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The effect of ALFALIFE™ in reducing low-grade inflammation

Submission date	Recruitment status	[X] Prospectively registered
18/05/2020	No longer recruiting	[ ] Protocol
Registration date	**Overall study status**	[ ] Statistical analysis plan
01/06/2020	Completed	[ ] Results
Last Edited	**Condition category**	[ ] Individual participant data
29/05/2020	Nutritional, Metabolic, Endocrine	[ ] Record updated in last year

Plain English summary of protocol

Background and study aims
Inflammation is an important part of how the body responds to infections and injuries. However, if inflammation occurs where it is not needed or the process goes on for too long, it can cause ill health. Low-grade chronic inflammation is thought to be linked to a number of diseases such as diabetes and heart disease. Some factors in the body that promote inflammation may be linked to diet, lifestyle, pollution exposure, and infections.

This study aims to investigate whether a supplement of a Cannabis sativa seed oil (ALFALIFE™) may reduce the level of low-grade inflammation in addition to a balanced diet.

The study will also look at whether there are preventive effects on cardiovascular and lung diseases, conditions such as diabetes, and low-grade inflammation, as well as inflammatory syndromes following viral infections such as COVID-19 (a condition caused by a coronavirus which can infect the respiratory system. This virus was first identified in 2019 and the outbreak was declared a pandemic by the WHO in March 2020).

The study will also assess if the ALFALIFE™ supplement is safe and tolerable and whether it improved the quality of life of the participants enrolled in the study

Who can participate?
Healthy adults aged 30 to 70 with no known medical conditions who also have an insufficient dietary intake of alpha-linolenic acid

What does the study involve?
Participants will be randomly assigned to one of two groups. One group will receive six capsules of ALFALIFE™ (1 capsule with their breakfast, 2 with their lunch, and 3 with their supper) for 60 days, whilst the second group will receive a placebo, that will look exactly as ALFALIFE™. At the start and end of the study, participants will have medical examinations, blood samples will be taken, and will be asked to complete some questionnaires.

What are the possible benefits and risks of participating?
The subjects participating in the study will be checked on a regular basis to asses their health

conditions, their quality of life as well as their acceptance to the treatment. They will freely receive their supplements and will receive medical advice during and at the end of the study, based also on the results of the medical examination and the response to the treatment.

As long as the intervention is based on supplements with no known side effects, there are no health safety issues related to the trial. However, the health status will be regularly checked and any side effects promptly recorded and resolved.

Where is the study run from?
The study will take place in primary care settings and subjects will be enrolled and followed-up in general practices. The lead centre is the UCCP Catanzaro Lido (Italy).

When is the study starting and how long is it expected to run for?
From June 2020 to October 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-01

Study information

Scientific Title

Interventional study to verify the efficacy of ALFALIFE™ administration in potentiating the effects of diet in patients with low-grade inflammation, and its associate conditions as prediabetes, diabetes, overweight and inflammatory response to viral infections

Acronym
H4H-01

Study objectives
The routine administration of ALFALIFE™ reduces the level of low-grade Inflammation improving conditions associated with chronic inflammation and hyperactivity of the immune response, resulting in an improvement in the inflammatory markers and associated conditions (like insulin resistance and glycaemic control in patients with diabetes, improvement or prophylaxis of the complications of the hyperactivation of the immune response in viral infections such as COVID-19, and improvement in overall quality of life).

Ethics approval required
Old ethics approval format

Ethics approval(s)
Approval 16/04/2020, the Calabria Region Ethics Committee (A.O.U. Mater Domini in Via Tommaso Campanella, 115 Catanzaro 88100 Italy; michelangelo.rossano@regcal.it; +39 0961 712111), ref: 129

Primary study design
Interventional

Study design
Multicentre longitudinal double-blind randomized clinical trial

Study type(s)
Prevention

Health condition(s) or problem(s) studied
Low-grade inflammation

Interventions
Participants will be randomly assigned to receive either ALFALIFE™ supplements or an identical placebo control. Both arms will be asked to take 6 capsules orally each day (1 at breakfast, 2 at lunch, and 3 at supper) for 60 days. Both groups will be required to maintain a balanced hypolipidemic and isocaloric diet. There will be a 15 day washout period following baseline measures being taken before the intervention begins. Immediately after the intervention, these measurements will be repeated at 60 days. Following this, there will be a 30 day washout period after which measurements are taken again at 90 days.

ALFALIFE™ is presented in soft capsules; the placebo contains edible oil and is presented in soft capsules that appear identical to ALFALIFE™. ALFALIFE™ contains cannabis sativa seed oil, extracted with mechanical process of cold pressing from dehulled seeds of Cannabis sativa. This oil has the following characteristics: high bioavailability of essential fatty acids such as α-linolenic acid (ALA) and linoleic acid, and of other polyunsaturated fatty acids such as γ-linolenic acid and stearidonic acid.

To assess the intervention, the investigators will measure the inflammatory and metabolic profiles of the participants, conduct medical examination of the participants, and ask the participants to complete questionnaires.

Blood samples:
The investigators will collect blood from the participants enrolled in the study and will send the samples for the measurement of:
1. Metabolic analytes: cholesterol total, LDLc, HDLc triglycerides, Lp (a), glycemia, glycated hemoglobin, HOMA-IR, HOMA-B, leptin, ghrelin, VCAM, ICAM, endothelin, homocysteine, fibrinogen, uricemia, PCR-HS, cytokines (13), total lymphocytes, ferritin, GOT, GPT, CPK, and creatinine
2. Measures of diabetes and prediabetes: insulin-resistance, and the lipidic profile
3. Measures of the general inflammatory response: level of immunomodulators that are known to be overexpressed in patients with SARS-CoV-2 infection: Lpa, VCAM, ICAM, Leptin, Ghrelin, endothelin, homocysteine, fibrinogen, and Hs-CRP

The samples will be taken at baseline before the 15 days washout period, and again at the end of the trial (60 days) and at the end of the following washout (90 days)

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, 3 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.

Medical examination:
To better assess the general condition and better evaluate the effect of the intervention, investigators will assess the general conditions of the enrolled participants and the level of perception of their health status. Investigators will perform a full medical examination before each blood collection.

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.

At the beginning of the study, the investigator will record the participant's 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 participants 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 participants during the whole trial. Any possible side effects should be reported on the medical records/files.

All participants will receive an ECG with RR interval recording lasting at least 5 minutes at baseline and 30 days.

BIA-ACC dual-frequency bioimpedance device will be used to assess body composition of all participants at baseline and 30 days

Questionnaires:
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.

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:
1. ANNEX I Questionnaire for patient recruitment
2. ANNEX II-IV 7-day recall questionnaires
3. ANNEX V Diet adherence questionnaire
4. ANNEX VI Physical activity monitoring questionnaire: IPAQ
5. ANNEX VII Capsule tolerance / adherence / intake questionnaire
6. ANNEX VIII Sleep Quality Questionnaire: PSQI
7. ANNEX IX Quality of Life Questionnaire: SF12
8. ANNEX X-XIX Isolipid diets while taking soft capsules

Intermediate questionnaires:
During the trial (after washout, during the active phase of the trial and at the end of the final washout), 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)
Improvement in low-grade inflammation measured by cholesterol total, LDLc, HDLc triglycerides, Lp (a), glycemia, glycated hemoglobin, HOMA-IR, HOMA-B, leptin, ghrelin, VCAM, ICAM, endothelin, homocysteine, fibrinogen, uricemia, PCR-HS, cytokines (13), total lymphocytes, ferritin, GOT, GPT, CPK, and creatinine levels from blood samples collected at baseline, 60, and 90 days.

Key secondary outcome(s)
1. Diabetes and prediabetes risk measured by insulin-resistance and lipid profile levels from blood samples collected at baseline, 60, and 90 days
2. General health and medical conditions measured through full medical history and examination at baseline, 60, and 90 days

3. Adverse events and side effects assessed through full medical history and examination, medical records, and recording of all supplements and drugs taken by participants at baseline, 60, and 90 days
4. Heart rate and heart rate variability measured using ECG with RR interval recording at baseline and 30 days
5. Body composition measured using BIA-ACC dual-frequency bioimpedance device at baseline and 30 days
6. Participant quality of life measured using the sleep quality questionnaire (PSQI), and quality of life questionnaire (SF12) at 30 and 60 days
7. Dietary adherence assessed through the diet adherence questionnaire at 30 and 60 days
8. Physical activity assessed through the 7-day recall questionnaire and physical activity monitoring questionnaire (IPAQ) at 30 and 60 days
9. Participant tolerance and compliance assessed through the capsule tolerance/adherence /intake questionnaire at 30 and 60 days

Completion date
31/10/2020

Eligibility

Key inclusion criteria
1. Patients with non-optimal ALA intake (<RDA of 0.5%)
2. Non-smokers
3. Adults, able to independently express informed consent
4. Aged 30 to 70 years
5. Possible or probable Low-grade inflammation as defined below (one or more of the following criteria met):
5.1. Detection of High sensitivity C-reactive protein (HS-CRP) between 3 and 10 mg/L (in asymptomatic patients with no known recent infection)
5.2. Evaluation via Galmes Genetic Score
5.3. Evaluation via INFLA score (Moli-Sani project)
5.4. Clinical, laboratory, and nutritional criteria as per the following conditions (any one, or more, of the criteria 5.4.1-5.4.4, and any one, or more, of the criteria 5.4.5-5.4.7):
5.4.1. Evaluation via Kaluza J 2020 nutritional questionnaire
5.4.2. BIA-ACC criteria (4 points) and PPG stress flow
5.4.3. Presence of other inflammatory markers (specific morphological fibrinogen ESR markers)
5.4.4. Previous diagnosis made by a specialist
5.4.5. Persistent or relapsing clinical symptoms related to chronic inflammation
5.4.6. Presence of frequently associated conditions (metabolic syndrome, overweight)
5.4.7. History of chronic or phased NSAID intake (to be discontinued during the trial)

Participant type(s)
Healthy volunteer

Healthy volunteers allowed
No

Age group
Adult

Sex

All

Key exclusion criteria
1. Taking over-the-counter self-prescribed drugs or supplements
2. Using generic dietary formats, which are not reliable from a scientific point of view
3. Currently being treated with drugs (any type) or nutritional supplements (any type) or who are expected to start treatments during the study period
4. Clinical symptoms or instrumental laboratory parameters such as to suggest the presence of acute or subacute viral/bacterial infection or other inflammation
5. Require 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.)
6. Menopausal, premenopausal and postmenopausal women under treatment or with active post-menopausal symptoms
7. Secondary metabolic diseases, endocrinopathies, and systemic diseases of any kind 8. Disabled or functionally limited patients
9. Severe depressive syndromes and/or 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. Previous bulimia/anorexia
12. Recent (within 3 months) strong decrease or increase in weight
13. Weight fluctuations greater than the sum of the analytical and pre-analytical physiological variability according to gender, age and weight
14. Weight trends on multiple measures constantly increasing or decreasing
15. BMI >30
16. Food restrictions due to food intolerances (unless these are attributable to LGI, with the exclusion of other causes, lactose, nickel, celiac disease, etc., intolerances) and vegan/vegetarian diets or for any other reason

Date of first enrolment
15/07/2020

Date of final enrolment
10/08/2020

Locations

Countries of recruitment
Italy

Study participating centre
UCCP Catanzaro Lido
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88100

Sponsor information

Organisation
Digitcal S.r.l.

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
Data sharing statement to be made available at a later date