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# The effect of ALFALIFE™ for the prevention of coronary heart disease in high-risk patients

**Submission date**
23/05/2020

**Recruitment status**
No longer recruiting

**Registration date**
10/06/2020

**Overall study status**
Completed

**Last Edited**
19/07/2023

**Condition category**
Circulatory System

[X] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[X] Results
[_] Individual participant data

## Plain English summary of protocol

Background and study aims
Coronary heart disease (CHD) is a major cause of death worldwide. This is due to atherosclerosis, a process that affects the walls of coronary arteries, leading to an obstruction of the blood supply to the heart and eventually to chronic damage. CHD is caused by a number of factors, including lifestyle, chronic metabolic conditions, genetic factors and inflammation. The aim of this study is to find out whether a supplement of Cannabis sativa seed oil (ALFALIFE™), along with a balanced diet, will improve the metabolic profile and the level of low-grade inflammation of patients with a high risk of developing CHD.

Who can participate?
Patients aged 30 to 69 with high blood cholesterol/lipoproteins with metabolic syndrome, who also have a low dietary intake of alpha-linolenic acid

What does the study involve?
Participants are randomly allocated to two groups, one receiving a daily dose of six capsules of ALFALIFE™ (one capsule with their breakfast, two with their lunch, and three with their supper) for 60 days, while the second group will receive a placebo that will look exactly the same as ALFALIFE™. The participants will be checked on a regular basis to assess their health conditions, their quality of life as well as their acceptance of the treatment.

What are the possible benefits and risks of participating?
Participants will receive their supplements for free and will receive medical advice during and at the end of the study based on the results of the medical examination and their response to the treatment. As the intervention is based on supplements with no known side effects, there are no health and safety issues related to the trial. However, the participants' health status will be regularly checked and any side effects promptly recorded and resolved.

Where is the study run from?
Lugo Medica (Italy)

When is the study starting and how long is it expected to run for?
January 2020 to December 2020

Who is funding the study?
Freia Farmaceutici s.r.l. (Italy)

Who is the main contact?
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## Additional identifiers

### Protocol serial number
H4H-02

## Study information

### Scientific Title
Interventional study to verify the efficacy of ALFALIFE™ administration in association with diet and standard therapeutic approach in reducing the metabolic risk profile for the prevention of coronary heart disease

### Acronym
H4H-02

### Study objectives
The routine administration of ALFALIFE™ improves the metabolic profile of patients with a Coronary Heart Disease (CHD) high-risk profile, reducing at the same time the level of low-grade Inflammation and improving those conditions associate to chronic inflammation and to the hyperactivity of the immune response. According to the study's hypothesis, ALFALIFE™ supplementation provides an improvement of the inflammatory markers, reduce broadly the risk of thrombosis and of other conditions associated to CHD (like insulin resistance resulting in better glycaemic control in patients with diabetes) and offer an improvement or prophylaxis of the complications of the hyperactivation of the immune response as in viral infections like the clinical syndrome of COVID-19, overall improving the quality of life.

### Ethics approval required
Old ethics approval format

### Ethics approval(s)
Approval pending

### Primary study design
Interventional

## Study design
Interventional multicentre longitudinal double-blind randomized clinical trial

## Study type(s)
Prevention

## Health condition(s) or problem(s) studied
Coronary heart disease (CHD)

## Interventions
This is a randomized double-blind versus placebo clinical trial based on the administration of ALFALIFE™ supplements in patients with a balanced hypolipidemic and isocaloric diet. ALFALIFE™ is presented in soft capsules; the placebo contains edible oil and is presented in soft capsules that appear identical to ALFALIFE™. Both arms will receive six capsules/day (one at breakfast, two at lunch, and three at supper) for 60 days.

The primary outcome is based on the assessment of the expected reduction of an improvement of the analytes that measure low-grade inflammation when compared to baseline parameters. To assess the primary outcome investigators will collect blood from the subjects enrolled in the study and will send the sample for the measurement of the analytes listed below. The baseline values are measured before the 15 days washout period. After the washout patient will receive daily the ALFALIFE™ or the placebo. The same analytes will be measured at the end of the trial (60th day) and again at the end of the following washout (90th day).

The blood samples will be collected according to the following procedure. Investigators will draw blood with the participant in a sitting position, from an antecubital vein of the arm, with a vacutainer system, in the absence of stasis, after 11-12 hours of fasting (water intake is allowed). The patient's name, surname, date of birth, date of sampling will be indicated on all the test tubes and in a special computer register, after completing the privacy forms for the entire duration of the study. The blood will be collected with a 10 ml vacutainer, preferably containing EDTA or heparin, without hemolyzing. The sample will be immediately centrifuged to separate the plasma. If it is not possible to perform the centrifugation immediately, it will be put on ice until the moment of centrifugation (15 min at 3000 xg). After centrifugation, three aliquots of 700 microliters of plasma will be taken, to be placed in 1.5 ml Eppendorf © tubes. The samples will then be placed immediately at -80°C. The samples will then be sent to the reference laboratories for the assay of the analytes. The same procedures will be repeated at each check, to allow maximum standardization of the procedure.

In addition, as inflammation is considered a major cause of the complication of the current COVID-19 pandemic, the investigators will assess the general inflammatory response expressed as a reduction of the level of those immunomodulators that are known to be overexpressed in patients with SARS-CoV-2 infection. Moreover, to better assess the general condition and better evaluate the effect of the intervention, investigators will assess the general conditions of the enrolled subjects and the level of perception of their health status. Investigators will perform a full medical examination before each blood collection. Patients will receive a mid-term questionnaire and a second questionnaire at the end of the trial to assess the level of acceptance defined as perceived easiness to take the capsules, and the daily and overall compliance.

Medical examination
Medical examination will be performed according to good medical practice standards. The

examiner will record the findings using a transferable or exportable electronic format. The examiner will always measure, according to shared standard, systolic pressure, diastolic pressure, mean heart rate, weight, and height (first check only), abdominal circumference. The same values will be measured at the end of the trial. At the beginning of the study, the investigator will record the subject medical history according to a standard template and will record in a form the findings with a special focus of the participant's diet. At the end of the study the compliance of the subjects in regularly taking the capsules will be assessed (specifying how many and which dose the subject has skipped). Investigators will record every diet supplementation and drugs taken by the subjects during the whole trial. Any possible side effects should be reported on the medical records/files. Each participant will be assessed at time 0 and on day 30th with an ECG with RR interval recording for at least 5 minutes - BIA-ACC. Each participant will be assessed at time 0 and on day 120th (before and at the end of the intervention) with a supra-aortic trunk, aortic, renal and femoral artery echo-Doppler (TSA) to evaluate the atheromatic risk, and a standard ECG.

Questionnaires
The questionnaires administered refer to the food history for adherence to the diet and evaluation of the pro-/anti-inflammatory diet, adherence to the administration of alpha-linolenic acid, and physical activity. Investigators will also assess the quality of life (SF-12), the quality and quantity of sleep, and any nutraceutical and supplements intake
The questionnaire and the table for the evaluation of the responses and for the evaluation of the diet composition are presented in the investigator's handbook that will be made available to every researcher. It will include the following annexes:
ANNEX I Questionnaire for patient recruitment
ANNEX II-IV 7-day recall questionnaires
ANNEX V Diet adherence questionnaire
ANNEX VI Physical activity monitoring questionnaire: IPAQ
ANNEX VII Capsule tolerance / adherence / intake questionnaire
ANNEX VIII Sleep Quality Questionnaire: PSQI
ANNEX IX Quality of Life Questionnaire: SF12
ANNEX X-XIX Isolipid diets while taking soft capsules

Intermediate questionnaires
During the activities, short questionnaires will be periodically administered to patients for the sole purpose of verifying the progress of food, physical behaviors, and other factors to keep attention to the protocol high.

## Intervention Type
Supplement

## Primary outcome(s)
1. Cholesterol Tot measured using enzymatic reaction at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
2. LDLc measured using enzymatic reaction at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
3. HDLc triglycerides measured using enzymatic reaction at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
4. Lp (a) measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
5. Glycemia measured using enzymatic reaction at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days

6. Glycated haemoglobin measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
7. HOMA-IR measured at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
8. HOMA-B measured at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
9. Leptin measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
10. Ghrelin measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
11. VCAM measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
12. ICAM measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
13. Endothelin measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
14. Homocysteine measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
15. Fibrinogen measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
16. Uricemia measured at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
17. CRP-HS measured at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
18. Cytokines measured using multiplex essay for nine cytokines, ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
19. Total lymphocytes measured using blood count at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
20. Ferritin measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
21. GOT measured using enzymatic reaction at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
22. GPT measured using enzymatic reaction at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
23. CPK measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days
24. Creatinine measured using ELISA at baseline, 30 days (end of wash-out phase), 90 days, 150 days, and 180 days

## Key secondary outcome(s)

1. The number of visualized plaques, as well as the total plaque area or total plaque volume, measured using supra-aortic trunk artery echo-Doppler at baseline and 120 days
2. The number of visualized plaques, as well as the total plaque area or total plaque volume, measured using aortic artery echo-Doppler at baseline and 120 days
3. The number of visualized plaques, as well as the total plaque area or total plaque volume, measured using renal artery echo-Doppler at baseline and 120 days
4. The number of visualized plaques, as well as the total plaque area or total plaque volume, measured using femoral artery echo-Doppler at baseline and 120 days

## Completion date

15/12/2020

# Eligibility

### Key inclusion criteria
1. Patients with non-optimal ALA intake (<RDA of 0.5% E; Questionnaire 01)
2. Adults, able to independently express informed consent
3. Age between 30 and 69 years
4. Participants with hyperlipoproteinemia sustained by an increase in Lp(a) (Lp(a) levels> 30 (patient with very high levels > 80 are also included and will be flagged for further subgroup analysis)
5. Patients with mixed hyperlipoproteinemia (type IIb) or hypercholesterolemia (type IIa), with metabolic syndrome (according to ATP III classification) or overweight

### Participant type(s)
Patient

### Healthy volunteers allowed
No

### Age group
Adult

### Sex
All

### Key exclusion criteria
1. Patients with secondary hypercholesterolaemia and endocrinopathies, even if borderline or if the condition is only suspected.
2. Patients with type 1 or 2 diabetes (or 1-5 according to the new Lancet Endocrinology 2018 classification)
3. Patients who usually take over-the-counter self-prescribed drugs or supplements, or who use generic dietary formats, which are not reliable from a scientific point of view
4. Patients being treated with drugs (any type) or nutritional supplements (any type) or who are expected to start treatments during the study period
5. Patients with clinical symptoms or instrumental laboratory parameters such as to suggest the presence of acute or subacute viral/bacterial infection or other inflammations
6. Patients who for ethical reasons need lipid-lowering or antithrombophilic therapy (patients with very high cardiovascular risk, patients with severe and/or unstable atheromasia, in any vascular district, patients with a history of angina, thromboembolism, TIA, heart infarction, stroke, etc)
7. Exclusion of menopausal, premenopausal and postmenopausal women under treatment or with active post-menopausal symptoms
8. Patients with secondary metabolic diseases, endocrinopathies, and systemic diseases of any kind, patients with disabilities/disabilities/functional limitations
9. Patients with severe depressive syndromes and other psychiatric diagnosis
10. Patients who for any reason cannot follow the periodic checks aimed to assess their diet and the adherence to the study
11. Patients with previous bulimia/anorexia, patients with recent (within 3 months) strong drop or weight increase, weight fluctuations greater than the sum of the analytical and pre-analytical variability physiological according to gender, age and weight, or weight trend on multiple measures constantly increasing or decreasing

12. Patients with BMI> 30, as per generic classification of obesity
13. Patients with food intolerances (unless these are attributable to LGI, with the exclusion of other causes, lactose, nickel, celiac disease, etc., intolerances) and vegan/vegetarian patients or for any other reason subject to food restrictions

### Date of first enrolment
15/07/2020

### Date of final enrolment
10/08/2020

## Locations

### Countries of recruitment
Italy

### Study participating centre
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## Sponsor information

### Organisation
Lugo Medica

## Funder(s)

### Funder type
Industry

### Funder Name
Freia Farmaceutici S.r.l.

## Results and Publications

### Individual participant data (IPD) sharing plan

The datasets generated and/or analysed during the current study during this study will be included in the subsequent results publication.

## IPD sharing plan summary

Other

## Study outputs

| Output type | Details | Date created | Date added | Peer reviewed? | Patient-facing? |
|---|---|---|---|---|---|
| Results article | | 04/08/2020 | 19/07/2023 | Yes | No |