Genetic basis for prediction of non-responders to dietary plant sterol intervention (GenePredict-PS): a study protocol for a double-blind, placebo-controlled, randomized two-period crossover study

CURRENT STATUS: UNDER REVIEW

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DOI:
10.21203/rs.2.469/v2

SUBJECT AREAS
General Medicine

KEYWORDS
Plant sterols, Cholesterol, Genetic, SNPs, Prediction
Abstract

Background: Functional food ingredients and natural health products have been demonstrated to reduce disease risk and thereby help to lower health care costs across populations at risk for chronic or degenerative diseases. However, typically a wide range of inter-individual variability exists in response across individuals to nutritional and natural health product bioactives, such as plant sterols (PS). This study aims to determine and utilize information on associations between genosets and the degree of responsiveness to dietary PS intervention, with a long-term objective of developing genetic tests to predict response to PS. Methods: This clinical trial is designed as a double blind, placebo controlled, randomized two-period crossover study. 64 eligible participants with the specific a priori -determined single nucleotide polymorphisms (SNPs) associated with responsiveness to PS will consume PS or a placebo treatment for two 4-week periods. The PS treatment consists of two daily single portions of margarine, each providing 1 g PS during the PS period (2.0 g/day of PS in total). The placebo will be an identical margarine containing no added PS. LDL-C responsiveness to controlled administration of PS will be investigated as the primary outcome and the associations between inter-individual genoset variabilities and response to PS consumption will be determined. Discussion: This research will provide further insight into whether the associations between previously identified SNPs and the response of LDL-C to PS consumption can be used in a predictive manner. It will also provide insight into the complexities of undertaking a nutrigenetic trial with prospective recruitment based on genotype. Trial registration: The study was registered at ClinicalTrials.gov (Identifier: NCT02765516). Keywords: Plant sterols, Cholesterol, Genetic, SNPs, Prediction

Background
Elevated blood concentrations of low density lipoprotein cholesterol (LDL-C) is an important risk factor for cardiovascular disease (CVD)(1). Evidence suggests that the incidence of coronary heart disease (CHD) is proportionally reduced through lowering LDL-C(2) and is the primary target for initiating lipid-lowering interventions in the current guidelines(3). LDL-C reduction strategies include diet and lifestyle changes, pharmaceutical therapies, intestinal bypass surgery, and lipid apheresis (3).

Changes in dietary habits can play a critical role in reduction of LDL-C concentrations, for example, supplementation with functional food ingredients such as dietary fibers and plant sterols (PS) have been demonstrated to reduce LDL-C(4) and thereby help to lower health care costs across populations with chronic or degenerative diseases (5). However, a wide range of inter-individual variability in responsiveness to natural health product bioactives, such as with soluble fibers PS has been reported (6). Understanding this existing inter-individual variability in responsiveness is important for both public health, and functional foods manufacturers, because it may help them predict which individuals might or might not receive benefits from consuming a particular functional food or natural health product.

Better understanding the cause of such inter-individual variability, especially the impact of genetics can help to inform individuals about optimal dosing strategies and can contribute to the development of a method that can determine, before starting to use a product, whether an individual will benefit from a particular bioactive. For instance, if an individual knows that they are a non-responder to a bioactive, they then may be able to choose other therapeutic products or approaches.

A series of predictive response tests for nutritional bioactives based on genotype would represent a genomics-derived solution to better understand and resolve the wide range of inter-individual variability in response to nutritional bioactives.
PS are a nutritional bioactive for which such a predictive test would be helpful. The clinical efficacy of consuming added PS for lowering LDL-C, is demonstrated in the vast number of clinical studies as summarized in several meta-analyses (Katan et al, 2003; Demonty et al 2009, Ras et al, 2014). The concept of a predictive responsiveness test for PS supplementation is based on our research findings of previously completed human nutrition intervention trials (7-9). In a recent intervention trial, the response of LDL-C to PS consumption was associated with SNPs in cholesterol 7 alpha-hydroxylase (CYP7A1, rs3808607) and apolipoprotein E (ApoE, rs7412 and rs429358) (Table 1)(10). A key discovery from these trials was that combinations of these SNPs (known as genosets) were found to interact with each other to form stronger associations with the magnitude of LDL-C lowering in response to PS consumption than for each SNP alone. However, these associations were established post-hoc in a trial that selected for individuals with high or low cholesterol synthesis. An a priori approach replicating these findings is required to provide evidence that these genosets could indeed be used as a predictive responsiveness test.

Table 1. Single nucleotide polymorphisms (SNPs) for plant sterol responsiveness testing.

| Gene | SNP | Function of gene | Association with plant sterol responsiveness |
|------|-----|-------------------|-----------------------------------------------|
| CYP7A1 | rs3808607 | The rate-limiting enzyme in the synthesis of bile acid in the classic pathway. | T/T = non-responsive  
G/T = responsive  
G/G = responsive |
| ApoE | Variant | Apolipoprotein E is a glycoprotein present in human plasma; ApoE is associated with triglyceride-rich lipoproteins (chylomicrons and VLDLs) and HDL. | ε2/- = Unknown  
ε3/ε3 = neutral  
ε4/- = responsive |

To the best of our knowledge, there is no clinical trial that has so far investigated the associations between certain SNPs and/or genosets and the degree of responsiveness to dietary PS intervention in an a priori fashion.

Therefore, the objective of this study is to determine and utilize information on
associations between SNPs and the degree of responsiveness to dietary PS intervention, with the long-term goal of developing a predictive responsiveness test.

The primary specific hypothesis of this study after these protocol amendments are therefore 1) the genoset formed from \(CYP7A1\) rs3808607T/T and \(APOE\) E3/3 predict non-response, and 2) \(APOE\) isoform \(\varepsilon4/-\) and \(CYP7A1\) rs3808607 G/- will independently predict response to PS consumption in a pattern which reflects the current gene-biomarker associations outlined in Table 1. For these hypotheses response is being defined as a reduction in LDL-C concentrations due to plant sterol consumption.

Methods

**Study design**

To formally validate if \(APOE\) isoform, formed by rs7412 and rs429358, and \(CYP7A1\) rs3808607 can predict responsiveness to PS consumption across the general population, the present proposal is to carry out a double blind, placebo-controlled, randomized two-period crossover study to investigate the LDL-C responsiveness to controlled administration of PS. The PS treatment will consist of two daily single portions of margarine, providing 1 g each of PS during the PS period (2.0 g/day of PS in total). The placebo treatment will be an identical margarine, except it will not contain any added PS. Both the PS and placebo margarine treatments will be coded by the industrial partner organization, Unilever, and provided to the research group to maintain blinding of both the researchers and participants throughout the clinical trial.

Our two original specific hypotheses were 1) \(APOE\) isoform and \(CYP7A1\) rs3808607 will independently predict the response to PS consumption in a pattern which reflects the current gene-biomarker associations as outlined in Table 1. \(APOE\) \(\varepsilon4/-\) will be more responsive to PS than \(\varepsilon3/\varepsilon3\). \(CYP7A1\) rs3808607 G allele will predict responsiveness to PS consumption in a dose responsive fashion, with T/T predicting non-response. 2) The
genosets formed by combinations of APOE isoform and CYP7A1 rs3808607 will follow the pattern as predicted in Table 2.

Table 2. Original plant sterol trial genotype recruitment targets and predicted response

| ApoE | CYP7A1 | Predicted response | Planned Recruitment |
|------|--------|--------------------|---------------------|
| ε2/- | T/T    | Non-responder      | n=8                 |
| ε2/- | G/-    | Responder          | n=8                 |
| ε3/ε3| T/T    | Non-responder      | n=8                 |
| ε3/ε3| T/G    | Responder          | n=8                 |
| ε3/ε3| G/G    | Responder          | n=8                 |
| ε4/- | T/T    | Responder          | n=8                 |
| ε4/- | G/G    | Responder          | n=8                 |

Due to the amount of time spent on recruitment, and difficulty in finding participants who were eligible with rare combinations of genosets, the APOE 2 groups were removed, and other groups were combined. To maintain the study power, we increased the n in the other groups as described in Table 3.

Table 3. Amended Plant sterol trial genotype recruitment targets and predicted response

| ApoE | CYP7A1 | Predicted response | Planned Recruitment |
|------|--------|--------------------|---------------------|
| ε3/ε3| T/T    | Non-responder      | n=20                |
| ε3/ε3| G/-    | Responder          | n=22                |
| ε4/- | -/-    | Responder          | n=22                |

The trial will use a priori recruitment of 64 individuals (Table 3) with the specific SNPs associated with responsiveness to PS at a) the University of Manitoba’s Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) and b) Seven Oaks General Hospital (SOGH) in Winnipeg, Manitoba, Canada. The present trial will therefore precisely select individuals from the general population with specific SNPs and then test their responsiveness to PS consumption. These responsiveness characterizations will generate the required data to validate the genoset-based classifications of responders and non-responders.

Each treatment period will consist of 28 days, with a minimum of washout of 21 days.
between periods. Figure 1 shows schematic flow diagram of the trial protocol.

Participants will be required to attend breakfast at the RCFFN or Seven Oaks General Hospital (SOGH) and consume a meal containing one daily portion of margarine under supervision from Monday to Friday; the additional daily portion will be consumed with their evening meal. Participants will be given their evening and weekend margarine portions to take home for consumption. Participants will be provided diaries in which they are instructed to record when they ate the margarine in the evenings and on the weekends. During the week, participants will be required to return the empty margarine tubs on the following day to help monitor compliance, with margarine tubs used on Saturday and Sundays’ being returned on Monday. The return of the empty tubs and the confirmation of recording the consumption in the study diaries will be verified by clinical coordinators using a compliance checklist. Additionally, serum non-cholesterol sterols, including sitosterol and campesterol, the two main PS in the margarine, will be measured to monitor compliance. Partial supervision of treatment consumption in person, and monitoring of non-supervised treatments, by return of empty container, as a compromised between participant compliance and participant burden.

Missed treatment consumption and return of margarine tubs will be recorded for each participant. Non-compliance will be defined as 1) missing supervision, 2) if participants fail to return at least 80% of the total empty margarine tubs per treatment period, and 3) missing 2 consecutive measurements or blood sampling days. Non-compliant participants will be asked to leave the trial; however, they will be compensated pro-rated based on the duration that they were involved in the trial. Participants were asked to maintain their typical diet and physical activity levels throughout the study.

Additionally, on a weekly basis, clinical coordinators will ask participants to report any changes in diet, lifestyle (sleep) or physical activity which may interfere with results of
the trial and any other health outcomes or symptoms they may experience during the trial. Fasting blood samples are collected from participants on two consecutive days at the beginning (Days 0 and 1) and at the end (Days 28 and 29) of each trial period as described in the Table 4.

Table 4: Schedule of enrolment, interventions and assessments.

| t' #  | Screening | 1, 2 | 3, 4 | 4-week wash out | 5, 6 | 7, 8 |
|-------|-----------|------|------|-----------------|------|------|
| week  | 0         | +    | +    | ( + 5 / -2 days) | ( +5 / -2 days) | ( +5 / -2 days) |
| emographic /Exclusion Criteria, | +        |      |      |                 |      |      |
| anthropometric Measures | +     | +    | +    |                 | +    | +    |
| Waist | +     | +    | +    |                 | +    | +    |
| Blood Pressure | + | + | + | | + | + |
| vons | + | + | + | | + | + |
| imples | + | | | | | |
| TC, LDL-C, HDL- | + | + | + | | + | + |
| stol Precursor | + | + | + | | + | + |
| tolerability | + | + | + | | + | + |
| on | + | | | | | |
| itility tion Checklist) | + | | | | | |

**Study participants**

Participants, 64 in total, will be recruited using various established methods, including
flyers around the University of Manitoba and Seven Oaks General Hospital (SOGH), newspaper advertisements, direct mail advertisements within the City of Winnipeg, and advertising digitally at the Active Living Center of University of Manitoba as well as SOGH social media, websites and newsletter advertising among 6500 members of SOGH. An internal list of previous volunteers who have expressed interest in participating in other clinical studies will also receive an advertisement. Participants will be initially screened for eligibility over the telephone by the study coordinator if they respond to advertisements. If eligible, potential participants will be invited to the clinical research unit at the RCFFN or SOGH for an information session to introduce the research staff and provide further information about the study. Those expressing further interest will be invited to consent to and have a blood sample taken to ensure they meet all other trial criteria as listed below. Blood samples will be taken by RCFFN or SOGH phlebotomists or registered nurses. Screening blood samples are analyzed for the following: fasting lipid profiles including LDL-C, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations, as well as, glucose, serum creatinine, blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), serum total protein, and serum albumin; all will be measured using the automated enzymatic methods on the Cobas 311 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) or measured by Diagnostic Services Manitoba (DSM) according to their standard protocols. DNA will be extracted from blood sample buffy coat using commercially available column-based DNA extraction kits (DNeasy Blood and Tissue Kit, QIAGEN Sciences) according to the manufacturer's instructions. The concentration and integrity of the genomic DNA will be assessed by micro-volume spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific). DNA samples will then be genotyped by TaqMan SNP genotyping assays (CYP7A1-rs3808607, assay identification (ID)
C2749212120; APOE rs7412, assay ID C2749212120; APOE-rs429358, assay ID C308479320; Life Technologies) on a StepOnePlus Real-Time PCR System (Applied Biosystems; Life Technologies). Data from the screening blood sample will be used to screen participants based on the predefined inclusion and exclusion criteria.

**Inclusion criteria:** Participants, men and women, aged 18 – 70 years with LDL-C concentrations of 3.0-4.9 mmol/L will be recruited into the trial. Participants must have a fasting glucose concentration of <6.1 mmol/L. A prospective recruitment scheme based on genotype will recruit 20-22 individuals in each of the three most common combinations, also called genosets, (outlined in Table 3). This would leave a minimum sample size of 20 participants for each individual genotype. The prospective recruitment based on genosets of interest will require screening of 200-400 potential participants. Such lengthy screening is required to find sufficient individuals who meet all the inclusion and exclusion criteria with the rarer genosets. Additionally, participants must be willing to: fast 10-12 hours before blood sampling; abstain from alcohol two days prior to blood sampling; and abstain from coffee and physical exercise for at least 12 hours before measurements and blood sampling. All participants must be able and willing to give informed consent to participate in the trial prior to their inclusion.

**Exclusion criteria:** Participants will be excluded if they are consuming, or have consumed in the last 3 months, medications or nutritional supplements which are known to affect lipid metabolism (such as cholestyramine, colestipol, niacin, clofibrate, gemfibrozil, probucol, HMG-CoA reductase inhibitors (statins), methotrexate, high-dose dietary fiber supplements, or plant sterols or stanols), or have any dietary restrictions which would prevent them from consuming the trial treatments. Participants who have a BMI >40 kg/m² will be excluded. Participants must not have self-reported weight gain or loss greater than 3 kg in the past three months. Participants must be free of active
cardiovascular disease including stroke, congestive heart failure, myocardial infarction, unstable angina pectoris, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, temporal ischemic attacks, anemia, abnormal electrolytes, proteinuria, and abnormal liver, kidney, or thyroid function. Participants will be excluded if they have clinically significant biochemistry defined as: LDL-C < 3.0 mmol/L or > 4.9 mmol/L; TC > 6.2 mmol/L; fasting glucose > 6.1 mmol/L, fasting TG > 4.52 mmol/L; AST > 100 U/L; ALT > 100 U/L or any other clinically significant abnormalities in hematology and/or biochemistry at the investigator’s discretion.

Participants will be excluded if they have phytosteroolemia, type 1 or type 2 diabetes, a history of cancer or malignancy in the last 5 years, or any metabolic disease, gastrointestinal disorder, or other clinically significant disease/disorder which could interfere with the results of the study or the safety of the participant. Participants will be excluded if they are smokers, tobacco/snuff/nicotine users, recreational drug users, or if they consume more than 14 alcoholic beverages a week. Participants who are pregnant or plan to become pregnant during the trial period will be excluded. Lactating women will also be excluded. Patients with unstable or serious illness, for example, dementia, terminal illness, recent bereavement, recent significant medical diagnosis will also be excluded. Employees of Unilever, Nutritional Fundamentals of Health (NFH) and the research institutes conducting the research will not be allowed to participate in the study.

Randomization

Eligible participants will be randomly allocated to two groups: the PS treatment group or the placebo group for the first period and then participants will switch treatments for the second period after the washout between periods. Randomization will be done by an assistant outside of the research team using a block randomization method through sealed envelopes with stratification by sex and genoset. Randomization in blocks of eight and
four, each with equal numbers of treatment orders will be used. This blocking is being done to minimize imbalances in treatment orders within each genoset group or by sex. Administration of the intervention will be conducted in a double-blind manner. Single portion tubs of PS treatment and placebo margarine are being created for this study by Unilever and are being delivered to the research team in identical packages labeled either A or B.

Remuneration

Study participants will receive up to a total of CAD $400 (i.e., $200/period x 2 periods) for study completion. This amount will be divided into 2 portions. Participants will receive $200 after completion of period 1 and another $200 after completion of period 2. If a participant withdraws early from the study, they will receive an appropriate pro-rated fraction of this amount.

Outcome measures

Primary outcome

Serum LDL-C concentration and its change in response to PS consumption, is considered the primary outcome of this trial. This was to measure plant sterol response, in terms lowering of LDL-C concentration between the placebo and plant sterol consumption period. Approximately, 20 mL blood samples on days 0, 1, 28 and 29, of the intervention period will be collected. Serum lipid profile (TC, LDL-C, HDL-C, and TG) will be measured using the Cobas 311 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Average values of days 0 and 1 will be used as baseline, and average of days 28 and 29 will be endpoint values.

Secondary outcomes

At baseline and at the end of the two intervention periods, anthropometric measurements including body weight, BMI, hip and waist circumference, and blood pressure will be
measured. Blood pressure will be measured in an office setting on. Days 0, 1, 28, and 29 of each treatment period. Participants will ask to rest 10 minutes prior to having their blood pressure taken, in the event they rushed in. This measurement will take place in a quiet room while the participant is in a seated position and arm rested on an armrest at heart level. Participants will be advised to rest quietly throughout the measurements. Blood pressure measurement will be performed four times at 2-minute intervals. Gastrointestinal tolerability questionnaires will be completed by participants at the beginning and at the end of each intervention period. The 10-year CVD risk score will be calculated for each participant during each intervention period utilizing the ACC/AHA Cholesterol Guideline risk calculator. Participants will be asked to complete a questionnaire upon completing the trial which asks them whether they think they know which treatment they received during with treatment period. This information will be used to verify participant blinding.

Fasting serum glucose, will be measured with the Cobas 311 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Plasma samples will be used to quantify concentrations of blood sterols and sterol precursors (non-cholesterol sterols, NCS) according to previously established method (8).Authenticated internal standards will be added to plasma samples, which will then be saponified with methanolic KOH solution. Sterols will then be extracted twice with petroleum ether. Extracted sterols will be derivatized using a trimethylsilylation (TMS) procedure. The TMS-derivatized samples and sterol analysis will be carried out by gas chromatography with flame ionization detection. Campesterol, sitosterol, campestanol, sitostanol, and cholestanol, as well as lanosterol, desmosterol, and lathosterol will be measured.

Fractional cholesterol synthesis will be measured by deuterium incorporation according to previously established procedures (7, 10, 11). 24 hours before the end of each treatment
period participants will be asked to consume deuterium water (D₂O) given at a dose of 0.7 g/kg body water (estimated at 60% of total body weight). D₂O is a stable isotopic tracer and poses no radiation hazard and can be safely administered to human participants. D₂O water will be administered orally. A fasted blood sample will be taken at baseline and on day 28 prior to isotope administration, as well as fasting samples on day 29. The change in deuterium enrichment within red blood cell free cholesterol will be determined as an index of cholesterol synthesis over days 28 and 29.

**Sample size calculation and statistical analysis**

The sample size, with a minimum of 20 participants for each individual genotype, and n=20-22 for each individual genoset is based on previous work performed by this research group (10). A power calculation was performed using PROC POWER SAS Institute (version 9.4) using the pairedmeans statement to model the AB/BA crossover design (corr =0.75, a = 0.05 and b = 0.80); based on an average reduction in LDL-C of 0.34 mmol/L resulting from PS consumption according to the meta-analysis findings of Demonty et al. (12), the standard deviations in LDL-C for placebo (0.67 mmol/L) and PS (0.7 mmol/L) from the MacKay et al(8) and a correlation in LDL-C values of 0.75, which was an estimate based on variability in LDL-C concentrations over time from MacKay et al(8) and within persons correlation in cholesterol response to plant sterols (13). From this power calculation, we determined that 18 participants would be needed for each genoset to detect a response, a significant reduction in LDL-C from placebo to plant sterol consumption period, in the group. Our objective was to test if each genoset would respond, with the hypothesis that the *CYP7A1 rs3808607T/T* and *APOE E3/3* genoset would not respond to plant sterol consumption with LDL-C lowering.

Given the crossover design the study outcomes measures will be analysed in a per-
protocol population where only participants who received both treatment and placebo are included. The effects of treatment, comparing the endpoint values of the treatment and placebo periods, will be analyzed by the SAS MIXED procedure. Sequence and sex will be included in the model as fixed factors, while participant will be included as a random and repeated factor. Genoset and treatment by genoset will be included as fixed factors to assess the impact of genoset on treatment. The impact of the individual genotypes will also be investigated individually. Significant treatment-by-genoset or treatment-by-genotype effects will be examined by the SAS SLICE function, with Bonferroni correction for the number of slices. Treatment effect sizes by genoset or genotype, from significant interactions, will be compared by t test or ANOVA using the difference in mixed-model least squares means summary statistics for the treatment effect slices, with Tukey-Kramer adjustment for multiple comparisons (10).

Discussion

In a recent clinical trial by our group, the response of LDL-C to PS consumption was associated with SNPs in cholesterol 7 alpha-hydroxylase (CYP7A1, rs3808607) and apolipoprotein E (ApoE, rs7412 and rs429358)(10). This ongoing GenePredict-PS clinical trial is investigating if this previous association identified between the SNPs and the LDL-C response to PS consumption can be used in a predictive manner. Individuals with genosets that fail to reach significant reductions in plasma LDL-C levels in response to PS consumption will be classified as non-responders; while those that do result in LDL-C lowering will be classified as responders (see predicted response in Table 3). Individuals who are classified by the genosets as responders could be advised to consider PS-added products for lowering their elevated blood total and especially LDL-cholesterol, while non-responders could be either recommended to modify the dose of PS or use other pharmaceutical or natural health products that may lower cholesterol through other
pathways. Very few studies in nutrigenetics and nutrition have yet to explore recruitment of participants a priori based on genotype, let alone combinations of genotypes (genosets). Previously, the impact of rs1801133, a variant in the methylenetetrahydrofolate reductase (MTHFR) gene, on riboflavin supplementation and blood pressure has been explored (14). In their trial, Wilson et al. were able to use an available population of 1427 patients with hypertension from which they were able to recruit individuals based on genotype. The strategy of recruiting directly from a previous genotyped population can be highly recommend given the difficulty that the current trial has faced with de novo recruitment from the general public. Recruitment of previously genotyped individuals may be the most suitable way a priori nutrigenetic studies can be carried out in a suitable fashion, specifically if the studies will be recruiting based on genosets or polygenic risk scores (15).

List Of Abbreviations

Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Biomedical Research Ethics Board (BREB), Blood Urea Nitrogen (BUN), Body Mass Index (BMI), Cardiovascular Disease (CVD), Cholesterol 7 Alpha-Hydroxylase (CYP7A1), Deoxyribonucleic Acid (DNA), Deuterium Water (D\textsubscript{2}O), Diagnostic Services Manitoba (DSM), Electrophoretic Mobility Shift Assay (EMSA), Gamma-Glutamyl Transferase (GGT), High Density Lipoprotein (HDL), Low Density Lipoprotein Cholesterol (LDL-C), Plant Sterols (PS), Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), Statistical Analysis Software (SAS), Seven Oaks General Hospital (SOGH), Single-Nucleotide Polymorphisms (SNPs), Total Cholesterol (TC), Triglycerides (TG), Tri-Methylsytlation (TMS), Very Low-Density Lipoprotein (VLDL)

Declarations

Ethics approval and consent to participate
The Bannatyne Campus Biomedical Research Ethics Board (BREB) in Winnipeg, Manitoba, Canada has approved this study protocol (HS19441 (B2016:011). All amendments to this studies protocol are reviewed and approved by the U of M BREB and changes to the protocol are updated on clinicaltrials.gov. This trial is registered at clinicaltrials.gov (Identifier: NCT02765516). All participants must provide a written informed consent before they are enrolled in this trial, these consent form will be dated and signed by participants and an appropriate trial staff prior to the participants entry into the trial. Participants sign a general consent to participate in the trial as well as a consent for genetic analysis. Participants receive a copy of the consent forms they have signed.

**Trial status**

This trial is ongoing and has been recruiting since July 2016. The trial is expected to continue until approximately June 2019. The current protocol number and date is version 5, July 20, 2018.

The recruitment began on April 2016 and will be completed by June 2019.

**Consent for publication**

All participants provide consent to publish or present their information gathered during the trial in a fashion where their personal information such as their name, address, telephone number and/or any other identifying information are not revealed.

**Availability of data and materials**

The de-identified datasets, that will be used and/or analyzed during the current study will be available from the corresponding author on reasonable request.

**Competing interests**

ET is employed by Upfield™ marketed food products with added plant sterols. All other authors declare that they have no competing interests.

**Funding**
This study is funded in part by the University of Manitoba, Mitacs (converge@mitacs.ca), Unilever R&D (now Upfield™ R&D), and Nutritional Fundamentals for Health (research@nfh.ca). The study sponsors, ET, is not involved in collection, management, analysis, and interpretation of data; and sponsors including ET will not be involved in the decision to submit the report for publication, however she is listed as a co-author.

Authors’ contributions

MS sought ethical approval and prepared the drafts of the manuscript and compiled feedback and changes from other authors. MJG assisted with the draft manuscript preparation and feedback. DM designed the study protocol, sought funding, and ethical approval. JH contributed to development of the study protocol, ethical approval and manuscript. DM designed the selection criteria of participants. All authors contributed, have read, made critical revisions, and all approved the final manuscript before submission.

Acknowledgement:

The authors would like to thank the volunteers that participated in the study for their time and willingness to contribute to the project. We would also like to acknowledge and thank the following members of the study team for their contributions to the project: Amanda Krueger, Rowan Clark, Itzel Vazquez-Vidal, Courtney Fitzpatrick, and Yongbo She.

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