including vanilla and spice. Values presented here are the average of both the flavors and there were no major differences between flavors for the above measures.

3.2.3 Subjects

3.2.3.1 Inclusion criteria

Participants aged 18-70 years were recruited. The participants were slightly overweight with BMI>25 kg/m^2, waist circumference for men > 94 cm and women > 80 cm, according to the International Diabetic Federation metabolic syndrome criteria for waist circumference and have to be deemed to be healthy by the study physician. Additionally, participants also met at least two of the following criteria. i) TG>1.7 mmol/L; ii) HDL-C<1 mmol/L for male and <1.3 mmol/L for female; iii) fasting glucose>5.6 mmol/L and iv) LDL-C>2.7 mmol/L, iv) blood pressure>130 mmHg for systolic and >85 mmHg for diastolic.

3.2.3.2 Exclusion criteria

Participants consuming lipid lowering medications or nutritional supplements, which are known to affect blood lipids, or have any dietary restrictions, which prevented them from consuming the study diet for 28 days during each phase, were excluded. Participants with a current or a history of any diseases and disorders that could interfere with fat absorption were excluded. Individuals were excluded from the study if they had a systolic blood pressure >150 mm Hg or diastolic blood pressure >100 mm Hg. Participants planning to become pregnant during the study period were excluded. Smokers and people consuming more than 1 alcoholic drinks/d or history of alcoholism or drug dependence were excluded from the study. Use of any experimental medication within 1 month prior to screening or as concomitant medications was one of the exclusion criteria.

3.2.4 Sample collection

Twelve-hour fasting blood samples were collected on day 1, 2, 29 and 30 of each intervention phase. Blood samples were centrifuged at 3000 rpm for 20 min at 4 °C, aliquoted to yield serum, plasma, and RBC, and then stored at -80 °C until analyses. For plasma lipid profile, average values of day 1 and 2 were considered as baseline and average values of day 29 and 30 were considered as the endpoint of each treatment phase.

3.2.5 Analytical methodology

Plasma and RBC fatty acid analyses: Fatty acid profiles were analyzed via direct transesterification of the washed erythrocyte (red blood cell) fraction of blood followed by gas chromatography (Flock et al., 2013). Methanol:toluene (2mL,4:1 vol:vol) [containing heptadecanoic acid (C17:0) (0.1mg/ml) as an internal standard] was added to the sample. Acetyl chloride (200 mL) was added while mixed on a vortex and heated (1 h; 100 ^{o}C) (Flock et al., 2013). Tubes were cooled in water (5 min), had K_2CO_3 6% (5 mL) added, and were subsequently centrifuged (1500 rpm; 5 min; 4^{o}C)(Flock et al., 2013). The upper toluene phase that contained fatty acid methyl esters was collected and stored in a gas chromatograph vial at -80^{o}C for analysis. Fatty acid methyl esters were separated on a Supelcowax 10 column (30m X 0·25mm with 0·25mm film thickness; Supelco, Bellefonte, PA, USA) using a gas chromatograph equipped with a flame ionization detector (Agilent Technologies, Mississauga, Ontario, Canada). 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.

3.2.6 Statistical analysis

Statistical analyses were performed using statistical software, SAS version 9.2. Baseline and endpoint measurements were compared using the analysis of variance (ANOVA) model for determination of treatment effects. Results were expressed as means and their standard errors. Normality of distribution of data was determined by a Shapiro-Wilk test and the non-normal variables were normalized before comparisons with other treatment by log transformation. Effects of dietary treatments were examined using a mixed model ANOVA procedure with diet, sequence and phase as fixed factors and subject as a random factor within the model. Statistical significance was set at $p < 0.05$ for all analyses. Significance of different dietary effects was examined with the Bonferroni adjustment for multiple comparisons.

3.3 Results

3.3.1 Baseline characteristics

Participant baseline characteristics are presented in Table 3.3. Eighty participants were screened for eligibility, and 35 eligible participants started the study. All participants had at least two MetS criteria in addition to central obesity, which characterized them as a population with, or, at risk for MetS. Twenty-nine subjects (12 males and 17 females) completed the study. There were 2 lean participants (BMI 23.6±0.9 kg/m^2), 9 overweight participants (BMI 27.8±0.37 kg/m^2), and 18 obese

participants (BMI 35.1± 1.1 kg/m^2). No significant differences were observed in baseline BMI between the participants.

Table 3.3. Baseline characteristic of participants (n=29)

	Control flour + control oil	Barley flour + control oil	Control flour + HOCODHA	Barley flour + HOCODHA
Age (years)		50±2		
Female (n)	17	17	17	17
Male (n)	12	12	12	12
Weight (kg)	87.3±3.2	89.5±3.2	88.4±3.0	89.6±3.2
BMI (kg/m2)	30.6±0.99	31.6±0.99	31.2±0.99	31.4±0.99
Total cholesterol (mmol/L)	5.34±0.13	5.27±0.13	5.36±0.13	5.33±0.13
HDL cholesterol (mmol/L)	1.19±0.039	1.16±0.039	1.25±0.039	1.24±0.039
LDL cholesterol (mmol/L)	3.34±0.12	3.23±0.12	3.33±0.12	3.31±0.12
TG (mmol/L)	1.70±0.13	1.90±0.13	1.67±0.13	1.70±0.13
Glucose (mmol/L)	5.56±0.12	5.46±0.12	5.62±0.12	5.66±0.12
SBP (mmHg)	118±5.1	120±5.1	118±5.1	120±5.1
DBP (mmHg)	79.6±3.1	81.1±2.1	80.5±3.1	79.6±2.1

All values are means ± SEM. HDL-High density lipoprotein, LDL-low density lipoprotein, TG- triglycerides, SBP-systolic blood pressure, DBP-diastolic blood pressure.

Thirty-five participants were recruited and randomly assigned to four different groups. Six participants withdrew from the study; two due to a relocation of residency and two lost interest. Two participants dropped out due to inability to comply with the study protocol. Participants did not report any change in physical

activity during the study protocol. No major side effects from the treatment were noted.

3.3.2 Plasma fatty acids

Total plasma fatty acid profiles of the completed participants at the end of all four treatments are presented in Table 3.4. All fatty acid concentrations were calculated as percentage values of total identified fatty acids measured. Changes in the plasma fatty acid concentrations (Table 3.3) reflected the fatty acid profile of the experimental diets (Table 3.2.1 & Table 3.2.2) indicating the participants' compliance. Since DHA was part of the dietary treatment, endpoint plasma DHA values were used to evaluate the overall compliance of the intervention study. The two-fold increase in DHA concentration after consumption of the two HOCODHA foods indicated high compliance to the study diet among the participants through the study. Plasma EPA and DHA concentrations were significantly elevated after consumption of HOCODHA-barley flour compared to control oil-barley flour ($P<0.0001$). Similarly, Plasma EPA and DHA concentrations were significantly higher after HOCODHA-control flour than control oil- control flour ($P<0.0001$).

No differences were observed in plasma EPA and DHA concentrations after consuming HOCODHA-control flour and the HOCODHA- β glucan foods. Similarly, no differences in plasma EPA and DHA concentrations were found between the control flour and β-glucan treatments.

Total PUFA decreased following the HOCODHA-control flour compared to the control oil-control flour group ($P<0.0001$). Similar PUFA reduction was observed

following the HOCODHA-barley flour compared to control oil-barley flour (P<0.0001). Total n-3 fatty acid increased by two-fold after consumption of the HOCODHA-control flour compared to control oil-control flour group (P<0.0001). Total n-3 fatty acid also increased by two-fold after consumption of HOCODHA-barley flour compared to control oil-barley flour (P<0.0001). No significant changes were found in total SFA levels across dietary treatments. The n-6 to n-3 ratio decreased both after consumption of HOCODHA-control flour and HOCODHA-barley flour (5.61%, P<0.0001 and 5.47%, P<0.0001 for HOCODHA-control flour and HOCODHA-barley flour, respectively) compared to the control oil-control flour group. Similarly the n-6 to n-3 ratio reduced by 5.81% and 5.68% after consumption of HOCODHA-control flour and HOCODHA barley flour treatment compared to control oil- barley flour treatment. In general, the n-6 to n-3 ratio also confidently explained the corresponding response to n-3 and n-6 rich dietary treatments, further indicating high compliance across the entire study among participants. Oleic acid levels were elevated after consumption of the HOCODHA-control flour compared to control oil-control flour treatment and control oil- barley treatment (1.26±0.38, P=0.0014 and 1.22±0.38, P=0.002, respectively); this confirmed the compliance of the study. No significant differences in fatty acid concentrations were found between control flour and barley flour groups.

Table 3.4. Plasma fatty acid concentrations at the end of each dietary phase in 29 participants (g/100g).

	Control oil + Control flour	Control oil+ Barley flour	HOCODHA + Control flour	HOCODHA+ Barley flour
C14:0	0.597±0.050	0.596±0.050	0.609±0.050	0.467±0.050
C14:1n5	0.157±0.021	0.141±0.022	0.168±0.024	0.160±0.023
C15:0	0.273±0.015	0.262±0.015	0.240±0.015	0.240±0.015
C16:0	30.3±0.42	30.6±0.42	31.0±0.42	31.0±0.42
C16:1n7	1.17±0.075	1.15±0.075	1.11±0.075	0.972±0.077
C18:0	14.4±0.22	13.6±0.22	14.4±0.22	14.0±0.22
C18:1n9	13.5±0.32^{a}	13.5±0.32^{a}	14.7±0.32^{b}	13.9±0.33^{ab}
C18:2n6	23.4±0.52^{a}	23.4±0.52^{a}	18.9±0.52^{b}	19.8±0.53^{b}
C18:3n6	0.264±0.026	0.279±0.027	0.285±0.033	0.280±0.032
C18:3n3	0.463±0.025	0.438±0.025	0.473±0.025	0.431±0.025
C20:0	0.265±0.068	0.310±0.061	0.384±0.069	0.304±0.065
C20:1n9	0.227±0.022^{a}	0.263±0.022^{ab}	0.302±0.024^{b}	0.285±0.021^{ab}
C20:2n6	0.349±0.019	0.329±0.018	0.360±0.019	0.316±0.019
C20:3n6	2.53±0.091	2.49±0.091	1.97±0.091	1.92±0.093
C20:4n6	8.48±0.30	8.68±0.30	8.20±0.29	8.65±0.30
C20:5n3	0.678±0.075^{a}	0.652±0.075^{a}	1.20±0.075^{b}	1.27±0.077^{b}
C22:0	0.313±0.024^{a}	0.394±0.024^{ab}	0.364±0.031^{ab}	0.424±0.029^{b}
C22:5n3	0.308±0.046^{a}	0.251±0.047^{a}	0.642±0.043^{b}	0.649±0.043^{b}
C22:5n6	0.576±0.029^{a}	0.568±0.029^{a}	0.283±0.029^{b}	0.256±0.030^{b}
C22:6n3	2.45±0.17^{a}	2.47±0.17^{a}	5.23±0.17^{b}	5.44±0.17^{b}
Total SFA	45.7±0.44	45.6±0.44	46.6±0.44	46.1±0.44

Total MUFA	14.7±0.36[a]	15.0±0.36[a]	16.2±0.36[b]	15.2±0.38[ab]
Total PUFA	39.1±0.55[ab]	39.2±0.55[a]	37.2±0.54[b]	38.6±0.56[ab]
Total n-3 PUFA	3.80±0.23[a]	3.72±0.23[a]	7.50±0.23[b]	7.74±0.23[b]
Total n-6 PUFA	35.5±0.51[a]	35.7±0.51[a]	29.7±0.51[b]	31.0±0.53[b]
n-6:n-3	9.72±0.31[a]	9.92±0.31[a]	4.11±0.31[b]	4.25±0.32[b]

Values are Means ± SEM; [a, b, c] Values within a row with different superscript letters were significantly different between treatment groups ($P<0.05$). Mixed-effects repeated-measures analysis of variance with treatment, age, gender and the dietary phase as fixed effect, the last diet (sequence effect) as a random effect, and the measures for each subject by phases were used for the data analysis.

3.3.3 RBC fatty acids

RBC fatty acid profiles of the completed participants at the end of all four treatments are presented in Table 3.5. No significant changes in total SFA were observed between the four treatments. Similar to plasma fatty acid profiles, RBC DHA levels increased after consumption of HOCODHA. HOCODHA-control flour group showed increased total n-3 PUFA ($P<0.0001$) and decreased n-6 PUFA ($P<0.0001$) as well as n-6 to n-3 ratio ($P<0.0001$) compared to control oil-control flour; similarly, HOCODHA-barley flour group showed increased total n-3 PUFA ($P<0.0001$) and decreased n-6 PUFA ($P<0.0001$) as well as n-6 to n-3 ratio ($P<0.0001$) compared to control oil-barley flour; this reflected the favourable changes in plasma fatty acid profiles. Total MUFA showed a trend of elevated levels after HOCODHA treatments. Moreover, the sum of EPA and DHA in RBC membranes (as a percent of total fatty acid content), which is denoted as the omega-3 index significantly increased following the HOCODHA-control flour compared to control oil-control

flour group (P<0.0001). Similarly, a significant increase in the omega-3 index was observed following HOCODHA-barley flour compared to control oil-barley flour (P<0.0001). Similar to the findings in plasma, no significant changes in fatty acids were found between control and β-glucan treatments.

Table 3.5. RBC fatty acid concentrations at the end of each dietary phase in 29 participants (g/100g).

	Control oil + Control flour	**Control oil+ Barley flour**	**HOCODHA + Control flour**	**HOCODHA+ Barley flour**
C14:0	0.574±0.024	0.563±0.024	0.768±0.024	0.559±0.024
C14:1n5	0.157±0.013	0.141±0.013	0.168±0.013	0.160±0.013
C15:0	0.187±0.0094	0.175±0.0094	0.170±0.0094	0.172±0.0095
C16:0	25.5±0.34	25.1±0.34	25.5±0.34	25.3±0.34
C16:1n7	0.471±0.037	0.514±0.037	0.483±0.037	0.468±0.038
C18:0	18.4±0.18	18.5±0.18	18.5±0.18	18.4±0.19
C18:1n9	14.8±0.23^{a}	14.9±0.23^{ab}	15.7±0.23^{c}	15.2±0.23^{bc}
C18:2n6	12.1±0.26^{a}	12.1±0.26^{a}	10.3±0.26^{b}	10.3±0.26^{b}
C18:3n3	0.236±0.016	0.228±0.016	0.254±0.016	0.22±0.016
C20:0	0.170±0.019^{a}	0.193±0.019^{ab}	0.218±0.018^{b}	0.201±0.018^{ab}
C20:1n9	0.306±0.013^{a}	0.336±0.013^{ab}	0.329±0.013^{ab}	0.356±0.013^{b}
C20:2n6	0.256±0.014	0.267±0.014	0.276±0.014	0.243±0.015
C20:3n6	1.84±0.30	1.65±0.30	1.39±0.29	1.44±0.30
C20:4n6	14.1±0.43	14.5±0.43	14.2±0.43	14.3±0.43
C20:5n3	0.606±0.047^{a}	0.597±0.047^{a}	0.779±0.047^{b}	0.832±0.048^{b}
C22:0	0.439±0.10	0.305±0.11	0.222±0.11	0.347±0.11
C22:2n6	2.78±0.18	2.51±0.18	2.61±0.18	2.58±0.18

C22:5n3	1.70±0.17	1.65±0.17	1.83±0.17	1.65±0.17
C22:5n6	1.84±0.073[ac]	1.90±0.073[ab]	1.75±0.073[c]	1.73±0.074[c]
C22:6n3	4.45±0.19[a]	4.42±0.19[a]	5.57±0.19[b]	5.87±0.19[b]
C24:0	0.665±0.11	0.733±0.12	0.582±0.11	0.646±0.11
C24:1n9	0.612±0.091	0.667±0.092	0.575±0.092	0.635±0.092
SAT	45.1±0.30	45.0±0.30	45.2±0.30	45.2±0.30
MUFA	16.2±0.25[a]	16.2±0.25[ab]	16.9±0.25[c]	16.6±0.25[c]
PUFA	38.7±0.29[a]	38.8±0.28[a]	37.9±0.29[b]	38.3±0.29[a]
n-3	6.92±0.24[a]	6.72±0.24[a]	8.51±0.24[b]	8.76±0.24[b]
n-6	31.8±0.42[a]	32.1±0.42[a]	29.4±0.42[b]	29.5±0.42[b]
n-6:n-3	4.82±0.30[a]	5.23±0.30[a]	3.58±0.30[b]	3.44±0.30[b]
EPA+DHA	5.83±0.31[a]	5.91±0.31[a]	7.22±0.31[b]	7.45±0.31[b]

Values are Means ± SE; [a, b, c] Values within a row with different superscript letters were significantly different between treatment groups ($P<0.05$). Mixed-effects repeated-measures analysis of variance with treatment, age, gender and the dietary phase as fixed effect, the last diet (sequence effect) as a random effect, and the measures for each subject by phases were used for the data analysis.

3.4 Discussion

The results of this study demonstrated that food enriched with HOCODHA consumed by men and women with MetS resulted in higher plasma and RBC total MUFA and n-3 PUFA as well as a lower n-6 to n-3 ratio compared to the control oil. The predictable increase of specific fatty acids representing the major dietary fatty acids in the treatment oils was observed as expected. Total n-3 PUFA increased while n-6 PUFA decreased significantly in both plasma and RBC with consumption of

the HOCODHA treatments. Total MUFA also were increased in both plasma and RBC after consumption of HOCODHA treatments. The observed consistent favorable shifts across the treatments highlight the successful compliance of the study. In addition, the increase in oleic acid levels in HOCODHA groups reflected the high oleic acids content in the dietary cookies and muffins.

Plasma total MUFA content increased after consumption of HOCODHA treatments. These results confirm the findings of Gillingham et al. (2011) that plasma MUFA concentration increased significantly with consumption of HOCO compared to flaxseed oil. Another randomized controlled trial examined the effect of DHA-enriched HOCO on CVD risk factors and showed higher plasma total MUFA and low blood triglyceride concentrations compared to the placebo after consumption of 60 g/day HOCO for 4 weeks (Senanayake et al., 2013). The two-fold increase of plasma DPA, EPA, DHA following HOCODHA dietary phases compared to the control oil dietary phases; confirmed the ability of DHA-enriched functional foods to increase plasma omega-3 concentrations. Research on effects of dietary DHA on circulating fatty acid profile has been widely conducted; the present study first demonstrates the effects of combination of HOCODHA and β-glucan on circulating fatty acid profile (Sagara et al., 2011; Rasmussen et al., 2006; Mensink et al., 1992; Rivellese et al., 2003 & Bjermo et al., 2012). A randomized controlled study involving 156 males showed a significant increase in plasma DHA and total n-3 PUFA (Sagara et al., 2011). Our finding of increases in plasma omega-3 concentration is consistent with previous data. The 2.5 fold increase in plasma EPA composition compared to the control oil groups supported the evidence that HOCODHA has

beneficial effects on cardiovascular health. Although DHA and EPA together have been proven to help reduce CVD risk, elevated plasma concentrations of EPA have been reported to exert independent actions in decreasing nonfatal cardiovascular endpoints (Mozaffarian & Wu, 2012; Yokoyama et al., 2007). The significant increase in EPA levels in HOCODHA groups could be explained by the higher EPA content in marine algal DHA that were used in the study. The present study has been the first to demonstrate effects of the combination of HOCODHA and β-glucan on circulating fatty acid profile.

Similar to plasma fatty acid profiles, total RBC MUFA slightly increased after consumption of HOCODHA enriched food products. RBC n-6 to n-3 ratio showed a significant reduction with total n-3 PUFA increased while n-6 PUFA decreased significantly. RBC fatty acid profiles reflected the tissue fatty acid profile indicating the long-term effects of dietary fatty acids. Omega-3 index was reported to act as an important risk factor for death caused by CVD (Harris, 2008; He et al., 2002; Harris & Clemens, 2004; Ramprasath, 2013). The EPA+DHA content of erythrocyte membranes is correlated with human cardiac membrane EPA + DHA levels (Giltay et al., 2003; Harris, 2008). In addition, the omega-3 index has been reported to be a stronger indicator in terms of sudden cardiac death than other risk factors including C-reactive protein, total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerol, and homocysteine (Albert et al., 2002; Albert et al., 2002; Harris, 2008) levels. The current study has shown the elevation of the omega-3 index after consumption of the HOCODHA for each 28 d phase, which indicates the cardioprotective effects it might deliver to the individual with MetS.

3.5 Conclusions

Consumption of novel foods containing HOCODHA and β-glucan significantly increased total plasma and RBC n-3 concentration, omega-3 index, and significantly reduced n-6: n-3 ratio. The results are of significance, further supporting the cardio protective effects of HOCODHA oils.

3.6 Acknowledgments

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Bridge to chapter IV

The following chapter comprises a manuscript, which presents one of the primary outcomes of the dietary intervention trial, body composition. In addition, the study secondary outcome was to investigate the fatty acids synthesis rate after consumption of foods containing high-oleic canola oil, DHA and β-glucan using whole-body indirect calorimetric and dual x-ray absorptiometry methods, the purpose of the following study was to simultaneously investigate effects of changes in fatty acid synthesis and body composition after consumption of high-oleic canola oil, DHA and β-glucan.

Chapter IV

Effects of food containing high oleic acid canola oil, DHA and β-glucan on fatty acids synthesis rate and abdominal fat mass in a population with metabolic syndrome: A randomized controlled trial

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4.1 Introduction

Cardiovascular disease (CVD) is the leading cause of death in Canada and worldwide (Statistics Canada, 2011 & WHO 2013). Over the past decade, obesity rate has increased dramatically (Jones et al., 2008). Abdominal obesity and insulin resistance are core features among the medical conditions (Aschbacher et al., 2014). Body weight reduction is one of the primary treatments for MetS (Grundy et al., 2005). Increasing evidence shows the beneficial effects of dietary monounsaturated fatty acids (MUFA) in regulating body fat distribution and cardiometabolic risk factors (Lui et al., 2016, Appel et al., 2005). Gillingham and colleagues (2012) showed a trend in lower android-to-gynoid ratio after consumption of high oleic canola oil (HOCO) for 28 days. Studies showed that dietary oleic acid and linoleic acid (LA) had higher absorption and oxidation rates than the saturated fatty acids (Jones et al., 1985). Fish oils also have been shown to stimulate fat oxidation in the human body (Couet et al., 1997). In addition, Wood and his colleagues reviewed 20 clinical trials estimating the dietary intervention on n-3 long-chain polyunsaturated fatty acid (PUFA) status, and concluded that both increases of α-linolenic acid (ALA) and decreases in LA intake resulted in significant increases in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) status (Wood et al., 2015). Studies have also shown that dietary fibre might reduce body weight and body fat through increasing satiety and reducing energy intake (Howarth et al., 2001; El Khoury et al., 2012). A random controlled study by Birketvedt et al. (2000), found that introducing dietary fibre to a low-calorie diet significantly improved body weight loss compared to the placebo.

The present study was conducted to evaluate the effects of the combination of HOCO, DHA oil along with barley β-glucan on body composition and cardiometabolic risk factors in subjects with MetS. Our hypothesis was that diets high in high oleic acid, DHA and β-glucan would deliver a synergetic effect on body fat distribution in individuals with MetS and reduce other cardiometabolic risk factors.

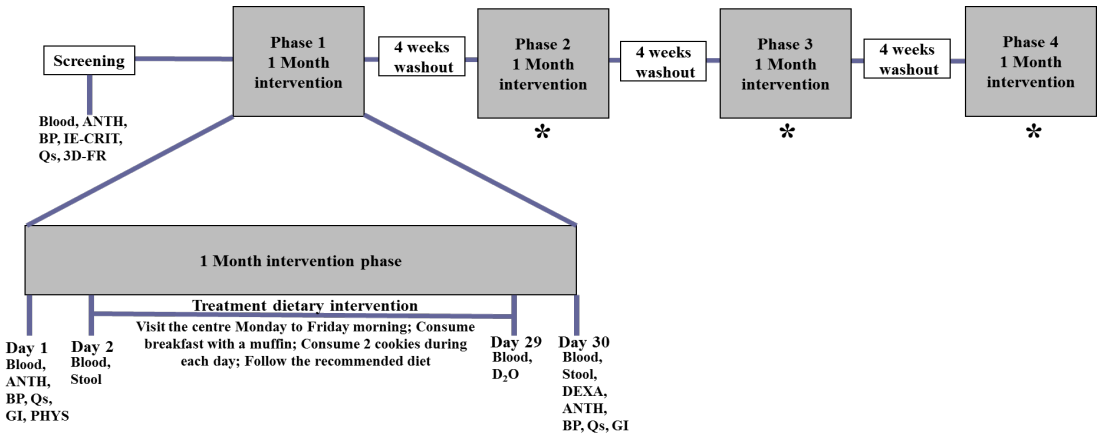
4.2 Methods

4.2.1 Human interventional trial

A randomized, single-blind, crossover trial was conducted at the Clinical Nutrition Research Unit at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), University of Manitoba. The study design consisted of 4 phases with 28 days per phase, and each phase was separated by 4-week washout periods (Figure 4.1). Participants consumed breakfast meals under supervision along with the treatment foods during weekdays from d 2 to 29 of each phase. Treatment muffins and cookies for the weekend were packed and provided to participants to take out on Fridays. Participants were strongly recommended to maintain consistency in their physical activities during the experimental period. Compliance with the treatment products was determined from checklists and by measuring the amount of leftover food if any in the containers returned by the participants the following day. Participants were given recommendations to follow a typical Western diet that meets the Canadian Recommended Nutrient Intake. Treatments were isocalorically incorporated into muffins and cookies and consumed as breakfast or evening snacks and at supper. Participants were required to consume one muffin along with their breakfast and two cookies during the rest of the day.

The study protocols (with ethical considerations) were reviewed and approved by research ethics boards at each participating institution, including the Biomedical Ethics Board at the University of Manitoba (B2014:029). The trial was registered at clinical trials.gov under the registration number NCT02091583.

Figure 4.1. Schematic representation of the experimental protocol



* - Procedures are same as Phase 1; Blood - 12 h fasted blood collection; ANTH - Anthropometric measurements; BP – Blood pressure; IE-CRIT - Inclusion and Exclusion criteria; Qs - Questionnaires (Fish and seafood consumption, concomitant medications, adverse events, physical activity); DEXA - Dual energy X-ray absorptiometry;

4.2.2 Test foods

Muffin and cookie recipes were developed at Agriculture and Agri-Food Canada laboratories at the RCFFN metabolic kitchen. Control oil (55% Ghee: 25% safflower oil: 20% sunflower oil), HOCODHA (85%: 15%) (Richardson Oilseed Limited; DSM Nutritional Products, Inc.), all-purpose wheat flour and barley flour (cultivar CDC Rattan; Alberta Barley Commission) were incorporated into cookies and muffins (Table 4.2.1, Table 4.2.2). Macronutrients including protein, carbohydrates and the fibre content of the foods were analyzed using laboratory methods at the Agriculture and Agri-Food Canada laboratories and results were incorporated into

FOOD PROCESSOR (ESHA Research, Salem, Oregon, United States). Total fat and fatty acid profile of the foods were measured at the RCFFN laboratories. Flavors of muffins including vanilla and spice while cookies were made with lemon, ginger and chocolate flavors. Flavors of muffins and cookies were provided in rotation with an equal number of days per flavor during each treatment period. Macronutrient contents of treatment foods were similar between different treatments and also there were no differences in the nutrition between different flavors of muffins or cookies.

Table 4.2.1. Nutritional composition of study muffins

Per muffin	Control flour + HOCODHA	Barley flour + control oil	Barley flour + HOCODHA	Control flour + control oil
Weight (g)	113	108	109	112
Energy (kcal)*	379	383	383	379
Total carbohydrates (g)*	32.7	36.6	36.6	32.7
Total protein (g)*	8.34	8.79	8.79	8.34
Total fibre (g)*	0.360	5.77	5.77	0.360
β glucan (g)*	0	2.18	2.18	0
Total fat (%Wt)	23.5	22.9	22.8	22.5
SFA (%)	13.2	34.2	14.8	34.1
MUFA (%)	56.7	27.2	52.4	27.9
PUFA (%)	30.1	38.6	32.8	38.0
n-3 PUFA (%)	5.59	1.01	5.64	0.860
n-6 PUFA (%)	24.5	37.6	27.2	37.1

*indicates that the values have been calculated based on values of the ingredients.

Muffins were made with two different flavors including vanilla and spice. Values presented here are the average of both the flavors and there were no major differences between flavors for the above measures.

Table 4.2.2. Nutritional composition of study cookies

Per cookie	Control flour + HOCODHA	Barley flour + control oil	Barley flour + HOCODHA	Control flour + control oil
Weight (g)	52.0	50.0	52.0	53.0
Energy (kcal)*	213	221	221	213
Total carbohydrates (g)*	23.0	25.0	25.0	23.0
Total protein (g)*	4.00	4.00	4.00	4.00
Total fibre (g)*	0.460	3.17	3.17	0.460
β glucan (g)*	0	1.09	1.09	0
Total fat (%Wt)	24.0	24.0	23.0	24.0
SFA (%)	12.5	35.4	12.6	33.8
MUFA (%)	63.4	29.0	64.2	31.1
PUFA (%)	24.1	35.7	23.2	35.2
n-3 PUFA (%)	6.44	0.610	6.28	0.480
n-6 PUFA (%)	17.5	34.9	16.9	34.7

*indicates that the values have been calculated based on values of the ingredients. Muffins were made with three different flavors including cocoa, ginger and lemon. Values presented here are the average of both the flavors and there were no major differences between flavors for the above measures.

4.2.3 Inclusion criteria

Participants aged 18-70 years were recruited. The participants were slightly overweight with BMI>25kg/m^2, waist circumference for men > 94 cm and women > 80 cm, according to the International Diabetic Federation metabolic syndrome criteria for waist circumference and were to be deemed to be otherwise healthy by the study physician. Additionally, participants met at least two of the following

criteria. i) TG>1.7 mmol/L; ii) HDL-C<1 mmol/L for male and <1.3 mmol/L for female; iii) fasting glucose>5.6 mmol/L and iv) LDL-C>2.7 mmol/L, iv) blood pressure>130 mmHg for systolic and >85 mmHg for diastolic.

4.2.4 Exclusion criteria

Participants consuming lipid lowering medications or nutritional supplements, which are known to affect blood lipids, or have any dietary restrictions which would prevent them from consuming the study diet for 28 days during each phase, were excluded. Participants with current or a history of any diseases and disorders that could interfere with fat absorption were excluded. Individuals were excluded from the study if they presented with a systolic blood pressure >150 mm Hg or diastolic blood pressure >100 mm Hg. Participants planning to become pregnant during the study period were excluded. Smokers and people consuming more than 1 alcoholic drinks/d or history of alcoholism or drug dependence were not included into the study. Use of any experimental medication within 1 month prior to screening or as concomitant medications was one of the exclusion criteria.

4.2.5 Sample collection

Twelve-hour fasting blood samples were collected on day 1, 2, 29 and 30 of each intervention phase. Blood samples were centrifuged at 3000 rpm for 20 minutes at 4 °C, aliquoted to yield serum, plasma, and RBC, and then stored at -80 °C until analyses. For plasma lipid profile, average values of day 1 and 2 were considered as baseline and average values of day 29 and 30 was considered as the endpoint of each treatment phase.

4.2.6 Clinical data collection

Whole body DEXA scanning (Lunar Prodigy Advance, Lunar Corp., Madison WI) was conducted to determine changes in body fat composition during the last week of each phase. Body fat percentage, gynoid fat, android fat, gynoid/android fat ratio, visceral fat were assessed for body fat composition. Body weight and waist circumference were monitored at beginning and end of each treatment period. Participants at the beginning and end of each intervention period completed gastrointestinal tolerability questionnaires.

4.2.7 Stable isotope tracer intake

Both fatty acid synthesis and metabolism involve water, which makes the deuterium water available to mark n-3, and n-6 fatty acid metabolism by measuring the deuterium enriched palmitic acid. To assess fatty acid synthesis rate, participants were asked to consume deuterated water at the end of each phase. On day 29, 0.7 g of D_2O /kg estimated body water was given orally prior to breakfast as a tracer to measure fatty acid synthesis rate over 24 hours. Fasting blood samples were obtained on day 29 (0 hours) and 30 (24 hours).

4.2.8 Calculation of de novo fatty acid synthesis rate

Based on the model for triglyceride-fatty acid synthesis rate using D_2O (Leitch & Jones, 1991), fractional synthesis rates of target fatty acids were calculated in response to the dietary oil treatments by the following equation:

$$\frac{FSR}{d} = \frac{\Delta FA}{\Delta Plasma \times 0.87 \times R(H)} \quad Eq.1$$

where FSR is the fractional synthesis rate over the study period (24 hours), ΔFA is the isotope enrichment in the plasma water, 0.87 is the correction factor observed by Jungas (1968), and R(H) represents the ratio of maximum incorporated deuterium atoms to total hydrogen atoms. Here, an assumption is made that the additional protons are all deuterated during the synthesis procedure (Leitch & Jones, 1991). Thus, the equation estimates the maximum fractional synthesis rate of certain fatty acids depending on their potential ^{2}H-^{1}H replacements.

The isotopic enrichment of deuterium on plasma water is calculated as below:

$$\Delta Plasma = \frac{R(sample)}{R\ (Standard)} - 1 \quad Eq.2$$

where R is the ratio of deuterium to hydrogen. R(sample) is the ratio of plasma water from the blood sample, and R(standard) is the reference standard. In the current study, Standard Mean Ocean Water (SMOW) equals to 1.5576×10^{-4} that refers to the ratio of D/H of standard mean ocean water was used as the reference standard.

4.2.9 Statistical analysis

Statistical analyses were performed using statistical software, SAS version 9.2. Baseline and endpoint measurements were compared using the analysis of variance (ANOVA) model for determination of treatment effects. Results were expressed as means and their standard errors (SEM). Normality of distribution of data was determined by a Shapiro-Wilk test and the non-normal variables were normalized before comparisons with other treatment by log transformation. Effects of dietary treatments were examined using a mixed model ANOVA procedure with diet,

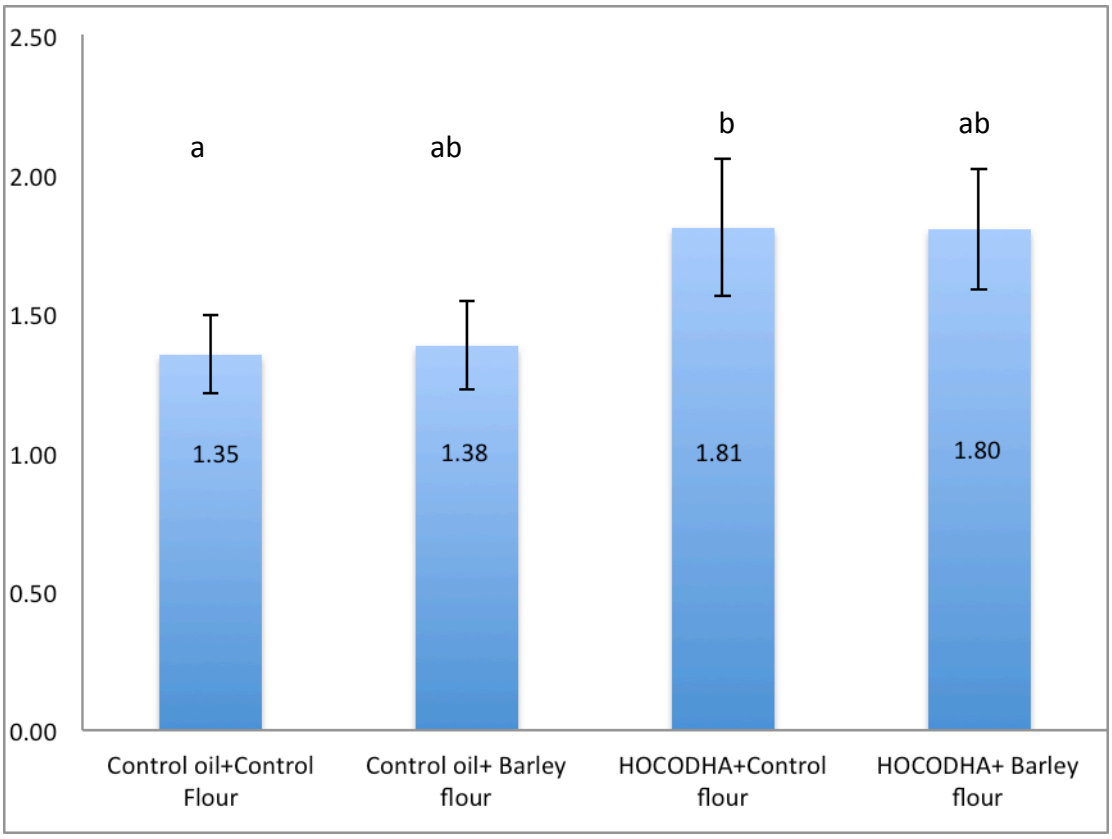
sequence and phase as fixed factors and subject as a random factor within the model. Statistical significance was set at $p < 0.05$ for all analyses. Significances between different dietary effects were examined with the Bonferroni adjustment for multiple comparisons.

4.3 Results

4.3.1 Fractional synthesis rates of palmitic acid

Results of deuterium enrichment of plasma palmitic acid were obtained in a total of 116 samples at 24 hours after consumption of the administered doses of deuterium water, presented in Figure 4.2. Overall, there were no major differences in de novo palmitic acid synthesis rate across the treatments. HOCODHA-control flour groups showed slightly higher de novo palmitic synthesis rate than

Figure 4.2. FSR of palmitic acid at the end of each dietary treatment (n=29)



Values with different superscript letters indicate significant differences between diets $p<0.05$

4.3.2 Between diet comparison for body composition at the end of the dietary treatments

Body composition results are presented in Table 4.2. Participants had no change in body weight after consumption of the dietary treatments. There were no significant changes observed in total, android and gynoid fat mass as well as android to gynoid ratio. No significant differences were observed in trunk fat mass, visceral fat mass and lean mass at the end of each dietary treatment. No differences were observed between different genders in body composition.

Table 4.2. Body composition at the end of each dietary treatment (n=29).

Body composition measures	Gender	Control oil+ Control flour	Control oil+ Barley flour	HOCODHA+ Control flour	HOCODHA+ Barley flour
Trunk Fat Mass(kg)	All	19.9±0.92	19.8±0.92	20.3±0.92	20.2±0.92
	Female	20.8±1.4	20.6±1.4	21.3±1.4	20.6±1.4
	Male	18.8±1.2	18.4±1.2	18.5±1.2	18.4±1.2
Android Fat Mass(kg)	All	3.54±0.19	3.52±0.19	3.54±0.19	3.60±0.19
	Female	3.66±0.26	3.62±0.26	3.59±0.26	3.64±0.26
	Male	3.40±0.27	3.37±0.27	3.43±0.27	3.32±0.27
Gynoid Fat Mass(kg)	All	5.58±0.36	5.49±0.36	5.62±0.36	5.66±0.36
	Female	6.37±0.53	6.18±0.53	6.37±0.53	6.24±0.53
	Male	4.54±0.34	4.48±0.34	4.47±0.34	4.51±0.34
A/G	All	0.667±0.026	0.670±0.026	0.667±0.026	0.665±0.026
	Female	0.598±0.025	0.620±0.025	0.588±0.025	0.604±0.025
	Male	0.762±0.040	0.765±0.040	0.794±0.040	0.764±0.040
Total Fat Mass(kg)	All	35.6±1.9	35.1±1.9	35.6±1.9	36.1±1.9
	Female	38.5±2.7	38.0±2.7	38.40±2.7	38.41±2.7
	Male	31.6±1.8	30.8±1.8	31.3±1.8	31.2±1.8
Trunk Lean Mass(kg)	All	23.6±1.1	23.5±1.1	23.7±1.1	23.5±1.1
	Female	20.7±0.80	20.1±0.80	21.2±0.80	20.3±0.80
	Male	27.9±1.5	28.0±1.5	28.1±1.5	28.5±1.5
Android Lean Mass(kg)	All	3.65±0.16	3.62±0.16	3.62±0.16	3.66±0.16
	Female	3.17±0.14	3.14±0.14	3.17±0.14	3.15±0.14
	Male	4.34±0.23	4.30±0.23	4.36±0.23	4.40±0.23
Gynoid Lean Mass(kg)	All	8.21±0.41	8.12±0.41	8.01±0.41	8.05±0.41
	Female	6.94±0.29	6.91±0.29	6.91±0.29	6.89±0.29
	Male	9.99±0.64	9.83±0.64	10.00±0.64	9.97±0.64

Total Lean Mass(kg)	All	51.1±2.3	50.9±2.3	50.5±2.3	50.6±2.3
	Female	43.9±1.6	43.5±1.6	44.0±1.6	43.5±1.6
	Male	61.5±3.1	61.5±3.1	61.8±3.1	62.0±3.1
Android Total Mass (kg)	All	7.24±0.28	7.18±0.28	7.21±0.28	7.30±0.28
	Female	6.87±0.38	6.81±0.38	6.80±0.38	6.84±0.38
	Male	7.81±0.39	7.75±0.39	7.83±0.39	7.68±0.39
Gynoid Total Mass (kg)	All	14.1±0.58	13.9±0.58	13.9±0.58	14.0±0.58
	Female	13.5±0.78	13.3±0.78	13.5±0.78	13.3±0.78
	Male	14.9±0.84	14.6±0.84	14.8±0.84	14.8±0.84
VAT Mass (kg)	All	1.52±0.12	1.47±0.12	1.51±0.12	1.52±0.12
	Female	1.38±0.12	1.30±0.12	1.37±0.12	1.34±0.12
	Male	1.90±0.19	1.86±0.19	1.90±0.19	1.85±0.19
Subcutaneous Fat Mass (kg)	All	32.3±2.1	32.1±2.1	32.4±2.1	32.8±2.1
	Female	37.1±2.6	36.8±2.6	37.0±2.6	37.1±2.6
	Male	29.7±1.7^{a}	28.9±1.7^{b}	29.4±1.7^{ab}	29.4±1.8^{ab}

Values are expressed as means ±SEM. Values in a row with different superscript letters indicate significant differences between diets $p<0.05$.

A/G: Android-to-gynoid fat mass ratio; VAT mass: Visceral fat mass

4.3.3 Correlations between palmitic acid synthesis and body composition (n=29)

Endpoint correlations between adipose tissue mass and fatty acid synthesis rate are presented in Table 4.3. Palmitic acid synthesis rate was negatively correlated with android fat mass ($r=0.20$, $p=0.04$), gynoid fat mass ($r=0.25$, $p=0.01$), subcutaneous fat mass ($r=0.25$, $p=0.01$), and total fat mass ($r=0.25$ $p=0.01$). Thus, as palmitic acid synthesis rate increased, there was a corresponding decrease in

android, gynoid fat mass as well as total fat mass after consumption of HOCODHA treatments.

Table 4.3 Correlations between palmitic acid synthesis and body composition (n=29)

	Android fat mass	Gynoid fat mass	VAT mass	Subcutaneous fat mass	Total fat mass
Palmitic acid synthesis rate	-0.199 P=0.044	-0.253 P=0.010	0.0148 P=0.88	-0.251 P=0.011	-0.253 P=0.0098

VAT mass: Visceral fat mass

4.4 Discussion

The present results demonstrate that consumption of the HOCODHA-rich diet for 28 days does not change fatty acid synthesis rate compared to control. Data further suggest that consumption of the experimental oils in the context of weight maintaining diets does not modulate body compositions. However, a negative relation was observed between FSR of palmitic acid and regional body fat mass. And a trend existed that consumption of HOCODHA may increase de novo lipogenesis. No changes in body composition between barley β-glucan groups and non-β-glucan groups were found at the end of the dietary phases.

Our finding of no effects of consumption of HOCODHA on body fat composition is not surprising. Piers et al. (2003) failed to see any changes in energy expenditure and substrate oxidation rates after substituted SFA with MUFA for 4 weeks in overweight and obese men. In addition, Liu et al. (2016) also failed to see any changes in android fat after consumption of HOCODHA even there was a reduction

in android fat after the HOCO diet. A randomized controlled trial conducted by Gillingham and colleagues (2012) did not observe changes in body composition, energy expenditure, and substrate oxidation after consumption of HOCO rich diet for 28 days. In addition, Flint et al. (2003) conducted a randomized crossover controlled study in 19 overweight men and observed no changes in energy expenditure after a fat-rich breakfast varying only in 18 carbon length fats. Another randomized controlled clinical trial by Noakes et al. (2006) found similar fat masses after consuming iso-caloric diets either high in fat or high in carbohydrate for 8 weeks. However, Jones et al. (1985) observed a significant increase oxidation of oleic acid higher than LA. Several studies showed an inverse association between a high MUFA diet and central obesity (Tortosa et al., 2007; Paniagua et al., 2007; Damasceno et al., 2013). However, a few human studies have examined the effect of dietary fatty acids varying in fatty acid profile on whole body fat oxidation, which includes fat oxidation and de novo lipogenesis. Despite the previous findings of others, no changes in body composition and de novo lipogenesis were noted in the present study.

A potential explanation for the present results observing no effect of dietary fat on body composition compared with previous studies may be related to differences in the caloric load in the diet and term of the treatment period. Previous studies may have maximized the metabolic response to dietary fat intake by limiting the calorie intake during the treatment period (Baxheinrich et al., 2012; Liu et al., 2016; Babio et al., 2014). The cause of body weight changes observed in the previous studies could be the limited background diets given together with the testing oil

(Baxheinrich et al., 2012; Liu et al., 2016; Babio et al., 2014; Kien, Bunn & Ugrasbul, 2005). In the present study, we tried to control the energy intake by engaging a registered dietician with the participants; still, full control of the background diets could not be assured. Even though we tried to counsel the participants to stay away from excessive fat intake as much as possible, most of the participants were consuming a typical western diet, which is high in calories and SFA. The tested cookies and muffins in total delivered 50g of oil per day which provides high calorie itself on top of the participants' daily background diet. The study conducted by Jones et al. (1985) investigating the oxidation of fatty acid and showed an increase oxidation of oleic acid higher than LA. However, the changes in fatty acid oxidation within the adipose tissue may cause the tissue uptake of fatty acids but may not have modulated fatty acid accumulation when oxidation was sufficient (Kien et al., 2015).

The present study also failed to observe any significant change in body composition after consumption of β-glucan. There are previous studies that have established body fat reductions after consuming β-glucan (Maki et al., 2010; Shimizu et al., 2008; Chang et al., 2013; McMillan-Price et al., 2006). A randomized controlled trial showed significant weight, BMI and visceral fat reduction after consuming 7 g/day of β -glucan for 12 weeks (Shimizu et al., 2008). Another randomized controlled study showed that body weight and BMI were significantly reduced with the consumption of 1.5g/day oat β-glucan for 12 weeks; the body fat percentage also decreased significantly (Chang et al., 2013). Potential reasons for the present study failing to see any changes in the body composition after

consuming the β -glucan diets could be the additional fat added to the daily consumption. In addition, the present study gave only 3.2g of β-glucan for 4 weeks, other studies gave either higher amount of β-glucan per day or a longer feeding time (Shimizu et al., 2008; Chang et al., 2013). As discussed in the previous section, the participants tend to eat more saturated fat in the daily background diet consumption. In the present study we aimed to investigate the synergetic effects combining HOCODHA and β-glucan, so we gave a total of 50g of either HOCODHA or control oil on top of their regular diets, in addition, there was no change in body composition after consuming HOCODHA, thus, our study did not show any changes in body composition after consumption of β-glucan rich diets. Previous studies tried to magnify the effects of β-glucan on body fat reduction by giving limited calorie intake and keeping low-fat diets during the testing period (Shimizu et al., 2008; Chang et al., 2013; McMillan-Price et al., 2006). The health claims that were allowed by various regulatory agencies recommending β-glucan intake (FDA, 2002; EFSA, 2006; Health Canada, 2012) were based on healthy low-fat diets rather than the western diets in the present study.

A possible explanation for why HOCODHA-control flour showed a higher FSR of palmitic acid than control oil-control flour could be due to the low SFA content in the HOCO treatment. The SFA content in HOCODHA oil was much lower than that in control oil and that typically found in participants' regular diets, so the human body would have synthesized more SFA through the endogenous pathway to balance the normal metabolism. Another possible reason could be that n-6 linoleic acids compete with palmitic acid for desaturation (Park et al., 2016). The n-6 content in

control oil was much higher than that in HOCO, so the inhibition of de novo synthesis of palmitic acid in control oil group was higher than that in HOCODHA group.

In summary, the present study demonstrated that in the context of current Western macronutrient intakes, altering the dietary fatty acid composition and adding β-glucan had no major effect on body composition during the 28 d controlled dietary intervention. The long-term effects of central obesity reduction require further research.

4.5 Acknowledgements

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Chapter V

Discussion and conclusion

5.1 Overall discussion and implications

The present study has important implications for dietary management of cardiovascular health. Dietary fatty acid is recognized to play an important role in cardiovascular disease (CVD). Therefore, specific fatty acid profiles including elevated omega 3 index in blood provide additional clinical information beyond plasma lipid levels for detecting population with metabolic syndrome (MetS) (Harris, 2008; He et al., 2002; Harris & Clemens, 2004; Ramprasath, 2013). Elevated plasma and tissue levels of EPA, docosapentaenoic acid (DPA), and DHA are associated with decreased CVD morbidity and mortality (Harris, 2008). The omega-3 index was reported to be a stronger indicator in terms of sudden cardiac death than other risk factors including C-reactive protein, total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerol, and homocysteine levels (Albert et al., 2002; Albert et al., 2002; Harris, 2008). Data from Chapter III demonstrated that after consumption of HOCODHA diets for 28 days, the omega-3 index increased indicating the bioavailability of cardioprotective effects of HOCODHA combination. Reductions in n-6: n-3 PUFA ratio after consumption of HOCODHA also support the fact that HOCODHA reduces the risk factors associated with CVD (Griffin, 2008; Schuchardt et al., 2011). The 2.5 fold increase in plasma EPA composition compared to the control oil groups further add the evidence that HOCODHA could benefit cardiovascular health. Elevated plasma concentrations of EPA were reported to be independently associated with decreased non-fatal cardiovascular endpoints (Mozaffarian & Wu,

2012; Yokoyama et al., 2007). These results are important, further supporting the cardioprotective effects of HOCODHA oils.

The impact of specific fatty acids on body weight balance and body composition are important considering the obesity prevalence in western populations. Previous literature demonstrated that unsaturated fatty acids are more rapidly metabolized for energy and fat oxidation as compared with saturated fatty acids (Jones & Schoeller, 1988; Kien, Bunn & Ugrasbul, 2005). The data presented in Chapter IV showing no effects of HOCODHA on body composition were not surprising. As discussed in Chapter IV, previous studies may have maximized the metabolic response to dietary fat intake by limiting the calorie intake during the treatment period (Baxheinrich et al., 2012; Liu et al., 2016; Babio et al., 2014). However, no change in body weight and body composition was observed after consuming HOCODHA rich diets challenged the evidence that MUFA and n-3 PUFA may help to maintain both energy and weight balance.

β-glucan treatment did not alter body composition in the present study according to the data showed in Chapter IV. As discussed in Chapter IV, previous research tested effects of β-glucan by giving a low fat diet and limiting the calorie intakes (Shimizu et al., 2008; Chang et al., 2013; McMillan-Price et al., 2006). Health claims that were allowed by various regulatory agencies recommending that an intake of a minimum of 3g of β -glucan from barley or oat can actively reduce risk factors for heart disease (FDA, 2002; EFSA, 2006; Health Canada, 2012) was based on a healthy low-fat diet.

The favorable changes in plasma and RBC fatty acid levels support the current United States Food and Drug Administration (US FDA) (2006) qualified health claims for canola oil and n-3 PUFA and health claim allowed by U.S. Food and Drug Administration (2006), European Food Safety Authority (EFSA) (2006) and Health Canada (2012) for CVD risk reduction. Furthermore, the present findings provide valuable information for a novel health claim on portfolio diets in Canada.

5.2 Limitation and future directions

The controlled, randomized and crossover study design employed in the current study is considered to be the "gold standard" for evaluating treatment intervention (AbuMweis, Jew & Ames, 2010). The design with introducing a registered dietician in the study tried to prevent the influence of habitual diet to the targeted participants.

Although the 4-week intervention design was sufficient in length to observe favourable changes in plasma and RBC fatty acid profiles, however, the length of treatment may not have been sufficient to observe a change in body composition. The testing foods add a total of 50 g of oils to the regular background diets which may have provided difficulties to the participants to limit calorie intake to compensate for the energy delivered by the treatment cookies and muffins. It is a challenge to fight against participants' food habit and reduce fat intake in typical western diets.

5.3 Final conclusion

In conclusion, the present study demonstrated that consumption of foods containing HOCODHA in the context of current Western macronutrient intake, altering the dietary fatty acid composition and adding β-glucan, had no major effect on body composition during the 28 day controlled dietary intervention. The consistent shifts among participants in their fatty acid profiles highlight the success of this controlled feeding clinical trial. Significant increases in plasma EPA and DHA levels, as well as the omega-3 index, provide evidence supporting the cardioprotective effects of HOCODHA.

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Appendices

Appendix I: Ethics approval for study

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UNIVERSITY OF MANITOBA | Bannatyne Campus
Research Ethics Board

BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF FINAL APPROVAL FOR AMENDMENTS AND ADDENDUMS

PRINCIPAL INVESTIGATOR: Dr. Peter Jones	INSTITUTION/DEPARTMENT: U of M/Richardson Centre for Functional Foods and Nutrceuticals	ETHICS #: HS 18201 (B2014:029)
BREB MEETING DATE (If applicable):	APPROVAL DATE: June 3, 2015	
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable): N/A		

PROTOCOL NUMBER: Appl#312802; NUT	PROJECT OR PROTOCOL TITLE: Developing and Evaluating Novel Food Supplements, CONFIDENCE (Canola Oil and Fibre with DHA Enhanced) Aiming at the Management of CVD Risk in a Population with Metabolic Syndrome
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: CIHR	

REMINDER: THE CURRENT BREB APPROVAL FOR THIS STUDY EXPIRES: **March 24, 2016**

REVIEW CATEGORY OF AMENDMENT:	Full Board Review ☐	Delegated Review ☒
Submission Date of Investigator Documents: May 26, 2015	BREB receipt date of Documents: June 1, 2015	

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol: Amendment per REB Amendment Form		May 22, 2015
Consent and Assent Form(s): Research Participant Information and Consent Form		26-May-2015
Other:		

CERTIFICATION
The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the amendment to the research study/project named on this ***Certificate of Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. The amendment and documents listed above were granted final approval by the Chair or Acting Chair, UM BREB.

BREB ATTESTATION
The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulation of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

grounds for research involving human participants. The study/project and documents listed above was granted final approval by the Chair or Acting Chair, UM BREB.

BREB ATTESTATION

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QUALITY ASSURANCE

The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONDITIONS OF APPROVAL:

1. The study is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the BREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM BREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Sincerely,

[redacted]

Lindsay Nicolle, MD, FRCPC
Chair, Biomedical Research Ethics Board
Bannatyne Campus

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

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BIOMEDICAL RESEARCH ETHICS BOARD (BREB)
CERTIFICATE OF FINAL APPROVAL FOR NEW STUDIES
Full Board Review

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. P. Jones	UofM / Richardson Centre for Functional Foods and Nutraceuticals	B2014:029
BREB MEETING DATE: March 24, 2014	**APPROVAL DATE:** April 2, 2014	**EXPIRY DATE:** March 24, 2015
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE:
NA	Developing and Evaluating a Novel Supplement Beverage, CONFIDENCE (Canola Oil and Fibre with DHA Enhanced) Aiming at the Management of CVD Risk in a Population with Metabolic Syndrome
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: CIHR	

Submission Date(s) of Investigator Documents:	REB Receipt Date(s) of Documents:
March 3 and April 2, 2014	March 3 and April 7, 2014

THE FOLLOWING ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol:		
Research Proposal received March 3, 2014		
Consent and Assent Form(s):		
Research Participant Information and Consent Form		2 Apr 2014
Additional Research Participant Information and Consent Form For Sample Storage Beyond 5 Years of Study for Genetic Analysis		March 10, 2014
Screening Consent		2 Apr, 2014
Other:		
Poster received April 7, 2014		
General Questionnaire Screening		2 Apr, 2014
Medical Questionnaire Screening		2 Apr, 2014
General Questionnaire		2 Apr, 2014
Medical Questionnaire D1/D29		2 Apr, 2014
Medical Questionnaire D2/D30		2 Apr, 2014
Side Effects Frequency Questionnaire		2 Apr, 2014
Side Effects Intensity Questionnaire		2 Apr, 2014

CERTIFICATION
The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the research study/project named on this ***Certificate of Final Approval*** at the ***full board meeting*** date noted above and was found to be acceptable on ethical

Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

QUALITY ASSURANCE

The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONDITIONS OF APPROVAL:

1. The study is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of annual approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the BREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM BREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Sincerely,

[redacted]

Lindsay Nicolle, MD, FRCPC
Chair, Biomedical Research Ethics Board
Bannatyne Campus

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BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF ANNUAL APPROVAL

PRINCIPAL INVESTIGATOR: Dr. P. Jones	**INSTITUTION/DEPARTMENT:** uofM/Richardson Centre for Functional Foods & Nutrceuticals	**ETHICS #:** B2014:029
BREB MEETING DATE (If applicable):	**APPROVAL DATE:** March 9, 2015	**EXPIRY DATE:** **March 9, 2016**
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER: NA	**PROJECT OR PROTOCOL TITLE:** Developing and Evaluating Novel Food Supplements, CONFIDENCE (Canola Oil and Fibre with DHA Enhanced) Aiming at the Management of CVD Risk in a Population with Metabolic Syndrome formerly Developing and Evaluating a Novel Supplement Beverage, CONFIDENCE (Canola Oil and Fibre with DHA Enhanced) Aiming at the Management of CVD Risk in a Population with Metabolic Syndrome
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: CIHR	

Submission Date of Investigator Documents: February 24, 2015	**BREB Receipt Date of Documents:** February 27, 2015

REVIEW CATEGORY OF ANNUAL REVIEW: **Full Board Review** ☐ **Delegated Review** ☒

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name(if applicable)	Version(if applicable)	Date

Annual approval

*Annual approval implies that the most recent **BREB approved** versions of the protocol, Investigator Brochures, advertisements, letters of initial contact or questionnaires, and recruitment methods, etc. are approved.*

Consent and Assent Form(s):

CERTIFICATION

The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the annual study status report for the research study/project named on this ***Certificate of Annual Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. Annual approval was granted by the Chair or Acting Chair, UM BREB, per the response to the conditions of approval outlined during the initial review (full board or delegated) of the annual study status report.

BREB ATTESTATION

The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in

QUALITY ASSURANCE
The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONDITIONS OF APPROVAL:
1. This amendment is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the BREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM BREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Sincerely,

[redacted]

Lindsay Nicolle, MD, FRCPC
Chair, Biomedical Research Ethics Board
Bannatyne Campus

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

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BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF FINAL APPROVAL FOR AMENDMENTS AND ADDENDUMS

PRINCIPAL INVESTIGATOR:	INSTITUTION/DEPARTMENT:	ETHICS #:
Dr. Peter Jones	U of M/Richardson Centre for Functional Foods and Nutrceuticals	B2014:029

BREB MEETING DATE (If applicable):	APPROVAL DATE:
	March 9, 2015

STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):
N/A

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE:
Appl#312802; NUT	Developing and Evaluating Novel Food Supplements, CONFIDENCE (Canola Oil and Fibre with DHA Enhanced) Aiming at the Management of CVD Risk in a Population with Metabolic Syndrome

SPONSORING AGENCIES AND/OR COORDINATING GROUPS:
CIHR

REMINDER: THE CURRENT BREB APPROVAL FOR THIS STUDY EXPIRES: March 24, 2015

REVIEW CATEGORY OF AMENDMENT:	Full Board Review ☐	Delegated Review ☒
Submission Date of Investigator Documents: February 24, 2015	BREB receipt date of Documents: February 27, 2015	

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol: Protocol		24-Feb-2105
Consent and Assent Form(s):		
Other:		

CERTIFICATION
The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the amendment to the research study/project named on this ***Certificate of Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. The amendment and documents listed above were granted final approval by the Chair or Acting Chair, UM BREB.

BREB ATTESTATION
The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulation of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

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

UNIVERSITY OF MANITOBA | BANNATYNE CAMPUS
Research Ethics Board

P126 - 770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Telephone 204-789-3255
Fax 204-789-3414

BIOMEDICAL RESEARCH ETHICS BOARD (BREB)

CERTIFICATE OF FINAL APPROVAL FOR AMENDMENTS AND ADDENDUMS

PRINCIPAL INVESTIGATOR: Dr. Peter Jones	INSTITUTION/DEPARTMENT: U of M/Richardson Centre for Functional Foods and Nutrceuticals	ETHICS #: B2014:029
BREB MEETING DATE (If applicable):	APPROVAL DATE: February 6, 2015	
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable): N/A		

PROTOCOL NUMBER: Appl#312802; NUT	PROJECT OR PROTOCOL TITLE: Appl#312802; NUT Developing and Evaluating a Novel Food Supplement, CONFIDENCE (Canola Oil and Fibre with DHA Enhanced) Aiming at the Management of CVD Risk in a Population with Metabolic Syndrome - formerly Developing and Evaluating a Novel Supplement Beverage, CONFIDENCE (Canola Oil and Fibre with DHA Enhanced) Aiming at the Management of CVD Risk in a Population with Metabolic Syndrome
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: CIHR	

REMINDER: THE CURRENT BREB APPROVAL FOR THIS STUDY EXPIRES: March 24, 2015

REVIEW CATEGORY OF AMENDMENT:	Full Board Review ☐	Delegated Review ☒
Submission Date of Investigator Documents: January 23, 2015	BREB receipt date of Documents: January 23, 2015	

THE FOLLOWING AMENDMENT(S) and DOCUMENTS ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol: Amendment per submission form		January 23, 2015
Consent and Assent Form(s): Research Participant Information and Consent Form		23-Jan-2015
Other:		

CERTIFICATION
The University of Manitoba (UM) Biomedical Research Board (BREB) has reviewed the amendment to the research study/project named on this ***Certificate of Approval*** as per the category of review listed above and was found to be acceptable on ethical grounds for research involving human participants. The amendment and documents listed above were granted final approval by the Chair or Acting Chair, UM BREB.

BREB ATTESTATION
The University of Manitoba (UM) Biomedical Research Board (BREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulation of Manitoba. In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in

In respect to clinical trials, the BREB complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

QUALITY ASSURANCE
The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

CONDITIONS OF APPROVAL:

1. This amendment is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the REB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the BREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the REB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM BREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Sincerely,

[redacted]

Lindsay Nicolle, MD, FRCPC
Chair, Biomedical Research Ethics Board
Bannatyne Campus

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

Appendix II: Study forms

UNIVERSITY OF MANITOBA | Richardson Centre for Functional Foods and Nutraceuticals

Room 106
196 Innovation Drive
Winnipeg, Manitoba
Canada R3T 2N2
Telephone (204) 474-8883
Fax (204) 474-7552
peter_jones@umanitoba.ca

Screening Consent

I have expressed an interest in participating in the above named study. I have been invited to have my health assessed to determine if I meet the requirements of the study.

Depending on my cholesterol level and other health indicators, and my availability over the next 7 months, I will be offered the opportunity to participate in this study.

To allow the necessary information to be obtained, I agree to provide fasting blood samples (approximately 10 ml or 2 teaspoons) for the measurement of blood cholesterol and other biochemical and hematological tests. I also agree to have a physical assessment done (blood pressure, weight, height, waist and hip measurement etc.), to complete a health history form.

Risks and Discomforts

As with any clinical trial, there may be as yet unknown or unforeseen risks of taking part in the study. 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. Shahrokh Nejad Ghaffar, will be available to contact at any time. Dr. Shahrokh Nejad Ghaffar can be reached at [redacted]

Confidentiality

Information gathered during screning study may be published or presented in public forums, however your name and other identifying information will not be used or revealed. Medical records that contain your identity will be treated as confidential in accordance with the

Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. All study documents related to you will bear only your assigned patient number (or code).

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as: The University of Manitoba Biomedical Research Ethics Board may review research-related records for quality assurance purposes.

All records will be kept in a locked secure area and only those persons identified will have access to these records. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave the Richardson Centre for Functional Foods and Nutraceuticals. With your permission your Family Physician (GP) will be notified about your participation in this study.

Prior to taking part in the study, I shall be given the specific study consent form to read and sign if I am still interested in participating.

I understand I can withdraw from this process at any time at my discretion.

Participant's Signature	**Participant's Name (please print)**
Date	
Investigator's Signature	**Investigator's Name (please print)**
Date	
(or signature of person who conducted consent discussion)	**(position)**

PARTICIPANT INFORMATION AND CONSENT FORM

UNIVERSITY OF MANITOBA | Richardson Centre for Functional Foods and Nutraceuticals

Room 106
196 Innovation Drive
Winnipeg, Manitoba
Canada R3T 2N2
Telephone (204) 474-8883
Fax (204) 474-7552
peter_jones@umanitoba.ca

196 Innovation Drive
University of Manitoba, SmartPark
Winnipeg, Manitoba R3T 6C5
Phone: 204-474-9787

You are being asked for permission to be contacted in the future for participation in research studies. Please take your time to review this consent form and discuss any questions you may have. You are free to discuss this form with your friends, family and others before you make your decision.

If you agree to be contacted in the future for research purposes, information about you will be entered into an electronic database. The database will be maintained by Dr. Peter Jones, (Principal Investigator) and Julia Rempel (Clinical Coordinator) at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN). You are only agreeing to be contacted about participating. For each study you will be given additional information including a consent form specific to that study.

The Database will have the following information about you: name, phone number, mailing address and email address.

Confidentiality of your information will be maintained in the following manner: Only the Principal Investigator and Clinical Coordinators at the RCFFN will have access to the electronic database which is on a password protected computer in a locked office. No outside clinics or institutions will have access to your information. Your contact information will be kept for 10 years. After 10 years your information will destroyed.

This consent form and the information in the database may be inspected by a University of Manitoba Research Ethics Board to ensure that your information is being collected and maintained in an ethical manner.

Your decision to allow your information to be in the database is completely voluntary. While there may be no benefit to you, your information will help researchers to find individuals that may be interested in participating in a research study. If you change your mind after agreeing to this, your information can be removed from the database. You will not be penalized in any way if you refuse to participate, or if change your mind and ask that your information be removed.

If you have any questions about this database, please contact:

Dr. Peter Jones at 204-474-9787 or Julia Rempel at [redacted]

If you no longer want to be part of the research database, please contact:

Julia Rempel at [redacted]

If you have questions about your rights as a research participant, you may contact The University of Manitoba, Bannatyne Campus Research Ethics Board Office at 204-789-3255.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to ask questions and discuss what is involved. I understand that my personal information will be kept confidential. By signing this consent form, I have not waived any of my legal rights.

Participant signature_____Date _____

Participant printed name: _____

I, the undersigned, have fully explained the relevant details of this research to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____ Date _____

Signature: _____

Role: _____

UNIVERSITY OF MANITOBA | Richardson Centre for Functional Foods and Nutraceuticals

Room 106
196 Innovation Drive
Winnipeg, Manitoba
Canada R3T 2N2
Telephone (204) 474-8883
Fax (204) 474-7552
peter_jones@umanitoba.ca

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: Developing and Evaluating Novel Food Supplements, Consisting of Canola Oil, Fibre and DHA, Aiming at the Management of CVD Risk in a Population with Metabolic Syndrome.

Principal Investigator: Peter Jones, PhD

Richardson Centre for Functional Foods and Nutraceuticals

University of Manitoba
196 Innovation Drive,
Winnipeg, Manitoba R3T 2N2
Phone: (204) 474-9787

Co-Investigators: Nancy Ames, PhD

Agriculture and Agri-Food Canada,

196 Innovation Drive

Winnipeg, Manitoba R3T 2N2
Phone: 204-474-7187

Vanu R Ramprasath, PhD

Richardson Centre for Functional Foods and Nutraceuticals

University of Manitoba

196 Innovation Drive,
Winnipeg, Manitoba R3T 2N2
Phone: 204 272 8383

Sijo Joseph, PhD

Agriculture and Agri-Food Canada,

196 Innovation Drive

Winnipeg, Manitoba R3T 2N2
Phone: 204 272 8383

Sponsors: Canadian Institute of Health Research
Room 97, 160 Elgin Steet,
Address locator: 4809A, Ottawa, (Ontario)
K1A 0W9 Tel.: (613) 941-2672

You are being asked to participate in a Clinical Trial (a human research study). Please take your time to review this 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 before you make your decision. 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. The study doctor (and or/ institution) is (are) receiving professional fees and financial support to conduct this study.

Purpose of Study

This Clinical Trial is being conducted to study the effects of consumption of a novel food supplement consisting in Canola Oil, Fibre and Docosahexaenoic acid (DHA), containing the most effective bioactives including n-3 fatty acid enriched dietary oil high in Monounsaturated Fatty Acids (MUFAs) and soluble dietary fibre, aiming at the management of heart disease risk factors and to test its efficacy and safety in humans. You are being asked to take part in this study because you are aged between 18-70 yrs, you have a Body Mass Index (BMI) from 25 to 40 kg/m^2, waist girth for men from 94 cm and women 80 cm and blood triglycerides >1.70 mmol/L. A total of 35 participants will participate in this study.

Study procedures

If you take part in this study, you will have the following tests and procedures: If you agree to take part in this study, as part of a 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. If you are eligible to participate based on your screening result you will begin the trial. Pregnancy tests will be performed for all pre-menopausal female participants at screening visits and at the beginning of each phase, if the test is positive at screening or during the study they will be asked to stop taking study treatment immediately and be withdrawn from the study. 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 4 phases of 4 weeks each during which you will consume morning breakfast prepared at the metabolic kitchen at RCFFN along with your assigned treatment under supervision. Foods for the rest of the day will be on your own and dietary advice will be available through a dietician. The four treatment phases will be interrupted by 4 weeks washout phases during which you follow your habitual diets. We will ask that you limit your consumption of alcohol and caffeinated beverages throughout the phases. A maximum of 2 caffeinated beverages per day and 14 alcoholic beverages per week will be permitted. You are allowed to consume no more than one serving of fish or seafood products per month during the study. Participants will be strongly recommended to maintain consistency in their physical activities during experimental period. A 3-day food record will be collected before and after each treatment phase.

The four treatments are listed as follows:

i) Muffins and cookies made with all purpose flour and containing 50g/day of a blend of high oleic acid canola oil (HOCO) and DHA.
ii) Muffins and cookies made with all purpose flour and containing 50g/day of a blend of sunflower oil, safflower oil and butter as a control oil.
iii) Muffins and cookies made with barley flour and containing 50g/day of a blend of high oleic acid canola oil (HOCO) and DHA.
iv) Muffins and cookies made with barley flour and containing 50g/day of a blend of sunflower oil, safflower oil and butter as a control oil.

Control oil comprised largely of saturated fat with substantial levels of n-6 linoleic acid, common to current North American intakes. Treatments will be incorporated into muffins and cookies and consumed in equal parts at breakfast, snacks and supper. No known risk of short term deficiency or excess in energy, protein, fat or carbohydrate is present with the experimental diets. This study is with a double blinded design which means neither you nor the clinical staff will know which diets you will be receiving. In an emergency, this information will be made available. You will receive all 4 diets.

Description of tests

Overall health status for eligibility: During recruitment, an overall assessment of your health will be done to ensure your eligibility for the study. A first screening visit of 30 minutes will be done to assess your eligibility. During this visit, we will check blood pressure, anthropometric measurements and a blood test. At a second visit, we will review your medical history and the medications you take and we will clearly explain the instructions of the study. These visits will be conducted by a nutritionist, under the supervision of our medical research team.

Resting blood pressure: Blood pressures at rest (following a 10-minute rest) will be taken early in the morning after a 12 hours fasting and will be repeated twice at an interval of three minutes. These measures will be taken at the first screening visit and at the beginning and at the end of each 4-weeks period.

Anthropometric measurement: Measures of your weight, your height (once), your waist and your hip circumference will be done at the screening visit and at the beginning and at the end of each 4-week period. Taking waist and hip circumference is done using a measuring tape and involves no pain. You will be weighed every day of the week during each 4-weeks period to monitor changes in your weight.

Body composition measurement (DXA): Body composition (fat mass and lean mass) will be measured with a procedure called dual energy x-ray absorptiometry (DXA). The examination takes place in a room specially designed for this test. A radiology technician or other person authorized to operate the device will perform the test. The test does not require any special preparation for the subject. You will be asked not to wear anything metal. The procedure takes about 15 minutes and the dose of radiation is very low (0.037 to 0.074 mrem according to the exposure time required to obtain results of high precision). The total exposure in this project with 5 measures of body composition with DXA will be a maximum of 0.185 mrem, which is 54 times less than a dental x-ray exposure which is estimated at 10 mrem. Exposure to cosmic rays during a 6 hours flight from Montreal to Vancouver is estimated at 3 mrem. The exposure in this project level is therefore quite safe.

Stable isotope tracer intake: To assess cholesterol synthesis, you will be asked to consume D_2O (heavy water) at the end of each phase. On day 29, 0.7 g of D_2O /kg estimated body water will be given orally prior to breakfast as a tracer to measure fractional cholesterol synthesis rate over 24 hours. Fasting blood will be obtained on days 29 and 30.

Microbial analysis: In addition, we would also study the influence of these diets on gastrointestinal microbial diversity in the current study population. For this analysis, you will be asked to provide your stool samples (4 or 5 scoops - 4g) at the beginning and end (days 2 and 30) of each phase of the trial. Stool sample collection kits including containers will be provided to collect stool samples. However, it is optional for you to provide the stool sample. It will not affect your study participation even if you do not select the option to provide stool samples.

Blood samples: At the screening visit, fasting blood sample of 2 teaspoons will be collected to check your blood counts, your lipid profile and to ensure the proper functioning of your liver, your kidneys and your thyroid gland. During days 1 and 30 of each phase fasting blood samples approximately 2 tablespoons will be taken on each blood draw day. On days 2 and 29 of each phase fasting blood samples approximately 1 tablespoon of blood will be taken on each blood draw day. The total amount of blood collected for all samples in the study will be about 600 ml (about 2.25 cups) spread over a period of about seven months. Generally a person can give up to 450 ml of blood every 2 months safely. The amount of blood collected in the context of this project is lower than that. However, you must refrain from donating blood during the study and for a

period of two months following your participation in this project. Finally, you should refrain from vigorous physical activity 3 days before each blood sampling.

These blood samples will be obtained for assessment of blood fat levels and fat metabolism. Each blood test will take approximately 5 minutes. For the purpose of subsequent analysis, we will store plasma and serum samples. At the end of the study, your records will be stored in accordance with Health Canada's regulations for 25 years at RCFFN. During the course of the study, processed blood will be stored at the RCFFN in -80ºC freezers, for a maximum of 5 years from the end of the study where possible. At this point the investigators will re-apply for a further extension of storage to accommodate new analysis indicated at that time. In the absence of such an application, all samples will be destroyed by autoclaving. During the storage period if you should change your mind about your samples being stored you have the opportunity to withdraw your consent. Simply call the centre at 204-480-1042 or any of the investigators listed on page 1 of this consent and inform them that you wish to withdraw your consent. The stored blood will be available for re-analysis of samples if values are called into question. In which case we may attempt to contact you by phone or letter requesting that you attend an information session where the new information will be discussed.

Participation in the study will be for 28 weeks. The researcher may decide to take you off this study if it is in the participant's medical best interest, participant's condition worsens, failure to follow the study protocol. You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study staff and your regular doctor first.

There are no serious health consequences of sudden withdrawal from the study for you. Your participation in this research project is entirely on a volunteer basis. You can refuse to participate or you can interrupt your participation anytime, throughout the study period, without any penalty or loss of benefits to which you would otherwise be entitled. Participants will receive a sealed and confidential letter which states their individual results of their blood tests, global physical health and dietary evaluation along with the mean values obtained from the entire study population. The letter will be sent by the principal investigator at RCFFN to the mailing address on the personal information form that participants fill out prior to enrolment to the study.

Risks and Discomforts

As with any clinical trial, there may be as yet unknown or unforeseen risks of taking part in the study. 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. Shahrokh Nejad Ghaffar, will be available to contact at any time. Dr. Shahrokh Nejad Ghaffar can be reached at (204) 805-1811.

Benefits

By participating in this study, you will be providing information to the study doctors that will show the effects of novel beverage supplement on heart health. There may or may not be direct medical benefit to you from participating in this study. We hope the information learned from this study will benefit other participants with increased risk for cardiovascular disease in the future. In addition to the above, you will also receive your results when they become available.

Costs

All clinic and professional fees, diagnostic and laboratory tests which 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. The study cost and honorariums will be covered by Canadian Institutes of Health Research, the study sponsor.

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 4 portions. You will receive $200 after the completion of each phase until the end of phase 3 and $400 after the completion of phase 4. 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.

Confidentiality

Information gathered in this research study may be published or presented in public forums, however your name and other identifying information will not be used or revealed. Medical records that contain your identity will be treated as

confidential in accordance with the Personal Health Information Act of Manitoba. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. All study documents related to you will bear only your assigned patient code number.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as: The University of Manitoba Biomedical Research Ethics Board may review research-related records for quality assurance purposes.

All records will be kept in a locked secure area and only those persons identified will have access to these records. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave the Richardson Centre for Functional Foods and Nutraceuticals. With your permission your Family Physician (GP) will be notified about your participation in this study.

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 not to participate or to withdraw from the study will not affect your other medical care at this site. If your study doctor feels that it is in your best interest to withdraw you from the study, your study doctor will remove you without your consent. We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

If you decide to participate, you will agree to co-operate fully with the study visit schedule, and will follow the study staff's instructions. If you are an employee of University of Manitoba, be sure that your performance evaluation will not be affected by your decision not to participate. Should you wish to withdraw your participation from the study, you must inform the study coordinators so that your file can be officially closed.

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. Shahrokh Nejad Ghaffar at [redacted] 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 or releasing the investigator(s) or the sponsor from their legal and professional responsibilities. If any health abnormalities are identified in the clinical tests conducted during this experiment, Dr. Shahrokh Nejad Ghaffar will be contacted, who will inform you of the results.

Questions

You are free to ask any questions that you may have about your treatment and your rights as a research participant. 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 listed in page 1. For questions about your rights as a research participant, you may contact The University of Manitoba Biomedical Research Ethics Board at (204) 789-3389. Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with Peter Jones and/or his study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statement or implied statements. Any relationship (such as employee, student or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this clinical trial is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of my medical records by the Food and Drug Administration, the Health Protection Branch, government agencies in other countries, and The University of Manitoba Biomedical Research Ethics Board. By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

I agree to being contacted in relation to this study. Yes ☐ No ☐

I agree to my family physician being notified of my participation in this study. Yes ☐ No ☐

I agree to being contacted for future studies at the RCFFN. Yes ☐ No ☐

Participant signature_____Date _____(day/month/year)

Participant printed name: _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____Date _____(day/month/year)

Signature: _____

Role in the study: _____

UNIVERSITY OF MANITOBA

Medical Questionnaire D1/D29

Diet 1 ☐ Diet 2 ☐ Diet 3 ☐ Diet 4 ☐

BLOOD PRESSURE AT REST (AFTER 10 MINUTES OF REST)

ARM :	☐ **LEFT**	☐ **RIGHT**
TYPE OF DEVICE :	☐ **Mercury**	☐ **Automatic**

10 min Systolic BP (mmHg) = _____ Diastolic BP (mmHg) = _____

13 min Systolic BP (mmHg) = _____ Diastolic BP (mmHg) = _____

16 min Systolic BP (mmHg) = _____ Diastolic BP (mmHg) = _____

Avg. Systolic BP (mmHg) = _____ **Avg. Diastolic BP (mmHg)** =_____

Heart rate / min = HR(1):_____ HR(2): _____ HR(3): _____ HR(mean):_____

Side effects questionnaires completed: Yes ☐ **No** ☐

ANTHROPOMETRIC MEASURMENTS

Body weight (kg): _____

Waist girth (cm) : WG(1): _____ WG(2): _____ WG(3): _____

Hip girth (cm) : HG(1): _____ HG(2): _____ HG(3): _____

Completed by: _____

Date of your last periods (1st day) : _____

Not applicable: ☐

BLOOD SAMPLES: CHECKLIST

Did you eat or drink anything except water in the last 12 hours?

☐ **Yes** ☐ **NO**

Have you consumed alcohol or a product containing alcohol in the last 48 hours?

☐ **Yes** ☐ **NO**

In the last 24 hours, have you done intensive exercise?

☐ **Yes** ☐ **NO**

BLOOD SAMPLES: ☐ LEFT ARM ☐ RIGHT ARM

BLOOD SAMPLES: ☐ **Yes** ☐ **NO**

EXTRA BLOOD SAMPLE (Keep frozen) ☐ **Yes** ☐ **NO**

Nurse's initials: _____

For visit D1 of diet 2, diet 3, diet 4:

Washout duration before this diet: _____ days

☐ **Not applicable (D1 of the first diet)**

☐ **Not applicable (visit D27)**

COMMENTS

_____
_____
_____
_____

UNIVERSITY OF MANITOBA

WEEK	DATE OF OBSERVATION			# SUBJECT(CODE)	
	DAY	MONTH	YEAR		

Medical Questionnaire D2/D30

Diet 1 ☐ Diet 2 ☐ Diet 3 ☐ Diet 4 ☐

ANTHROPOMETRIC MEASURMENTS

Body weight (kg): _____

BLOOD SAMPLES: CHECKLIST

Did you eat or drink anything except water in the last 12 hours?

☐ **Yes** ☐ **NO**

Have you consumed alcohol or a product containing alcohol in the last 48 hours?

☐ **Yes** ☐ **NO**

In the last 24 hours, have you done intensive exercise?

☐ **Yes** ☐ **NO**

BLOOD SAMPLES: ☐ LEFT ARM ☐ RIGHT ARM

BLOOD SAMPLES: ☐ **Yes** ☐ **NO**

EXTRA BLOOD SAMPLE (Keep frozen) ☐ **Yes** ☐ **NO**

Nurse's initials: _____

For visit D30 of all diets:

Number of days on the diet: _____ days ☐ **Not applicable (visit D2)**

3-Day food records collected ☐ **Yes** ☐ **NO**

Fecal samples collected ☐ **Yes** ☐ **NO**

FISH INTAKE

Did you consume one serving of fish or seafood products during the last month?

☐ **Yes** ☐ **NO**

If yes;

7.2 When? _____

7.3 What kind? _____ 7.4 Amount: _____

CHANGE IN MEDICATION **Yes** ❑ **No** ❑

Name of drug	Dose	Freq.	Indication	Start	Stop
_____	_____	_____	_____	___/___/____	___/___/___
_____	_____	_____	_____	___/___/____	___/___/___

CHANGE IN NATURAL HEALTH PRODUCTS Yes ❑ No ❑

Name of drug	Dose	Freq.	Indication	Start	Stop
_____	_____	_____	_____	___/___/____	___/___/___
_____	_____	_____	_____	___/___/____	___/___/___
_____	_____	_____	_____	___/___/____	___/___/___
_____	_____	_____	_____	___/___/____	___/___/___

PHYSICAL ACTIVITY

During the last month, has your level of physical activity changed?

- ❑ **Yes, my level increased**
- ❑ **Yes, my level decreased**
- ❑ **Yes, I didn't exercise**
- ❑ **No**

COMMENTS

____

UNIVERSITY OF MANITOBA

WEEK	DATE OF OBSERVATION			# SUBJECT(CODE)	
	DAY	MONTH	YEAR		

Medical Questionnaire
SCREENING S1

Sex : ☐ M ☐ F

Date of birth (dd/mm/yyyy): _____Age: _____

BLOOD PRESSURE AT REST (AFTER 10 MINUTES OF REST)

ARM : ☐ **LEFT** ☐ **RIGHT**

TYPE OF DEVICE : ☐ **Mercury** ☐ **Automatic**

10 min Systolic BP (mmHg) = _____ Diastolic BP (mmHg) = _____

13 min Systolic BP (mmHg) = _____ Diastolic BP (mmHg) = _____

16 min Systolic BP (mmHg) = _____ Diastolic BP (mmHg) = _____

Avg. Systolic BP (mmHg) = _____ **Avg. Diastolic BP (mmHg)** =_____

Heart rate / min = HR(1):_____ HR(2): _____ HR(3): _____ HR(mean):_____

ANTHROPOMETRIC MEASURMENTS

Body weight (kg): _____ Height (m): _____ BMI (kg/m^2):_____

Waist girth (cm) : WG(1): _____ WG(2): _____ WG(3): _____

Hip girth (cm) : HG(1): _____ HG(2): _____ HG(3): _____

Completed by: _____

ETHNICITY

- ❑ Caucasian (eg North America, Europe, Middle East, North Africa, etc.)
- ❑ African and Afro-American (eg Jamaica, Caribbean, Niger, Haiti, etc.)
- ❑ Fist Nations
- ❑ Metis
- ❑ Inuit (eg Eskimo)
- ❑ Asian (eg Far East, Southeast Asia, Cambodia, China, etc.)
- ❑ Hispanic (eg Mexico, Cuba, Dominican Republic, etc.)
- ❑ Other: _____

WEEK	DATE OF OBSERVATION	# SUBJECT(CODE)

BLOOD SAMPLES: CHECKLIST

Did you eat or drink anything except water in the last 12 hours?

☐ **Yes** ☐ **NO**

Have you consumed alcohol or a product containing alcohol in the last 48 hours?

☐ **Yes** ☐ **NO**

In the last 24 hours, have you done intensive exercise?

☐ **Yes** ☐ **NO**

BLOOD SAMPLES: ☐ LEFT ARM ☐ RIGHT ARM

BLOOD SAMPLES: ☐ **Yes** ☐ **NO**

EXTRA BLOOD SAMPLE (Keep frozen) ☐ **Yes** ☐ **NO**

Nurse's initials: _____

Comments:

_____

_____

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	DAY	MONTH	YEAR		

Medical Questionnaire/Life habits
SCREENING S2

Sex : ☐ M ☐ F

MEDICAL HSITORY

Family history	YES	NO	Don't Know	If Yes, specify
GP=grand				F=father, M=mother, S=siblings, parents, U=unknown (ex : adopted)
Type 1 Diabetes	❑	❑	❑	_____
Type 2 Diabetes	❑	❑	❑	_____
Cardiovascular diseases	❑	❑	❑	_____
(Class 1)** Cardiovascular diseases	❑	❑	❑	_____
(Class 2)*** Hypertension	❑	❑	❑	_____
Dyslipidemia	❑	❑	❑	_____
Endocrine disorders	❑	❑	❑	_____
Cancers	❑	❑	❑	_____

Others	❑	❑	❑	_____

Personal history (dx) *	**YES**	**NO**	**Don't Know**	**If Yes, specify**	**Age**
Type 1 Diabetes	❑	❑	❑	_____	
Type 2 Diabetes	❑	❑	❑	_____	
Cardiovascular diseases (Class 1)**	❑	❑	❑	_____	
Cardiovascular diseases (Class 2)***	❑	❑	❑	_____	
Hypertension	❑	❑	❑	_____	
Dyslipidemia	❑	❑	❑	_____	
Endocrine disorders	❑	❑	❑	_____	
Gastrointestinal disorders	❑	❑	❑	_____	
Hepatic diseases	❑	❑	❑	_____	
Cancer	❑	❑	❑	_____	
Surgeries (past or future)	❑	❑	❑	_____	
Other(s)	❑	❑	❑	_____	
Kidney diseases	❑	❑	❑	_____	

* *Age at diagnosis*

** *Class 1: heart attack, **Ischemic cardiomyopathy**, angina, "bypass"…*

*** *Class 2: stroke, aneurysm, thrombophlebitis, cerebral hemorrhage, peripheral vascular disease …*

MEDICATION: **Yes ❑** **No ❑**

Name of drug	Dose	Freq.	Indication	Start	Stop	*
_____	_____	_____	_____	___/___/____	__/___/___	❑
_____	_____	_____	_____	___/___/___	___/___/___	❑
_____	_____	_____	_____	___/___/___	___/___/___	❑
_____	_____	_____	_____	___/___/___	___/___/___	❑
_____	_____	_____	_____	___/___/___	___/___/___	❑
_____	_____	_____	_____	___/___/___	___/___/___	❑

***** *Check this box if the participant has not stopped using the drug.*

Drug Allergies: Yes ❑ No ❑

If yes, specify: _____

Natural health products

Do you consume dietary supplements, vitamins and / or minerals, homeopathic remedies and other natural products (probiotics, medicinal plants, omega-3 ...):

Yes ❑

No ❑

Would you be willing to stop taking these supplements at least 6 weeks before the beginning of the study? **Yes ❑** **No ❑** **Not applicable ❑**

Name of product	Dose	Freq.	Indication	Start	Stop	*
_____	_____	_____	_____	___/___/___	___/___/___	❑

_____ _____ _____ _____ ___/___/___ ___/___/___❑

_____ _____ _____ _____ ___/___/___ ___/___/___❑

_____ _____ _____ _____ ___/___/___ ___/___/___❑

_____ _____ _____ _____ ___/___/___ ___/___/___❑

_____ _____ _____ _____ ___/___/___ ___/___/___❑

_____ _____ _____ _____ ___/___/___ ___/___/___❑

_____ _____ _____ _____ ___/___/___ ___/___/___❑

***** *Check this box if the participant has not stopped using the natural health product*

WOMEN ONLY (PREGNACY-GYNECOLOGY)

Are you postmenopausal? Yes ❑ No ❑

Premenopausal women

Are you currently taking oral contraceptives or other forms of hormonal contraception?
Yes ❑ No ❑

Do you have a regular menstrual cycle? Yes ❑ No ❑ Not applicable ❑

Average length of your menstrual cycle: _____
Date of your last periods (1st day) : _____
Next expected menstruation date: _____

Are you pregnant? Yes ❑ No ❑

Are you planning a pregnancy soon? Yes ❑ No ❑

Postmenopausal women

Age at menopause: _____ Yrs Don't know ❑

Type of menopause:
Natural ❑ *Hysterectomy* ❑ *Hysterectomy + partial oophorectomy (1 ovary)* ❑
Hysterectomy + total oophorectomy (2 ovaries) ❑ *Tubal ligation* ❑
Caused by chemotherapy or other treatment ❑ *Don't know* ❑

Are you currently taking hormone replacement? Yes ❑ No ❑
If yes, start date: ___/___/___
If yes, type of hormones: _____

Have you ever taken hormone replacement in the past? Yes ❑ No ❑
If yes, stop date : ___/___/___
If yes, type of hormones: _____

LIFE HABITS

Alcohol **Yes ❑ No ❑**

_____

_____

Type(s) of alcohol:

Quantity consumed:

How often : _____

Tobacco **Yes ❑ No ❑**

_____

_____

Date stopped (if any) :

If yes, how often:

Street drug **Yes ❑ No ❑**

_____

_____

Date stopped (if any):

If yes, how often:

NUTRITION / EATING HABITS

	YES	NO	Don't know	If yes, specify
Allergies:				
Foods	❑	❑	❑	_____
Others	❑	❑	❑	_____
Intolerances	❑	❑	❑	_____
Special dietary habits (Ex: vegetarianism)	❑	❑		_____

Consumption of:				
Soft drinks	❑	❑	❑	_____
Energy drinks	❑	❑	❑	_____
Coffee	❑	❑	❑	_____
Tea	❑	❑	❑	_____

Number of meal /day : _____

Number of snack (s)/day: _____

PHYSICAL ACTIVITY

Type	*Frequency*	*Duration*
_____	_____ _____	
_____	_____ _____	
_____	_____ _____	
_____	_____ _____	

COMMENTS

_____

_____

_____

_____

_____

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DATE OF OBSERVATION			# SUBJECT(CODE)	
DAY	MONTH	YEAR		

QUESTIONNAIRE ON SIDE EFFECTS-FREQUENCY

Diet 1 ☐ Diet 2 ☐ Diet 3 ☐ Diet 4 ☐

Day 1 ☐ **Day 28** ☐

Indicate if you experienced side effects listed below over the **last four weeks** and if so, how hard was the intensity.

Side effects	**Frequency**			
	Never[0]	Rarely[1]	Sometimes[2]	Often[3]
1. Headache	☐	☐	☐	☐
2. Anxiety	☐	☐	☐	☐
3. Fatigue / exhaustion	☐	☐	☐	☐
4. Lack of energy	☐	☐	☐	☐
5. Tend to become exhausted quickly	☐	☐	☐	☐
6. Decreased appetite	☐	☐	☐	☐
7. Increased appetite	☐	☐	☐	☐
8. Hiccup	☐	☐	☐	☐
9. Nausea	☐	☐	☐	☐
10. Vomiting	☐	☐	☐	☐
11. Indigestion	☐	☐	☐	☐
12. Stomach or abdominal pain	☐	☐	☐	☐
13. Constipation	☐	☐	☐	☐
14. Diarrhea	☐	☐	☐	☐
15. Flatulence	☐	☐	☐	☐
16. Abdominal bloating	☐	☐	☐	☐
17. Palpitations	☐	☐	☐	☐
18. Balance disorders	☐	☐	☐	☐
19. Decreased ability to concentrate	☐	☐	☐	☐
20. Flushing	☐	☐	☐	☐
	☐	☐	☐	☐

21. Feeling cold				
22. Joint or members pain	☐	☐	☐	☐
23. Numbness, burning or itching (feet/hands)	☐	☐	☐	☐
24. Dark or depressing thoughts	☐	☐	☐	☐
25. Other	☐	☐	☐	☐

UNIVERSITY OF MANITOBA

DATE OF OBSERVATION			# SUBJECT(CODE)	
DAY	MONTH	YEAR		

QUESTIONNAIRE ON SIDE EFFECTS-INTENSITY

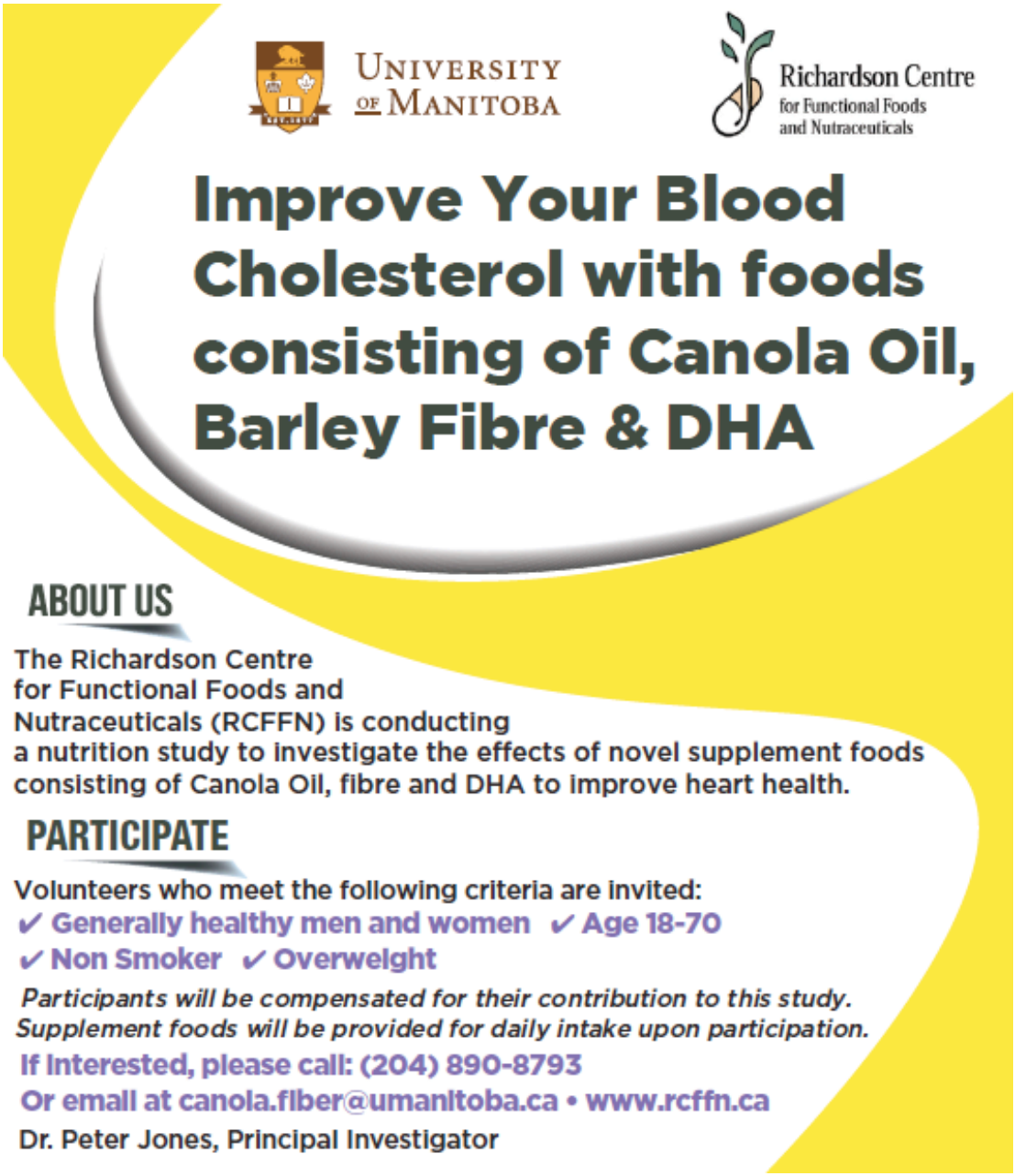
Diet 1 ☐ Diet 2 ☐ Diet 3 ☐ Diet 4 ☐

Day 1 ☐ **Day 28** ☐

Indicate if you experienced side effects listed below over the **last four weeks** and if so, how hard was the intensity.

Side effects	**Intensity**			
	None[0]	Mild[1]	Moderate[2]	Severe[3]
26. Headache	☐	☐	☐	☐
27. Anxiety	☐	☐	☐	☐
28. Fatigue / exhaustion	☐	☐	☐	☐
29. Lack of energy	☐	☐	☐	☐
30. Tend to become exhausted quickly	☐	☐	☐	☐
31. Decreased appetite	☐	☐	☐	☐
32. Increased appetite	☐	☐	☐	☐
33. Hiccup	☐	☐	☐	☐
34. Nausea	☐	☐	☐	☐
35. Vomiting	☐	☐	☐	☐
36. Indigestion	☐	☐	☐	☐
37. Stomach or abdominal pain	☐	☐	☐	☐
38. Constipation	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐

39. Diarrhea				
40. Flatulence				
41. Abdominal bloating				
42. Palpitations	☐	☐	☐	☐
43. Balance disorders	☐	☐	☐	☐
44. Decreased ability to concentrate	☐	☐	☐	☐
45. Flushing	☐	☐	☐	☐
46. Feeling cold	☐	☐	☐	☐
47. Joint or members pain	☐	☐	☐	☐
48. Numbness, burning or itching (feet/hands)	☐	☐	☐	☐
49. Dark or depressing thoughts	☐	☐	☐	☐
50. Other	☐	☐	☐	☐



Appendix III. Standard operation procedure for fatty acid extractions

SOP Name/Title: **Standard Operating Procedure for One-Step plasma / RBC Fatty Acid Methylation in Dr. Jones Lab**			
Document I.D.:	**SOP Version #:**	**Effective Date:**	**Next Review Date:**
Prepared By (Name): Shuo Yang		Signature:	*Date Prepared:*
Reviewed By (Name): Stephanie Jew		Signature:	*Date Reviewed:*
Approved By (Name):		Signature:	*Date Approved:*

Purpose:

All staff and students who are associated with analysis or system operating under RCFFN will be evaluated based on the standard operating procedures. This evaluation will qualify the operating procedure to ensure the precision of the analysis at RCFFN.

Scope:

This procedure covers all chemicals, systems, equipment used under RCFFN for one step fatty acid extraction.

Responsibilities:

All personnel working in the lab for fatty acid extraction at RCFFN need to follow the SOP.

Definitions:

Safety, Environmental and Precautionary Measures:

- Lab coat, gloves, safety goggles
- Fume hood

Materials and Equipment:

1) 200 µL, 400 µL, 10 mL pipettes
2) Centrifuge
3) Incubator
4) Nitrogen flush tank
5) 15 mL test tubes
6) Chloroform (2 mL per sample)
7) Methanol (6 mL per sample)

8) Toluene (400 µL per sample)

9) Acetyl chloride (200 µL per sample)

10) K2CO3 : 6% (5 mL per sample)

Procedure:

1. Preparation

1.1 Test tube preparation

1) Rinse 15 mL test tubes and caps with 2 mL chloroform, wrap Teflon tape around the top of the test tube 3-5 turns
2) Label each tube properly on the top area of the tube, then tape the labeling area
3) Let dry for 20 mins
4) Pre-heat the incubator dry bath to 80^{o}C

1.2 Sample preparation

1) Take plasma/ RBC samples out of -80^{o} C freezer
2) Thaw completely
3) Vortex the samples before use

1.3 Internal standard preparation (75uL per sample, prepare in small batch as the solvent evaporates)

1) Weigh 5 mg C17:00 (Heptadecanoic acid) in a beaker
2) Pipette 5 mL chloroform into the beaker
3) Stir until completely dissolved
4) Transfer the C17:00 chloroform solution to 5 test tubes (1 mL each), mark the level of solution on the tube, cap tightly
5) Fully cover tubes with aluminum foil and store in a dark cool area

* If solvent evaporated, dry the tube with nitrogen and add 1 mL chloroform/hexane into the tube

2. Protocol

2.1 Extraction

- Cover bench with absorbent paper.
- Pipette 0.2-0.25 g of blood samples (either plasma or RBC) into a prepared test tube (test tubes should be prepared as per step 1.1 of this SOP before use) and record volume/ weight, try reach the bottom of the tube without touching the tube wall to leave any residue
- Transfer each chemical into proper labeled beaker with volume needed for one set of samples, discard the left over into waste bottle
- Ensure pipette tips do not touch the tube wall, if pipette tips touch the tube wall, the tip should be changed before any further extraction
- Vortex the tube while adding 200 uL acetyl chloride, add slowly to protect sample overheat and spit (hold the pipette vertically to avoid partially overheat)

2.2 Incubation

2.2.1 Flush tube with nitrogen

1) Turn on the nitrogen, wash flush needles with a tube filled with chloroform for 10 seconds before use
2) Pull down the needle, make sure needles stay at least 2 cm away from the top of the sample or touch the tube
3) Cap immediately after flushing
4) Turn off all the needles and nitrogen tank after use
5) Place tubes in to dry bath for 1 hour, use the proper size of the holders to ensure adequate heating temperature, set the timer for 1 hour

2.2.2 Further preparation

1) While waiting, prepare potassium carbonate (K_2CO_3)
   - Weigh 6g K_2CO_3, add into volumetric flask
   - Add 100 mL DD water
   - Put into sonic mixer for 2 minutes
2) Label GC vials with ID, date, and sample source
3) Prepare Pasteur pipets for next step

2.3 Transfer

1) Transfer top layer from the extracting tube into a GC vial using Pasteur pipets

   * Ensure nothing from aqueous layer are taken

2) Freeze the vials in -80^{o}C freezer for GC analysis; analyze samples within 2 weeks after extraction.

2.4 Cleaning

- Discard residue in the tube to an appropriate biological waste container
- Let tubes sit in the fume hood for 10 mins to evaporate organic solvent
- Soak the tubes with detergent and bleach (1:1 water : bleach), make sure the tube completely immersed

3. Examination and Results

3.1 Gas chromatograph flame ionization detector (GC FID) operations

1) Set up sequence
   - Define the right method for individual's analysis
   - Label properly for each sample
   - Fill in proper run ID
   - Compare with standard to determine the correct retention time
   - Add overnight method at the end of the process to ensure proper wash of the column
2) Determine the correct injection volume according to the sample's concentration of fat
3) Check the gas tank before running the machine
4) Place the sample in to correct position; **Never** spin the sample tray

3.2 GC FID maintenance

1) File in the excel sheet on the computer, record the personnel, date and sample quantity of each operation to ensure the proper maintenance
2) Change the liner and septum every 100 run cycles
3) Discard the waste vial timely
4) Exchange the toluene and hexane vial timely to ensure efficient wash after each run cycle
5) Develop a new retention time list after each time changing the column

3.3 Data collection

1) Integrate the chromatograph according to standard
2) Reprocess
3) Delete the internal standard from the exported file
4) Recalculate the area percentage

4. Quality Control

4.1 Use timer while vortexing to ensure adequate time

4.2 *** All steps** have to be operated in the fume hood

Test Limitations and Troubleshooting:

1) Extraction protocol applied to only blood samples, not for food mixture fatty acids analysis.

2) Report to the facility manager when issues happened.

References/Related Documents:

A. Related documents

Manuals
Protocol
etc.

B. References

C. Attachments

<u>One-step Fatty Acid Methylation method</u>

<u>Chemicals</u>

- Internal standard (C17:00 1mg/ml in chloroform)
- Methanol
- Toluene
- Acetyl chloride

- K_2CO_3 : 6%

Procedure

- Weigh 0.2 -0.25 of RBC in a glass tube (0.05g if oil).
- Add 1.6 ml methanol and vortex for 20s.
- Add 75ul of internal standard and vortex for 5s.
- Add 400ul of toluene and vortex for 20s.
- While vortexing, slowly add 200ul of acetyl chloride in the tube.
- Flush the tube under nitrogen for 10s and cap tightly.
- Vortex tubes for 10s.
- Heat tubes in dry bath at 80^{o}C for 1 hour.
- Let cool.
- Add 5ml K_2CO_3 6% and vortex for 20s.
- Centrifuge tubes at 2500 rpm for 5 min.
- Transfer top layer in a GC vial with insert. Cap to seal for GC analysis.

End of Document