Study of the effects of periodic cycles of the fasting-mimicking diet or recommendations for a continuous Mediterranean Longevity diet on body composition and health markers in adolescents: a randomized controlled trial

07/2026

A. Project presentation (protocol for submitting the research project)

A.1 Project title

Study of the effects of periodic cycles of the fasting-mimicking diet or recommendations for a continuous Mediterranean Longevity diet on body composition and health markers in adolescents: a randomized controlled trial

Acronym: FARO-A (Fasting & Alimentary Regimens for Obesity in Adolescents)

A.2 Project leader/Promoter (curriculum, Annex 1)

Dr. Vincenzo Bottino (local leader)

A.3 Other researchers involved, their institutions and contact details

coordinating center Fondazione Valter Longo;

PI Dr. Romina Cervigni; Co-PI Prof. Valter Longo

A.3.1 Collaborators

Mohamad Maghnie, MD, PhD (Clinica Pediatrica, Endocrinologia, IRCCS Istituto Giannina Gaslini, Università degli Studi di Genova; Dipartimento di Neuroscienze, Riabilitazione, Oftalmologia, Genetica e Scienze Materno-Infantili - DINOGMI, Università degli Studi di Genova)

Anna Claudia Romeo, MD, PhD (Department of Paediatrics, UniCamillus - Saint Camillus International University of Health and Medical Sciences, Rome, Italy)

Sonja Chiappetta, PD Dr. med. habil. (Direttore UOC Chirurgia Generale e Laparoscopica, Centro di Eccellenza Chirurgia Bariatrica e Metabolica, Ospedale Evangelico Betania)

Antonio Maria Salzano, PsyD (Clinical Psychologist and Psychotherapist, Head of the Clinical Psychology Service, Evangelical Hospital Betania, Naples, Italy. Seminar Lecturer at Postgraduate Schools of Psychotherapy)

A.4 Research site(s)

Evangelical Hospital Betania, Via Argine, 604, 80147 Naples (NA)

A.5 Is authorization from other bodies (e.g., hospitals, schools, prisons) required to access data or involve participants? If yes, attach a copy of the authorization letter.

No

A.6 Is the consent of a legal representative (parent, guardian, holder of parental responsibility) required? If yes, attach a copy of the consent letter (Appendix N1, N2, optional Appendix P).

Yes

A.7 Does the head of the research have sufficient time, equipment, facilities and personnel to conduct the research? If yes, attach a declaration countersigned by the head of the structure to which he/she belongs (All 2).

Yes

A.8 The head of the research undertakes to provide the Ethics Committee (CEA) with a final report at the conclusion of the project and to communicate any extensions.

Protocol Summary

Protocol number	
Brief title	Mediterranean Longevity Diet and Fasting Mimicking Diet in adolescents with overweight or obesity
English title	Study of the effects of periodic cycles of the fasting-mimicking diet or recommendations for a continuous Mediterranean Longevity diet on body composition and health markers in adolescents: a randomized controlled trial
Sponsor	Regione Campania
Investigation type	Diet
Study type	Interventional
Primary Objective(s)	Change from baseline to end of study in BMI Z score.
Secondary Objectives	Change from baseline to end of study in percentage and absolute value of fat mass, lean body mass, phase angle, total body water, extracellular water, muscle mass, body weight, BMI, circumference, blood pressure, serum lipids, glucose, HbA1C, CV biomarkers, insulin resistance, sleep quality and duration, diet and lifestyle quality, Fatty Liver Index, % Total Body Weight Loss (%TBWL).
Study design	This is a randomized, open-label trial in 330 adolescent subjects between the age of 14 and 18: 110 randomized to the control group (arm 1) that will continue their usual diet and lifestyle; 110 subjects randomized to receive instructions and supervision to follow a Mediterranean Longevity Diet program (MLD) for a 12-month period (arm 2); 110 subjects randomized to the Fasting Mimicking Diet (FMD) arm will be instructed to complete as many cycles as possible of a 5-day FMD recommended once every three months, for a 12-month period (arm 3); Participants belonging to the control arm, after 6 months, will be given an opportunity to follow a 6-month MLD and FMD program.
Rationale	Twenty years of extensive preclinical and clinical studies sponsored by the National Institutes of Health (NIH) and conducted at the Longevity Institute and Diabetes and Obesity Research Institute of the University of Southern California (USC) resulted in the development of two very promising dietary interventions effective in extending not only longevity but also the healthy life span: the Fasting Mimicking Diet

Commentato [CN1]: Or DMD

	(FMD) and the Longevity Diet (LD). However, because the LD was developed for adults and the mediterranean diet is much more validated on adolescents and adults, we have developed dietary recommendations that will implement the safe and nourishing content of the standard Mediterranean Diet with personalized changes featured in the LD (example avoidance of ingredients that may promote inflammation, limitation of high starch and sugary foods, limitation of animal proteins and fats, etc) The objective of the study is to determine the effects of two different dietary interventions, FMD and MLD, on body composition and cardiovascular (CV) biomarkers (body weight, BMI, blood pressure, serum lipid levels and dysglycemia blood measurements).
Study population	Adolescents aged 14-18 years recruited from the general population
Key Inclusion/exclusion criteria	Inclusion Criteria • Adolescents (14-18 years of age) with overweight/obesity defined by WHO BMI criteria: Overweight: ≥+1SD (equivalent to BMI 25 kg/m^2 at 19 years) Obesity: ≥+2SD (equivalent to BMI 30 kg/m^2 at 19 years) Exclusion Criteria • individuals with a family member already included in the study; • Individuals with severe obesity (WHO BMI ≥ 3 SD equivalent to BMI 35kg/m^2 at 19 years); • Individual with BMI > 25 kg/m^2 but with high percentage of muscle mass and less than 25% of fat mass for male subjects and less than 30% of fat mass for female subjects • individuals who are allergic to tree nuts (macadamia, cashew, almond, pecan), soy, oats, sesame, or celery/celeriac; • Pregnant or possibly pregnant females; • No evidence of pubertal delay requiring medical evaluation; • pharmacological treatments known to interfere with the study treatment; all concomitant medications (including, but not limited to, levothyroxine, asthma medications, etc.) will be evaluated by the study's responsible pediatrician; • use of hypoglycemic agents;

Commentato [NF2]: Feedback Sonja: Cari colleghi,

1. va aggiustato la nomenclatura: adolescents with obesity (non obese adolescents) in punto 2 - defined with a BMI...
2. sono 2 criteri: adolescents with obesity and age 14-18 - avete poi scritto

And at least one of the following:
• Obesity (Body mass index equal to or greater than 30 kg/m2).

Se rimane cosi in realta gia si possono arruolare senza una comorbidità

Se lasciate invece solo il giallo come at least one of the following -
penso che si dovrebbe anche aggiungere: PCOS, OSAS and MAFLD (altre 3 importanti comorbidità)

Sicuramente un adolescente con obesità patologica poi ha uno di questi criteri

Per i secondary outcomes: registrerei sicuramente i farmaci perché è fondamentale (chi poi non prende non si deve registrare - ma purtroppo vedo tanti adolescenti che sono già in terapia per il sindrome metabolico)

Un caro saluto,

Sonja

Commentato [cr3]: Valutiamo di escludere pazienti con proven adrenal gland dysfunction, pituitary pathology, congenital metabolic disease, treatment with medication causing IR, Genetic hypercholesterolemia, History of nephro or cholelithiasis, of pancreatitis,
eating disorders or any psychiatric disease, pharmacological treatments known to interfere with the study treatment, history of bariatric surger

	• No history of recurrent syncope or unexplained hypotensive episodes. • Diagnosis of a condition listed among the exclusion criteria
Study treatment	*Fasting Mimicking Diet.* A plant-based diet consists of ingredients which are Generally Regarded As Safe (GRAS). It was initially designed to attain fasting-like effects on the serum levels of IGF-1, IGFBP-1, glucose, and ketone bodies while providing both macro- and micronutrients to minimize the burden of fasting and adverse effects. Day 1 of the FMD supplies ~1000 kcal (~9% protein, 61% fat, and 30% carbohydrate), whereas days 2 to 5 provide ~700 kcal (~8% protein, 52% fat, and 40% carbohydrate) per day. The FMD comprises proprietary formulations of vegetable-based soups, energy bars, energy drinks, chip snacks, tea, and a supplement providing high levels of minerals, vitamins, and essential fatty acids. All items to be consumed per day were individually boxed to allow the subjects to choose when to eat while avoiding accidentally consuming components of the following day. *Mediterranean Longevity Diet (MLD).* MLD is a dietary model derived from the traditional Mediterranean diet, from which it fully retains the qualitative and structural principles, including food variety, consumption frequencies, nutritional recommendations, and the limitation of ultra-processed foods. It is a complete Nutritional Program that includes all essential nutrients for adolescents, including plant and animal proteins, carbohydrates, and fats. It is modified as follows to enhance fat loss, protect lean mass and growth and reduce inflammation (see Valter Longo, et al, Cell 2022). Protein consumption should be 0.9 grams per kilogram of ideal weight (most of the protein coming from plant proteins and fish limiting red meat to one meal a week, and limiting poultry, eggs, dairy to 5 meals a week); vegetable monounsaturated and polyunsaturated fats are predominant; complex Carbohydrates from vegetables, whole grains and legumes; However, the MLD will implement the safe and nourishing content of the standard Mediterranean Diet with personalized changes featured in the LD (example avoidance of ingredients that may promote allergies or intolerances (tomato skin, nuts, soy, etc) limitation of high starch and sugary foods, limitation of animal proteins and fats, etc); + a 12 hour time-restricted eating. All food must be consumed within 12 hours each day.

Commentato [CN4]: Vogliamo mettere una grammatura più alta visto che parliamo di minori?

Efficacy assessments	1. *Primary endpoint*: change from baseline to end of study in BMI Z score. 2. *Secondary endpoints*: change from baseline to end of study in percentage and absolute value of fat mass, lean body mass, phase angle, total body water, extracellular water, muscle mass, body weight, BMI, waist circumference, WHR, change of blood parameters when abnormal (blood pressure, serum lipids, glucose, HbA1C, cardiovascular biomarkers, insulin resistance), sleep quality and duration in people with sleep issues, dietary change assessment, Fatty Liver Index (FLI), % Total Body Weight Loss (%TBWL).
Key safety assessments	AEs and AEs leading to discontinuation
Key words	Mediterranean Longevity Diet, Fasting Mimicking Diet, Overweight, Obesity, BMI, Abdominal circumference, Fat mass

Commentato [CN5]: manterrei BMI z score e inserirei come secondari % Total Body Weight Loss (%TBWL) e % Excess Weight Loss (%EWL). Grazie Sonja per il suggerimento.
(più usati in interventi chirurgia bariatrica ma soprattutto per adulti -che non crescono- ma rischia di penalizzare chi cresce in altezza, non distingue tra perdita di grasso vs perdita di massa magra, non correggere per sesso ed età).

Commentato [CN6]: La ripartizione di perdita di punti BMI può essere analizzata per sottogruppi di BMI

Commentato [CN7]: Total Weight Loss in % (che in tutti i studi chirurgia bariatrica viene usato e da più informazione omogenea)
Risultati più omogenei, valore robusto

Commentato [CN8]: Specifica: per quelli che hanno valori overlimit

1. Background Information and Scientific Rationale

1.1. Background Information

Overweight and obesity pose significant public health challenges worldwide, particularly among children and adolescents. According to the World Health Organization (WHO), in 2022, an estimated 37 million children under the age of 5 years were overweight, and over 390 million children and adolescents aged 5–19 years were affected by overweight (including obesity). The prevalence of overweight (including obesity) among children and adolescents aged 5–19 has risen dramatically from just 8% in 1990 to 20% in 2022. The rise has occurred similarly among both boys and girls: in 2022 19% of girls and 21% of boys were overweight. (https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight).

Commentato [CN9]: Aggiornato a maggio 2025

The prevalence of obesity has risen dramatically worldwide in both adults and children, becoming a global epidemic. Most children with obesity remain overweight through adolescence, and many initially present as metabolically healthy obese (MHO). However, longitudinal studies indicate that approximately 30-50% of these individuals transition over time to metabolically unhealthy obesity (MUO), which carries a higher risk of serious complications. The accumulation of abdominal and visceral fat plays a key role in this shift, promoting insulin resistance, inflammation, and the development of metabolic syndrome (MetS). MUO is strongly associated with cardiovascular diseases, type 2 diabetes, certain cancers, and increased overall mortality. Central obesity, rather than total body fat, is the main driver of these risks, as it accelerates atherosclerosis and can reduce life expectancy, especially if not properly managed through sustained lifestyle and medical interventions (Engin A. Adv Exp Med Biol. 2024).

Commentato [CN10]: Ho aggiornato con la versione del 2024 dello stesso studio. Ha IF basso, valutiamolo insieme

Beyond these cardiometabolic consequences, obesity is also strongly linked to metabolic dysfunctions such as insulin resistance, dyslipidemia, and metabolic dysfunction–associated steatotic liver disease (MASLD, formerly NAFLD), which may progress to metabolic dysfunction–associated steatohepatitis (MASH) and, in advanced stages, to hepatocellular carcinoma (HCC). These effects arise from an imbalance between energy intake and expenditure, leading to excessive adipose tissue (AT) accumulation. AT acts as an endocrine organ, secreting various adipokines—including leptin, adiponectin, and resistin—as well as inflammatory cytokines that modulate insulin sensitivity and promote chronic low-grade inflammation. Dysregulated adipokine secretion alters metabolic homeostasis and contributes to carcinogenesis, influencing tumor initiation, progression, metastasis, and treatment response. Consequently, understanding the molecular mechanisms linking obesity, adipokine activity, and liver pathology is essential for identifying new therapeutic targets. Current research increasingly focuses on these mechanisms and their role in the development of obesity-related metabolic disease and liver cancer (Rajesh Y et al. Int J Mol Sci. 2021).

Commentato [CN11]: Aggiornato al 2021

Despite being located in the cradle of the Mediterranean Diet (MD), considered one of the healthiest dietary strategies, Italy ranks among the top three European countries with the highest percentages of overweight children and adolescents, placing it among the nations with the most concerning rates globally among high-income countries (Hruby A et al. Pharmacoeconomics, 2015), (ISTAT, 2019), (Stival C et al. Nutrients. 2022).

A 2023 report shows that approximately 23.1% of Italian children are classified as overweight, with 8.9% considered obese, which includes 3.6% severely obese (https://www.who.int/europe/news/item/09-12-

2020-italy-over-20-of-children-are-overweight-says-new-report). Misinterpretation of the Mediterranean Diet has contributed to this epidemic, as many individuals consume excessive quantities of starchy foods, leading to high starch intake that converts into sugars in the intestine (Archero F et al. Nutrients, 2018), (Zhang X et al. JAMA Pediatr. 2024).

This issue is particularly pronounced in southern Italy, where childhood obesity rates are among the highest in the country. In Campania, approximately 37.6% of children and adolescents aged 3 to 17 were reported as overweight in the 2020-2021 period, significantly above the national average of 27% (Nardone P et al., "Okkio alla salute" 2019). The percentage of children with excess weight in Campania peaked at 44.2% in 2019, starkly contrasting with regions like Valle d'Aosta, which reported only 14% (Rapporto Osservasalute 2022).

The increasing prevalence of childhood obesity in Campania has severe health implications, as overweight children and adolescents are at a higher risk of developing chronic conditions such as type 2 diabetes, hypertension, and dyslipidemia, which often continue into adulthood. This phenomenon highlights not only individual health concerns but also broader social issues, including sedentary lifestyles and unhealthy eating habits. Given its rich culinary tradition, Campania faces the urgent challenge of promoting healthy eating habits and active lifestyles among its youth to combat this growing epidemic.

Commentato [NF12]: Potete verificare che questi dati siano corretti e aggiornati?

Dietary restriction (DR), implemented as chronic and coordinated reduced intake of all dietary constituents except vitamins and minerals, was first shown more than 90 years ago to extend lifespan in model organisms and in humans. Dietary interventions that avoid unrealistic levels of self-deprivation and pharmacological interventions that recapture the beneficial effects of DR are therefore goals for improving human health. Despite its potential for disease prevention and treatment, prolonged fasting is difficult to implement in human subjects, especially in children and adolescents and may exacerbate pre-existing nutritional deficiencies, making it not feasible and/or safe for the majority of children, the elderly, frail individuals, and even most of the healthy adults. Twenty years of extensive preclinical and clinical studies sponsored by the National Institutes of Health (NIH) and conducted at the Longevity Institute and Diabetes and Obesity Research Institute of the University of Southern California (USC) resulted in the development of two very promising dietary interventions effective in extending not only longevity but also the healthy life span: the Fasting Mimicking Diet® (FMD) and the Longevity Diet (LD).

1.2. Fasting Mimicking Diet

The Fasting Mimicking Diet (FMD) (Appendix A) is a 5-day meal program to be consumed every 1 to 6 months based on an authorized healthcare professional's recommendation. It is designed to promote the body's natural ability to protect itself and potentially activate multi-system regenerative processes as shown in rodent models (Brandhorst S et al. Cell Metab. 2015).

In clinical studies, FMD has been shown to reduce abdominal fat and maintain healthy levels of blood glucose, C-reactive protein (CRP), and insulin-like growth factor 1 (IGF-1) (Wei et al., 2017). Multiple studies have also shown that FMD cycles can cause obesity and diabetes regression leading to both A1C reduction and reduced drug doses/use (Micarelli A et al. Cell Rep Med. 2025), (Sulaj A. et al. J Clin Endocrinol Metab. 2022), (Van den Burg EL et al. Diabetologia. 2024).

1.3. Mediterranean Longevity Diet (MLD).

The Mediterranean Longevity Diet (MLD) is a dietary model derived from the traditional Mediterranean diet, which fully retains its qualitative and structural principles, including food variety, consumption frequency, nutritional recommendations, and the limitation of ultra-processed foods. Although, the MLD is based on the Mediterranean diet it is also influenced by and modified based on epidemiological, clinical and basic research studies focused on healthy longevity (Brandhorst S et al. Cell Metab. 2015).

Like the Mediterranean diet it is characterized by a high intake of foods of plant origin, with particular emphasis on vegetables, legumes, and whole grains, a regular consumption of fish as the primary source of animal protein, with a very limited consumption of red and processed meat, and a limited consumption of dairy products, and eggs, and the predominant use of healthy unsaturated fats. The MLD which will be recommended to teen agers in this clinical trial recommends a protein intake of 0.9 g/Kg/Day with approximately 50% derived from animal sources and 50% from plant based sources and mostly whole grains, nuts, seeds, and legumes, in order to ensure an adequate and individualized protein intake while maintaining a high nutritional quality of protein sources. In cases where the child or adolescent presents a clinical indication for body weight reduction, food quantities will be personalized, with particular attention to the controlled reduction of portions of starchy foods, without exclusion of any food groups. The MLD is planned and monitored to ensure, at all stages, compliance with age-, sex-, and growth stage–specific energy and nutritional requirements, thereby guaranteeing adequate intake of essential macro- and micronutrients and safeguarding normal growth and development.

Mediterranean Longevity Diet program (Appendix G), described by Dr. Longo, is based on the common denominator of traditional foods that people consumed in longevity areas of the world including Okinawa, Southern Italy, Loma Linda (California) in the 1950's and 1960's and modifications based on epidemiological, clinical and laboratory longevity studies. It is a complete Nutritional Program that includes all essential nutrients for children, including proteins, carbohydrates, fats and micronutrients. (Valter Longo, La Longevità inizia da Bambini, Vallardi, 2019).

Adolescents in the trials will be asked to consume all food within a 12-hour window, which represents a much safer and feasible version of the Time Restricted Eating regimen, which normally involves over 14 hours of daily fasting and which is effective in causing weight loss (Longo 2025 Nat Rev Endocrinol, 2025), (Longo VD et al. Cell Metab 2016).

Commentato [CN13]: Controlliamo che tutte le appendici corrispondono

Commentato [CN14]: Appendice MLD?

The MLD will also be personalized to limit sugar and starch consumption to avoid allergens or other ingredients that may promote inflammation in a particular subject.

Also, the MLD proposes limited fruit consumption (1-3 fruit servings per day) versus the high consumption normally recommended by the standard Mediterranean diet.

General guidelines for children and adolescents (Mediterranean Longevity Diet – MLD) of this nutritional regimen can be summarized as follow:

- Protein content should be 0.9 grams per kilogram of ideal weight. Sources of protein include both vegetable proteins (from legumes and nuts) and animal proteins (fish 2-3 times a week, limit red meat, white meat, and eggs to once a week, ideally organic).
- Vegetable monounsaturated and polyunsaturated fats are predominant and only very few amounts of saturated and trans-fats are allowed.
- Include complex carbohydrates from vegetables, whole grains, and legumes to create meals with a relatively low glycemic index. Low consumption of high-starch foods such as pasta, bread, pizza, potatoes, and rice, as well as fruit juices, snacks, and sweetened drinks; minimal consumption is recommended rather than complete elimination. Limited fruit consumption is also recommended (1-3 servings of fruit per day). Additionally, increase food volume by substituting high-starch foods with fiber-rich vegetables and legumes to promote healthier eating habits and effectively manage body weight. Fiber Caution: consume whole wheat and high-fiber foods cautiously; consult a pediatric gastroenterologist if there are signs of discomfort.
- Addition of vitamins, minerals and Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA) (Omega3) fats, when necessary.
- 30 grams per day of either walnuts, hazelnuts, or almonds.
- Among the foods listed in this protocol we recommend those selected from the healthy traditional food consumed by their grandparents/ancestors. Prioritize local, seasonal, and organic ingredients.
- Time-restricted eating. All food must be consumed within 12 hours each day.
- Meal Frequency: limit meals to 3 and healthy snacks based on the recommendations above to once/twice a day.
- Flexibility with dietary rules to accommodate individual preferences, with the guidance of a nutritionist.

Commentato [CN15]: Stessa cosa di sopra

Commentato [CN16]: Aggiungiamo “quando necessario?”

2. Study Objectives/Endpoints

2.1. Objectives

The objective of the study is to determine the effects of two different dietary interventions: FMD and Mediterranean Longevity Diet (MLD), on BMI Z score.

Secondary objectives include the assessment of the effects of two different dietary interventions on: percentage and absolute value of fat mass, lean body mass, phase angle, total body water, extracellular water, muscle mass, body weight, BMI, circumference, blood pressure, serum lipids, glucose, HbA1C, CV biomarkers, insulin resistance, proportion of participants who receive antihypertensive/ glycemic medications, sleep quality and duration in people with sleep issues, dietary change assessment.

2.2. Endpoints

2.2.1. *Primary endpoint*: change from baseline to end of study in BMI Z score.

2.2.2. *Secondary endpoints*: Change from baseline to end of study in percentage and absolute value of fat mass, lean body mass, phase angle, total body water, extracellular water, muscle mass, body weight, BMI, waist circumference, WHR, change of blood parameters when abnormal (blood pressure, serum lipids, glucose, HbA1C, cardiovascular biomarkers, insulin resistance), sleep quality and duration, diet and lifestyle quality, Fatty Liver Index (FLI), % Total Body Weight Loss (%TBWL).

Commentato [CN17]: manterrei BMI z score e inserirei come secondari % Total Body Weight Loss (%TBWL) e % Excess Weight Loss (%EWL). Grazie Sonja per il suggerimento.
(più usati in interventi chirurgia bariatrica ma soprattutto per adulti -che non crescono- ma rischia di penalizzare chi cresce in altezza, non distingue tra perdita di grasso vs perdita di massa magra, non correggere per sesso ed età).

Commentato [CN18]: La ripartizione di perdita di punti BMI può essere analizzata per sottogruppi di BMI

Commentato [CN19]: Total Weight Loss in % (che in tutti i studi chirurgia bariatrica viene usato e da più informazione omogenea)
Risultati più omogenei, valore robusto

Commentato [CN20]: Body circumferences

Commentato [CN21]: Specifica: per quelli che hanno valori overlimit

3. Study Design

This is a randomized, open-label trial in 330 adolescent subjects: 110 subjects randomized to control group who will continue following their usual diet for 6 months (arm 1), after which they will have an opportunity (optional) follow either the recommendations of arms 2 or 3, 110 randomized to recommendations and monitoring for MLD program for a 12-month period (arm 2), and 110 randomized to a Fasting Mimicking Diet (FMD) plan with a 5-day meal program once every three months for a maximum of 5 cycles (25 days total) in a 12-month period (arm 3).Figure 1). The enrolment period will last 24-30 months and the patients will be followed for 12 months from study entry. This trial uses a control group to provide robust evidence of the effects of nutritional interventions on the primary and secondary endpoints.

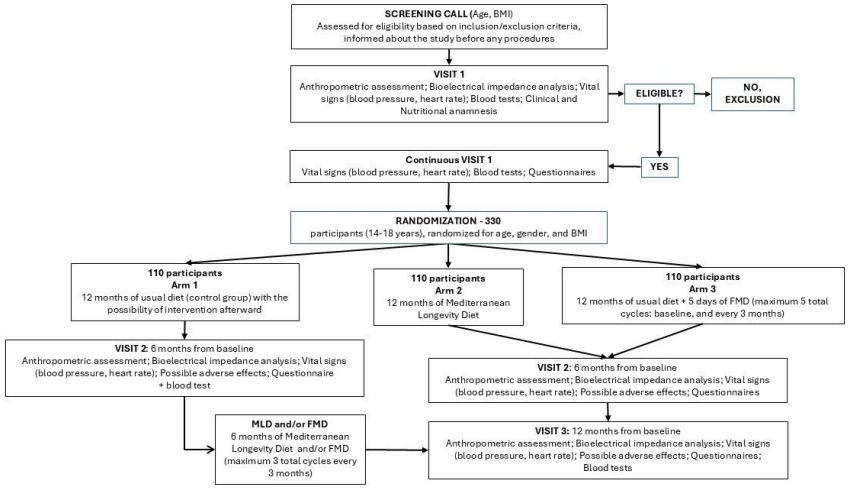


Figure 1. Schematic representation of study design.

3.1 Selection and withdrawal of subjects

3.1.1 Inclusion Criteria

- Subjects of 14-18 years of age;
- with overweight/obesity defined by WHO BMI criteria: Overweight: ≥+1SD (equivalent to BMI 25 kg/m^2 at 19 years); Obesity: ≥+2SD (equivalent to BMI 30 kg/m^2 at 19 years)

3.1.2 Exclusion Criteria

- individuals with a family member already included in the study;
- Individuals with obesity ≥ 3 SD (equivalent to BMI 35kg/m^2 at 19 years);
- Individual with BMI > 25 kg/m^2 but with normal waist circumference or less than 20% of fat mass for male subjects and less than 25% of fat mass for female subjects
- individuals who are allergic to tree nuts (macadamia, cashew, almond, pecan), soy, oats, sesame, or celery/celeriac;
- Pregnant or possibly pregnant females;
- No evidence of pubertal delay requiring medical evaluation;
- pharmacological treatments known to interfere with the study treatment; all concomitant medications (including, but not limited to, levothyroxine, asthma medications, etc.) will be evaluated by the study's responsible pediatrician;
- use of hypoglycemic agents;
- No history of recurrent syncope or unexplained hypotensive episodes.

3.1.3 Medical History and Concurrent Diseases

- A history of allergies or intolerance to study products. Detailed descriptions of study product are included in Sections 4.1 and 4.2, appended to the Study Informed Consent;
- Clinically significant vital sign abnormalities (systolic blood pressure <90 mmHg or >200 mmHg, diastolic blood pressure <50 mmHg or >105 mmHg or resting heart rate of <50 or >100 bpm) at screening visit;
- any documented cancer diagnosis within the past 5 years;
- chronic steroid use (longer than 45 consecutive days);
- Individuals with severe hypertension (systolic greater than 200 mmHg and or diastolic greater than 105 mmHg;
- proven adrenal gland dysfunction,
- pituitary pathology,
- congenital metabolic disease,
- treatment with medication causing IR,
- Genetic hypercholesterolemia,
- History of nephropathy or cholelithiasis, of pancreatitis,
- history of bariatric surgery
- Known infection with HIV, TB or Hepatitis B or Hepatitis C.
- A current diagnosis or personal history of:
    - any cardiovascular disease including myocardial infarction, angina, cardiovascular surgery (within 5 years), congestive heart failure, cardiac arrhythmias or conduction abnormalities, cerebrovascular accident, transient ischemic attack (TIA), or peripheral vascular disease, deep vein thrombosis or pulmonary embolus. Diabetes mellitus requiring inhaled or injected insulin.

- Any autoimmune disease such as inflammatory bowel disease (including Crohn's disease and/or ulcerative colitis), multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, polymyositis, scleroderma and/or thyroiditis.
- Any significant liver or kidney disease such as cirrhosis or non-alcoholic fatty liver disease, glomerulonephritis, and/or ongoing dialysis treatment.
- Any malignancy (with the exception of adequately treated malignancies with no known recurrence for >2 years).

- Any serious mental illness including a history of attempted suicide.
- Eating disorders or any psychiatric disease.
- Any medical condition that in the opinion of the primary care doctor or a specialist would preclude safe participation in this study or interfere with compliance.

3.1.4 Substance Use

- Use of drugs of abuse (such as marijuana, cocaine, phencyclidine [PCP] and methamphetamine) 15 days prior to Day 1 and for the duration of the study.
- History of regular intake of alcoholic drinks

3.1.5 Technical reasons

Any condition in which bioelectrical impedance testing would be impossible or uninterpretable (e.g. prostheses in extremities on both sides, limb amputation, implanted pacemaker, inability to lie still or supine, or skin defects on preferred electrode placement sites.

3.1.6 Other Exclusion Criteria

- Inability to comply with study and/or follow-up visits/blood tests.
- Any concurrent condition (including clinically significant abnormalities in medical history, physical examination or laboratory evaluations) that, in the opinion of the PI, would preclude safe participation in this study or interfere with compliance.
- Any sound medical, psychiatric and/or social reason which, in the opinion of the PI, would preclude safe participation in this study or interfere with compliance.
- Abnormal laboratory findings including: abnormal blood counts (hematocrit < 33% or > 47%; WBC < 3.0 or > 12.0 x103/mm3; platelets < 140 or > 500 x 109/L); abnormal kidney function test (creatinine > 2.5 mg/dL) or liver function test(s) (AST, ALT, alkaline phosphatase) > 1.5X the upper limit of normal; serum calcium > 11 mg/dL); serum K < 3.5 mEq/L; Na < 134 or > 148 mmolL-1

3.1.7 Women of Childbearing Potential

Contraception: the effects of the study products on the developing human fetus have not been studied extensively. For this reason, if a woman becomes pregnant or suspects she is pregnant while participating in this study, she should inform study staff and her primary care physician immediately.

Pregnancy: because there is an unknown but potential risk for adverse events in pregnant women during treatment with the study products, pregnant women are not eligible for study participation.

Breast-feeding: Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the study products, breastfeeding mothers are not eligible for study participation.

3.1.8 Co-enrollment Guidelines

Co-enrollment in other trials is restricted other than for observational studies. Study staff should be notified of co-enrollment as it may require the approval of the investigator.

3.2 Recruitment

Participants will be identified and recruited by doctors and health professionals within the hospital and schools in 3 years. If potentially eligible and interested, participants' parents will then be than contacted by the study staff directly or by telephone call to determine their initial eligibility (screening phone call visit) after signing the privacy and consent form (Appendix N1, Appendix O1). Final confirmation of eligibility and subsequent consenting will be carried out at the visit 1(Appendix N2, Appendix O2). The visits will take place at the hospital or authorized healthcare facilities located throughout the Campania region.

Commentato [CN22]: Quindi vogliamo levare le scuole?

Commentato [NF23]: Aggiustare secondo quanto riportato nel bando. Il reclutamento sarà nelle scuole?

Commentato [CN24R23]: Abbiamo detto sia scuole che ospedale

Commentato [CN25]: Speificare reclutamento 3 anni

3.3 Scheduled clinical visits

The assessment schedule (Table 1) lists all the assessments and when they are to be performed. Subjects will meet with study staff. During the clinical visits, staff will review calendars, assess for signs and symptoms of adverse events, review compliance with the study and answer any questions from the subject. Eligible participants will receive a baseline visit (t_0) and two follow-up visits (after 6 and 12 months from the baseline). Participants in the control arm (arm 1) who decide to follow a 6-month MLD program or 3 FMD cycles after the second visit at 6 months, will receive the MLD nutritional protocol or the FMD kits. Participants in arm 3 (FMD) will also be contacted to assess signs and symptoms of adverse events and compliance with FMD (Appendix B). Throughout the study period, participants will receive monthly monitoring by a nutritionist to evaluate their clinical course and adherence to the nutritional intervention but also to receive help related to the dietary changes to be followed. The nutritionist will attempt to speak with both students and parents each month to help implement the dietary interventions.

Table 1 reports the timeline of the clinical visits and the scheduled activities. All data obtained from these assessments must be supported in the participant's source documentation.

Table 1. Scheduling of the activities.

	Screening Call	Baseline Visit 1 (T_0)	Visit 2 (T_1)	Visit 3(T_2)
Informed Consent	√			
Review Eligibility Criteria	√	√		
Start of intervention				
Physical examination[1]		√	√	√
Vital Signs[2]		√	√	√
BIA		√	√	√
Hematological analyses[3]		√	√[4]	√
AE and SAE collection			√	√
Primary and secondary endpoints assessment		√	√	√
Medications	√	√	√	√

[1] height, weight (undressed with underwear only), waist, hip and other body circumferences. [2] Blood pressure, pulse. [3] complete blood count (CBC), glucose (FBG), insulin, HOMA-IR, Glycated hemoglobin (HbA1c), lipid profile (total cholesterol, HDL, LDL and

Commentato [CN26]: Da valutare se fare sleep quality anche a metà del percorso o solo inziio e fine

Commentato [VD27]: Solo vita e fianchi come circonferenze corporee?

triglycerides), ferritina, ALT (GTP), AST (GOT), GGT, C-reactive protein (CRP), uric acid, creatinine, albumin, alkaline phosphatase, TSH, FT4, FT3, Citochine (TNF-α, IL-6, IL-1ß), Vitamin D. [4]*blood test at 6 months only for arm 1.*

Subjects in the control group (arm 1) will be asked to make no changes in dietary programs, exercise programs and nutritional supplements during the first 6 months of the study and before being offered to begin MLD or FMD. The expectation is that subjects will maintain their current weight and not engage in behaviours intended to produce weight loss.

3.3.1 Screening call

The purpose of the screening call is to determine volunteer eligibility for study participation (Appendix H). Subjects will be screened for eligibility for the study, ascertaining inclusion criteria and only if they don't meet some of its permanent exclusion criteria (e.g. morbidities, medication use, known allergies, etc.). Once a participant meets all inclusion/exclusion criteria, the study staff will offer them the opportunity to learn more about the research opportunity before any procedures are performed. Screening will include the investigation of height, weight, review of medical history and current medications (Appendix H).

3.3.2 Baseline, visit 1 (T_0)

During the visit, staff will administer the Clinical Anamnesis Questionnaire (Appendix I) for participants and parents, the Lifestyle and Dietary Habits Questionnaire (Appendix L) including the IPAQ and the SIO questionnaire for obesity-related disabilities (TSD-OC) for participants and parents and the Nutritional Questionnaire (Appendix M). Baseline visit will also include body composition assessment using Bioelectrical Impedance Analysis (BIA), measurement of height, weight, and vital signs (Appendix Q), and assessment of sleep quality and disturbances with the Pittsburgh Sleep Quality Index (Appendix C) and the risk associated with sleep apnea with the Berlin Questionnaire (Appendix D), and collection of fasting blood.

Blood testing will include: complete blood count (CBC), glucose (FBG), insulin, HOMA-IR, Glycated haemoglobin (HbA1c), lipid profile (total cholesterol, HDL, LDL and triglycerides), ferritin, ALT (GTP), AST (GOT), GGT, C-reactive protein (CRP), uric acid, creatinine, albumin, alkaline phosphatase, TSH, FT4, FT3, Cytochine (TNF-α, IL-6, IL-1β), Vitamin D. *Optionally* blood test will include: IGF-1, ghrelin, adipokines (leptin, adiponectin), GLP-1, methionine, FGF21, circulating stem cells.

Subjects in the MLD arm will be asked to limit the food consumption period to 12 hours a day and fast for 12 hours/day (refraining from consumption of food and beverages though unlimited consumption of water is allowed and encouraged).

Optional: During this visit, ring sizes of participants will be obtained for all fingers, from which a ratio of appropriate ring sizes for this population group will be established. 10 subjects for each study group reporting a sleep problem on the basis of the score obtained at the Pittsburgh Sleep Quality Index questionnaire (Appendix C) will also receive an Oura Ring device for the continuous monitoring of sleep state and wakefulness for a 2-week period (30 participants in total). Study staff will ensure the participants wear the rings properly and that the ring and Oura mobile app will be connected the following 2 weeks to upload the data. Thirty second epoch by epoch data will be then obtained from Oura's cloud.

Optional: Study participants will undergo a FibroScan examination (transient liver elastography), a non-invasive method for the assessment of liver stiffness and hepatic steatosis. The examination will be

Commentato [NF28]: Decidere se vogliamo inserire fibroscan (opzionale) al baseline e alla fine?

Commentato [cr29]: Ferritin as an Inflammatory Marker in Pediatric Metabolic Syndrome: Links to Obesity and Liver Ultrasound Alterations
by Mihaela-Andreea Podeanu
Int. J. Mol. Sci. 2025,

Commentato [cr30]: POTREMMO ANCHE SOLO EFFETTUARE TSH REFLEX

Commentato [CN31]: Chiedi conferma

Commentato [cr32]: A tutti gli altri partecipanti potremmo calcolare il FATTY LIVER INDEX a d inzio e fine studio
Si calcola qui usando BMI, CIRC VITA, TRIGLICERIDI , GGT
https://www.mdcalc.com/calc/10001/fatty-liver-index#evidence

Commentato [CN33R32]: Da aggiungere outcome sec

performed by qualified and appropriately trained medical staff, in accordance with the facility's standard operating procedures.

Pubertal Assessment (Tanner Staging) Optional: An optional pediatric assessment of pubertal status will be performed in participants, subject to prior informed consent, by qualified medical personnel in accordance with the site's standard operating procedures.
Assessment of pubertal stage (Tanner classification) may be performed optionally at baseline and/or follow-up visits by qualified medical personnel.
Purpose:

- To ensure that participants are not experiencing pubertal delay
- To assist interpretation of hormonal and body composition outcomes
- To support safety monitoring in cases of excessive weight loss or menstrual irregularity

Pubertal assessment will:

- Be performed only after specific informed consent
- Be conducted respecting privacy and participant comfort
- Not be used as an exclusion criterion unless clinically indicated

If pubertal delay or regression is suspected, the participant will be referred for endocrine evaluation and the dietary intervention may be suspended.

An exploratory optional, three meetings with the psychology team for a portion of the enrolled adolescents will be conducted at each study visit (Visit 1, Visit 2, and Visit 3). These sessions will consist of a structured interview aimed at improving adherence to the clinical study and providing psychological support throughout the 12-month study period, with the objective of improving the participant's relationship with food. Two questionnaires, the Binge Eating Questionnaire (Appendix E) and the Eating Attitudes Test (EAT-26; Appendix F), will be administered. The interview and questionnaires will be repeated at each follow-up visit (Visit 2 and Visit 3). Additionally, at 3 and 6 months from study initiation, video conference interviews will be conducted with the adolescent participant and a parent or legal guardian, subject to the availability and consent of both parties. Participants who score above clinical cut-offs on EAT-26 or Binge Eating assessments at baseline will not be eligible. We will attempt to carry out these meetings for at least 20 subjects per group (at least 60 total).

Participants will then be randomised into either one of the intervention arms (MLD or FMD) or into the control arm using a web-based (RedCap) randomisation system that will be controlled by the researchers of the Valter Longo Foundation in Milan (see section 8)

After the baseline visit, subjects in arm 3 (FMD) will be given one box of FMD together with a ketone test strips to self-monitor urinary ketone bodies during the FMD cycles. Subjects in arm 2 will receive informative material and instructions during the Mediterranean Longevity Diet. Detailed information on the respective diet plans will be given to subjects during the visit.

3.3.3 Visit 2 (after six months or at least 7-10 days after the third FMD cycle)

After 3 and 6 months from the randomization, subjects in arm 3 will be contacted to receive the FMD boxes for the second and third FMD cycles, respectively, together with the ketone test strips to self-monitor urinary ketone bodies during the FMD cycles.

Commentato [CN34]: Chiedi conferma

Commentato [CN35]: Chiedi conferma

Commentato [CN36]: Chiedi conferma

Commentato [CN37]: Ketostix: li facciamo?

Commentato [NF38R37]: Io li includerei per essere sicuri della compliance

Commentato [MF39]: are these ketone body data collected or just informative during visit call?

if collected:
where? diary?
Will be them instructed on frequency of testing, time of day (e.g., morning fasting sample)?

Commentato [CN40R39]: Vengono consegnati ai partecipanti insieme al kit FMD, 5 per partecipante da utilizzare con le prime urine del mattino dal giorno 1 a giorno 5. I partecipanti provvederanno a mandare la foto (dopo aver atteso la colorazione per 15-30 secondi) al nutrizionista e poi potranno buttare il campione.

Visit 2 includes body composition assessment using Bioelectrical Impedance Analysis (BIA), measurement of height, weight, circumference and vital signs, questionnaires such as the Clinical Anamnesis Questionnaire (Appendix I), Nutritional Questionnaire (Appendix M), Lifestyle and Dietary Habits Questionnaire (Appendix L) and, only for arm 3, FMD's questionnaire (Appendix B).

If performed, Visit 2 includes meetings with the psychology team with questionnaire: Binge Eating Questionnaire (Appendix E) and EAT-26 (Appendix F).

Visit 2 includes the final blood test only for arm 1. At this point, subjects belonging to the control arm will be given an opportunity to follow a MLD or FMD program for a 6-month period (optional).

3.3.4 Visit 3 (after twelve months or 7-10 days after the fifth FMD cycle)

Subjects will meet with the study staff. During the clinical visit, staff will assess for signs and symptoms of adverse events, review compliance with the diet programs and questionnaires such as the Clinical Anamnesis Questionnaire (Appendix I), Nutritional Questionnaire (Appendix M), Lifestyle and Dietary Habits Questionnaire (Appendix L) and, only for arm 3, FMD's questionnaire (Appendix B).

If performed, Visit 3 includes meetings with the psychology team with questionnaire: Binge Eating Questionnaire (Appendix E) and EAT-26 (Appendix F).

Visit 3 will also include body composition assessment using Bioelectrical Impedance Analysis (BIA), measurement of height, weight, circumference and vital signs, medical history and current medications and a collection of fasting blood for both hematological as previously described for the baseline visit (see Table 1).

Only subjects who received the Oura Ring device at the baseline visit will also receive an Oura Ring device for the continuous monitoring of sleep state and wakefulness for a 2-week period as previously mentioned. After this period, study staff will assess sleep quality and disturbances with the Pittsburgh Sleep Quality Index (Appendix C), the risk associated with sleep apnea with the Berlin Questionnaire (Appendix D) and will answer any questions from the subject.

If performed, both the FibroScan assessment and the pediatric visit will be repeated at Visit 3.

All subjects from control Arm 1 will also undergo Visit 3 and blood tests, whether they continued the control diet or switched to MLD or FMD.

3.4 Oura Rings (optionally)

The study will also include a continuous monitoring of sleep state and wakefulness by providing Oura Ring devices: the rings are already documented in the literature for their potential in the measurement of sleep parameters with results comparable to polysomnography, the gold standard for the assessment of sleep disturbances, but making it also available on a larger scale and for a longer period of time (The Sleep of the Ring: Comparison of the ŌURA Sleep Tracker Against Polysomnography; Behav Sleep Med. Mar-Apr 2019;17(2):124-136.). The ring is a "sleep tracker" that comes in different sizes (US standard ring size 6–13). It is waterproof, weights approximately 15 g and has a battery life of approximately 3 days. It automatically connects via Bluetooth and transfers data to a mobile platform in the dedicated App. The ring continuously collects data and processes them through an algorithm, to determine the stages of sleep (wakefulness, "light", "deep" and REM sleep): each night it monitors the latency sleep onset, time spent in "deep sleep", time spent in "light sleep", total time spent asleep and periods of wakefulness after starting sleep. It also adds information regarding sleep patterns, an analysis of heart rate and its variations, physical activity and body temperature. Oura ring fulfils the essential requirements of the applicable CE marking directives and regulations.

The design plans to randomly allocate 10 rings among 30 subjects in the 3 study groups that will result with sleep problems at the screening visit on the basis of the score obtained at the Pittsburgh Sleep Quality Index questionnaire (Appendix C). Continuous monitoring of each parameter and sleep marker will last for a 2-week period. At each check point, questionnaires will also be distributed to assess sleep quality

and disturbances using the Pittsburgh Sleep Quality Index (Appendix C) and to assess the risk associated with sleep apnea using the Berlin Questionnaire (Appendix D).

3.5 Fiber Scan (optionally)

Optionally, the study will include a FibroScan examination (transient liver elastography), a non-invasive method for the assessment of liver stiffness and hepatic steatosis. The examination will be performed by qualified and appropriately trained medical staff, in accordance with the facility's standard operating procedures.

The Fatty Liver Index (FLI) will be used as an assessment tool for hepatic conditions. (Bedogni, G., Bellentani, S., Miglioli, L. *et al.* The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 6, 33 (2006). https://doi.org/10.1186/1471-230X-6-33)

3.6 Prohibited Medications and Procedures

No concomitant prescription medications, over-the-counter medications, medical foods, and nutritional supplements are to be started or doses changed during the study unless they are prescribed by the PI (or the subject's primary care giver) for treatment of a specific clinical event. The pediatrician (Dr. Claudia Romeo) will be available to advise the PI and others on this matter.

3.7 Rescue Medications during FMD

Paracetamol 500 mg – 1000 mg may be administered for the treatment of mild headache or myalgia, if deemed necessary and in accordance with the indications of the study pediatrician.

3.8 Subject Compensation

Subjects will not be compensated for participation in this trial.

4 Study Product:

Formulation, Packaging, Labelling, Preparation, Administration, and Dosage of Study Products

4.1 Arm 1 (control group)

Participants in arm 1 will undergo the same testing measures as the intervention groups and will be recommended to continue their usual diet. Participants belonging to this group will be given an opportunity to follow a 6-month MLD and/or FMD program at month 6.

4.2 Arm 2 (MLD plans)

General guidelines for adolescents (Mediterranean Longevity Diet – MLD) of this nutritional regimen can be resumed as follow:

- Protein content should be 0.9 grams per kilogram of ideal weight. Sources of protein include both vegetable proteins (from legumes and nuts) and animal proteins (fish 2-3 times a week, limit red meat, white meat, and eggs to once a week, ideally organic).
- Vegetable monounsaturated and polyunsaturated fats are predominant and only very few amounts of saturated and trans-fats are allowed.
- Include complex carbohydrates from vegetables, whole grains, and legumes to create meals with a relatively low glycemic index. Low consumption of high-starch foods such as pasta, bread, pizza, potatoes, and rice, as well as fruit juices, snacks, and sweetened drinks; minimal consumption is recommended rather than complete elimination. Limited fruit consumption is also recommended (1-3 servings of fruit per day). Additionally, increase food volume by substituting high-starch foods with fiber-rich vegetables and legumes to promote healthier eating habits and effectively manage body weight. Fiber Caution: consume whole wheat and high-fiber foods cautiously; consult a pediatric gastroenterologist if there are signs of discomfort.
- Addition of vitamins, minerals and Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA) (Omega3) fats, when necessary.
- 30 grams per day of either walnuts, hazelnuts, or almonds.
- Among the foods listed in this protocol we recommend those selected from the healthy traditional food consumed by their grandparents/ancestors. Prioritize local, seasonal, and organic ingredients.
- Time-restricted eating. All food must be consumed within 12 hours each day.
- Meal Frequency: limit meals to 3 and healthy snacks based on the recommendations above to once/twice a day.
- Flexibility with dietary rules to accommodate individual preferences, with the guidance of a nutritionist.

Commentato [CN41]: Stessa cosa di sopra

Commentato [CN42]: Aggiungiamo “quando necessario?”

4.3 Arm 3 (FMD plan)

The subjects will be instructed to follow their usual diet plan, except for 5 days when they eat the foods in the FMD box. The FMD diet consist of *nut* bars, dehydrated soups, tea, olives, kale crackers, electrolyte beverages, and a chocolate crisp bar.

- Nut bars: Almond, macadamia nuts, pecans, vegetable inulin fibre (chicory root fiber), honey, coconut flour, flaxseed, natural flavour, sea salt, rosemary extract;
- Almond and Kale crackers: almonds, sesame seeds, tapioca flour, chia seeds, golden flax seeds, sunflower seeds oil, kale, sea salt, coconut sugar, coconut vinegar, onion powder, chili pepper, cumin seeds, black pepper, antioxidant: extract rich in tocopherol, garlic, origan, acidifier: citric acid;
- Electrolyte beverages containing water, natural vegetable glycerin and natural flavor;
- Olives;
- Spearmint tea, spearmint lemon tea, hibiscus tea.
- There are 5 different soups, which contain various combinations of rice flour, rice starch, potato flakes, tomato concentrate, sweet red pepper, basil, onion, leek, extra virgin olive oil, parsley, peas, yeast extract, savoy cabbage, carrot, garlic, spinach, celery, turmeric, black and white beans, champignon mushroom powder: minestrone, mushroom, tomato, vegetable, black beans soup;
- Chocolate crisp bar: inulin, almond butter, brown rice, cocoa powder, almonds, chocolate chips (brown sugar, cocoa mass, cocoa butter), rolled oats, brown rice syrup, flaxseed oil, rice dextrin, grape juice, salt.

In order to evaluate adherence to the FMD and its metabolic effects, patients in arm 1 will receive ketone test strips together with each FMD kit to self-monitor urinary ketone bodies.

4.3.1 Study Product Storage and Stability

Subjects in arm 3 will be given one box of FMD at the end of the baseline visit and the other 2 boxes before the start of the other FMD cycles during the study. Subjects in arm 2 (MLD) will be given a nutritional protocol of the Mediterranean Longevity Diet at the baseline visit. The study products will be handled and dispensed to subjects at ambient temperature at the study site according to good clinical practices.

4.3.2 *Study Product Accountability Procedures*

It is the responsibility of the PI to ensure that a current record of study product disposition is maintained at the Study Site, and that records and logs include:

- amount of study product received and placed in the storage area;
- dates and initials of the individual responsible for investigational product inventory entry/movement;
- amount dispensed to and returned by each subject, including unique subject identifiers;
- amount transferred to another area for dispensing or storage;
- non-study disposition (e.g. lost, broken, wasted);
- amount destroyed at the site (if applicable).

The unused study products from this study will be disposed of according to good clinical practices.

5 Potential Risks and Benefits

5.1 Potential Risks

There is, however, a possibility that a subject could be sensitive to food ingredients in one or more of the food formulas and have an allergic or intolerant reaction. Potentially serious reactions may include face and throat swelling, trouble breathing, anaphylaxis (shock), convulsions, and death. Such reactions are possible if a subject has an intolerance or allergy (known or unknown) to the ingredients in the FMD. TheMediterranean Longevity diet will be based on a selection of the traditional food of Southern Italy and thus the possibility of allergic reactions is very low.

Because FMD includes temporary caloric restriction, the following additional precautions apply:

- Participants will receive a safety check-in call on Day 2–3 during the first FMD cycle.
- If moderate symptoms occur (persistent dizziness, weakness, nausea), the FMD cycle will be discontinued.
- Participants will transition to a balanced, normocaloric diet if discontinuation is required.
- Urinary ketone testing is used for metabolic monitoring only and is not a therapeutic target.
- No participant will be encouraged to achieve a specific ketone level.

5.2 Potential Benefits

The FMD is designed to achieve the beneficial effects of fasting while providing micronutrient nourishment (vitamins, minerals and others) of which the body is deprived during fasting. It minimizes the psychological burden of pure fasting. The Mediterranean Longevity diet has the advantage of being easy to follow as a lifestyle element, with appropriate foods available at multiple venues. The subjects will receive extensive medical testing with results made available to the subjects after completion of their participation for their ongoing care.

5.3 Assessment of Subject Compliance with Study Product

Compliance will be measured using both a subject calendar provided by the investigator and direct verification of consumption of the FMD and adherence to the Mediterranean Longevity diet. Subjects will not be asked to return FMD boxes.

6 Description of Specific Testing Modalities

6.1 Hematological analyses

Blood testing will include: complete blood count (CBC), glucose (FBG), insulin, HOMA-IR, Glycated haemoglobin (HbA1c), lipid profile (total cholesterol, HDL, LDL and triglycerides), ferritin, ALT (GTP), AST (GOT), GGT, C-reactive protein (CRP), uric acid, creatinine, albumin, alkaline phosphatase, TSH, FT4, FT3, Cytochine (TNF-α, IL-6, IL-1β), Vitamin D.

Optionally the blood test will include: IGF-1, ghrelin, adipokines (leptin, adiponectin), GLP-1, methionine, FGF21, and circulating stem cells.

A total of 25 mL of blood will be collected at each scheduled blood draw for laboratory analyses. Therefore, participants will undergo two blood draws, corresponding to a total blood volume of 50 mL over the study period: over 12 months for Arms 2 and 3, and over 6 months for Arm 1.

Optionally, on a voluntary basis, participants assigned to the fasting-mimicking diet (FMD) protocol may consent to an additional blood sampling performed on day 5 of the FMD cycle and to a subsequent sampling at least 7–10 days later. In this case, the total blood volume collected will not exceed 25 mL overall.

The total volume of blood collected is within limits considered safe and acceptable for pediatric and adolescent populations.

6.1.1 Range for Haematological analyses

triglycerides ≥ 150 mg/dL, low HDL-C as HDL-C < 40 mg/dL for children aged 6–15 years and < 40 mg/dL for boys and < 50 mg/dL for girls aged 16 years or older, high BP as SBP/DBP ≥ 130/85 mmHg for those aged 10 years or older, high FBG as FBG ≥ 100 mg/dL, high LDL-C (≥ 130mg/dL), HOMA-IR cut off 2.5.

6.2 Blood Pressure Measurements

Subjects will not consume any caffeine or alcohol or use tobacco products within 6 hours of the blood pressure measurement. Subjects' blood pressure will be measured with a mercury sphygmomanometer at each visit. Subject's blood pressure will be measured in the left arm in the sitting position three times at 2-minute intervals, following the American Heart Association (AHA) guidelines for blood pressure measurement. Heart rate will be measured with each blood pressure measurement.

6.3 Body Impedance Analysis

The BIA technique is based on the fact that lean tissues have a high water and electrolyte content thereby providing a good electrical pathway. Fat contains a lower percentage of body water and thus is a poor conductor of the electrical signal. Utilizing a low energy, high frequency, electrical signal (50 kHz, 500 micro amp), a measurement of the baseline resistance and reactance to the flow of electrical current can

Commentato [cr43]: Ferritin as an Inflammatory Marker in Pediatric Metabolic Syndrome: Links to Obesity and Liver Ultrasound Alterations
by Mihaela-Andreea Podeanu
Int. J. Mol. Sci. 2025,

Commentato [cr44]: POTREMMO ANCHE SOLO EFFETTUARE TSH REFLEX

Commentato [CN45]: La misuriamo?

Commentato [NF46R45]: Secondo me sì

be made. The measurement relates directly to the volume of the conductor, which is used to determine total body water, lean body mass, and finally, fat mass and percent body fat.

7 Safety

7.1 Definition of an Adverse Event (AE)

An AE is any unanticipated or unintended medical occurrence or worsening of a sign or symptom (including an abnormal laboratory finding) or disease in a study subject, including those events which do not necessarily have a causal relationship with the study condition, procedures or Study Product(s), that occurs after the informed consent is obtained.

Pre-existing conditions or illnesses that are expected to exacerbate or worsen are not considered adverse events and will be accounted for in the subject's medical history.

7.2 Definition of a Serious Adverse Event (SAE)

A SAE is defined as an AE meeting one of the following outcomes:

- Death during the period of protocol defined surveillance.
- Life Threatening Event (defined as a subject at immediate risk of death at the time of the event).
- Inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance.
- A persistent or significant disability/incapacity.
- Any other important medical event that may not result in one of the above outcomes may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.3 Assessing, Recording, Analyzing, and Managing Safety Parameters

7.3.1 Methods and Timing for Assessment

Adverse or clinical events will be assessed at each clinical visit. Each visit is conducted by a clinician. All reported events will be recorded in the subject's medical record.

At any time during the study, in the event of a body weight reduction exceeding 10% over a 30-day period, or if the psychology team identifies clinically relevant changes in the participant’s mood, an additional monitoring visit will be promptly scheduled to evaluate the appropriateness of continued participation in the study.

All participants will be monitored for clinical safety throughout the study period. Safety evaluation includes:

- Clinical assessment at each in-person visit
- Monthly remote monitoring (phone or video call)
- Additional contact during FMD cycles. Participants in the FMD arm will receive additional safety monitoring during each 5-day FMD cycle.

- Laboratory monitoring at baseline and 12 months (or earlier if clinically indicated)

Vital Sign Criteria
- Systolic blood pressure ≥ 90 mmHg and ≤ 160 mmHg
- Diastolic blood pressure ≤ 100 mmHg
- Resting heart rate between 50–110 bpm

Laboratory Criteria
- Fasting glucose ≥ 70 mg/dL
- Sodium ≥ 134 mmol/L
- Potassium ≥ 3.5 mEq/L
- Creatinine within normal age-adjusted range
- AST/ALT ≤ 2× upper limit of normal
- No clinically significant anaemia
- Investigators may exclude any participant if clinical judgment indicates potential safety concerns.

Stopping Rules
The dietary intervention will be suspended if any of the following occur:
- 10% body weight loss within 30 days
- Symptomatic hypotension or syncope
- Hypoglycaemia (<70 mg/dL)
- Significant electrolyte abnormalities
- Clinically significant lean mass reduction
- Development of eating disorder symptoms
- Secondary amenorrhea (≥3 months)

Immediate discontinuation occurs in case of:
- Severe allergic reaction
- Serious adverse event
- Severe psychiatric deterioration

If confirmed, permanent/temporary discontinuation will be considered. Re-initiation of dietary intervention will occur only after medical reassessment.

7.3.2 AE Severity — Grading Scale

Each adverse event will be graded for severity. All laboratory and clinical AEs that occur in a subject, will be assessed for severity and classified into one of the categories below following a standard criterion of Mild, Moderate or Severe.

- Mild Adverse Event — event requires minimal or no treatment and does not interfere with the subject's daily activities.

- Moderate Adverse Event **—** event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe Adverse Event — event interrupts a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

7.3.3 AE/SAE Causality — Relatedness Scale

For all collected AEs, the clinician who examines and evaluates the subject will determine the adverse event's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below:

- Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs within a plausible time frame following study product administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the product (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.
- Possibly Related: there is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the study product). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse event may be rated only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded as appropriate.
- Unlikely: a clinical event, including an abnormal laboratory test result, whose temporal relationship to the study product makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study product) and in which other drugs or chemicals or underlying diseases provide plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).
- Unrelated/None: the AE is completely independent of study product administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.
- Expected Events Related to Disease Process: expectedness refers to the awareness of adverse events previously observed, not on the basis of what might be anticipated from the pharmacological properties of the Study Product.

7.3.4 Recording/Documentation

At each contact with the subject, information regarding adverse events will be elicited through appropriate questioning and examinations and immediately recorded in a source document. Source documents will include: progress notes, laboratory reports, consult notes, phone call summaries, survey tools and data collection tools. Source documents will be reviewed in a timely manner by the research

team. All reportable adverse events that are identified will be recorded on an appropriate case report form (CRF) if applicable. The start date, the stop date, the severity of each reportable event, and the PI's judgment of the AEs relationship to the Study Product/intervention will also be recorded in the Subject's progress notes or on the CRF if applicable.

7.3.5 Specific Serious Adverse Event Requirements

SAEs will be handled according to good clinical practices associated with Serious Adverse Events. SAEs will be recorded in the study's SAE form and clinician progress notes, and require expeditious handling and reporting to the Sponsor. Follow-up information which becomes available as the SAE evolves, as well as supporting documentation, will be collected subsequently and reported to the Sponsor. The PI will report SAE events to the IRB if applicable, in compliance with the IRB's requirements.

7.3.6 Reporting of Pregnancy

Should a woman become pregnant or suspect she is pregnant while participating in this study, she is instructed to inform study staff and her primary care provider immediately.

7.4 Type and Duration of the Follow-up of Subjects after Adverse Events

7.4.1 Monitoring of Subjects

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination.

AEs may be observed by the Investigator and/or study staff, elicited from the subject and/or family member, or volunteered by the study subject. Adverse events that had previously been reported by study subject will also be reassessed for duration, intensity and possible reoccurrence. Assessment of safety will include clinical observation and monitoring of hematological, chemical, and immunological parameters.

Any AE that occurs between the times a study Subject signs the informed consent form and the time s/he departs the study at the end of the final visit (or at the time of early discontinuation of the subject from the study for any reason) will be captured and recorded.

Due to the nature and composition of the study product, no delayed toxicities or withdrawal effects are expected after a subject has discontinued participation in the study. Therefore, no collection of safety information will be done after the subject's discontinuation from the study granted that the subject does not have any unresolved AEs.

7.4.2 Follow-up of Subjects after Adverse Events

All SAES and non-serious AEs reported in this study will be followed until resolution or until the investigator and the clinical/medical monitor are in agreement that the AE/SAE has stabilized and no further follow-up is required. This requirement indicates that follow-up may be required for some events after the patient discontinues participation in the study.

7.4.3 Modification of Study Product(s)/Intervention(s) for a Subject

No food substitutes are permitted. A reduction of food products, a hold of food or discontinuation of food product is allowed at the discretion of the study clinician.

7.4.4 Halting Rules for the Protocol

The PI will closely monitor and analyze study data as they become available and will make determinations regarding the presence and grading of adverse events. Evaluation of adverse events will be analyzed for the study products and with regard to the known complications associated with administration. The study will be halted (no new enrollments and no further administration of product) by the investigators and a report will be submitted to the IRB (if applicable) if a safety issue is identified.

7.4.5 Stopping Rules for an Individual Subject

A study subject will be discontinued from further Study Product(s) administration for: any clinical adverse event, laboratory abnormality, concurrent illness, or other medical condition.

7.4.6 Premature Withdrawal of a Subject

A subject may decide to withdraw informed consent for any reason. Premature discontinuation by a subject will be evaluated to assess the status of the subject at termination and will be recorded in the subject's medical record and in the CRF.

7.4.7 Replacement of a Subject Who Discontinues Study Treatment

Subjects may be replaced at the PI's discretion.

7.5 Research Use of Stored Human Samples, Specimens or Data

7.5.1 Use of Stored Data

Any other research or experimental treatments will be done under additional protocols for which separate signed informed consent documents will be obtained.

7.5.2 Disposition of Stored Samples and Data

Data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only authorized members of the study team will have access to the samples and data.

7.6 Assent or Informed Consent Process in Case of a Minor

Minors will be represented by their parents or legal guardians through the appropriate informed consent.

7.7 Subject Confidentiality

The investigator will ensure that the subject's anonymity is maintained. Subjects will not be identified in any publicly released reports of this study. All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to medical records.

The investigator will inform the subjects that the above-named representatives will review their study-related records without violating the confidentiality of the subjects. All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number in order to maintain subject confidentiality. All records will be kept locked and all computer entries and networking programs will be done with coded numbers only. Clinical information will not be released without the written permission of the subject, except as necessary for monitoring by the FDA, the Sponsor, or the sponsor's designee.

7.8 Study Discontinuation

The study will be discontinued for the following reasons:

- any clinical adverse event or serious adverse event, laboratory abnormality or inter-current illness which, in the opinion of the PI, indicates the continued treatment with study therapy is not in the best interest of the subjects.
- Termination of the study by the Sponsor.

7.9 Safety Philosophy of the Trial

This study prioritizes:

- Gradual metabolic improvement
- Preservation of lean body mass
- Protection of pubertal development
- Psychological well-being

No rapid weight loss targets are pursued.

8. Statistical Analysis Plan

8.1 Study Randomization

This is a randomized, open-label three-arm study. After the screening visit, participants will be randomized through a pre-stratified randomization method to achieve balance between the three groups (interventions and control group) according to baseline characteristics (covariates) that include gender, age (14-15; 16-18), and BMI (25-29,9; 30 and over) obtaining 8 strata. The block-based randomization will then be performed separately within each layer using a web-based randomisation system (https://www.project-redcap.org/) that will be controlled by the researchers not involved in the study from the Valter Longo Foundation in Milan.

8.2 Study Records Retention

The PI will retain investigational product disposition records, electronic database files, and source documents for the maximum period required by applicable regulations and guidelines, or as specified by the Sponsor, whichever is longer. Records with individual patient identifiers will be maintained for only 10 years. After this date, all records will be identified by subject number only with no connection to individual patients. The Sponsor will inform the PI when records are no longer needed.

8.3 Clinical Monitoring Plan

Representatives of the Sponsor will be allowed to visit the study site periodically to assess the data, quality and study integrity. This will include: reviewing study records, comparing records with source documents, discussing conduct of study with the PI and verifying the conditions of the facility. In addition, the study may be evaluated by the Sponsor's auditors and government inspectors. They will be allowed access to source documents, the electronic database, and other study files.

8.4 Sample Size Consideration

The sample size was determined to ensure adequate statistical power to detect an effect of the fasting-mimicking diet (FMD) nutritional intervention on BMI over the 6-month treatment period compared with the control group. The primary hypothesis will be tested by evaluating the time × intervention interaction using a repeated-measures design with two time points (baseline and 6 months).

Sample size estimation was performed using G*Power software. For the sample size calculation, we referred to a previous study on the same dietary interventions, which reported a time × intervention interaction effect size of partial eta squared (ηp^2) = 0.253 (corresponding to Cohen's f ≈ 0.58). To obtain a conservative estimate for the planning of the present trial, we assumed a small effect size for the intervention (Cohen's f = 0.20), a correlation of 0.50 between repeated measurements, and a two-sided significance level of $\alpha = 0.05$. Under these assumptions, a sample size of 34 participants per group provides at least 90% power to detect a statistically significant time × intervention interaction.

To account for an anticipated 30% dropout rate, a minimum sample size of 49 participants per group would be sufficient to achieve adequate statistical power. However, to ensure a conservative study design, improve the robustness of the results, and account for potential variability and dropout, the planned sample size was substantially increased. Accordingly, the present study will recruit 110 participants per arm, resulting in a total sample size of 330.

8.5 Source Documents and Access to Source Data/Documents

Study data will be collected on CRFs designed for the study if required by the PI. The PI is responsible for ensuring that the collected data is complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of a piece of data) should support the data collected on the case report form. The CRFs will be signed and dated by the person who records and/or reviews the data. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Data for CRFs will be collected during patient visits, phone calls with subjects and health care providers, and subject diaries (if applicable). The CRF form may act as the source document for the following study procedures: Subject Scheduling, Vital Signs, Clinical Assessments (adverse event and medication logs). It is not acceptable for the CRF to be the only record of a patient's participation in the study. This is to ensure that anyone who would access the patient's medical record has adequate knowledge that the patient is participating in an event, based upon the 2016 American Heart Association and American College of Cardiology recommendations, compliance and age are planned. Additional subgroup analyses may be performed.

8.6 Safety Review

A review of the collected safety data will be ongoing by the study coordinator. These reports will be reviewed by the PI.

8.7 Study conclusions

Collected data from subjects will be reviewed to demonstrate that favourable changes are noted in biomarkers of body composition and age-related biomarkers with administration of the proprietary food products. Data will be evaluated for changes from baseline over time within arms and for differences between the intervention and control arms.

8.7.1 Analysis supporting primary objectives

Generalized mixed linear models will be used to compare arm 3 (FMD) to arm 1 (control group) sitting abdominal circumference expressed in terms of change from baseline to the end of the FMD cycles (month 12, visit 3). The analysis will include the fixed categorical effect of treatment, a fixed term modelling changes between baseline and other time periods, and a random term accounting for repeated measurements on the same patients. The same analyses will be used to compare arm 2 to arm 1, sitting abdominal circumference expressed as change from baseline to the end of the study (month 12).

8.7.2 Analysis supporting secondary objectives

Generalized mixed linear models will be used as previously described to compare arm 2 and arm 3 vs arm 1, sitting the following parameters expressed in terms of change from baseline to the end of the study (month 12): inflammatory biomarkers (CRP, etc), IGF-1, blood pressure, serum lipids, glucose, CV biomarkers and, body weight, BMI, body composition, insulin resistance, sleep quality and duration, and number of participants who receive antihypertensive/hypoglycaemic medications.

We will also evaluate the effect of FMD and MLD treatments on risk factors for CVD and metabolic syndrome, defined as three of the following five conditions: abdominal obesity, elevated fasting glucose, elevated blood pressure, high serum triglycerides, and low HDL cholesterol.

8.7.3 Safety and Tolerability/Acceptability Evaluation

Collected data from subjects will be reviewed for the occurrence of AEs and for data concerning hedonics, tolerability and acceptability of products. CMP and CBC data will be tallied and evaluated. This information will be considered in drafting any product instructions and warnings.

8.7.4 Subgroup analyses

To explore the effect of treatment within subgroups, the estimated effect of the nutritional interventions will be derived for each subgroup listed below. No adjustment for multiple comparisons will be made. Additionally, the frequency and percentage of subjects reaching the primary endpoint will be presented by treatment group for each subgroups based on the baseline measurements: gender, age groups (14-15; 16-18), and BMI (25-29,9; 30 and over).

9. Ethical Considerations

9.1. Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the International Conference for Harmonization Good Clinical Practice regulations and guidelines, whichever affords the greater protection to the subject.

9.2. Informed Consent Process

Informed consent is a process initiated before the individual agrees to screen for and participate in the study and it continues throughout the individual's participation. An extensive discussion of the risks and possible benefits of this therapy will be provided to the subjects. Consent forms describing in detail the Study Product, the study procedures and associated risks will be given to the Subject and written documentation of informed consent is required prior to screening for the study and if qualified, prior to starting the study. The subject will be asked to read and review the document. Upon reviewing the document, the investigator or designee will explain the research study to the Subject and answer any questions that may arise. The subjects will sign the informed consent document prior to any procedures being done specifically for the screening process and if qualified, for the study. The subjects will have sufficient opportunity to discuss the study and process the information in the consent process prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the trial. A copy of all informed consent documents will be given to the subjects for their records. Minors will be represented by their parents or legal guardians through the appropriate informed consent.

The acquisition of informed consent will be documented in the subject's medical records. The informed consent form will be signed and personally dated by the subject and the person who conducted the informed consent discussion. The original signed informed consent form will be retained in the medical chart and a copy will be provided to the subject.

The study poses minimal risk for the participants. The anonymized information collected in the study will be used for research and publication purposes, always safeguarding the right to privacy and anonymity according to the rules of General Data Protection Regulation (GDPR) of the European Union (EU) 2016/679.

This study will be initiated only after all required legal documentation has been reviewed and approved by asl Napoli 1 Centro, according to the national and international regulations. The same applies to the implementations of changes introduced by amendments.

Individual patient medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited with the exception noted below. Patient confidentiality will be ensured by using patient identification code number.

The biological samples will be stored for 10 years at -80° C at Betania Hospital, Ponticelli, NA.

Commentato [CN47]: Chiedere a BOTTINO

Type of premises (specify)	Address	Number
Legal/operational headquarters	Via Argine n. 604 – 80147 Naples	6
Scientific and technological laboratories	Via Argine n. 604 – 80147 Naples	3
Other premises (other site, e.g., organizational secretariat)	Via E. Gianturco n. 66 – 80146 Naples	2

They will undergo a coding process with measures taken to ensure that specimens are kept under correct conditions always when they are stored. Each sample will be assigned an identification code, which will be used by researchers to prevent third parties from identifying study participants from the samples. Encryption and access passwords will be adopted to protect and safeguard the stored samples.

References

- Engin A. The Definition and Prevalence of Obesity and Metabolic Syndrome: Correlative Clinical Evaluation Based on Phenotypes. Adv Exp Med Biol. 2024;1460:1-25. doi: 10.1007/978-3-031-63657-8_1. PMID: 39287847

- Rajesh Y, Sarkar D. Association of Adipose Tissue and Adipokines with Development of Obesity-Induced Liver Cancer. Int J Mol Sci. 2021 Feb 22;22(4):2163. doi: 10.3390/ijms22042163. PMID: 33671547; PMCID: PMC7926723

- Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics. 2015 Jul;33(7):673-89. doi: 10.1007/s40273-014-0243-x. PMID: 25471927; PMCID: PMC4859313

- https://www.istat.it/it/files/2019/11/Stili-di-vita-bambini-EN.pdf?utm

- Stival C, Lugo A, Barone L, Fattore G, Odone A, Salvatore S, Santoro E, Scaglioni S, van den Brandt PA, Gallus S, Hbsc Lombardy Committee, OKKio Alla Salute Lombardy Committee. Prevalence and Correlates of Overweight, Obesity and Physical Activity in Italian Children and Adolescents from Lombardy, Italy. Nutrients. 2022 May 28;14(11):2258. doi: 10.3390/nu14112258. PMID: 35684058; PMCID: PMC9182936.

- Archero F, Ricotti R, Solito A, Carrera D, Civello F, Di Bella R, Bellone S, Prodam F. Adherence to the Mediterranean Diet among School Children and Adolescents Living in Northern Italy and Unhealthy Food Behaviors Associated to Overweight. Nutrients. 2018 Sep 18;10(9):1322. doi: 10.3390/nu10091322. PMID: 30231531; PMCID: PMC6165180

- Zhang X, Liu J, Ni Y, et al. Global Prevalence of Overweight and Obesity in Children and Adolescents: A Systematic Review and Meta-Analysis. JAMA Pediatr. 2024;178(8):800–813. doi:10.1001/ jamapediatrics.2024.1576

- Brandhorst S, Choi IY, Wei M, Cheng CW, Sedrakyan S, Navarrete G, Dubeau L, Yap LP, Park R, Vinciguerra M, Di Biase S, Mirzaei H, Mirisola MG, Childress P, Ji L, Groshen S, Penna F, Odetti P, Perin L, Conti PS, Ikeno Y, Kennedy BK, Cohen P, Morgan TE, Dorff TB, Longo VD. A Periodic Diet that Mimics Fasting Promotes Multi-System Regeneration, Enhanced Cognitive Performance, and Healthspan. Cell Metab. 2015 Jul 7;22(1):86-99. doi: 10.1016/j.cmet.2015.05.012. Epub 2015 Jun 18. PMID: 26094889; PMCID: PMC4509734.

- Micarelli A, Mrakic-Sposta S, Vezzoli A, Malacrida S, Caputo S, Micarelli B, Misici I, Carbini V, Iennaco I, Granito I, Longo VD, Alessandrini M. Chemosensory and cardiometabolic improvements after a fasting-mimicking diet: A randomized cross-over clinical trial. Cell Rep Med. 2025 Feb 18;6(2):101971. doi: 10.1016/j.xcrm.2025.101971. PMID: 39970875; PMCID: PMC11866515.

- van den Burg EL, Schoonakker MP, van Peet PG, van den Akker-van Marle EM, Lamb HJ, Longo VD, Numans ME, Pijl H. Integration of a fasting-mimicking diet programme in primary care for type 2 diabetes reduces the need for medication and improves glycaemic control: a 12-month randomised controlled trial. Diabetologia. 2024 Jul;67(7):1245-1259. doi: 10.1007/s00125-024-06137-0. Epub 2024 Mar 28. PMID: 38546821; PMCID: PMC11153305.

- Sulaj A, Kopf S, von Rauchhaupt E, Kliemank E, Brune M, Kender Z, Bartl H, Cortizo FG, Klepac K, Han Z, Kumar V, Longo V, Teleman A, Okun JG, Morgenstern J, Fleming T, Szendroedi J, Herzig S, Nawroth PP. Six-Month Periodic Fasting in Patients With Type 2 Diabetes and Diabetic Nephropathy: A Proof-of-Concept Study. J Clin Endocrinol Metab. 2022 Jul 14;107(8):2167-2181. doi: 10.1210/clinem/dgac197. PMID: 35661214; PMCID: PMC9282263.

- Longo, V.D. Intermittent and periodic fasting in the treatment of obesity and type 2 diabetes mellitus. Nat Rev Endocrinol 21, 73–74 (2025). https://doi.org/10.1038/s41574-024-01078-5

- Longo VD, Panda S. Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. Cell Metab. 2016 Jun 14;23(6):1048-1059. doi: 10.1016/j.cmet.2016.06.001. PMID: 27304506; PMCID: PMC5388543.

- Brandhorst S, In Young C. A Periodic Diet that Mimics Fasting Promotes MultiSystem Regeneration, Enhanced Cognitive Performance, and Healthspan. Cell Metabolism 22, 86-99, July 7, 2015 Elsevier Inc

- Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res. 2009 Feb;37(3):e21. doi: 10.1093/nar/gkn1027. Epub 2009 Jan 7. PMID: 19129229; PMCID: PMC2647324.

- Houston M, Hayes L Acute effects of an oral nitric oxide supplement on blood pressure, endothelial function, and vascular compliance in hypertensive patients. J Clinical Hypertension (Greenwich) 2014;16(7):524-9.

- Knoops KTB, de Groot LC, et al. Mediterranean Diet, Lifestyle Factors, and 10-year Mortality in Elderly European Men and Women. JAMA 2004;292(12):1433-9.

- Levine ME, Suarez JA, Brandhorst S, Balasubramanian P, Cheng CW, Madia F, Fontana L, Mirisola MG, Guevara-Aguirre J, Wan J, Passarino G, Kennedy BK, Wei M, Cohen P, Crimmins EM, Longo VD. Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. Cell Metab. 2014 Mar 4;19(3):407-17. doi: 10.1016/j.cmet.2014.02.006. PMID: 24606898; PMCID: PMC3988204.

- Pollack MN. Insulin, insulin-like growth factors, insulin resistance, and neoplasia. Am J Clin Nutr. 2007 Sep;86(3):s820-2. doi: 10.1093/ajcn/86.3.820S. PMID: 18265475.

- Wei M, Brandhorst S, Shelehchi M, Mirzaei H, Cheng CW, Budniak J, Groshen S, Mack WJ, Guen E, Di Biase S, Cohen P, Morgan TE, Dorff T, Hong K, Michalsen A, Laviano A, Longo VD. Fasting-mimicking diet and markers/risk factors for aging, diabetes, cancer, and cardiovascular disease. Sci Transl Med. 2017 Feb 15;9(377):eaai8700. doi: 10.1126/scitranslmed.aai8700. PMID: 28202779; PMCID: PMC6816332.

B.7 Declaration of absence of conflict of interest (All 3)

C. INFORMATION ON PARTICIPANTS

C.1 What types of subjects will take part in the study?

- ☐ Students
- ☐ Adults (over 18 years of age and able to give their consent)
- ☒ Children and adolescents aged 18 or under
- ☐ Elderly (over 65 years of age and able to give their consent)
- ☐ Non-native Italian speakers
- ☐ Subjects with cognitive/mental deficit, NOT able to give their consent
- ☐ Other persons whose capacity to give consent may be impaired (indicate why)
- ☐ Subjects with physical disability (specify what type)
- ☐ Institutionalized subjects (e.g., prisoners, hospitalized patients, etc.)
- ☐ Patients and/or clients referred by doctors, psychologists or other professionals
- ☐ It is not possible to determine the type of subjects (e.g., administration via the internet)

C.2 Might some of the subjects be in a position of dependence on the researcher or one of his/her collaborators, such that it could be assumed that their consent to participate is not entirely free and without any kind of pressure (e.g., student/professor, patient/doctor, employee/employer)? If so, indicate how you intend to prevent the subject from feeling obliged to take part in the research.

No

C.3 Inclusion/exclusion criteria for research participants

3.1.1 Inclusion Criteria

- Subjects of 14-18 years of age;
- with overweight/obesity defined by WHO BMI criteria: Overweight: $\geq$+1SD (equivalent to BMI 25 kg/m^2 at 19 years); Obesity: $\geq$+2SD (equivalent to BMI 30 kg/m^2 at 19 years)

3.1.2 Exclusion Criteria

- individuals with a family member already included in the study;
- Individuals with obesity $\geq$ 3 SD (equivalent to BMI 35kg/m^2 at 19 years);
- Individual with BMI > 25 kg/m^2 but with normal waist circumference or less than 20% of fat mass for male subjects and less than 25% of fat mass for female subjects
- individuals who are allergic to tree nuts (macadamia, cashew, almond, pecan), soy, oats, sesame, or celery/celeriac;
- Pregnant or possibly pregnant females;
- No evidence of pubertal delay requiring medical evaluation;
- pharmacological treatments known to interfere with the study treatment; all concomitant medications (including, but not limited to, levothyroxine, asthma medications, etc.) will be evaluated by the study's responsible pediatrician;
- use of hypoglycemic agents;
- No history of recurrent syncope or unexplained hypotensive episodes.

3.1.3 *Medical History and Concurrent Diseases*

- A history of allergies or intolerance to study products. Detailed descriptions of study product are included in Section 4.1 and 4.2, appended to the Study Informed Consent;

- Clinically significant vital sign abnormalities (systolic blood pressure <90 mmHg or >200 mmHg, diastolic blood pressure <50 mmHg or >105 mmHg or resting heart rate of <50 or >100 bpm) at screening visit;
- any documented cancer diagnosis within the past 5 years;
- chronic steroid use (longer than 45 consecutive days);
- Individuals with severe hypertension (systolic greater than 200 mmHg and or diastolic greater than 105 mmHg;
- proven adrenal gland dysfunction,
- pituitary pathology,
- congenital metabolic disease,
- treatment with medication causing IR,
- Genetic hypercholesterolemia,
- History of nephro or cholelithiasis, of pancreatitis,
- history of bariatric surger
- Known infection with HIV, TB or Hepatitis B or C.
- A current diagnosis or personal history of:
    - any cardiovascular disease including myocardial infarction, angina, cardiovascular surgery (within 5 years), congestive heart failure, cardiac arrhythmias or conduction abnormalities, cerebrovascular accident, transient ischemic attack (TIA), or peripheral vascular disease, deep vein thrombosis or pulmonary embolus. Diabetes mellitus requiring inhaled or injected insulin.
    - Any autoimmune disease such as inflammatory bowel disease (including Crohn's disease and/or ulcerative colitis), multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, polymyositis, scleroderma and/or thyroiditis.
    - Any significant liver or kidney disease such as cirrhosis or non-alcoholic fatty liver disease, glomerulonephritis, and/or ongoing dialysis treatment.
    - Any malignancy (with the exception of adequately treated malignancies with no known recurrence for >2 years).
- Any serious mental illness including a history of attempted suicide.
- Eating disorders or any psychiatric disease.
- Any medical condition that in the opinion of the primary care doctor or a specialist would preclude safe participation in this study or interfere with compliance.

3.1.4 *Substance Use*

- Use of drugs of abuse (such as marijuana, cocaine, phencyclidine [PCP] and methamphetamine) 15 days prior to Day 1 and for the duration of the study.
- History of regular intake of alcoholic drinks

3.1.5 Technical reasons

Any condition in which bioelectrical impedance testing would be impossible or uninterpretable (e.g. prostheses in extremities on both sides, limb amputation, implanted pacemaker, inability to lay still or supine, or skin defects on preferred electrode placement sites.

3.1.6 *Other Exclusion Criteria*

- Inability to comply with study and/or follow-up visits.
- Any concurrent condition (including clinically significant abnormalities in medical history, physical examination or laboratory evaluations) which, in the opinion of the PI, would preclude safe participation in this study or interfere with compliance.
- Any sound medical, psychiatric and/or social reason which, in the opinion of the PI, would preclude safe participation in this study or interfere with compliance.
- Abnormal laboratory findings including: abnormal blood counts (hematocrit < 33% or > 47%; WBC < 3.0 or > 12.0 x103/mm3; platelets < 140 or > 500 x 109/L); abnormal kidney function test (creatinine > 2.5 mg/dL) or liver function test(s) (AST, ALT, alkaline phosphatase) > 1.5X the upper limit of normal; serum calcium > 11 mg/dL); serum K < 3.5 mEq/L; Na < 134 or > 148 mmolL-1

C.4 How will the information and the invitation to participate in the research be disseminated? (Attach a copy of any flyers or letters to be sent.)

C.5 Is any form of compensation foreseen for the healthy volunteer participant?

No

N.B. The investigator is required to communicate any changes in the way participants are involved.

D. Risk and risk management

D.1 The research involves

- ☒ use of questionnaires (attach a copy)
- ☐ structured or semi-structured interviews (attach a copy of the questions to be asked; where this is not possible, indicate the topics that will be addressed)
- ☐ in-depth interviews
- ☐ focus groups
- ☐ autobiographical narratives
- ☐ collection of diaries (diary keeping)
- ☐ observation of subjects' behavior without their knowledge
- ☐ observation of subjects' behavior
- ☐ audio or video recordings of subjects
- ☐ administration of stimuli, tasks or procedures and recording of behavioral responses, opinions or judgments
- ☐ administration of stimuli, tasks or procedures that the subject might find annoying, stressful, or physically or psychologically painful, either during or after the study
- ☐ eye movement recording
- ☐ use of TMS (Transcranial Magnetic Stimulation)
- ☐ immersion in virtual reality environments

- ☐ recording of evoked potentials
- ☐ administration of tests, questionnaires or experimental protocols via the internet (web, e-mail)
- ☐ use of neuropsychological tests
- ☐ neuroimaging techniques
- ☐ behaviors that might lower subjects' self-esteem, or induce embarrassment, distress or depression
- ☐ deception of subjects
- ☐ administration of substances or agents (e.g., drugs, alcohol)
- ☒ collection of human tissue or fluid samples (e.g., blood tests)
- ☒ participation in a clinical trial
- ☒ collection of genetic material
- ☒ other (specify)

We are carrying out this study to understand whether two balanced diets, already tested in other studies, are able to reduce fat mass and reduce/slow down the effects of aging. These are, in particular, a hypocaloric diet and a longevity diet. The study will take place in Campania, in the 5 provinces of the region. With the support of nutrition specialists, 330 volunteers will be recruited with the aim of verifying (the effects of two different dietary interventions on FMD, alone or in combination with the longevity diet (FMD + LD), on body composition. Secondary objectives include assessing the effects of two different dietary interventions on: cardiovascular (CV) biomarkers, antihyperglycemic and antihypertensive drugs, and sleep quality.

D.2 If the research involves the use of procedures that could be stressful or dangerous for participants, describe the nature of the risks and the reasonably expected consequences of the procedures used

There are risks and discomforts associated with the fasting-mimicking diet, such as hunger, anxiety, drowsiness, dizziness, headache, muscle pain, fatigue, low blood pressure and, in rare cases, fainting. These dietary interventions may also cause abnormal heart rhythms, short-term nutrient deficiencies and a weakened immune response. A long period of hypocaloric diet can be particularly dangerous in people who are already malnourished. There are also the risks and discomforts associated with any allergies/intolerances to the foods present in the diet (not known before participating in the study). At the end of the diet, participants should gradually resume their normal diet, starting with liquid foods, such as soups and fruit juices, accompanied by light meals.

The risks and discomforts associated with the longevity diet are very rare but could include episodes of allergies/intolerances to the foods present in the diet that were not known before participating in the study. Participants may feel lightheaded from the blood draw. In rare events, participants may experience bruising, excessive bleeding, infection, dizziness and fainting. Participants may stop the blood draw procedure at any time. Participants must contact their personal doctor or seek immediate medical attention if bleeding and infections related to the blood draw occur.

D.3 Is a specific civil liability insurance policy foreseen in addition to that of the University? If yes, attach a full copy of the insurance contract.

A specific civil liability insurance policy is foreseen, for which a quote has been produced that also indicates the relevant coverage. The contract will be signed and submitted to the CEA after approval of the study.

D.4 How do you plan to deal with any complications or adverse reactions?
During the period of the fasting-mimicking diet, participants should drink adequate water to prevent dehydration and avoid strenuous activity/exercise. Participants should avoid using motor vehicles and heavy machinery. Participants should avoid exposure to high-temperature environments, such as hot showers or baths, and avoid alcohol consumption.
Participants must contact the investigator, consult a personal doctor or seek immediate medical attention if they have any questions or experience discomfort. Participants may stop the blood draw procedure at any time. Participants must contact their personal doctor or seek immediate medical attention if bleeding and infections related to the blood draw occur.
D.5 Are any benefits expected for those taking part in the research? Which ones?
By joining the study, the participant will have the opportunity to follow either a balanced hypocaloric diet or the longevity diet, which could improve their general health with a reduction in fat mass, blood pressure and markers of inflammation.
By joining the study, the participant will contribute to the development of knowledge on the effects of hypocaloric and longevity diets on human health. These diets could, in fact, have beneficial effects on their own health and on the health of the population by slowing/reducing the adverse effects on human health of already known risk factors (hypertension, body mass index, inflammation) for human aging.
Other expected results: increased knowledge, change in eating and lifestyle habits, improved health, awareness of consumption, consumption of healthy foods and consolidation of the values of healthy longevity.

E. Information and consent

E.1 Summary of the planned information process (interview, signing of consent, return of results…)
The researchers and nutritionists involved will complete the medical history form together with the study participants and will assess their eligibility to take part in the study according to the inclusion/exclusion criteria indicated in this project. Subjects eligible to take part in the study will have to sign the informed consent and the privacy notice for the medical history and personal data collected during the screening visits, and will then be randomized and assigned to one of the 3 arms of the study. For each visit, each participant will be contacted by telephone to be informed of the results of the tests they underwent.
E.2 Information Form and Declaration of Consent (Appendix N1, N2, O1, O2)
E.3 If subjects unable to give consent are involved, indicate whom you will ask to consent to participation, specifying their role and the reasons
Subjects unable to give consent will not be involved.
E.4 If, for the conduct of the study, it is methodologically appropriate not to inform participants of its objective before the start of the trial, specify how the subsequent clarification interview will be conducted
Participants will always be informed about all the procedures of the study.

Informed consent for the participation of the minor patient – Clinical study

Official title of the clinical study
Study of the effects of periodic cycles of the fasting-mimicking diet or recommendations for a continuous Mediterranean Longevity diet on body composition and health markers in adolescents: a randomized controlled trial

Title of the study in terms more understandable to the patient
Analysis of the effects of the Mediterranean longevity diet and of a hypocaloric protocol in reducing risk factors in adolescents with overweight or obesity.
Structure/context in which the study will take place
Participants will be recruited in the Campania region, with the support of nutrition specialists. After enrollment, each participant will follow the assigned diet at home.
Coordinating center and coordinators of the clinical study
Coordinating center: Fondazione Valter Longo
Study coordinators: Dr. Romina Inès Cervigni (Valter Longo Foundation, European Longevity Institute, Milan)
Protocol registration and identification code
Identification code: _____
Protocol registration: _____
Principal investigator (local leader)
Name: Dr. Romina Inès Cervigni
Affiliation: Fondazione Valter Longo, European Longevity Institute, Milan
Promoter
Name: Dr. Vincenzo Bottino
Funding body
Regione Campania
Ethics committee
CET Campania 1
Structure of the document
This document includes: A. Introduction; B. Information section – Study summary: main information; C. Information section – Further information; D. Consent section.

A. Introduction

Dear Madam / Sir,
We are proposing that your son/daughter/the minor take part in the clinical study described below.
It is your right, as well as that of your son/daughter/the minor, to be informed about the purposes and characteristics of the study, so as to be able to make a free and informed decision about participation.
This document is intended to inform you about the nature of the study, its objectives, what participation will entail, and the related rights and duties. We invite you to read the following carefully.
The researchers indicated at the beginning of the document are available to answer any questions. No question is trivial: do not hesitate to ask for clarification.
You may discuss the proposal with your son/daughter/the minor, with your family doctor, with family members or with trusted persons. Take all the time you need to decide. You may take home an unsigned copy of the document to reflect on it or to discuss it with others before deciding.
If you decide not to participate in the study, you will still be able to benefit independently from the activities organized by the Fondazione Valter Longo.
Refusal will in no way be interpreted as a lack of trust.
After reading the document, receiving answers to your questions and deciding to participate, you will be asked to sign the consent form, of which you will receive a paper copy.

The Principal Investigator

B. Information section – General summary of the study – Main information

This section summarizes the fundamental aspects of the study. The following sections will provide further details to allow you to give fully informed consent.

Why is your son/daughter/the minor being asked to participate?

We are asking you to take part in a clinical study funded by the Evangelical Hospital Betania and the Fondazione Valter Longo, because your son/daughter/the minor has certain clinical characteristics that will be specified further in the following sections.

Objectives of the study and number of participants

The study aims to answer the question: is it possible to obtain beneficial effects on body composition and slow down aging through two specific controlled nutritional interventions?

The following will be evaluated:

- Fasting-mimicking protocol
- Mediterranean longevity diet

The study will take place at the Evangelical Hospital Betania in Naples, enrolling participants from Campania, under the responsibility of:

- Dr. Romina Inès Cervigni
- Prof. Valter Longo
- Dr. Vincenzo Bottino

330 volunteers will be recruited to verify whether these nutritional interventions can:

- modify body composition
- reduce fat mass
- reduce cholesterol, IGF-1 and inflammatory and cardiovascular markers
- induce changes that protect against biological aging
- reduce the use of hypoglycemic or antihypertensive drugs, if they are being used
- improve sleep quality

Blood tests will be performed at: Evangelical Hospital Betania – Naples

Is participation my free choice?

You and your son/daughter/the minor can freely decide whether or not to participate. Even after agreeing, you can change your mind at any time.

What happens if I decide to have my son/daughter/the minor participate?

If you decide to participate, your son/daughter/the minor will be **randomly** assigned to one of three groups:

1. **Usual diet** (control group)
2. **Mediterranean longevity diet** for 12 months
3. **Fasting-mimicking protocol**: up to 5 cycles (one every three months) over 12 months

After the first 6 months, participants in the control group may **optionally** follow the Mediterranean longevity diet and/or fasting-mimicking protocols for another 6 months.

Duration of the project:

- 36 months overall
- 12 months of participation for each subject

- up to 12 months for the control group that chooses the nutritional intervention

During the study:

- **peripheral blood draws (25 mL) (n=2)** will be required
- no invasive procedures (biopsies, bone marrow, etc.) are planned

Expected benefits

Participation may:

- improve general health
- reduce: fat mass; blood pressure; inflammation and cardiovascular markers; biological age

In addition, it will help deepen scientific knowledge about the effects of hypocaloric and longevity diets on human health.

Potential risks of the fasting-mimicking diet:

- hunger
- drowsiness
- dizziness
- headache
- body aches
- tiredness
- hypotension
- rarely fainting
- arrhythmias
- temporary nutritional deficiencies
- reduced immune response

Additional risks:

- unknown food allergies/intolerances
- dehydration (if not drinking enough)
- pre-existing malnutrition

Precautions:

- drink adequately
- avoid intense exertion
- avoid driving vehicles or operating machinery
- avoid very hot environments, such as saunas or Turkish baths
- avoid alcohol

After the diet:

- gradually resume eating with liquid and light foods.

Potential risks of the Mediterranean longevity diet: incompatibility of the diet with allergies/intolerances unknown before participation in the study. The diet will be personalized according to food preferences, allergies and intolerances present at the start of the study or arising later.

Potential risks of the blood draw:

- dizziness
- hematomas
- bleeding
- infection

- fainting (rare)

Can I withdraw my son/daughter/the minor from the study?

Yes.

You and/or your son/daughter/the minor may express the wish to **leave the study at any time**, without giving reasons.

The doctor may request a final visit to ensure the safety of your son/daughter/the minor.

Early termination by the doctors

Participation may be discontinued if:

- health conditions worsen
- excessive weight loss occurs in a limited time
- unfavorable new information emerges
- the rules of the study are not followed
- pregnancy occurs
- the study is suspended by the authorities or by the promoter

Informed consent – Section C: further information

Purpose of the study

The increase in life expectancy makes it essential to **age in good health**. For this reason, after 15 years of preclinical and clinical studies (NIH, USC), the following were developed:

- **Fasting Mimicking Diet (FMD)**
- **Longevity Diet (LD)**

The objective of this study is to evaluate the effects of these two nutritional interventions on: body composition; cardiovascular biomarkers; blood parameters; inflammation; biological age; use of hypoglycemic and antihypertensive drugs; sleep quality.

Which groups of patients are compared in the study? Which types of nutritional intervention are tested?

If you decide to have your son/daughter/the minor participate in the trial, he/she will be randomly assigned to one of three different groups that will be formed during the study:

- the first group will continue to follow the usual diet (control group)
- the second group will follow the Mediterranean longevity diet for 12 months;
- the third group will carry out up to 5 cycles of hypocaloric/fasting-mimicking diet (one cycle every three months) for a duration of 12 months;

Once 6 months have elapsed, participants in the control group will be offered, on a voluntary basis, the possibility of following the Mediterranean longevity diet and/or the fasting-mimicking protocol for a further 6 months.

The overall duration of the project will be 36 months, but each subject's participation will last 12 months.

During the study period, on two occasions, participants will be asked to provide a peripheral blood sample on which clinical and molecular-genetic investigations will be performed.

330 participants will be enrolled, randomized into the three arms of the study in a 1:1:1 ratio, who meet the following inclusion and exclusion criteria.

Main inclusion criteria

- healthy volunteers
- age 14–18 years
- BMI ≥ 25
- Giving of consent

Main exclusion criteria

- individuals with a family member already included in the study
- individuals with BMI > 35 kg/m²
- individuals with BMI > 25 kg/m² but with a high percentage of muscle mass and less than 25% fat mass in men, 30% in women.
- allergy to tree nuts (macadamia nuts, pecans, almonds)
- pregnancy or breastfeeding
- No evidence of pubertal delay requiring medical evaluation;
- Pharmacological treatments known to interfere with the study treatment; all concomitant medications (including, but not limited to, levothyroxine, asthma medications, etc.) will be evaluated by the study's responsible pediatrician;
- Use of hypoglycemic agents;

- No history of recurrent syncope or unexplained hypotensive episodes.
- History of allergies or intolerances to the study products. Detailed descriptions of the study products are given in sections 4.1 and 4.2, attached to the study's informed consent;
- Clinically significant abnormalities of vital signs (systolic blood pressure <90 mmHg or >200 mmHg, diastolic blood pressure <50 mmHg or >105 mmHg, or resting heart rate <50 or >100 bpm) at the screening visit;
- Any documented diagnosis of neoplasia in the last 5 years;
- Subjects with severe hypertension (systolic pressure >200 mmHg and/or diastolic >105 mmHg);
- Documented adrenal gland dysfunction;
- Pituitary pathology;
- Congenital metabolic diseases;
- Treatment with drugs that cause insulin resistance;
- Genetic hypercholesterolemia;
- History of nephrolithiasis or cholelithiasis, or of pancreatitis;
- History of bariatric surgery;
- Known infection with HIV, tuberculosis, hepatitis B or C;
- Any serious psychiatric disorder, including a history of attempted suicide;
- Eating disorders or any other psychiatric disorder;
- Any medical condition that, in the judgment of the primary care physician or a specialist, could preclude safe participation in the study or interfere with adherence to the protocol.
- A current diagnosis or personal history of:
    - any cardiovascular disease, including myocardial infarction, angina, cardiovascular surgery (within the last 5 years), congestive heart failure, cardiac arrhythmias or conduction abnormalities, cerebrovascular accident, transient ischemic attack (TIA) or peripheral vascular disease, deep vein thrombosis or pulmonary embolism. Diabetes mellitus requiring inhaled or injected insulin.
    - any autoimmune disease such as inflammatory bowel disease (including Crohn's disease and/or ulcerative colitis), multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, polymyositis, scleroderma and/or thyroiditis.
    - any significant liver or kidney disease such as cirrhosis or non-alcoholic fatty liver disease, glomerulonephritis and/or ongoing dialysis treatment.
    - any malignant neoplasm (except adequately treated neoplasms with no known recurrence for >2 years).
    - any serious mental illness, including a history of attempted suicide.
- Use of drugs (such as marijuana, cocaine, phencyclidine [PCP] and methamphetamine) in the 15 days preceding the start of the study and for the entire duration of the study.
- History of regular consumption of alcoholic beverages
- Any condition in which measurement by bioimpedance would be impossible or uninterpretable (e.g., prostheses in the limbs on both sides, limb amputation, implanted pacemaker, inability to remain still or in a supine position, or skin defects at the preferred electrode placement sites).
- Inability to comply with the study and/or follow-up visits.
- Any concomitant condition (including clinically significant abnormalities in medical history, physical examination or laboratory tests) that, in the judgment of the Principal Investigator (PI), could preclude safe participation in the study or interfere with adherence.

- Any valid medical, psychiatric and/or social reason that, in the judgment of the PI, could preclude safe participation in the study or interfere with adherence.

Description of the diets

The nutritional interventions to which your son/daughter/the minor may be subjected are **the fasting-mimicking diet** or **the Mediterranean longevity diet**.

1. **The fasting-mimicking diet**, to be repeated every 3 months, consists of a food protocol scheduled over 5 consecutive days designed to bring about a simulated fast. This program aims to encourage the adoption of a healthier diet, thanks to which it is possible to reduce the effect of risk factors in cardiometabolic and chronic-degenerative diseases.
   This food program provides about 1100 kcal on the first day of the diet and about 800 kcal from the second to the fifth day. It includes various components formulated with organic ingredients, including bars, olives, soups, drinks and supplements, entirely plant-based, gluten-free and lactose-free.
   The kit developed to carry out this food program simply and practically is divided into five small packages, one for each day of the diet. In particular, the nutritional composition is divided into about 45% carbohydrates, of which 9% simple, about 45% lipids, mainly unsaturated, and 9–10% plant proteins.
   From the second to the fifth day of the fasting-mimicking diet, a drink based on vegetable glycerin is provided, which must be diluted according to body weight, as indicated on the label. All instructions are given in the Diet Card supplied inside the package.
2. **The Mediterranean longevity diet**, an adaptation of the Longevity Diet for children, is based on the diet of the longest-lived centenarians in the world but, thanks to scientific evidence, experiments and results obtained, its fundamental principles have been developed.
   It is a diet that comes as close as possible to a vegetarian-type diet (legumes, vegetables, fruit) with the addition of fish, limiting fish consumption to 2–3 times a week and avoiding fish with a high mercury content (swordfish, tuna). The daily protein intake is about 0.9 g/kg of body weight.
   It is essential to eat meals within 12 hours (starting after 8 a.m. and ending before 8 p.m., or in any case before 9 p.m.), with a preference for complex carbohydrates (tomatoes, broccoli, legumes, carrots, whole-wheat bread, etc.) and good-quality fats, minimizing saturated fats and simple sugars.
   At the base of a good diet there must be an adequate amount of quality unsaturated fats, such as the mono- and polyunsaturated fats found in oily fish (salmon, mackerel, sardines), in dark chocolate > 85%, in nuts (almonds, walnuts, etc.) and in extra virgin olive oil, with a low content of hydrogenated saturated and trans fats.
   The Longevity Diet is not just a dietary scheme, but an approach intended to be followed by all the inhabitants of the world; many long-term diets are abandoned by patients because they are too restrictive, not filling enough, or because they require lifestyle changes that are too radical. This dietary approach, by contrast, is easily applicable, because it shares many elements with the Mediterranean Diet and allows your son/daughter/the minor to eat more and not less.

What exams, tests and procedures are planned if my son/daughter/the minor participates in the study?

If you decide to have your son/daughter/the minor participate in the trial, he/she will be randomly assigned to one of three different groups that will be formed during the trial. Regardless of the group to which your son/daughter/the minor is assigned, he/she will undergo 4 medical visits: the pre-screening visit (by telephone), the enrollment/baseline visit, and a visit at 6 and 12 months from the start of the nutritional intervention (fasting-mimicking diet or longevity diet). If you decide to participate in the study, you will be given a calendar of visits with the tests to be performed, which will be booked directly by the center. During these visits the following will be carried out:

- bioimpedance analysis to study body composition;
- assessment of vital signs, including blood pressure;
- venous blood draw for biochemical analyses. The volume foreseen in this study is 25 mL per single draw; 50 mL in total.

The tests will be carried out in collaboration with the Evangelical Hospital Betania in Naples. The sharing of samples will take place through couriers in compliance with current regulations on privacy, sample identification and transport (management of sample shipping according to IATA regulations).
The blood tests will include: complete blood count, fasting glucose, insulin, HOMA-IR, glycated hemoglobin (HbA1c), lipid profile (total cholesterol, HDL, LDL and triglycerides), ferritin, ALT (GPT), AST (GOT), GGT, C-reactive protein (CRP), uric acid, creatinine, albumin, alkaline phosphatase, TSH, FT4, FT3, cytokines (TNF-α, IL-6, IL-1β), vitamin D.
Optionally, the blood tests may include: IGF-1, ghrelin, adipokines (leptin, adiponectin), GLP-1, methionine, FGF21, circulating stem cells.

What risks might my son/daughter/the minor face if he/she participates in the study?
There are risks and discomforts associated with the fasting-mimicking diet, such as hunger, anxiety, drowsiness, dizziness, headache, body aches, fatigue, low blood pressure and, in rare cases, fainting. These dietary interventions may also cause abnormal heart rhythms, short-term nutritional deficiencies and a weakened immune response. A long period of hypocaloric diet can be particularly dangerous in people who are already malnourished. There are also risks and inconveniences associated with any allergies/intolerances to the foods present in the diet (not known before participating in the study). During the diet period, participants must drink an adequate amount of water to prevent dehydration and avoid strenuous activities/exercise. Participants must avoid using motor vehicles and heavy machinery. Participants must avoid exposure to high-temperature environments, such as hot showers or baths, and avoid alcohol consumption.
Participants must contact the investigator, consult their own physician or seek immediate medical assistance in case of questions or if discomfort appears.
At the end of the diet, participants must gradually resume their normal diet, starting with liquid foods, accompanied by light meals.
The risks and inconveniences associated with the Mediterranean longevity diet are very rare but could include episodes of allergies/intolerances to foods in the diet not known before participating in the study.
Participants may feel lightheaded following the blood draw. In rare events, participants may experience bruising, excessive bleeding, infections, dizziness and fainting. Participants may stop the blood draw procedure at any time. Participants must contact their own physician or seek immediate medical assistance if bleeding or infections related to the blood draw occur.

What information must my son/daughter/the minor and I know regarding contraception and pregnancy?
You must agree not to begin a pregnancy or conceive a child during participation in this study. It is recommended to use an appropriate contraceptive method. Such methods include total abstinence or use of oral/injectable/implantable contraceptives.
Contraception (including abstinence from sexual intercourse) is required because it is not known whether the study treatment may cause harm to the fetus. Inform the study doctor or the team immediately if you think you may be pregnant or have conceived a child before receiving further study treatments.

How will you and your son/daughter/the minor be notified of any unexpected results following the diagnostic investigations?
In the current state of knowledge, including that relating to epigenetic clocks, the analyses foreseen by the study will not produce results relating to your susceptibility to developing particular diseases or to being a carrier of genetic diseases that could potentially lead to the birth of sick children.
Is it useful/necessary to inform the family doctor of my son/daughter/the minor?
Given the design of the study, if you decide to participate, it is important to inform your general practitioner. For this purpose, we have prepared a letter that you can give him/her, explaining the procedures of the study.

What will be the commitment of my son/daughter/the minor, and what will be the responsibilities of my son/daughter/the minor if I decide to have him/her participate?

- Scrupulously follow the instructions and requests of the health personnel who follow the study and ensure attendance at appointments.
- Inform the doctor who follows the study:
    - of all the medications being taken, including non-conventional medicine;
    - of any visit or hospitalization at facilities other than the study center;
    - of current or previous participation in other clinical studies;
    - to avoid pregnancy or breastfeeding during the study;
    - of any side effect that arises during the study.

Will my son/daughter/the minor have to bear costs for participating in the study? Will my son/daughter/the minor be reimbursed for any expenses? Will I receive compensation?
There are no costs to you arising from participation in the study, since these are entirely covered by the Sponsor. No financial compensation is foreseen for participation in the study.

What happens if my son/daughter/the minor suffers harm as a result of participating in the study?
Participation in a clinical study may involve inconveniences and risks that cannot be determined in advance. For this reason, the clinical study provides insurance coverage to protect your participation. In accordance with current legislation, insurance is provided to cover any damages suffered as a result of participation in the study, for the entire duration of the study, covering the civil liability of the investigator and the promoter.
INSURANCE COMPANY: Chubb European Group SE, maximum coverage 1,000,000.00.

Please note that, pursuant to the Ministerial Decree of 14 July 2009, the insurance policy does not cover the amount exceeding the maximum coverage and is effective only for damages whose claim for compensation was submitted within the period provided by the policy (36 MONTHS). However, this limitation does not prejudice your right to obtain compensation from the party responsible for any damages (for the protection of the study subject).

How will the health data of my son/daughter/the minor, including identifying data, be processed and who will have access to it during the study?

Your personal and health data, and only to the extent that they are indispensable with respect to the objective of the study and for pharmacovigilance purposes, will be processed in accordance with EU Regulation 2016/679, known as the GDPR (General Data Protection Regulation), and Legislative Decree no. 101 of 10 August 2018. In practical terms, the documents relating to the participant will be kept in a secure place and will not bear your name in clear text, known only to the researchers, but an identification code.

The data, made anonymous, may be subject to inspection by regulatory bodies and may be used for scientific publications (journals, conferences).

Your clinical data collected for the purposes of the study, as well as the results of the tests performed, will be kept for a maximum period of 15 years and then destroyed. They will not be destroyed only if a) it is no longer possible to trace them back to your identity, because they were anonymized during the study itself; b) with your specific informed consent.

If personal data are transferred to a third country or an international organization, all the guarantees provided by Article 46 of GDPR 679/2016 relating to the transfer will be adopted.

Further information is included in the attached form authorizing the processing of data.

How will the biological samples of my son/daughter/the minor collected for the purposes of the experiment be processed and who will have access to them?

As with your health data, your biological samples, pseudonymized (a technique that makes it possible to modify and mask the personal and sensitive data of a natural person, so as not to make them directly and easily attributable to that person), will be used for the purposes of the experiment.

Once the experiment is completed, the samples will be labeled with a numerical code and stored at -80 °C at the laboratory of the Evangelical Hospital Betania in Naples. Access to the samples and data will be reserved exclusively for personnel involved in the study. With this form we also ask for your authorization to store the DNA samples for a maximum period of 15 years from the conclusion of the study, for future research on the aging process. In that case, the opinion of the Ethics Committee on new genetic research studies will be requested.

How can my son/daughter/the minor and I have access to the results of the study?

Once the experiment is completed and all the resulting data have been collected, they will be analyzed to draw conclusions. The investigators and the promoter undertake to make them available to the scientific community.

The law provides for the possibility of access by participants to the results of the experiment. Therefore, you may ask the investigating doctor to communicate the general results of the study to you.

Has the study been approved by the Ethics Committee?

The study protocol that has been proposed to you has been examined and approved by the Ethics Committee of Campania 1. Among other things, the Ethics Committee has verified the conformity of the study with the Rules of Good Clinical Practice and the ethical principles expressed in the Declaration of Helsinki and that your safety, your rights and your well-being have been protected.

Whom can my son/daughter/the minor and I contact for further information on the clinical study we are invited to participate in?
DR. ROMINA INÈS CERVIGNI, Chief Scientific Officer of Valter Longo Foundation, Milan, Lombardy, Italy. Tel. +39 3397805607
PROF. VALTER LONGO (Professor of Biogerontology and Director of the Longevity Institute at the University of Southern California – Davis School of Gerontology, Los Angeles)

If you consider it appropriate to report events or facts relating to the study you have joined to persons not directly involved in the study itself, you and your son/daughter/the minor may contact the Ethics Committee that approved the study.
Name and surname of the doctor who provided the information _____ Date **//**____ Time _____ Signature _____