TITLE OF THE STUDY

"Combined interventions of amino acid supplementation, diet and exercise in patients

Older Adults With Dyanpenic Obesity: Effects on Physical Frailty Syndrome – MAYBE Dyna Study-

Ob".

ABBREVIATED TITLE: MAYBE- Dyna -Ob

SIGLA: MAYBE

PROMOTER: Azienda Ospedaliera Universitaria Integrata Verona (AOUI-VR)

UOC Geriatria B

Principal Investigator

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In collaboration with

- University of Brescia (UNIBS)

- University of Padua (UNIPD)

- University of Milan (UNIMI)

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List of abbreviations: BMI =Body Mass Index; miRNA = micro RNA; Exe-L =

exercise with moderate intensity levels; Exe-H = exercise with intensity levels

High; AA-1 = low dose of amino acid mixture; AA-2 = full dose of mixture

aminoacidica; Dyna-Ob = dynapenic obese; SPPB = Short Physical Performance Battery; BIA

= bioimpedenziometria; DXA = dual-energy X Ray Absorptiometry; PASE = Physical Activity

Scale for the Elderly; PBMC = cellule mononucleate del sangue periferico.

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BACKGROUND

Although life expectancy has increased in industrialized countries, older people are spending more

several years of their lives in conditions of disability, and especially women.

Among the many diseases and chronic conditions that can lead to disability

of the elderly, frailty syndrome is perhaps the most significant. The

fragility is a condition characterized by a decreased functional reserve and a reduced

resilience and resilience following pathological events (Clegg et al., 2013; Rodríguez-Mañas

et al., 2013). Early diagnosis and prevention are crucial in this scenario, but unfortunately not

Health care programs or drug treatments for seniors are available

largely due to the lack of a precise and operational definition of the syndrome

of fragility, due in turn to the multidimensional nature of this condition.

In the literature it has been shown that sarcopenia, a condition of quantitative alteration and

of skeletal muscle mass (Cruz-Jentoft et al., 2010), represents the

central biological substrate of fragility (Landi et al., 2015). The prevalence of sarcopenia

increases with age and has been estimated to be in the order of 5-13% in subjects between 60 and 70 years of age for

increase by up to 50% among individuals aged 80 years and older (Janssen, 2011). In addition, the

reduction in muscle mass is significantly and independently associated with disability

in older subjects and in particular in women (Landi et al., 2015). The most advanced studies

argue that the phenotype of physical frailty substantially overlaps with that of sarcopenia

(Morley et al., 2013; Calvani et al., 2015) and pathogenic mechanisms are hypothesized to be common

to the two clinical conditions.

At the same time, the global geriatric population is witnessing a global health challenge

linked to the epidemiological increase in obesity even in the elderly population. The index of

Average body mass (BMI) is increasing in the elderly population in both sexes (Finucane

et al., 2011). Importantly, obesity exacerbates related functional decline

age and also contributes to frailty (Buch et al., 2016). In addition, obesity in the elderly

visceral (characterized by excess abdominal fat) is associated with fat dysfunction

subcutaneous, with spillover of fats to other organs, and ultimately induction of insulin resistance

(Tchernof and Després, 2013). Intramyocellular lipid accumulation and/or

formation of intermuscular adipocytes that are associated with obesity contribute to the deterioration of

functional. Intermuscular fat deposition is considered a key responsible factor

of decreased muscle function even in non-obese elderly subjects. It has been hypothesized that

that the most common frailty phenotype in the coming decades may be that of a

obese, sarcopenic and disabled, i.e. suffering from so-called sarcopenic obesity (Villareal et al.,

2011). In this context, in fact, the loss of lean mass, together with the accumulation of visceral fat and

with the consequent metabolic alterations, would represent key mechanisms in the

pathogenesis of frailty in geriatric age (Buch et al., 2016). While the complex mechanisms

etiopathogenetic disorders still remain to be clarified, it has been hypothesized that the age-related decrease of the

mitochondrial function plays a key role in the frailty phenotype (Buch et al., 2016). In

mitochondrial alterations (changes in biogenesis and mitochondrial dynamics)

they participate in the energy decline of the elderly subject (Nisoli and Valerio, 2014; Valerio and Nisoli,

2015) and their contribution to the pathophysiology of frailty deserves to be investigated. In addition, in the

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specific miRNAs (small non-coding RNAs that

regulate gene expression by interaction between miRNAs and mRNAs), called myo-miRNAs

which could play a significant role in muscle atrophy and regeneration

skeletal (Horak, 2016). My-miRNAs would in fact be able to modulate different processes

pathophysiological factors, including the switching of the type of muscle fibre, the activity of the

neuromuscular junction, the degree of inter- and intramuscular lipid infiltration, and the activation of

satellite cells (Brown and Goljanek-Whysall, 2015). In addition, miRNAs have a regulatory capacity

translational within mitochondria and some mitochondrial miRNAs have recently been described

characteristics of human skeletal muscle (Latronico and Condorelli, 2012). Potentially, the

muscle and/or mitochondrial miRNAs could be modulated in aging. Therefore

studies aimed at exploring the role of miRNAs in sarcopenia and their

contribution in the pathogenesis of frailty. In addition, the dosage of these miRNAs in the blood

could provide biomarkers of disease and monitoring of therapeutic interventions.

The efforts of geriatric clinicians and basic researchers in gerontology to identify

interventions useful for preventing the frailty syndrome of the elderly are hindered by the lack of

a single, standardised and universally accepted operational definition for this condition. A

The critical point remains that of the variety of clinical phenotypes that makes it difficult to define

diagnostic tools useful to support diagnosis, monitor the progression of the disease in the

time and response to interventions (Calvani et al., 2015). Although various clinical-diagnostic definitions

have been proposed so far, the identification of biomarkers could lead to a

significant progress in this context (Calvani et al., 2015) particularly in a

prevention perspective, to identify sarcopenic individuals at risk of physical frailty

before functional decline reaches a critical threshold, beyond which recovery is not

possible.

The present study is part of a larger project1 that aims to respond to this ambitious

thanks to the multidisciplinary effort involving researchers from different disciplines and

geriatricians.

Preclinical studies using mouse models of aging are currently underway to

evaluate different measurable aspects of physical frailty (mitochondrial bioenergetics in muscle and

circulating peripheral blood cells, circulating miRNAs, inflammatory cytokines), in parallel with the

multidimensional assessment of physical performance similar to that used in humans.

Statistical approaches of multivariate analysis and data mining will be applied to lead to the

definition of a multidimensional tool for the diagnosis of physical frailty. The panels

diagnostic tests identified in preclinical studies will then be applied in elderly subjects for

confirm their reliability in the early diagnosis of physical frailty syndrome,

allowing it to be monitored over time.

In parallel, preclinical studies are currently investigating interventions that can restore

mitochondrial efficiency in conditions of muscle dysfunction, based on previous studies

published by the research group of the study coordinator (Nisoli et al., 2005; D'Antona et al.,

2010). In particular, the ability of dietary supplementation with formulas is being investigated

1 “Multicomponent Analysis of phYsical frailty BiomarkErs: focus on mitochondrial health – MAYBE”

(Fondazione Cariplo, cod. 2016-1006)

balanced amino acids, similar to those that have been shown to restore mass and function

mitochondrial deficiency in aging muscle (D'Antona et al., 2010), in addition to the

caloric and exercise restriction in normalizing biomarkers and reversing the phenotype of

frailty in aged mice.

In the present clinical trial geriatricians will recruit sarcopenic-obese elderly subjects of both

to assess response to treatment with a balanced amino acid mixture

(branched-chain amino acids and essential amino acids) in addition to the standard interventions for this

clinical condition, based on diet and exercise (Villareal et al., 2011; Villareal et al., 2017).

In collaboration with partner centres, in parallel with the multidimensional and

geriatric physical performance (Villareal et al., 2011) the

modifications of the biomarkers mentioned above (mitochondrial bioenergetics in circulating cells

of peripheral blood, circulating miRNAs and cytokines).

1. STUDY DESIGN

Single-center, open-label, 6-arm randomized experimental trial with ratio of

allocation 1:1:1:1:1:1. Participants will be randomized into two groups with protocol of

exercise at different intensity levels: at high intensity (Exe-

H). For each level of exercise, participants will be further randomized into groups

who will receive (double-blind) dietary supplementation with placebo,

or full dose of amino acid mixture (AA-2).

Summary of the study

The study will be conducted in a group of obese dynapenic patients (Dyna-Ob) aged > 65 years in

moderately low-calorie diet therapy aimed at comparing the effect of different levels of exercise

combined or not with dietary supplementation with amino acids, on physical performance,

muscle strength, body composition, muscle oxidative metabolism, and biomarkers of

sarcopenia. The supplementation intervention will be conducted in a double-blind manner. In Figure 1 it is

the scheme of the study is represented.

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2. POPULATION

Obese dynapenic subjects (Dyna-Ob) on moderately low-calorie dietotherapy afferent to

Nutrition clinics of the Clinical Nutrition Service of Geriatrics B of the AOUI of Verona

with characteristics capable of meeting the following eligibility criteria:

Inclusion Criteria

· diagnosis of Dyna -Ob: definition (see materials and methods)

· male and female subjects

· Age between 65 and 80 years

· BMI > 30 kg/m2

· residents of Verona (Italy)

· stable weight (in the last 2 months)

· previously sedentary (less than an hour of exercise per week in the previous 6

months)

· signing of informed consent for participation in the study

Exclusion Criteria

· Unstable angina or recent myocardial infarction

· Malignant or unstable arrhythmias (ventricular tachycardia, second or third degree AV block, atrial flutter,

junctional rhythm)

· NYHA class > II heart failure

· Severe respiratory failure

· Severe heart valve disease (severe aortic stenosis)

· Abdominal and/or thoracic aortic aneurysm

· Recent intracerebral or subdural hemorrhage

· Poorly controlled arterial hypertension

· Presence of pacemakers or metal prostheses

· Severe chronic renal failure

· Symptomatic musculoskeletal pathology

· Symptomatic herniated disc

· Symptomatic osteoarthritis

· Acute joint, tendon and ligamentous injuries and pathologies

· Hip, knee prosthesis of recent placement (<6 months) or with joint instability

· Symptomatic or large inguinal or abdominal hernia

· Acute retinal detachment or bleeding

· Recent eye surgery (laser, cataract, retinal surgery, glaucoma surgery)

· History of malignant oncological disease within the previous 5 years

· Diagnosis of dementia

· Eating disorders

Ongoing exit from the study and deviations to the protocol

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Subjects may withdraw from the study via withdrawal of informed consent. It will be recorded

any deviation from the clinical protocol, such as the subject's decision to

not to continue the follow-up, to change the group to which they belong or to stop the treatment.

3. DESCRIPTION OF THE CHARACTERISTICS OF THE SUBJECTS UNDER CONSIDERATION

Subjects with BMI > 30 kg/m2 and low

BMI-indexed lean mass values (Lee et al., 2013; Morley et al., 2011)

4. OBJECTIVE OF THE STUDY

In general, the objective of the study is to evaluate the effect of different types of supplementation

amino acid (placebo, supplementation with amino acids at different doses) on performance

physical and biomarkers of frailty in obese dynapenic subjects aged > 65 years in diet

moderately low-calorie. The primary outcome is the quantitative Short Physical Performance Index

Battery (SPPB).

Similar studies published in the literature conducted on dynapenic-obese elderly subjects of both

who evaluated the response to a treatment with a balanced amino acid mixture and with

diet and exercise (Villareal et al., 2011; Villareal et al., 2017) support an assessment

after 5 months of intervention on the primary outcome of our study.

Primary Objectives

The aim of the experiment is to evaluate after 5 months of treatment, compared to the primary outcome

(SPPB), the effect of the interaction between:

A. the "amino acid intake" factor (3 levels) and the "exercise level" factor (2 levels);

B. the "amino acid intake" factor (3 levels) and the "gender" factor (2 levels).

Secondary Objectives

To evaluate the effect of the above interactions (A, B) after 9 months of treatment on SPPB and after

5 and 9 months of treatment with respect to biomarkers of fragility such as:

- muscle strength (dynamometer)

- muscle mass and muscle quality (bioimpedance analysis and DEXA)

- miRNA assay in circulating blood

- mitochondrial bioenergetics in PBMC

- blood levels of cytokines

- telomere length in PBMC

5. STUDY ENDPOINTS

Endpoint primari

- Short Physical Performance Battery (SPPB score) in T0, T5.

Secondary endpoints

· Short Physical Performance Battery (SPPB score) in T9,

· Muscle strength of the dominant limb (kg) in T0, T5, T9

· Bio-impedance analysis: skeletal muscle mass (kg and %) and fat mass (kg and %) in T0,

T5, T9

· DEXA: Total Body Fat free Mass (Kg e %), Total Body Fat mass (Kg e %) e Appendicular

Fat free Mass (Kg e.%) in T0, T5, T9

6. DESCRIPTION OF THE TREATMENTS

Recruitment of Obese Dynapenic Subjects (Dyna -Ob)

Subjects evaluated for admission to the study will be selected from among the afferent subjects

to the Geriatrics B Nutrition clinic of the AOUI of Verona who will be contacted

telephonically. A study-specific screening visit (baseline visit) will then be scheduled during

which will evaluate the inclusion and exclusion criteria of the study and the

subject to sign informed consent.

In case of confirmation of participation in the study and consequent signing of consent, the subjects

will be phenotypically characterized as obese dynapenic subjects in the

screening.

Random assignment of subjects to one of the three supplementation treatment groups will be

made by means of a block randomization list of 8 with an allocation ratio of 1:1:1

subjects stratified by level of exercise (2 levels). The list will be created using the program

Stata 14.2 (StataCorp. 2015).

Procedure

Dyna -Ob patients of both sexes aged 65-80 years will be recruited. All

Subjects who will undergo nutritional intervention with moderately dieting

aimed at achieving weight loss of 5% at 6 months and 8-10% at 12 months. In addition, all the

Participants will be randomized into groups with exercise protocol at different levels

intensity: at high intensity (Exe-H). For each level of

exercise, participants will be further randomized into groups that will receive (in

double-blind) a dietary supplementation with placebo or full dose of amino acid mixture (AA-2).

All subjects will undergo:

-T0 (enrolment): medical examination and anamnesis collection, functional and psychological questionnaires,

anthropometry, body composition (BIA and DEXA), Short Physical Performance Battery

(SPPB), measurement of muscle strength in the upper limb (handgrip), blood sampling (for

blood chemistry tests, miRNA assay, PBMC extraction), indirect calorimetry for

assessment of basal energy expenditure.

- T5 and T9: same evaluations (except calorimetry)

Nutritional intervention

All obese dynapenic subjects will undergo moderately low-calorie diet therapy

aimed at achieving a weight loss of 5% compared to the initial weight in 6 months and 8-10% compared to the

to the initial weight in one year. The calorie restriction initially proposed will be 500 Kcal per day

below the resting energy expenditure calculated by indirect calorimetry, multiplied by

1.4. Each subject will receive nutritional claims that provide 60% carbohydrates, 25%

fat, 15% protein and 20 g of fiber, divided into three meals and two snacks; in particular, the share

protein will be calculated in such a way as to guarantee 1 g of protein per kg of ideal body weight. The

compliance with dietary treatment will be assessed by clinical-nutritional follow-up every 3

Months. The prescription of calorie restriction will be adjusted in order to achieve the decline

expected weight.

Amino acid blend supplementation

Patients will be randomized into double-blind groups who will receive different dosages of a

mixture of oral amino acids (Amino-Ther see attached data sheet) or a product

isocaloric containing maltodextrin instead of amino acids (see attached data sheet) (Aquilani

et al., Arch Gerontol Geriatr 52:e123–e128, 2011).

Especially:

- Placebo groups: one sachet of Placebo in the morning and one in the afternoon, for a total of 0 g

aminoacidi/die;

- Groups AA: one sachet of amino acids (4 g) in the morning and one sachet of amino acids (4 g) per day

afternoon, for a total of 8 g amino acids/day.

The sachets will be dissolved in half a glass of water and taken between meals, mid-morning

and mid-afternoon. On the days when the exercise is carried out, they will be taken 1 hour before

exercise sessions.

Physical activity

All subjects will participate in a supervised muscle strength training program

lasting 9 months at the Sports Science facilities of the University of Verona. The goal

The main one is the increase in strength and muscle mass. In dynapenic patients, training at the

Strength can provide benefits in terms of body composition, muscle strength and functional capacity

(Liao et al., 2017). To achieve this goal, strength training will be prescribed following

le linee guida dell’American College of Sport Medicine (ACSM, 2009).

The proposed exercises will mainly stimulate the development of resistant strength and

marginally of maximal strength. Some exercises will stimulate large muscle groups

(quadriceps, glutes, pectorals). Other exercises will stimulate smaller muscle groups (triceps,

biceps, calf). Together with the exercises on isotonic machines, some exercises will be carried out at

floor exercise. The exercises will remain the same for each intervention group, but the intensity at the

which will be carried out will be modulated differently.

The proposed training modality has been repeated in numerous intervention studies and has been shown to be

effective and safe. In particular, for the type of patients to whom this study is addressed, it is not possible to

presents risk because: it is a type of activity in which contacts are avoided, the speed of

performance of the exercises are limited, patients are monitored during the entire duration of the

training sessions and workloads increased progressively (the patient has the

time to adapt to different workloads based on the improvements achieved in the

previous training) (Huang et al., 2017).

Patients will be involved in:

- High intensity group (Exe-H): 3 times a week, 3 sets will be performed for each exercise

8-12 repetitions at an intensity between 70-85% of 1 RM, 1-2 minutes of recovery between

series and the other. The measurement of 1 RM will be carried out for each type of exercise by means of a test at

indirect loading, safer and less risky for the patient. The exercises will be the same as the group

Exe-L. Squats, leg presses and bench press will be the exercises proposed to stimulate the development of the

maximal and resistant strength on large muscle groups. As for the Exe-L group, in addition, other

exercises will be carried out "free body" and using elastic bands for load modulation (small

muscle groups - resistant strength).

At the beginning of each session, all subjects will perform a warm-up phase (5-10 minutes) composed of

by dynamic stretching exercises, in preparation for the next strength work, while at the end of the

The session will be followed by 5-10 minutes of cool-down and static stretching. In total, each session of

training will last about 60 minutes.

7. PROCEDURE

Study participants prior to starting the intervention and 5 and 9 months after the start of the

intervention program will be subjected to:

Clinical evaluation

All subjects will undergo clinical evaluation, with blood pressure measurement.

A complete medical history will be collected with particular attention to the presence of pathologies of

taking drugs and weight changes. This assessment is part of the

normal clinical practice as part of the follow-up of the nutrition clinic at the Nutrition Service

Clinical Nutrition of Geriatrics B.

All subjects will undergo the PASE (Physical Activity Scale for the Elderly) questionnaire

for the assessment of the degree of physical activity at baseline and at time T0, T5 and T9.

Anthropometric Evaluations

Each patient will be subjected to body weight measurement, after being stripped of the

heavy clothing and shoes, with an approximation of 0.1 kg (Salus Scale, Milan), and to

Height measurement using a stadiometer, to the nearest 0.5 cm

(Salus Stadiometer, Milan). Body mass index (BMI) will be calculated as the ratio of

weight and height squared (Kg/m2). Anthropometric measurements will then be taken with the

patient in orthostatism, using a tape measure. The waist circumference will be calculated in

feet, halfway between the superior iliac crest and the last rib. The

hip circumference. These assessments are routinely carried out as part of the investigations

for the clinical classification of the obese patient at the Clinical Nutrition Clinic from

medical staff and dietitians of the Clinical Nutrition Service of Geriatrics B.

Body composition assessment (BIA and DXA)

Impedance analysis (BIA) is an easy-to-perform, rapid, non-invasive test that finds

elective indication for the measurement of total body water and its distribution among the

intra- and extracellular spaces and allows hydration to be assessed in any clinical condition and

regardless of body weight. As with other methods that analyze the composition

it depends on numerous static and dynamic laws concerning the electrical properties of the

of the body; composition, hydration, density; as well as age, race, sex and conditions

clinics of the people evaluated.

Human body fluids are broken down into total body water (TBW)

distributed in the intracellular (LIC) and extracellular (LEC) compartments, this in turn

divided into intravascular and interstitial. With the exception of the intravascular space and any

pathological collections, the fluids are not free, but bound and distributed in the structure of the mass

Body. Body mass can be thought of in terms of a bi-compartmental model, as

fat mass (FM) and fat-free mass (FFM). Lean mass can be

further divided into muscle mass (Lean Body Mass) and bone mineral content, obtaining

a three-compartmental model of body mass.

DXA uses an X-ray source that emits photons at two different energy levels. In

The study conducted will use the DXA Hologic QDR 4500, already supplied by Geriatrics B,

equipped with analysis software V5.674 (Waltam, USA)44. Patients will undergo total examination

body, which will be used for the assessment of total lean mass, total fat mass and

body fat percentage.

The radiant exposure is less than 8 Sv and the total measurement time (in

array scan) is equal to about 7 minutes in the total body examination. All scans will be

examined by a single, specially trained operator.

Such assessments (BIA and DXA) will be performed at baseline, 5 and 9 months post-start assessment

Study.

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Dynamometer test and performance test

The evaluation of physical performance will be carried out by means of "Short Physical Performance

Battery (SPPB)". The Guralnik SPPB scale is derived from a short battery of tests used to

evaluate the functionality of the lower limbs. This battery consists of three tests:

a) Balance Test (test dell’equilibrio)

This test assesses balance with three tests: maintaining the position with feet together for 10

seconds, then in a semi-tandem position (heel of one foot next to the big toe of the other foot) and

finally tandem (heel of one foot in front of the other foot) again for 10 seconds.

b) Gait Speed Test (test del cammino)

This test assesses the time taken by the subject to walk a 4-meter distance at a pace

habitual. The use of aids (e.g., cane) is permitted if necessary. In the evaluation it is

used the fastest of the 2 tests performed. The cut-off used to assess the decrease in

The speed of the march is independent of gender and corresponds to a speed > 0.8 meters/second.

c) Chair Stand Test

This test measures the time it takes for the subject to get up 5 times from a chair with arms

Folded. The test must be carried out as quickly as possible. Its evaluation begins in

the moment in which the subject assumes the upright position for the fifth time.

Each of these three tests is assigned a score from 0 to 4, with 0 indicating complete inability to

Test E 4 indicates a high level of performance. The sum of the values obtained from the three tests

Determine a total score (between 0 and 12). Values between 0-3 indicate severe

functional limitation, between 4-6 a moderate limitation, between 7-9 a slight limitation and between 10-12

a minimal limitation.

Assessment of the strength of the flexor muscles of the dominant hand will be performed by

Portable hydraulic dynamometer: Handgrip test (JAMAR, JA Preston Corp, Ontario, Canada). For

Each subject will be performed three measurements and will be considered the best of the three

Measurements. The cut-off used to assess the decrease in force was assigned based on the

sex. For men there is a cut off < 30 kg, for women a cut off < 20 kg.

This test, along with the "Short Physical Performance Battery" (SPPB), is a strong predictor of

disability and other adverse outcomes.

Finally, flexibility and elasticity will be evaluated by means of a Eurofit Test method, functional tests to be

field. These assessments are routinely carried out as part of the investigations for

the clinical classification of the obese patient at the Clinical Nutrition Outpatient Clinic by staff

physician and dietitians of Geriatrics B and will be performed at baseline, 5 and 9 months assessment

from the start of the study.

Laboratory evaluations

Blood chemistry analysis

In all participants will be determined at the start of the study, on venous blood sampling at

fasting carried out at the Clinical Nutrition Service of Geriatrics B, blood count, ionemia and

creatinine, fasting blood glucose, cholesterolemia, LDL cholesterol, HDL cholesterol, triglycerides,

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insulin, hs-CRP. These dosages are part of the blood chemistry routine to which the

patients belonging to the Clinical Nutrition clinic of the AOUI of Verona. An aliquot of serum

will be stored at -80 °C at the LURM of the University of Verona (Geriatrics Section) and up to

a maximum of two years from the time of sampling (in case it is necessary to repeat some

analysis). The Principal Investigator will be responsible for storage. The share of whey

any excess remaining after the dosages will be disposed of according to company procedures.

Study of bioenergetics and telomere length in PBMC and dosage of inflammatory cytokines

In the absence of data from the literature as well as in consideration of the cost of the methods, in a

subgroup of 72 male and female subjects (N=6 for each experimental group reported in tab.

1), after acquiring ad hoc informed consent, a blood sample will be taken on an empty stomach

during the screening visit and subsequent checks (T5 and T9) for the study of parameters

of bioenergetics and telomere length in peripheral blood mononuclear cells

(PBMC) and for the assay of inflammatory cytokines (TNF-α, IL-6). For the separation of PBMCs

blood will be collected in tubes from blood count with EDTA. Samples will be immediately

sent to the Department of Molecular and Translational Medicine (DMMT) of the University of

Studies in Brescia where the PBMCs will be separated quickly. Purified cells

will be immediately used for the measurement of cellular bioenergetics parameters

by respirometry with the Seahorse XFe24 Analyzer (Agilent) instrument. A share of PBMC

it will be centrifuged, rapidly frozen and stored at -80°C at the certified cryogenic bank

ISO9001 of the DMMT for the subsequent measurement of telomere length by means of a

Quantitative PCR. For the dosage of inflammatory cytokines, blood will be collected in test tubes

non-pyrogenic in the absence of anticoagulants. The test tubes will be immersed in ice for 30 min to

allow coagulation, then centrifuge for 10 min at 3000 g at a temperature of 4 °C. The

serum will be separated, rapidly frozen and stored at -80°C at the cryogenic bank

certified ISO9001 by the DMMT for subsequent dosages. The person responsible for the conservation will be the

Prof. Alessandra Valerio. Samples will be stored for up to two years from the date

of the withdrawal. The excess sample remaining after the dosages will be disposed of according to the

company procedures.

Assay of circulating miRNAs

In the absence of data from the literature as well as in consideration of the cost of the methods, in a

subgroup of 48 male and female subjects (N=6 for the following groups: EXE-L + placebo; EXE-L

+ AA2; EXE-H + placebo; EXE-H + AA-2) after obtaining ad hoc informed consent,

will take a fasting blood sample at the screening visit and in subsequent

controls (T5 and T9) for the measurement of miRNAs in circulating blood. The blood will be collected in

test tubes with sodium citrate. Samples will be immediately frozen in liquid nitrogen and

stored at -80 °C at the AUOI Analysis Laboratory in Verona. Head of

will be the Principal Investigator. The samples will then be sent on ice

to the Department of Biology of the University of Padua where they will be kept at -

80 °C until the moment of miRNA dosing via digital PCR. Head of

conservation at the Department of Biology will be prof. Gerolamo Lanfranchi. The champions

will be kept for up to a maximum of two years from the date of collection. The sample share

any excess remaining after the dosages will be disposed of according to company procedures.

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The procedures of the study are summarized in the Table of Procedures.

Procedures Table

Procedure

Clinical Practice (PC)

o Specific Study

(SS)

SS clinical evaluation

SS Functional Testing

PC Anthropometric Evaluations

PC Indirect Calorimetry

Assessment of Body Composition (BIA and DXA) SS

Dynamometer Test and PC Performance Test

PC blood chemistry analysis

Study of bioenergetics and telomere length in PBMC and dosage of

inflammatory cytokines

SS

Assay of circulating SS miRNAs

SS Functional Questionnaires

8. SAMPLE SIZE

Analysis conducted with Stata 14.0 (power twoway) and G\*Power 3.1.9.2

The aim of the experiment is to evaluate (Primary Aim A) the effect of the interaction between the factor

"amino acid intake" (3 levels) and "gender" (2 levels) and (Primary Aim B) the effect

the interaction between the "amino acid intake" factor (3 levels) and the "exercise level" factor

(2 levels) compared to the primary outcome, i.e. the quantitative Short Physical Performance Index

Battery (SPPB). To assess the sample size of the experiment, we estimated the

sample size for each of the two experimental designs, then choosing, in a

conservative, the largest sample size.

At. The interaction between the "amino acid intake" factor (3 levels) and the "amino acid intake" factor (3 levels)

exercise" (2 levels) compared to the primary outcome (SPPB) will be assessed using a twoway

ANOVA. A p-value of less than 0.05 will be considered significant. One dimension

total sample of 84 (14 subjects for each group) allows us to obtain a

power not less than 90% to reject the null hypothesis of absence of interaction when

the actual effect size is 0.4.

B. The interaction between the "amino acid intake" factor (3 levels) and the "gender" factor (2

levels) compared to the primary outcome (SPPB) will be assessed using a two-way

ANOVA. A p-value of less than 0.05 will be considered significant. One dimension

sample size of 96 (16 subjects for each group) allows us to obtain a

power not less than 90% to reject the null hypothesis of absence of interaction when

the actual effect size is 0.38.

For Primary Objective A, a larger effect size has been assumed than for

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Headline Objective B as the "exercise level" effect is expected to be greater

with respect to the "gender" effect, so we can reasonably assume a greater

difference between the means of SPPB observed in the 6 groups and a variability less or at most equal

intra-group compared to what was hypothesized for the interaction factor "amino acid intake" and

"gender" factor.

In conclusion, the sample size of the study was chosen as 96 subjects; however

calculating a drop-out percentage of about 10%, the recruitment will be 108 patients, with 2

additional patients for each study subgroup.

9. STATISTICAL ANALYSIS

The data collected will be summarized through descriptive statistics. The level of statistical significance is

set at 5%, the threshold for 95% confidence intervals. All analyses will be carried out with

the statistical program Stata 14.0.

The analysis of the objectives will be done considering the ITT (intention-to-treat) population. The result of

this analysis will be compared with those of the PP (per-protocol) analysis. The number and percentage

of subjects who decided to exit the study after randomization will be reported compared to

different treatment groups. These subjects will be further described to highlight the

characteristics that distinguish them, the moment of leaving the studio and the reason for leaving.

Primary endpoint analysis

The interaction between the "amino acid intake" factor (3 levels) and the "exercise level" factor (2

levels) compared to the primary outcome (PPT) will be assessed using a two-way ANOVA or the

respective nonparametric Kruskal Wallis test.

The interaction between the "amino acid intake" factor (3 levels) and the "gender" factor (2 levels)

with respect to the primary outcome (PPT) will be assessed using a two-way ANOVA or the respective

test non parametrico at Kruskal Wallis.

Secondary endpoint analysis:

A two-way mixed design ANOVA will be used for secondary endpoint analysis or,

if more appropriate, the nonparametric Kruskal Wallis test evaluated with respect to each time point

expected.

As an exploratory analysis, if the expected assumptions apply, a linear model can be used

mixed in which to insert any covariates or effect modifiers considered statistically or

clinically relevant.

10. ETHICAL ASPECTS AND GOOD CLINICAL PRACTICE

The latest revision of the Helsinki Declaration and the Oviedo Declaration are the basis for the

for the ethical conduct of the firm.

The study protocol is designed and will be conducted to ensure adherence to the principles and procedures

of Good Clinical Practice and to comply with Italian laws, as described in the following

documents and accepted, with their signature, by the study investigators:

- ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996

- Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community

- Legislative Decree no. 211 of 24 June 2003.

- D. L.vo n.200 6 November 2007

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- Ministerial Decree of 21 December 2007.

- AIFA determination of 20 March 2008.

All essential clinical documents to prove the validity of the study will be kept, and

the integrity of the data collected.

11. DATA OWNERSHIP AND DISCLOSURE AGREEMENTS

The results will be owned by the Geriatrics B Unit of the AOUI Verona. Any publications

of the results of the study will refer to the present protocol.

12. DATA MANAGEMENT AND PROCEDURES TO ENSURE CONFIDENTIALITY

OF DATA

Databases will be used and the entry of data from paper to the computer will be carried out with

il “double data entry”.

Paper materials related to clinical evaluations will be kept in cabinets, the keys to which

will only be in the possession of persons authorized by the study managers at Geriatrics B

(Management).

The paper copies will be kept for at least 10 years in compliance with Legislative Decree 200/2007

For databases, it is necessary to enter a username and password to access the computers where

data are entered. In addition, access to the database in order to start a data entry session is

protected by username and password.

To ensure data secrecy, as well as to avoid data manipulation and loss

The following precautionary measures are taken:

i)Access to data is restricted only to members authorised by the study lead

ii) The network is protected by firewalls

iii) The Internet connection is encrypted with a digital certificate (SSL technology)

iv) The database is located on a server, protected by a password that is changed periodically.

v) Access to the database is password protected and is only accessible to persons responsible for the

center.

vi) Periodic back-ups are performed.

vii) Each patient is registered and identified by means of a code in order to ensure anonymity.

13. DURATION OF THE STUDY

Recruitment: 2 years...

Patient involvement: 10 months...

Total duration: 3 years...

Data retention period: 7 years

14. ECONOMIC ASPECTS

The present study is part of the project "Multicomponent Analysis of physical frailty BiomarkErs:

focus on mitochondrial health‐MAYBE" which has obtained funding from Fondazione Cariplo

in 2016. For participation in this project, the AOUI Verona will receive € 55,000.

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