The Combined Portfolio Diet and Exercise Study (PortfolioEx)

NCT 02481466

Protocol - September 2, 2022

Protocol

12B) PROPOSED TRIAL

12B) I. ***What is the proposed trial design?*** This is a randomized 2x2 factorial design with four experimental arms of 3 years duration (Figure 2) to estimate the effect of the enhanced dietary portfolio, with or without increased physical activity under real world conditions. Participants will be recruited in 3 academic centers in 3 cities across Canada (Quebec, Toronto, Vancouver) from newspaper advertisements providing they do not require pharmacological intervention according to current CCS guidelines (1) and are within 30% of their target LDL-C concentrations for the low or moderate risk category according to current Canadian Working Group recommendations (2), i.e. those who would normally be considered for initial treatment with lifestyle alone. All participants will first be screened by ultrasound for the presence of plaque in the carotid arteries and will then be randomized to one of the four experimental arms:

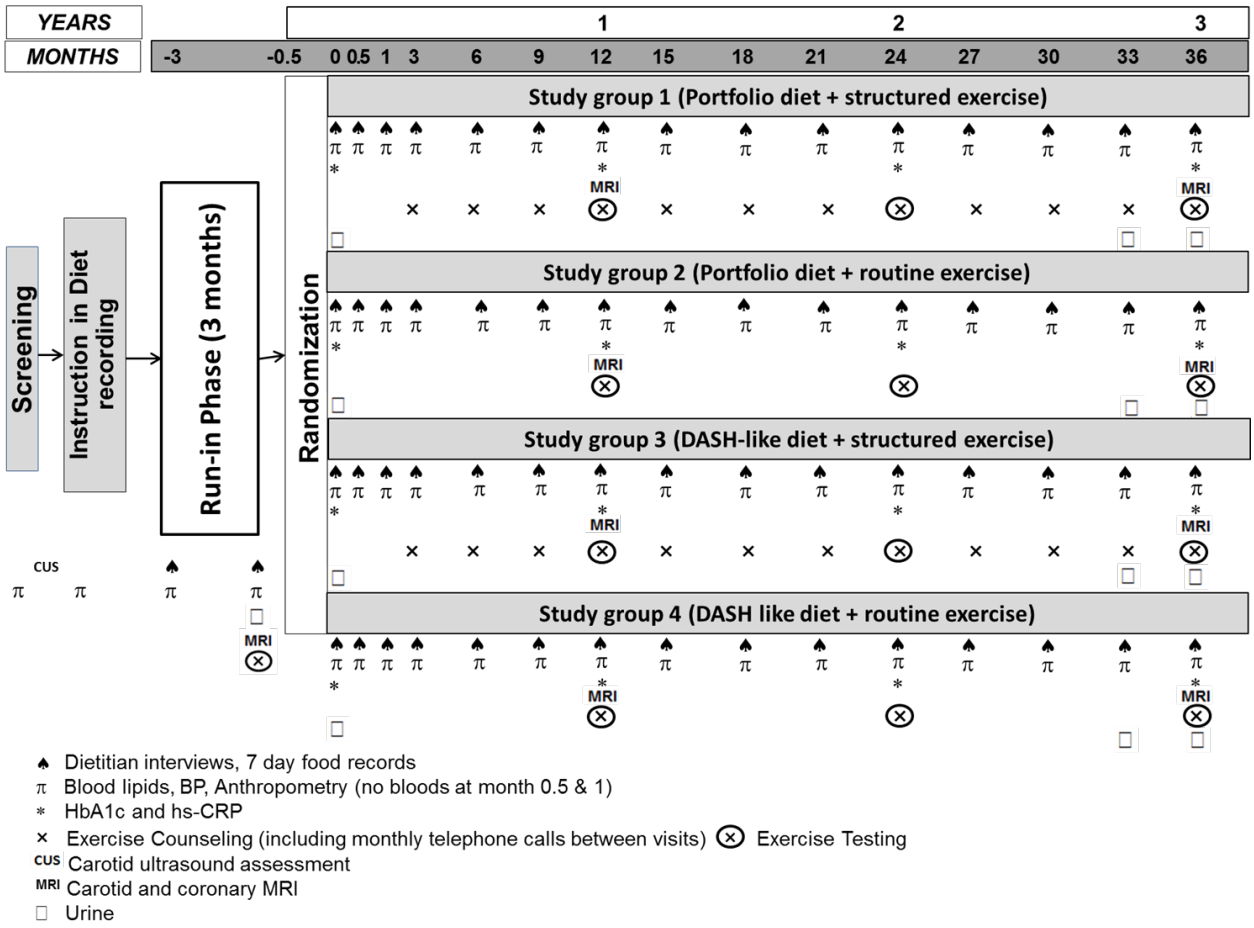
1) enhanced dietary portfolio (Appendix 4- Table 1) and an intensive increased physical activity program with follow up at 3-month intervals for the remainder of the trial,

2) enhanced dietary portfolio and a copy of Health Canada Physical Activity Guidelines with advice to increase physical activity

3) control diet (modified DASH, Appendix 4- Table 2) given as routine clinical advice as used in our recent studies (JAMA 2008, 2011) (3, 4)
and an intensive increased physical activity program with follow up at 3 month intervals for the remainder of the trial

4) control diet (modified DASH) as described in (3) above and a copy of Health Canada Physical Activity Guidelines with advice to increase physical activity.

Figure 2: Schematic Design of Combined Study



For the treatments with intensive increased physical activity the standardized physical activity/exercise component will be supervised by trained kinesiologists (exercise physiologists) for the 4 visits followed by monthly phone calls for the first year for the exercise component, when the major exercise training is provided. This "dose" of activity/exercise is one quarter the frequency used in the first year of the Look AHEAD and PREDIMED studies (5, 6) for the Test treatment. 7-day Exercise diaries will be collected with 7-day food records. Using well-established procedures (7) standardized across centres by the Quebec Heart and Lung Institute, the baseline visit will be used to provide a broad qualitative assessment of participants' lifestyle habits and preferences. Standardized physical activity questionnaires (8) will be completed and participants will be asked to wear a pedometer for seven days prior to the intervention to quantify baseline physical activity (daily step count Cardiorespiratory fitness (CRF) is assessed using a submaximal treadmill test adapted from a progressive submaximal power output test performed

on a cycle ergometer (9). The protocol begins with a warm-up workload of 2.5 mph with a 0% slope. The second stage is performed at a speed of 3.5 mph with a 2% slope. The third stage is adjusted in an attempt to reach 75% of the age-estimated maximal heart rate (HR). If necessary, a 4th stage is performed. Estimated VO2max is predicted by extrapolation to age-predicted maximal heart rate (DHR at a standardized submaximal treadmill stage (3.5 mph, 2% slope) and estimated maximal oxygen consumption (VO2max) are the variables considered as indicators of CRF in the present study. For treatments with routine advice to increase physical activity Health Canada Physical Activity Guidelines for adults 18-64 years or for older adults 65 years & older will be provided. They will be seen at the start and the end of each year for their formal exercise testing and will bring with them completed physical activities questionnaires. They will receive no other physical activities instruction. Prior to starting either diet, participants will undergo screening ultrasound examination of both right and left carotids to enable selection of those individuals whose intima-media thickness (IMT) would be 5-30% below the cut point considered by the Mannheim Consensus (10) as relevant arterial thickening to ensure a relatively low risk group, yet with some measurable arterial thickening. The **main outcome** will be **MRI** assessment of maximum vessel wall volume. This assessment will be repeated at year 1 and 3. It will be emphasized at the outset that both the dietary portfolio (11-14) and the DASH-like diets (15, 16) have been associated with benefits in terms of cholesterol reduction to provide equal encouragement for those randomized to the test and control groups. Portfolio and DASH-like dietary advice will consist of half hour individual sessions with the dietitian at baseline, and at 3-month intervals throughout the trial. Prior to starting each diet, instruction will be given on achieving the dietary goals. Visits or telephone calls to reinforce dietary advice and optimize retention will be conducted at 2 weeks and 4 weeks after randomization. At subsequent follow-up visits, the participants' completed 7-day diet records will be discussed and the original advice will continue to be reinforced. Every effort will be made to obtain study blood samples and carotid imaging data from all subjects at the designated times regardless of adherence to the dietary aspects of the study protocol. All subjects will be included in the intention-to-treat analysis.

During pandemics and/or lockdowns, clinic visits will be conducted by telephone (with the exception of final visit activities) and study length may be extended until in-person clinic visits can resume and study participants feel comfortable returning to clinic. Study length may be extended for those who are out of the city or country at the time of their final visit.

Table 1A. Target Active Ingredients in Supplements for a 2000 kcal/d Diet

	Portfolio	DASH
Soy protein (g/d)	45	
Plant sterols (g/d)	2	
Viscous fiber (g/d)	18	
Almonds (g/d)	45	

Table 1B. Ideal Macronutrient Profile for a 2000 kcal/d Diet

	Portfolio	DASH
Total protein (%)	24	23
Vegetable protein (%)	24	8
Total dietary fat (%)	34	27
SFA (%)	6	7
MUFA (%)	15	10
PUFA (%)	13	10
Dietary cholesterol (mg/d)	0	52
Carbohydrate (%)	43	50
Total Dietary Fibre (g/d)	52	48

%= % of total enery: SFA, MUFA and PUFA= saturated, mono and polyunsaturated fatty acid

12B) II. ***What are the planned trial interventions?*** The dietary portfolio advice will conform to current therapeutic diets appropriate for hypercholesterolemic subjects (<7% of energy saturated fat, <200 mg/d cholesterol) PLUS the combination of viscous fibres, soy protein, plant

sterols and nuts, 5% extra monounsaturated fat, and selection of low glycemic index foods (Table 1A). The DASH-like diet will advise intake of whole grains, and low-fat dairy (Table 1B). Advice on both diets will emphasize current recommendations for fruit and vegetable intakes (5-10 servings/d) (15, 17). Foods on the dietary portfolio plan will contribute 9 g/1000 kcal viscous fibre as β-glucan (oats, barley, oat bran breads and soups) and psyllium (cereal), 1 g plant sterol/1000 kcal diet (in sterol margarine and sterol liquid supplement), 22.5 g soy protein/1000 kcal (soy burgers, dogs, links, other soy meat analogues, soy milks, yogurts and cheese) and 22.5 g almonds or equivalent of other nuts/1000 kcal (Table 1A) and increased MUFA (as olive and canola oils, avocados, nuts, margarine and salad dressings). The glycemic index will be reduced from 83 to 70 GI units (bread scale). The DASH-like diet group will be encouraged to follow a diet of whole grain foods (brown rice, whole wheat breads, muffins and breakfast cereals), reduce meat consumption, choose low fat dairy foods and a control margarine.

Exercise: The physical activity/exercise program is based on the program used at the Quebec Heart and Lung Institute (Please see section 2.1) (18). As mentioned above, a short submaximal treadmill test will be used to assess cardiorespiratory fitness (Attah an appendix) together with a measure of Rating of Perceived Exertion on the Borg Scale (http://www.cdc.gov/physicalactivity/everyone/measuring/exertion.html;).

The sub-maximal test will be completed within 10 min. and fitness will be determined using two different indices as mentioned previously. (Individuals unfamiliar with walking on a treadmill will have a few minutes practice session on a treadmill, at each site, prior to the sub-maximal test at baseline.) The Quebec experience indicates that the key factor associated with success of the intervention is the adherence to the planned visits with a kinesiologist.

The kinesiologist will work with participants in the structured exercise arms using behavioral strategies to develop and support a plan that aims to achieve the target of 150 min/week of physical activity or exercise in 3 or 5 sessions weekly (vigorous, moderate or mild depending of goals and tolerance) in keeping with maximum achievable goals of participants (19). When necessary, appropriate walking speed/intensity will be demonstrated on a treadmill by the kinesiologist. The program will also target a goal of achieving at least 10,000 steps daily recorded on their pedometers. We expect from previous experience that the vast majority of participants will have a much lower step count than the 10,000 step target at baseline but that most of them will be able to reach this target within 3 months (20). The routine exercise groups will receive standard of care for individuals being seen by a general practitioner. Specifically they will be advised to achieve the current recommended targets for daily physical activity and educational material published by Health Canada (Canada's Physical Activity Guide, Health Canada, Please see Appendix 4). Participants in the routine exercise arms will receive an exercise diary and the 7-D diet histories including the treadmill tests at the start and end of each 1 year period. They will not receive the more frequent, targeted visits with a certified kinesiologist.

12B) III. ***What are the proposed practical arrangements for allocating participants to trial groups?*** Participants who meet inclusion criteria will be randomized after stratification by centre and sex, due to the later onset of CHD in women. Random permuted blocks of varying

sizes (≤10) will be used to eliminate the possibility of predicting a future allocation leading to selection bias. The treatment allocation will be revealed to the dietitian using the web-based system after stratum entry, in the presence of the participant during the zero time visit.

12B) IV. ***What are the proposed methods for protecting against other sources of bias?***
Blinding or masking the participants or the dietitians in this study is not possible due to the very different nature and physical form of the foods. The statistician will be blinded to the nature of the treatment when analyzing the data (i.e. will not know what treatment assignment 1 and 2 refer to). Physicians will not be informed of patient randomization when requested to make clinical decisions. Technicians who measure study outcomes will be blinded to the treatments, as will those who will be scoring the MRI images at the central location. It will be stressed to the participants that both dietary treatments have been considered to confer benefits. In this way the expectation of benefit from either treatment will be balanced (equipoise) (Please also see section 12B) I.).

12B) V. ***What are the planned inclusion/exclusion criteria?***
Inclusion criteria: (Please also see 12 B) I for basic inclusion criteria) Eligible participants will be men and postmenopausal women with a BMI ≤40 kg/m^2 and who have measurable arterial thickening (>1.2mm) at screening (please see details of risks, Sections 12A) and 12B) I***).***They should also have at least 1 of the following characteristics:

- Type 2 diabetes;
- Non-diabetic subjects post MI or post percutaneous coronary intervention (angioplasty) on statin therapy;
- Hypercholesterolemic and treated with statins or have been prescribed statins but are not taking it because of either statin intolerance or have refused statin treatment.
- Raised blood pressure (>140/90 untreated)

Exclusion criteria will include: any participant with cardiovascular disease that precludes exercise (recent stroke or myocardial infarction), or cardiac condition that compromises normal function (e.g. mitral valve disease, heart failure NYHA grades 2-4, severe angina or other conditions preventing exercise), The exercise program will be based on participants baseline levels of physical activity and fitness, secondary causes of hypercholesterolemia (hypothyroidism unless treated and on a stable dose of L-thyroxine, renal (that precludes dietary change) or liver disease), LDL-cholesterol ≤1.42mmol/L, uncontrolled blood pressure (>150/95mmHg, treated), major disability or disorder requiring continuous medical attention (on Coumadin) and treatment, such as chronic heart failure, liver disease, renal failure or cancer (except non-melanoma skin cancer—basal cell, squamous cell) chronic infections, autoimmune disease, chronic inflammatory diseases (eg lupus, ulcerative colitis, celiac disease or gluten sensitivity); with major surgery <6 months prior to randomization, or newly diagnosed with diabetes, or alcohol consumption >2 drinks/d, will be excluded (5, 21). Individuals with food allergies or sensitivity to study foods or study food components (e.g. tree nuts, peanuts, soy, wheat, gluten, oats, eggs, milk) will not be recruited. Those not suitable for MRI examination (e.g. have metal implants or are claustrophobic) will be excluded. Also, individuals who already follow a portfolio-like diet and are not prepared to change or have a structured exercise program which they cannot increase any further will be excluded. Individuals who do not have a family doctor will be excluded.

12B) VI. ***What is the proposed duration of treatment period?*** Each arm of the study will last for 3 years (Please see Study flow chart –Figure 2).

12B) VII. ***What is the proposed frequency and duration of follow-up?*** (Please see section 12B) I. and Figure 2) The MRI imaging assessment of carotid arteries will be done at baseline, year 1 and year 3. Previous assessments using the 1-3 year time frame for angiographic and MRI assessments of diet (22-24) and statin trials have shown benefits (25-27) which have increased over time (28). An assessment of recruitment and retention will be made at 1year for future grant writing and as progress report for CIHR.

12B) VIII. (a) ***What are the proposed primary and secondary outcome measures?***
Primary: Maximum Vessel Wall Volume by MRI at years 1 and 3.
Secondary: Lipid rich necrotic core and intra plaque hemorrhage assessed by MRI at years 1 and 3; blood pressure and pulse rate at year 3; and treatment difference in initiation of statin therapy according to current CCS guidelines (1).
Tertiary: Completeness of clinic attendance (and data collection) and provision of treadmill testing, exercise histories, and pedometer records and change in formal measures of physical activity including heart rate on standardized testing. Changes in fitness/daily step counts will also be recorded (see methods below (12b-IX).

12B) VIII. (b) ***What is the justification for the primary and secondary outcome measures?:*** Primary and secondary outcomes should relate to preclinical CHD, be non-invasive, not involve ionizing radiation, and allow conclusions to be drawn from small sample sizes in finite periods of time. We believe that MRI the imaging techniques fulfill these criteria.

12B) IX. ***How will the outcome measures be measured at follow-up?***
Diet Recording Methods: All subjects will be instructed on diet history recording and also supplied with printed booklets in which to record food items and their amounts, either as actual weights or amounts using common measures e.g. cups or teaspoons etc., during the periods when diet histories are recorded (29, 30).

Physical activity/Exercise tolerance (or cardiorespiratory fitness); Physical activity will be assessed using a waist mounted pedometer collected over a period of 7 days every month for the first 12 months of the trial. As mentioned previously cardiorespiratory fitness will be assessed using a sub-maximal treadmill test annually. A standardized 7 day physical activity journal will also be recorded and continuous pedometer recording for the year on the test, and at the start and end of the year only (31) for the control.

Dietary Assessment: Diets will be analyzed using a program based on USDA data (ESHA) (32) (Please see Appendix 8A for details) and through metabolomics assessment, to help identify objective biomarkers of specific foods consumed by the participants which is not prone to the

same measurement and reporting errors of traditional self-report diet tools at baseline and year one.

Analytical Techniques: Serum lipids and C-reactive protein (CRP) will be measured at each centre on fasting serum. LDL-C will be calculated using the Friedewald equation (33). Serum lipid standards (Solomon Park Research Laboratories, Kirkland, Washington) will be used to quality control the lipid analyses at the 3 collaborating sites. (Please see Appendix 8A for details).IMT will be measured by B-mode Carotid Ultrasound at 12 carotid artery segments (1-cm long) of the near and far walls of the internal, bifurcation and common left and right carotid arteries. Screening will follow the protocol of the SECURE Trial (34, 35). Magnetic Resonance Imaging (MRI) will follow standardized protocol in terms of Scanner, Coils, Sequences, and Software: MRI carotid scans will be performed at the 3 centres using 3.0 T whole-body scanners and using a standard protocol with a phased array neurovascular coil and read at a central location (Sunnybrook). (Cardiovascular imaging software: MRI-PlaqueView 2.0, VP Diagnostics, Inc., Seattle, WA). The carotid flow divider will be used as the iso centre of the data volume and carotid wall parameters 25mm above and below this point will be included in the analysis. Semi-automated vessel wall boundaries will be defined using Q-plaque software (Medis, the Netherlands) (Please see Appendix 5).

Metabolomics analysis for dietary assessment: Metabolites will be analyzed in de-identified plasma samples at the Broad Institute at MIT and Harvard. The metabolites will be measured in plasma by LC-MS, using HILIC-pos method to measure polar metabolites and the C8-pos method to measure lipids.

Fecal microbiome and metagenomic analyses: Participants will be asked to collect a fecal sample for their 3 year visit. Prior to their visit, they will be consented, provided with a fecal sample collection kit, and instructions on how to do so. DNA will be extracted from fecal samples and compositional analysis will be done by 16S or shotgun generation sequencing. The fecal samples will have DNA extracted by study staff at the Medical Science Building, University of Toronto, and then sent to an external lab for sequencing.

12B) X. ***Will health service research issues be addressed?*** Although we believe our numbers are too small to assess these issues reliably, subjects will be asked to estimate the cost of their one week diet prior to the start and at Years 1 and 3 of the diet. We will attempt to obtain some idea of how satiating, palatable and sustainable the diets are using numeric scales. The Medical Outcomes Study 36-Item Short Form Questionnaire (SF-36) administered pre-study and at Year 1 and 3 will gain insights on how the diets influence overall quality of life while the Montreal Cognitive Assessment (MoCA) and Mild Behavioural Impairment scale (MBI-C) will attempt to measure how cognitive function and mild behavioural impairment could be influenced by the diets. Our current study is too small in size to address health services research issues but the future large multi-centre event trial will be able to assess questions in this area.

12B) XI. ***Sample Size and Justification***

i) Effect Size

We have selected -106.5mm^3 as the effect size treatment difference at year 3 in MRI assessed plaque volume based on a study by Migrino et al. that demonstrated a reduction in plaque volume following increased statin dosage (36). This study demonstrated a 60mm^3 reduction in plaque volume for a 0.31mmol reduction in LDL-C (36) over a 6-month period. Our own recent portfolio study (**JAMA 2011**) demonstrated a 0.49mmol/L LDL-C reduction at 6 months on the portfolio arm after allowance was made for 0.16mmol/L LDL-C reduction on the control. Our planned study will last for 3 years and our unpublished open label single phase dietary portfolio study of 61 participants indicated a further reduction of 0.06mmol/L in LDL-C over the 6 month to 3 year period. We have added this further reduction to our JAMA 2011 value (4), giving an overall 0.55mmol/L LDL-C reduction. This 0.55mmol/L LDL-C reduction would correspond to a between treatment difference of 106.5mm^3 in plaque volume, according to the findings of Migrino and colleagues and therefore will be our effect size (36).

ii) SD was determined to be 276mm^3 by using a pooled SD from the Migrino paper (baseline: 300mm^3, end of treatment: 331m m^3, n=26) (36), a Fenster cross-sectional assessment (210mm^3, n=10) (37), and our own preliminary data from our CIHR diabetes study at baseline and 1 year (baseline: 227mm^3, 1-year: 225mm^3, n=16) (pooled data, blinded to treatment). We used the two papers since they assessed individuals with more modest plaque, similar to the population to be recruited. These SDs spanned the range of SDs emerging from our current CIHR funded glycemic index MRI study.

iii) Dropout: Our recent portfolio diet study had a dropout rate of 23% (**JAMA 2011**) (4). However, our current CIHR funded low GI diet study with similar ultrasound and MRI protocol has only had a 10% (7/69) dropout in the 1-year period (6/7 dropped out in the first 3 months). We therefore consider our dropout rate will be between 10% and 25% and most likely below 20% (i.e. >80% retention), which has traditionally been considered as providing a high level of evidence, e.g. National Institute for Health and Clinical Excellence (NICE), Grading of Recommendations Assessment, Development and Evaluation (GRADE), etc (38-41). However, we have allowed for 30% dropout in the sample size calculation to ensure that we have adequate power.

iv) Screening: Only when there is measurable thickening in intima-media thickness (>1.0mm) can significant change be reliably detected as shown with 2 recent statin trials (42, 43) and a diet trial (22). Since it is estimated that between 30 to 50% of the non-diabetic population in our participants' age range have measurable arterial thickening (22, 44-46), we should be prepared to recruit and screen up to 3 times as many participants as we finally randomize to ensure measurable arterial thickening in our participants.

v) Sample size Calculation: We are using our previous power calculation (see below) for the present factorial design since we are advised that the final numbers required remain the same if there is no intended interaction between the two treatments. Using two-sample t-test, we would require 105 participants per treatment arm to detect a significant effect size of 106.5mm^3 (i.e. an end of treatment difference based on the expected relative cholesterol reduction in test vs. control, considering study length and dropout) with an end of treatment SD of 276mm^3 (α=0.05, 1-β=0.80)(47). However, since our final data will be analyzed by ANCOVA, we have used the ANCOVA sample size calculation to determine our final sample size. Due to the lack of r-values in the literature, and our expected recruitment of participants with preclinical vascular disease in a narrow range of screening arterial thickening, we anticipate only a modest degree of correlation

between end and baseline values. We have therefore used a correlation of 0.6 in our ANCOVA sample size calculation which yields 67 participants required per arm (48). Therefore, assuming 30% dropout rate over 3 years (Please see Section 12B) XIV), we will need to randomize a minimum of 194 participants (81 per arm) for the proposed study. We will therefore screen and scan three times this number to ensure measurable arterial thickening, i.e. 576 theoretically eligible participants of whom one third will be randomized.

Secondary Measures: A published power calculation from a major meta-analysis of the literature indicated that with 130 completers we will be able to detect a ≈ 10% between treatment difference in maximum percentage lipid rich necrotic core (49). Reliable estimates for intraplaque hemorrhage are not currently available due to the newness of this now clinically relevant MRI measurement. However from biological considerations, power requirements are likely to be similar to those for the lipid rich necrotic core. In previous studies 120 participants have been sufficient to detect 4% changes in BP and pulse (**Arch Intern Med. 2012**) (50). No participant with previously acceptable LDL-C levels rose above the predetermined cut-point requiring statin treatment. Data were therefore not available on which to base a power calculation.

Tertiary Measures With 194 participants we will be sufficiently powered to detect between treatment differences of of 8.5% in LDL-C, 6.9% in total cholesterol:HDL-C ratio, and 4.5% in CRP (**JAMA 2011**, **JAMA 2003**) (4, 14).

12B) XII. (a) ***What is the planned recruitment rate?*** We plan to screen 582 subjects/1.5y, 292 in Toronto and 145 in each of the other centres (to randomize 194).

Although our study was closed at the time we reached our recruitment number of 194, a further 60-70 potential participants were still in the pipeline due to the length of time the recruitment process takes. Since they had signed the consent form, we felt it inappropriate to tell them they were no longer needed. Therefore our new total for the Toronto site is 160 for a total of 260.

12B) XII. (b) ***How will the recruitment be organized?*** -4 methods of recruitment will be used:

1) Advertising in newspapers and on the Toronto subway system.
2) From previous studies—contacting past study participants who had consented to being contacted for future studies. They will be contacted by mail followed by a telephone call in 2 weeks if no response.
3) Advertising (using REB approved study poster) in family doctors clinics and specialized clinics at St. Michael's Hospital and hospitals in the Greater Toronto Area having first obtained permission from the relevant clinics. The specialized clinics will include Lipid and Diabetes clinics, family doctors' clinics, cardiology, vascular (CATH) labs, and cardiac rehabilitation clinics.
   Initial screening will take place by telephone.

   A. Participants will also be recruited through the UTOPIAN (University of Toronto Practice-Based Research Network): All physicians at North York Family Health Team

(NYFHT) and other community family heath teams and clinics in the Greater Toronto Area (GTA) will be informed about the study and asked for permission to access their patients' record on the EMR (Electronic Medical Record). St. Michael's Hospital Academic Family Health Team **will not** be included in this process. Physicians that are a part of UTOPIAN-CPCSSN project will be approached first as it will allow for a quicker access to their de-identified patient data and querying to identify eligible patients.

Physicians who agree to participate in the recruitment drive will sign a consent form to this effect. This recruitment method has now been approved by the University of Toronto Research Ethics Board.

We will arrange evening information sessions at each centre for groups of 10 to 20 potential volunteers and their spouses. Those showing interest will be recruited to the study if inclusion and exclusion criteria are satisfied and if those responsible for their medical care see no contraindication, are not anticipating changing lipid treatment in the next 6 months, and will contact the research team for discussion before any change is initiated (we ask for a physician letter of support). We anticipate running 1-2 evening recruitment sessions per week for the first 1.5 years.

12B) XII. (c) ***Over what time period will recruitment take place?*** Volunteers will be recruited during the first 1.5y of the 5y term of the study.

12B) XII. (d) ***What evidence is there that the planned recruitment rate is achievable?*** From hospital clinics and newspaper advertisements we have recruited 96 hypercholesterolemic study participants in a 12 month period (July 2007 to July 2008) at the Toronto site for a study using this diet with only 25% of the advertising planned for the present study. We believe that, with the extra advertising and interest generated by MRI assessment of the carotid artery, our recruitment, participant retention and enthusiasm for the study are all likely to be greatly increased, as demonstrated by our current diabetes study retention.

12B) XIII. ***Are there likely to be any problems with compliance?*** Similar to our recent portfolio study, we expect on average participants to consume 41-46% of the recommended dietary components (adherence) which previously resulted in 13-14% LDL-C reductions (**JAMA 2011**) (4). This level of compliance is similar to the 52.5% adherence seen in our open-label unrandomized 1 year study (12). We anticipate that significant LDL-C reductions will be maintained over the 3 year study period. Therefore, to support adherence to the interventions over the duration of the study, optional activities will be offered to participants. These may include: 60-90-minute workshops, which will include 20-minute presentation of new research on foods encouraged on the respective diets, question and answer period, recipe tasting and discussion on modifications; cooking classes, which will be organized in collaboration with the Loblaws cooking school; quarterly info-graphic newsletters which will feature new research on specific foods included in the diets. We would also like to include a feedback survey to be collected from attendees at the end of each activity in order to assess how they were received and what can be improved upon for subsequent activities. Study participants will also be able to use a diet app, designed by our research team, to help with study compliance. It will include various resources including recipes, tip sheets, graphs summarizing their progress in following the dietary advice and personal messages to direct participants to resources to assist them in areas of

difficulty. The app will be rolled-out over a few months in order to assess whether it has increased compliance and to review feedback on its use including addressing any technical issues. Metabolomics assessment will also occur at baseline and year one, to help identify objective biomarkers of specific foods consumed by the participants which is not prone to the same measurement and reporting errors of traditional self-report diet tools.

12B) XIV. ***What is the likely rate of loss to follow-up?*** Based on our recent dietary portfolio study (JAMA 2011) and our current CIHR funded trial experience in type 2 diabetes using the same diagnostic protocol, we believe our dropout rate will be <20% (Please see Section12B)XI p. 11o). We will make every effort to minimize dropouts including emphasis in the original consent for the need for a final visit, as recommended in US FDA and European Guidelines (38, 39). We will make phone calls to establish contact, make sure participants feel they are collaborators in the study, educate them about the respective dietary issues, and express appreciation for their time (51). These strategies have resulted in 86% retention in a recent 5-year cohort study (we have consulted with the senior author of the NEJM 2011 study, Dr. Margaret Herridge) (51, 52). We will also offer $250 to offset possible inconvenience in attending the final visit (this amount will be provided to all participants to facilitate their final attendance) (Please also see Budget Justification- "Participant Final Year 3 Visit Compensation"). We will ask participants, unwilling/unable to attend (e.g. those who have left the district), to have LDL-C measured by their local physician or at least make available their most recent LDL-C measurement to assess whether dropout was associated with non-adherence.

a. **12B) XV.** ***How many centres will be involved?*** Three recruitment sites will be involved: Institute of Nutraceuticals and Functional Foods, Laval University, Quebec; Healthy Heart Lipid Clinic, St. Paul's Hospital, Vancouver; and the Clinical Nutrition and Risk Factor Modification Centre, St Michael's Hospital or the David Naylor Building at the University of Toronto, and North York Family Health Team, North York General Hospital will be the recruitment clinics in Toronto. The MRI unit, Sunnybrook Health Science Centre, Toronto, Ontario, will be responsible for the interpretation of MRI scans. Robarts Institute, Western University, will be interpret and analyze screening US data. The Department of Biostatistics, McMaster University will oversee statistical aspects of the trial. The Nutritional Sciences Department, Faculty of Medicine, University of Toronto will also be involved in the assessment and quality control of recorded dietary data for all centres. We are adding an additional stress test clinic site, Toronto Cardiac Clinic because the stress test clinic at St. Michael's Hospital is not able to accommodate all of our research study patients because of the Covid-19 pandemic. Participants randomized (as opposed to screened) will be 98 at the Toronto site and 48 each at the other sites.

12B) XVI. ***What is the proposed type of analysis?*** An ANCOVA linear regression model will be used with the 3 year value as the outcome, treatment and sex as main effects and baseline as a covariate. In the intention-to-treat analysis, we will assume that data are multivariate, normally distributed and missing data are missing at random (MAR). For multiple imputations, the mean of 5 sets of generated data will be taken; this process will generate data for missing values, standard errors, and 95% confidence intervals [CI]. For example, using LDL-C

at 24 weeks in our recent study, the strongest predictors of missingness at 24 weeks were age ($\beta = 0.0454$, $P = .01$) and sex (women vs. men) ($\beta = -0.2807$, $P = .11$) (4), and these, along with the observed values, were used to inform the imputations. Site and diet assignment did not predict missingness in that study ($P \geq .16$) (4). As a sensitivity analysis, we will compare the results using the ITT with those of the completers-only. The 10-year Framingham CHD risk score will also be calculated (53) for this predominantly white cohort. Pearson correlation will be used to calculate the correlation coefficient between dietary adherence and change in maximum vessel wall volume (plaque volume).

12B) XVII. ***What is the proposed frequency of analyses?*** The imaging analyses will be made at baseline and year 3. Assessment of recruitment and retention will be made at 1y to inform further grant applications

12B) XVIII. ***Are there any planned subgroup analyses?*** No subgroup analyses are planned. However, we may conduct exploratory analyses by sex, site, or within groups meeting specified LDL-targets. Since we are not primarily powered for these analyses, we will treat these as secondary, exploratory analyses only.

12B) XIX. ***Has any pilot study been carried out using this design?*** A number of dietary portfolio studies (12) including the CIHR funded study of 6 months duration have now been completed (**JAMA 2011**) (4) demonstrating 13-14% LDL-C reductions with 40% adherence to all the recommended dietary components which are the same as those being tested in present study. Similarly, our CIHR funded low glycemic index study (**JAMA 2008**) (3) and our CIHR monounsaturated fat enriched portfolio study (**CMAJ 2010**) have also provided feedback (54). These studies have influenced the design of the present study in terms of power, magnitude of expected effect, and dropout rate, together with considerable feedback on participants' dietary preferences to facilitate overall adherence. The exercise protocol has been tested successfully in participants at high risk of CVD (55).

12C) TRIAL MANAGEMENT

12C) I. ***What are the arrangements for day-to-day management of the trial?*** Each clinical centre will have a centre dietitian who will be responsible for preparing hospital ethics committee protocols, protocol modification and adverse event reporting. The dietitians will also alert the centre physicians to any concerns, which may need medical action. They will arrange advertising, answer resulting phone calls and screen potential participants, set up information sessions, and participant screening visits and later study visits, coordinate physician and blood technician schedules, and make appointments for diagnostic tests (e.g. MRI). At clinic visits they will encourage consumption of the appropriate amounts of the key ingredients on the dietary portfolio and adherence to whole grain cereals on the control diet, check 7-day diet records and, if necessary, alter the diet plan accordingly. Tests in each clinical center will be under the supervision of a physician with an expertise in exercise testing. Previous cardiac history as well general health information will be made available for the safe conduct of exercise testing and to those conducting exercise testing. The patients' personal health information will be transferred through File Transfer to the alternate stress test location (Toronto Cardiac Clinic) and the results of the exercise testing will be sent back to us via study ID and ONE Mail. The physician does not

have to be a cardiologist and does not necessarily have to be present in the exercise lab while the testing is being done; as long as he/she is present on the floor and easily reachable. Each center will also have a certified kinesiologist (0.15 FTE) who will provide assistance to the centre dietitian for the conduct/coordination of the study. In addition, the kinesiologist will be responsible for the assessment of baseline physical activity levels, cardiorespiratory fitness and readiness to change for all local participants and prepare an individualized intervention aiming at reducing sedentary behaviors, increasing the number of daily steps to 10,000 and increase cardiorespiratory fitness through the prescription of 150 weekly minutes of moderate to vigorous intensity endurance exercise depending on baseline fitness. Together with the part-time clerk, they will enter dietary, laboratory, anthropometric and blood pressure data on the data templates provided by the Applied Health Research Centre (AHRC) of the Li Ka Shing Institute which is responsible for electronic data storage and quality control of the data. Together with the part-time clerk, they will enter dietary, laboratory, anthropometric and blood pressure data on the data templates provided by the Applied Health Research Centre (AHRC) of the Li Ka Shing Institute which is responsible for electronic data storage and quality control of the data. The dietitian at the Toronto site will also act as the study coordinator responsible for coordination between the 4 sites.

Study charts will be organized by the part-time study clerk together with physician notes and consent forms. They will assist the dietitians with all aspects of recruitment, with diet history coding, data entry, and quality control. Centre PIs will be available throughout the study to discuss study issues with the site dietitian as they arise and will meet on at least a daily basis.

Blinded to treatment, MRI scan data will be read and the data processed by the research assistant, Postdoctoral Fellow and MSc students under Dr. Alan Moody at the Sunnybrook Vascular Imaging core facility.

Food Supply:

Study participants will be able to pick up study bread and other food supplies from our clinic centre in-person or by curbside pick-up during pandemics as availability permits from study sponsors. We anticipate centre pick-ups will be no more than once per month and will likely replace a normal shopping outing. The study bread will also be available for purchase directly from a local baker in store or by curbside pick-up; and we will cover the bread cost so no contact is necessary for payment during pandemics. Study participants may be also be able to order food products directly from study sponsors using a study code for a subsidized cost in order to have products delivered to their home.

Data Management: Data will also be organized by the recently initiated (2009) AHRC (www.ahrconline.com) for clinical trial support, of the Li Ka Shing Knowledge Institute of St. Michael's Hospital, which has a clinical trials unit designed precisely to support such studies. The MRI studies will generate a very large volume of imaging data. Storage of anonymised, catalogued, image data will be located at the Sunnybrook Health Sciences Centre MRI Research Unit with links to the AHRC, using blade-server technology to permit ease of upsizing when future projects are added to the MRI program. Post-processing will take place at the Sunnybrook Vascular Imaging core facility. Image data will be reduced to quantitative data that will be entered into the Case Report Forms that will be developed prior to study commencement. The clinical database will be held centrally at St. Michael's Hospital's AHRC in collaboration with the Li Ka Shing Institute. The data management system also allows for MRI images to be

uploaded onto a web-based system with centralized servers in a secure data centre. Both the data management system and the physical infrastructure where the hardware will reside exceed international privacy and security standards. This system ensures direct, secure access to data via a dedicated portal to the Institute server, which can be accessed by the internet, and enables information storage, retrieval and sharing. The system will be organized by the Li Ka Shing Knowledge Institute IT group, whose statistical group will also provide support. AHRC, will provide overall clinical data management support through the MediData RAVE™ data management software (www.mdsol.com) which is being used for over 10 studies run through AHRC, including 3 currently funded CIHR multi-centre trials.

12C) II. ***What will be the role of each principal applicant and co-applicant proposed?*** David Jenkins, Jiri Frohlich, and Benoit Lamarche will have overall responsibility for running the studies at the 3 recruitment sites. Frank Sullivan will be responsible, with his team, for recruitment through UTOPIAN at the Toronto site. David Jenkins, supported by Cyril Kendall, Julia Wong, John Sievenpiper, and Livia Augustin will be responsible for coordination between sites, with Seyed-Arash Mir-Rahimi and Laura Chiavaroli (PhD Students), as HQP, identifying issues at the larger Toronto site with potential implications for all sites and coordinating with the CUS and MRI reading centers and the data management Centre (AHRC at St. Michael's Hospital). Jean-Pierre Despres will chair the exercise group that will include: Scott Lear, Simon Fraser University; and Scott Thomas, University of Toronto. Alan Moody, together with Kim Connolly, Andrew Yan, and Johnathon Leipsic will form the cardiovascular imaging group and together with the MRI expertise of Anish Kirpalani and Walter Montanera will be responsible for all MRI components of the study, including transference and analysis of MRI images from collaborators (Eric Larose at Laval, , and Alex MacKay and Johnathon Leipsic at UBC). Likewise, Alan Moody (as a Co-principle investigator of the Canadian Atherosclerosis Imaging Network), Aaron Fenster (Robarts Institute, Western University), and Sammy Chan at UBC, will be responsible for technical issues, measurements, and interpretation. Joseph Beyene, at McMaster, Department of Clinical Epidemiology & Biostatistics, supported by Russell de Souza and John Sievenpiper, will be responsible for the statistical aspects of the study together with the AHRC team. Muhammad Mamdani will be responsible, with his AHRC team, for the data management of the study. All the above, including Lawrence Leiter, Robert Josse, Patrick Couture, and Vladimir Vuksan representing the endocrinology and blood pressure group, have had input in drafting this application and will be responsible for study advice, interpretation and manuscript preparation related to their respective interests. (Please see Appendix 10 and 11 "Letters of Collaboration" and "Applicant Table")

12C) III. ***Description of the trial steering committee and data safety and monitoring committee.*** The steering committee and the Data Safety Monitoring Committee (DSMC) will be modeled on current practice for our CIHR funded diabetes trial. The steering committee will be made up of the site PIs who will discuss initially monthly by conference call problems as they arise, especially recruitment goals and later with quarterly conference calls. For specific issues, e.g. MRI, etc, those responsible (Please see above) will be involved. Adverse events will be reviewed by a physician independent of the study. The DSMC will involve physicians from other centres experienced in clinical trials (our current CIHR diabetes trial DSMC members will be

approached first for membership) who will review serious adverse events immediately and minor events at 6 month intervals.

OTHER ISSUES

Details of risks to the safety of participants involved in the trial. We see little risk associated with the dietary components of the trial. The foods to be used are available for purchase in supermarkets except for a psyllium-enriched oatbran bread which we have had provided by local bakers in our previous studies (JAMA 2011) (1). Those with food allergies to the dietary components will not be recruited. The exercise program is a structured program. The work intensity corresponds to brisk walking. Therefore, the risks of our program are very low, even among high risk individuals. For instance, we have had no recurrent CHD events experienced by our high risk participants who were exposed to this intervention for one year. We are therefore fully confident in the wide applicability of our physical activity/moderate endurance exercise prescription as it essentially corresponds to current public health recommendations. Furthermore, in addition to the 3-monthly visits, participants will have access to the kinesiologist (phone, e-mail, etc) to obtain further support/assistance if need be.

Health Economics: The proposed first trial is relatively small to allow detailed assessment of health economics. However, it will be possible to obtain an assessment of the cost of dietary change from participants' food records and from participants' weekly food costs before and during the diet and relate these costs to the LDL-C reduction. The cost of dietary LDL-C reduction can in turn be compared with the cost of statin usage for a similar LDL-C reduction. Meaningful economic measures will be derived from a future large hard endpoint multi-centre trial.

Quality of Life: The Medical Outcomes Study 36-Item Short Form Questionnaire (SF-36) will be administered pre-study and at Year 3. We have also included taste, satiety and sustainability (preparedness to continue on the diet) questions in the protocol which we believe will go some way to addressing this issue. The SF-36 has been used in numerous clinical studies (56, 57) with very good psychometric properties, and provides a comprehensive and subject friendly tool to assess quality of life.

Cognitive Assessment: The Montreal Cognitive Assessment (MoCA) (version 7.1 original version) will be administered at months 0, 12, 24 and 36. This tool measures different cognitive domains such as attention, concentration, memory, language etc. (ref. Z. Nasreddine MD; www.mocatest.org). **The Mild Behavioural Impairment scale (MBI-C) will be administered at months 0, 12, 24, and 36 months.** The MBI-C is designed to detect behavioural pre-dementia at-risk states with a high level of sensitivity and specificity (ref: Ismail, Z. https://mbitest.org/). The MBI-C is a free validated rating scale consisting of 34 questions, which takes around 10-15 minutes to complete and is designed to be completed by either participant, close informant or clinician at clinic or over the phone.

Age: Women will be post-menopausal. The lower limit on age for men is of little significance since men in our subject pool are predominately in the 50y plus age range. We have no upper limit providing volunteers are in good health.

Consumer Involvement in Trial Development: We have not formally asked our previous or potential future participants to assess our current plans but have used the comments made by participants in our recent dietary portfolio and glycemic index CIHR funded trials. Their

suggestions are reflected in our use of a positive control diet rather than simply the standard of care. The control is both high fibre and a DASH-type diet with additional potential benefits on lipids and blood pressure.

Genetic Testing: This aspect of the study will be optional.
Objective: To determine whether there are differences between individuals in their response to exercise and dietary change, e.g. changes in plant oil intake, low versus high glycemic index foods or a lower carbohydrate diet.

Background

There is considerable interest in the scientific community to study how genes may influence response to diet / exercise. We know for example that carriers of the apo E3 / E4 allele tend to show better cholesterol lowering responses to soy in terms of LDL-C reduction (Gaddi et al. AJCN 1991;53 (5):1191-96) and also better responses to viscous fiber (Jenkins DJA, Hegele RA, et.al. Metabolism 1993;42 (5):585-93). We have recently found that equol producers (from the soy isoflavone, genestein) also appear to respond to soy by raising HDL-C, suggesting that in this group soy may be particularly cardio protective. In terms of taste our diets tend to be bland containing lower salt and also less sugar. The genes controlling these tastes may therefore determine intake and adherence to therapeutic diets (Dias AG et. al. Chem Senses. 2013 Feb; 38(2):137-45, Eny KM et al. Physiol Genomics. 2008;33(3):355-60, Garcia Bailo et al. OMICS. 2009;13(1):69-80). A variant in the APOA2 gene has also been shown to modify the relationship between saturated fat intake and BMI (Corella et al. Arch Intern Med. 2009;169(20):1897-906. These are a few examples of why comprehensive genetic analysis, due to the implication of a growing number of genes of interest, would be helpful. We will, however limit our analyses to specific genes currently known to affect dietary response. Generally, the effect of genes on diet is very modest and usually requires significant numbers of people to detect the difference. It therefore has very limited clinical relevance. We therefore plan to pool data from across our studies (typically 140-340 participants per study) which have the same high fiber treatment for the control groups, and the dietary portfolio in the test groups, to provide the larger numbers required to detect a difference if one exists. A bio-bank will be established for the proper storage and management of these samples

We hope to make our initial findings known to participants at the end of the study, especially if we discover specific groups for which certain types of dietary advice may prove advantageous. Genetic testing results are solely for use in this study and will not be shared with any third parties (i.e. insurance companies or the hospital)

Analyses

The DNA will be extracted from “buffy coat” of white blood cells obtained after plasma has been removed from citrated blood samples (blue tops) drawn at week 0 or any time point during the main study. Therefore, no additional blood samples will be required for this study.

The genes of interest will include those related to the effectiveness of dietary change on blood glucose, cholesterol and associated measurements including blood pressure, taste preference,

compliance (dopamine receptor polymorphisms) and other related genes which may influence response to diet.

Incidental findings

> ***An incidental finding is a finding concerning an individual research participant that has potential health or reproductive importance and is discovered in the course of conducting research but is beyond the aims of the study.***
> **(Managing Incidental Findings in Human Subjects Research: Analysis and Recommendations. Wolf SM et al J Law Med Ethics. 2008; 36(2):219-48)**

Any incidental findings (as defined above) obtained from screening or during the course of the main study from laboratory tests, ultrasound or MRI images will be provided to the family physician for further investigation. The participant will be notified in reassuring terms and advised to follow-up with their family physician. Furthermore, if from the Carotid ultrasound a participant is found to have a luminal narrowing greater than 50% the individual will have the option of referral to a vascular surgeon for further assessment. However, in a similar cohort of 160 patients being screened by ultrasound none were found to have an incidental stenosis of this magnitude.

In this sub-study, if genes identified have clinical implications the advice of experts in that particular field will be sort to arrange appropriate counseling for the participant. The family physician's involvement would be sought if the participant consents to it. However, the chances of incidental findings are limited since only specific genes known to affect dietary response will be studied.

Banking of Samples: Blood samples (de-identified) will be stored at the University of Toronto (at the Medical Science Building or the MaRS centre) until all planned analyses have been undertaken-usually about 3 to 5 years. Optional longer-term banking will be made available to all participants for storage periods of 25 years after study completion to allow for use in future secondary analyses in relation to the objectives of the current research project. At the end of each storage period, samples will be destroyed by autoclaving.
We are currently investigating the process of setting up a Biobank to efficiently and securely store and manage these samples; the necessary application will be sent to the St. Michael's REB once the project proposal is drawn up.
Presently the use and ultimate destruction of these samples will be the responsibility of the following investigators, individually or collectively: Dr. David Jenkins, Dr. Cyril Kendall and Dr. Sievenpiper. However, detailed information on the organization and management will be in the biobank proposal that will be submitted to the REB in the near future.

International Collaboration: None.

Patient Information Sheet: To be developed after grant review.

Industrial Partners: If awarded, Loblaw Ltd. and Unilever have committed to provide unrestricted funds of $360,000 and $50,000 respectively. (Please see Appendix 7 for letters of support**)**

Trial Significance: If this trial demonstrates a 10% or better estimated CHD risk reduction based on the primary (anatomical) outcome (MRI Vessel Wall Volume), it will both encourage the use of this combined dietary and exercise strategy in clinical practice and provide the necessary stimulus for an international hard endpoint (cardiovascular events) trial for which the trialists' network will form the nucleus.

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