Title: **Effects of Short-term Curcumin and Multi-polyphenol Supplementation on the Anti-inflammatory Properties of HDL**

ClinicalTrials.gov ID: **NCT02998918**

Study Protocol and Statistical Analysis Plan

Date: 03/14/2018

Specific Aims

Atherosclerosis is a chronic inflammatory disease[1] underlying coronary artery disease, driven in part by the innate immune system, particularly macrophages.[2] The adhesion of leukocytes to the vascular endothelium, mediated by endothelial cellular adhesion molecules including vascular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), is one of the crucial initial steps in atherogenesis. Elevated levels of high-density lipoprotein cholesterol (HDL-C) are associated with reduced risk for cardiovascular disease (CVD);[3] however, interventions designed to increase HDL-C concentration in humans have yet to lead to reductions in cardiovascular events.[4] A possible explanation for the failure of recent clinical trials is the structural and functional complexity of HDL particles, which have multiple cardioprotective properties, including anti-inflammatory, antioxidative, and reverse cholesterol transport activities. The anti-inflammatory effects of HDL include reduction of inflammatory cytokines and vascular leukocyte adhesion molecules.[5] A recent study showed that dietary composition can affect HDL's anti-inflammatory properties, namely the ability to inhibit the expression of ICAM-1 and VCAM-1.[6]

Numerous studies have shown that polyphenols, including curcumin, quercetin, and resveratrol, exhibit multiple health benefits, including anti-inflammatory properties.[7-13] Curcumin is a flavonoid polyphenol that is the active ingredient in the spice turmeric. Quercetin is one of the most abundant dietary flavonoids and is found in many fruits, vegetables, and beverages. Resveratrol is a non-flavonoid polyphenol present in a limited number of plant-derived foods, including grapes and peanuts. *In vitro* studies show these three polyphenols independently decrease VCAM-1 and ICAM-1 expression induced by tumor necrosis factor alpha (TNFα) in human endothelial cells,[14-16] as well as increase cholesterol efflux to apolipoprotein A-I (apoA-I) and HDL in macrophages.[17-20] However, previous *in vitro* models used direct incubation with each polyphenol (i.e., HDL was directly exposed to the polyphenol in the cell culture, as opposed to incubation with plasma after consumption of the polyphenol), with doses much higher than found in typical human diets or supplements.

The health effects of polyphenols in humans are limited by their poor bioavailability, as they are rapidly metabolized and excreted. Recent studies have found that formulating poorly-absorbed molecules with phosphatidylcholine via phytosomes increases their bioavailability. For example, recent studies comparing curcumin phytosome (Meriva®) and standard curcumin formulations in humans found that the curcumin phytosome formulation increased curcuminoid bioavailability between 8- to 29-fold.[21,22] To our knowledge, no study has examined the effects of polyphenol supplementation, particularly phytosome-formulated polyphenols, in humans on the ability of circulating plasma to inhibit the expression of cellular adhesion molecules or enhance cholesterol efflux capacity *in vitro*. Furthermore, it is unknown whether polyphenol supplementation modulates the ability of HDL particles to perform these same functions.

Therefore, the purpose of this study is to examine whether acute and short-term (1-week) polyphenol supplementation in humans affects inflammation measured at the whole plasma level, as well as the inflammatory and cholesterol efflux properties of HDL particles. We will test the effects of two supplements in a cross-over design: a curcumin phytosome and a multi-polyphenol supplement (containing curcumin phytosome, quercetin phytosome, and trans-resveratrol). We hypothesize that one of the mechanisms by which polyphenols exert a beneficial effect on inflammation and atherosclerosis is through its modulation of HDL particles. Specifically, *we hypothesize that polyphenol supplementation will improve the quality of HDL particles by increasing their anti-inflammatory and cholesterol efflux capacity properties, and that this benefit will be larger in the multi-polyphenol supplement compared to curcumin alone*. We will test this in the following aims: **Aim 1: Examine the acute (1 hr) and short-term (1 wk) effects of curcumin and poly-resveratrol supplementation in adults on VCAM-1 and ICAM-1 expression measured in (A) whole plasma and (B) the plasma HDL fraction.** *Hypothesis: Both acute and short-term polyphenol supplementation will significantly decrease VCAM-1 and ICAM-1 expression in whole plasma, which will be partly mediated by HDL-specific reductions in VCAM-1 and ICAM-1 expression*. *The acute effects of polyphenol supplementation at baseline will be attenuated but still significant after one-week of supplementation*.

Aim 2: Examine changes in cholesterol efflux capacity in J774 macrophages after one-hour and one-week of curcumin and poly-resveratrol supplementation in adults. *Hypothesis: Both acute and short-term polyphenol supplementation will significantly increase HDL cholesterol efflux capacity.*

Significance. Polyphenols are among the most studied dietary supplements, with a plethora of health benefits demonstrated in animal and in vitro models. However, human trials have been limited by the poor bioavailability of these compounds. Most human studies have only measured circulating inflammatory markers (e.g., TNFα, IL-6) and HDL-C levels, ignoring the possible effects of polyphenol supplementation on *in vitro* inflammatory properties such as VCAM-1 and ICAM-1 expression and HDL function. Given the shift in the HDL

field from quantity to quality, identifying dietary supplements that positively affect HDL functionality is important. The proposed study will overcome these limitations by employing phytosome formulations that increase bioavailability and measuring the effects of supplementation on the *in vitro* inflammatory properties of whole plasma and plasma HDL.

Research Strategy

Cross-over design. We plan to recruit 20 adults (aged 18-40 yrs) for this study. Subjects will be healthy, light drinkers (1-3 drinks/day or less), non-smokers, and not taking any medications or dietary supplements. Each volunteer will undergo a screening visit to ensure eligibility. Following consent, each volunteer will complete 2 one-week trials in a randomized, blinded order separated by two weeks (**Figure 1**). Half the participants will be randomized to curcumin followed by polyresveratrol, the other half to polyresveratrol followed by curcumin.

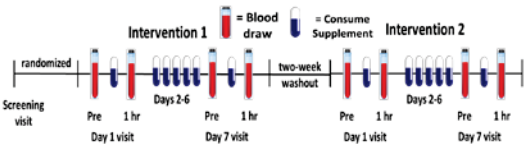

Figure 1. Cross-over design of study.

Polyphenol supplementation. During each intervention period, the subjects will take two capsules of either curcumin phytosome (500 mg, Meriva-SR®, Thorne Research) or PolyResveratrol-SR® (100 mg curcumin phytosome, 100 mg quercetin phytosome, 100 mg green tea phytosome, 100 mg trans-resveratrol, 100 mg trans-pterostilbene; Thorne Research) twice daily for one week. Subjects will be advised to maintain their current diet and exercise patterns, while avoiding alcohol intake and restricting certain foods with high content of polyphenols, such as grapes, olive oil, curry, coffee, etc.

Laboratory measurements. Blood draws will be performed at baseline before and one hour after ingestion of one dose of the supplement, followed by the same sequence on the final day (Day 7) of the intervention. Thus, a total of 4 in-person visits including 8 blood draws will be performed over the 4-week trial period (**Figure 1**). All blood samples will be taken in the morning after an overnight fast and processed using the techniques described below. All measurements will be performed in Dr. Sarzynski's laboratory unless otherwise stated. The standard lipid profile will be measured using enzymatic methods. Whole plasma and plasma HDL concentrations of TNF-α and interleukin (IL)-6 will be determined by ELISA.

HDL isolation. The isolation of HDL particles from human plasma will be performed using gel filtration chromatography. Specifically, 370 µL of plasma will be applied to two Superdex 200 10/300 GL increase columns arranged in series on an ÄKTA Pure 25 L FPLC system (GE Healthcare), as previously described.[23] The HDL fractions will be combined, aliquoted, and stored for the various assays (see below).

Cell adhesion molecule expression. Human umbilical vein endothelial cells (HUVECs) will be purchased and cultured as previously described.[24,25] Confluent HUVECs will be incubated for 16 hrs with whole plasma or plasma HDL and then stimulated by TNF-α. After 4-5 hrs the cell-surface expression of VCAM-1 and ICAM-1 will be measured by flow cytometry.

Flow cytometry. Cell surface expression of VCAM-1 and ICAM-1 will be performed in the Flow Cytometry and Cell Sorting Core at the USC School of Medicine, as previously described.[25]

Cholesterol efflux assay. Cholesterol efflux capacity will be assessed by measuring efflux of ^{3}H-labeled cholesterol from J774 macrophages to HDL plasma, as previously described.[26] Samples are run in triplicate on 96-well plates and results normalized to pooled control plasma included in each assay.

Statistical analysis. All analyses will be performed using SAS 9.3 (Cary, NC). The individual effects of each supplement will be tested using paired t-tests, while unpaired t-tests and mixed linear models will be used to compare the treatment effects between supplements. The effects of acute (1 hr) and short-term (1 wk) supplementation will be tested separately. Probability levels less than 0.05 will be considered significant.

Translation of Results into an R01 application. The successful completion of this pilot project will result in extensive preliminary data to be used for subsequent NIH R01, ADA junior faculty, and AHA scientist development grant applications focused on lifestyle factors (diet & exercise) and HDL function in the prevention and treatment of CVD and type 2 diabetes and their pathological precursors. The proposed research will provide data on the ability of resveratrol to decrease inflammation at the level of whole blood, as well as by modulation of the functional properties of HDL particles. Establishing the mechanisms by which resveratrol improves inflammation and HDL function is a necessary next step. As such, future studies are needed that examine the compositional changes to HDL in response to resveratrol supplementation, including changes in the HDL proteome, HDL lipidome, and HDL-associated microRNAs. These findings will need to be extended to other polyphenols and dietary supplements. This work will lead to new research initiatives on the biological mechanisms responsible for changes in the function and composition of HDL particles, particularly their anti-inflammatory properties, with dietary supplements.

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Protection of Human Subjects

This Human Subjects Research meets the definition of a clinical trial.

1. Risks to Human Subjects

a. Human Subjects Involvement, Characteristics, and Design

The proposed study involves human subjects undergoing two separate one-week dietary supplement trials. As this is a pilot study, we are not including a placebo control group. Instead, the subjects will be randomly assigned to one of the two supplements for the first week and after a two-week washout period consume the other supplement for one week. We anticipate recruiting 20 adults (18-60 yrs), with an equal number of males and females, that are healthy, light drinkers (1-3 drinks/day or less), non-smokers, and not taking any medications or dietary supplements. Our sampling strategy will be a convenience sample, although ideally we would prefer to recruit overweight/obese, middle-aged adults that are more likely to have an increased inflammatory profile. We will recruit participants continuously by advertising at the university (e.g., e-mail, listservs, newsletters, posters). Volunteers will initially be screened via telephone. The following are lists of inclusion and exclusion criteria.

Inclusion	Exclusion
• Age 18-60 years • 19 < BMI ≤ 40 kg/m^2 • Non-smoking for last year • Light drinker (1-3 drinks/day or less) • Able to provide own transportation to study visits and intervention • Not currently involved in any other research study • Willing and able to participate in all aspects of the trial including research testing • Willing to give informed consent to participate	• Pregnant women • Self-reported significant cardiovascular disease including but not limited to serious arrhythmias, cardiomyopathy, congestive heart failure, myocardial infarction, stroke • Other self-reported medical conditions including but not limited to diabetes, uncontrolled hypertension, chronic or recurrent respiratory problems, active cancer, thyroid disorders, or any serious medical condition that may affect adherence to the protocol, or be aggravated by dietary supplements • Medications known to affect metabolism or inflammation (e.g. NSAIDs, thyroid medication, β-blockers, or stimulants) • Excess use of curcumin in diet

Potential participants will be invited to complete screening visits. All participants will sign a consent form approved by the University of South Carolina Institutional Review Board before any measurement will be taken. Height and weight will be measured to calculate body mass index (BMI). Blood pressure and waist circumference will be measured. Fasting venous blood will be drawn for metabolic assays. Exclusion criteria will be assessed during screening visits. Those who remain eligible will be invited to join the study and randomly assigned to one of the two supplements to begin.

During each intervention period, the subjects will take two capsules of either curcumin phytosome (500 mg, Meriva-SR®, Thorne Research) or PolyResveratrol-SR® (100 mg curcumin phytosome, 100 mg quercetin phytosome, 100 mg green tea phytosome, 100 mg trans-resveratrol, 100 mg trans-pterostilbene; Thorne Research) twice daily for one week. After taking the supplement for one week, there will be a two week washout period, followed by one week of taken the second supplement. Aside from the first and last doses, which will be administered in the clinic, all other doses will be self-administered. Subjects will be advised to maintain their current diet and exercise patterns, while avoiding alcohol intake and restricting certain foods with high content of polyphenols, such as grapes, olive oil, curry, coffee, etc.

All participants will receive clear instructions for participation in the study. We plan to give each volunteer a total of $125 for completing the study, which breaks down to $25 per visit to the clinic (screening, baseline visit for supplement 1, post-supplement 1 visit, baseline for supplement 2, post-supplement 2 visit).

b. Sources of Materials

Blood draws will be performed at baseline before and one hour after ingestion of one dose of the supplement, followed by the same sequence on the final day (Day 7) of the intervention. This sequence will be repeated during the trial with the second supplement. Thus, a total of 4 in-person visits including 8 blood draws

will be performed over the 4-week trial period. All blood samples will be taken in the morning after an overnight fast from the antecubital vein. The blood will be immediately spun down and aliquoted into plasma and serum fractions and frozen for future use. At the baseline visit, anthropometric data will be measured including height, weight, waist circumference, blood pressure. Data on physical activity and family history will be ascertained via questionnaire and results will be recorded on a paper form or in a computer database. Paper forms will be stored in locked file cabinets and electronic files will be password-protected in an office that is locked when not in use.

All participants will be randomly assigned a subject number. Thus, all data will be recorded using the anonymous subject number. Only the PI (Dr. Sarzynski) will have access to the identifiable information that links the subject id number to the individual. Blood samples will be stored in a freezer in the PI's laboratory, which is locked when not in use. The samples will be labeled using deidentified id numbers. Only personnel authorized by the PI will have access to the database and biological samples. All materials and data will be obtained specifically for research purposes.

c. Potential Risks

As this study involves the ingestion of dietary supplements and blood draws, there are potential for risks to the participants, which include:

- There is a risk when having blood drawn. Participants may experience discomfort, bruising and/or bleeding where the needle is inserted. Occasionally some people become dizzy, lightheaded, or feel faint. Infection may occur on rare occasions. Only trained staff will collect blood samples.
- There may be contraindications for the dietary supplements to be ingested as part of this study:
    - Meriva-SR is contraindicated in an individual with a history of hypersensitivity to any of its ingredients. Meriva-SR contains curcumin phytosome, a plant extract complexed with phosphatidylcholine, which is derived from soy. Phosphatidylcholine contains no soy protein. There are no other soy-based ingredients in Meriva-SR.
    - PolyResveratrol-SR is contraindicated in an individual with a history of hypersensitivity to any of its ingredients. PolyResveratrol-SR contains green tea phytosome and curcumin phytosome, plant extracts complexed with phosphatidylcholine, which is derived from soy. Phosphatidylcholine contains no soy protein. There are no other soy-based ingredients in PolyResveratrol-SR.
    - Although the doses used in the proposed study for both supplements is low (1g/day) and has a large safety threshold, it is possible for the participant to experience mild side effects, including headache, skin rash, minor flatulence and a yellowing of the stool. Although unlikely to happen, these side effects will stop after cessation of the supplement.

No alternative treatments or procedures are available to participants. Thus, if a participant is adverse to having blood drawn or experiences side effects from the supplementation, all procedures will be stopped and they will be removed from the study.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent

Potential participants who are eligible via telephone screening, and who agree to go through the screening process, will be invited to a screening visit. The informed consent process will follow the procedures of the University of South Carolina Institutional Review Board (IRB). The study interviewers will explain the purpose, methods and extent of the study to prospective participants. The potential participant will be given an opportunity to read the informed consent form and ask questions. The form will be written in simple easy-to-understand language and will be approved by the IRB. We will require study staff to review all of the key aspects of the study verbally with the potential participants, and staff will be provided with a structured checklist for this purpose. Participants will be encouraged to ask questions, and staff will ensure that they fully understand the study. If a participant cannot read the consent form, a staff member will read it to her or him, review the checklist, provide an opportunity for questions, and then ask the participant if s/he would like to sign the consent form. A copy of the signed and dated consent form will be given to participants, and the original document will be placed in their individual study files, which will be stored in a secure location. In compliance with the Health Insurance Portability and Accountability Act (HIPAA) and the Standards for Privacy of Individually Identifiable Health Information of the Department of Health and Human Services, we will access personal health information only after obtaining informed consent.

According to the NIH and the University of South Carolina IRB guidelines, the informed consent will contain the following elements:

1. A statement that the study involves research
2. An explanation of the purposes of the research
3. The expected duration of the subject's participation
4. A description of the procedures to be followed
5. Identification of any procedures which are experimental
6. A description of any reasonably foreseeable risks or discomforts to the subject
7. A description of any benefits to the subject or to others that may reasonably be expected from the research
8. A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject
9. A statement describing the extent to which confidentiality of records identifying the subject will be maintained
10. An explanation of whether any compensation and any medical treatments are available if a research-related injury occurs and, if so, what these consist of, or where further information may be obtained
11. An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject
12. A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits, to which the subject is otherwise entitled
13. Anticipated circumstances under which subject participation may be terminated by the investigator
14. Any additional costs to the subject that may result from participation in the research
15. The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject
16. A statement that significant new findings developed during the course of the research, which may relate to the subject's willingness to continue participation, will be provided to the subject
17. The approximate number of subjects involved in the study

b. Protection against risk

Safety measures during the measurements: All study assessments will be conducted by trained and certified staff. Safety precautions will be taken during all blood draws by applying standardized stopping criteria. If a participant reports pain, feels faint, lightheaded or dizzy, or other significant medical problems, the procedure will be stopped.

Safety measures during the interventions: The initial dietary supplementation trial will begin in the Department's Clinical Exercise Research Center, with the subjects ingesting one dose of the supplement then undergoing a blood draw one hour later. All sessions will be supervised by trained staff who will monitor potential adverse experiences and symptoms. On-site staff, who are trained in advanced cardiac life support, are available to deal with medical emergencies. If at any point during a visit or off-site, participants develop side effects from the supplement or adverse reactions to the blood draw, they will be monitored by staff and subsequent supplements or blood draws will not be taken until the issues are resolved and the participant consults with their physician. For the portions of the study during which the subject is responsible for the self-administration of the dietary supplements, they will be instructed to contact the study staff and their physician if any symptoms or side effects occur. These individuals will be instructed to see their physicians before continuing with the trial.

Confidentiality: Data will be used only in aggregate and no identifying characteristics of individuals will be published or presented. Confidentiality of data will be maintained by using research identification numbers which uniquely identify each individual. The information collected from participants in this study has a low potential for abuse because the data does not address sensitive issues. Nevertheless, appropriate measures will be taken to prevent unauthorized use of study information. Data other than demographic information will not use names as an identifier, but rather the study ID number. The research records will be kept in locked file cabinets and password-protected computer files. The files matching the participants' names and demographic information with the study ID numbers will be kept in a separate room and locked in a file that uses a different key from that of all other files. Files may not be obtained from the research unit by persons other than research personnel, who are asked to sign a document agreeing to maintain the confidentiality of the information. After the study is completed, the local data will be stored with other completed research studies in a secured storage area.

Storage and disposal of biological material: Biological samples will be stored at the University of South Carolina for up to ten years after the end of the study, at which time the samples will be destroyed. Samples will be stored in locked –80°C alarmed freezers located in a locked room. The laboratory coordinator and the PI have access to the keys to the freezers. The alarm system is triggered by opening the freezer, power outage or critical temperature rise, and is connected to the laboratory coordinator by means of an automatic telephone dialer. We have devised emergency procedures to salvage the samples in case of power outage. All samples will have study IDs with no personal identifiers of the participants. Participants can request to have their samples destroyed at any time by contacting the PI in writing.

3. Potential benefits of the proposed research to human subjects and others

Participants may experience clinical benefits from knowing some of their physiological parameters, such as blood lipid profile and blood pressure. Participants are expected to experience health benefits from ingesting the dietary supplements, particularly reduced inflammation. Individuals who do not qualify will be told the reasons for disqualification and referred for appropriate care. Therefore, the risk/benefit ratio is acceptable since potential risks of these research procedures are minimal and/or infrequent, and possible complications will be carefully monitored. Potential risks are reasonable in relation to anticipated benefits to health from the interventions.

4. Importance of the Knowledge to be Gained

Systemic inflammation and HDL dysfunction is common in overweight, sedentary adults. These conditions lead to more serious health risks and diseases such as diabetes and cardiovascular disease. Polyphenols are among the most studied dietary supplements, with a plethora of health benefits demonstrated in animal and in vitro models. However, human trials have been limited by the poor bioavailability of these compounds. Most human studies have only measured circulating inflammatory markers (e.g., TNFα, IL-6) and HDL-C levels, ignoring the possible effects of polyphenol supplementation on *in vitro* inflammatory properties and HDL function. Given the shift in the HDL field from quantity to quality, identifying dietary supplements that positively affect HDL functionality is important. The proposed study will overcome these limitations by employing phytosome formulations that increase bioavailability and measuring the effects of supplementation on the *in vitro* inflammatory properties of whole plasma and plasma HDL.

5. Data and Safety Monitoring Plan

A Data and Safety Monitoring Plan (DSMP) will be implemented as described below to ensure the safety of all participants involved in the study and to ensure the validity and integrity of the data. If required by NIH, a Data Safety Monitoring Board (DSMB) will be established, with responsibility to monitor all aspects of the study, including those that require access to any blinded data. The operational plan and all members will be approved by the NIH Program Scientist.

The PI and the Project Coordinator will meet regularly on a continuous basis to review study progress and to examine reports of adverse incidents as well as participant recruitment and follow-up. All serious adverse events will be reported to the IRB within the required time frame, and if the event occurred as a direct result of participation in this study, an amendment may be made to the study protocol and consent form and the PI will request new IRB approval.

Adverse events include any event that occurs during the course of the study that results in a participant suffering physical or mental injury, pain or suffering. Adverse events can be major, such as a subject who suffers significant gastrointestinal pain/fluctuation from the supplementation, or minor such as a subject experiencing minor flatulence from supplementation. Deviations from the study's protocol are also considered an unexpected or notable event.

This study will follow the IRB guidelines for reporting Adverse Events/Unanticipated Problems. Both major and minor events will be reported using the study's Adverse, Unexpected, or Notable Event Reporting Form. A description, date and location of the event will be recorded on this form, which will be kept in the subject's research file. Any major event, i.e., any serious injury or life-threatening event, will be reported to the IRB immediately after completing any and all actions that are necessary to protect the subject's health and safety.

Inclusion of Women and Minorities

Inclusion of women. The proposed study will include both men and women with equal number (50% men and 50% women).

Inclusion of minorities. There will be no exclusion based on ethnic or racial status. Subjects will be recruited from the Greater Columbia area. Based on the recruitment of prior clinical trials and the ethnic distribution in our recruitment area, we anticipate that to be approximately (racially) 65% white, 31% African-American, 3% Asian, and 1% other races, or (ethnically) 6% Hispanic and 94% non-Hispanic.

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Curcumin and multi-polyphenol supplementation on HDL anti-inflammatory properties

Total Enrollment: 20 **Protocol Number:**

Grant Number:

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Sex/Gender Unknown or	Total
Hispanic or Latino	1	1		2 **
Not Hispanic or Latino	9	9		18
Unknown (individuals not reporting ethnicity)				
Ethnic Category: Total of All Subjects*	10	10		20 *
Racial Categories				
American Indian/Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American	3	3		6
White	7	7		14
More Than One Race				
Unknown or Not Reported				
Racial Categories: Total of All Subjects*	10	10		20 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Sex/Gender Unknown or	Total
American Indian or Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White	1	1		2
More Than One Race				
Unknown or Not Reported				
Racial Categories: Total of Hispanics or Latinos**	1	1		2 **

* These totals must agree.

** These totals must agree.

Inclusion of Children

As we are interested in systemic inflammation and the protocol involves consuming a dietary supplement, our proposed research involves young-to-middle aged adults. No children will be included in the proposed study.

Vertebrate Animals
None.

Select Agent Research
None.

Resource Sharing Plan

We intend to make the data and results generated by the proposed study as broadly available as possible. We will actively pursue dissemination of our findings both in scientific meetings and peer-reviewed journals, as well as seeking collaboartion wirh other scientists and studies pursuing similar research questions.

Authentication of Key Biological and/or Chemical Resources
None.