

# **The Effect of Soya Beverage Fortified with Plant Sterol on Major Serum Lipids in Normocholesterolemic, Healthy Southern Chinese Individuals**

Brief Title: The Effect of 3-Week Consumption of Soya Beverage Enriched With Plant Sterols on Serum LDL-C

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## 1 LIST OF ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
BMI	Body Mass Index
CRF	Case Report Form
CTC	Clinical Trial Centre
CVD	Cardiovascular Disease
eCRF	Electronic Case Report Form
DMP	Data Management Plan
EDC	Electronic Data Capture
EU	European Union
GCP	Good Clinical Practice
HDL-C	High-Density Lipoprotein Cholesterol
HKU	The University of Hong Kong
ICH	The International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IRB	Institutional Review Board
ITT	Intention-to-Treat
LDL-C	Low-Density Lipoprotein Cholesterol
PI	Principal Investigator
PP	Per-Protocol
TAG	Triglycerides
TC	Total Cholesterol
UP	Unanticipated Problems
US FDA	United State Food and Drug Administration

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### 3 PROTOCOL SYNOPSIS

**Title:** The Effect of Soya Beverage Fortified with Plant Sterol on Major Serum Lipids in Normocholesterolemic, Healthy Southern Chinese Individuals

**Phase:** Phase II

**Study Site:** Queen Mary Hospital, The University of Hong Kong

**Study Design:** This is a local, single-center, two-arm, randomized, double-blind, placebo-controlled study that examine the effect of the consumption of a soya beverage enriched with plant sterol for 3 weeks. Subject will be randomly assigned to receive either the investigational or control product. The randomization will be stratified by both gender (male and female) and age group (18-40 years and >40 years).

Blood specimens will be collected at the baseline and at the end of the consumption period, which will be subjected to laboratory analysis.

**Study Objectives:** **Primary Objective:** To examine the effect of the consumption of a plant sterols fortified soya beverage on serum LDL cholesterol in healthy, free-living normocholesterolemic individuals

**Secondary Objective:** To examine health-benefits by consuming the plant sterols fortified soya beverage in terms of serum total triglyceride, total and HDL cholesterol, other cardiometabolic risk factors and musculoskeletal-related traits.

**Study Population:** Two hundred (200) Southern Chinese male or female in Hong Kong who aged 18 or above with good general health will be recruited in this study.

**Investigational and Control Product Administration:** The investigational product is a soya drink fortified with 2g plant sterols per pack and the control product is the same soya drink except without plant sterols.

Subjects will be asked to consume 1 pack of either investigational or control product daily for 3 consecutive weeks, each pack consumed once with main meal. Subjects consuming test product will therefore consume 2 g of plant sterols daily.

**Study Duration:** This study is estimated to last for 12 months (from subject enrollment until completion of data analyses)

**Subject Participation Duration:** 3 weeks

**Estimated Time to Complete Enrollment:** 9 months

**Study Endpoints:** **The primary endpoint** is the mean serum LDL-C at the end of week 3 after the consumption of plant sterols fortified soya beverage or control product.

**The secondary endpoints** include the changes of serum LDL-C, HDL-C, total cholesterol, triglycerides (TAG), serum creatinine, fasting blood glucose, other cardiometabolic risk factors and musculoskeletal-related traits from baseline to week 3.

**Statistical Analysis:** **Sample size rationale:**

The sample size calculation is based on ANCOVA with baseline serum LDL-C as independent covariate. According to a previous study (36), the standard deviation of LDL-C at week 4 is approximately 0.85 mmol/l and the estimated correlation coefficient between the outcomes measured at baseline and week 4 is about 0.7. In addition, a minimum difference of 0.28 mmol/l in LDL-C between the groups is considered clinically important.

By using PASS 12 for the sample size calculation, a power of 0.8 and a 5% of the maximum tolerable false positive rate, 75 subjects per group is required to detect the minimum difference between the mean values of LDL-C.

Considering an attrition rate of 25%, a total of 200 subjects will be recruited.

**Data set analyzed:**

The intention-to-treat (ITT) population is consisted of all randomized subjects. The per-protocol (PP) population includes all eligible subjects who have 80% of study product compliance, efficacy data collected within the pre-specified allowable visit window, and no protocol violation that is clinically significant.

**Handling of missing or invalid data:**

No data imputation will be performed to replace missing or invalid data.

**Efficacy Analysis:****Primary Efficacy Analysis:**

ANCOVA will be carried out to compare the serum LDL-C level at week 3 between the groups. The baseline of the response outcome will be used as a covariate to control for initial group differences. A log transformation will be performed if the normality assumption for the ANCOVA model is violated. And if the normality assumption is still not sustained, non-parametric approach might be performed. The primary efficacy analysis will be performed on both the ITT and the PP populations.

**Secondary Efficacy Analyses:**

Stratified analysis for the primary endpoint will be performed using the ANCOVA model adjusted for gender and age group. Subjects will be classified to one of the following strata:

1. Male, aged 18-40 years
2. Male, aged >40 years
3. Female, aged 18-40 years
4. Female, aged >40 years

The changes of the following response outcomes from baseline to week 3 will be summarized by descriptive statistics:

- Blood Tests: LDL-C, HDL-C, total cholesterol, TAG, creatinine and fasting glucose.
- Cardiometabolic risk factors: blood pressure, body temperature, weight, BMI, waist and hip circumferences.
- Musculoskeletal-related traits: handgrip strength, peak expiratory flow rate, bio-impedance, gait speed and body balance

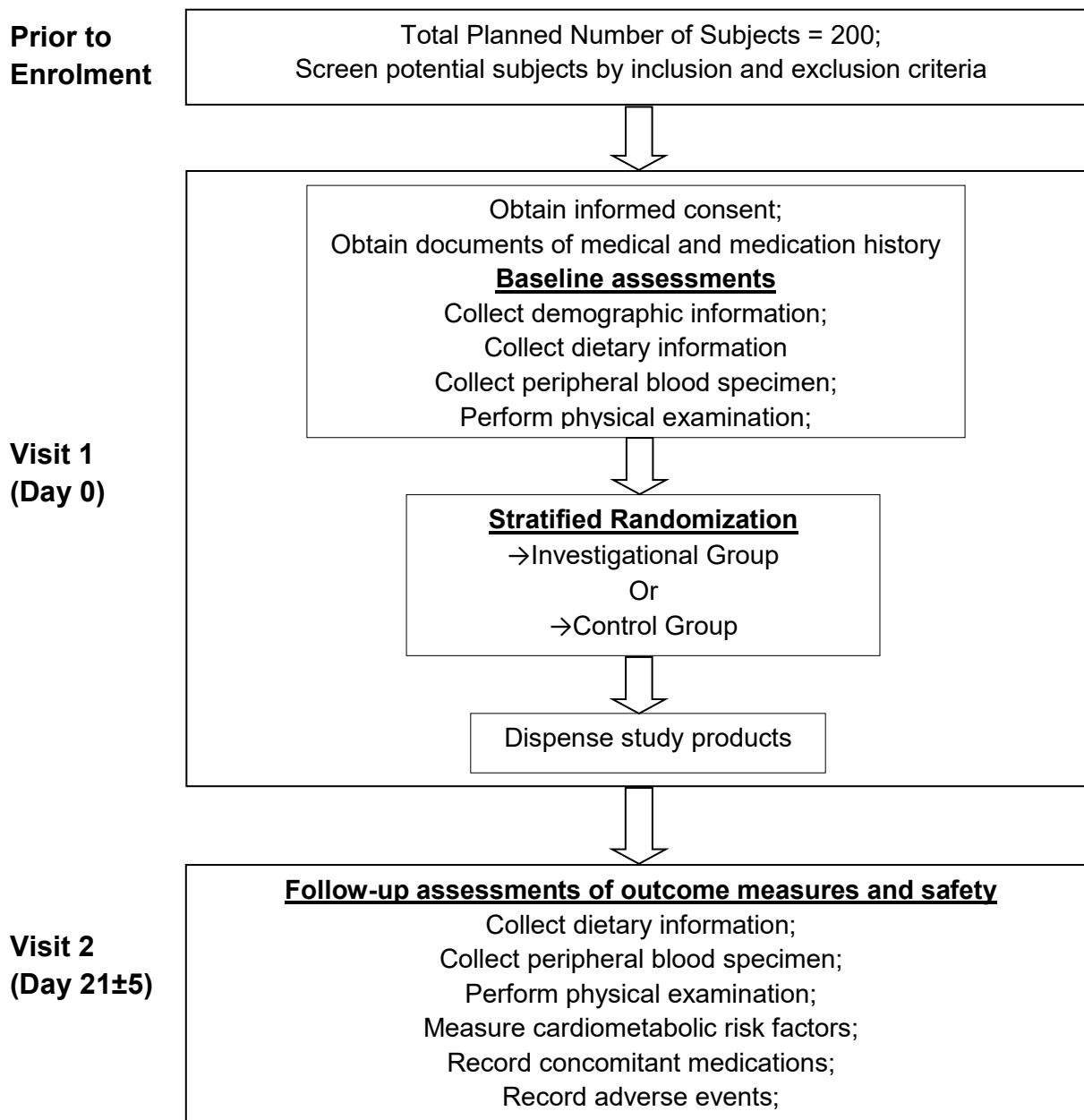
The secondary efficacy analyses will be performed on the ITT population only.

***Safety Analysis:***

The incidence of AE such as nausea, abdominal cramps, vomiting, diarrhea, and flatulence will be summarized by frequency tables per study group. Data listings will be reported.



#### 4 SCHEMATIC OF STUDY DESIGN



#### Remarks

Subjects will be asked to refrain from drinking 2 alcoholic or caffeinated beverages per day and advised to maintain their typical diet and physical activity levels. They will also be asked to report any changes in diet or physical activity and symptoms or changes in health and medications throughout the trial.

## 5 SCHEDULE OF STUDY ASSESSMENTS / PROCEDURES

Assessments / Procedures	Screening & Baseline (Day 0)	End of Study (Day 21 ± 5)	Early Withdrawal
Informed Consent Form Signed	X		
Eligibility Criteria	X		
Demographic Information	X		
Medical History	X		
Physical Examination	X	X	X
Cardiometabolic Risk Factors <sup>1</sup>	X	X	X
Blood Test <sup>2</sup>	X	X	X
Concomitant Medications	X	X	X
Dispensing Study Product	X		
Adverse Events		X	X
Study Product Compliance		X	X
Dietary Information	X	X	X

### Remarks:

- Cardiometabolic risk factors include blood pressure, body temperature, body height and weight, waist and hip circumferences. Body height will be measured at the baseline only.
- Blood tests include:
  - Lipid profile: Serum level of LDL-C, HDL-C, total cholesterol and TAG
  - Serum creatinine, fasting blood glucose
- Physical examination includes measurement of bioimpedance. Participants with metal implant will be excluded from this measurement.

## 6 INTRODUCTION

### 6.1 Background Information

#### 6.1.1 *Disease Background Relevant to Clinical Study*

##### The Relationship Between Cardiovascular Disease (CVD) and Low-Density Lipoprotein Cholesterol (LDL-C)

A cardiovascular disease (CVD) is a major health problem as the third leading causes of mortality in Hong Kong (3). A number of CVD risk factors have been identified, such as hypertension, obesity, tobacco exposure and physical inactivity. Among these risk factors, elevated plasma LDL-C levels play a significant role in CVD development. The deposition of cholesterol in the arterial wall by the infiltration of LDLs is a key step in the development of atherosclerotic CVD such as coronary artery disease, stroke and peripheral vascular disease. The consensus of LDL as the major proatherogenic lipoproteins in the circulation provides a valuable parameter for assessing CVD risk (4). Indeed, blood LDL-C level is also one of the predictors in the Framingham Risk Score, a risk assessment tool widely used in clinical setting for estimating of 10-year cardiovascular risk of an individual (5).

The blood cholesterol level is controlled by cholesterol absorption from the diet and by endogenous cholesterol synthesis that occurs through a series of pathways. The main strategy of conventional lipid lowering therapy is to interfere with some of these pathways by pharmacological agents and dietary components (6). For example, statins are a class of highly effective cholesterol-lowering drugs by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver (7). Despite the various lipid lowering agents for the management of hyperlipidemia, a significant number of patients do not reach their LDL-C target goals due to various reasons such as low drug compliance and resistant to lifestyle change (7-8). The often poor achievement of LDL-C goals among high risk patients warrants the need of other cholesterol lowering measures with higher efficacy and patient acceptance.

### **6.1.2 Investigational Product Background Relevant to Clinical Study**

#### Plant Sterols and their Physiological Characteristics

Plant sterols are the naturally occurring functional equivalent of mammalian cholesterol. The most common plant sterols include sitosterol, campesterol, and stigmasterol. Plant sterols differ structurally from cholesterol by a methyl or ethyl group in their side chains and are not synthesized by the human body. These structural differences render them minimally absorbable in the intestine, of the fraction of 0.5-2% for plant sterols (9). As a result of low absorption and efficient excretion after uptake by the liver, circulating levels are low, varying from 0.3-1.0 mg/dL for plant sterols. Phytosterol-enriched foods and dietary supplements have been marketed for decades. The main food sources of plant sterols are vegetable oils, margarines, breads, cereals, and vegetables, contributed 50-80% of the daily plant sterol intake (11-12).

#### Lipid-Modifying Effect of Plant Sterols

The cholesterol lowering effect of plant sterols were demonstrated more than 50 years ago (13). Earlier studies demonstrated that pure plant sterol supplementation lowered both total serum cholesterol and LDL-C in individuals with hypercholesterolemia (14). A number of clinical trials have been examined the efficacy of plant sterols/stanols as a cholesterol lowering agent when incorporated into low fat foods including yoghurt, bread and cereal (15). Results from several epidemiological studies consistently reported that dietary incorporation of plant sterols/stanols (1.5-2 g/day) reduces serum LDL-C levels 5% to 15% with minimal adverse events (16-18). In addition, the impact of the consumption of plant sterols/stanols-enriched foods is relatively consistent in both normolipidemic and dyslipidemic individuals including those treated with lipid-lowering agents, regardless of background diet and that there is little or no effect on high-density lipoprotein (HDL) cholesterol or triglyceride (TAG) levels (19-20). It has now been recognized by the United States Federal Drug Administration (US FDA) and the American Heart Association that dietary incorporation of plant sterols is recommended for blood cholesterol reduction and thereby reducing the risk of CVD (21-22).

## Potential Mechanisms of Action of Plant Sterols

*Several studies have investigated the mechanism of action of phytosterols on reducing serum LDL-C level (23-24). The initial stage of intestinal handling of phytosterols from diet is essentially similar to the absorption of cholesterol, involving emulsification, hydrolysis and micellar transport (25). However, most of plant sterols/stanols are pumped back into the gut lumen via the ATP-binding cassette co-transporters G5 and G8 (ABCG5/ABCG8), resulting in the minimal entry of these plant-derived molecules into the circulation. The hypocholesterolemic effects of plant sterols/stanols is explained by an inhibition of cholesterol absorption, which is ascribed to a competition with intestinal cholesterol for incorporation into mixed micelles (26), although other possible mechanistic explanation have been proposed (27).*

## Clinical Significance

The goal of this study is to determine the efficacy of regular consumption of plant sterols-enriched soya beverage in reducing serum LDL-C level among Southern Chinese individuals. The hypocholesterolemic effects of plant sterols with soya milk as carrier has only been investigated in few studies, the beneficial effects of soya beverage fortified with plant sterols on Southern Chinese individuals are not well-understood and necessitate investigation. Results from this study will have important clinical implications in the control and management of blood LDL-C level among normolipidemic individuals and will promote further development of novel soya beverage or related products containing plant sterols for future use in cholesterol-lowering initiatives. In addition to a role in primary prevention in the general population, functional food enriched with plant sterols may provide additional LDL-C lowering benefit in dyslipidaemic patients at high CV risk as an adjunct to traditional pharmacologic therapy.

## **6.2 Study Rationale**

Eligible Southern Chinese in Hong Kong aged 18 or above with good health condition will be asked to consume 2g of plant sterols orally per day for 3 consecutive weeks as provided by one pack of plant sterols-containing soya beverage, each pack consumed during the main meal every day. The dosage regime is designed in accordance with the recommendations from European Union (EU) Register of Nutrition and Health claims as well as previous clinical trials and epidemiological studies regarding the same study ingredient (13-18). It is hypothesized that daily consumption of 2g of plant sterols in a soya beverage results in a significant reduction of serum LDL-C.

*Result from this study encourages further development of novel soya beverage or related products containing plant sterols for future use in cholesterol-lowering initiatives*

*among Chinese population.*

## *Potential Risks and Benefits*

### **6.2.1 Potential Risks**

- This study does not involve any pharmacological intervention. Pre-clinical and previous clinical trials (13-18) indicated that the study product is generally safe and will not affect the health status of the subjects. Therefore, additional risk will be minimal.
- Blood samples will be taken at the start and the end of the study by a registered nurse. Venipuncture may result in pain or bruises. The chance of infection associated with venipuncture is very low.

### **6.2.2 Potential Benefits**

- Subjects will receive an assessment of their current health status and they will be informed if any test result shows a potential risk to their health condition.
- Previous studies have demonstrated that regular consumption of plant sterols may reduce total blood cholesterol and LDL-C level. Reduction of LDL-C levels has been demonstrated to significantly reduce CVD morbidity and mortality (13-18).
- While it is possible, however, that subjects may not obtain any direct benefits in this clinical study, the information obtained from this study maybe useful to scientific community to examine the efficacy of plant sterols for future use in cholesterol-lowering initiatives and prevention of cardiovascular disease.

## 7 OBJECTIVES

### 7.1 Study Objectives

- **The primary objective** of this study is to assess the efficacy of daily consumption of 2g plant sterols as provided by one pack of 250 ml soya beverage product each taken during the main meal for 3 consecutive weeks, in lowering the blood LDL-C level among Southern Chinese.
- **The secondary objective** is to examine if there is other health-benefits by consuming the plant sterols-enriched soya beverage, such as glucose and TAG lowering effect.



## 8 STUDY DESIGN

The study is a 3-week, single-center, double-arm, randomized, double-blind clinical study. Healthy, normocholesterolemic Southern Chinese in Hong Kong will be recruited and screened by the inclusion and exclusion criteria.

Two hundred (200) eligible and consented subjects will be assigned to consume either the investigational or control product by stratified randomization, according to the instructions as follows:

- Investigational Group: Daily consumption of 2g of plant sterols as provided by one pack of the plant sterols-enriched soya beverage for 3 consecutive weeks, each pack consumed during the main meal
- Control Group: Daily consumption of one pack of the soya beverage without plant sterols for 3 consecutive weeks, each pack consumed during the main meal

The randomization will be stratified by both gender (male and female) and age group (18-40 years and >40 years).

Subjects will be invited to attend a baseline visit (Day 0), after which study products will be administered for 21 days. A follow-up assessment (Day 21±5) at the end of the study intervention will be performed for outcome assessment. Clinical data and blood specimen will be obtained by trained personnel in both visits. All blood specimens will be analyzed in a centralized and certified clinical laboratory.

Subjects will be asked to refrain from drinking 2 alcoholic or caffeinated beverages per day and advised to maintain their typical diet and physical activity levels. They will also be asked to report any changes in diet or physical activity and symptoms or changes in health and medications throughout the trial.

## **9 STUDY POPULATION**

### **9.1 Inclusion Criteria**

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Southern Chinese male or female  $\geq 18$  years.
- In good general health as evidenced by medical history
- Have the ability to understand the requirements of the study, provide written informed consent, including consent for the use and disclosure of research-related health information, and comply with the study data collection procedures. Provide signed and dated informed consent form

### **9.2 Exclusion Criteria**

An individual who meets any of the following criteria will be excluded from participation in this study:

- Subject with familial hypercholesterolemia (31)
- On regular medication(s) which affect gastrointestinal functions and blood lipids level for the past 3 months, such as, but not limited to:
  - Antibiotics of  $>1$  week duration
  - GI related medications such as antacids
- Having blood lipid lowering medications such as statins, selective cholesterol absorption inhibitors (e.g. ezetimibe), fibrates, niacin, resins, omega-3
- Heavy-smokers (more than 1 pack per day)
- Subject with heavy alcohol intake ( $>40$  g/day for men ;  $>30$  g/day for women) (32), or having history of alcohol abuse within 12 months prior to the study
- Subjects with taste aversion to placebo/intervention soya beverages
- Subject refusing to stop the consumption of plant sterols-enriched products if any during the study (other than the studied product) or having regular consumption of sterols/stanols cholesterol-lowering supplements/functional foods or other related products such as:
  - Sterol/ Stanol - containing margarines, milk, yoghurt drink and soya beverages
  - Sterol / Stanol supplements
  - Fish oils & omega-3 supplements
- Subject receiving systemic treatment or topical treatment likely to interfere with evaluation of the study parameters

- Subject currently involved in a clinical trial or in an exclusion period following participation in another clinical trial
- Pregnancy or lactation
- Having soy allergy
- Being a vegan
- Being an athlete
- Concurrently participating in weight management or dietary program
- On diet pills, such as, but not limited to chitosan and dulcolax
- With history of sitosterolemia
- Having history of hypercholesterolemia, diabetes, thyroid disease, severe kidney diseases, cardiovascular diseases, chronic gastrointestinal disorders, cancers and AIDs
- Having history of malabsorption syndrome arising from diseases such as, but not limited to celiac disease, short bowel syndrome, cystic fibrosis, pancreatitis, diseases of gall-bladder, liver or pancreas, intestinal infection, injury, surgery and radiotherapy
- Taking over-the-counter Chinese medications or supplements with cholesterol/lipidlowering and related claims
- High blood cholesterol at screening [Total cholesterol level  $\geq 6.22$ mmol/L]
- High LDL cholesterol [LDL cholesterol level  $\geq 4.15$ mmol/L]
- High blood triglyceride at screening [TG  $\geq 3.39$ mmol/L]
- Anything that would place the individual at increased risk or preclude the individual's full compliance with or completion of the study.

## **9.3 Subject Withdrawal**

### **9.3.1 Reasons for Withdrawal**

Subjects are free to withdraw from participation in the study at any time upon request. Withdrawal from this study will not affect subjects' ability to receive normal medical care and will not lose any benefits to which they would otherwise be entitled.

An investigator may terminate a study subject's participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- The subject do not follow instructions regarding to the study
- The research investigators or ethic committee(s) stop this research project due to any reasons

For subjects meeting violation of exclusion criteria for baseline laboratory values (i.e. lipid profiling) after randomization, subjects' participation will be continued at the discretion of the investigator.

### **9.3.2 Handling of Subject Withdrawals or Subject Discontinuation of Study Intervention**

Effort will be made to collect the safety and efficacy outcome measures of the withdrawn subjects or subjects who discontinue study intervention by undertaking safety follow-up visit to capture adverse events (AEs), unanticipated problems (UPs), and to collect biological specimens for laboratory evaluations. The participation of the withdrawal visit is entirely voluntary.

Since 25% dropout rate is assumed in the calculation of sample size, no replacement of subjects who withdraw or discontinue early will be made.

#### **9.4 Premature Termination or Suspension of Study**

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the study investigators and regulatory authorities. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Insufficient adherence to protocol requirements.
- Data that is not sufficiently complete and/or evaluable.
- Determination of futility.

## **10 GROUP ASSIGNMENT PROCEDURES**

### **10.1 Subject Number**

Prior to study intervention at Day 0 (Screening / Baseline visit), the investigator assigns a uniquely identified subject number to each eligible subject chronologically (e.g. 001, 002, etc). The subject number will not be reused. The actual subject number allocated will be recorded on a subject number allocation log.

### **10.2 Randomization Schemes**

The Data Management and Medical Statistics unit in CTC, HKU, will define randomization specifications and generating the randomization codes, prior to the start of the study.

Block randomization with varying block sizes will be performed separately within each stratum to maintain approximately 1:1 allocations of the two study groups.

Four strata are constructed as follows:

#### **Strata:**

1. Male, aged 18-40 years
2. Male, aged >40 years
3. Female, aged 18-40 years
4. Female, aged >40 years

### **10.3 Group Allocation**

A web-based randomization system will be developed by CTC, HKU to carry out the on-line group allocation.

At Day 0 visit, each subject who fulfils all the eligibility criteria will be given a subject number by the Investigator. The Investigator will prescribe the study intervention according to the subject number by completing a prescription form to the unblinded personnel. Once the unblinded personnel receive the prescription form for the subject number, the unblinded personnel will login the web-based randomization system and randomize the subject using the particular subject number. The information about the group assignment to the subject will be printed out [Group Allocation Sheet] from the system as a source document.

#### **10.4 Masking**

This clinical trial is double-blinded in which neither the subjects nor the investigators know which of the subjects belong to the control group or the investigational group. The product manufacturer will help to ensure the products are packaged, coded and labelled in a manner that will not make reference to group allocation.

The whole process of group allocation, study product preparation and dispensing will only be carried out by the designated personnel with privilege of using the web-based randomization system. The blinding of the trial will be maintained throughout the trial until all data entry and processing are complete and the database has been locked.

#### **10.5 Emergency Unblinding**

Breaking of randomization codes will be performed in a medical emergency where knowledge of the blinded treatment is necessary, for the treatment of a safety event, or requested by the PI. In the event of a code break, the person requesting the code break, subject number, reasons for unblinding, date and time should be documented.

## **11 INVESTIGATIONAL AND CONTROL PRODUCT ADMINISTRATION**

### **11.1 Product Description**

#### ***11.1.1 Acquisition***

The investigational and control products will be supplied by VITASOY International Holdings Ltd.

#### ***11.1.2 Formulation, Packaging, and Labeling***

The investigational product is a plant sterols-enriched soya beverage packed in *cartons* and the control product is the same soya beverage except without plant sterols.

All study products will be labeled with a subject number, bar code, manufacturer, instructions for administration, contact information of study staff, caution statements indicating the product is strictly for subject's use and product storage.

#### ***11.1.3 Product Storage and Stability***

The study products have to be stored in cool (around room temperature) and dry place.

### **11.2 Dosage, Preparation and Administration of Study Products**

Investigational Group: Subjects will orally consume 2g of plant sterols daily as provided by one pack of the soya beverage for 3 consecutive weeks, each pack consumed during the main meal.

Control Group: Subjects will orally consume one pack of the soya beverage except without plant sterols for 3 consecutive weeks, each pack consumed during the main meal.

Subjects will be advised to consume the products immediately once the package is opened and store the products in cool place.

### **11.3 Accountability Procedures for the Study Products**

Study products will be distributed to subjects during their screening and baseline visit (Day 0).

All unused products have to be returned to the study staff in the final visit (Day 21  $\pm$ 5) which will be used as a count of subject's compliance with the study products.



## **12 STUDY SCHEDULE AND PROCEDURE**

### **12.1 Study Visit**

#### ***12.1.1 Screening and Enrollment/Baseline (Visit 1, Day 0)***

- Obtain and document signed informed consent form
- Review medical history to determine eligibility based on inclusion/exclusion criteria
- Review and record medications history to determine eligibility based on inclusion/exclusion criteria
- For eligible subject, perform medical examinations
- For eligible subject, collect detailed demographic information, dietary information, medical history, medication history, alcohol, and tobacco use history
- For eligible subject, collect fasting blood specimen and cardiometabolic risk factors
- Schedule follow-up visit for subject who is eligible and available for the duration of the study
- Provide subject with instructions of the administration of study products
- Provide subject with instructions needed to prepare for follow-up study visit; For instance, return the excess product as a measure of product compliance

#### ***12.1.2 End of Study (Visit 2, Day 21 ± 5)***

- Perform physical examinations
- Record adverse events as reported by subject or observed by investigator
- Record concomitant medications as reported by subject
- Collect fasting blood specimen and cardiometabolic risk factors
- Record subject's compliance with the study product

### **12.1.3 Early Withdrawal**

If subject withdraws early or investigator terminates subject's participation, following evaluations will be offered to the subject:

- Perform physical examinations
- Record adverse events as reported by subject or observed by investigator
- Record concomitant medications as reported by subject
- Collect fasting blood specimen and cardiometabolic risk factors
- Record subject's compliance with the study product

## **12.2 Procedures and Evaluations**

### **12.2.1 Clinical Laboratory Evaluations**

42ml peripheral blood sample will be collected from all subjects. The following laboratory assessments will be assessed at baseline and after the 3-week study period.

Biological specimens will be stored under the subject's consent for any genetic and cell analysis related to risk factors of cardiometabolic disease in the future.

### **12.2.2 Special Assays or Procedures**

#### **Fasting lipid profile**

Blood will be collected into EDTA-coated tubes and the plasma levels of total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) and triglycerides will be measured automatically by electrophoresis (Architect ci8200 analyzer; Abbott Diagnostics, Wiesbaden, Germany) at the certified Clinical Laboratory.

## 13 STUDY MEASUREMENTS AND VARIABLES

### 13.1 Efficacy Measurements

#### 13.1.1 Primary Endpoint

The **primary endpoint** is the mean serum LDL-C at the end of the week 3 after the consumption of plant sterols fortified soya beverage or control product.

#### 13.1.2 Secondary Endpoints

The **secondary endpoints** include the changes of serum LDL-C, HDL-C, total cholesterol, triglycerides (TAG), serum creatinine, fasting blood glucose, other cardiometabolic risk factors and musculoskeletal-related traits from baseline to week 3.

##### Blood Tests:

- Serum LDL-C, HDL-C, total cholesterol, and triglycerides (TAG)
- Serum creatinine and fasting blood glucose

##### Cardiometabolic risk factors:

- Blood pressure, body temperature, body height and weight, waist and hip circumferences

##### Musculoskeletal-related traits:

- Handgrip strength, peak expiratory flow rate, bio-impedance, gait speed and body balance

### 13.2 Safety Measurements

#### 13.2.1 Adverse Events

An adverse event (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

The relationship of each AE to the study product is defined as below:

- **Unrelated:** There is no temporal sequence of the AE onset relative to the administration of study product, and /or there is evidence of other cause such as concurrent medication or illness contributed to the event.

- **Possibly related:** A temporal sequence of the AE onset relative to the administration of trial treatment is not clear, alternative causes are also possible.
- **Probably related:** There is a clear temporal sequence of the AE onset relative to the administration of study product, and potential alternative etiology is not apparent.
- **Definitely related:** There is a clear temporal sequence of the AE onset relative to the administration of study product, and no other possible cause.

The severity of each AE will be assessed as described below:

- **Mild:** The AE is transient and easily tolerated by the subject.
- **Moderate:** The AE causes the subject discomfort and interrupts the subject's usual activities.
- **Severe:** The AE causes considerable interference with the subject's usual activities and may be incapacitating or life threatening.

### 13.3 Demographic Information

Demographic information including age, sex and socioeconomic status will be collected during the baseline visit.

### 13.4 Medical History

Medical history including medical and medications history, alcohol, and tobacco use history will be obtained by interview or from medical records during the screening and baseline visit.

### 13.5 Dietary Information

Structured dietary habit questionnaire will be used to evaluate the dietary habit within one month before the screening and end of study visit.

### 13.6 Study Product Compliance

At the end of the study intervention period, subjects need to return all the excess soya beverage packs for counting the measure of compliance.

The study product compliance is calculated by the following equation:

- Study Product Compliance (%) =  $\frac{\text{Observed Consumption}}{\text{Expected Consumption}} \times 100\%$
- Observed consumption = Pack of soy milk dispensed – Pack of soy milk returned
- Expected consumption =  $\sum$  (Expected daily consumption × Number of treatment days)

### **13.7 Prior/Concomitant Medications**

Complete medications history of prescription and over-the-counter medications within 3 months before the screening and baseline visit will be collected.

Detailed information regarding to subject's concomitant medication records including medication name and total dosage will be collected during the baseline and follow-up visit. Any subject receiving the following medications will be excluded from the study:

- On diet pills, such as chitosan and dulcolax
- Ingestion of over-the-counter Chinese medications or supplements with cholesterol/lipidlowering and related claims
- On medications which affect gastrointestinal functions and blood lipids level for the past 3 months, such as,
  - Antibiotics of >1 week duration
  - GI related medications such as antacids
  - Blood lipid lowering medications such as statins, selective cholesterol absorption inhibitors (e.g. ezetimibe), fibrates, niacin, resins, omega- 3

### **13.8 Protocol Deviations**

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or manual of procedures requirements. The noncompliance may be on the part of the subject, the investigator, or study staff. All deviations from the protocol will be recorded in the study subject source documents.

## **14 STATISTICAL SECTION**

### **14.1 Sample Size Rationale**

The primary endpoint is the mean serum LDL-C at the end of the 3-week treatment period. The sample size calculation is based on ANCOVA with baseline serum LDL-C as independent covariate.

According to a previous study (36), the standard deviation of LDL-C at week 4 is approximately 0.85 mmol/l and the estimated correlation coefficient between the outcomes measured at baseline and week 4 is about 0.7. In addition, a minimum difference of 0.28 mmol/l in LDL-C between the groups is considered clinically important.

By using PASS 12 for the sample size calculation, a power of 0.8 and a 5% of the maximum tolerable false positive rate, 75 subjects per group is required to detect the minimum difference between the mean values of LDL-C.

Considering an attrition rate of 25%, a total of 200 subjects will be recruited.

### **14.2 Data Set Analyzed**

The intention-to-treat (ITT) population is consisted of all the cases that are randomized.

The per-protocol (PP) population includes all eligible subjects who have:

- 80% of study product compliance
- Primary efficacy data collected within the pre-specified allowable visit window
- No protocol violation that is clinically significant.

### **14.3 Handling of Missing or Invalid Data**

No data imputation will be performed to replace missing or invalid data.

### **14.4 Demographics and Baseline Characteristics**

Demographics data and other baseline characteristics including medical history, dietary information, physical examination and prior medication will be summarized by appropriate descriptive statistics.

#### **14.5 Primary Efficacy analysis**

ANCOVA will be carried out to compare the serum LDL-C level at week 3 between the groups. The baseline of the response outcome will be used as a covariate to control for initial group differences. A log transformation will be performed if the normality assumption for the ANCOVA model is violated. And if the normality assumption is still not sustained, non-parametric approach might be performed.

The primary efficacy analysis will be performed on both the ITT and the PP populations.

#### **14.6 Secondary Efficacy analyses**

Stratified analysis for the primary endpoint will be performed using the ANCOVA model adjusted for gender and age group. Subjects will be classified to one of the following strata:

1. Male, aged 18-40 years
2. Male, aged >40 years
3. Female, aged 18-40 years
4. Female, aged >40 years

The changes of the following response outcomes from baseline to week 3 will be summarized by descriptive statistics:

- Blood Tests: LDL-C, HDL-C, total cholesterol, TAG, creatinine and fasting glucose.
- Cardiometabolic risk factors: Blood pressure, body temperature, weight, BMI, waist and hip circumferences.
- Musculoskeletal-related traits: Handgrip strength, peak expiratory flow rate, bio-impedance, gait speed and body balance

The secondary efficacy analyses will be performed on the ITT population only.

#### **14.7 Safety Analysis**

The incidence of AE such as nausea, abdominal cramps, vomiting, diarrhea, and flatulence will be summarized by frequency tables per treatment group. Data listings will be reported.

#### **14.8 Compliance of the study products**

The compliance of the study products will be summarized by descriptive statistics per study group.

#### **14.9 Concomitant Medications**

Data listing of concomitant medications will be reported per study group.

#### **14.10 Interim Analyses**

No interim analyses will be planned for this study.

## **15 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS**

### **15.1 Source Documents**

The Investigators will maintain source documents for each subject in the study.

Source documents are defined as original documents, data and records. This may include hospital records, clinical and office charts, laboratory data/information, subjects' diaries or evaluation checklists, pharmacy dispensing and other records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays. The Investigator could create source data worksheets and document information that are not available on the hospital records.

Designated investigator staff will document all the information required by the protocol. It is the investigator's responsibilities to ensure the compliance of protocol and the completeness and accuracy of collected data. Investigator will only instruct qualified site personnel to make any required corrections or additions of collected data. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

The Investigator(s)/institution(s) will permit any trial-related monitoring, audit, IEC/IRB review, and regulatory inspection(s), providing direct access to source data documents.



## **16 DATA HANDLING AND RECORD KEEPING**

### **16.1 Data Management Responsibilities**

Data management will be carried out by data management personnel at CTC, HKU. A data management plan (DMP) will be developed to outline all works related to data management activities, including but not limited to Electronic-Case report forms (eCRF) design, data collection, data cleaning and data storage.

However, data collection and accurate documentation are the responsibility of the study staff under the supervision of the principal investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems and adverse events must be reviewed by the investigator or designee.

### **16.2 Data Capture Methods**

An Electronic Data Capture (EDC) system will be setup by CTC, HKU. eCRFs will be developed and to collect information for this study. All information on the eCRFs must also be reflected in the subject source documents. The principal investigator will review the eCRFs for completeness and accuracy of eCRFs.

Data entered by the site personnel on the web-based EDC system will be submitted and stored securely in the study database. The data management personnel could access and review the study database for completeness and logical consistency. A comprehensive validation check program is built in the system for identifying missing data, out-of-range data and other data inconsistencies. The data management personnel will also perform regular validation check to generate discrepancy reports and individual data query to investigators for solution of data.

Once all the study data are cleaned, the data manager will lock the study database. Once the database is locked, the randomization codes will be unblinded and the data is made available for data analyses.

### **16.3 Study Records Retention**

Study documents should be retained for a minimum of 25 years after the end of the study. No records will be destroyed without the written consent of the sponsor-investigator, if applicable.

The study database will be retained in CTC, HKU for retrieval for at least three years from the date of the clinical study report is submitted to sponsor-investigator.

## **17 ETHICS/PROTECTION OF HUMAN SUBJECTS**

### **17.1 Ethical Standard**

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6.

### **17.2 Institutional Review Board**

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study.

### **17.3 Informed Consent Process**

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to subjects and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the subject. Consent forms will be IRB-approved, and the subject is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the subject and answer any questions that may arise. The subject will sign the informed consent document prior to any study-related assessments or procedures. Subjects will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The consent process will be documented in the clinical or research record.

#### **17.4 Subject Confidentiality**

Subject confidentiality is strictly held in trust by the sponsor-investigator, study staff, and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to any study information relating to subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor-investigator.

The study monitor or other authorized representatives of the sponsor-investigator may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study subjects. The clinical study site will permit access to such records.

#### **17.5 Future Use of Stored Specimens and Other Identifiable Data**

Subjects can choose freely if they agree to the future use of his/her specimens for any genetic or cell analysis related to risk factors of cardiometabolic disease in the future. Residual specimens and personal data will be maintained for at least 25 years after the study is completed only under subject's consent. All data collected and written assignment for this study will be stored in a secured server. The server will then be locked in a cabinet in a locked room. Only principal investigator (Professor Cheung Bernard Man Yung) and his delegates will have access to this locked cabinet. Confidentiality will be protected as all specimens will be coded and anonymized. All data will be destroyed after the storage period.

## **18 PUBLICATION/DATA SHARING POLICY**

The clinical trials registration policy will be adopted for this study. Before the start of the study, registration will be done in an acceptable public trials registry such as *ClinicalTrials.gov*.

Vitasoy International Holdings Ltd. and the University of Hong Kong are the legal co-owners of the data. Vitasoy International Holdings Ltd. is free to use the data and/or any results derived from the data or publications based on that data for marketing purposes, scientific purposes, internal or educational uses.

Publications describing study results must be submitted to Vitasoy International Holdings Ltd at least 30 days before planned manuscript submission and at least 14 days prior to planned abstract submission. If the Vitasoy International Holdings Ltd has no objection to publication requests within the review-period, the submission for publication will proceed.

## 19 LITERATURE REFERENCES

1. Miettinen TA & Vanhanen H. Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. *Atherosclerosis*. 1994;105, 217–226.
2. Hallikainen MA & Uusitupa MIJ. Effects of 2 low fat stanol ester containing margarines on serum cholesterol concentrations as part of a low fat diet in hypercholesterolemic subjects. *Am. J. Clin. Nutr.* 1999;69, 403–410.
3. Centre for Health Protection, HKSAR. 2014. No-communicable diseases and risk factor. Retrieved from <http://www.chp.gov.hk/en/content/9/25/57.html>
4. Jialal I & Remaley AT. Measurement of low-density lipoprotein cholesterol in assessment and management of cardiovascular disease risk. *Clin Pharmacol Ther.* 2014;96(1):20-2.
5. D'Agostino RB.; Sr., Vasan, RS, Pencina, MJ, Wolf, PA, Cobain, M, Massaro, JM, Kannel, WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation*. 2008;117(6): 743–753.
6. Ros E. Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. *Atherosclerosis*. 2000;151, 357–379.
7. Gitt AK, Drexel H, Feely J, et al. Persistent lipid abnormalities in statin-treated patients and predictors of LDL-cholesterol goal achievement in clinical practice in Europe and Canada. *Eur J Prev Cardiol*. 2012;19:221-30.
8. Kotseva K, Wood D, De Backer G, et al. Cardiovascular prevention guidelines in daily practice: a comparison of EUROASPIRE I, II, and III surveys in eight European countries. *Lancet*. 2009;373:929-40.
9. Ostlund Jr RE, McGill JB, Zeng C-M, et al. Gastro-intestinal absorption and plasma kinetics of soy D5-phytosterols and phytosterols in humans. *Am J Physiol Endocrinol Metab*; 2002;282:E911-6.
10. Björkhem I, Boberg KM, Leitersdorf E. Inborn errors in bile acid biosynthesis and storage of sterols other than cholesterol. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. *The metabolic and molecular bases of inherited disease*. 8th ed. New York: McGraw-Hill; 2001. pp. 2961--88
11. Klingberg S, Andersson H, Mulligan A, et al. Food sources of plant sterols in the EPIC Norfolk population. *Eur J Clin Nutr*. 2008;62:695-703.
12. Piironen V, Lampi AM. Occurrence and levels of phytosterols in foods. In: Dutta PC, editor. *Phytosterols as functional food components and nutraceuticals*. New York: Marcel Dekker, Inc; 2004. pp. 1-32
13. Peterson, DW. Effect of soybean sterols in the diet on plasma and liver cholesterol in chicks. *Proc. Exp. Biol. Med.* 1951;78,
14. Farquhar JW, Smith RE, Dempsey ME. The effect of beta sitosterol on the serum lipids of young men with arteriosclerotic heart disease. *Circulation*.

- 1956;14:77-82.
15. Clifton PM, Noakes M, Sullivan D, Erichsen N, Ross D, Annison G, Fassoulakis A, Cehun M, Nestel P. Cholesterol lowering effects of plant sterol esters differ in milk, yoghurt, bread and cereal. *Eur J Clin Nutr* 2004;58:503-9.
  16. Andersson SW, Skinner J, Ellegård L, et al. Intake of plant sterols is inversely related to serum cholesterol concentration in men and women in the EPIC Norfolk population: a cross-sectional study. *Eur J Clin Nutr* 2004;58:1378-85.
  17. Klingberg S, Ellegård L, Johansson I, et al. Inverse relation between naturally occurring dietary plant sterols and serum cholesterol in northern Sweden. *Am J Clin Nutr*. 2008;87:993-1002.
  18. Wang P, Chen YM, He LP, et al. Association of natural intake of dietary plant sterols with carotid intima-media thickness and blood lipids in Chinese adults: a cross-section study. *PLoS ONE* 2012;7:-32736.
  19. Demonty I, Ras RT, van der Knaap HC, et al. The effect of plant sterols on serum triglyceride concentrations is dependent on baseline concentrations: a pooled analysis of 12 randomised controlled trials. *Eur J Nutr*. 2013;52:153-60.
  20. Plat J, Mensink RP. Plant stanol esters lower serum triacylglycerol concentrations via a reduced hepatic VLDL-1 production. *Lipids*. 2009;44:1149-53.
  21. FDA (2000): Food labeling: health claims; plant sterol/stanol esters and coronary heart disease. Food and Drug Admin. Fed. Reg. 65, 54686–54739
  22. Lichtenstein AH & Deckelbaum RJ. AHA Science Advisory. Stanol/sterol ester-containing foods and blood cholesterol levels. A statement for healthcare professionals from the Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. *Circulation*. 2001. 103,1177–1179.
  23. Berge KE, von Bergmann K, Lutjohann D, Guerra R, Grundy SM, Hobbs HH & Cohen JC. Heritability of plasma noncholesterol sterols and relationship to DNA sequence polymorphism in ABCG5 and ABCG8. *J. Lipid Res*. 2002. 43, 486–494.
  24. Plat J & Mensink RP. Increased intestinal ABCA1 expression contributes to the decrease in cholesterol absorption after plant stanol consumption. *FASEB J*. 2002;16, 1248–1253.
  25. Lu K, Lee M & Patel SB. Dietary cholesterol absorption; more than just bile. *TRENDS Endocrinol. Metab*. 2001;12, 314–320.
  26. Ikeda, I., Tanabe, Y., and Sugano, M. (1989) Effects of sitosterol and sitostanol on micellar solubility of cholesterol. *J. Nutr. Sci. Vitaminol*. 35, 361–369
  27. De Smet E, Mensink RP, Plat J. Effects of plant sterols and stanols on intestinal cholesterol metabolism: suggested mechanisms from past to present. *Mol Nutr Food Res*. 2012;56:1058e72.

28. Abumweis SS, Barake R, Jones PJ. Plant sterols/stanols as cholesterol lowering agents: A meta-analysis of randomized controlled trials. *Food Nutr Res.* 2008;52
29. Salen G, von Bergmann K, Lütjohann D, et al., Multicenter Sitosterolemia Study Group. Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. *Circulation* 2004;109:966-71.
30. Calkin AC, Tontonoz P. Liver x receptor signaling pathways and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2010 Aug;30(8):1513-8.
31. Consumer Reports; Drug Effectiveness Review Project (2013). "Evaluating statin drugs to treat High Cholesterol and Heart Disease: Comparing Effectiveness, Safety, and Price". Best Buy Drugs (Consumer Reports): 9.
32. Thomsen AB, Hansen HB, Christiansen C, Green H, Berger A. Effect of free plant sterols in low-fat milk on serum lipid profile in hypercholesterolemic subjects. *Eur J Clin Nutr.* 2004 Jun;58(6):860-70.
33. Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci.* 1993; 694:72-7.
34. Evans AM, DeHaven CD, Barrett T, et al. Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. *Anal Chem* 2009;81:6656-6667.
35. Ohta T, Masutomi N, Tsutsui N, et al. Untargeted metabolomic profiling as an evaluative tool of fenofibrate-induced toxicology in Fischer 344 male rats. *Toxicol Pathol* 2009;37:521-535.
36. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000 Feb 29 - . Identifier NCT02541201, The Effect of a Plant Sterols-Enriched Low-Fat Milk on Lipid Profile in Healthy Southern Chinese (COME-PASS); 2015 Aug 26. Available from: <http://clinicaltrials.gov/ct2/show/NCT02541201>