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Follow up Study of the Effects of the Longevity and the Fasting Mimicking Diet on Body Composition, Risk Factors Age Correlated and Biomarkers of Aging in a Randomized Study 1 and 2

1. Background Information and Scientific Rationale

1.1. Background Information

With a growing aging population all over the world, healthy ageing is an important goal for public health. Dietary restriction (DR), implemented as chronic and coordinate reduced intake of all dietary constituents except vitamins and minerals, was first shown more than 80 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 beneficial effects of DR, are therefore important goals to improve human health during aging. Despite its potential for disease prevention and treatment, prolonged fasting is difficult to implement in human subjects and may exacerbate pre-existing nutritional deficiencies, making it not feasible and/or safe for children, the elderly, frail individuals, and even most of the healthy adults. Fifteen 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 a very promising dietary interventions effective in extending not only longevity but also the healthy life span: the fasting mimicking diet® (FMD).

1.2. Fasting Mimicking Diet

The fasting-mimicking diet (FMD) 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, regenerate and rejuvenate itself. 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). Well-designed clinical trials, such as the study conducted by Houston et al. utilizing an independent review board (IRB) approved protocol, have demonstrated the safety and short-term benefits of plant-based nutraceuticals and food restriction in individuals with chronic hypertension (Houston et al., 2014).

A large ongoing randomized, open-label trial aimed at evaluating the effects of two different dietary interventions, FMD and LD, on body composition and cardiovascular (CV) biomarkers in a real word population (NCT05698654) is actually ongoing. This trial started in January 2024 will enrol 501 adult subjects between the ages of 30 and 65: 167 subjects randomized to the FMD arm with a 5-day meal program once every three months for a 6-month period (arm 1); 167 subjects randomized to follow the FMD plus a Longevity Diet program (FMD+LD) for a 6-month period (arm 2); 167 randomized to the control group (arm 3) that will continue their usual diet. On 2024,410, participants were enrolled and randomly assigned to FMD, FMD + LD, or control arm. Although preliminary data demonstrated the beneficial effects of such nutritional plans on body weight, BMI, body composition, and cardiovascular (CV) biomarkers, limited data is available on the long-term effects of these powerful nutritional interventions.

2. Study Objectives/Endpoints

2.1. **Objectives** The objective of the study is to determine long-term trends of a 5-day FMD meal program in patients already subjected to FMD in a previous trial (NCT05698654) and that will continue an FMD-based plan to assess its long-term effect on fat mass maintenance. The secondary objectives are to determine long-term trends of a 5-day FMD meal program in patients already subjected to FMD who will continue an FMD-based plan to assess its long-term effect on the maintenance of body weight, body mass index, blood pressure, IGF-1, haemoglobin A1c, fasting glucose, LDL, total cholesterol, C-Reactive Protein, and the proportion of participants who received medications at baseline.

2.2. Endpoints

2.2.1. *Primary endpoint*: maintenance of at least 50% of the fat mass lost during the previous trial and measured after one follow up year on FMD every 3 months

2.2.2. *Secondary endpoints:* maintenance of at least 50% of one or more risk factors improvements achieved during the previous clinical trial and measured after one year of follow up FMD cycles every 3 months including improvements in: body mass, body mass index, blood pressure, IGF-1, haemoglobin A1c, fasting glucose, LDL, total cholesterol, and C-Reactive Protein. Determine long-term trends in the reduction/stabilization in the use of antihypertensive and glucose-lowering drugs or other commonly used medications.

3. Study Design

Follow up clinical trial including participants of the approved studies "*Studio degli effetti della dieta mima-digiuno e della dieta della longevità sulla composizione corporea, fattori di rischio per malattie età-correlate e marcatori dell'invecchiamento in uno studio randomizzato" and "Studio degli effetti della dieta mima-digiuno e della dieta della longevità sulla composizione corporea, fattori di rischio per malattie età-correlate e marcatori dell'invecchiamento in uno studio randomizzato-2"* that will follow 5-day of the FMD meal program every three months, for one year.

Selection and withdrawal of subjects of the previous clinical study

3.1.1. *Inclusion Criteria*

Individuals already included in the aforementioned previous studies *who were assigned to one of the 2 intervention arms: Fasting mimicking diet or fasting mimicking diet plus Longevity diet* and who completed the study less than 6 months prior to starting the new study. Blood tests and a nutritional assessment will be conducted, serving as the baseline for the new study.

3.1.2. *Exclusion Criteria*

- individuals who are allergic to tree nuts (macadamia, cashew, almond, pecan), soy, oats, sesame, or celery/celeriac;
- pregnant females;
- any documented cancer diagnosis within the past 5 years;
- documented myocardial infarction within past 5 years;
- documented cerebrovascular accident within past 5 years;
- chronic steroid use (longer than 45 consecutive days);
- insulin-dependent diabetes mellitus;

- individuals taking insulin or insulin-like drugs and individuals taking hypoglycemic agents other than metformin. In this last case, close attention will therefore be paid to the self-monitoring of blood glucose during the FMD cycles;
- Individuals with severe hypertension (systolic greater than 200 mmHg and or diastolic greater than 105 mmHg).

3.1.3. Prohibited Medications, Medical Foods or Nutritional Supplements

- Change in prescription medications, over-the-counter (OTC) medications, medical foods, and nutritional supplements within 30 days prior to the start and for the duration of the study.
- Use of medications classified as narcotics 15 days prior start and for the duration of the study.
- Use of prescription medications and/or over-the-counter medications for acute and semi-acute medical conditions 15 days prior to start and for the duration of the study. Use of acetaminophen is permitted on an as-needed basis.
- Use of an investigational drug or participation in an investigational study within 30 days prior to the start and for the duration of the study.
- Use of anticoagulant medications (heparin compounds or warfarin) within 30 days prior to the start and for the duration of the study. Use of aspirin 400 mg once daily is permitted
- Subjects will not be allowed to discontinue prohibited prescription medications to meet enrolment criteria.

3.1.4. *Medical History and Concurrent Diseases*

- A history of allergy 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.
- A serious, unstable illness including cardiac, hepatic, renal, gastrointestinal, respiratory, endocrinologic, neurologic, immunologic, or hematologic disease.
- 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 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.

- 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.

3.1.5. *Substance Use*

- History of regular intake of >14 alcoholic drinks per week for females, and >21 drinks per week for males (1 drink = 35 cl. beer, 12 cl. wine, or 30 ml. hard liquor).

3.1.6. 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.

3.1.7. *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 (haematocrit < 33% or > 47%; WBC < 3.0 or > 12.0 $x10^3/mm^3$; platelets < 140 or > 500 x 10^9/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 $mmolL^{-1}$

3.1.8. *Women of Childbearing Potential*

Contraception: the effects of the study products on the developing human fetus have not been studied extensively. For this reason, women of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Females of childbearing potential will have a pregnancy test prior to receiving study products. Should a woman become pregnant or suspect 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.

3.2. Recruitment

The participants will be identified within the intervention groups of the previous clinical studies (*Studio degli effetti della dieta mima-digiuno e della dieta della longevità sulla composizione corporea, fattori di rischio per malattie età-correlate e marcatori dell'invecchiamento in uno studio randomizzato1 and 2*) who are willing to start a new study for other 12 months, and who completed the study less than 6 months prior to starting the new study and achieved the study's objectives. Blood tests and a nutritional assessment will be conducted, serving as the baseline for the new study. The recruitment period will last up to 3 years

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. When the visit doesn't include blood collection or BIA the visit can be made remotely. During the clinical visits, staff will review calendars, assess for signs and symptoms of adverse events, review compliance to the study product and answer any questions from the subject. Eligible participants will receive 3 different visits (Baseline visit (t0), and two follow-up visits (after 6 and 12 months from the baseline). Eligible participants will be telephonically contacted at the end of each FMD cycle (day 6) in order to assess signs and symptoms of adverse events, compliance to the nutritional intervention (see Appendix A, B), to answer to the questionnaire for dietary recall (see Appendix C).

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

	Screening Visit	Baseline visit (T_0)	Follow-up visit 1 (T_1)	Follow-up visit 2(T_2)
Informed Consent	√			
Review Eligibility Criteria	√			
Start of intervention				
Physical examination[1]		√	√	√
Vital Signs[2]	√	√	√	√
BIA		√	√	√
Peripheral Blood for genetic analysis		√		√
Hematological analyses[3]		√		√
Pregnancy test	√	√		√
AE and SAE collection		√	√	√
Primary and secondary endpoints assessment		√	√	√
Medications	√	√		√

[1] height, weight (dress weight), waist and hip circumferences. [2] Body temperature, blood pressure, pulse, respiratory rate. [3] Lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides, serum/plasma, albumin, alkaline phosphatase, insulin-like growth factor 1 (IGF-1), Insulin-like growth factor-binding protein 1 (IGFBP1), creatinine, insulin, C-reactive protein (CRP),

aspartate aminotransferase (AST), alanine aminotransferase (ALT), homocysteine, glucose, glycosylated hemoglobin (HbA1c).

Screening and Baseline visit

Potential participants already subjected to a 5-day fasting-mimicking diet (FMD) program either alone or in combination with the longevity diet previously enrolled in one of the two interventional arms and who completed the clinical study NCT05698654 less than three months before the enrolment period will be contacted *via* telephone to determine preliminary eligibility and interest. Once participants meet all inclusion/exclusion criteria, they will be offered by study staff the opportunity to learn more about the research opportunity before any procedure is performed. Participants meeting eligibility will be then invited to attend a baseline and two follow-up visits

Baseline visit will include measurement of height, weight, and vital signs (body temperature, blood pressure, pulse, respiratory rate), completion of medical history questionnaire, review of medical history and current medications, and collection of fasting blood (12 mL: 6 mL for serum, 6 mL for plasma). Blood testing will include: complete blood count (CBC), glycated hemoglobin (HbA1c), glucose, insulin, lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides), AST (GOT), ALT (GTP), alkaline phosphatase, creatinine, reactive C protein (PCR), albumin, insulin, IGF-1, IGFBP1, homocysteine as well as a urine pregnancy test in females of child-bearing potential. Fasting is defined as 12 hours of refraining from consumption of food and beverages though unlimited consumption of water is allowed and encouraged. Participants will be given three boxes of FMD together with ketone test strips to self-monitor urinary ketone bodies during the FMD cycles. Detailed information on the FMD plans will be also given during the visit. Baseline visit will also include body composition assessment using Bioelectrical Impedance Analysis (BIA), measurement of height, weight, and vital signs, medical history, current medications and a collection of fasting blood for the genetic analysis (25 mL: 7.5 mL for serum, 7.5 mL for plasma, 10 mL for white blood cells). Note, the typical draw volume for blood donation in Italy is ~450 mL. Blood testing information will be also retried from the screening visit.

Follow-up visits

The follow-up visits will be carried out after three (follow-up visit 1) and five (follow-up visit 2) FMD cycles (6 and 12 months), in order to evaluate the progress of the trial.

Follow-up visit 1

During the first follow-up visit, the study staff will assess the signs and symptoms of adverse events and review compliance to the FMD program. The visit will also include body composition assessment using BIA, measurement of height, weight, and vital signs, medical history and current medications. Participants will receive the FMD boxes for the remaining two cycles together with the ketone test strips for self-monitoring of urinary ketone bodies during the FMD cycles.

Follow-up visit 2

During the second follow-up visit, the study staff will assess the signs and symptoms of adverse events and review compliance to the FMD program. This visit will also include body composition assessment using BIA, measurement of height, weight, and vital signs, medical history and current medications. In addition, a collection of fasting blood for haematological and for the genetic analyses will be performed.

4.1. Additional assessments

4.1.1. Exploratory Genetics: Telomere length and epigenetic biological age (optional)

The study includes genetic sample at the screening visit that requires a separate informed consent form if the participant agrees to participate.

Exploratory genetic research studies are planned at the end of the study with the aim to evaluate the impact of FMD on telomere length used as a biomarker of individual ageing.

4.2. 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 subject's primary care giver for treatment of a specific clinical event.

4.3. Rescue Medications

Acetaminophen, may however, be used for mild headache or myalgia at a dose of 650 mg three times daily as needed.

4.4. Subject Compensation

Subjects will not be compensated for participation in this trial.

5. Study Product:

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

The subjects will be instructed to follow their usual diet plan, except for the 5 days when they will be eating the foods included in the FMD box. The FMD diet is made up 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 glycerine, natural flavour,

• 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.

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

5.1.1. Study Product Storage and Stability

Subjects will receive the FMD boxes before the start of the next FMD cycle. The study products will be handled and dispensed to subjects at room temperature at the study site, according to good clinical practices.

5.1.2. 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 products received and placed in storage area;
- dates and initials of 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 for this study will be disposed off according to good clinical practices.

5.1.3. Potential Risks

There is a possibility, however, that a subject could be sensitive to food ingredients in one or more of the food formulas and have an allergic or intolerance reaction. Potentially serious reactions may include face and throat swelling, trouble breathing, anaphylaxis (shock), convulsions, and death.

5.1.4. Potential Benefits

The FMD is designed to achieve the beneficial effects of fasting while providing micronutrient nourishment (vitamins, minerals and others) from which the body is deprived during fasting. It minimizes the psychological burden of pure fasting. The subjects will receive extensive medical testing

and these results will be available to the subjects after completion of their participation for their ongoing care.

5.1.5. 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. Subjects will not be asked to return FMD boxes.

6. Description of Specific Testing Modalities

6.1. Hematological analyses

Complete metabolic and lipid panels (after overnight fasting) will be completed at the Istituto Clinico "Prof. Dr. R. De Blasi" srl, Via Torrione Prol.to n. 55, in Reggio Calabria and analyzed immediately after the blood draw of each visit. Clinical and biochemical evaluations include lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides), serum/plasma, albumin, alkaline phosphatase, insulin-like growth factor 1 (IGF-1), Insulin-like growth factor-binding protein 1 (IGFBP1), insulin, C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), homocysteine, glucose, glycosylated hemoglobin (HbA1c).

6.2. Blood collection and storage

Blood samples will be collected and stored for future biomarker analyses. In particular, two 6.0 ml ethylenediaminetetraacetic acid tube of whole blood samples will be collected for each participant. One tube will be spun at 1300 g for 20 minutes at room temperature to generate supernatant (plasma), buffy coat and red blood cells which will be individually aliquoted into 1 ml, polypropylene, cryovials and stored at -80 °C for later analysis. The other 6.0 ml tube of whole blood will individually aliquoted into 1 ml and stored at -80 °C for later analysis as previously mentioned.

6.3. Blood Pressure Measurements

Subjects will not consume any caffeine or alcohol or use tobacco products within 6 hours of the blood pressure measurement. Subjects will have their blood pressure on each visit. Subject's blood pressure will be measured in the left arm 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.

6.4. Body Impedance Analysis

The BIA technique is based on the fact that lean tissues have a high water and electrolyte content, and thus provide 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 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 measurement on the Tanita® scale.

6.5. Telomere length (optional)

Telomere length will be measured in genomic DNA extracted from stored buffy coat samples by quantitative polymerase chain reaction (qPCR) using a method adapted from the one originally

described by Cawthon (2009). Telomere length will be performed on the blood samples collected before the dietary intervention and at the end of the treatment. Telomere length measurements will be carried out at the laboratory of Genetics of Department of Biology, Ecology and Earth Sciences, University of Calabria or at the University of Palermo.
Epig. Clock could also be evaluated.

6.6. Clinical and Research Laboratory Evaluations and Specimen Collection

6.6.1. Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products; appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health. The samples will be collected by specialized personnel.

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 which 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 congenital anomaly or birth defect
- 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.

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 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 in a plausible time relationship to 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 rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to 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 aetiology. There must be an alternative, definitive aetiology 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 by appropriate questioning and examinations and will be immediately recorded on 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 Pl'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 IRB requirements.

7.3.6. Reporting of Pregnancy

Should a woman become pregnant or suspects 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 haematological, chemical, and immunologic 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 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 more follow-up is required. This requirement indicates that follow-up may be required for some events after the patient discontinues participation from 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 it becomes 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:

a significant reduction of BMI greater than 15% from baseline measurement and in any case of a BMI measurement lower than 22 Kg/m^2 for women and 23 Kg/m^2 for men during the follow-up visits; any clinical adverse event, laboratory abnormality, concurrent illness, 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.

7.5.3. Assent or Informed Consent Process in Case of a Minor

Minors are excluded from this study.

7.6. 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 entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA, the Sponsor, or the sponsor's designee.

7.7. 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.

8. Statistical Analysis plan

8.1.

All participants meeting the inclusion criteria will be considered. Based on the current drop-out rate of 21% and 18%, for FMD or FMD+LD, respectively, observed during the completed study **"Studio degli effetti della dieta mima-digiuno e della dieta longevità sulla composizione corporea, fattori di rischio per malattie età-correlate e marcatori dell'invecchiamento in uno studio randomizzato" and assuming a similar drop out in the study: Studio degli effetti della dieta mima-digiuno e della dieta longevità sulla composizione corporea, fattori di rischio per malattie età-correlate e marcatori dell'invecchiamento in uno studio randomizzato-2" not yet completed, we can hypothetical foresee a maximum number of subjects of 267.**

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 10 years only. 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 Sponsor will be allowed to visit the study site periodically to assess the data, quality and study integrity. This will include reviewing study records, compare 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 Sponsor's auditors and government inspectors. They will be allowed access to source documents, electronic database, and other study files.

8.4. Source Documents and Access to Source Data/Documents

Study data will be collected on CRFs designed for the study if required by the Pl. The PI is responsible for assuring that the data collected 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 recording and/or reviewing 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 medical record has adequate knowledge that the patient is participating in a event based upon the 2016 American Heart Association and American College of Cardiology recommendations, compliance and age are planned. Additional subgroup analysis may be performed.

8.5. Safety Review

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

8.6. 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 change from baseline over time within arms and for differences between the intervention arm and the control arm of previous study.

8.6.1. Analysis supporting primary objectives

Generalized mixed linear models will be used to compare the fat mass percentage change between the value measured at the end of the trial vs the baseline value. The analysis will include a fixed term modeling the change of fat mass between baseline and the second follow-up visit (after 1 year), as well as a random term accounting for repeated measurements on the

same participants. We hypothesize that the coefficient associated with the fixed term (fat mass) will be either equal to zero (no change) or negative (reduction of fat mass).

8.6.2. Analysis supporting secondary objectives

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

We will select clinically relevant cutoffs and compared normal and at-risk subjects for each risk factor: total cholesterol >199 mg/dl and LDL cholesterol levels >130 mg/dl are associated with an increased risk for CVD, a fasting glucose >99 mg/dl indicates impaired fasting glucose/prediabetes, and triglyceride levels >100 mg/dl as well as CRP >1 mg/liter are associated with increased risk for CVD. For serum IGF-1, no clinically relevant risk level has been established, but a number of epidemiological studies have associated IGF-1 levels above 200 ng/ml with various cancers (Levine et al., 2014; Pollack et al., 2007). We will therefore compare the effect of FMD cycles on subjects in the highest quartile of IGF-1 expression (>225 ng/ml) with that on subjects with IGF-1 levels ≤225 ng/ml. The effect of FMD and FMD+LD treatments on these risk factors will be assessed also stratifying our sample by baseline BMI, SBP and DBP, fasting glucose, cholesterol, CRP and IGF-1.

8.6.3. Safety and Tolerability/Acceptability Evaluation

Collected data from subjects will be reviewed for 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.

8.6.4. Subgroup analyses

To explore the effect of treatment in subgroups, the estimated effect of the nutritional interventions will be estimated for each of the subgroups listed below will be derived. 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 of the subgroups based on the baseline measurements: baseline fat mass (≥ 20% and ≥25% for men; ≥ 30% and ≥ 35% for women), age groups (30-50, 51-70 years), gender, baseline BMI (25-29.9; 30 and over);

8.7. Interim analysis

We will perform an interim analysis of the results obtained by participants enrolled who have completed the 6 months of treatment in order to determine the efficacy of the treatment to positively affect primary and/or secondary endpoints of the study. Statistical analysis will be performed as mentioned in section 8.6.1 and 8.6.2

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 that is initiated prior to the individual agreeing to screen for and participate in the study and continuing throughout the individual's study participation. Extensive discussion of 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 previous 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.

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, and always safeguarding the right of privacy and anonymity according to the rules of General Data Protection Regulation (GDPR) of the European Union (UE) 2016/679.

This study will be initiated only after all required legal documentation has been reviewed and approved by the University of Palermo Ethic Committee, Comitato Etico Locale, CEL) according to the national and international regulations. The same applies for 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 in liquid nitrogen or at minus 80°C for 15 years under liquid nitrogen at the Laboratory of Genetics of the University of Calabria or University of Palermo and thereafter destroyed. They will undergo a coding process with measures taken to ensure that

specimens are kept under correct conditions always when it is stored. Each sample will be assigned to an identification code, which will be used by researchers and will prevent third parties from identifying the study participants from their samples. Encryption and access passwords will be adopted to protect and safeguard the stored samples.

10. References

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INFORMATION SHEET AND INFORMED CONSENT FORM FOR PATIENT PARTICIPATION IN A CLINICAL TRIAL

Official title of the trial Follow up on the effects of the mimic fasting diet on metabolic risk factors in individuals who participated in the previous study REF NCT05698654.
Official title of the trial in terms more understandable to the patient Analysis of the effects of an additional four cycles of mimic fasting on body composition
Structure-context in which the trial will take place Participants will be recruited from participants of the previous clinical trial.
Coordinating centre *(if different from the facility where* **the trial** *will take place)* **and trial coordinator** Coordinating Centre **Valter Longo Foundation** Trial Coordinator Dr. Romina Cervigni (Fondazione Valter Longo, European Longevity Institute, Milan), Prof. Valter Longo (*IFOM-Fondazione Istituto FIRC di Oncologia Molecolare, Milan)*
Principal Investigator *(please indicate the local Head of the trial)* Name: Dr Romina Inès Cervigni
Sponsor/Funding body Valter Longo Foundation
Ethics Committee University of Palermo

This document consists of the following sections:

A. PREMISE
B. INFORMATIVE SECTION. SUMMARY OF THE TRIAL: KEY INFORMATION
C. INFORMATIVE SECTION. FURTHER INSIGHTS
D. EXPRESSION OF CONSENT SECTION

ANNEXES

ADDITIONAL DOCUMENTS

Dear Ms/Mrs, the information contained in the following information sheet is very detailed. We ask you to agree to take part in the trial ONLY after you have read this information sheet carefully and have had an EXTREMELY CLEAR talk with a member of the trial team who will have to take the TIME REQUIRED to fully understand what is being proposed to you.

A. PREMISE

Dear Madam/Sir,

We propose that you take part in the clinical trial, which is described below.

It is your right to be informed about the purpose and characteristics of the trial so that you can make an informed and free decision whether to participate.

The purpose of this document is to inform you about the nature of the trial, its purpose and what participation will entail for you, including your rights and responsibilities.

Please read the following carefully. The researchers involved in this project, indicated at the beginning of this document, are available to answer your questions. No question that comes to your mind is trivial: do not be afraid to ask it!

In addition to us, you can discuss the proposal contained in this document with your family doctor, your relatives and other people you trust. Take all the time you need to decide. You can take an unsigned copy of this document home to think about it or to discuss it with others before making a decision.

If you decide not to participate in the trial you will still receive the best possible care for patients with your condition/disease.

Your refusal will in no way be interpreted as a lack of trust.

Once you have read this form, had any questions answered and possibly decided to participate in the trial, you will be asked to sign a consent form, a paper copy of which you will receive.

The Principal Investigator

B. INFORMATION SECTION.
GENERAL SUMMARY OF THE TRIAL: KEY INFORMATION

The purpose of this section is to briefly present the key aspects of the trial we are proposing you to join. Subsequent sections will provide more detail in order to give you the opportunity to give or not to give fully informed consent to your participation in the trial.

- What are the objectives of the trial? How many centres and patients will take part?

The trial is a continuation of the previous clinical study carried out in Varapodio investigating the effects of the mimic fasting protocol alone or in combination with the longevity diet.

This new study involves the continuation of four cycles of mimic fasting over a 12-month period, with visits at month six and month twelve.

- Is it my free choice whether or not to participate?

You can freely choose whether or not to participate in the trial. Even after accepting, you can change your mind at any time.

- What happens if I decide to take part in the trial?

If you decide to take part in the trial, you will be given the mime fasting protocol and will continue to be followed step by step by the Valter Longo Foundation Scientific Team.
***No** planned invasive procedures (e.g. biopsies, bone marrow sampling, etc.) are planned during the trial.*

The full schedule of examinations and tests scheduled during the trial is given in the section next 'What examinations, tests and procedures are involved in the trial?

- What are the risks and benefits if I participate in the trial?

Both risks and benefits may arise from participating in the trial. It is important to weigh these carefully before making a decision.

Expected benefits
1) *By joining the trial you will have the opportunity to continue the nutritional intervention*
This nutritional intervention could improve your overall health with a reduction in fat mass, blood pressure, markers of inflammation status and a reduction in your biological age.

2) *Joining the trial will contribute to the development of knowledge on the effects of low-calorie and longevity diets on human health. Such diets, in fact, could have beneficial effects on her health status and the health status of the population by slowing down/reducing the adverse effects on human health of already known risk factors (hypertension, body mass index, inflammation) for human ageing.*

Potential risks

We want to make sure you understand from the outset what some of the possible risks are: additional information can be found in the following section 'What risks may I face if I participate in this trial'?

There are risks and discomforts associated with the mimic fasting diet, such as hunger, anxiety, drowsiness, dizziness, headaches, muscle pain, fatigue, low blood pressure and, in rare cases, fainting. These dietary interventions can also cause abnormal heart rhythms, short-term nutrient deficiency and a weakened immune response. A long period of low-calorie dieting can be particularly dangerous in people who are already malnourished. There are also the risks and discomforts associated with possible allergies/intolerances to foods in the diet (not known prior to participation in the study).
During the diet period, participants should drink an adequate amount of water to prevent dehydration and avoid strenuous activities/exercises. 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 should contact the investigator, consult
n personal physician or seek immediate medical attention if they have any questions regarding the study or in case of need.
At the end of the diet, participants should gradually resume their normal diet, starting with liquid foods such as soups and juices, accompanied by light meals.

Participants may feel dizzy when blood is drawn. In rare events, participants may experience bruising, excessive bleeding, infection, dizziness and fainting. Participants may stop the blood sampling procedure at any time. Participants should contact their personal physician or seek immediate medical attention if bleeding or infection related to the blood draw occurs.

- Is consent final? Can I decide to withdraw from the clinical trial (voluntary exit)?

You may decide to withdraw from the trial at any time and for any reason, without having to justify your decision. If you decide to stop participating, please let one of the investigating doctors know as soon as possible: it is important to stop the treatment safely. The doctor may consider a final check-up/examination appropriate.

The investigators will keep you informed of any changes in the trial that may affect your willingness to participate.

- Are there any reasons why the trial could be terminated not of my own volition (early termination)?

Yes, the investigators may decide to terminate your participation in the trial if:

- *his health condition should change and participation in the trial would be potentially harmful.*
- *new information became available and the trial was no longer in his best interest*
- *you do not follow the agreed rules for participation in the trial.*
- *For women: you happened to start a pregnancy during the trial.*
- *The trial is terminated by the competent authorities or the sponsor.*

IN ANY CASE MAKE EXPLICIT THE NECESSITY/APPROPRIATENESS OF CONTINUING PLANNED FOLLOW-UP VISITS IN CASE OF WITHDRAWAL OF CONSENT, DISCONTINUATION OF THE TRIAL, PREGNANCY OR OTHER.

C. INFORMATION SECTION. FURTHER DETAILS

1. What is the purpose of the trial?

With the increase in life expectancy in Western countries over the last century, ageing in good health is an important public health goal.

Fifteen years of extensive pre-clinical and clinical studies sponsored by the National Institutes of Health (NIH) and conducted at the Longevity Institute and the Diabetes and Obesity Research Institute of the University of Southern California (USC) have led to the development of two very promising dietary interventions effective in slowing down the human longevity ageing process, but also in improving the quality of life: the Diet for Minimal Fasting (FMD).

The aim of the study is to determine the effects of these two different dietary interventions on body composition, cardiovascular (CV) biomarkers (body weight, BMI, blood pressure, serum lipid levels and blood measurements of dysglycaemia) and biological ageing, on the reduction in the use of hypoglycaemic or antihypertensive drugs and on sleep quality.

2. What examinations, tests and procedures are envisaged if I participate in the trial?

During the visits the following will be carried out

- bioimpedance analysis for the study of body composition;

- assessment of vital parameters including blood pressure

- venous blood sampling including fasting blood glucose, glycated haemoglobin, lipid profile, C-reactive protein, albumin, creatinine, IGF-1, IGFBP3, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, homocysteine, and complete blood count.

- Telomere length on DNA extracted from peripheral blood by RT-PCR.

The blood samples, approximately 25 ml per visit, will be used both for haematological analysis and as a source for DNA extraction.

3. How will I be informed of any unexpected results following in-depth diagnostics?

In the current state of knowledge, including that relating to epigenetic clocks, no results will emerge from the performance of the tests envisaged in the trial concerning your predisposition to the future development of particular diseases or your status as a carrier of a genetic disease, which could lead to the generation of sick children.

4. What will be my commitment and responsibility if I decide to participate? (section no longer than ½ page)

- *Scrupulously observe the instructions and requests of the medical personnel following the trial and ensure attendance at appointments.*
- *Inform the doctor supervising the trial:*
  - *of all the drugs he/she is taking, including drugs from unconventional medicine;*
  - *of any side effects arising during the course of the trial;*
  - *of any visit or hospitalisation in facilities other than the investigating centre;*
  - *current or previous participation in other clinical trials.*
  - *avoidance of pregnancy or lactation during the trial.*

o

5. Will I incur any costs for participating in the trial? Will I be reimbursed for any expenses? Will I receive compensation?

You will not incur any costs from taking part in the trial as these are fully covered by the Valter Longo Foundation. There is also no provision for financial compensation for taking part in the trial.

6. What happens if I suffer damage as a result of taking part in the trial?

Participation in a clinical trial may entail inconveniences and risks which cannot be determined beforehand. This is why the clinical trial provides for insurance coverage to protect your participation.

In accordance with the laws in force, an insurance policy is in place to cover any damages suffered as a result of participation in the trial, for the entire period of the trial, covering the civil liability of the investigator and the promoter.

It should be noted that, according to the Ministerial Decree of 14 July 2009, the insurance policy does not cover the value in excess of the maximum sum insured and is only operative for damages for which a claim has been made no later than the period provided for in the policy (36 MONTHS). *However, this limitation does not affect his right to obtain compensation from the party responsible for the eventual damage* (to protect the trial subject).

7. How will my health data, including identification data, be processed and who will have access to them during the trial?

His or her data, in particular personal and health data and only to the extent that they are indispensable in relation to the objective of the trial and for pharmacovigilance purposes, will be processed in compliance with EU Regulation 2016/679, known as 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 safe place and will not bear his or her name in plain text, known only to researchers, but an identification code.

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

Your clinical data collected for the purpose of the trial, as well as the results of the examinations performed, will be kept for a maximum period of 15 years and subsequently destroyed. They will only be destroyed if a) it is no longer possible to trace them back to his identity, because they have been anonymised in the course of the trial itself;
b) in the presence of his specific informed consent.
In the event that personal data is transferred to a third country or international organisation, all safeguards provided for in Article 46 of GDPR 679/2016 relating to the transfer will be adopted.

Further information is included in the attached data processing authorisation form.

8. How will my biological samples taken for the purposes of the trial be processed and who will have access to them?

In the same way as for your health data, your biological samples, pseudonymised (a technique that allows personal and sensitive data of a natural person to be modified and masked so that they cannot be directly and easily attributed to that person), will also be used for the purposes of the trial.
Once the trial is over, its samples will be labelled with a numerical code and stored at -80°C in the Genetics laboratory of the University of Calabria. Access to the samples and data will be restricted to the personnel involved in the study. With this form we are also asking you for

authorisation to keep your DNA samples for a maximum of 15 years after the end of the study, for future research on the ageing process. In this case, the opinion of the Ethics Committee on new genetic research studies will be requested.

9. How will I get access to the results of the trial?

Once the trial is over and all the resulting data have been collected, they will be analysed to draw conclusions. The investigators and the sponsor undertake to make them available to the scientific community.

The standard provides for participants' access to the results of the trial. Therefore, you may ask the researchers involved in the trial to inform you of the general results of the trial.

10. Has the trial been approved by the Ethics Committee?

The protocol of the trial proposed to you has been examined and approved by the Ethics Committee of the University of Palermo. Among other things, the Ethics Committee verified that the trial complied with the Standards of Good Clinical Practice and the ethical principles expressed in the Declaration of Helsinki and that your safety, rights and well-being were protected.

11. Who can I refer to for more information about the clinical trial in which I am invited to participate?

DR. ROMINA INÈS CERVIGNI
SCIENTIFIC RESPONSIBLE VALTER LONGO ONLUS FOUNDATION
EUROPEAN LONGEVITY INSTITUTE
MILAN, LOMBARDY, ITALY TEL.
3397805607

PROF VALTER LONGO
IFOM-FIRC Institute of Molecular Oncology Foundation
MILAN, LOMBARDY, ITALY
TEL. 0257430 3801

12. If I take part in the trial, who can I contact in case of need?

For any doubts and unplanned or unplanned events during the trial (doubts about the treatment being carried out, side effects, decision to abandon the trial, etc.), you may contact

DR ROMINA INÈS CERVIGNI
SCIENTIFIC RESPONSIBLE VALTER LONGO ONLUS FOUNDATION MILAN,
LOMBARDY, ITALY
TEL. 3397805607

PROF VALTER LONGO
IFOM-FIRC Institute of Molecular Oncology Foundation
MILAN, LOMBARDY, ITALY
TEL. 0257430 3801

If you feel it is appropriate to report events or facts relating to the trial you have joined to subjects not directly involved in the trial itself, you may refer to the Ethics Committee of the University of Calabria which approved the trial.

_____/___/_____

Full name of researcher Date Time Signature

who delivered the information

D. EXPRESSION OF CONSENT SECTION

(Notes: 1 copy for the participant, 1 copy for the trial manager)

I, the undersigned _____

born_____on __/___/_____

DECLARE

▢ that I have received from Dr. _____exhaustive explanations regarding the request to participate in the research in question, according to the information section, which is part of this consent, a copy of which was delivered to me on _____at _____*(indicate date and time of delivery*);

▢ that the nature, purpose, procedures, expected benefits, possible risks and inconveniences, and alternative treatment modalities to the proposed clinical trial have been clearly explained to me and I understand;

▢ to have had the opportunity to ask any questions to the investigator of the study and to have received satisfactory answers;

▢ had sufficient time to reflect on the information received

▢ that I have had sufficient time to discuss it with others

▢ that I have been informed that the protocol of the trial and all the forms used have had the favourable opinion of the relevant Ethics Committee

▢ that I am aware that the research may be interrupted at any time, by decision of the person in charge of the research;

▢ that I have been informed that I will be made aware of any new information that may compromise the safety of the research and that, for any problems or further questions, I may refer to the doctors under whose care I am being treated;

▢ that for the best protection of my health I am aware of the importance (and my responsibility) of informing the general practitioner of the trial in which I agree to participate. I am aware of the importance of providing all the information (medication, side effects, etc.) concerning me to the investigator;

▢ that I have been informed that the results of the study will be made known to the scientific community, protecting my identity in accordance with current privacy legislation

▢ that I am aware that any choice expressed in this consent form may be revoked at any time without justification;

▢ that I have received a copy of this consent form.

I therefore DECLARE that

▢ I wish		participate in the trial
▢ WANT	▢ DO NOT wish to	be informed of any unexpected information concerning my health present or future that may incidentally arise from the investigations foreseen in the trial, including genetic investigations, when this may lead to possible benefits
▢ want	▢ DO NOT want	be informed of unexpected news concerning my health present or future only when it may be useful for my health care or to enable me to make informed reproductive choices
▢ want	▢ DO NOT want	to be contacted again after the end of the trial to provide information about my health status (applies only for contacts not foreseen as follow-up in the study protocol)

_____/___/_____

Full name of adult patient Date Time Signature