

**EFFECTS OF “FOODS FOR HEALTH” PORTFOLIO OF FUNCTIONAL FOOD
PRODUCTS ON LIPID MANAGEMENT IN INDIVIDUALS UNWILLING TO USE
STATIN THERAPY**

by

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A thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

DEPARTMENT OF FOOD AND HUMAN NUTRITIONAL SCIENCES

University of Manitoba

Winnipeg

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ABSTRACT

The objective of this study was to examine the effect of a range of proprietary products containing a portfolio of bioactives (5 g fiber, 1000 mg alpha-linolenic acid, 1000 mg phytosterols and 1800 μ mol antioxidants per serving), on cholesterol levels, in statin reluctant participants. A dual-center, randomized, double-blind, free-living cross-over study composed of 2 phases of 4 weeks each, separated by a 4 week washout, was conducted. Participants (n=54) consumed two servings per day from an assortment of products including oatmeal, pancakes, bars, sprinkles, and smoothies. Control products were market drawn like-items. Lipid profile, glucose, insulin and high sensitivity C-reactive protein concentrations were analyzed at baseline and endpoint of both phases. After consumption of study foods, in comparison to control foods, there were significant reductions in total cholesterol ($5.08 \pm 8.24\%$ or -0.28 ± 0.47 mmol/l) and LDL-C levels ($8.80 \pm 12.5\%$ or -0.29 ± 0.39 mmol/l) observed at endpoint. There were no significant changes in any other outcome.

ACKNOWLEDGEMENTS

I am deeply grateful to my supervisor Dr. Peter Jones for his professional guidance and support; I am very indebted to your patience and invaluable advice that inspired me to see things positively and felt honored with your confidence and trust on my ability.

I would like to thank my advisor committee Dr. James House, Dr. Michael Aliani and Dr. Todd Duhamel for their guidance through this process; your discussion, ideas, and feedback have been invaluable.

I thank Dr. Vanu Ramprasath who inspired me to start up the clinical phase of the research saying, “If you wait until you’re ready, you’ll never get started.”

Thanks to the clinical team and support staff. Special thanks to Stephanie Jew, for going far beyond the call of duty, assisting throughout my research.

I am grateful to Dr. Steven Kopecky and Jessica Bauman for the wonderful opportunity to collaborate. Thanks to Dr. Elizabeth Klodas for the encouragement and support.

I also acknowledge the funding support from Manitoba Agri-Health Research Network Inc., Step One Foods and the international graduate student entrance scholarship from the University of Manitoba.

I would like to thank my fellow graduate students and everyone who contributed to this research. I am very grateful to all of you.

Finally, but by no means least, thanks go to my family for almost unbelievable support. This journey would not have been possible with you all.

DEDICATION

I humbly dedicate this work to

Those Mighty Hands

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LIST OF ABBREVIATIONS

ACAT- 2 -Acyl-coenzyme A cholesterolacyltransferase-2

AHA - American Heart Association

ALA - alpha-linolenic acid

APOE - Apolipoprotein E

BMI – Body mass index

CVD - Cardiovascular disease

CYP7A1 - Cholesterol 7 α -hydroxylase

DHA - Docosahexaenoic acid

EFSA - European Food Safety Authority

EPA - Eicosapentaenoic acid

FDA - Food and Drug Administration

HDL-C - High-density lipoprotein cholesterol

HMG Co-A reductase - 3-Hydroxy-3-methyl-glutaryl-coenzyme A reductase

hsCRP -High sensitivity C-reactive protein

LDL-C - Low-density lipoprotein cholesterol

LXR - Liver X receptors

MTP - Microsomal triglyceride transfer protein

NCEP - National Cholesterol Education Program

PUFA - Polyunsaturated fatty acids

SNP - Single-nucleotide polymorphisms

TC - Total cholesterol

TG - Triglyceride

TICE - Trans-intestinal cholesterol excretion

VLDL - Very Low-density lipoprotein

1.BACKGROUND AND INTRODUCTION

1.1 INTRODUCTION

Cardiovascular diseases (CVDs), the major avertible cause of mortality in the world, are to blame for 17.3 million deaths per year according to the health statistics report 2015 by the American Heart Association (AHA).^{1,2} The improvement of cardiovascular health is, thus, a global necessity and the AHA points out ‘control of cholesterol’ as one of the seven key health factors to move towards this goal.³ The underlying disease process of CVD is mainly ‘atherosclerosis’, which finds its path through behavioral risk factors including less physical activity and unhealthy diets rich in fat and calories. The metabolic risk factors such as elevated blood lipids and hypertension aggravate the problem leading to cardiovascular disease conditions.² Dietary solutions can play a potential role in modifying lipid profile and, thus, ameliorate CVD risk factors.^{4,5}

1.2 ROLE OF FUNCTIONAL FOODS IN HYPERLIPIDEMIA MANAGEMENT

Dyslipidemia, characterized by alterations in serum lipoprotein levels, is one of the major culprits of atherosclerosis and chronic heart disease.⁶ This is supported by the fact that a 1mmol/l (38.6 mg/dL) decrease in the low-density lipoprotein cholesterol (LDL-C) level decreases the rate of total mortality by 12% and coronary events by 20%, regardless of the risk at baseline.⁷

Statins constitute a major class of cholesterol-lowering medications that act by inhibiting HMG Co-A reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase), the rate-controlling enzyme of cholesterol synthesis in the body^{8,9}. Efficacy of statins is demonstrated through

excellent supportive data, placing them as a first-line drug of choice for patients with mixed hyperlipidemia, hypercholesterolemia and a history of CVD.⁷ However, 10-30 % of patients treated with statins suffer from muscle-related side effects in a dose-dependent manner.¹⁰ These side effects range from myalgia to myositis and muscle rhabdomyolysis, ultimately causing renal failure and death. Studies also reveal that statins can increase the risk of diabetes mellitus (10–12 %). Here, side effects result in statin intolerance in certain populations creating hindrance against the preventive goal targets for CVD.⁷

A conveniently formulated functional food combination aimed at reducing LDL-C can be utilized especially by four categories of individuals who are at risk of developing CVD, including individuals who are statin intolerant, individuals who are reluctant to take statin due to other reasons, individuals who are unable to achieve LDL-C targets at maximum tolerated dose of statin, and individuals who are unable to respond to statins.^{11,12}

The well studied functional food ingredients; phytosterol, fiber, omega -3 fatty acids and antioxidants, could play a key role in creating a diet conducive to heart health that could be effectively utilized by this population. These functional food ingredients have been extensively documented in the literature for their beneficial role in cholesterol levels individually, and are discussed in the literature review section.^{6,12} There are only a limited number of studies looking into combinatory hypocholesterolemic effects of the nutritional bioactives outlined in the following literature review section.¹³

A marked inter-individual variability in cholesterol response, linked to heterogeneity of metabolic pathways, has been evidenced by observational and dietary intervention studies.^{14–16} Two single-nucleotide polymorphisms (SNPs) in genes coding for important enzymes within

pathways of cholesterol metabolism have been repeatedly found to explain the variability in response to phytosterol and or fiber consumption, which are also discussed in the following literature review section.^{17,18}

2. LITERATURE REVIEW

2.1 PHYTOSTEROLS

Phytosterols refer to plant-derived sterols and stanols compounds that are structurally similar to cholesterol. Beta-sitosterol, campesterol, and stigmasterol represent the main sub-classes of the plant-sterol family whereas the saturated forms; sitostanol, campestanol and stigmastanol, represent the stanol group.^{19,20}

Phytosterols and/or stanols are well recognized cholesterol-lowering agents that facilitate primary and secondary prevention of cardiovascular diseases.²¹ This is underlined by the fact that various national and international guidelines including The American Heart Association²² and the European Atherosclerosis Society consider phytosterols as an effective treatment option for lowering cholesterol.²³ According to these guidelines, an addition of 2 g/d phytosterols as part of a healthy diet decreases CVD risk in subjects with elevated LDL-C concentrations. Up to a 10% reduction in LDL-C can be accomplished through this approach.²⁴

The mechanisms by which phytosterols and/or stanols exert their cholesterol-lowering effects are due to their similarity to cholesterol in structure and metabolic pathway. Dietary cholesterol undergoes cleavage from esterified into free form and is solubilized in the lumen by the action of bile acids; incorporated into mixed micelles, then penetrates across the mucosal barriers including the aqueous diffusion layer and the striated border to enter into enterocytes through the Niemann-Pick C1-like transporter. After absorption into the enterocyte, intestinal enzyme, acyl-coenzyme A cholesterolacyltransferase-2 (ACAT-2) re-esterifies cholesterol. Microsomal triglyceride transfer protein (MTP) then incorporates these cholesteryl esters into chylomicrons which are then secreted into the lymph.

Phytosterols exhibit competitive inhibition in this pathway, primarily by displacing cholesterol from mixed micelles. Unlike cholesterol, after absorption into enterocytes, phytosterols remain in free form, being poor substrates for ACAT-2. This interference in cholesterol absorption results in excretion of unabsorbed cholesterol through faeces.^{11,25}

Competition for incorporation into chylomicrons, competition of the involved transporters, and activation of liver X receptors (LXR) target genes are the other suggested theories explaining the mechanism of action of phytosterols. Recently a trans-intestinal cholesterol excretion (TICE) pathway theory has also been postulated as a rationale for the cholesterol reducing action of phytosterols. Stimulation of TICE accelerates faecal sterol loss.²⁴

2.2 FIBER

Dietary fibers are broad group of functionally active carbohydrate polymers and include naturally occurring edible as well as extracted and synthetic carbohydrate polymers.⁶

Consumption of dietary fibers found in oats, barley, and psyllium can reduce LDL-C and total cholesterol (TC) levels at statistically significant levels, as has been scientifically substantiated by epidemiologic studies, randomized clinical trials, and subsequent meta-analyses. Most studies could not establish any advantageous effect of fiber consumption on high-density lipoprotein cholesterol (HDL-C) or triglyceride (TG) levels.^{12,26,27}

Dietary fiber, in general, is classified into two categories i.e. water-insoluble or less fermented fibers; cellulose, hemicellulose, lignin and the water-soluble or well-fermented fibers; pectin, beta glucan, and mucilage.

Water-soluble fibers are important in imparting a hypocholesterolemic effect as evident through clinical studies and which are in turn supported by the proposed mechanisms by which fiber lowers cholesterol. A major proposed mechanism is that water-soluble fibers interfere with the absorption of dietary and bile acid derived cholesterol, enhancing its excretion through feces. This might be through creating a barrier on the intestinal cell surface hindering cholesterol absorption, entrapment of cholesterol in its own local matrix, and or directly binding to cholesterol molecules. In addition, soluble fiber absorbs water in the gastrointestinal tract resulting in increased bulk of stools and decreasing the intestinal transit time. These actions can have advantageous effects on satiety by sustaining a 'fullness' feeling. Another proposed theory is that soluble fibers hinder the absorption of glucose and nutrients which will, in turn, result in a low glycemic response and thus, a decline in insulin levels. The hormone insulin has a significant role in the synthesis of hepatic cholesterol through activation of HMG-Co A reductase enzyme. Thus, a low insulin level can slow down hepatic cholesterol synthesis. An additional theory is based on colonic fermentation of soluble fiber resulting in short chain fatty acids, especially propionate, that can hinder hepatic cholesterol metabolism through various pathways. The latter two mechanisms are less supported as a dominant mechanism of fiber action. On the other hand, the only effect water-insoluble fiber can exert in this scenario is that it substitutes for foods containing saturated fats and cholesterol.^{28,29} Although there are studies showing cholesterol-lowering effects of fibers from as little as 3g per day consumption; 10g per day is the minimal recommended dose to attain a clinically significant reduction of 3-5% in LDL-C levels.¹²

There are numerous studies on LDL-C and TC lowering effects of fiber.^{28,30,31} However, the advantages of this ingredient as a functional food alongside other major functional food actives

that can exert a similar effect through other mechanisms require further studies in order to reveal any beneficial effects it can have on cholesterol lowering.

2.3 ALPHA-LINOLENIC ACID (ALA)

Dietary polyunsaturated fatty acids (PUFA) can be categorized into omega-6 (n-6) and omega-3 (n-3) PUFA. Two important n-6 PUFA are linoleic acid and arachidonic acid. Major n-3 PUFA are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and are present abundantly in fish, shellfish, and sea mammals.³² Microscopic water-plants called phytoplankton, synthesize EPA and DHA and are the primary producers of marine life. On the other hand, plants of the land also offer a rich source of n-3 PUFA, the 18-carbon ALA.³³

ALA is an essential fatty acid and is present in canola, flaxseed, soybean, and walnuts.³⁴ Humans can convert ALA into EPA. ALA may also confer additional health benefits by itself.³³ The conversion rates of ALA to EPA ranges between 0.2% - 21% with the average being 8%, and that of ALA to DHA ranges between 0%-9% with the average being <1%, and affected by multiple factors.³²

Epidemiologic studies support use of ALA for prevention of arrhythmias. The n-3 PUFA have anti-inflammatory and antithrombotic properties. Further, these fatty acids may trigger endothelial-derived nitric oxide, and hence are vasoprotective. In addition, n-3 PUFA have hypolipidemic properties with effects on triglycerides and very low-density lipoproteins (VLDLs), and inhibit atherosclerosis.³³ A meta-analysis reviewed 27 studies associating ALA to CVD events and concluded that there is 10% lowered risk of mortality from chronic heart disease with each 1 g per day increase in the consumption of ALA³⁵. Thus, ALA is a potential

functional ingredient present in plant sources that can be used in a portfolio diet for CVD risk prevention.

2.4 ANTIOXIDANTS

The advantages of functional foods compared to supplements are that functional foods can confer additional benefits due to the presence of antioxidants. Antioxidants can beneficially interfere with disease-related oxidative stress and modify risk factors. There exist a variety of sites and modes of action of antioxidants conferring cardiovascular benefits, which include anti-oxidative and anti-inflammatory effects, autonomic adrenergic response modulation and endothelial function modification.³⁶⁻⁴⁰

Fruits, especially berries are well-studied antioxidant-containing food groups of recent interest. They contain several phenolic compounds with antioxidant activities which includes: vitamin C, anthocyanins, carotenoids, flavonols and ellagitannins. Berries, especially members of two families; Rosaceae (strawberry, raspberry, blackberry) and Ericaceae (blueberry, cranberry), belong to the best dietary sources of bioactive compounds.⁴¹

Anthocyanins are one of the most powerful natural antioxidants and berries are one of the major sources of anthocyanins among all the fruits.⁴² Saskatoon berries are a major source with significantly higher levels of anthocyanins among berries with potential health benefits. Saskatoon berries have comparatively higher levels of antioxidants than other berries such as wild blueberries, strawberries and raspberries. The major classes of flavonoids that impart anti-oxidant activity in this fruit-berry includes; flavonols (quercetin and rutin), flavanes (proanthocyanidin compounds) and anthocyanins.⁴³

Anthocyanins impart the purple, blue and red color of many fruits, including berries. Structurally, anthocyanins belong to phenolic group and are categorized into six classes; cyanidin, malvidin, peonidin, petunidin, pelargonidin and delphinidin. Studies utilizing in-vitro, in-vivo models as well as human clinical trials with dietary supplementation of a range of berry products has reported reductions in levels of several biomarkers of oxidative stress. Earlier studies have also demonstrated that the consumption of berries rich in antioxidant phenolic compound can increase plasma total antioxidant status in humans.⁴²

2.5 PORTFOLIO OF FUNCTIONAL FOODS

David Jenkins is the pioneer who introduced the portfolio diet. He demonstrated that combining plant sterols, viscous fibers, soy protein, and nuts is far advantageous in modifying lipid profiles than using a single functional food active.⁴⁴⁻⁴⁹ Five major dietary portfolio studies have been completed which are discussed below.

The first portfolio study in 2002 hypothesized the additive effect of the portfolio diet. The intervention was 4 weeks, with 13 participants, with a low saturated fat diet high in plant sterols (1 g), viscous fibers (8.2 g), soy protein (22.7 g) and almonds (2.9g) per 1000 kcal sourced on foods obtained from health food stores and supermarkets. The study utilized NCEP step 2 diet as background diet. The design included full feeding control, except for low calorie fruits and vegetables. A substantial reduction of 29% was observed in LDL-C level, which matched the efficacy of statins. However, the study used only historical control readings and no control arm, the effect of the low-fat diet was not assessed, and food acceptability was scored only 6.3 out of 10; which meant “acceptable with minor modifications”.⁴⁴ As such, this study possessed a number of limitations.

In 2003 Jenkins et al. conducted another study to ascertain the effect of a portfolio low-fat diet modifying the study design to include a control arm. A total of 12 versus 13 participants completed control and study arms of this parallel open labelled study, respectively. The very low saturated fat diet utilized the same ingredients, although there were some variations in amounts, being high in plant sterols (1.2 g), viscous fibers (8.3 g), soy protein (16.2 g), and almonds (16.6 g) per 1000 kcal. A 2-week pre-wash period minimized any previous statin effects that participants were consuming. The study demonstrated an LDL-C reduction of 35% following the treatment arm and 12.1% following the control arm. The low sample size and complex diet were the major limitations of the study.⁴⁵

Further in 2005, Jenkin's group reported a study giving a direct comparison of the effects on lipids of consumption of a portfolio diet versus taking a statin drug for lipid level control. The study arms included a low-fat control diet, a low fat plus statin diet, and the portfolio diet. An open labeled crossover design was used where the portfolio diet was very low in saturated fat, diet high in plant sterols (1.0 g), soy-protein foods (21.4 g), almonds (14 g), and viscous fibers (10 g) per 1000 kcal. The study diet showed a 29.6% reduction in circulating LDL-C levels as compared to 33% with statin, which were significantly different with each other.⁴⁷

Another long-term study by Jenkins in 2006 demonstrated the effects of a self-selected portfolio by study participants over a duration of 1 year. Sixty-six hyperlipidemic participants were advised to follow a portfolio diet high in plant sterols (1.0 g), soy protein (22.5 g), viscous fibers (10 g), and almonds (23 g) per 1000 kcal for 1 year. The study reported a 12% LDL-C reduction which was lower than expected, for which low compliance was a major reason. Although 55 participants completed the study, only 26 participants showed greater than 55% compliance.⁴⁸

Furthermore, a portfolio study in 2011 used a participant self-selected approach to consuming a portfolio diet at 2 levels of intensity; routine portfolio and intensive with 2 and 7 clinic visits for dietary counseling, respectively, over a period of 6 months. The study reported no additional benefits with the intensive visit portfolio having 13.8% LDL-C reduction as compared to 13.1% reduction observed with routine portfolio. The study was not metabolically controlled, thus helped to mimic real world effects of possible compliance limitations. However, the dropout rates observed in the study were 22.6%.⁴⁹

In summary, portfolio studies have shown significant beneficial effects on lipid profiles suggesting advantages of combining functional ingredients to reduce CVD risk. However, this dietary pattern requires a strong commitment that results in poor compliance by participants across these studies. Further, no studies have examined the effect of such a combination in statin reluctant people. A portfolio of ready to eat healthy, tasty foods that can confer the beneficial effects of fiber, phytosterol, omega -3 fatty acids and antioxidants might be able to accomplish cardiovascular risk reduction in a patient population that cannot or are unwilling to tolerate statins.

2.6 HETEROGENEITY IN CHOLESTEROL RESPONSE ACROSS INDIVIDUALS: EFFECTS OF GENETICS

Although there are genetic variants that have been associated with phytosterol response, the available data on gene-response associations are mostly inconsistent. Some single-nucleotide polymorphisms have been linked with variability in cholesterol response to phytosterol consumption and only two of them have been replicated by interventional studies so far. These are rs3808607 in cholesterol 7 α -hydroxylase (CYP7A1) and apolipoprotein E (APOE) isoform.

A study by De Castro-Oros et al. demonstrated that rs3808607 in the promoter region of the CYP7A1 gene is linked phytosterol response.⁵⁰ MacKay et al. replicated this finding and concluded an allelic dose effect of CYP7A1 with T/T carriers giving least response and an upsurge in response with each G-allele.¹⁷ In addition, Wang et al. found a similar association of the same SNP in LDL-C lowering by a high molecular weight soluble fiber of β -glucan.¹⁸

Miettinen and Vanhanen et al., found an improved cholesterol lowering with phytosterol consumption in APOE4 carriers compared to E2 and E3, which was also replicated by MacKay et al. These findings suggest the possibility of predictable cholesterol responses to functional bioactives in food affecting cholesterol metabolism; with the aid of these SNPs.^{17,51,52}

3. RATIONALE, HYPOTHESIS AND OBJECTIVES

3.1 RATIONALE AND HYPOTHESIS

Despite the recognition that dietary interventions can ameliorate cardiovascular disease risk in statin reluctant patients^{53,54}, that is those who cannot or are unwilling to tolerate statins. There have been no studies conducted that explore the use of a portfolio of healthy appetizing foods in improving health in this population. We hypothesized that a portfolio of recommended ready to eat healthy foods can improve serum lipid profiles and reduce markers of cardiovascular disease (CVD) risk in a patient population who are unable or unwilling to tolerate a medication-based approach for risk factor reduction.

3.2 OBJECTIVES OF THE RESEARCH

The overall objective of this project was to investigate the ability of a portfolio diet to affect CVD risk factors in a statin reluctant population, through a novel easily implemented functional food-based approach. The specific objectives were:

- To evaluate the effect of consuming a range of specifically formulated proprietary products on serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), total cholesterol (TC), glucose, insulin and high sensitivity C-reactive protein (hsCRP) concentrations and on plasma fatty acid profiles.
- To associate potential SNPs affecting cholesterol metabolism with the level of LDL-responsiveness to the portfolio of foods tested.

4. MANUSCRIPT

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4.1 ABSTRACT

OBJECTIVES: Dietary approaches can ameliorate cardiovascular disease risk factors, especially dyslipidemia. Yet no studies have been undertaken to explore if a portfolio approach with healthy appetizing ready-to-eat foods having functional food ingredients can improve cardiovascular health in patients who cannot or will not take statin drugs. The objective of our study was to evaluate the effect of a range of proprietary products, which contain four functional bioactives, on cholesterol levels in statin reluctant participants.

METHODS: A multi-center, randomized, double-blind, free-living cross-over study composed of 2 regimented phases of 4 weeks each, separated by a washout of 4 weeks, was conducted. Participants (n=54) ingested two servings per day from an assortment of packaged shelf-stable food products as a partial substitute for some foods they were already consuming. Treatment food products consisted of oatmeal, pancakes, cranberry bars, chocolate bars, sprinkles, and smoothies specially formulated in such a way as to provide at least 5g of fiber, 1000 mg of alpha-linolenic acid, 1000 mg of phytosterols and 1800 µmol antioxidants per serving. Control products were similar-use calorie-matched items drawn from the general grocery marketplace.

OUTCOME MEASURES: High-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), total cholesterol (TC), glucose, insulin and high sensitivity C-reactive protein (hsCRP) concentrations were analyzed at baseline and endpoint of both phases. Plasma fatty acid profiling was carried out at the endpoint of both phases to ascertain alpha linolenic acid (ALA) levels to confirm compliance with the ingestion of the study foods. Single nucleotide polymorphisms in CYP7A1-rs3808607 and APOE genes were analyzed for associations with variability in LDL-C responses to treatment.

RESULTS: A reduction of 5.08 ± 8.24 % was observed in total cholesterol (p-value < 0.0001) levels with LDL-C levels being reduced by 8.80 ± 12.5 % (p-value < 0.0001) after the consumption of study foods compared to control at the endpoint. Circulating HDL-C, TG levels, and glucose concentrations and hsCRP were not influenced by dietary treatment.

CONCLUSIONS: Consumption of a portfolio of ready-to-eat bioactive containing foods significantly improves serum lipid profiles in patients who are unable or unwilling to take statin drugs. This novel, easily adaptable food-based approach is anticipated to have extensive implications for clinical practice.

4.2 INTRODUCTION

Cardiovascular disease (CVD) remains the primary cause of death worldwide and according to World Health Organization (WHO) at least 2 million premature deaths from CVD could be prevented each year by achieving risk-factor targets.² Currently, HMG-Co A reductase inhibitors are the primary medications used in the prevention and treatment of CVD through cholesterol lowering. However, a large proportion of individuals prescribed statins are reluctant to take them for various reasons. This statin reluctance is principally due to intolerance from muscle side effects as well as a growing concern about several other adverse effects including potentiating the risk for diabetes mellitus upon statin consumption. In addition, some patients are unable to achieve their target goals despite taking a maximum dose of statins, and non-responders to statins also exist.^{7,11,12,55}

Lowering low-density lipoprotein cholesterol (LDL-C) is a key therapeutic approach for reducing the CVD risk and dietary therapy should play a vital role in this. Evidence-based guidelines of the World Health Organization (WHO), the American Heart Association (AHA), as well as the Canadian Cardiovascular Society, recommend dietary modification as a critical part of CVD risk reduction strategies.^{2,3,56} Extensive research has been conducted through randomized clinical trials and cohort studies to document the effects of individual dietary components on CVD risk factor reduction.⁵⁷⁻⁵⁹

A novel approach to lipid lowering based on a plant-based dietary pattern termed the “portfolio diet” combining four core food components (phytosterols, viscous fibre, plant protein and nuts) was introduced in the 2000s by Jenkins et al.⁴⁴ All of these ingredients have health claims for CVD risk reduction or cholesterol-lowering from Health Canada, Food and Drug Administration

(FDA), and/or the European Food Safety Authority (EFSA). The study was accompanied by further portfolio attempts with slight modifications in the ingredients and study designs reinforcing its benefits on CVD risk. A recent meta-analysis pooled the existing 7 portfolio trials and concluded an additive effect of the portfolio ingredients with an average 17% LDL-C reduction, being 21% in efficacy (ie compliance controlled) and 12% in effectiveness (ie not compliance controlled) trials.¹³

The portfolio dietary pattern was originally designed to supply 2 g of plant sterols, 20 g of viscous soluble fiber, 50 g of plant protein and 42 g of nuts on a 2000 kcal diet basis. All the 5 portfolio trials made use of background nutritional intakes based on National Cholesterol Education Program (NCEP) Step II diet having limits of 7% energy from saturated fat, 30% energy from total fat, and 200 mg per day of cholesterol. Thus, all portfolio diet trials required the entire diet of participants to fit these recommendations resulting in poor overall compliance.^{13,45} The low feasibility of imparting such marked dietary changes indicates the need for developing other more acceptable portfolio approaches that can be easily implemented and widely utilized.

In our study, a two snack per day substitution of shelf stable ready to serve foods, having commensurable amounts of plant sterols, soluble fibers, alpha-linolenic acid (ALA) and antioxidants, was utilized to deliver the portfolio approach provided as a healthy appetizing array of products. Systematic reviews and meta-analyses of randomized controlled trials have reported reductions in LDL-C levels on an average of 7% for soluble fibers at a consumption of 5-10 g per day and 7% for plant sterols when consumed 2 g per day.¹³ A meta-analysis on the effect of phytosterol across various dose ranges found LDL-C reductions ranging 6-12% for doses ranging 0.6-3.3 g per day.⁶⁰ ALA, the plant-based n-3 PUFA, undergoes conversion to

eicosapentaenoic acid (EPA) at a rate of 5–10%. Epidemiologic studies and meta-analyses report that ALA may have hypolipidemic properties with effects on triglycerides (TGs) and very low-density lipoproteins (VLDLs) and can inhibit atherosclerosis.^{33,61,62} Antioxidants, on the other hand, may confer cardiovascular benefits which include anti-inflammatory effects, autonomic adrenergic response modulation and endothelial function modification.^{36,37,40} Thus, combining these four ingredients may yield synergistic beneficial effects on CVD risk, especially within a statin reluctant population. To our knowledge, no other studies have explored if a portfolio approach with ready-to-eat foods containing functional food ingredients can improve cardiovascular health in this population.

Generalized dose-response curves need to be redefined in this era of nutrigenetics in order to achieve personalized nutrition strategies. Clinical trials by De Castro-Oros et al., MacKay et al. and Wang et al. suggest varied cholesterol uptake and metabolizing abilities across individuals possessing different genetic variants for CYP7A1-rs3808607 and APO-E. These studies have demonstrated that CYP7A1-rs3808607 and APOE single nucleotide polymorphism heterogeneity affects variability in response to phytosterol or fiber intake for LDL-C or TC lowering. Thus, it may be possible to predict LDL-C responses to these functional ingredients by examining the role of CYP7A1 and APOE gene polymorphisms.^{15,17,18,50}

The objective of the present study was to, therefore, examine the effect of a range of proprietary products containing four functional bioactives; plant sterols, soluble fibers, alpha-linolenic acid and antioxidants; on cholesterol levels in statin reluctant participants. Our specific aim was to evaluate the changes in serum high-density lipoprotein cholesterol (HDL-C), LDL-C, TG, total cholesterol (TC), glucose, insulin and high-sensitivity C-reactive protein (hsCRP) concentrations

over a 4 week regimen in response to a portfolio diet. In this study, we also aimed to correlate the LDL-C responses as a function of CYP7A1 and APOE polymorphisms.

4.3 EXPERIMENTAL METHODS

4.3.1 STUDY DESIGN

This dual-center international study followed a randomized, free-living crossover design composed of 2 regimented phases of 4 weeks each, separated by a 4 week washout. The clinical trial was conducted at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), University of Manitoba, Winnipeg, Manitoba, Canada; and at Mayo Clinic, Rochester, Minnesota, USA.

Trial participants followed a run-in period of 4 weeks with their usual dietary patterns; maintaining their normal levels of physical activity throughout the study. A maximum of 2 alcoholic beverages per day was permitted during the study. After a washout period of 4 weeks, participants received a randomized supply of packaged shelf-stable food products presented in a single-serve format requiring minimal to no preparation. The treatment products were provided by Step One Foods and consisted of items including oatmeal, pancakes, cranberry bars, chocolate bars, smoothies, and a sprinkle offering which could be added to almost any food to enhance its nutritional impact. All products were interchangeable in terms of their nutrients of interest and possessed a minimum of 5 g of fiber, 1000 mg of omega-3 fatty acids, 1000 mg of phytosterols and 1800 μ mol antioxidants per serving (*Table 1*). Calorie counts of the products ranged from 110-190 kcal per serving. Control products were like-items drawn from the general grocery marketplace. These products were identical in type, packaging, appearance and preparation requirements, and were matched for calories to the treatment products. Participants were

instructed to consume 2 items over the course of each day as a substitute for breakfast, as a snack, or as a component of the main meal.

All participants received printed instructional materials outlining test product use and were verbally encouraged to follow directions provided in the written materials. Participants were also contacted once a week over the course of intervention to encourage compliance. They were also specifically instructed not to change other parts of their diet or activity levels. During intervention periods, participants were instructed to fill out a checklist of the number of products consumed. Compliance to the intervention protocol was determined using the completed checklists and food return counts. Medication use during the trial was also monitored. The trial plan is summarized in *Table 2*.

4.3.2 ETHICAL CONSIDERATIONS

The study procedures were approved by the biomedical research ethics board (BREB) of the University of Manitoba (Ethics File Number B2014:113) and Mayo Clinic Institutional Review Board. Written informed consent was obtained from all participants accepted into the study. The study was registered in the clinicaltrials.gov registry (Identifier: NCT02341924; www.clinicalTrials.gov).

Table 1. Summary of key nutrients and ingredients in the study food products

	Oatmeal	Pan cakes	Cranberry Bar	Chocolate Bar	Smoothie	Sprinkle
Fiber (g)	5	6	5	6	5	5
ALA (mg)	1700	1018	1210	1002	1875	1910
Plant sterols (mg)	1062	1059	1002	1025	1025	1010
Anti oxidants (µmol)	2281	2372	2652	3322	3320	1556
Ingredients	Oat bran, flax seed, dried blueberries (blueberries, apple juice concentrate, sunflower oil), dried cranberries (cranberries, apple juice concentrate, sunflower oil), almonds, walnuts, raisins, chia seeds, plant sterols, cinnamon, Saskatoon berries.	Ground whole oats, raisins, oat bran, chia seeds, flax seeds, walnuts, almonds, aluminum-free baking powder, carrot powder, bananas, arrowroot flour, dried cranberries, dried egg white, plant sterols, pea fiber, cocoa, vanilla, cinnamon, Saskatoon berries.	Dried cranberries, date paste (dates, water), almonds, pecans, chia seeds, oat bran, flax seeds, walnuts, raisins, plant sterols, Saskatoon berries.	Bitter sweet chocolate [unsweetened chocolate, sugar, cocoa butter, soy lecithin, vanilla], almonds, whole pinto bean flour, chia seeds, oat bran, walnuts, dried cranberries, raisins, flax seed, plant sterols, Saskatoon berries.	Strawberries, bananas, oat bran, dried cranberries, almonds, walnuts, raisins, chia seeds, flax seeds, plant sterols, Saskatoon berries.	Oat bran, dried cranberries, almonds, walnuts, raisins, chia seeds, flax seeds, plant sterols, Saskatoon berries.

Table 2. Summary of trial plan

	Screening WK -4		V1 - PID1(±0 d) WK1	V2 - PID2 (±0 d) WK1	V3 - PID29 (+5/-2d) WK4	V4 - PID30 (+5/-2d) WK4		V5 - P2D1 (±0 d) WK9	V6 - P2D2 (±0 d) WK9	V7 - P2 D29 (+5/-2d) WK 12	V8 - P2D30 (+5/-2d) WK 12		
Informed consent	•	INITIAL WASHOUT					WASHOUT						
Inclusion-exclusion criteria	•												
Anthropometrics	•		•			•		•				•	
Blood pressure	•		•			•		•				•	
Concomitant medications	•		•			•		•				•	
HDL-C, LDL-C, TG, TC, Glucose, Insulin	•		•	•	•	•		•	•	•	•	•	•
Plasma fatty acid								•				•	
Treatment dispense					•					•			
GI tolerance								•				•	
Treatment checklist								•				•	
Adverse events								•				•	
End of study form													•

4.3.3 PARTICIPANT SELECTION CRITERIA

Inclusion criteria: Participants aged $21 \geq$ to ≤ 65 years were recruited based on their ability to give informed consent and being unwilling to use or intolerant of at least one statin medication.

Exclusion criteria: Participants were excluded if they were unable to speak or read English or unable or unwilling to temporarily withhold all statin or lipid-lowering therapies including supplements throughout the study period.

Participants with diabetes (on oral hypoglycemic agents and/or insulin) or severe obesity (body mass index (BMI) $>35 \text{ Kg/m}^2$) were also excluded. Women who were pregnant or planning to become pregnant during the study period were excluded.

Participants with food allergies or intolerances, food restrictions due to medical, religious or philosophical reason including Kosher, vegan, vegetarian, high protein, low-carbohydrate, low-phosphorus diet were excluded. These exclusion criteria did not apply for participants attempting to follow an eating plan as generally advocated by the American Heart Association (low sodium, low cholesterol, reduced fat).

Participants were not eligible if their baseline fasting LDL-C was $\leq 2.6 \text{ mmol/l}$ (100 mg/dL) or $\geq 4.9 \text{ mmol/l}$ (190 mg/dL); or showed evidence of tissue cholesterol deposition based on a medical examination; or if their baseline fasting TG was $\geq 4.5 \text{ mmol/l}$ (400 mg/dL) or baseline fasting blood glucose was $\geq 7 \text{ mmol/l}$ (126 mg/dL). Clinical values obtained through screening or the previously available values for up to 6 months prior to screening were used to ascertain this.

Consumption of more than 2 alcoholic drinks per day or history of alcoholism or drug dependence also served as an exclusion criterion. Smokers were excluded from the study.

Individuals with history of non-skin cancer, melanoma, rheumatoid arthritis or other chronic rheumatologic condition and advanced cardiovascular disease including moderate or greater valvular disease, congestive heart failure or known coronary artery disease, dysrhythmias requiring medical or surgical intervention were excluded. Subjects with known chronic liver or renal disease, diabetes, inflammatory bowel disease, celiac disease, uncontrolled thyroid disease, hormonal supplementation (other than thyroid), pancreatitis, gallbladder or biliary disease, neurological or psychological disease, and gastrointestinal disorders that could interfere with fat absorption were also excluded.

Individuals with uncontrolled hypertension having systolic blood pressure >150 mm Hg or diastolic blood pressure >100 mm Hg were also excluded from the study.

4.3.4 OUTCOME MEASURES

During baseline and end point of the two intervention phases, anthropometric measurements including body weight, height, BMI, hip and waist circumference and blood pressure were recorded. Blood pressure was measured with an automated blood pressure device in the non-dominant arm according to AHA instructions for measuring blood pressure.

Gastrointestinal tolerability questionnaires were completed by participants at the end of each intervention period.

Approximately 20 ml of blood samples were collected on days 1, 2 (baseline) and 29, 30 (endpoint) of each of the two intervention phases. Samples were centrifuged at 1300 g for 10 min, separated and stored as 0.5 ml aliquots at -80°C until further analysis.

Serum lipid profile (HDL-C, TG levels, and TC) and fasting serum glucose levels were measured using a Vitros Chemistry System 350 (Ortho-Clinical Diagnostics, Johnson & Johnson). LDL-C concentrations were calculated using the Friedewald equation.

Serum insulin levels were measured in duplicate by radioimmunoassay using MilliporeSigma (Etobicoke, Ontario) Human Insulin Specific RIA Kits with ^{125}I as a tracer. Radioactivity was determined by gamma counter (LKB Wallac, 1282 compugamma CS, Fisher Scientific, Montreal, QC, Canada) and expressed as counts per minute. Serum insulin concentrations were quantified by constructing a standard curve.

For hsCRP, serum samples were analyzed in duplicate, using Meso Scale Discovery (MSD) electrochemiluminescence-based multiplex assays (MSD, Gaithersburg, MD) and the assay results were read using a Sector Imager 2400. Serum hsCRP concentrations were obtained by interpolation on log calibrator curves.

Plasma fatty acid profiles including alpha linoleic acid levels were measured by extracting total lipids from plasma and fatty acids and converting them to their methyl esters. Fatty acid concentrations were then determined by gas chromatography with flame ionization (Agilent Technologies, Mississauga, Ontario, Canada). Heptadecanoic acid was used as an internal standard and pure standards for each fatty acid were used to confirm retention times.

DNA was extracted from white blood cells using a column-based DNA extraction kit (DNeasy Blood and Tissue Kit, QIAGEN Sciences). Integrity of the genomic DNA was ascertained by micro-volume spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific Inc., Waltham, MA, USA). TaqMan SNP genotyping assays (Life Technologies, Burlington, ON) were used for

genotyping DNA samples on Step-One-Plus Real-Time PCR System (Applied Biosystems, Life Technologies, Burlington, ON).

4.3.5 STATISTICAL ANALYSIS

The main driver for determining enrollment levels for the study was the ability to demonstrate a clinically significant difference in circulating LDL-C response between consuming 2 servings of products per day. A previous study of a similar patient population reported a 2-week change in LDL-C of -8.5% (standard deviation of 11%) among participants who underwent a control diet. Using these estimates, a sample size of 21 in each study period was expected to have 80% power to detect a 10% group difference in LDL-C change (additional 10% reduction in treatment arm; and assuming that the common standard deviation of LDL-C change is 11%) using a 2-group t-test with a 0.050 two-sided significance level. Powering the trial to detect a 10% difference between interventions was therefore appropriate. Assuming the same standard deviation of 11%, detecting a 10% 4 week decrease in LDL-C from utilizing any food intervention was expected to have a power of over 99% for the 25 participants per center in the trial so enrollment numbers should have been more than adequate to detect a significant LDL-C change from baseline. In order to ensure adequate participant numbers in each group at the end of the trial, we aimed to recruit 30 participants at each center, considering the possibility of a 20% drop-out rate (or unexpected ineligibility based upon baseline laboratory values).

The study and control foods were identically packaged and coded by Step One Foods as Blue and Orange foods respectively to mask the treatments. Randomization codes with blocks of 10 were prepared in RCFN centre using Excel function to randomize Blue and Orange treatments for Phase I and II. Once participants were found eligible for the study after screening procedures

and upon providing written informed consent, each subject was randomly assigned to treatment or control phase on a first come basis according to these codes, by the clinical coordinators of respective study centres.

Statistical analyses were performed using statistical software, SAS. Baseline and endpoint measurements were compared using the analysis of variance (ANOVA) model for determination of treatment effects using the SAS PROC MIX procedure with diet, sequence, sex and center as fixed factors and participant as a random factor within the model utilizing Bonferroni correction for multiple comparisons. The Shapiro Wilk test was used to analyze normality of distribution of data, and non-normal variables were normalized by log transformation. Results were expressed as least square means and standard errors unless stated otherwise. Statistical significance was set at $p < 0.05$ for all analyses. Only the results of endpoint analyses are detailed as this approach avoids the noise from baseline points.

4.4 RESULTS

4.4.1 PARTICIPANT CHARACTERISTICS

Participants were recruited with the help of advertisements for participants disseminated via local media and official websites of study centres *Figure 1.* shows participant flow during the study. Both the RCFFN and Mayo clinic study centre recruited 30 participants each. One drop out occurred at the RCFFN due to unwillingness to provide blood samples and consume study foods after phase 1. Five dropouts occurred during phase-1 at Mayo Clinic for participants who failed to comply with the study protocol. Fifty four participants successfully completed the study and were included in the analyses except for hsCRP, where participants with hsCRP-values above 95.2nmol/L were excluded as levels this high are generally attributed to infection.^{63,64}

Characteristics at the baseline for participants who completed the study are presented in *Table 3*. The study included 18 men and 36 women of mean age 49 ± 12 yrs. Mean body weight was 76.8 ± 13.9 kg and mean BMI was 27.2 ± 3.4 kg.

4.4.2 COMPLIANCE AND SIDE EFFECTS

Compliance was determined from checklists filled in by participants and by counting the returned food packets at the end of each intervention phase. There was overall good compliance of 95.0% with the treatment food and 96.5% with the control food. Compliance did not differ statistically between the treatment and control foods. The gastrointestinal side effects experienced by participants during the study food phase are summarized in *Table 4*.

4.4.3 ANTHROPOMETRICS AND BLOOD PRESSURE

Endpoint analysis outcomes of the study are summarized in *Table 5* and baseline-endpoint analyses are summarized in *Table 6*. No change in physical activity was reported by study participants, and no significant changes in body weight or BMI were noticed at the completion of the study period at endpoint analysis. Neither waist circumference nor waist-to-hip ratio were observed to have undergone any changes. Blood pressure did not differ after four weeks with treatment food compared to control.

4.4.4 BLOOD LIPIDS

After 4 weeks of treatment foods compared to that of control at the endpoints, we found a significant improvement in the lipid parameters measured in that LDL-C ($8.80\pm 12.5\%$ or -

0.29±0.39 mmol/l) and TC (-5.08±8.24% or -0.28±0.47 mmol/l) levels were reduced (P<.0001). No changes in HDL-C levels were observed.

4.4.5 GLUCOSE, INSULIN AND hsCRP

Serum glucose, insulin and hsCRP levels remained unaltered at treatment endpoint compared to that of control.

4.4.6 FATTY ACIDS

Endpoint analyses of total plasma fatty acid profiles for the 54 participants who completed the study are presented in *Table 7*. Fatty acid concentrations are expressed as percentages of total identified fatty acids measured. Increases in ALA (P-value <.0001) and EPA (P-value = 0.0348) at the end of the treatment phase compared to that of control phase were observed. Additionally, decreases in palmitic acid (P-value = 0.0116), and total saturated fatty acid (P-value = 0.0405) levels after 4 weeks with treatment food were observed, compared to that of control at the endpoint. No other significant differences were observed across the rest of the fatty acid profile including DHA with treatment phase compared to that of control at the endpoint.

4.4.7 GENETIC POLYMORPHISM INTERACTIONS

Serum LDL-C responsiveness to study foods compared to control, categorized by SNPs, are presented in *Table 8*. No statistically significant influence of CYP7A1 or APOE SNPs on the observed LDL-C changes were found. All “adverse responders” (those who have not shown positive deviations in LDL-C in treatment phase compared to control at the endpoint) were found to have one or more T alleles for CYP7A1 while the homozygous G group was found to have no adverse responders although there were 2 non-responders in the group.

Figure 1. Participant flow chart

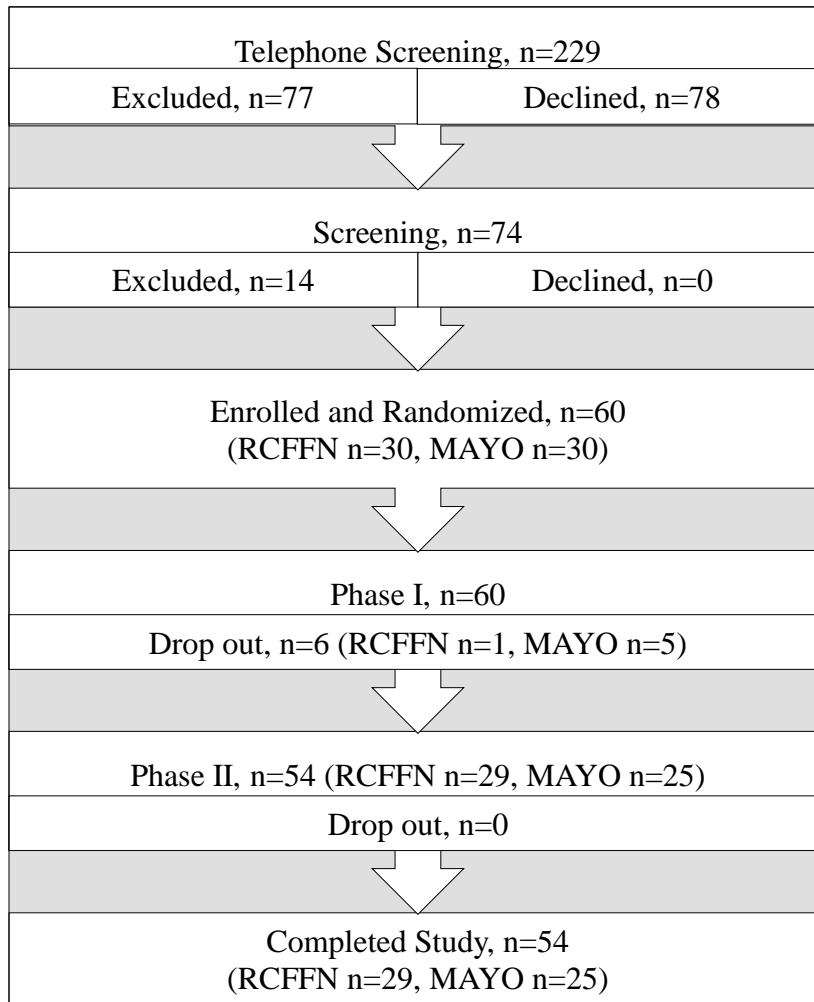


Table 3. Baseline characteristics of study participants

	Baseline values
Male (n)	18
Female (n)	36
Age (years)	49±12
Body Weight (kg)	76.8±13.9
BMI (kg/m²)	27.2±3.4
Waist Girth (cm)	91.3±9.27
Waist Hip Ratio	0.9±0.07
Office Blood Pressure- Systolic (mmHg)	118±11.7
Office Blood Pressure-Diastolic (mmHg)	79.2±7.8
HDL-C (mmol/L)	1.46±0.36
LDL-C (mmol/L)	3.38±0.83
TG (mmol/L)	1.76±0.93
TC (mmol/L)	5.65±0.95
Glucose (mmol/L)	5.28±0.47
Insulin (μU/mL)	15±5.76
hsCRP (nmol/L)	21.6±18.4*

Values in Means±SDs

*n=38

n=54 unless specified

Table 4. Summary of side effects with consumption of treatment foods

	Number of participants reported side effects*
Stomach pain	1
Constipation	2
Diarrhea	2
Flatulence	5
Abdominal bloating	8

*During study food phase

Table 5. Endpoint analyses of cardiovascular risk factors in response to study food consumption compared to control

	Control Endpoint*	Treatment Endpoint*	P-value	Percentage difference (%)
Body Weight (kg)	78.6±1.84	79.0±1.84	0.075	0.56
BMI (kg/m²)	27.1±0.51	27.2±0.51	0.197	0.52
Waist Girth (cm)	92.1±1.3	92.2±1.3	0.866	0.08
Waist Hip Ratio	0.91±0.01	0.91±0.01	0.529	-0.32
Blood Pressure- Systolic (mmHg)	118±1.64	119±1.64	0.506	0.91
Blood Pressure-Diastolic (mmHg)	78.7±1.21	79.9±1.21	0.178	1.52
HDL-C (mmol/L)	1.42±0.04	1.43±0.04	0.552	0.95
LDL-C (mmol/L)	3.28±0.11	3.0±0.11	<.0001**	-8.80
TG (mmol/L)	1.84±0.13	1.85±0.13	0.819	0.32
TC (mmol/L)	5.55±0.13	5.28±0.13	<.0001**	-5.08
Glucose (mmol/L)	5.39±0.07	5.38±0.07	0.821	-0.17
Insulin (µU/mL)	16.0±0.89	15.5±0.89	0.577	-2.99
hsCRP (nmol/L) ***	26.7±3.94	26.9±3.94	0.705	0.72

*Values in LS-means ± SEs

**Significantly different

***n=38

n=54 unless specified

Table 6. Baseline-to-endpoint analyses of cardiovascular risk factors in response to study foods compared to control

	Changes in Treatment period*	Changes in control period*	P-value	Percentage difference (%)
Body Weight (kg)	-0.17±0.17	-0.31±0.17	0.492	0.19
BMI (kg/m²)	0.02±0.07	-0.01±0.07	0.793	0.09
Waist Girth (cm)	-0.47±0.43	0.26±0.43	0.212	-0.79
Waist Hip Ratio	-0.01±0.00	0.00±0.00	0.333	-0.74
Blood Pressure- Systolic (mmHg)	-0.79±1.06	1.09±1.06	0.843	0.32
Blood Pressure- Diastolic (mmHg)	-0.04±0.93	-0.70±0.93	0.597	0.85
HDL-C (mmol/L)	0.01±0.02	-0.02±0.02	0.307	-0.95
LDL-C (mmol/L)	-0.35±0.06	-0.03±0.06	<.001**	-10.16
TG (mmol/L)	0.05±0.08	-0.01±0.08	0.550	3.92
TC (mmol/L)	-0.32±0.07	-0.05±0.07	0.002**	-4.96
Glucose (mmol/L)	0.03±0.05	0.11±0.05	0.155	-1.45
Insulin (μU/mL)	-0.10±0.70	0.54±0.70	0.475	-4.11
hsCRP (nmol/L) ***	4.47±3.57	7.79±3.57	0.492	-18.90

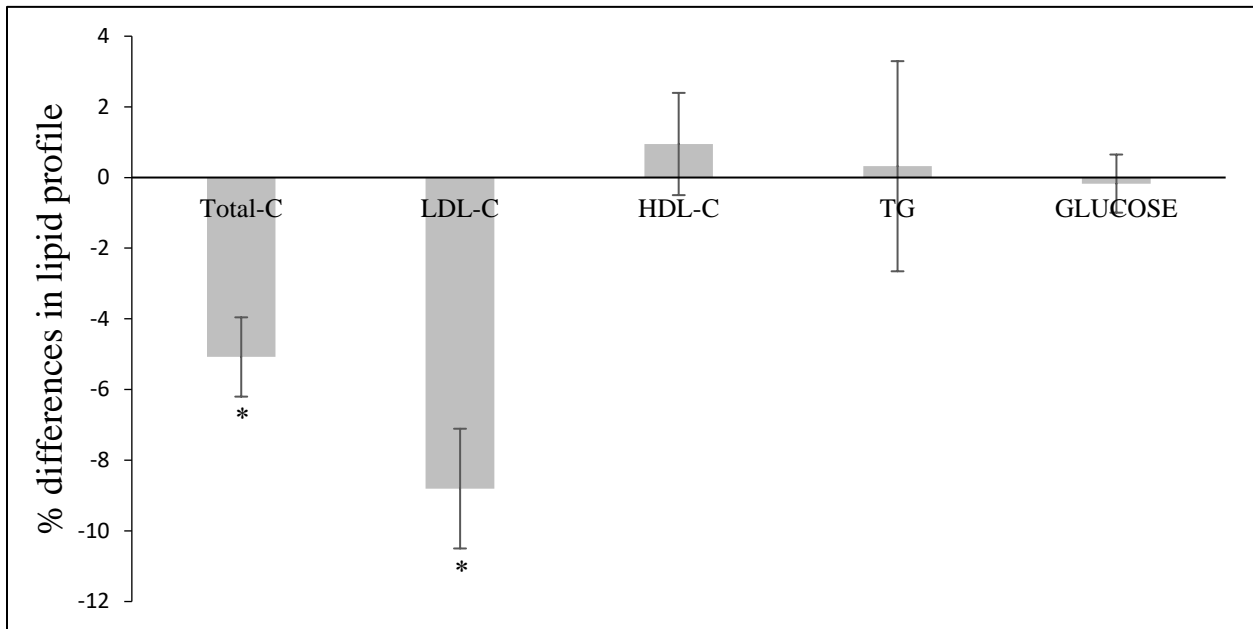
*Values in LS-means ± SEs

**Significantly different

***n=38

n=54 unless specified

Figure 2. Percentage changes in lipid profile in response to study foods compared to control at endpoints



* P value significantly different

Figure 3. Waterfall plot of individual percentage changes in LDL-C in response to study foods compared to control at endpoints

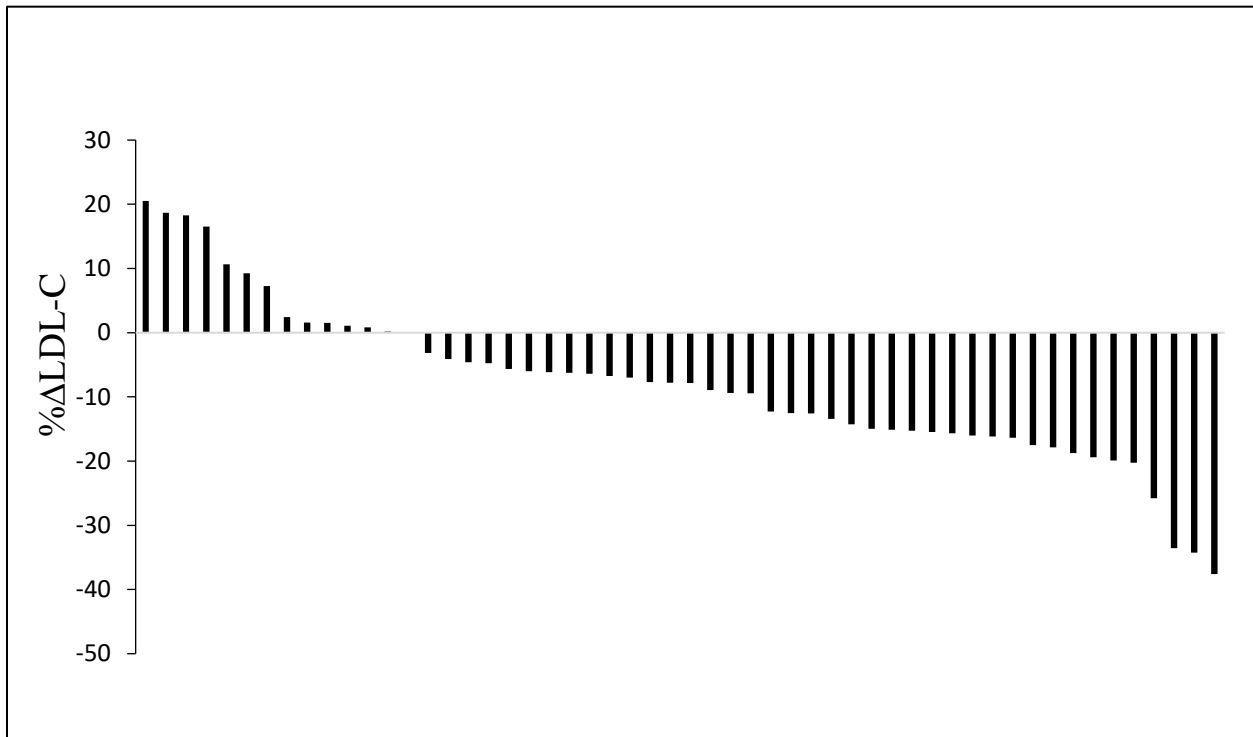


Table 7. Endpoint analyses of plasma fatty acid profile in response to study foods compared to control

	Control*	Treatment*	P-value
C14:0	0.63±0.03	0.63±0.03	0.842
C15:0	0.22±0.01	0.22±0.01	0.358
C16:0	31.21±0.24	30.79±0.24	0.012**
C16:1	0.95±0.07	1.02±0.07	0.406
C18:0	13.62±0.17	13.65±0.17	0.852
C18:1n9	13.44±0.28	13.82±0.28	0.166
C18:2n6	22.8±0.36	23.08±0.36	0.320
C18:3n3	0.4±0.02	0.48±0.02	<.001**
C20:0	0.06±0	0.06±0	0.704
C20:1n9	0.11±0	0.1±0	0.911
C20:4n6	9.71±0.21	9.51±0.21	0.133
C20:5n3	0.7±0.06	0.79±0.06	0.035**
C22:5n3	0.35±0.03	0.35±0.03	0.981
C22:6n3	2.73±0.11	2.67±0.11	0.196
SFAtotal	45.84±0.24	45.46±0.24	0.041**
MUFAtotal	14.54±0.31	14.98±0.31	0.164
PUFAtotal	39.62±0.28	39.56±0.28	0.814

*Values in LS-means ± SEs.

** significantly different

Table 8. Serum LDL-C responsiveness to study foods compared to control categorized by SNPs

	LDL-C response to treatment compared to control at endpoint* (mmol/l)	Average percentage LDL-C response to treatment compared to control at endpoint (%)	P-value
APO_E3	-0.25±0.07	-6.27	0.230
APO_E4	-0.31±0.08	-9.95	
CYP7A1-rs3808607 GG	- 0.28±0.09	-7.75	0.650
CYP7A1-rs3808607 TG	-0.28±0.09	-9.17	
CYP7A1-rs3808607 TT	-0.28±0.08	-6.98	

*Values in LS-Means±SE

Figure 4. Individual percentage changes in LDL-C in response to study foods compared to control at endpoints stratified by Apo E isoform

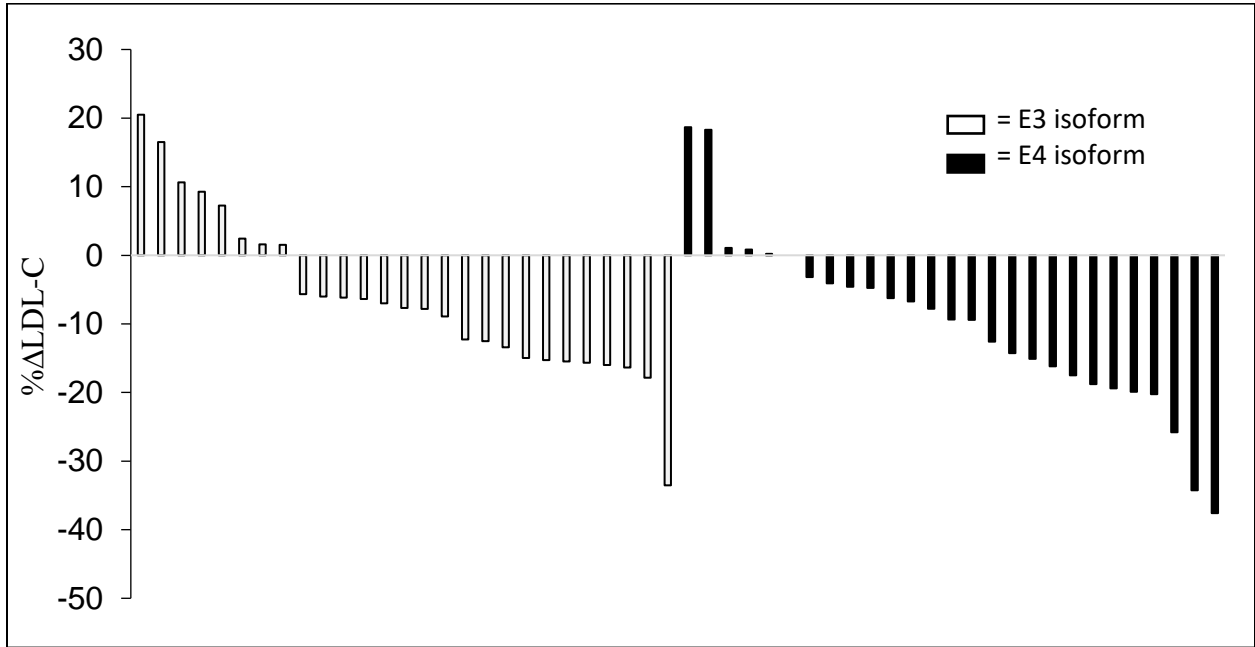
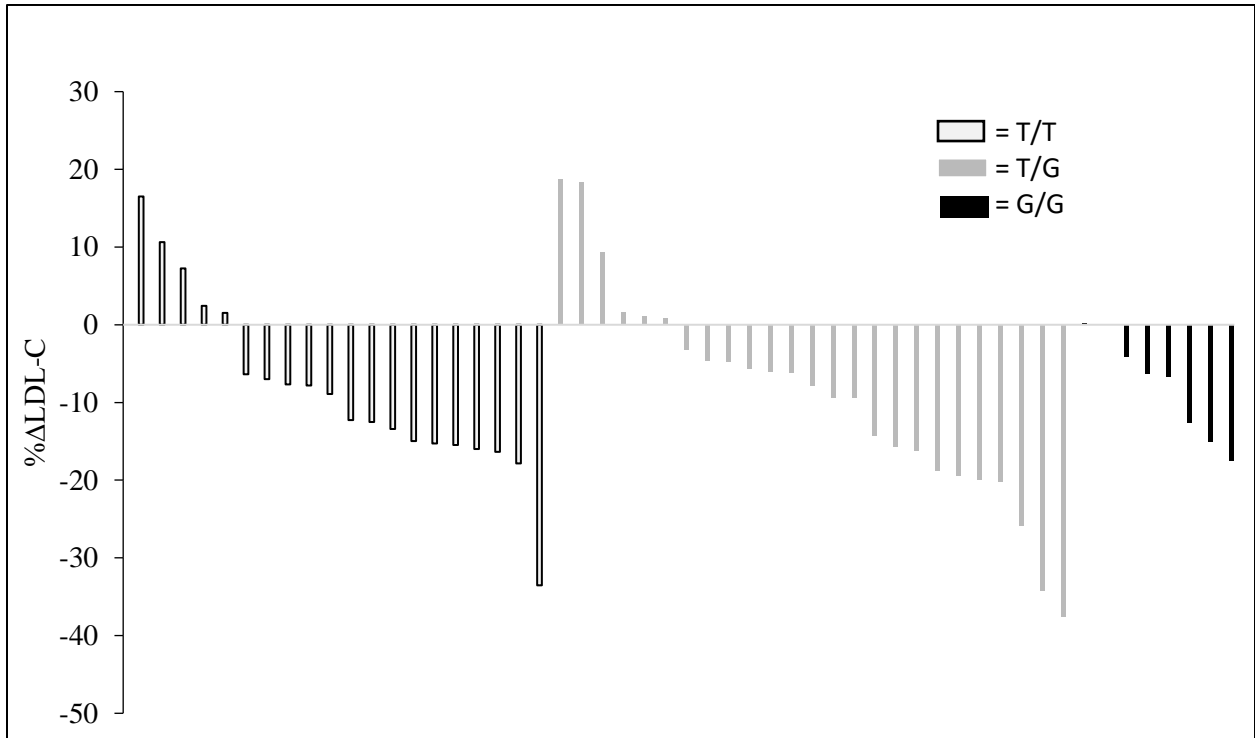


Figure 5. Individual percentage changes in LDL-C in response to study foods compared to control at endpoints stratified by CYP7A1 genotype



4.5 DISCUSSION

In this study, we aimed to assess the efficacy of a combination of functional food components provided through ready-to-eat proprietary products to reduce LDL-C levels. The study foods were consumed without altering background diet or physical activity and thus, our study was a weight maintenance study. Serum LDL-C and TC concentrations were decreased by 8.80 ± 12.5 % (-0.291 mmol/l) and 5.08 ± 8.24 % (-0.2843 mmol/l), respectively, by study foods consumption compared to control at the endpoint. The magnitude of decrease in LDL-C in just 4-weeks with two ready-to-serve or minimal preparation snacks is promising, especially since some participants experienced 30% or greater LDL-C reductions. A 1% reduction in total mortality for each 1 mg/dl or 0.0259 mmol/l decrease in LDL-C has been reported by a meta-analysis that considered the results of 30 trials⁶⁵ underscoring the clinical significance of the reduction achieved. The comparatively lower reduction in TC is understandable as there was a slight, though statistically insignificant, increase in HDL-C.

The LDL-C reductions of 17% reported in prior portfolio diet studies can be in part attributed to the extensive food substitution levels deployed during those interventions. This is in contrast to the minimal substitutions required in the present study, suggesting that the lipid-lowering effects seen in the presently are likely directly attributable to the active nutritional ingredients in the intervention foods.^{13,57}

Significantly, a recent meta-analysis of effectiveness in previous portfolio-diet studies revealed that adherence to the full dietary pattern was less than 50% and was directly associated with LDL-C reductions trials.¹³ Dietary patterns in Canada, the United States and Europe presently fail to meet the suggested target portfolio dietary pattern. Improvement strategies are thus being

widely discussed. The overall high compliance of 95% with study foods in statin reluctant participants reported in our free-living study is a major advantage of this approach, underscoring the feasibility and ease of including two servings of hedonically acceptable snacks towards achieving risk reduction goals.¹³

However, our LDL-C reduction results were lower than those observed in portfolio diet studies which distributed the functionally active ingredients throughout the entire diet of participants. Other studies have evaluated the cholesterol-lowering action of phytosterols in a fiber matrix. A randomized incomplete-crossover study of a 3-week intervention period with 58 participants compared the effects of phytosterol added to milk, yoghurt, bread and cereal providing 1.6 g phytosterols daily. LDL-C lowering levels of 15.9%, 8.6%, 6.5% and 5.4% were observed with enriched milk yoghurt, bread and cereal, respectively. The sterol-enriched bread and cereal were both significantly less efficacious than was the milk. The study results demonstrated that varying the food matrix results in different cholesterol-lowering effects using the same dose of phytosterols.⁶⁶ Another study on rye bread containing 2 g per day of phytosterols demonstrated an 8% lowering of LDL-C.⁶⁷ A recent study utilizing a plant sterol-enriched whole grain breakfast cereal biscuit found only 5.6% LDL-C reduction as compared to standard wholegrain breakfast cereal biscuit.⁶⁸ These studies suggest that phytosterol has comparatively less efficacy in a fiber matrix.

In our study, five different study foods were used, each of them providing similar levels of the nutrients of interest. This variety acknowledges the different palatability needs of participants and likely contributed to the higher compliance rates compared with previous portfolio studies. However, variations may exist in bioavailability of the bioactives, especially phytosterol and ALA, across the different food matrices used in the study. Since participants consumed their own

choice of combinations from the study food varieties; we could not account for this source of variability, which was a limitation of the present study.

Although dietary guidelines from WHO and AHA advocate high intakes of fiber for cardiovascular risk reduction; the average dietary consumption of fiber in North American adults remains well below recommended levels. Our study foods provide a novel way of delivering 10 g of whole food fiber, 2 g phytosterol and 1 g ALA in two daily food servings; thereby partly bridging the gap between actual and advised dietary consumptions for CVD risk prevention.^{5,69} Water-soluble fibers are proposed to trap dietary and bile acid cholesterol, by the swollen matrix formed in the intestine, enhancing its excretion through feces.^{28,29} Phytosterols, on the other hand, are suggested to sequester cholesterol in the intestine, resulting in competitive inhibition of cholesterol absorption and reabsorption.^{11,25}

We observed a significant increase in ALA in circulating plasma with treatment foods which affirms that study foods were consumed. The concurrent increase in EPA assures ALA elongation and desaturation as proven by other studies.^{70,71} However, the intervention was ineffectual in imparting changes in plasma DHA, identical to observations of some other studies that used comparable or even higher doses of ALA.^{9,71-74} On the other hand, we observed a decrease in plasma fatty acid, palmitic acid, resulting in an overall reduction in total saturated fatty acid at treatment endpoint compared to that of control. A recent study that evaluated effects of ALA consumption on fatty acid composition of serum phospholipids in 81 overweight and obese patients found a similar decrease in palmitic acid and total saturated fatty acids.⁷⁵ ALA consumption may confer cardiovascular benefits; a meta-analysis including 27 studies suggests that each 1 g per day increased intake of ALA results in 10% lowered risk of chronic heart disease.³⁵

We observed large inter-individual variability in LDL-C responses which are in line with observations of previous studies utilizing phytosterols¹⁴. The baseline cholesterol levels and genetic polymorphisms affecting cholesterol trafficking inside the body are two potential possible reasons behind this variability.^{15,17,18} Interestingly, in our study we found no adverse responders in CYP7A1- GG group and all the adverse responders of the study foods had at least one CYP7A1 T allele. This supports the earlier observations by De Castro-Oros et al., MacKay et al. and Wang et al suggesting that G allele has positive associations with LDL-C lowering response to phytosterol and/or fiber.^{15,17,18,50} Although no significant effects of the genetic polymorphisms in CYP7A1 and APOE isoforms were observed in our study, it is noteworthy that the sampling size was not tailored for the examination of genetic associations. In particular, the CYP7A1- GG carriers were represented by only n=8 compared to n=21 for CYP7A1- TT carriers. Further, the present study utilized a combination of functional additives in 5 snack alternatives, adding more experimental noise to the result values. A targeted study on these SNPs in the future might help to reveal if G allele carriers could benefit more from this food intervention, compared to other heterogenetic groups, and hence predict LDL-C responses to a certain extent. It is noteworthy that even with these variabilities, 80% of the participants observed positive deviations in their lipid profile even with an intervention trial of 4 weeks.

4.6 STRENGTHS AND WEAKNESSES OF THE STUDY

To our knowledge, this is the first study that demonstrated the impact of portfolio functional additives in a statin reluctant population. One of the major strengths of our study was double blinding of foods, being packaged to look identical to the participant as well as researcher which helped to avoid bias. A run-in period of 4 weeks was another design strength that enabled avoidance of effects of statins or supplements affecting lipid profiles. Moreover, the dual centre

model not only eased participant recruitment, but also contributed to the heterogeneity in lipid profile responsiveness by representing Canadian as well as U.S populations.

Conversely, the free-living design of the study limits control over the background diet. Although expectations are that participants maintained a stable background diet throughout the study, there might have been variations in diet affecting tested outcomes. Another drawback is the use of varied food matrices in the study to provide alternatives assuring compliance. This could have affected the overall results. Although we aimed to correlate variations in LDL-C responses with CYP7A1 and APOE SNPs, the study sample size was likely insufficient for identification of differences in such responses. A targeted study in the future, with a balanced distribution of participants for the tested genetic alleles, is required to illuminate any associations of these SNPs with lipid profile responsiveness of the study foods.

4.7 CONCLUSION

Recommendations to the public to adopt a more whole-food diet must be supported by better availability of high-quality foods varieties that can measurably contribute to health while being highly palatable. In the present study, we found reductions in serum LDL-C by 8.80 ± 12.5 % and TC concentrations by 5.08 ± 8.24 % with the consumption of a portfolio of ready-to-eat bioactive foods compared to control. The clinical outcomes observed in our study help expand the breadth of options available for treatment of hyperlipidemia, especially benefitting the statin reluctant population.

5. GENERAL DISCUSSION AND CONCLUSION

Statins are the leading cardiovascular risk preventive drugs in clinical practice; however, statin intolerance and reluctance pose a great hindrance to effective treatment in patients afflicted with cardiovascular disease. About 20% of prescribed individuals experience some degree of intolerance, and 40–75% of patients discontinue their medication within 1–2 years after initiation of statin therapy.⁷⁶ Availability of alternative targeted nutrition interventions could be highly beneficial to these individuals and at least part of these populations could achieve targeted cholesterol levels through specific dietary changes.

Much of the North American population fails to eat a healthy diet conducive to heart health.⁷⁷ Added to this, the continued significant food consumption pattern outside the home reduces the likelihood that patients will make appropriate dietary decisions on their own or with general dietary education alone. Physician-derived advice platforms regarding diet provided are generally non-specific and the limited choices of healthy, pre-packaged foods that are clinically impactful for physicians to prescribe contribute to poor outcomes in dietary interventions. Thus, practical easy-to-use food items, which incorporate whole-food ingredients shown to favorably impact cholesterol levels without needing to replace all meals, represent an effective tangible dietary choice which physician would be able to “prescribe” to contribute to heart health of statin-reluctant patients. Thus, the significant reductions in LDL-C and TC levels achieved with 4 weeks consumption of a portfolio ready-to-eat bioactive foods can have extensive implications in cardiovascular risk prevention, especially in a statin reluctant population.

We have also observed significant increases in the plasma fatty acid ALA, recognizing the study foods as a potential source of this essential fatty acid. A concurrent EPA increase assures its

bioavailability transformation in the body. A significant decrease in palmitic acid and total saturated fatty acids were found in the study warranting the need for further targeted studies on the underlying mechanisms.

The genetic polymorphisms in CYP7A1 and APO-E isoforms might be affecting the variability in study food responses and a targeted study with a balanced distribution of participants for these genetic alleles in the future might be able to predict LDL-C responses to the study foods to a certain extent.

To conclude, even with these variabilities, we observed significant reductions in total cholesterol ($5.08 \pm 8.24\%$ or -0.28 ± 0.47 mmol/l) and LDL-C levels ($8.80 \pm 12.5\%$ or -0.29 ± 0.39 mmol/l) with 4 weeks study foods consumption compared to control at the endpoint.

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8. APPENDICES

8.1 ETHICS APPROVAL FORMS



UNIVERSITY
OF MANITOBA

BANNATYNE CAMPUS

Research Ethics Board

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

P126-770 Bannatyne Avenue
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PRINCIPAL INVESTIGATOR: Dr. Peter Jones	INSTITUTION/DEPARTMENT: U of M and Richardson Centre for Functional Foods and Nutraceuticals/Department of Food Sciences	ETHICS #: B2014:113
BREB MEETING DATE: October 27, 2014	APPROVAL DATE: November 26, 2014	EXPIRY DATE: October 27, 2015
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable): N/A		

PROTOCOL NUMBER:	PROJECT OR PROTOCOL TITLE: Validating the "Foods for Health" Portfolio of Functional Food Products: Effects on Lipid and Blood Glucose Management in Individuals Intolerant of Statin (HMG-CoA reductase inhibitor) Therapy
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: MB Agri-Health Research Network Inc., MB Jobs & the Economy, TM Therapeutics, StepOne Canada	

Submission Date(s) of Investigator Documents: October 6 and November 25, 2014	REB Receipt Date(s) of Documents: October 6 and November 25, 2014
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THE FOLLOWING ARE APPROVED FOR USE:

Document Name	Version(if applicable)	Date
Protocol:		
Protocol	2	November 25, 2014
Consent and Assent Form(s):		
Research Participant Information and Consent Form	2	November 25, 2014
Additional Research Participant Information and Consent Form for Genetic Analysis	2	November 25, 2014
Other:		
Case Report Form	2	November 25, 2014
Poster	2	November 25, 2014
Telephone Screening Form	1	October 6, 2014
Doctor Study Approval Form	1	October 6, 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 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

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 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 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,



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



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 and RCFFN/Food Sciences	ETHICS #: B2014:113 (HS18262)
BREB MEETING DATE (If applicable):		APPROVAL DATE: September 17, 2015
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER: NA	PROJECT OR PROTOCOL TITLE: Validating the "Foods for Health" Portfolio of Functional Food Products: Effects on Lipid and Blood Glucose Management in Individuals Intolerant of Statin (HMG-CoA reductase inhibitor) Therapy
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: MB Agri-Health Research Network Inc., MB Jobs & the Economy, TM Therapeutics, StepOne Canada	

REMINDER: THE CURRENT BREB APPROVAL FOR THIS STUDY EXPIRES: **October 27, 2015**

REVIEW CATEGORY OF AMENDMENT:	Full Board Review <input type="checkbox"/>	Delegated Review <input checked="" type="checkbox"/>
Submission Date of Investigator Documents: August 26 and September 17, 2015	BREB receipt date of Documents: September 8 and September 17, 2015	

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

Document Name	Version(if applicable)	Date
Protocol: Revised Protocol	V. 3	August 26, 2015
Consent and Assent Form(s): Research Participant Information and Consent Form	V. 4	September 17, 2015
Additional Research Participant Information and Consent Form for Genetics Analysis	V. 3	August 26, 2015
Other: Case Report Form	V. 3.0	August 26, 2015
Poster	V. 3	August 26, 2015
Telephone Screening Form	V. 2	August 26, 2015
Weekly Follow-Up Calls Script	V. 1	August 26, 2015
Product Use Log Blue Group	V. 1	August 26, 2015
Product Use Log Orange Group	V. 1	August 26, 2015
The American Heart Association's Diet and Lifestyle Recommendations	V. 1	August 26, 2015
Product Use Instructions: Blue Group	V. 1	August 26, 2015
Product Use Instructions: Orange Group	V. 1	August 26, 2015

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.

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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,



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



UNIVERSITY
OF MANITOBA

Research Ethics - Bannatyne
Office of the Vice-President (Research and International)

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 and RCFN/Food Sciences	ETHICS #: HS18262 (B2014:113)
BREB MEETING DATE (If applicable):		APPROVAL DATE: November 9, 2015
STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):		

PROTOCOL NUMBER: NA	PROJECT OR PROTOCOL TITLE: Validating the "Foods for Health" Portfolio of Functional Food Products: Effects on Lipid and Blood Glucose Management in Individuals Intolerant of Statin (HMG-CoA reductase inhibitor) Therapy
SPONSORING AGENCIES AND/OR COORDINATING GROUPS: MB Agri-Health Research Network Inc., MB Jobs and the Economy, TM Therapeutics, StepOne Canada	

REMINDER: THE CURRENT BREB APPROVAL FOR THIS STUDY EXPIRES: October 27, 2016

REVIEW CATEGORY OF AMENDMENT:	Full Board Review <input type="checkbox"/>	Delegated Review <input checked="" type="checkbox"/>
Submission Date of Investigator Documents: October 2, 2015	BREB receipt date of Documents: November 6, 2015	

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

Document Name	Version(if applicable)	Date
Protocol: Revised Protocol	V. 4	November 2, 2015
Consent and Assent Form(s): RCFFN Preliminary Trial Screening Consent	V. 1	November 2, 2015
Other: Case Report Form	V. 4	November 2, 2015
Telephone Screening Form	V. 3	November 2, 2015

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

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7. The UM BREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report**.

Sincerely,



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

8.2 RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: **Validating the “Foods for Health” Portfolio of Functional Food Products: Effects on Lipid and Blood Glucose Management in Individuals Intolerant of Statin (HMG-CoA reductase inhibitor) Therapy**

Investigator: Peter J.H. Jones, PhD
Richardson Centre for Functional Foods and Nutraceuticals
University of Manitoba
196 Innovation Drive
Winnipeg, Manitoba, Canada R3T 6C5
Phone: [REDACTED]

Sponsors: Manitoba Agri-Health Research Network Inc.
13-59 Scurfield Boulevard
Winnipeg, Manitoba, Canada R3Y 1V2

Manitoba Jobs and the Economy
1010-259 Portage Avenue
Winnipeg, Manitoba, Canada R3B 3P4

TM Therapeutics
13-59 Scurfield Blvd
Winnipeg, Manitoba Canada R3Y 1V2

StepOne Canada
c/o Campbell, Marr LLP
10 Donald Street
Winnipeg, Manitoba, Canada R3C 1L5

You are being asked to participate in a research study. Please take your time to review this Information and Consent Form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand. Should you decide to participate in this clinical study, please be advised that you are not allowed to participate in any other study for the duration of this clinical study. The study staff and institution are receiving professional fees and financial support to conduct this study.

Purpose of study

The main purpose of this study is to investigate the ability to affect cardiovascular (CVD) risk through a novel, easily implemented functional food-based approach in a statin intolerant group. Statin intolerant individuals include those who have side effects such as muscle aches, pains, weakness as well as liver abnormalities. The goal of the proposed project is to evaluate the changes in blood fat and sugar levels, as well as CVD biomarkers over a 4 week regimen using healthy tasty foods which will be self-selected by a patient group who are statin intolerant and /or statin unwilling. It is anticipated that consuming these food products will result in a favorable modification of blood fat, and sugar level, as well as CVD biomarkers alter body composition, specifically through the reduction of android (stomach) fat.

Study procedures

Pre-screening procedures

If you agree to take part in this study, you will be asked to give a fasting (nothing to eat or drink 12 hours before the test) blood sample (approximately two teaspoons) to measure your blood lipid levels and additional biochemistry parameters. In addition, we will measure your blood pressure and waist circumference.

Prior to beginning the study, the study staff will review medical history and ask questions to determine whether you are eligible to participate. Any change in your health status at any point during the study needs to be reported to the study investigators.

Study procedures

The study will consist of 2 phases of 30 days each during which you will consume your assigned treatment foods. Consumption of treatment foods will be from days 2 to 29. There will also be a washout period of 4 weeks between the 2 treatment phases where you can consume your habitual diets. The entire study is designed to take 12 weeks from start to completion. During each study phase, you will be provided with packaged, shelf-stable food products which will be in a single-serve format. These products will be ready-to-eat or required minimal preparation. You will be required to consume 2 items over the course of each day as a substitute for breakfast, as a snack or as part of a meal. We ask that you return any leftovers on day 30 of each phase.

The 2 phases of treatment will include:

- 1) Treatment phase: Treatment products will consist of foods such as oatmeal, pancakes, cranberry bars, chocolate bars, smoothies, and a sprinkle offering which can be added to almost any food to enhance its nutritional impact. All products are interchangeable in terms of their nutrients of interest and contain a minimum of 5 g of fibre, 1800 mg of omega-3 fatty acids, 1000 mg of phytosterols and 1800 µmol antioxidants per serving.
- 2) Control phase: Control products will be identical in type to the treatment products as mentioned above, similar in appearance and preparation requirements and will be matched for calories.

This study is a double-blind design, which means that neither you nor the clinical study staff will know which treatment you will be receiving in each phase. You will receive both treatments, however, it will be unknown the order you will be given in what phase. In the unlikely event of an emergency, this information will be made available.

During days 1, 2, 29 and 30 of each of the treatment phases of the trial, fasting blood samples (approximately 4 teaspoons will be taken on each blood draw day) will be obtained for assessment of blood fatty acid composition blood lipid profile, and blood glucose. Each blood test will take approximately 5 minutes.

On days 2 and 30 of each phase we will also measure your body weight, hip and waist circumference, take your blood pressure, and you will be asked to fill out gastro-intestinal tolerability questionnaires. Any change in your health status at any point during the study needs to be reported to the study investigators.

No alcoholic beverages are to be consumed within 48 hours prior to blood draws during the study periods. No caffeinated beverages consumption within 12 hours prior to blood draws during the study periods

Risks and discomforts

As with any clinical trial, there may be as yet unknown or unforeseen risks of taking part.

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, contact the study coordinator at [REDACTED] or the principal investigator at [REDACTED]

Benefits

You may not benefit from participation in this research; however, the study should contribute to a better understanding of the effect of combining specific food or food ingredients on blood fat and sugar levels as well as CVD biomarkers. You will also receive access to your test results when they become available.

Costs

All clinic and professional fees, diagnostic and laboratory tests that will be performed as part of this study are provided at no cost to you. There will be no cost for the study treatment that you will receive.

Remuneration for participation

You will receive up to a maximum of \$200.00 at completion of this study for your time and inconvenience of the study schedule. This amount will be divided into 2 portions. You will receive \$100 after completion of phase 1 and another \$100 after completion of phase 2. If you withdraw early from the study, you will receive an appropriate pro-rated fraction of this amount.

Alternatives

You are not obligated 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

Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. The RCFFN staff involved with your care may review/copy medical information that may reveal your identity. With your permission, the study staff will also write to your Family Doctor to tell him/her that you are taking part in a study or to obtain further medical information if needed. The Biomedical Research Ethics Board at the University of Manitoba may also review your research-related records for quality assurance purposes. If the results of the trial are published, your identity will remain confidential. Personal information such as your name, address, telephone number and/or any other identifying information will not leave the RCFFN. The Principal Investigator will maintain the codes linking your personal information under lock and key.

You will be assigned a participant code. The coding system of the study for participant identification will be a three-digit number. The three-digit number will be based on chronological order of participant selection. The identification codes corresponding to the study participants will be on the written documents which will only be available to the RCFFN staff.

Study samples will be stored in the freezer at the RCFFN. Only the study coordinators and the principal investigator will have access to the samples. Your samples will not be used for any additional analyses, nor stored for any longer than 5 years, nor shared with any other group, other than is indicated in the protocol, without your specific consent.

Voluntary participation/withdrawal from the study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision to not participate or to withdraw from the study will not affect your other medical care.

Your participation in this study may be terminated without your consent by the study coordinators, physician or principal investigator. The study staff will withdraw you if he/she feels that participation is no longer in your best interest, or if you fail to follow the directions of the study staff.

If you decide to participate, you will agree to cooperate fully with the study visit schedule, and will follow the study staff's instructions.

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study.

Should you wish to withdraw your participation from the study, you must inform the study coordinators so that your file can be officially close.

Medical care for injury related to 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 principal investigator or study coordinator or go to your nearest emergency room to receive necessary medical treatment. You are not waiving any of your legal rights by signing this consent form nor releasing the investigator or the sponsor from their legal and professional responsibilities. If any health abnormalities are identified in the clinical tests conducted during this experiment, the principal investigator or study coordinator 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 staff.

Investigator:	Dr. Peter Jones	Tel No.	██████████
Coordinator:	Soumya Alias	Tel No.	██████████

For questions about your rights as a research participant, you may contact:

The Biomedical Research Ethics Board, University of Manitoba at 204-789-3389

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

This study is registered on a publicly available Registry Databank at Clinicaltrials.gov. ClinicalTrials.gov is a website that provides information about federally and privately supported clinical trials. A description of this clinical trial will be available on <http://ClinicalTrials.gov>. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Consent

I agree to allow the principal investigator to inform my family doctor that I am participating in this study or to obtain information regarding my medical history.

Yes No

1. I have read and understood this Information and Consent Form, and I freely and voluntarily agree to take part in the clinical trial (research study) described above.
2. I understand that I will be given a copy of the signed and dated Information and Consent Form. I have received an explanation of the purpose and duration of the trial, and the potential risks and benefits that I might expect. I was given sufficient time and opportunity to ask questions and to reflect back my understanding of the study to study personnel. My questions were answered to my satisfaction.
3. I agree to cooperate fully with the study coordinator and the principal investigator; and will tell him if I experience any side effects, symptoms or changes in my health.
4. I am free to withdraw from the study at any time, for any reason, and without prejudice to my future medical treatment.
5. I have been assured that my name, address and telephone number will be kept confidential to the extent permitted by applicable laws and/or regulations.

6. By signing and dating this document, I am aware that none of my legal rights are being waived.

Signature: _____ Date: _____

Printed name of above: _____

I confirm that I have explained the purpose, duration etc. of this clinical trial, as well as any potential risks and benefits, to the participant whose name and signature appears above. I confirm that I believe that the participant has understood and has knowingly given their consent to participate by his/her personally dated signature.

Signature: _____ Date: _____

Printed name of above: _____ Study role: _____

ALL SIGNATORIES MUST DATE THEIR OWN SIGNATURE

8.3 ADDITIONAL RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM FOR GENETICS ANALYSIS

ADDITIONAL RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM FOR GENETICS ANALYSIS

Title of Study: **Validating the “Foods for Health” Portfolio of Functional Food Products: Effects on Lipid and Blood Glucose Management in Individuals Intolerant of Statin (HMG-CoA reductase inhibitor) Therapy**

Investigator: Peter J.H. Jones, PhD
Richardson Centre for Functional Foods and Nutraceuticals
University of Manitoba
196 Innovation Drive
Winnipeg, Manitoba, Canada R3T 6C5
Phone: [REDACTED]

Sponsors: Manitoba Agri-Health Research Network Inc.
13-59 Scurfield Boulevard
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1010-259 Portage Avenue
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TM Therapeutics
13-59 Scurfield Blvd
Winnipeg, Manitoba, Canada R3Y 1V2

StepOne Canada
c/o Campbell, Marr LLP
10 Donald Street
Winnipeg, Manitoba, Canada R3C 1L5

You are being asked to participate in a research study. Please take your time to review this Information and Consent Form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this clinical trial and you may discuss it with your regular doctor, friends and family. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand.

The study staff and institution are receiving professional fees and financial support to conduct this study.

Nature and duration of procedure

From the blood drawn during the clinical study as outlined in the Research Participant Information and Consent Form, we would like to extract metabolomics information and genetic information from your blood cells. The extracted genetic information will be used to perform analyses to 1) determine how your genetic makeup influences the efficiency of your body in responding to the portfolio of functional foods, and 2) determine how the intake of the portfolio of functional foods changes your gene expression profile. Genetic information which includes DNA and RNA are molecules found in the cells of your body and are organized into genes. These genes contain all of the information needed to make the proteins that perform specific biological functions in your body.

Confidentiality and safekeeping of biological samples containing genetic information

All of the information obtained about you and the results of the research will be treated confidentially. We will protect your confidentiality by assigning your samples containing genetic information a specific code. This code will link you to your samples containing genetic information and can only be decoded by the principal researcher or an individual authorized by the latter. Samples containing your genetic information will be kept at the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, under the supervision of Dr. Peter Jones for a 5-year period following the end of the research project. After this time, all samples will be destroyed. Your samples containing genetic information will only be used for the purpose of this research project.

Your participation and the results of the research will not appear in your medical record. Although the results of this study may be published or communicated in other ways, it will be impossible to identify you. Unless you have provided specific authorization or where the law permits or a court order has been obtained, your personal results will not be made available to third parties such as employers, government organizations, insurance companies, or educational institutions. This also applies to your spouse, other members of your family and your physician. However, for the purposes of ensuring the proper management of research, it is possible that a member of an ethics committee, a Health Canada representative, or a representative from the Richardson Centre for Functional Foods and Nutraceuticals may consult your research data and record. You can communicate with the research team to obtain information on the general progress or the results

of the research project. Project updates will be mailed at the end of the project. However, we will not communicate any individual results to you.

Potential risks and/or benefits

Receiving information regarding susceptibility to genetic disease or identification of blood relationships may cause distress. Genetic counselling will be available upon request.

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 contact the study coordinator or the principal investigator.

Investigator:	Dr. Peter Jones	Tel No.	██████████
Coordinator:	Soumya Alias	Tel No.	██████████

While there may be no direct benefits to you for taking part in these additional analyses, we hope that these results will provide us with the information on genetic characteristics of people in response to the intake of the portfolio of functional foods being consumed in this trial.

Signature of participant

The content of the procedure and procedures associated with it have been fully explained to me, and all experimental procedures have been identified. I have had the opportunity to ask questions concerning any and all aspects of the project and procedures involved, and may continue in the future to ask further questions at any time, as it is my right to do so. I am aware that I may refuse to participate as well as withdraw my consent at any time. I acknowledge that no guarantee or assurance has been given by anyone as to the results to be obtained and that my participation in this study is completely voluntary. I understand that although genetic testing is usually accurate, as with all testing some inaccuracies may occur. Also genetic testing is ongoing and new research may mean that the interpretation of the test results may change over time. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. Samples will not be utilized for any additional analyses, nor stored for any prolonged period, nor shared with any other group, other than is indicated in the protocol, without my specific consent.

I, _____, have read the above description. I have been made aware of all the procedures, advantages and disadvantages of the study, which have been explained to me.

Signature of Participant

Date

I confirm that I have explained the purpose, duration etc of this clinical trial, as well as any potential risks and benefits, to the participant whose name and signature appears above. I confirm that I believe that the participant has understood and has knowingly given their consent to participate by his/her personally dated signature.

Signature: _____ Date/Time: _____

Printed name of above: _____ Study role: _____

8.5 TELEPHONE SCREENING FORM

Participant code:	Date of call:
Eligible for study: Y or N	Reason if not eligible:
Name: _____	
Telephone number: (home): _____	(other): _____
Email: _____	
Age: _____	Birth Date (Y/M/D): _____
Weight: _____ kg (2.2 lb/kg)	BMI: _____ kg/m ² (exclude of > 35)
Height: _____ m (2.54 cm/in)	
Smoker: Y or N	

Medial History:

Have you been part of any clinical studies within past 1 month? Y or N

Do you have high blood cholesterol? Y or N

Are you statin intolerant / statin unwilling? Y or N

Have you ever taken any cholesterol-lowering, TG lowering/blood lipid lowering medications? (frequency and length of time): Y or N

If yes, are you able and willing to temporarily hold all statin/lipid-lowering therapies including supplements throughout the study period? Y or N

Are you taking any other prescription medication? (frequency and length of time): (Systemic antibodies, corticosteroids, androgens or phenytoin) Y or N

Have you been diagnosed with any medical conditions (i.e., rheumatoid arthritis or other chronic rheumatologic condition, known chronic liver or renal disease, diabetes, inflammatory bowel disease, celiac disease, uncontrolled thyroid disease, hormonal supplementation, pancreatitis, gallbladder or biliary disease, neurological/psychological disease, and gastrointestinal disorders that could interfere with fat absorption)?

Y or N

(If yes, specify and mention from when?)

Do you have a history of advanced cardiovascular disease (moderate or greater valvular disease, history congestive heart failure, known coronary artery disease), history of dysrhythmias requiring medical or surgical intervention?

Y or N

Do you have uncontrolled hypertension having systolic blood pressure >150 mm Hg or diastolic blood pressure >100 mm Hg? Y or N

Have you been diagnosed with cancer (i.e., non-skin cancer, melanoma, etc.)? Y or N
If yes, occurrence or any therapy within past 1 year?

Do you have any other underlying health issues? Y or N

(Only if female)

Pregnant, breastfeeding or planning to become pregnant during the course of this trial? Y or N
Postmenopausal? Y or N

Diet:

Have you taken lipid-lowering supplements (e.g., omega-3 supplements, plant sterols/stanols foods or supplements, fiber, etc.) within last 6 months? Y or N

Do you consume alcohol or have a history of alcoholism? (exclude if > 2 alcoholic drink/day) Y or N

Fiber or stimulant laxatives? (exclude if >2 dose/day) Y or N

Are you taking vitamin supplements or any natural health/herbal/food supplements? (units/day) (Multi, Vit E, Vit A, beta-carotene, etc.) Y or N

Allergies (food or medication):

Food intolerances:

Do you follow a special diet:

Vegetarian: Y or N

Note: Participants with any food allergies/intolerances, food restrictions due to medical, religious or philosophical reason - including Kosher, vegan, vegetarian, high protein, low-carbohydrate, low-phosphorus, etc. other than attempting to follow an eating plan as generally advocated by the American Heart Association (low sodium, low cholesterol, reduced fat, etc.) will be excluded.

Do you a history of eating disorders? Y or N

Eligible for study: Y or N	Participant Code:
-----------------------------------	--------------------------

8.6 WEEKLY CALL SCRIPTS

Weekly Follow-up calls:

Participant No: _____

Please call the participant once a week and fill the details below:

Date (dd/MON/yyyy): __ __ / __ __ __ / __ __ __ __ Assessed by: _____

1. Did you have an adverse event during the last week? Yes No

If "Yes" fill out adverse event form

2. Did you consume the study food products according to the instructions? Yes No

3. In the past week has your exercise level changed? Yes No

If Yes, was it: More Active Less Active No Exercise

4. Have you taken any prescription or non-prescription drugs in the past week? Yes No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____

5. Have you taken any vitamins, minerals or other supplements in the past week? Yes No

If Yes, specify:

Name	Dosage	Frequency	Reason	Start Date	End Date
_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____

6. Have you had any changes in a medical condition, new illness or injury in the past week?

Yes No

If Yes, describe: _____

7. Comments on study foods: _____

8. Any other

Comments: _____

Please remind the participant the following instructions:

- **Please make sure to consume 2 items over the course of each day as a substitute for breakfast, as a snack, or as a component of a main meal.**
- **Fill out the product use log (checklist) on the amount and type of products consumed.**
- **If you forgot to consume an item for the day and notice the omission the day after, do not take the forgotten item and continue with the foods of the day where you are.**
- **Except for this dietary inclusion, you must keep the same eating habits and physical activity wherever possible.**
- **You can consume a maximum of two serving/drink of alcohol per day (beer, glass of wine or 1 oz liquor). Low-alcohol beer (0.5%) is permitted.**
- **Dietary supplements and natural health products such as fish oil Omega- 3 or Omega-6 supplements are forbidden.**

Action points

- Remind the participant the next visit date and hour.
- Fasting 12 hours before the blood sampling and no alcohol the 2 days preceding blood sampling.
- Remind the participant to bring the product use log (checklists) at your next visit to the clinic.

8.7 THE AMERICAN HEART ASSOCIATION'S DIET AND LIFESTYLE RECOMMENDATIONS

The American Heart Association's Diet and Lifestyle Recommendations

A healthy diet and lifestyle are your best weapons to fight cardiovascular disease. It's not as hard as you may think! Remember, it's the overall pattern of your choices that counts. Make the simple steps below part of your life for long-term benefits to your health and your heart.

Use up at least as many calories as you take in.

Start by knowing how many calories you should be eating and drinking to maintain your weight. Nutrition and calorie information on food labels is typically based on a 2,000 calorie diet. You may need fewer or more calories depending on several factors including age, gender, and level of physical activity.

If you are trying not to gain weight, don't eat more calories than you know you can burn up every day.

Increase the amount and intensity of your physical activity to match the number of calories you take in.

Aim for at least 150 minutes of moderate physical activity or 75 minutes of vigorous physical activity – or an equal combination of both – each week.

Regular physical activity can help you maintain your weight, keep off weight that you lose and help you reach physical and cardiovascular fitness. If it's hard to schedule regular exercise sessions, try aiming for sessions of at least 10 minutes spread throughout the week.

If you would benefit from lowering your blood pressure or cholesterol, the American Heart Association recommends 40 minutes of aerobic exercise of moderate to vigorous intensity three to four times a week.

Eat a variety of nutritious foods from all the food groups.

You may be eating plenty of food, but your body may not be getting the nutrients it needs to be healthy. Nutrient-rich foods have minerals, protein, whole grains and other nutrients but are lower in calories. They may help you control your weight, cholesterol and blood pressure.

Eat an overall healthy dietary pattern that emphasizes:

a variety of fruits and vegetables,

whole grains,

low-fat dairy products,

skinless poultry and fish

nuts and legumes

non-tropical vegetable oils

Limit saturated fat, trans fat, sodium, red meat, sweets and sugar-sweetened beverages. If you choose to eat red meat, compare labels and select the leanest cuts available.

One of the diets that fits this pattern is the DASH (Dietary Approaches to Stop Hypertension) eating plan. Most healthy eating patterns can be adapted based on calorie requirements and personal and cultural food preferences.

Eat less of the nutrient-poor foods.

The right number of calories to eat each day is based on your age and physical activity level and whether you're trying to gain, lose or maintain your weight. You could use your daily allotment of calories on a few high-calorie foods and beverages, but you probably wouldn't get the nutrients your body needs to be healthy. Limit foods and beverages high in calories but low in nutrients. Also limit the amount of saturated fat, trans fat and sodium you eat. Read Nutrition Facts labels carefully — the Nutrition Facts panel tells you the amount of healthy and unhealthy nutrients in a food or beverage.

As you make daily food choices, base your eating pattern on these recommendations:

Eat a variety of fresh, frozen and canned vegetables and fruits without high-calorie sauces or added salt and sugars. Replace high-calorie foods with fruits and vegetables.

Choose fiber-rich whole grains for most grain servings.

Choose poultry and fish without skin and prepare them in healthy ways without added saturated and trans fat. If you choose to eat meat, look for the leanest cuts available and prepare them in healthy and delicious ways.

Eat a variety of fish at least twice a week, especially fish containing omega-3 fatty acids (for example, salmon, trout and herring).

Select fat-free (skim) and low-fat (1%) dairy products.

Avoid foods containing partially hydrogenated vegetable oils to reduce trans fat in your diet.

Limit saturated fat and trans fat and replace them with the better fats, monounsaturated and polyunsaturated. If you need to lower your blood cholesterol, reduce saturated fat to no more than 5 to 6 percent of total calories. For someone eating 2,000 calories a day, that's about 13 grams of saturated fat.

Cut back on beverages and foods with added sugars.

Choose foods with less sodium and prepare foods with little or no salt. To lower blood pressure, aim to eat no more than 2,400 milligrams of sodium per day. Reducing daily intake to 1,500 mg is desirable because it can lower blood pressure even further. If you can't meet these goals right now, even reducing sodium intake by 1,000 mg per day can benefit blood pressure.

If you drink alcohol, drink in moderation. That means no more than one drink per day if you're a woman and no more than two drinks per day if you're a man.

Follow the American Heart Association recommendations when you eat out, and keep an eye on your portion sizes.

Also, don't smoke tobacco — and avoid secondhand smoke.

8.8 PRODUCT USE INSTRUCTION SHEETS

Product Use Instructions: Blue Group

Eat 1 (one) serving of your chosen study food twice (2 times) per day **AS A SUBSTITUTION** for a meal or snack you would have otherwise consumed. You can mix and match the foods as you like.

It is best to spread out the study foods at least 4 hours apart over the course of a day (one food serving for breakfast, one as an afternoon snack, for example). However, it is more important to make sure you eat two servings of the foods assigned each day than to worry about the time between servings. So, if you forget to have a serving, it is better to makeup the serving at the next meal (even if that means doubling up on the study foods) than to skip a serving altogether.

Log your study food intake on the provided log sheets every day.

Product	Preparation Instructions
Study Fruit Bar	Open pouch and enjoy.
Study Chocolate Bar	Open pouch and enjoy.
Study Food Sprinkle	Open pouch and sprinkle onto 8 ounces of your favorite yogurt.
Study Smoothie	Place $\frac{1}{4}$ cup cold water into a blender. Add contents of smoothie packet and $\frac{1}{2}$ cup of your favorite yogurt drink or kefir. Blend until smooth.
Study Pancake	Empty packet contents into a small bowl and add $\frac{1}{3}$ cup water. Stir until just combined. Batter will be slightly lumpy (do not overmix). Let mixture rest while you heat griddle to 375°F (medium heat). Spray griddle with cooking spray and pour batter onto griddle dividing into 3 pancakes. Make sure top bubbles before flipping. Cook until golden brown, about 1-1 $\frac{1}{2}$ minutes per side.
Study Oatmeal	Empty packet contents into a small bowl and add $\frac{1}{3}$ cup boiling water. Stir and let sit for 1 minute.

May contain one or more of the following allergens: EGGS, MILK, PEANUTS, SOY BEANS, TREE NUTS, WHEAT

Product Use Instructions: Orange Group

Eat 1 (one) serving of your chosen study food twice (2 times) per day **AS A SUBSTITUTION** for a meal or snack you would have otherwise consumed. You can mix and match the foods as you like.

It is best to spread out the study foods at least 4 hours apart over the course of a day (one food serving for breakfast, one as an afternoon snack, for example). However, it is more important to make sure you eat two servings of the foods assigned each day than to worry about the time between servings. So, if you forget to have a serving, it is better to make up the serving at the next meal (even if that means doubling up on the study foods) than to skip a serving altogether.

Log your study food intake on the provided log sheets every day.

Product	Preparation Instructions
Study Fruit Bar	Open pouch and enjoy.
Study Chocolate Bar	Open pouch and enjoy.
Study Food Sprinkle	Open pouch and sprinkle onto 8 ounces of your favorite yogurt.
Study Smoothie	Place ¼ cup cold water into a blender. Add contents of smoothie packet and ½ cup of your favorite yogurt drink or kefir. Blend until smooth.
Study Pancake	Empty packet contents into a small bowl and add 1/3 cup water. Stir until just combined. Batter will be slightly lumpy (do not overmix). Let mixture rest while you heat griddle to 375°F (medium heat). Spray griddle with cooking spray and pour batter onto griddle dividing into 3 pancakes. Make sure top bubbles before flipping. Cook until golden brown, about 1-1 ½ minutes per side.
Study Oatmeal	Empty packet contents into a small bowl and add 1/3 cup boiling water. Stir and let sit for 1 minute.

May contain one or more of the following allergens: EGGS, MILK, PEANUTS, SOY BEANS, TREE NUTS, WHEAT

8.9 PRODUCT ACCOUNTABILITY LOGS

Participant #: _____ **Product** Disbursement **Date:** _____

Product Use Log Blue Group

Instructions: Please log the date and product use each day.

Date	Serving 1	Serving 2
Day 1 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 2 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 3 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 4 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 5 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 6 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 7 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 8 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 9 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving

Date	Serving 1	Serving 2
Day 10 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 11 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 12 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 13 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 14 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 16 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 17 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 18 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 19 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 20 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 21 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving

Date	Serving 1	Serving 2
Day 22 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 23 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 24 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 25 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 26 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 27 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 28 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 29 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 30 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 31 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 32 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving

Date	Serving 1	Serving 2
Day 33 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 34 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 35 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 36 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 37 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 38 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 39 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 40 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 41 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 42 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving

Participant #: _____ **Product** Disbursement **Date:** _____

Product Use Log Orange Group

Instructions: Please log the date and product use each day.

Date	Serving 1	Serving 2
Day 1 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 2 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 3 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 4 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 5 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 6 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 7 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 8 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 9 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving

Date	Serving 1	Serving 2
Day 10 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 11 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 12 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 13 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 14 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 16 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 17 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 18 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 19 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 20 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 21 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving

Date	Serving 1	Serving 2
Day 22 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 23 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 24 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 25 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 26 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 27 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 28 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 29 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 30 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 31 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 32 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving

Date	Serving 1	Serving 2
Day 33 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 34 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 35 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 36 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 37 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 38 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 39 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 40 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 41 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving
Day 42 Date:	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving	<input type="checkbox"/> Ate full serving <input type="checkbox"/> Ate partial serving <input type="checkbox"/> Missed serving

8.10 CASE REPORT FORM

Richardson Centre for Functional Foods and Nutraceuticals

Validating the “Foods for Health” Portfolio of Functional Food Products: Effects on Lipid and Blood Glucose Management in Individuals Intolerant of Statin (HMG-CoA reductase inhibitor) Therapy

Case Report Form

Version: 4.0

Release Date: November 2, 2015

Participant Code: _____

General CRF:

Visit #			1, 2	3, 4		5, 6	7,8
Study week	Screening	4 wk wash out	0 (± 0 d)	4 (+ 5 / -2 d)	4 wk wash out	9 (+5 / -2 d)	12 (+5 / -2d)
Informed Consent, Demographic Information, Inclusion/Exclusion Criteria, and Medical History	+						
Vital Signs (Body Weight, BMI, Blood Pressure)	+		+	+		+	+
Concomitant Medications	+		+	+		+	+
Fatty Acid Composition in Plasma			+	+		+	+
Serum Lipid Profile (TG, TC, LDL-C, HDL-C), and Glucose	+		+	+		+	+
Serum Insulin and hsCRP			+	+		+	+
Gastrointestinal (GI) Tolerability Questionnaires			+	+		+	+
Treatment Dispensation			+			+	
Treatment Accountability				+			+
Treatment Checklists				+			+
Adverse Events				+			+
Study Termination							+

Screening

4. Exclusion Criteria:

		YES	NO	COMMENT
4.1	Unable to speak/read in English			
4.2	Unable or unwilling to temporarily hold all statin/lipid lowering therapies including supplements throughout the study period			
4.3	Diabetic on oral hypoglycemic agents and/or insulin			
4.4	Severe obesity, i.e., body mass index (BMI; in kg/m ²) >35			
4.5	Currently pregnant or planning to become pregnant during the study period.			
4.6	Participants with any food allergies/intolerances, food restrictions due to medical, religious or philosophical reason - including Kosher, vegan, vegetarian, high protein, low-carbohydrate, low-phosphorus, etc. other than attempting to follow an eating plan as generally advocated by the American Heart Association (low sodium, low cholesterol, reduced fat, etc.)			
4.7	Baseline fasting: <ul style="list-style-type: none"> LDL-cholesterol (LDL-C) < 100 mg/dL or ≥ 190 mg/dL (or evidence of tissue cholesterol deposition), or triglycerides (TG) ≥ 400 mg/dL, or • glucose >126 mg/dL 			Complete following Lab analysis results (item 7)
4.8	Consumption of more than 2 alcoholic drinks/day or history of alcoholism or drug dependence.			
4.9	Smokers (tobacco products for the last 6 months).			
4.10	History of non-skin cancer, history of melanoma, history of rheumatoid arthritis or other chronic rheumatologic condition, history of advanced cardiovascular disease (moderate or greater valvular disease, history congestive heart failure, known coronary artery disease, history dysrhythmias requiring medical or surgical intervention), , known chronic liver or renal disease, diabetes, inflammatory bowel disease, celiac disease, uncontrolled thyroid disease, hormonal supplementation (other than thyroid), pancreatitis, gallbladder or biliary disease, neurological/psychological disease, and gastrointestinal disorders that could interfere with fat absorption			
4.11	Uncontrolled hypertension having systolic blood pressure >150 mm Hg or diastolic blood pressure >100 mm Hg			
4.12	Consume or planning to consume lipid lowering medications or supplements.			
4.13	Use of any experimental medication within 1 month prior to screening or as concomitant medications.			

To meet exclusion criteria, ALL items must be “NO”

5. Anthropometrics

5.1 Body weight (kg): _____

5.2 Height (m): _____

5.3 BMI (kg/m²): _____

5.4 Waist girth 1: _____ 5.7 Hip girth 1: _____

5.5 Waist girth 2: _____ 5.8 Hip girth 2: _____

5.6 Waist girth 3: _____ 5.9 Hip girth 3: _____

5.10 Ave. waist girth (cm): _____ **5.11 Ave. hip girth (cm):** _____

5.12 Waist/hip ratio: _____

Completed by: _____

6. Blood Pressure (after 10 minutes of rest)

6.1 Arm: Left Right

6.2 Type of device: Automatic Mercury

6.3 Reading 1 systolic BP (mmHg): _____ 6.4 Reading 1 diastolic BP (mmHg): _____

6.5 Reading 2 systolic BP (mmHg): _____ 6.6 Reading 2 diastolic BP (mmHg): _____

6.7 Reading 3 systolic BP (mmHg): _____ 6.8 Reading 3 diastolic BP (mmHg): _____

6.9 Ave. Systolic BP (mmHg): _____ **6.10 Ave. diastolic BP (mmHg):** _____

6.11 Heart rate (beats/min): _____

Completed by: _____

Comments _____

7. Lipids Profile

7.1 Fasting blood samples collected Yes No

7.2 When did you last eat (hour)? ____ : ____

7.3 Time the blood sample was taken (hour): ____ : ____

7.4 LDL-C levels: _____ (mg/dL)

7.5 TG levels: _____ (mg/dL)

7.6 Blood glucose levels: _____ (mg/dL)

Please complete page 2 item 4.7 accordingly

Please document the blood results output in the patient file

8. Does the participant use any concomitant medication and/or supplement YES NO If yes, please specify:

	MEDICATION / SUPPLEMENT	INDICATION*	ROUTE ^A	DOSE	FREQ ^B	DATE STARTED (dd/MON/yyyy)	DATE STOPPED (dd/MON/yyyy)	ONGOING
8.1								<input type="checkbox"/>
8.2								<input type="checkbox"/>
8.3								<input type="checkbox"/>
8.4								<input type="checkbox"/>
8.5								<input type="checkbox"/>
8.6								<input type="checkbox"/>
8.7								<input type="checkbox"/>

* If a new indication, please complete Adverse Event form **A-Route:** 1=oral; 2=intravenous; 3=subcutaneous; 4=topical; 5=inhalation; 6=transdermal; 7=rectal; 8=intramuscular **B-Frequency:** 1=once daily; 2=twice daily; 3=thrice daily; 4=four times daily; 5=once weekly; 6=as needed

Participant is eligible for trial Yes No

Investigator: _____ Date: ___ / ___ / ___ (dd/MON/yyyy)

Checklist

- Informed Consent Form
- Demographic Details
- Inclusion/ Exclusion Criteria
- Anthropometrics
- Blood Pressure
- Blood samples (lipids profile)
- Concomitant medications

Study logs check list:

- ICF log
- Screening log

Visit 1

Visit 1

1. Date of Visit:

___/___/___ (dd/MON/yyyy)

2. Anthropometrics

2.1 Body weight (kg): _____

2.2 Height (m): _____

2.3 BMI (kg/m²): _____

2.4 Waist girth 1: _____ 2.7 Hip girth 1: _____

2.5 Waist girth 2: _____ 2.8 Hip girth 2: _____

2.6 Waist girth 3: _____ 2.9 Hip girth 3: _____

2.10 Ave. waist girth (cm): _____ 2.11 Ave. hip girth (cm): _____

2.12 Waist/hip ratio: _____

Completed by: _____

3. Blood Pressure (after 10 minutes of rest)

3.1 Arm: Left Right

3.2 Type of device: Automatic Mercury

3.3 Reading 1 systolic BP (mmHg): _____ 3.4 Reading 1 diastolic BP (mmHg): _____

3.5 Reading 2 systolic BP (mmHg): _____ 3.6 Reading 2 diastolic BP (mmHg): _____

3.7 Reading 3 systolic BP (mmHg): _____ 3.8 Reading 3 diastolic BP (mmHg): _____

3.9 Avg. Systolic BP (mmHg): _____ 3.10 Ave. diastolic BP (mmHg): _____

3.11 Heart rate (beats/min): _____

Completed by: _____

Comments _____

4. Medication and/or Supplement Yes No

If yes, please specify:

	MEDICATION / SUPPLEMENT	INDICATION*	ROUTE ^A	DOSE	FREQ ^B	DATE STARTED (dd/MON/yyyy)	DATE STOPPED (dd/MON/yyyy)	ONGOING
4.1								<input type="checkbox"/>
4.2								<input type="checkbox"/>
4.3								<input type="checkbox"/>
4.4								<input type="checkbox"/>
4.5								<input type="checkbox"/>
4.6								<input type="checkbox"/>
4.7								<input type="checkbox"/>

* If a new indication, please complete Adverse Event form **A-Route:** 1=oral; 2=intravenous; 3=subcutaneous; 4=topical; 5=inhalation; 6=transdermal; 7=rectal; 8=intramuscular **B-Frequency:** 1=once daily; 2=twice daily; 3=thrice daily; 4=four times daily; 5=once weekly; 6=as needed

Date: ___ / ___ / ___ (dd/MON/yyyy)

5. Blood Samples

5.1 Fasting blood samples collected Yes No

5.2 When did you last eat (hour)? ___ : ___

5.3 Time the blood sample was taken (hour): ___ : ___

Please verify that the blood samples are labeled with subject no., date and hour.

Checklist

- Anthropometrics
- Blood Pressure
- Concomitant Medication/ Supplements
- Blood Samples

Study logs check list:

- Enrollment log
- Patient ID log
- Randomization log

Visit 2

Visit 2

1. **Date of Visit:** (dd/MON/yyyy)

2. Blood Samples

2.1 Fasting blood samples collected Yes No

2.2 When did you last eat (hour)? ____ ____ : ____ ____

2.3 Time the blood sample was taken (hour): ____ ____ : ____ ____

Please verify that the blood samples are labeled with subject no., date and hour.

3. Study Product Dispensation

3.1 Dispensation date : (dd/MON/yyyy)

3.2 Randomization number: _____

***The participant must be instructed to return study product package
(including all remaining food items)***

Comments:

Next schedule visit (4 weeks \pm 5 days): ____ / ____ / ____ (dd/MON/yyyy)

Checklist

- Blood Samples
- Study Product Dispensation

Study logs check list:

- Accountability log

Visit 3

Visit 3

1. Date of Visit:

___ / ___ / ___ (dd/MON/yyyy)

2. Blood Samples

2.1 Fasting blood samples collected Yes No

2.2 When did you last eat (hour)? ___ : ___

2.3 Time the blood sample was taken (hour): ___ : ___

Please verify that the blood samples are labeled with subject no., date and hour.

Checklist

Blood Samples

Visit 4

Visit 4

1. Date of Visit: __ __/__ __ __/__ __ __ __ (dd/MON/yyyy)

2. Adverse Events

2.1 Did the participant have any adverse event(s) since the last visit? Yes No

If yes, fill out “adverse events” form

3. Severe Adverse Events

3.1 Did the participant suffer from any severe adverse event(s) since the last visit? Yes No

If yes, fill out “Severe adverse events” form and make sure the proper documents are filed in the CRF

4. Concomitant Medications/Supplements

4.1 Has the participant started or ceased consuming concomitant medications or supplements since the last visit? Yes No

If yes, fill in the concomitant medications form

5. Anthropometrics

5.1 Body weight (kg): _____

5.2 Height (m): _____

5.3 BMI (kg/m²): _____

5.4 Waist girth 1: _____

5.7 Hip girth 1: _____

5.5 Waist girth 2: _____

5.8 Hip girth 2: _____

5.6 Waist girth 3: _____

5.9 Hip girth 3: _____

5.10 Ave. waist girth (cm): _____

5.11 Ave. hip girth (cm): _____

5.12 Waist/hip ratio: _____

Completed by: _____

6. Blood Pressure (after 10 minutes of rest)

6.1 Arm: Left Right

6.2 Type of device: Automatic Mercury

6.3 Reading 1 systolic BP (mmHg): _____ 6.4 Reading 1 diastolic BP (mmHg): _____

6.5 Reading 2 systolic BP (mmHg): _____ 6.6 Reading 2 diastolic BP (mmHg): _____

6.7 Reading 3 systolic BP (mmHg): _____ 6.8 Reading 3 diastolic BP (mmHg): _____

6.9 Avg. Systolic BP (mmHg): _____ **6.10 Ave. diastolic BP (mmHg):** _____

6.11 Heart rate (beats/min): _____

Completed by: _____

Comments _____

7. Gastrointestinal (GI) Tolerability Questionnaire

Participant Code _____

Study Phase _____

Treatment (i.e., **Orange, Blue**) _____

Start Date _____

End Date _____

Indicate if you experienced GI symptoms listed below over the last four weeks and if so, what was the severity (on a scale of 0 to 3, i.e., 0 = none, 1 = mild, 2 = moderate, 3 = severe).

GI symptoms

Severity

None (0) Mild (1) Moderate (2) Severe (3)

7.1 Hiccup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.2 Nausea	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.3 Vomiting	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.4 Indigestion	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.5 Stomach or abdominal pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.6 Constipation	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.7 Diarrhea	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.8 Flatulence (Gas)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.9 Abdominal bloating	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.10 Cramping	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.11 Heartburn	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.12 Other (please describe) _____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

8. Well-being Questionnaire

Participant Code _____

Study Phase _____

Treatment (i.e., **Orange, Blue**) _____

Start Date _____

End Date _____

Please rate the degree of improvement (if any) in your memory, concentration and mood after using the food items since the last visit (on a scale of 0 to 3, i.e., 0 = none, 1 = minor, 2 = moderate, 3 = considerable).

8.1 Degree of improvement

None (0) Minor (1) Moderate (2) Considerable (3)

8.1.1 Memory

8.1.2 Concentration

8.1.3 Mood

8.2. Degree of worsening

None (0) Minor (1) Moderate (2) Considerable (3)

8.2.1 Memory

8.2.2 Concentration

8.2.3 Mood

9. Food Items Accountability

9.1 Product Reconciliation Product Group: Orange Blue

<u>Product</u>	<u>Number Servings Provided</u>	<u>Number Servings Returned</u>	<u>Number Servings Consumed</u>
9.1.1 Fruit Bar	20		
9.1.2 Chocolate Bar	20		
9.1.3 Sprinkle	20		
9.1.4 Smoothie	20		
9.1.5 Pancake Mix	20		
9.1.6 Oatmeal	20		
9.1.7 Total	120		

9.2.1 Number of returned food items: _____

9.2.2 Number of days that the study product was not taken: _____

10. Food Items Acceptability

	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
10.1. Using this eating system was as simple as taking a medication					
10.2. I prefer using food rather than medications to manage my cholesterol					

11. Blood Samples

11.1 Fasting blood samples collected Yes No

11.2 When did you last eat (hour)? ____ : ____

11.3 Time the blood sample was taken (hour): ____ : ____

Please verify that the blood samples are labeled with subject no., date and hour.

Comments:

Next schedule visit (4 weeks +5/-2 days): ____ / ____ / ____ (dd/MON/yyyy)

Checklist

- Adverse Events
- Concomitant Medications/Supplements
- Anthropometrics
- Blood Pressure
- Gastrointestinal Tolerability Questionnaire
- Well-Being Questionnaire
- Food Items Accountability
- Food Items Acceptability
- Blood Samples

Study logs check list:

- Accountability log

Visit 5

Visit 5

1. Date of Visit

___/___/___ (dd/MON/yyyy)

2. Anthropometrics

2.1 Body weight (kg): _____

2.2 Height (m): _____

2.3 BMI (kg/m²): _____

2.4 Waist girth 1: _____ 2.7 Hip girth 1: _____

2.5 Waist girth 2: _____ 2.8 Hip girth 2: _____

2.6 Waist girth 3: _____ 2.9 Hip girth 3: _____

2.10 Ave. waist girth (cm): _____ 2.11 Ave. hip girth (cm): _____

2.12 Waist/hip ratio: _____

Completed by: _____

3. Blood Pressure (*after 10 minutes of rest*)

3.1 Arm: Left Right

3.2 Type of device: Automatic Mercury

3.3 Reading 1 systolic BP (mmHg): _____ 3.4 Reading 1 diastolic BP (mmHg): _____

3.5 Reading 2 systolic BP (mmHg): _____ 3.6 Reading 2 diastolic BP (mmHg): _____

3.7 Reading 3 systolic BP (mmHg): _____ 3.8 Reading 3 diastolic BP (mmHg): _____

3.9 Avg. Systolic BP (mmHg): _____ 3.10 Ave. diastolic BP (mmHg): _____

3.11 Heart rate (beats/min): _____

Completed by: _____

Comments _____

4. Medication and/or Supplement Yes No

If yes, please specify:

	MEDICATION / SUPPLEMENT	INDICATION*	ROUTE^A	DOSE	FREQ^B	DATE STARTED (dd/MON/yyyy)	DATE STOPPED (dd/MON/yyyy)	ONGOING
4.1								<input type="checkbox"/>
4.2								<input type="checkbox"/>
4.3								<input type="checkbox"/>
4.4								<input type="checkbox"/>
4.5								<input type="checkbox"/>
4.6								<input type="checkbox"/>
4.7								<input type="checkbox"/>

* If a new indication, please complete Adverse Event form **A-Route:** 1=oral; 2=intravenous; 3=subcutaneous; 4=topical; 5=inhalation; 6=transdermal; 7=rectal; 8=intramuscular **B-Frequency:** 1=once daily; 2=twice daily; 3=thrice daily; 4=four times daily; 5=once weekly; 6=as needed

Physician: _____ **Date:** ___ / ___ / ___ (dd/MON/yyyy)

5. Blood Samples

5.1 Fasting blood samples collected Yes No

5.2 When did you last eat (hour)? ____ ____ : ____ ____

5.3 Time the blood sample was taken (hour): ____ ____ : ____ ____

Please verify that the blood samples are labeled with subject no., date and hour.

Checklist

- Anthropometrics
- Blood Pressure
- Concomitant Medication/ Supplements
- Blood Samples

Visit 6

Visit 6

1. **Date of Visit:** (dd/MON/yyyy)

2. Blood Samples

2.1 Fasting blood samples collected Yes No

2.2 When did you last eat (hour)? ____ ____ : ____ ____

2.3 Time the blood sample was taken (hour): ____ ____ : ____ ____

Please verify that the blood samples are labeled with subject no., date and hour.

3. Study Product Dispensation

3.1 Dispensation date :
____ / ____ / ____ (dd/MON/yyyy)

3.2 Randomization number: _____

***The participant must be instructed to return study product package
(including all remaining food items)***

Comments:

Next schedule visit (4 weeks +5/-2 days): ____ / ____ / ____ (dd/MON/yyyy)

Checklist

- Blood Samples
- Study Product Dispensation

Study logs check list:

- Accountability log

Visit 7

Visit 7

1. Date of Visit:

___/___/___ (dd/MON/yyyy)

2. Blood Samples

2.1 Fasting blood samples collected Yes No

2.2 When did you last eat (hour)? ___ : ___

2.3 Time the blood sample was taken (hour): ___ : ___

Please verify that the blood samples are labeled with subject no., date and hour.

Checklist

Blood Samples

Visit 8

Visit 8

1. Date of Visit:

___/___/___ (dd/MON/yyyy)

2. Adverse Events

2.1 Did the participant have any adverse event(s) since the last visit? Yes No

If yes, fill out “adverse events” form

3. Severe Adverse Events

3.1 Did the participant suffer from any severe adverse event(s) since the last visit? Yes No

If yes, fill out “Severe adverse events” form and make sure the proper documents are filed in the CRF

4. Concomitant Medications/Supplements

4.1 Has the participant started or ceased consuming concomitant medications or supplements since the last visit? Yes No

If yes, fill in the concomitant medications form

5. Anthropometrics

5.1 Body weight (kg): _____

5.2 Height (m): _____

5.3 BMI (kg/m²): _____

5.4 Waist girth 1: _____

5.7 Hip girth 1: _____

5.5 Waist girth 2: _____

5.8 Hip girth 2: _____

5.6 Waist girth 3: _____

5.9 Hip girth 3: _____

5.10 Ave. waist girth (cm): _____

5.11 Ave. hip girth (cm): _____

5.12 Waist/hip ratio: _____

Completed by: _____

6. Blood Pressure (after 10 minutes of rest)

6.1 Arm: Left Right

6.2 Type of device: Automatic Mercury

6.3 Reading 1 systolic BP (mmHg): _____ 6.4 Reading 1 diastolic BP (mmHg): _____

6.5 Reading 2 systolic BP (mmHg): _____ 6.6 Reading 2 diastolic BP (mmHg): _____

6.7 Reading 3 systolic BP (mmHg): _____ 6.8 Reading 3 diastolic BP (mmHg): _____

6.9 Avg. Systolic BP (mmHg): _____ **6.10 Ave. diastolic BP (mmHg):** _____

6.11 Heart rate (beats/min): _____

Completed by: _____

Comments _____

7. Gastrointestinal (GI) Tolerability Questionnaire

Participant Code _____

Study Phase _____

Treatment (i.e., **Orange, Blue**) _____

Start Date _____

End Date _____

Indicate if you experienced GI symptoms listed below over the last four weeks and if so, what was the severity (on a scale of 0 to 3, i.e., 0 = none, 1 = mild, 2 = moderate, 3 = severe).

GI symptoms

Severity

None (0) Mild (1) Moderate (2) Severe (3)

7.1 Hiccup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.2 Nausea	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.3 Vomiting	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.4 Indigestion	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.5 Stomach or abdominal pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.6 Constipation	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.7 Diarrhea	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.8 Flatulence (Gas)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.9 Abdominal bloating	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.10 Cramping	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.11 Heartburn	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7.12 Other (please describe) _____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

8. Well-being Questionnaire

Participant Code _____

Study Phase _____ Treatment (i.e., **Orange, Blue**) _____

Start Date _____ End Date _____

Please rate the degree of improvement (if any) in your memory, concentration and mood after using the food items since the last visit (on a scale of 0 to 3, i.e., 0 = none, 1 = minor, 2 = moderate, 3 = considerable).

8.1 Degree of improvement

None (0) Minor (1) Moderate (2) Considerable (3)

8.1.1 Memory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8.1.2 Concentration	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8.1.3 Mood	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

8.2. Degree of worsening

None (0) Minor (1) Moderate (2) Considerable (3)

8.2.1 Memory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8.2.2 Concentration	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8.2.3 Mood	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

9. Food Items Accountability

9.1 Product Reconciliation Product Group: Orange Blue

<u>Product</u>	<u>Number Servings Provided</u>	<u>Number Servings Returned</u>	<u>Number Servings Consumed</u>
9.1.1 Fruit Bar			
9.1.2 Chocolate Bar			
9.1.3 Sprinkle			
9.1.4 Smoothie			
9.1.5 Pancake Mix			
9.1.6 Oatmeal			
9.1.7 Total			

9.2.1 Number of returned food items: _____

9.2.2 Number of days that the study product was not taken: _____

10. Food Items Acceptability

	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
10.1. Using this eating system was as simple as taking a medication					
10.2. I prefer using food rather than medications to manage my cholesterol					

11. Blood Samples

11.1 Fasting blood samples collected Yes No

11.2 When did you last eat (hour)? ____ : ____

11.3 Time the blood sample was taken (hour): ____ : ____

Please verify that the blood samples are labeled with subject no., date and hour.

Comments:

Checklist

- Adverse Events
- Concomitant Medications/Supplements
- Anthropometrics
- Blood Pressure
- Gastrointestinal Tolerability Questionnaire
- Well-Being Questionnaire
- Food Items Accountability
- Food Items Acceptability
- Blood Samples

Study logs check list:

- Accountability log

Additional Forms

- **End of Study Form (EOS)**
- **Concomitant Medication/Supplement Form**
- **Adverse Event (AE) Form**

1. Did the participant complete the trial according to the protocol?		Yes <input type="checkbox"/> No <input type="checkbox"/>
If No, specify a reason:		
1.1. <input type="checkbox"/>	Adverse Event (AE) – fill an AE form	
1.2. <input type="checkbox"/>	Severe Adverse Event (SAE) – fill an SAE form	
1.3. <input type="checkbox"/>	Protocol violation, specify _____	
1.4. <input type="checkbox"/>	Voluntary withdrawal.	
1.5. <input type="checkbox"/>	At the request of the investigator or sponsor.	
1.6. <input type="checkbox"/>	At the request of patient’s family physician or study physician.	
1.7. <input type="checkbox"/>	Lost contact with the participant.	
1.8. <input type="checkbox"/>	Other: _____	

End of Study Form (EOS)

Investigator’s statement:

I certify that all information entered in this case report form by myself, my associates or designates is complete and accurate to the best of my knowledge.

Investigator: _____ **Date:** __ __/ __ __/ __ __ __ __ (dd/MON/yyyy)

Concomitant Medication/Supplement Form

MEDICATION / SUPPLEMENT	INDICATION*	ROUTE ^A	DOSE	FREQ ^B	DATE STARTED (dd/MON/yyyy)	DATE STOPPED (dd/MON/yyyy)	ONGOING
1		<input type="checkbox"/>		<input type="checkbox"/>			<input type="checkbox"/>
2		<input type="checkbox"/>		<input type="checkbox"/>			<input type="checkbox"/>
3		<input type="checkbox"/>		<input type="checkbox"/>			<input type="checkbox"/>
4		<input type="checkbox"/>		<input type="checkbox"/>			<input type="checkbox"/>
5		<input type="checkbox"/>		<input type="checkbox"/>			<input type="checkbox"/>
6		<input type="checkbox"/>		<input type="checkbox"/>			<input type="checkbox"/>

* If a new indication, please complete Adverse Event form **A-Route:** 1=oral; 2=intravenous; 3=subcutaneous; 4=topical; 5=inhalation; 6=transdermal; 7=rectal; 8=intramuscular **B-Frequency:** 1=once daily; 2=twice daily; 3=thrice daily; 4=four times daily; 5=once weekly; 6=as needed

In case the above mentioned medications / supplements prevent further participation; fill a trial termination form

Investigator: _____ **Date:** __ __ / __ __ / __ __ __ __ (dd/MON/yyyy)

Adverse Event (AE) Form

ADVERSE EVENT	DATE ONSET (dd/MON/yyyy)	SEVERITY ^A	SAE YES NO	RELATIONSHIP ^B	OUT- COME ^C	DATE RESOLVED (dd/MON/yyyy)	ACTION TAKEN ^D
1		<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>
2		<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>
3		<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>
4		<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>
5		<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>
6		<input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>

A- Severity: 1=Mild; 2=Moderate; 3=Sever **B-Relationship to study medication:** 0=Not related; 1=Unlikely; 2=Possible; 3=Probable; 4=Related

C-Outcome: 1=Recovered; 2=Recovered with sequelae ; 3=Ongoing; 4=Death.

D-Action taken: 0=None; 1=Drug treatment; 2=non drug treatment specify:

Investigator: _____ **Date:** ___/___/___ (dd/MON/yyyy)

8.11 SUMMARY OF KEY NUTRIENTS AND INGREDIENTS IN THE CONTROL FOOD PRODUCTS

	Oatmeal ^a	Pan cakes ^b	Cranberry Bar ^c	Chocolate Bar ^d	Smoothie ^e	Sprinkle ^f
Fiber (g)	4	< 1	4	2	0	2
Omega 3 (mg) *	0	0	26	< 50	< 50	< 400
Plant sterols (mg) *	< 50	< 50	0	0	< 20	0
Anti-oxidants (µmol) *	< 250	< 50	< 250	< 50	< 1000	< 200
Serving size (g) **	46	46	49	41	32	24

*Estimated based on listed ingredients

**Adjusted for calorie match

^a**Kashi® Heart to Heart Apple Cinnamon Oatmeal**

Whole grain rolled oats, evaporated cane juice, dried apples (apples, apple juice concentrate), chicory root fiber, cinna-mon, guar gum, potassium chloride, salt, natural flavors, decaffeinated green tea extract, calcium carbonate, decaffeinated white tea extract, alpha tocopherol acetate (natural vitamin E), ascorbic acid (vitamin C), activin grape seed extract, pyridoxine hydrochloride (vitamin B6), zinc oxide, ferrous fumarate (iron), beta carotene (source of vitamin A), folic acid, vitamin B12.

^b**Bisquick® Heart Smart Pancake Mix**

Enriched bleached flour (wheat flour, niacin, iron, thiamin mononitrate, riboflavin, folic acid), canola oil, leavening (baking soda, sodium aluminum phosphate, monocalcium phosphate), dextrose, sugar, tricalcium phosphate, salt, DATEM, corn starch.

^c**Kellogg's® Nutri-Grain Bar**

Crust: Whole grain oats, enriched flour (wheat flour, niacin, reduced iron, vitamin B1 [thiamin mononitrate], vitamin B2 [riboflavin], folic acid), whole wheat flour, soybean oil, high fructose corn syrup, soluble corn fiber, sugar, calcium carbonate, whey, wheat bran, salt, cellulose, potassium bicarbonate, mono- and diglycerides, soy lecithin, natural and artificial flavor, wheat gluten, niacinamide, vitamin A palmitate, carrageenan, zinc oxide, reduced iron, guar gum, vitamin B6 (pyridoxine hydrochloride), vitamin B1 (thiamin hydrochloride), vitamin B2 (riboflavin), folic

acid. Filling: high fructose corn syrup, corn syrup, strawberry puree concentrate, glycerine, sugar, sodium alginate, modified corn starch, citric acid, natural and artificial flavor, dicalcium phosphate, methylcellulose, caramel color, malic acid, red 40.

^dQuaker® Chewy Chocolate Granola Bar

Granola (whole grain rolled oats, brown sugar, crisp rice [rice flour, sugar, salt, malted barley extract], whole grain rolled wheat, soybean oil, dried coconut, whole wheat flour, sodium bicarbonate, soy lecithin, caramel color, nonfat dry milk), corn syrup, brown rice crisp (whole grain brown rice, sugar, malted barley flour, salt), peanut butter spread (peanuts, sugar, palm oil, salt), semisweet chocolate chips (sugar, chocolate liquor, cocoa butter, soy lecithin, vanilla extract), invert sugar, peanut flavored chips (sugar, palm kernel and palm oil, partially defatted peanut flour, lactose, dry whey, dextrose, corn syrup solids, soy lecithin, salt, vanillin [artificial flavor]), corn syrup solids, glycerin. contains 2% or less of calcium carbonate, sorbitol, salt, water, natural and artificial flavor, BHT (preservative), citric acid.

^eEnsure® Nutrition Powder

Corn syrup, corn maltodextrin, sugar (sucrose), corn oil, sodium and calcium caseinates, soy protein isolate, artificial flavor, potassium citrate, magnesium chloride, calcium phosphate, sodium citrate, potassium chloride, soy lecithin, ascorbic acid, choline chloride, zinc sulfate, dl-alpha-tocopheryl acetate, niacinamide, ferrous sulfate, calcium pantothenate, manganese sulfate, cupric sulfate, thiamine hydrochloride, pyridoxine hydrochloride, riboflavin, vitamin A palmitate, folic acid, biotin, chromium chloride, sodium molybdate, potassium iodide, sodium selenate, phyloquinone, cyanocobalamin, and vitamin D3.

^fBear Naked® Granola Fruit and Nut

Whole grain oats, honey, canola oil, almonds, coconut, raisins (raisin, glycerine, sunflower oil), sweetened dried cranberries (cranberries, sugar, glycerin, sunflower oil), maple syrup, pecans, walnuts, oat bran, ground flax seeds, toasted sesame seeds.