THE EFFECTS OF THE DIETARY SUPPLEMENT CARDIOFLEX Q10 ON REDUCING CARDIOVASCULAR DISEASE RISK FACTORS IN ADULTS

ID: CardioFLex Q10
NCT03826914 | *September,1 2020*

STUDY RATIONALE

CVD is the leading cause of death worldwide (Mc Namara, Alzubaidi, & Jackson, 2019). CVD accounted for 24% of all deaths in the United States in 2014 (Benjamin et al., 2019) and 19% of all deaths in Canada in 2018 (Canada, 2020). The prevailing view by cardiovascular researchers is that most cases of CVD can be minimized and/or prevented by good nutrition, choosing not to smoke, regular physical activity, a healthy body weight, stress reduction, and limited alcohol consumption. Despite these recommendations, adherence to government health guidelines is low and a lot of patients at high risk for developing CVD do not make the lifestyle changes recommended. Given the prevalence of CVD worldwide, it is critical to explore new strategies aimed at reducing CVD prevalence and mortality.

Dietary supplements may be a way to reduce CVD prevalence and risk. The sparse and inconsistent epidemiologic data relating dietary supplement use to the risk of CVD can be partially explained by the differences in the supplement given, genotypic differences in individuals, and baseline intake of nutrients. For example, a case-control study in Sweden, where fruits and vegetable consumptions are low, showed an inverse association between intake of multi-vitamin-mineral supplement and the incidence of myocardial infarction (Holmquist, Larsson, Wolk, & de Faire, 2003). Generally speaking, study

participants who report taking dietary supplements generally have a healthier lifestyle; they report eating more nutritious food, less likely to smoke or drink alcohol, and exercise more (Chen et al., 2019). It is plausible to hypothesize that individuals who aren't following government health guidelines will benefit more from dietary supplements more than individuals following healthier lifestyles.

In particular, antioxidant supplements are a promising area of research in the prevention and treatment of CVD. Observational data have identified associations between intake of carotenoids, folic acid, and vitamin E and CVD risk (Lichtenstein, 2009). Research shows that antioxidants have the ability to inhibit the oxidation of cholesterol and improve endothelial function (Malekmohammad, Sewell, & Rafieian-Kopaei, 2019). High antioxidant intake has been shown to prevent atherosclerosis (Nunez-Cordoba & Martinez-Gonzalez, 2011).

Despite biological plausibility about the effects of antioxidants to reduce CVD risks as suggested with some observational studies, data derived from most nutrient supplement trials have been disappointing (Monsen, 2000). The discrepancies between observational and interventional data may be due to confounding diet and lifestyle patterns and unaccounted for genotypic variations. It may also be that specific nutrients need to be given together to translate into significant changes. Specific nutrients working in concert may produce a health benefit greater than the sum of the individual parts. For example, human studies have shown convincingly the dose-dependent enhancing effects of ascorbic acid (vitamin C) on iron absorption (Lynch & Cook, 1980). Whether it is because they enhance each other's absorption or because they have more potent physiological effects when taken together, pairing nutrients that have a synergistic effect may lead to more favourable outcomes. It may be that combinations of nutrient supplements need to be given together to translate into significant changes.

Although direct comparisons between multi-ingredient supplements and single ingredients have never been done, a substantial body of evidence suggests that multi-ingredient formulations greatly increase the efficacy of the product (Harty et al., 2018). For example, there is research to suggest that multi-ingredient supplements can improve lipid blood profiles in as little as 30 days (Hobbs, Caso, McMahon, & Nymark, 2014). However, the body of literature evaluating dietary supplements to reduce CVD prevalence and prognosis is minimal. The view by most health care practitioners and government agencies is that currently there is insufficient data to recommend the routine use of nutrient supplements to prevent or treat CVD. It is only in the last decade that dietary supplement databases with open access for the public have existed in the USA (Coates, 2016). Although a great deal of progress has been made in recent years, much remains to be done to ensure that dietary supplements are safe, efficacious, and reasonable in cost

so that they contribute positively to the public's health. Given the potential benefit of these products, more research needs to be done.

Cardioflex is a multi-ingredient supplement comprised of amino acids (L-lysine, L-proline, L-glutamine, L-threonine), vitamins (vitamins A, C, E, folate, vitamin B12), minerals (potassium, magnesium, selenium) and CoQ10. The individual ingredients in Cardioflex have shown or been hypothesized to show, positive results in reducing CVD and CVD risk factors. For example, the literature demonstrates that amino acids can play a significant role in cardiovascular health. L-threonine supplementation has been shown to lower blood pressure in patients with CVD (Tuttle, Milton, Packard, Shuler, & Short, 2012). High antioxidant intake has been shown to prevent atherosclerosis (Nunez-Cordoba & Martinez-Gonzalez, 2011). Low blood serum levels of magnesium have been associated with an increased risk for several chronic diseases including diabetes, hypertension, coronary heart disease, and osteoporosis (Kass, Weekes, & Carpenter, 2012). Multiple randomized, placebo-controlled, clinical trials show that daily supplementation of CoQ10 can cause significant antihypertensive effects in as little as 4 weeks of treatment (Ho, Bellusci, & Wright, 2009).

This clinical trial will help clarify the role of multi-ingredient supplements containing amino acids, vitamins, and minerals in the prevention and management of CVD and CVD risk factors.

RESEARCH OBJECTIVES

The overall objective of this study is to determine whether daily supplementation with the dietary supplement Cardioflex for 90 days will reduce risk factor biomarkers for CVD in adults aged 30–65 years old.

The primary objective is to determine whether daily supplementation with the dietary supplement Cardioflex will improve their plasma lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, lipoprotein A).

The secondary objectives are to determine if Cardioflex supplementation will improve anthropometric measures (waist circumference, body mass index), cardiac measures (heart rate, heart rate variability, accelerated plethysmography (APG age, which measures the biological age of arteries), blood pressure), and inflammatory and endothelial function biomarkers (C-reactive protein, IL-6, sICAM-1, sVCAM-1,) and assess the safety of the supplement by measuring serum levels of liver and kidney enzymes (alanine transaminase; ALT, aspartate transaminase; AST, lactate dehydrogenase; LHD, blood urea nitrogen; BUN, and creatinine). To quantify confounding variables, participants were asked to record everything they ate and drank in a food log and wore a pedometer to

track total steps on day 1 (beginning), day 45 (mid-point) and day 90 (end) of the study.

HYPOTHESES

- Participants who consume one serving (10 g) of Cardioflex per day, the label recommended dosage, will have improved lipid profile, as reflected by lower levels of total cholesterol, LDL-cholesterol, triglycerides, and lipoprotein A, and higher HDL-cholesterol than participants who consumed the placebo.
- Participants who consume one serving of Cardioflex per day will have improved cardiac health, as measured by lower blood pressure, higher HRV, lower APG age, and lower levels of inflammation biomarkers (C-reactive protein, IL-6, sICAM-1, and sVCAM-1) than participants who consumed the placebo.
- No significant changes will be seen in anthropometric measures (waist circumference, body mass index) or liver and kidney functions biomarkers (ALT, AST, LHD, BUN or creatinine) between the Cardioflex and the placebo group.

STUDY DESIGN

This was a randomized, double-blind, placebo-controlled, parallel design study where participants were assigned to receive one serving (10g) of Cardioflex or 10g of a maltodextrin placebo daily for 90 days and independent of any other diet or exercise interventions. The study was conducted at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), located at the University of Manitoba Ft. Garry Campus. The study was registered on clinicaltrial.gov with ID number NCT03826914 and approved by the University of Manitoba Joint-Faculty Research Ethics Board (JFREB) with protocol number J2018:010 (HS21609).

The current lack of accepted guideline recommendations for nutrient supplements is explained by the lack of clinical evidence showing nutrient therapy's effectiveness and safety. Hence, a placebo-controlled randomized clinical trial is warranted. Randomly assigning the intervention eliminates the influence of unknown confounding variables or bias that could lead to an incorrect measurement of treatment effect. Randomization eliminates confounding baseline variables, thus eliminating the possibility that the observed effects are because one treatment group had a more advantageous starting point. The placebo control allows participants, investigators, and study staff to be blinded. The advantage of a placebo-controlled trial over an observational study is the ability to demonstrate causality.

Participants were given the treatments as individual stick packs so there was no variance in the daily dosage consumed. Participants were told to mix the supplement with water

and consume it before eating breakfast daily. The group supplementing with Cardioflex ingested one serving (10g) of Cardioflex daily, whereas the placebo group ingested 10g of maltodextrin placebo. We chose this as our placebo supplement because it was likely to have minimal effect on CVD health biomarkers. A log was given to the participants to record their supplement intake. Compliance with the supplement protocol was assessed by measuring blood levels of CoQ10 and providing participants with more treatment packages than the total number of days of the study and asking the participants to return any unused stick packs to the supplement coordinator at the end of the study. We chose blood levels of CoQ10 as our main indicator of compliance because it is not readily found in food and previous research found a significant increase in plasma CoQ10 concentrations when participants were given Coq10 supplements daily (Niklowitz, Sonnenschein, Janetzky, Andler, & Menke, 2007). Thus, it is anticipated that a participant's CoQ10 blood values should increase with Cardioflex supplementation and would indicate compliance with taking the supplement. In addition, participants were given a randomized number of stick packs between 93 and 100, giving every participant at least a few stick packs to return at the end of the clinical trial. Participants were not told the number of stick packs they were given.

Participants were instructed to follow the same diet and maintain the same level of physical activity throughout the clinical trial. To access dietary intake and physical activity, participants were asked to record everything they ate and drank in a food log and wore a pedometer to track total steps on day 1 (beginning), day 45 (mid-point) and day 90 (end) of the study. A 90-day study length was chosen because previously published data saw significant changes in triglycerides, LDL-cholesterol, HDL-cholesterol, and total cholesterol when participants were given a multi-ingredient supplement for 90 days (Lombardo et al., 2013). A sample size calculation was completed using previously published data (Hobbs et al., 2014) to ensure enough participants were recruited. We calculated that we would need 60 subjects to reach statistical significance. With an expected attrition rate of 20%, we determined that 72 participants needed to be recruited.

To determine the effects of Cardioflex on CVD risk, the dependent variables tested included anthropometrics (weight, height, waist circumference), cardiovascular parameters (blood pressure, heart rate, HRV, APG age), dietary intake, plasma lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, lipoprotein A), and inflammatory and endothelial function biomarkers (C-reactive protein, IL-6, sICAM-1, sVCAM-1). Anthropometrics were included because large scale epidemiological studies have shown correlations between CVD mortality and BMI (Valavanis, Mougiakakou, Grimaldi, & Nikita, 2010) and increased waist circumference is recognized as a major cardiometabolic risk factor (Tchernof & Despres, 2013). Cardiovascular parameters carry valuable information for the prediction of long-term and near-term CVD risk. Blood

pressure is a consistent risk factor for the development of atherosclerosis (Lu, Cassis, & Daugherty, 2007). A higher resting heart rate is linked with greater CVD risk (Fox et al., 2007). Reduced HRV is associated with an increased risk for coronary heart disease and CVD mortality (Liao et al., 1997). Arterial elasticity is an established risk factor to predict future CVD events (Glasser et al., 1997). Lipid profile is recognized as an established risk factor in the development and progression of CVD (Graham, Cooney, Bradley, Dudina, & Reiner, 2012; Jellinger et al., 2012). Inflammation and endothelial function play a significant role in developing atherosclerosis, and risk of future cardiovascular events (Jarvisalo, Juonala, & Raitakari, 2006).

PARTICIPANTS

Recruitment of study participants employed several strategies including email, flyers, and in-person presentations. The study eligibility criteria were adult men and women between the age of 30-65 years old, perform less than 150 minutes of moderate or vigorous physical activity per week, have not used prescription cholesterol or blood pressure medication in the last 3 months, be willing to stop taking any dietary supplements during the study period of 90 days, not pregnant nor planning on getting pregnant during the study, and an APG type of D, E, F or G and/or an HDL-cholesterol to total cholesterol ratio of $\leq$24 percent.

Participants were stratified (male/female) and randomized (block size = 5) to the study treatments using a computer-generated randomization (www.randomizer.org) numbering system.

SAMPLE SIZE

A sample size calculation was completed using a standard sample size in equation 1 to determine the number of participants needed for the study. With α = .05 and β = .90 and a 12% mean decrease of cholesterol using previously published data (Hobbs et al., 2014) in the supplementation group, we calculated that we would need 60 subjects to reach statistical significance. With an expected attrition rate of 20%, we determined that 72 participants needed to be recruited.

$$n = \frac{2\left(Z_{\alpha} + Z_{1-\beta}\right)^2 \sigma^2}{\Delta^2}$$

Equation 1 - Estimated sample size equation
$Z_{\alpha} = contasnt\ t\ othe\ accepted\ error, Z_{1-\beta} = constant\ according\ to\ power,$
$\sigma = standard\ deviation,$ Δ= effect size

Overall, 90 adults were screened for eligibility, with 75 adults being deemed eligible. At the time of starting the study 69 adults chose to participant, and 6 eligible adults chose not to participate for personal reasons not related to the study.

STUDY PROCEDURES AND ASSESSMENTS

BLOOD COLLECTION

Blood was drawn at 3 time points in the study – the assessment for eligibility for entry into the study, baseline, and end of the 90-day supplement period. All blood samples were collected in the morning after at least 8 hours overnight fast. The participants reported to the RCFFN at the University of Manitoba Ft. Garry Campus. Blood samples were taken by venipuncture from an antecubital vein into serum tubes by a certified phlebotomist. For eligibility assessment, one 5mL serum tube was withdrawn. For clinical trial baseline and endpoint measurements, two 10mL serum tubes, and one 5mL serum tube, totaling 25mL of blood was withdrawn from each participant. Samples were centrifuged at 4100g for 10 minutes. The plasma was allocated into Eppendorf tubes and frozen at -86 ° C for later analysis.

Cardiac Measurements

Participants were required to sit for a minimum of 5 minutes rest at the beginning of each assessment session. Blood pressure was measured at the brachial artery of the left arm, using an automatic sphygmomanometer (Series 10, Omron). The blood pressure of each participant was taken three times, and the average was recorded. Accelerated plethysmograph (APG) type, HR, and HRV were assessed using a Meridian digital pulse-wave analyzer (DPA) machine (Long Life Cardio LLC, USA). HRV was measured using the standard deviation of all of the RR intervals (the distance between each heartbeat) after 5 minutes of recording.

ANTHROPOMETRICS

All anthropometric measurements were collected with minimal clothing, without shoes and all metals removed from the body. Height in cm was measured using a standard stadiometer, body mass was measured using a digital scale (7562EF, Taylor Precision Products, USA). BMI calculation was performed by dividing weight in kg by height in meters squared (kg/m^2). Waist circumference was measured at the level of the iliac crest with the participants in a standing position.

Dietary Intake Records and Analysis

Detailed dietary data and total steps were collected from the participants on 3 occasions; that is, data was collected on day 1 (beginning), day 45 (mid-point) and day 90 (end) of the study using a 1-day food record method to determine participants' intake of all food, and drink, and a pedometer to track daily total steps. Participants recorded all details of food and drink type, method of preparation, and estimated portions. A 1-day food record was chosen to minimize respondent burden. Food and activity records were collected from participants during the final assessment session.

Basal metabolic rate (BMR) was calculated by Equation 2 for input into Goldberg's energy cut-off equation. The Goldberg's energy cut-off equation (Black, 2000) with a perceived activity level of 1.5 and 95% confidence interval was used to remove outliers who provided diet records of poor validity. Three outliers were removed from the dietary analysis; one in the Cardioflex group and two in the placebo group.

Men: BMR = 88.362 + (13.397 x weight in kg) + (4.799 x height in cm) - (5.677 x age in years)

Women: BMR = 447.593 + (9.247 x weight in kg) + (3.098 x height in cm) - (4.330 x age in years)

Equation 2 – Basal metabolic rate calculation*
*Information adapted from (Harris & Benedict, 1918)

Dietary records were analysed for differences in energy intake, protein, carbohydrate, fat, and micronutrients among treatments. Dietary analysis was completed by inputting all food record data into Food Processor Nutrition Analysis Software V11.7 (ESHA Research, USA).

Blood Analysis

Serum concentrations of total cholesterol, triglycerides, HDL-cholesterol, BUN, creatinine, AST, ALT, and LDH were measured using automated enzymatic methods on a Vitros-350 chemistry analyzer. LDL-cholesterol levels were estimated using the Friedewald formula: LDL-cholesterol = Total cholesterol - HDL-cholesterol - triglycerides/2.2 (Rifai N, 2006).

CRP, IL-6, SiCAM, and SvCAM were measured using Meso Scale Discovery V-PLEX assays (MSD, Rockville, Md., USA). 25 μL of sample, calibrator, or control was added to each well of a 96-well plate. The calibrator and control were provided by Meso Scale Discovery. The plate was sealed and incubated at room temperature for 2 hours on a plate shaker set to 400 rpm. Next, 25 μL of detection antibody was added to each well. The plate was sealed and incubated for 1 hour at room temperature on a plate shaker set to 400 rpm. Finally,

150 μL of read buffer was added to each plate and the plates were read on the Meso Scale Discovery QuikPlex SQ 120 instrument. All blood analysis was done in duplicates, with the average of the two values being recorded.

Lipoprotein A was measured using ELISA assay (Human lipoprotein A No.ab212165, Abcam Trading Company, Ltd, China). 50 μL of sample or standard and 50 μL of antibody were added to each well of a 96-well plate. The plate was incubated at room temperature for 1 hour on a plate shaker set to 400 rpm. Next, 100 µL of TMB substrate was added to each well, followed by incubating the plate for 10 minutes on a plate shaker set to 400 rpm. Finally, 100 µL of stop solution was added to each well and the plate was read with a microplate reader set to 450 nm (autoreader EL311; Bio-tek instruments). All blood analysis was done in duplicates, with the average of the two values being recorded.

Coenyzme Q10 was measured using ELISA assay (Human CoQ10 No. CSB- E14081h, Cusabio® Biotech Co., Ltd, China). 50µl of sample or standard and 50 of HRP-conjugate was added to each well of a 96 well plate. The plate incubated for 40 minutes at 37°C. Next, 90 µl of TMB substrate was added to each well. The plate incubated for 20 minutes at 37°C. Finally, 50µl of stop solution was added to each well, and the plate was read with a microplate reader set to 450 nm (autoreader EL311; Bio-tek instruments). The blood analysis was done in duplicates, with the average of the two values being recorded.

STATISTICAL ANALYSIS

Data were analyzed using IBM SPSS Statistics (v.25). Significance was determined using the treatment effect between groups. The significance value was set at $p \leq 0.05$. An independent t-test showed that there were no statistical differences between the starting points of the two groups (P>0.05), indicating that the two groups were indistinguishable from each other.

The primary analysis was conducted using the post-treatment biomarker value, comparing the two groups, and controlling for their baseline values by using analysis of covariance. Analysis of covariance examines the differences in the mean values of the dependent variable that is related to the effect of the controlled independent variable while taking into account the influence of the uncontrolled variables. In this study, the dependent variable was the biomarkers being tested, the controlled independent variable was the treatment given and the uncontrolled variables were the baseline differences between participants. Analysis of covariance makes the assumption that the data is normally distributed in equal variances. To check the assumption of normal distribution, a Shapiro–Wilk test was performed. To check the assumption of equal variance, a Levene's test was performed.

Results are given as mean $\pm$ standard deviation (SD). Outliers were determined as values greater or less than the mean $\pm$ 3*SD (Jones, 2019). If outliers were present, they were removed, and the results were recalculated. For each parameter, the percent change was calculated by equation 3.

$$\%\ Change = \frac{Final\ Value - Starting\ Value}{Starting\ Value}\ x\ 100$$

Equation 3: Percent change calculation

CONSENT FORM

University of Manitoba

Faculty of Agriculture and Food Sciences

Food and Human Nutritional Sciences

Title: The effect of the dietary supplement Cardioflex on reducing cardiovascular disease risk factors in adults

Investigators: Semone Myrie, RD, PhD (Semone.Myrie@uamanitoba.ca; 204-474-7290) and Trevor Kouritzin, MSc candidate (KouritzT@MyUmanitoba.ca; (780) 239-4993)

This study is being conducted by Trevor Kouritzin, a Masters student in the Department of Food and Human Nutritional Sciences as part of his thesis, under the supervision of Dr. Semone Myrie, RD, PhD.

This is a clinical trial. The study will be registered with 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.

Sponsors: Mitacs Accelerate Grant Program (Government of Canada research program), and Innotech Nutrition Solutions, Winnipeg, MB (Winnipeg-based company that manufactures the dietary supplement Cardioflex).

Disclosure: Trevor Kouritzin has worked for Innotech Nutrition Solutions in the past, and as part of the Mitacs grant program is required to complete an internship period with

Innotech Nutrition Solutions during his MSc program. Precautions will be taken to reduce the potential for bias during the study. For example, the study treatments will be blinded to the researchers throughout the study.

Introduction

You are being asked to participate in a research study. Please take your time to carefully review this Consent Form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this study and you may discuss it with your friends, family or (if applicable) your doctor before you make your decision. 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**.**

Purpose of Study

The purpose of this study is to determine whether 90 days of supplementation with the dietary supplement Cardioflex can reduce cardiovascular risk factors in healthy adults.

Study procedures

Pre-screening:

If you would like to take part in this study, you will be required to first complete a pre-screening questionnaire to provide us with some information about your health and to make sure you meet the eligibility criteria to participate in this study. The pre-screening questionnaire will ask questions about your general health, medical history, use of supplements and current level of physical activity.

Also, as part of the screening criteria, a Meridian digital pulse wave analyzer (DPA) machine will be used to measure the health of your arteries. The machine uses a technique known as accelerated plethysmogram (APG) waveform to measure the stiffness of your arterial walls (the "biological age" of your arteries). The pulse wave analyzer machine is non-invasive. The study coordinator will place a small clip device over your index finger, which shines infrared LED light through your fingertip and obtains pulse wave information based on the light interaction with the blood cells in your finger. The DPA machine converts the changes of transmitted light into an electrocardiogram waveform (measure of arterial stiffness) and pulse rate.

If you meet the first round of preliminary study requirements, then you will need to meet with a phlebotomist to get blood drawn to ensure you also meet the lipid blood marker requirements. If you also meet the preliminary lipid blood marker requirements, then you meet all the inclusion criteria requirements and you are allowed to participate in the study.

Study:

The study will be 90 days, and you will be randomized to receive a study treatment, which means you will be randomly assigned to receive either the experiment or the placebo group. The study will use a parallel design therefore you will only receive one treatment

throughout the study. You do not get to choose which group you are in; the treatments are randomly assigned by the principal investigator using a randomization program. The study will use a double-blinded design, which means you will not be told which treatment group you are assigned until after the study is complete. The investigators in the study will also not know your assigned group until after the study is complete. Participants in the experimental group will be given 1 serving (10g) of Cardioflex each day for the study duration. Participants in the control group will be given a flavored 10g placebo that looks and tastes similar to Cardioflex. You will be instructed to consume the beverage in 500 mL of water upon waking each morning, prior to eating or drinking anything else. The study coordinator will review this in detail with you. You will be instructed to maintain your normal dietary and exercise habits over the course of the study.

The ingredients in Cardioflex are:

Ingredients per 10 g / Ingrédients par 10 g

Calories / Calories 28 | Carbohydrate / Glucides 0.5 g | Fat / Lipides 0 g

L-Lysine HCl / L-lysine HCl	2800 mg
Vitamin C (Ascorbic Acid, Magnesium Ascorbate) / Vitamine C (acide ascorbique)	2000 mg
L-Proline / L-proline	1000 mg
L-Glutamine / L-glutamine	500 mg
L-Threonine / L-thréonine	500 mg
Potassium (Potassium Gluconate) / Potassium (gluconate de potassium)	40 mg
Magnesium (Magnesium Ascorbate) / Magnésium (ascorbate de magnésium)	33 mg
Coenzyme Q10 (Ubiquinol) / Coenzyme Q10 (Ubiquinol)	30 mg
Folate (L-5-Methyltetrahydrofolate) / Folate (L-5-méthyltétrahydrofolate)	1000 mcg
Vitamin B12 (Methylcobalamin) / Vitamine B12 (Methylcobalamin)	300 mcg
Selenium (Selenomethionine) / Sélénium (sélénométhionine)	100 mcg
Vitamin D (Cholecalciferol) / Vitamine D (cholécalciférol)	12.5 mcg or 500 IU
Vitamin E (d-alpha tocopheryl acetate) / Vitamine E (acétate de d-alpha tocophéryl)	100 IU

Non-Medicinal Ingredients: Inulin (Pre-biotic), Blueberry Juice Powder, Cranberry Juice Powder, Beetroot Powder, Calcium Citrate, Citric acid, Natural Flavour, Stevia rebaudiana leaf, Tapioca, Silica.

Ingrédients non médicinaux: Inuline (pré-biotique), poudre de jus de bluets, poudre de jus de canneberge, poudre de betterave rouge, citrate de calcium, acide citrique, arôme naturel, feuille de Stevia rebaudiana, tapioca, silice.

Any changes in your health status at any point during the study needs to be reported immediately to the study investigators.

Should you choose to participate, you will be asked to complete 2 testing sessions [baseline (i.e. day 0 or day 1) and day 90] during the 90 days study period. All testing sessions will occur at the Richard Centre for Functional Foods and Nutraceuticals (RCFFN), located in the Smart park on the Fort Gary campus at the University of Manitoba.

Each testing session will require approximately 30-40 minutes of your time. Each testing session will include measurement of your blood pressure (about 10-15 minutes), your arterial status using the DPA machine (about 5 minutes), anthropometric measurements (about 5 minutes) and collection of blood samples (about 5-10 minutes). For blood collection, you will need to fast (no food or beverage, except water) for a minimum of 8 hours before your appointment. Approximately 25 ml of blood will be taken. Blood will be used to measure various biomarkers, including blood lipid profile, kidney and liver functions, inflammatory and endothelial functions – see specific biomarkers below.

Overall, health markers that will be tested in this study include your waist circumference, body weight, height, atherosclerosis risk factors (blood pressure, heart rate, arterial stiffness, total blood lipid profile (total cholesterol, high density lipoprotein (HDL cholesterol), low density lipoprotein (LDL cholesterol), triglycerides), lipoprotein A, and inflammatory and endothelial function biomarkers (C-reactive protein (CRP), Interleukin-6 (IL-6), soluble intercellular cell adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1).

Kidney and liver functions will be assessed to measure the safety of the supplement. These assessments include testing for alanine aminotransferase (ALT), aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), and Blood Urea Nitrogen (BUN), creatinine). Blood coenzyme Q10 levels will also be tested as a measure of treatment compliance.

To monitor your progress during the study, you will be required to complete the treatment feedback sheets, dietary food records and wear a pedometer at certain times throughout the study. First, you will be given a 90-day compliance sheet document to be used to record what time of the day you took the supplement and if you experienced any side-effects such as gastrointestinal distress. Second, you will be required to complete a 3-day food record at day 1, day 45 and day 90 of the study to help us assess if any major changes in your diet/dietary habits during the study. Finally, you will be required to wear a pedometer that tracks total steps and exercise intensity on week 1, week 6 and week 13 (the final week of the study) to allow us to assess your level of physical activity during the study.

Risks and Discomforts

There are no known serious side effects for any of the procedures proposed in this study. However, as with any clinical trial, there might be as yet unknown or unforeseen risks of taking part.

Cardioflex supplement: No adverse effects have been reported. The active ingredients in Cardioflex are within acceptable levels known to be safe and effective and in accordance with Health Canada licensing guidelines. Health Canada has issued the product a natural health product (NHP) number and the supplement has already been available for purchase to the commercial market.

Blood draw: Blood sampling may have some rare risks, like placing a needle into a vein which may contribute to infection, perforation or penetration of the needle through the vein, and bleeding, pain, or bruising at the site. As part of the process for blood sampling, to help minimize risks for infection, the phlebotomist will start by performing his/her hand hygiene (wash with soap and water) and wearing clean from you. Next, the phlebotomist will disinfect the site on you will be used for blood collection using 70% isopropyl alcohol swab. After blood is drawn and the needle removed from the vein, the phlebotomist will apply gentle pressure to the puncture site using clean gauge or cotton ball to stop bleeding, and a bandage is applied. A cream called EMLA can be

applied to the puncture site to numb the skin to and help reduce infection. Infection risk is also minimized by the use of prepackaged sterilized equipment, and all needles are disposed after a single use.

There are no known risks associated with the blood pressure measurement and measuring arterial stiffness using the Digital pulse wave analyzer (DPA**)** machine.

Benefits

You may not directly benefit from participation in this research; however, this study will contribute to a better understanding of the effects of the dietary supplement Cardioflex on reducing cardiovascular disease risk factors. All the procedures that will be performed as part of this study are provided at no cost to you. You will receive test results when they become available. You will receive your test results based on your preference of either electronic format or as a hard copy available from the study coordinator.

Confidentiality

Medical / research records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. All records will be kept in a locked secure area and only those persons identified as requiring access to your records will have opportunity to review or copy your medical / research records. Information gathered in this research study may be published or presented in public forums; however, your name and other identifying information will not be used or revealed. Personal information such as your name, address, telephone number and/or any other identifying information will be protected. If the results of the study are published, your identity will remain confidential. No information revealing any personal information such as your name, address or telephone number will be made publicly available.

The University of Manitoba may review records related to the study for quality assurance purposes.

All records such as questionnaires will be kept in a locked cabinet, in a secure area at the RCFFN and only those persons identified as researchers on this study will have access to these records. Study biological samples will be stored in locked freezers at the RCFFN or the Duff Roblin Building, University of Manitoba. Your samples will not be used for any additional analyses, nor stored for any longer than 5 years after the completion of the study, nor shared with any other groups, other than is indicated in the protocol, without your specific consent.

Remuneration and Feedback

You will receive up to a maximum of $50 remuneration at the completion of this study. You will receive $10 for showing up to the pre-screening phase, $20 for showing up to the

first testing session and $20 for returning the study pedometer and showing up to the final testing session.
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.

Feedback about the research results will be provided to you as an electronic document or a paper copy through regular mail (see dissemination section below).

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. Should you wish to withdraw your participation from the study please inform the study coordinators so that your file can be officially close. If the study staff feels that it is in your best interest to withdraw you from the study, they will remove you without your consent.

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

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. Semone Myrie Semone.Myrie@umanitoba.ca	Tel No.	204 474 7290 or 204-272-1555
Co-investigator	Trevor Kouritzin KouritzT@MyUmanitoba.ca	Tel No.	204 -272-1555 or 780-239-4993

This research has been approved by the Joint-Faculty Research Ethics Board at the University of Manitoba. If you have any concerns or complaints about this project you may contact any of the above-named persons or the Human Ethics Coordinator 204-474-7122, or e-mail humanethics@umanitoba.ca.

A copy of this consent form has been given to you to keep for your records and reference.

Dissemination

The results of the study will be written up in Trevor Kouritzin's master's thesis and may be published in recognized scientific journals and presented to public groups such as at scientific meetings and seminars. Additionally, all study participants will receive their individual results along with the mean value obtained from the whole study population and a summary of findings. However, participants will not be able to have access to the individual results of other study participants.

Please indicate below how you would like to receive your results and a summary of the study findings:

Email:

Ground mail (provide mailing address):

Medical Care for Injury Related to the Study

In the event of an injury that occurs to you as a direct result of participating in this study, or undergoing study procedures you should immediately 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.

I am aware that there are risks associated with placing a needle into a vein for blood sampling, including risk of bleeding, pain or bruising at the site, and possible infection.
Yes ☐ No ☐

Consent

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 have been assured that my name, address and telephone number will be kept confidential to the extent permitted by applicable laws and/or regulations.
4. By signing and dating this document, I am aware that none of my legal rights are being waived.

Participant signature:_____ Date: _____

Printed name of above:_____

I confirm that I have explained the purpose, duration etc. of this study, 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.

Research staff signature: _____ Date: _____

Printed name of above: _____ Study role: _____

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