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Title of the thesis

Lifestyle Medicine for Longevity (LSM4Long) pragmatic randomized controlled trial: development and implementation of a combined lifestyle intervention for older people in Long-Term Care

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SUMMARY:

Background:

Worldwide the population is rapidly aging, but this increasing in longevity does not completely mean an extension of healthy lifespan. Population ageing is an inevitable irreversible demographic transition resulting towards longer life and smaller families, as a result, the utilization of long-term care (LTC) facilities is soaring. Innovative and comprehensive strategies are needed to support the well-being and quality of life among LTC older individuals, such as optimizing individuals' intrinsic capacity, including domains in cognition, psychological, sensory function, vitality and movement. Lifestyle medicine intervention combining physical, nutritional, and psychological components have been found effective in general older population and exert a profound impact on short- and long-term health and quality of life. In long-term care facilities the burden of disability, morbidity and mortality is mostly attributable to cardiovascular diseases, sarcopenia, dehydration, and osteoporosis. Implementation of lifestyle medicine interventions in LTC is limited by personal or environmental barriers but also by barriers to organizing those programs. Nursing home residents are people with advanced disease and/or limited prognoses, and evidence about the implementation of lifestyle medicine interventions are somewhat limited.

Methods:

In order to design Lifestyle Program (LP) based on Lifestyle Medicine interventions to promote longevity in elderly people admitted in a Long-Term Care setting, an overview of reviews and a systematic review were conducted to retrieved effective components of the intervention. A Lifestyle Medicine for Longevity (LSM4Long) program has been developed and the effectiveness of the intervention assessed designing a pragmatic randomized controlled trial. The study took place in real-world setting (Nursing home) and residents living in the LTC facility for one or more years, able to discern and to express informed consent, and requiring nursing care were considered eligible. The three-months multicomponent intervention combined bi-weekly physical exercise groups, a healthy diet, and weekly psychological wellbeing sessions. Patients of the control group were subjected to usual care. At the end of the intervention differences between participants were evaluated

through Barthel Index, Katz Activities of Daily Living, and Tinetti scales. Blood samples were taken from participants, in the morning in fasting conditions, for the evaluations of oxidants/antioxidants, as thiobarbituric acid reactive substances, 8-hydroxy-2-deoxyguanosine, 8-isoprostanes, superoxide dismutase activity, glutathione and vitamin D. In vitro, human umbilical vascular endothelial cells (HUVEC) were used to examine the effects of plasma on viability, ROS release and mitochondrial membrane potential.

Results

Fifty-four patients (females = 39, 72.22%) with a mean age of 84 years took part to the study. Physical exercise was attended by all the subjects of the intervention group, with an average participation to 9 out of the 13 total sessions (68.98 %, SD 21.75). Supportive therapy registered an average participation to 5 out of 7 sessions (82.0 %, SD 25.02). Both groups took less calories than planned in the diets (control group 1615.54 Kcal, SD = 152.31; intervention group 1456.61 Kcal, SD = 212.16; p-value=0.01) in addition, the intervention group showed a lower energy and carbohydrates intake than the control group. At the end of the study, the intervention group showed a significant improvement in the total scores of all the scales, in particular: Barthel index (control group: mean=30.04, SD=29.54; intervention arm: mean=44.96, SD=26.72; p-value=0.02). After the interventions: SOD, GSH, 25OHD, and JC1 were significantly higher in the lifestyle arm compared to the control arm. Also, TBARS were significantly lower in intervention group compared to control arm. No significant differences were found in Thymosin β plasma levels between groups.

Conclusion:

Due to a synergistic effect of the interaction of the intervention components, the LSM4Long improves functionality and oxidative stress biomarkers levels in older people living in the LTC setting. These results were achieved in a short timeframe. However, a further exploration of underlying factors is needed to better understand the barriers that interfered with a complete nutritional intervention in this context. Moreover, the results obtained will help in advance the knowledge and inform policies for health promotion in these particular settings.

BACKGROUND

RATIONALE

Healthy Ageing (HA) is defined by the World Health Organization (WHO) as the process of developing and maintaining the Functional Ability (FA) that enables well-being in older age. FA is largely determined by the individual's Intrinsic Capacity (IC) (i.e. combination of the individual's physical, mental, and psychological capacities) and its interaction with the environment in which they have lived (i.e. in its broadest sense, the sum of physical, social and policy environment) (1,2)

Worldwide the population is rapidly aging (3,4), but this increasing in longevity does not completely mean an extension of an healthy lifespan (5). More emphasis should be placed on promoting a healthy life and to improving its quality, rather than solely on prolonging lifespan (5).

The "*Global strategy and action plan on ageing and health"*, provided by the WHO, offers a policy framework aimed at ensuring that health systems meet the needs of older populations. It calls for tailoring health systems to guarantee affordable and equitable access and integrated services that fulfill the needs and rights of aged population. Meaning that health care professionals and policy makers should ensure effective interventions for the prevention of disease and the maintenance of functionality, using an holistic approach (2,6).

Population ageing is an inevitable irreversible demographic transition resulting towards longer life and smaller families, as a result, the utilization of long-term care (LTC) facilities is soaring and public spending in most countries has been insufficient to cover the growing demand. The data reported by Organization for Economic Co-operation and Development (OECD) show a reduction of average funding allocated to the long-term care services: 1.5% of GDP in 2019 against 1.7% of GDP in 2017. In consideration of the demographic projections, a significant worsening of this trend is expected in coming years and the insufficient funding could lead to a poor quality of care due to inadequate, undervalued, underpaid care or rely on informal services. Furthermore, concerns about the sustainability of public expenditure for health care or pension has been raised, and the recent COVID-19

economic crises negatively impacted the economic burden on families and care-givers (7). Innovative and comprehensive strategies to support the well-being and quality of life among LTC older individuals are needed, such as optimizing individuals' intrinsic capacity, including domains in cognition, psychological well-being, sensory function, vitality, and movement (8).

Personal healthy lifestyle (adequate physical activity, mental wellbeing, healthy food habits) significantly contributes to fostering longevity and preventing negative outcomes of non-communicable diseases (NCDs). Several studies demonstrated that nutrient-dense dietary patterns (which were characterized by higher intake of vegetables, fruits, legumes, nuts, whole grains, unsaturated vegetable oils, fish, and lean meat or poultry) were associated with a decrease in all-cause mortality in older individuals (9). Physical activity (PA) is considered as an essential element for an healthy and long life in older people. Moreover, due to its protective factor for chronic conditions, PA is associated with improved mental health and delay of mild-cognitive impairment (10). This approach has been defined “Lifestyle Medicine”, a medical specialty that uses therapeutic lifestyle interventions as the primary approach to promote health and wellbeing, and to treat chronic conditions (11).

Lifestyle (i.e. daily habits and actions) habits and practices exert a profound impact on short- and long-term health and quality of life. The strength of the body of evidence overwhelmingly proves lifestyle measures’ effect on prevention of major NCDs, such as cardiovascular disease, diabetes, cancers. Hence, lifestyle interventions are incorporated into, virtually, every evidence-based clinical practice guideline (CPG) addressing the prevention of NCDs (11).

The U.S. Institute of Medicine (IOM) defines CPG as “statements that include recommendations intended to optimize patient care, that are informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options” (12). Over the past 2 decades CPGs have become an increasingly popular tool for synthesis of the ever-expanded and often contradictory best practices information and the number of organizations creating such guidelines has been proliferating exponentially (13). At the end

of 2011 more than 2,700 CPGs were archived in the Agency for Healthcare Research and Quality's National Guideline Clearinghouse (NGC) and over than 6,800 GPCs were stored in the Guidelines International Network (G-I-N,), a network of guidelines developers composed by 105 organizations representing 34 nations (14–16).

In order to find CPGs that meet their needs, physicians, healthcare decision-makers and general population had to surf into this dizzying array of clinical information in a -time-wasting research that often produces two or more inconsistent recommendations, generating confusion (16–18).

A noteworthy example of divergence in CPG recommendations are the recently glycemic targets for patients with type 2 diabetes developed by Conlin and colleagues from the U.S Department of Veterans Affairs (VA) and the U.S. Department of Defense (DoD) that differ from those developed by the American Diabetes Association (ADA) and the American Association of Clinical Endocrinologist (AACEE) (18–21).

Health care professionals relies heavily on the translation of evidences into CPG because, by definition, evidence-based CPGs are perceived to be unbiased and valid, but several concerns about their trustworthiness have been recently raised: a conservative estimate is that 50% of current evidence-based CPGs suffer from either methodological flaws, limited panel composition or conflicts of interest (COI) making their conclusion untrustworthy (16,22,23). In 2000 only 5% (22/431) CPGs fulfilled 3 basic quality criteria, in 2012 49.1% (56/114) CPGs met more the 50% of IOM standard criteria. In this last sample, information about COI was given in 29.8% (34/114) and were present in 71.4% and 90.5% of committee chairperson and co-chairperson (13,16).

The GIMBE foundation and Istituto Superiore della Sanità (ISS) retrieved 359 (50%) CPGs from 712 documents that were published throughout 2000-2016 by Italian scientific societies (81/403). The G-I-N standard of quality criteria were met by 21% (75/359) of CPGs and COI disclosure was given in only 3% (24).

It is essential to restrain the unintended and harmful clinical effects triggered by the adoption of flawed CPGs and the consequences of their use for the definition of care pathways (17). The AGREE(II) instrument (Appraisal of Guidelines for Research and Evaluation II) was drawn for the purpose of evaluating the process of CPGs development and the quality of reporting (25).

Healthcare decision-makers also need high-quality evidence to make a decision: evidence-informed policy-making (EIP) aims to ensure that the best available data, information and research evidence are used to formulate policies to improve the health of individuals and populations (26). On the assumption that policy-makers will make better decision if they are provided with higher-quality information, EIP might contribute to stronger health systems (27).

Given the increasing prevalence of frailty - a clinical geriatric syndrome defined as presence of weakness, slow gait, exhaustion, unintended weight loss, and low physical activity - appropriate actions to reduce frailty, disability, and dependence among older adults should be at the forefront of all policies to tackle the challenges of an aging population. While it may pose a significant challenge and burden, it also presents opportunities for concerted action to foster effective policies and initiatives in community frailty prevention and reduction. There has not been a comprehensive review of interventions and/or policies that can prevent or reduce the level of frailty in long-term care older adults (28).

In long-term care facilities the burden of disability, morbidity and mortality is mostly attributable to cardiovascular diseases (29), sarcopenia (30), dehydration (31,32), and osteoporosis (33).

In fact, it has been estimated that in a nursing home the prevalence of hypertension is 70.79%, moreover, 30.69% of older residents live with diabetes, coronary acute disease is present in 25.74% of older elderly, peripheral arterial disease affects 12.87%, prevalence of ischemic-stroke is 11.38% and finally the prevalence of congestive heart failure is 11.88% (34).

Sarcopenia is characterized by progressive loss of muscle mass with decline in strength and/or physical function. It has been estimated that a sedentary lifestyle is independently and positively associated with sarcopenia (OR=1.36; 95% CI 1.18 to 1.58) (35). The prevalence of sarcopenic residents in a nursing home has been estimated to be 51% (95% CI: 37-66%) in men and 31% (95% CI: 22-42%) in women (36). The annual cost of muscle weakness has been estimated to be £2,707 per person in the UK, with an overall estimated cost of £2.5 billion in 2018 (37). Individuals with sarcopenia have greater odds of hospitalization (OR, 1.95; $p<0.001$), the total estimated cost of hospitalizations in

individuals with sarcopenia is USD $40.4 billion; in Europe, sarcopenia increased hospitalization costs by € 721 (95% CI: € 13-1,429) in patients aged 65 years and over (38,39).

Prevalence rates of dehydration (chronic or acute) in residents of Nursing Homes ranges between 0.8 – 38%. Significant risk factors associated with dehydration are fever and cognitive impairment. There are no data available regarding annual health care costs related to hospitalizations for Europe, but it has been estimated that dehydration reached $4.97 billion, in 2008, in the United States (31).

Osteopenia and osteoporosis are defined as a T-score (measured by DEXA) equal to or less than -1 and -2.5 standard deviations, respectively, below the peak bone mass of a young healthy cohort or in the presence of a minimal-trauma fracture (37). The prevalence of osteoporosis in nursing home is estimated to be 13.5% (40).

"Osteosarcopenia" is a geriatric syndrome characterized by low bone and muscle mass and increased ectopic fat which increases the risk of frailty, hospitalizations, and death (41). The prevalence of osteosarcopenia increases with age: in male older adults from 14.3% at60–64 years to 59.4% at ≥75 years; in older women from 20.3% to 48.3% at ≥75 years, $P < 0.05$ (42).

Resistance training, adequate protein and calcium intake, associated with maintenance of appropriate levels of vitamin D, exert a positive effect on bone and muscle, reducing falls, fractures, and, consequently, mitigating, delaying or reverting the worsening of those conditions (43,44).

However, implementation of lifestyle medicine interventions in LTC is limited by personal or environmental barriers but also by barriers for organizing those programs such as: unknowledge of recommendations reported in CPGs, preconception that recommendations are not feasible, understaffing, low salaries or lack of specialized personnel (45–47). By definition, nursing home residents are people with advanced disease and/or limited prognoses and, evidence about the implementation of lifestyle medicine interventions are somewhat limited (48).

Essential Elements of Aging Process

Aging arises from the accumulation of oxidative damage to cells and tissues, which is associated with a progressive increase in the chance of morbidity and mortality (49). The imbalance between peroxidative factors and reactive oxygen species (ROS), over antioxidants (50) would lead to oxidative damage of carbohydrates, lipids, proteins, mitochondria, as well as, DNA (51,52)**,** which, finally, could trigger the aging process by not clearly defined mechanisms (53). It is also to note that the efficiency of the endogenous antioxidant system in elderly people often shows a decline, which could account for the high prevalence of NCDs (54).

Several theories have been proposed to explain aging process as explained in table 1 (55).

Table 1: Aging process theories (55)

Author. Years	**Theory**	**definition**
Peter Medawar, 1952	Evolutionary theory	deleterious mutational accumulations
George Williams, 1957	Antagonistic pleiotropy	Aging is caused by specific alleles and mutations that display either positive or no effects in early development but can be detrimental in later life as natural selection fails to remove them
Denhan Harman, 1956	Free radical theory	When accumulation of oxygen species overwhelms anti-oxidatave mechanism, cellular components are damaged
Valter Longo, 2005	Programmed theory	Aging and death are results of a genetic program that evolved to benefit future generations
Mikhail Blagosklonny, 2008	Hyperfunction theory	Sustained hyperactivity of genes during the reproductive age window causes a state of cellular hypertrophy that results in aging, but is not primarily related to molecular damage

Human aging is the progressive loss of organ and tissue functions over time developed from an accumulation of physical, environmental, and social factors, and consequently loss of the ability to resist to stress, damage and disease (56).

Oxidative stress

Superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($^1O_2$) are commonly defined as reactive oxygen species (ROS). Those biological elements are mainly produced by mitochondrial respiratory chain during both physiological or pathological conditions, in particular as a result of electron transport and the reduction of the oxygen molecule. As a results of an excessive level of ROS production or accumulation in cells and tissues, important cellular structures like proteins, lipids, and nucleic acids are subjected to a harmful effect (57,58).

Mitochondrial reactive oxygen species (mitoROS) are carefully regulated in a continuous process required for the regulation of many life activities, such as cell differentiation, senescence, signal transduction, hypoxic adaptation (59)

Mitochondria release H_2O_2 under physiological hypoxia to activate the hypoxia inducible factor 1 (HIF-1), to initiate a protective mechanism of cells against hypoxia. Subsequently, it was found that mitochondrial release of H_2O_2 can activate c-Jun N-terminal kinase 1 (JNK1), p53, and NF-κB; thus, the release of mitoROS has evolved as an adaptative physiological method to maintain homeostasis and promote adaptation to stress (58,60). Hypoxia is not the only cell stimuli upregulating mitoROS levels. In fact, immunoreceptor ligation, and cytokine stimulation, cytosolic calcium concentration and activation of PI3K can lead to increased mitoROS levels. Down-regulation of mitoROS production can be induced by antioxidant protein expression (such as activation of Sirt3 and FOXO) or through activation of BNIP3 that induce mitophagy, a process for the removal of damaged mitochondria that produce more ROS. Finally, increased expression of uncoupling proteins (UCPs) can decrease mitoROS levels. UCPs belongs to mitochondrial anion carrier proteins that are present in the mitochondrial inner membrane. UCPs have a physiological central role in thermogenesis lowering mitochondrial membrane potential and dissipating metabolic energy as heat, but also in oxidative phosphorylation and ROS level regulation (58,61,62).

ROS play several beneficial roles if the concentrations of those free-radicals are maintained at a low or moderate level. They are involved in physiological process such as: synthesis

of cellular structures, host defense against pathogens and cellular signaling pathways. As an example, free radicals are produced and stored by phagocytes and then released against pathogens. Nitric oxide (NO) is the most well-known free radical acting as a signaling molecule, its function is required to properly regulate blood flow, thrombosis and normal neural activity (57).

Both endogenous or exogenous sources are involved in the process of free-radicals production. Endogenous events responsible of free-radicals production are: immune cells activation, inflammation, ischemia, infection, cancer, excessive exercise, mental stress, and aging. The metabolism or degradation of external compounds generated free radicals as by-products. Some exogenous sources are: exposure to environmental pollutants, heavy metals (Cd, Hg, Pb, Fe, and As), certain drugs (cyclosporine, tacrolimus, gentamycin, and bleomycin), chemical solvents, cooking (smoked meat, used oil, and fat), cigarette smoke, alcohol, and radiations (57).

Endogenous antioxidants, like the enzymes of catalase family, glutathione group, thioredoxin-related group, and superoxide dismutase, together with exogenous antioxidant as reduced glutathione, carotenoids, and vitamins C and E, constitute the indispensable ROS detoxifying system (56).

It is known that ROS play a pivotal role in the onset and progression of chronic disease as hyperlipidemia, diabetes mellitus, hypertension, ischemic heart disease, and chronic heart failure (63). Superoxide radicals ($O_2^{\bullet -}$) can itself exert effects on vascular function but it also play a pivotal role in generating other reactive species (63)

When free radicals and oxidants are in excess overwhelming antioxidants a phenomenon named oxidative stress (OS) occurs (63). OS is a state produced by the imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the improper function of a biological system to clear these reactive products and at chronic high levels can produce a cellular damage correlated with aging and age-related disease. (56,57,64).

As mentioned above, sarcopenia is an age-associated progressive loss of muscle strength and mass that causes functional loss and predisposes to frailty, loss of independence, and physical disability (36–39,65). Although muscle loss is a multifactorial and not completely understood condition, maintenance of skeletal muscle mass is regulated by a delicate equilibrium between anabolic and catabolic factors. Thus, loss of mass muscle is generally attributable to a disproportionate decrease in muscle protein synthesis and/or increase in protein breakdown. It is known that aging is related to a reduced anabolic drive leading to a decrease in synthetic capacity (66). One of the numerous pathways proposed to be involved in sarcopenia is the accumulation of ROS in skeletal muscles as they consume large amount of oxygen. In older elderly antioxidants defenses are compromised and OS contribute to muscle mass loss by stimulating proteolysis and down-regulating muscle protein synthesis. Furthermore, OS may reduce acetylcholine release at the synaptic cleft and may modify the morphology of the neuromuscular junction, leading to a failure in action potential generation or reduction of innervation and number of fibers. OS chronic exposure and damage may also induce structural modification of actin and myosin (67).

Thus, sarcopenia is considered to be both the biological substrate of physical frailty or a predictor of the adverse outcomes related to frailty (68–70) and so sarcopenia could be a target for muscle-building interventions (67).

Role of mitochondria in aging

Aging and age-related disease are associated and correlated with mitochondrial quality and activity decline, it is also known that mitochondrial mutations increase in frequency with age (71).

Since mitochondria regulate different metabolic and signaling pathways and also play an important role in programmed cell death, mitochondrial dysfunction has been hypothesized to be the physiopathology substrate for aging, lifespan and age-related diseases. The primary function of mitochondria is to produce ATP through the process of oxidative phosphorylation, which is conducted by electron transport chain, a protein complex located in the inner mitochondrial membrane made of four RC complexes (complexes I–IV) and the ATP synthase (complex V) (72–74). A known peculiarity of mitochondria compared to other cellular organelles, is they contain their own genetic information, named mtDNA

(74). Since mitochondrial oxidative phosphorylation system is the main producer of ROS, it has been suggested that the close proximity of mitochondrial ROS production places mtDNA to the oxidative stress (OS) negative effects that are considered strongly and directly correlated with aging and lifespan (67,75–77).

ROS readily attack DNA and generate a variety of DNA lesions leading to genomic instability and 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the most highly mutagenic and prevalent DNA lesions caused by increase production of ROS (72). Since mtDNA is not protected by histones, mtDNA is very susceptible to oxidative mutagenic damage. The increase of mtDNA mutation frequency contributes fairly to accumulation of mutated mtDNA; rather, mtDNA replication errors of DNA Polymerase Gamma (PolG) are considered the main source of mtDNA mutations (72,74,78,79).

Autophagy, a lysosomal-mediated intracellular protein degradation pathway, plays a key role in the turnover and quality control of cellular proteins and organelles, clearing damaged cellular contents avoiding cellular damage and apoptosis. Since this process has been described to occur as a selective removal pathway of defective mitochondria, the term mitophagy has been introduced (80–82).

Consequently, impaired or decreased cellular autophagic and mitophagic activity leads to accumulation of abnormal mithocondrial, mutated mtDNA copies and dysfunctional mitochondria activity, thus to an increased oxidative stress (78,80,81).

CIRCULATING OXIDANTS

Due to their evanescent nature (i.e. short half-life), ROS do not meet completely the criteria used to define a biomarker. In fact, the WHO and the National Institute of Health define a biomarker as any substance, structure or process that is objectively measured in the body and evaluated as an indicator of a normal biological process or as a predictor of a pathogenic process, or incidence outcome (pharmacological response or disease outcome) (83,84). Several markers of oxidative stress met those criteria but clinically they failed to be relevant in terms of specificity, prognostic value and disease activity (85).
For those reasons it is more suitable to estimate oxidative stress by measuring their oxidant target products (86,87).

Thiobarbituric acid-reactive substances (TBARS)

Malondyaldeide (MDA) is one of the most studied final products derived from peroxidation of polyunsaturated fatty acids (PUFAs). Since oxidants act by extracting a hydrogen, lipids, especially polyunsaturated fatty acids, are the most affected biomolecules in oxidative-stress-induced impairments leading the formation of unstable lipid radicals (L-) and production of MDA. MDA interacting with proteins or by itself, has been shown an atherogenic activity promoting atherosclerosis due to impaired interaction between oxidized low-density lipoprotein (OxLDL) and macrophages. For those reasons MDA is considered to be a marker of oxidative stress in various cardiovascular diseases through the quantification of thiobarbituric acid-reactive substances (TBARS) (88,89). The evidence of the correlation within TBARS and oxidative stress is strong, that means that lower levels of TBARS are indicative of decreased oxidative stress (90). TBARS show also a clinical relevance: serum levels of TBARS from patients ($N = 634$) with documented coronary artery disease (CAD) enrolled in a RCT, have been found to be strongly predictive of major cardiovascular events (fatal/nonfatal myocardial infarction, stroke) [RR 2.94; 95% CI 1.75 to 4.94; $P<0.0001$] and of major vascular procedure [RR 2.1; 95% CI 1.61 to 2.84; $P<0.0001$] in a 3-year follow-up period (91). Furthermore, elevated TBARS levels predicted carotid atherosclerotic plaque progression over 3 years as assessed by carotid wall thickness on ultrasound (92).

Results of cross-sectional studies show a significant ($P<0.001$) higher level of plasma hydroperoxide (measured ad MDA) in frail geriatric people than in non-frail geriatric subjects (93). A recent systematic review of RCT´s which aimed to assess the effectiveness of lifestyle interventions on oxidative stress (94), found that diet alone (mediterranean diet) improved oxidative biomarkers, decreasing MDA levels ($P<0.01$) (95) and also that combined nutritional and physical activity could lead to a significant reduction of MDA levels ($P<0.05$) (96). Also physical activity alone has been found to lower MDA plasma levels in active older people compared to non-active older people (μmol/g Hb= 0.23 ± 0 03; vs μmol/g Hb= 0 25 ± 0 02, $p<0.01$) (97).

Isoprostanes

Another oxidative product class of peroxidation of polyunsaturated fatty acids are isoprostanes (IsoPs). IsoPs are prostaglandin-like molecules independently generated by the cyclooxygenase (COX) as they could be initially formed by arachidonic acid in situ, such as in platelets. One of the most used biomarkers to measure IsoPs levels linked to oxidative stress is F_2-IsoPs (98–101).

Higher levels of F_2-IsoPs has been found to positively correlate with high BMI in obese adults ($\beta= 0.127$; $P<0.001$) and other CVD risk factors (102). Diabetic patients showed a significant increased plasma levels of F_2-IsoPs compared with non-diabetic patients (mean= 33.4 pg/mL; SEM= ±4.8 pg/mL - mean= 22.2; SEM= ±1.9 pg/mL; $P<0.02$) (103). Increased lipid peroxidation, and therefore higher levels of F_2-IsoPs, are linked to reduced mineral bone density/osteoporosis assessed trough dual energy X-ray absorptiometry (DEXA) or quantitative ultrasound (QUS) in adults ($\beta= -55$; SD= 26; $P< 0.03$) (104).

Thus, increased levels of IsoPs are considered to be detrimental to human health; as seen for TBARS, physical activity has been found to lowering F_2-IsoPs plasma levels in active older people (65-75 years) when compared to non-active older people (pg/ml = 421 ± 31; vs pg/ml 555 ± 42C; $P<0.01$) (97).

Advanced oxidation protein products (AOPP)

Advanced oxidation protein products (AOPPs) are a family of di-tyrosine-containing cross-linking proteins, formed through the H_2O_2-myeloperoxidase (MPO) as a reaction between plasma albumin and chlorinated oxidants (such as hypochlorous acid). AOPPs are mainly carried by albumin in the circulation and rapidly cleared by liver or spleen uptake. AOPPs are released during excessive production of ROS, but evidence shows that AOPPs is also a potential inducer of oxidative stress leading, definitely, to tissue damage and perpetuating oxidative stress exposure (105–107).

The atherogenic effects of elevated AOPPs plasma levels has been investigated and since AOPPs increases the risk of abnormal changes of the carotid intima-media thickness (CIMT) (OR=2.038, 95% CI = 1.441 to 2.883; $P<0.001$), higher levels of AOPP are considered to be a cardiovascular risk factor. (106).

AOPPs levels have been found to be directly related to age and frailty ($P<0.001$) (108)

8-hydroxy-2′-deoxyguanosine (8-OH-2dG)

As seen above 8-hydroxy-2′-deoxyguanosine (8-OH-2dG) is one of predominant mitochondrial-DNA and also cellular-DNA lesion caused by free radical-induced oxidative stress (79). 8-OH-2dG is consider to be a biomarker of increased oxidative stress since it has been found to positively correlate to Alzheimer’s Disease ($P<0.0001$) (109). Moreover, it increases the risk of osteoporosis (OR=1.54; 95% CI = 1.14 to 2.31, P=0.003) (110) and 8-OH-2dG levels greater than 0.523 ng/mL ($P < 0.0001$) are significantly associated with severe coronary artery lesions in elderly with diabetes (111).

ANTIOXIDANTS

Antioxidants are endogenous or exogenous compounds that scavenge, neutralize or buffer free radicals, protecting biological systems from their toxicity, in fact they are named "free radical scavengers" (67). An imbalance in the oxidant/antioxidant ratio leads to a state of OS (112).

The antioxidant pathway, that form the components of this defensive system against the OS, is divided into enzymatic and nonenzymatic systems and exogenous/endogenous sources.

The external sources (exogenous) for antioxidants comes from the diet (dietary antioxidants), mostly from fruits, vegetables, and grains. Some examples of dietary antioxidants are: beta-carotene, lycopene, and vitamins A (retinol), C (acid ascorbic), E (alpha-tocopherol) and selenium (67,113).

Most studies showed an association between aging and decreased levels of molecular antioxidants, in fact, as an example, elderly people (65 to 74 years old) show lower plasma levels of vitamin E than younger adults, aged less than 50 years (42 to 47 µM vs 51 µM), and a similar profile has been found for vitamin A, vitamin C and carotenoids. Furthermore, higher levels of exogenous antioxidants are relevant for longevity and an adequate intake of both macro and micronutrients are claimed to be responsible for an healthy aging, as found in centenarians (114).

Micronutrients could be administered as a supplementation to prevent OS damage, but evidence do not support this hypothesis in healthy adults or as secondary prevention intervention. Furthermore, a systematic review demonstrated that antioxidant supplements significantly increased mortality [18,833 dead/146,320 (12.9%) versus 10,320 dead/97,736 (10.6%); RR 1.04, 95% CI 1.01 to 1.07] in a pool of 56 trials with a low risk of bias (115).

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) are some of the most studied antioxidant enzymes. Radical superoxide anion ($O_2^{\cdot}$) is converted by SOD to hydrogen peroxide (H_2O_2) that is not a free radical but it is able to form the highly reactive ROS hydroxyl ion ($OH^{\bullet}$). CAT converts H_2O_2 in water (H_2O) and oxygen (O_2) preventing hydroxyl-radicals production. Moreover H_2O_2 is detoxified in H_2O and O_2 by GSH-Px oxidating glutathione (GSH); indeed an efficient antioxidant enzymatic system

is needed to prevent an excessive formation of free radicals or reactive species and fostering healthy aging or delaying NCDs (67,116).

Those enzymes are thought to be upregulated by transcriptional factors as an adaptative oxidative-stress response induced by inflammatory process. Although the mechanisms are not clearly understood, physical exercise is known to reduce inflammation in young adults increasing resistance to oxidative stress, whereas the effects of exercise in older populations are uncertain (97).

Single bout of exercise, endurance, and resistance training types of physical exercise that can be performed at various level of intensity. Both inactivity and high-intensity physical exercise (>80% of VO_2Max - the maximum rate of oxygen consumption measured during incremental exercise) seem to growth oxidative stress deleterious effects in older people, on the other hand moderate intensity exercise appears to reduce oxidative stress levels in the same population (97,117).

Therefore, due to the age-related decline of antioxidant defenses, single bout of exercise in older people might induce a free-radicals production that could not be completely cleared by the concomitant increase in antioxidants (97).

The beneficial effects (i.e. better functioning, better health status and decreased mortality) of regular exercise in elderly could be induced by the upregulation of antioxidant enzymes (97), as moderate exercise might induce an adaptive oxidative-stress response (118).

Endurance and resistance training might have a synergistic positive effect on oxidative stress in elderly people. In fact, endurance training, that is an aerobic exercise (such as walking, jogging, swimming, biking and jumping rope) able to increase heart rate and breathing frequency and so improving overall fitness (119), balances oxidative/antioxidant effects when is performed at intensities between 50% and 80% of VO2max (the maximum rate of oxygen consumption measured during incremental exercise) and with a frequency of two to three sessions per week. At this moderate level of intensity endurance training can lower the plasma levels of TBARS at lest of 20% and the basal production of H_2O_2 and fostering the adaptative response to the oxidative stress (120).

Resistance training exercises are built to improve strength, anaerobic and size of skeletal muscles performing muscular contraction against a resistance (such as person's own body weight or free weights, weight machines or resistance bands) (121). The health benefits in

older adults coming from resistance training are achieved following a training protocol based at least on 3 to 5 sets, 10 repetitions of contraction for each muscle group at intensities between 50% and 80 % of one-repetition maximum, the maximum amount of force that can be generated in one maximal contraction (67,120).
For those reasons moderate physical endurance and resistance training are suggested to have a pivotal role in age-related sarcopenia prevention or mitigation in older adults, as they positively affect oxidative/antioxidant balance (67).

The non-enzymatic endogenous antioxidants are products of the body's metabolism that take part in the inhibition of formation of new reactive species terminating the free radical chain reactions. The main contribution to non-enzymatic antioxidant capacity is attributable to metal-binding proteins (MBPs) such as plasma circulating albumin, bilirubin, ferritin, transferrin and ceruloplasmin. The detoxifying propriety of those molecules involve their ability to bind metal ions (122). Albumin direct and indirect (i.e. binding unsaturated fatty acids) antioxidant proprieties, account approximately for the 40 to 70% of the total antioxidant activity of the human serum. Lower concentration levels of albumin, that are typical in aging, and elevated glycemic value (i.s elevate blood glucose) could lead to a reduced antioxidant capacity of serum albumin (67,122).

Superoxide dismutase (SOD)

As reported above, superoxide dismutase (SOD) converts superoxide to H_2O_2, which is further degraded by GSH-Px, (CAT), and thioredoxins. There are 3 isoforms of SOD: SOD1 (cytoplasm and inner mitochondrial membrane), SOD2 (mitochondrial matrix), and SOD3 (extracellular) (112).

A recent systematic review found that dietary interventions alone or combined with physical activity increase SOD activity 77.7% ($p < 0.05$), in particular in metabolic syndrome and diabetes patients (94).

Glutathione (GSH)

Glutathione (GSH), an important intracellular non-protein thiol, is often referred to as scavenging antioxidant, it prevents cellular oxidative stress-induced damage, but also detoxifies xenobiotics and is involved in drug metabolism. GSH scavenges active radicals to inhibit chain initiation and break chain propagation reactions (123).
Decreased GSH levels are associated with the common features of aging, as well as, of a wide range of pathological conditions, including neurodegenerative disorders, in particular Parkinson's disease (124). At the best of actual knowledge GSH levels seem not to be affected by lifestyle interventions (94).

Thymosin Beta-4 (Tβ4)

Thymosin beta 4 (Tβ4) is a ubiquitous protein with many biological functions. Tβ4 was originally isolated and characterized from the thymus gland, and is involved in cell proliferation (epithelialization, angiogenesis), promotion of differentiation stem cell (adult epicardial stem cell), prevention of cell death, and modulate inflammatory mediators counteracting the so called immunesenescence. By sequestering G-actin, Tβ4 promote the reorganizing of actin cytoskeleton fostering cell migration. Platelets, white blood cells, wound fluids contain high concentration of Tβ4 and is released after any injury but is not considered a growth factor (125,126).

With regard to the role of Tβ4 in oxidative stress, it might protect cells upregulating antioxidant enzymes (SOD and CAT), significantly reducing ROS production with the restoration of mitochondrial membrane potential and modulating the expression of anti-apoptotic genes (125,126)**.**

Vitamin D

Vitamin D [25(OH)D], corticosteroid hormone, is not only essential for the physiological process of musculoskeletal metabolism, but also is involved in modulating inflammation and excessive intracellular oxidative stress. Vitamin D deficiency is defined as serum 25(OH)D concentrations < 20 ng/mL (50 nmol/L). In regard of oxidative stress Vitamin D modulate the mitochondrial respiratory function, and thus is involved in aging process. Suboptimal fail to control oxidative stress conditions, augment intracellular oxidative

damage and the rate of apoptosis. Adequate levels of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] (the active hormonal form) induce Vitamin D genes transcription and interacting with its intracellular receptor might activate a second messenger systems protecting to excessive oxidative stress (127).

ROLE OF EXERCISE AND DIET IN AGING

Role of exercise in aging

A large amount of epidemiological and experimental studies demonstrates the effectiveness of physical activity (PA) and exercise training (ET) to struggle consequences of aging. Epidemiological evidence links many chronic diseases to physical inactivity. The benefits of increased physical activity and exercise and the harmful effects of inactivity are well recognized (128,129).

PA is defined as any movement of the body coordinated by skeletal muscles, which increases energy expenditure beyond resting condition (130), ET is a planned, structured and repetitive subcategory of physical activity aimed at improving or maintaining health or physical fitness (131).

An active lifestyle is usually recommended to all groups of ages because ET leads to a beneficial functional, morphological, metabolic and biological effects thus increasing overall physical performance and promoting well-being. To ensure a significant effect, PA and ET should be tailored to individual characteristic because not every training is suitable for everyone and inappropriate intensity or volume of exercise can also be risky (132).

While there is substantial evidence supporting the positive effects of physical activity (PA) on the physical and psychological health of adults, studies focusing on older elderly (people aged ≥ 75 years) are relatively few. This is because the physiological changes and prevalence of multiple morbidities in this age group make the health effects of PA or exercise training (ET) more complex to study.
However, adults aged ≥ 75 years take advantage on the maintenance of muscle strength, power, endurance and flexibility if they are physical active (129,133). On the other hand, due to lack of activity, the likelihood of reduced bowel function, falls and deterioration on daily living activity (such as dressing, climbing stairs or rising from a chair) is greater in older elderly (129).
Physical inactivity has been identified by the World Health Organization (WHO) as the fourth leading risk factor for global mortality. In order to define the adequate age-specific

level of PA and ET, the WHO provides detailed recommendations that are relevant for the following age-associated health outcomes (130):

- Cardiorespiratory health (coronary heart disease, cardiovascular disease, stroke and hypertension).
- Metabolic health (diabetes, obesity and metabolic syndrome).
- Musculoskeletal health (bone health, osteoporosis, sarcopenia).
- Cancer (breast and colon cancer).
- Functional health and prevention of falls.
- Mental Health (depression, anxiety and cognitive impairment).

WHO recommendations on Physical Activity for health addressed to adults aged over 65 years and above are reported in BOX 1

BOX 1: Global Recommendations on Physical Activity for Health – Age Groups: 65 years old and above (130)

1. Adults aged 65 years and above should do at least 150 minutes of moderate-intensity aerobic physical activity throughout the week or do at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week or an equivalent combination of moderate- and vigorous intensity activity.
2. Aerobic activity should be performed in bouts of at least 10 minutes duration.
3. For additional health benefits, adults aged 65 years and above should increase their moderate intensity aerobic physical activity to 300 minutes per week, or engage in 150 minutes of vigorous intensity aerobic physical activity per week, or an equivalent combination of moderate-and vigorous-intensity activity.
4. Adults of this age group, with poor mobility, should perform physical activity to enhance balance and prevent falls on 3 or more days per week.
5. Muscle-strengthening activities should be done involving major muscle groups, on 2 or more days a week.
6. When adults of this age group cannot do the recommended amounts of physical activity due to health conditions, they should be as physically active as their abilities and conditions allow.

The overall evidence unequivocally reveals an inverse association with physical activity and all-cause mortality, with a hazard ratio (HR)=0.60 (95% CI: 0.45 to 0.82). Physical active women and men aged 65 years and above, compared to less active or inactive individuals, have at least 30% lower rates of all-cause mortality, and moreover lower rates of NCDs (such as coronary heart disease, high blood pressure, stroke, type 2 diabetes, colon

cancer, breast cancer). A minimum amount of 1,000 kilocalories per week or 10 to 12 MET-hours per week (approximately equivalent to 2.5 hours per week of moderate-intensity activity) are needed to significantly lowering the risk (134,135).

The health benefits observed in adults aged 65 years reflect in a better cardiorespiratory and muscular fitness, healthier body mass and composition, enhancement of bone health and biomarker profile, thus preventing NCDs and fostering better quality of life and healthy aging, independently of a pre-existing NCD (134–136).

Elderly people who, due to existing functional or health limitations, are not able to meet the required level of PA, should be active at least to the level their abilities and health condition allow (134–137). In fact, it has been estimated that 31% of seniors aged 65 – 74 years living with disabilities (defined as any deficit in overall health that affects a person's ability to do tasks of everyday life) fail to walk a quarter of mile, as well as 69% of those aged 85 years and over. Combined aerobic (especially walking), muscle strengthening and flexibility activities, performed in 3 to 5 session per week lasting from 30 to 90 minutes at a moderate-vigorous intensity, show moderate evidence in the maintenance or improvement of functional ability in older adults with existing functional limitation (134).

Moreover, institutionalized people are by definition residents of a Long-term care facilities, living with disabilities, and often have a poor level of physical activity (less than 600 MET mins·week^{-1}), and mostly with low intensity. On one hand elderly adults are more likely to be introverted and reluctant to join physical activities, on the other hand, as above referred, nursing home have limited space, few physiotherapists, and people with reduced physical function. Overall, these barriers may explain why people in nursing homes overall have a more sedentary lifestyle, in fact, residents spend 12.9 hours/day sitting (excluding sleeping hours), involved in reading, playing games, chatting, and watching television, and perform physical activity at a low or light intensity. Eighty-five percent of LTC residents' time is sedentary, and very little time is dedicated to physical activity (12% low intensity, 2% light intensity, and 1% moderate-to-vigorous intensity) (138,139).

Since self-efficacy might be a mediating factor able to promote a better adherence to adequate levels of physical activity in nursing homes residents, reminiscence, defined as

the recall of personally experienced events from one's past, could foster the acceptance of oneself and might arouse residents to be more inclined to participate in physical activity interventions (138,139).

Thirty-three percent of 70 years and older will fall within any given year and fall-related injuries can have a large adverse effect on functional ability in older adults. Sessions performed 3 time/week of balance training and moderate intensity muscle-strengthening activities reduce the risk of falls by nearly 30% (134).

Role of dietary pattern in aging

Dietary habit is one of the main modifiable lifestyle factors to improve health and wellbeing in older population and to prevent age-related diseases (8). A dietary pattern is defined as the frequency with which the quantities, proportions, variety, or combination of different foods, drinks, and nutrients are consumed in diets (140).

The Dietary Guidelines Advisory Committee states that strong evidence demonstrates that all-cause of mortality decreases in adults and older adults if the dietary patterns are characterized by (140): vegetables, fruits, legumes, nuts, whole grains, unsaturated vegetable oils, and fish, lean meat or poultry when meat was included.

These patterns should be also relatively low in red and processed meat, high-fat dairy, and refined carbohydrates or sweets. Some of these dietary patterns also could include alcoholic beverages in moderation. Meanwhile the relationship between macronutrient or micronutrient and all-cause of mortality remains uncertain. This recommendation comes from a systematic review that included 153 single studies; although heterogenic in the methodology to examine or derive dietary patterns, the findings of the included studies consistently highlighting the statistically significant relationships between dietary patterns and all-cause mortality risk (140).

For this reason, adults and older adults that follow a similar dietary pattern are defined as having a healthy eating habit.

There are also dietary patterns that significantly increase the risk of all-cause of mortality and should be avoided, in particular the consumption of (140):

- meat or meat products such as beef, pork, sausage; red meat or meat products; red meat and processed meats; fresh and processed meats and seafood, and/or
- high-fat dairy products such as ice cream, cheese, whole milk, and/or
- refined grains and flour-based foods such as pastries, and/or
- sweets and desserts such as cake, cookies, chocolate and candy and/or
- lower intake of low-fat dairy products, rice, and pasta, lower intake of fruit, fish, other seafood, and dark green vegetables.

A healthy eating pattern is also positively related with the reduction of NCDs specific mortality, such as CVD (141), is associated with favorable bone health outcomes in adults, decreasing the risk of hip fracture (142), and might lower the risk of age-related cognitive impairment and/or dementia (143). The relationship with sarcopenia remains uncertain, in fact a systematic review of 16 studies (3 RCT and 13 observational design studies), overall judged to be at high level of risk of bias, shows no association between a healthy dietary pattern and reduced risk of sarcopenia (OR=0.95; 95% CI: 0.85 to 1.06) (144,145).
Overall, a healthy dietary pattern for older adults should ensure about 20 kcal/kg/day (146) and the Dietary Reference Intakes (DRIs), defined as the reference values used to plan and assess nutrient intake of healthy people, for their reference age. Hence, 1.8 g/day of sodium and chloride, 1,200 mg/day of calcium, 20 µg/day (800 IU) of Vitamin D, fibers intake should be 21g/day in females and 30g/day in males (147).

As above reported malnutrition sarcopenia and dehydration are known to be common problems in LTC homes and there has been inadequate detailed research on the dietary intake of the residents (148). It has been estimated that 63% of residents in a nursing home routinely consume a regular (normal texture) diet, 13% eat puréed/liquidized foods and in the 24% is needed to chop foods. The intakes of nutrients are below the recommendations at least in 80% of females and males, in particular for fiber, potassium, calcium, magnesium, zinc, iodine, vitamins D, E, folic acid. In 59% of older elderly the mean protein intake is below the recommended intake in older people (1–1.2 g/kg/day) (148–150). The likelihood risk of a poorer dietary pattern is 3.2-fold higher for the females than for the males ($p = 0.005$) and 4.6-fold lower for those consuming a regular versus chopped diet ($p<0.001$). Elderly people should consume at least 21–30 g of proteins during the meals, but has been demonstrated that this recommendation is rarely met in LTC facilities, in fact, most of protein intake, that is essential for the militainment of muscle mass, is mainly assumed at lunch (18.80g; 95% CI: 18.16 to 19.43 in women - 21.20g; 95% CI: 20.09 to 22.30 in men) (148–150). Hence, the nutritional intake of the residents of LTC homes are inadequate (148,151) explaining the high prevalence of sarcopenia and the overall prevalence of malnourished residents (~50%) (148,152).

In addition to sarcopenia and malnutrition, older people are at greater risk of dehydration: to compensate for daily fluid depletion (by breathing, exudation, urine and feces) older women should be offered at least 1.6 L/day of liquids, while older men should be offered at least 2.0 L/day of liquids (foods water excluded), unless water restriction is needed (146).

It has been estimated that 85.5% of older adults residing in LTC homes had a mean total fluid intake of 1,104.1 mL/day (± 379.3) that is well below the recommended daily adequate intake for total water (153).

Although evidence is not consistent, it has been estimated that 38% of LTC residents are dehydrated (serum osmolality >300 mOsm/kg) and a further 30% have impending dehydration (serum osmolality = 295 to 300 mOsm/kg) (154). While, age, gender, body max index, functional status (such urinary o fecal continence), energy intake, beverage consistency (thickened drinks), total water intake, snack frequency, and type of oral nutritional supplement seem not to be factors associated with dehydration, otherwise diabetes, poor kidney function or lower cognitive function are considered to be risk factors for insufficient fluid intake (155). In older adults the raise of osmolality should stimulate cell membrane osmoreceptors triggering thirst, but thirst is no longer associated with this feedback mechanism, leaving older adults without a physiological response and obviously at higher risk of hospitalization for delirium and other consequences (154). Plain water is not the only fluid useful when residents need to be hydrated, most of non-alcoholic drinks are suitable for hydration, but no intervention to support adequate drinks have been clearly shown to prevent dehydration due to high risk of bias present in many studies (154,156).

LONG TERM CARE AND AIRWAYS INFECTION

Large population observational studies consistently highlight an inverse association between lifestyle intervention and inflammatory biomarkers, leading to a reduction in risk of viral upper respiratory tract infections (URTI) (11). By suppressing the IL-17 inflammatory pathway, an unhealthy dietary pattern can reduce the host immune system's response against airway pathogens (157). Immune cells function are influenced by Vitamin D, people with Vitamin D concentration beyond the optimal circulating level (75 nmol/l) showed a lower incidence of URTI than those with a recommended vitamin D concentration of 50 nmol/l (158).

Regular, moderate physical activity improves immunosurveillance against pathogens lowering URTI risk (11), in fact, epidemiological data demonstrate a dose-response relationship between physical activity performed before infection and a reduction in the incidence of URTI (incidence rate ratio (IRR)=0.82, 95% CI=0.69 to 0.98) (157,159). Regular exercise induces interleukin (IL)-6 production at the skeletal muscle level. IL-6 triggers an anti-inflammatory cascade via the induction of the anti-inflammatory cytokines interleukin-1 receptor antagonist (IL-1ra) and IL-10, and also inhibits tumour necrosis factor (TNF)-α. The immunosenescence phenomenon (i.e. the decreased of cell-mediated immune function) might contribute to higher risk of URTI and related mortality in older population, such as due to influenza (158).

As Covid-19 infection, which was declared as pandemic by the World Health Organization on 11 March 2020 (38) and has accumulated over with over 7,044,637 recorded deaths and 775,293,630 confirmed global infections (as of 7 April 2024) (160), also highly pathogenic influenza viruses are often associated with dysregulation of cytokine and an excessively exaggerated immune response, commonly known as cytokine storm (161). In association with an increase ROS release, cytokine storm may activate apoptosis of epithelial and endothelial cells and, subsequently, vascular leakage, abnormal immune system response leading to acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) or death (161,162).

The clinical and economic burden of influenza is significant, particularly in vulnerable populations. The risk of influenza-related complications increases with age and, influenza-

associated hospitalizations are most common among elderly aged ≥ 65 years. Up to 90% of influenza-attributable mortality is seen in this cohort (163), approximately 5 million cases of severe illness and up to 640,000 deaths are caused by influenza each year (164). Globally, the estimated mean annual influenza-associated respiratory excess mortality rate ranges from 2.9 to 44.0 per 100,000 in those between 65 and 74 years, and 17.9 to 223.5 per 100,000 in those ≥75 years of age (165). Estimated direct cost of influenza amounts to € 2.3 million (vaccinated and unvaccinated) each year in Europe (164).

According to the OECD, there were in 2020 1.4 million older people accommodated in 15,600 LTC homes in the United States. Similarly, in 2013 there were an estimated 5,153 nursing homes and 12,525 residential care homes in the United Kingdom, of which over 90% of residents were 65 years of age or older. Likewise, as of 2020, approximately 965.800 people in Japan lived in LTC homes (152). Thus, taking in account that in Italy there are more than 423,876 LTC recipients in institutions (other than hospital) (153) and for all the above reasons, influenza has a relevant impact on long term care residents. In fact, due to their overall frailty, people residing in a long-term care facility are considered to be very susceptible to influenza infection. The environmental characteristics of a LTC place this setting at risk of an elevated number of influenza outbreaks with explosive effects (166) as the pathogen can be transferred by staff members, visitors or new residents. The complications of influenza infection (such as bronchitis, pneumonia, ALI and ARDS) growth the risk of hospitalization (RR=1.43; 95% CI: 0.99 to 2.08) and death (RR=2.77; 95% CI: 1.55 to 4.91) of residents exposed to influenza virus (166).

For these reasons it is important to understand which residents are more susceptible to flu-like syndromes and which strategies are most useful for preventing or reducing the likelihood of LTC seasonal influenza outbreaks.

OBJECTIVES:

1. **Project 1 - Lifestyle Medicine for Longevity (LSM4Long) study:**
   - To develop and implement a combined lifestyle medicine intervention (nutritional, physical and psychological) aimed to improve functionality and oxidative stress biomarkers of residents of a LTC facility.
   - To assess the effectiveness of the LSM4Long intervention aimed to improve functionality and its effects on oxidative status.

2. **Project 2 - UPO SoGuD Primary Prevention:**
   - To systematically summarize best practices related to NCD prevention retrieved from the best clinical practice guidelines found in scientific literature.
   - To develop a diagnostic-preventive pathway for clinicians, general population and policy makers.

3. **Project 3 – Flu-like syndrome/COVID-19 onset in LTC:**
   - To assess the presence of an association between plasma redox state/mitochondria function and flu-like syndrome/COVID-19 in the elderly admitted to a long-term care unit.

4. **Project 4 – Vaccination Strategies against Seasonal Influenza in Long Term Care**

   - To study the model of diffusion of influenza virus outbreaks in a typical long-term care setting for the development of a mathematical model.

PROJECT 1 - LIFESTYLE MEDICINE FOR LONGEVITY STUDY (LSM4LONG)

Materials and methods - planning the study

A systematic overview of lifestyle interventions to improve longevity indicators in elderly patients hospitalized in long-term care facilities

In order to develop a lifestyle intervention that can be effective in preventing or mitigating sarcopenia, dehydration, malnutrition, osteoporosis and risk of falls, an overview of systematic review has been designed and performed. The aim of an overview of systematic review is to answer questions related to the prevention or treatment of various disorders using an explicit and systematic method to search for and identify multiple systematic reviews in the same topic area (167).

The protocol has been registered in the PROSPERO database of systematic reviews (CRD42020206090)
https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020206090

The research question was formulated using the PICO format as shown in table 2:

Table 2: Overview of systematic review PICO format

Population:	Adult/elderly participants (≥60 year), resident in long-term care facilities. No or Mild cognitive impairment was included
Intervention:	Dietary, physical activity, nutritional supplementation
Comparison:	usual care
Outcome:	Changes in body composition (lean mass, fat mass) defined as % lean mass, appendicular skeletal muscle mass, phase angle, BMI, % fat mass Hydratation, defined as total body water. Bone health, defined as cortical bone thickness, t-score

PubMed, Scopus and Cochrane databases were searched on 16th November 2020 using the search strings reported in table 3:

Table 3:Umbrella Review Strings Search

PubMed
("sarcopenia"[MeSH Terms] OR "sarcopenia"[All Fields]) AND (physical activities[All Fields] OR physical activitiy[All Fields] OR physical activity[All Fields] OR physical activity,[All Fields] OR physical activity All Fields]) AND (systematic[sb] AND "humans"[MeSH Terms] AND English[lang])
SCOPUS
TITLE-ABS-KEY (diet AND sarcopenia AND ageing) AND (LIMIT-TO (DOCTYPE,"re")) AND (LIMIT-TO (LANGUAGE,"English"))
Cochrane Database
(supplementation or "physical activity" or exercise or rehabilitation or hydration or mobilization) and (sarcopenia or osteoporosis or "body composition" or "lean mass" or "muscular strength") and (aged or ageing or elderly or "long-term care" or "residential care")

Table 4 shows the inclusion/exclusion criteria used for the selection of Systematic Reviews

Table 4: Overview of systematic reviews inclusion and exclusion criteria

Inclusion Criteria
Systematic review of the literature with or without metanalysis Patients' age over 60 years old; Patients living in nursing home residence or similar structures (LTCFs); Sarcopenia or lean mass or fat mass Hydration Bone health No or mild cognitive impairment (as described by a MMSE score > 20, or by a score on the Mini-Cog ≥ 3, or similar results among other tests for the assessment of cognitive impairment)
Exclusion criteria
Narrative literature reviews Case reports, Editorials, Book comments and chapters, Articles not in English language

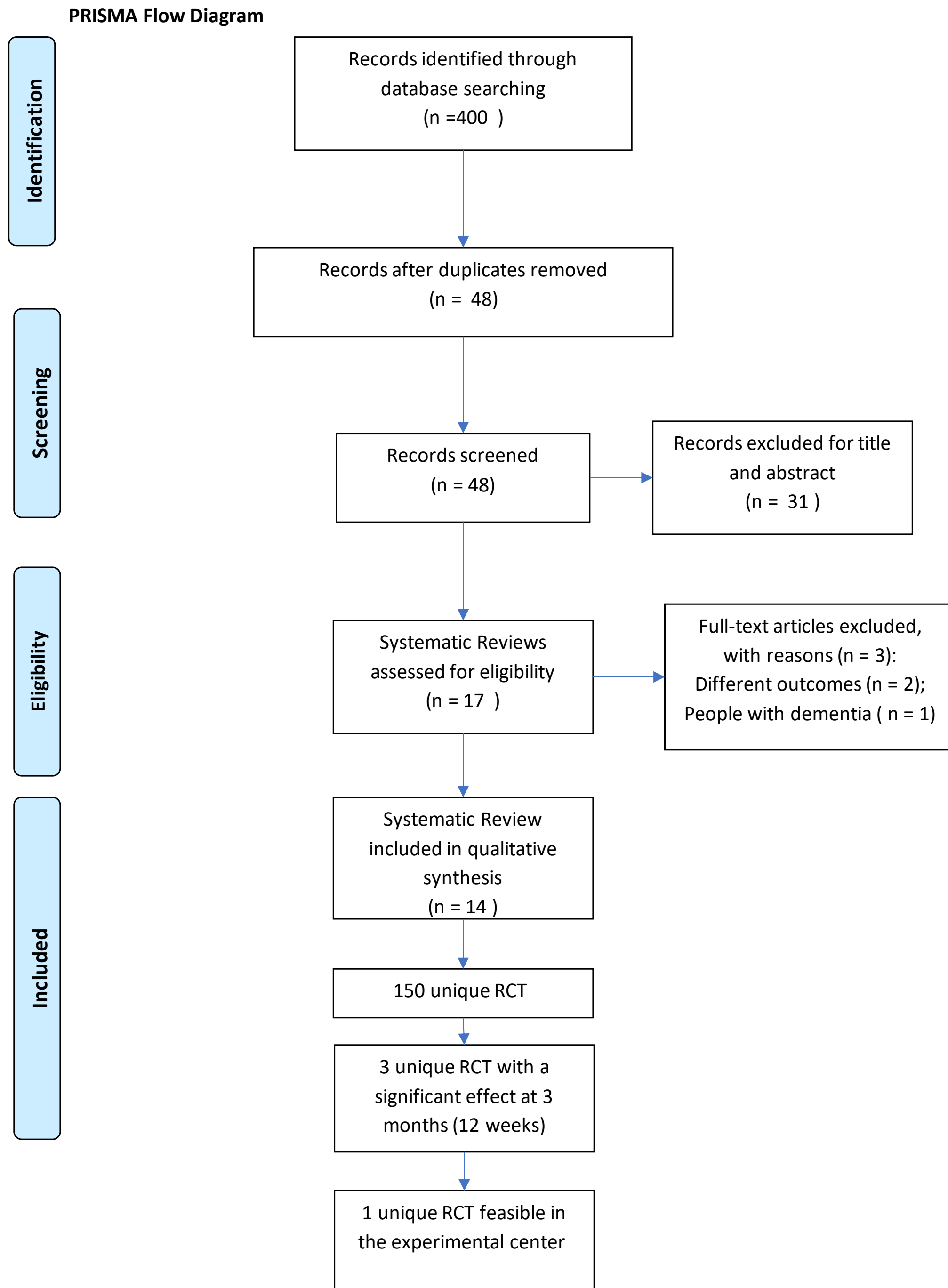
Since implementations of a lifestyle intervention can last several months or years (168,169) and the median length of stay in a Nursing Home might be brief: only 5 months (170), in order to develop an economically sustainable intervention for a LTC facility, only the results assessed at a follow-up period less or equal to 12 weeks (3 months) were included. Inclusion and exclusion criteria are reported in table 4.

Table 5:Overview of systematic reviews inclusion and exclusion criteria for single RCT

Inclusion Criteria
Randomized Controlled Trial
Free body exercise intervention to be adaptable for people with physical disability and supervised by trained personnel
Nutritional intervention that can be cooked in a Nursing Home
Effect of intervention assessed at Follow-up less or equal to 12 weeks (3 months)
Exclusion criteria
Non-Randomized Controlled Trial
Exercise intervention with exercise machines
Nutritional intervention only with supplementations
Any intervention with a significant effect only at Follow-up greater than 12 weeks

Following the Preferred Reporting Items for Systematic Reviews and MetaAnalyses (PRISMA, 2009) flowchart (171) (figure 1), two researchers (D.C., G.C.) independently examined the eligibility of titles and abstracts of systematic reviews identified by the search strategy, a third reviewer (V.A.) resolved any discrepancies that emerged between the reviewers.

Figure 1: Overview of systematic reviews Prisma Flow Diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Systematic review quality assessment has been rated using AMSTAR 2 checklist (172): AMSTAR 2 is a validated revision of the original appraisal instrument with a total of 16 items against the 11 previous tool. Systematic review critically appraised are classified as:

- High: no or one critical-weakness, findings of studies included in the Systematic Review provides are accurate and comprehensive (SCORE 11-16).
- Moderate: Systematic Reviews is flawed by more than one non-critical weakness, summary results of the included studies may be accurate (SCORE 6-10)
- Low: there is a critical weakness with or without non-critical flaws that reduced the confidence in interpreting the findings (SCORE 0-5).
- Critically low: more than one critical weakness, the Systematic Review findings are not reliable

Two researchers (DC, G.C.) developed an abstraction form to collect data from each included Systematic Review.

Non-pharmacological approaches to depressed elderly with no or mild cognitive impairment in long-term care facilities: a systematic review of the literature

In order to identify which interventions are effective and feasible in the LTCFs context to increase self-confidence and motivation, a systematically review of non-pharmacological approaches for depressed older adults with no or mild cognitive impairment (as described by a Mini Mental State Examination [MMSE] score > 20) living in LTCs facilities has been developed and performed.

The research question was formulated using the PICO format as shown in table 6

Table 6: Systematic review PICO Format

Population	Elderly people (age over 65 years old) residing in LTCFs, with no or mild cognitive impairment
Intervention	Any non-pharmacological approach
Comparison	No-intervention group (when applicable)
Outcome	Depression, as assessed by validated psychometric measures

PubMed and Scopus databases were searched on 16th November 2020 using accurate search strings (see table 7):

Table 7: Systematic review - string search used in PubMed and Scopus databases

Search string used in PubMed database:
(((((“aged”[MeSH] OR “aging”[MeSH] OR “Aged, 80 and over”[Mesh] OR “old age people” OR elder* OR adult* OR retired OR ancient*))))) AND ((((((((((((((“Assisted Living Facilities”[Mesh]) OR “Community Health Nursing”[Mesh]) OR “Group Homes”[Mesh]) OR “Halfway Houses”[Mesh]) OR “Health Facility Environment”[Mesh]) OR “Homes for the Aged”[Mesh]) OR “Institutionalization”[Mesh]) OR “Long-Term Care”[Mesh]) OR “Nursing Care”[Mesh]) OR “Nursing Homes”[Mesh]) OR “Rehabilitation Centers”[Mesh]) OR “Rehabilitation Nursing”[Mesh]) OR “Residential Facilities”[Mesh]) OR “Housing for the Elderly”[Mesh]) OR “Geriatric Nursing”[Mesh])) AND ((depression [MeSH] OR depression [text word] OR depress* [text word] OR depressive symptoms [text word] OR emotional depression [text word] OR depressive disorder [MeSH] OR depressive disorder [text word] OR depressive disorder, major [MeSH] OR major depression [text word] OR MDD [text word] OR major depressive disorder [text word]))
String search used in Scopus database:
(ALL (“Assisted Living Facilities” OR “Community Health Nursing” OR “Group Homes” OR “Halfway Houses” OR “Health Facility Environment” OR “Home(s)* for the Aged” OR “Institutionalization” OR “Long-Term Care” OR “Nursing Care” OR “Nursing Homes”)) AND (ALL (“aged” OR “aging” OR “Aged, 80 and over” OR “old age people” OR elder* OR adult* OR retired OR ancient*)) AND (TITLE-ABS-KEY (“Depression”) OR TITLE-ABS-KEY (“Depressive symptoms”) OR TITLE-ABS-KEY (“Emotional depression”) OR TITLE-ABS-KEY (“Depressive disorder”) OR TITLE-ABS-KEY (“Major depression”) OR TITLE-ABS-KEY (“MDD”) OR TITLE-ABS-KEY (“Major depressive disorder”) OR TITLE-ABS-KEY (“Depress*”))

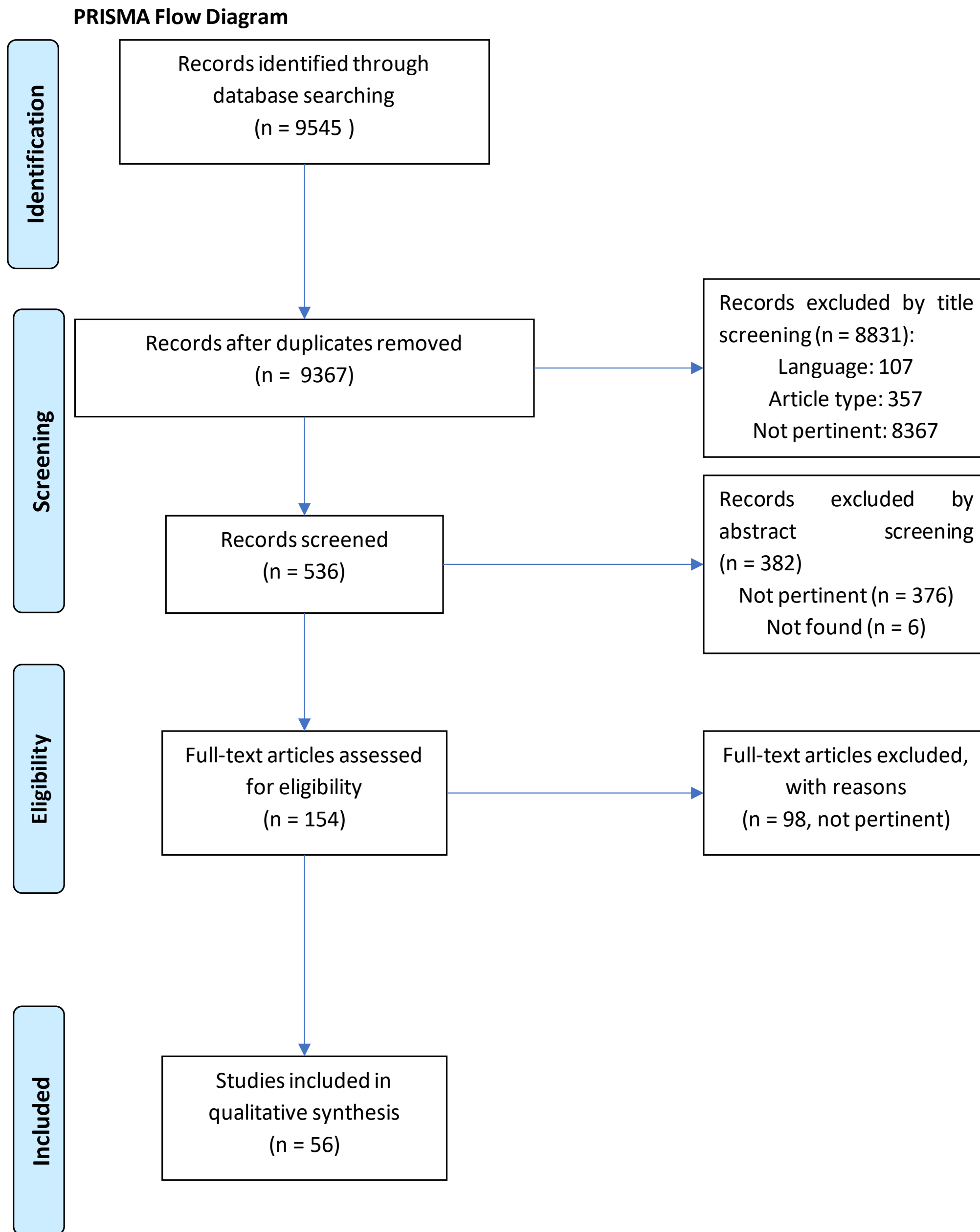
Table 8 shows the inclusion/exclusion criteria used for the selection of studies

Table 8:Systematic review - inclusion and exclusion criteria

Inclusion Criteria
Patients' age over 65 years old; Patients living in nursing home residence or similar structures (LTCFs); Diagnosis of depression, either based on clinical assessment or on the administration of questionnaires or scales; No or mild cognitive impairment (as described by a MMSE score > 20, or by a score on the Mini-Cog ≥ 3, or similar results among other tests for the assessment of cognitive impairment).
Exclusion criteria
Narrative literature reviews Case reports, Editorials or book comments or chapters, Articles not in English language

In collaboration with Eleonora Gattoni (E.G.), Debora Marangon (D.M.) and Prof Carla Gramaglia (C.G.) studies selection was made following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, 2009) flowchart (171), Two independent reviewers (D.M., E.G.) assessed the articles identified by the search strings (see above); a third reviewer (C.G.) resolved any discrepancies that emerged between the reviewers.

Figure 2: Systematic review - Prisma Flow Diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

A data abstraction form has been developed to collect data from each included study (see Table 9) and to assess the quality of each study included using Jadad modified scale (173) for Randomized Control Trial (RCT), Newcastle-Ottawa Scale (NOS) (174) for Quasi-experimental Design and Non-experimental studies. The first one is a 8 point-score tool that assess the risk of bias of studies evaluating randomization, double-blinding, withdrawals and dropouts, inclusion and exclusion criteria, statistical analyses used (173). The NOS is a 9 point-score scale used for assessing the quality of QES and NE evaluating selection and comparability of cohorts and assessment of outcome (174).

Table 9: Systematic review- Main information extracted from each study included

Author and year of publication, sample size, demographic data (mean age, gender), dropout rate
Type of study design (RCT: Randomized Controlled Trial; QED: Quasi Experimental Design; NE: Non-experimental),
Setting: residential care type
Outcomes measures for dementia or depression
Type of intervention qualification of the intervention provider, setting, monotherapy/augmentation
Main findings

In order to estimate the effect size of each single intervention, standardized mean differences (SMDs) with 95% CIs were calculated to assess depression severity across different depression scales. As recommended by Cochrane Hedges' adjusted g statistic was used to calculate SMDs (it is very similar to Cohen's d statistic, but includes an adjustment for small sample bias). Standard mean differences were calculated using RevMan 5.4 (167).

Relevant findings from the systematic overview of lifestyle interventions to improve longevity indicators in elderly patients hospitalized in long-term care facilities

As figure 1 shows: after deduplication 48 single records (out of 400 records – 12%) were screened for title and abstract where 64.58% (n=31) were excluded because judged not relevant. Fourteen out of 17 Systematic Reviews assessed for eligibility met the inclusion criteria and were included in qualitative analysis.

Only 1 out of 14 Systematic Reviews provided a confident summary of findings (AMSTAR 2: High), the remaining (n=13) were rated as moderate quality (table 10 – 11).

13 Systematic Reviews (93%) performed a selection of studies in duplicate, whereas 2 (14%) performed data extraction in duplicate, 12 reviews (86%) have not provided complete information on excluded studies. 12 studies (86%) have assessed and documented the quality of included studies and 8 (57%) reviews appropriately combined the findings of studies using Meta-analysis methods. 25% of the items were rated as "partially yes" for at least one article. Out of 14, 8 reviews (57%) have had defined methods a priori. Among the 14 SRs, all (100%) did not completely or explicitly declare the inclusion and exclusion criteria and 14% (n=2) of the reviews failed to give a satisfactory explanation for any heterogeneity observed in the results of the reviews.

AMSTAR CHECKLIST

Table 10: Overview of reviews - Methodological Quality of systematic reviews or meta-analyses using AMSTAR 2

Title	Author	Publication Year	AMSTAR SCORE
Exercise alone or combined with dietary supplements for sarcopenic obesity in community-dwelling older people: A systematic review of randomized controlled trials	Martinez-Amat A	2018	7
Effectiveness of nutritional and exercise interventions to improve body composition and muscle strength or function in sarcopenic obese older adults: A systematic review	Theodorakopoulos C	2017	6
Nutrition and physical activity in the prevention and treatment of sarcopenia: systematic review	Beudart C, Dawson A	2017	6
Increasing Fluid Intake and Reducing Dehydration Risk in Older People Living in Long-Term Care: A Systematic Review	Bunn D	2015	6
Effects of protein, essential amino acids, β-hydroxy β-methylbutyrate, creatine, dehydroepiandrosterone and fatty acid supplementation on muscle mass, muscle strength and physical performance in older people aged 60 years and over. A systematic review of the literature	Beaudart C, Rabenda V	2017	10
Effects of multi-domain interventions in (pre)frail elderly on frailty, functional, and cognitive status: a systematic review	Dedeyne	2017	6
Exercise interventions in healthy older adults with sarcopenia: a systematic review and meta-analysis	Vlietstra L	2018	8

Title	Author	Publication Year	AMSTAR SCORE
Oral nutritional support with or without exercise in the management of malnutrition in nutritionally vulnerable older people: A systematic review and meta-analysis	Wright J	2018	8
Effects of protein supplementation combined with resistance exercise on body composition and physical function in older adults: a systematic review and meta-analysis	Liao CD	2017	10
Systematic review and meta-analysis of the effect of protein and amino acid supplements in older adults with acute or chronic conditions	Cheng H	2017	9
The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: a systematic review and meta-analysis	Antoniak AE	2016	11
Vitamin D and vitamin D analogues for preventing fractures in postmenopausal women and older men	Avenell A	2016	10
Exercise for preventing and treating osteoporosis in postmenopausal women (Review)	Howe TE	2011	9
Physical Exercise Interventions for Improving Performance-Based Measures of Physical Function in Community-Dwelling, Frail Older Adults: A Systematic Review and Meta-Analysis	Gine'-Garriga M	2014	10

Table 11:Overview of systematic reviews - AMSTAR 2 Assessment according to quality items

Quality items	Yes n (%)	No n (%)	PY n (%)
1. Did the research questions and inclusion criteria for the review include the components of PICO?	14 (100)	0	0
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	8 (57)	0	6 (43)
3. Did the review authors explain their selection of the study designs for inclusion in the review?	0	14 (100)	0
4. Did the review authors use a comprehensive literature search strategy?	11 (79)	0	3 (21)
5. Did the review authors perform study selection in duplicate?	13 (93)	1 (7)	0
6. Did the review authors perform data extraction in duplicate?	13 (93)	1 (7)	0
7. Did the review authors provide a list of excluded studies and justify the exclusions?	2 (14)	12 (86)	0
8. Did the review authors describe the included studies in adequate detail?	12 (86)	0	2 (14)
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	8 (57)	3	3 (43)

Quality items	Yes n (%)	No n (%)	PY n (%)
10. Did the review authors report on the sources of funding for the studies included in the review?	0	14 (100)	0
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	7 (50)	7 (50)	0
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	6 (43)	8 (57)	0
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	9 (64)	5 (36)	0
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	12 (86)	2 (14)	0
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	1 (7)	13 (93)	0
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	12 (86)	2 (14)	0

Lifestyle interventions addressed in the reviews were grouped into three categories based on the clinical outcome targeted by the intervention.

1. interventions targeting body composition as lean mass and/or fat mass (12 reviews)
2. interventions targeting hydration (1 review)
3. interventions targeting bone health (2 reviews)

Overall, findings of the included Systematic Reviews came from 300 studies. Randomized study design was the most representative study design (n=286, 95.33%), few quasi-experimental designs contribute to the results of Systematic Reviews (n=8, 2.66%), less than 1% of the included studies in this sample adopted a cross-over or cross-sectional or retrospective cohort study designs.
Outcomes examined included changes in bone health and /or body composition (lean mass and/or fat mass) and/or hydration.

The aim of this milestone was to identify a nutritional and a physical exercise intervention that could be implemented in the setting of Nursing Home, thus all single studies provided by each systematic review were collected.

Overall, after deduplication 150 single RCTs out of 286 were retrieved, and only 3 RCT showed a significant effect at 12 weeks Follow-up.

Only 1 study meet all the criteria previously defined for the selection of intervention.

1 RCT (KIM 2016) (175) has been excluded because the nutritional intervention was a fortified Tea with catechin supplementation and it cannot be produced in a LTCs kitchen. The physical exercise intervention was a progressive sequence of resistance and weight-bearing exercises with hydraulic exercise machine (chair exercise, resistance band exercise and hydraulic exercise machine) and aerobic training using a stationary bicycle (175) that were not usually used in all the LTCs facilities or were not tailored for people with physical disability.

In the study of Arnarson, 2013 (176), the resistance exercise program was designed involving exercise machines during each training session, in fact participants performed seated leg extension, seated leg curl, seated leg press, seated chest fly, seated row, seated

pull-down, seated biceps curl, seated triceps curl, seated lower back, extension and seated abdominal curl. The nutritional intervention associated with physical exercise was a two drinks supplementation made of whey proteins and carbohydrates (176).

Rondanelli 2016 (177) designed a RCT with a combined nutritional and a comprehensive physical fitness intervention. The training program was supervised by trained personnel and participants performed strengthening, balance and gait exercise sessions with a cool-down period. No exercise machines were needed, participants performed a free body progressive sequence from seated to standing position in an increasing intensity. A fixed ankle weight was used during lower body strengthening exercises in accordance with participant's ability, while a resistance band was used for upper body strengthening and resistance exercises (177).

The nutritional intervention consists of an oral essential aminoacid, whey-protein and vitamin D mixture (177). Since the author reported the content of the nutritional supplements (Table 12), it was possible to translate nutrients and minerals in a Healthy diet as a part of a menu that could be cooked in a LTC facility.

Table 12: Nutritional content of the dietary supplement used by Rondanelli 2016 (177)(177)

	Energy value		% RDA per 32-g dose
	Per 100 g	Per 32 g	
Kilojoules	1466	469	
Kilocalories	351	112	
Nutrients, g			
Whey protein	68.9	22	
Lipids	1.1	0.4	
SFAs	0.2	0.0	
Total carbohydrates	14.8	4.7	
Simple carbohydrates	2.6	0.8	
Complex carbohydrates	3.9	1.2	
Polyols	8.3	2.7	
Fiber	6.9	2.2	
Fructo-oligosaccharides	3.2	1.0	
Minerals, mg			
Calcium	25.8	8.3	1
Phosphorus	76.3	24.4	3
Sodium	917.4	293.6	
Magnesium	140.7	45.0	12
Iron	0.8	0.3	2
Vitamins, D3 (cholecalciferol) mg (IU)	7.8 (312)	2.5 (100)	50

RDA, Recommended Dietary Allowance

Relevant results obtained from the non-pharmacological approaches to depressed elderly with no or mild cognitive impairment in Long-Term Care facilities systematic Review

As reported in figure 2, after deduplication 9,367 articles were retrieved and after exclusion by title or abstract screening 154 single studies were assessed for eligibility. 56 single studies were included in the systematic review: the intervention most used were psychoeducation/rehabilitation in 15 studies (26,8%) and reminiscence and story sharing in 14 studies (25%), physical exercise, pet therapy, horticulture/gardening and psychotherapy were the interventions used in n = 9, n = 4, n = 3 studies, respectively. For 8 studies the intervention did not match any of categories above.

The overall quality of the included studies RCTs assessed with the Jadad and NOS for RCTs and QED or NE studies respectively, was quite poor (some studies involving small samples and/or with a poor methodological approach). This result is consistent with those reported by the previous review by Simning and Simons which highlighted an overall lack of quality of the studies in the field (178).

Overall included studies showed heterogeneity in sample size (from 5 to 403 participants) as in eligibility criteria. Regarding study design, 53.57% (n=30/56) were Randomized Controlled Trials, 39.28% (n=22/56) were Quasi-Experimental Design (QED) and 4 out of 56 were Non-Experimental design (NE).

The Geriatric depressive scale (GDS) was the most validated psychometric tool used in the sample of studies included, authors measured outcomes with the 30-item version but even more in its short (15-item) version.

53.57% (n=30) studies were conducted in western nations primarily in the Americas (n=17/56; 30.35%), European (n=13/56; 23.21%) and Oceania (n=3/56; 5.36%) regions. The remaining studies (41%) were performed mostly in Taiwan (14/26; 53.85%). None of studies was experimented in Africa. Horticulture was the intervention used in all the articles from the Asian continent, while 75% of the articles dealing with pet therapy came from Italy.

Overall, the non-pharmacological intervention lasted 8 weeks with a weekly session of 30 to 60 minutes.

Findings of the systematic review showed that usually the qualification of the intervention provider had no impact on its effectiveness, so the treatment can be administered by an external expert or with the cooperation of a staff member (such as a psychologist).

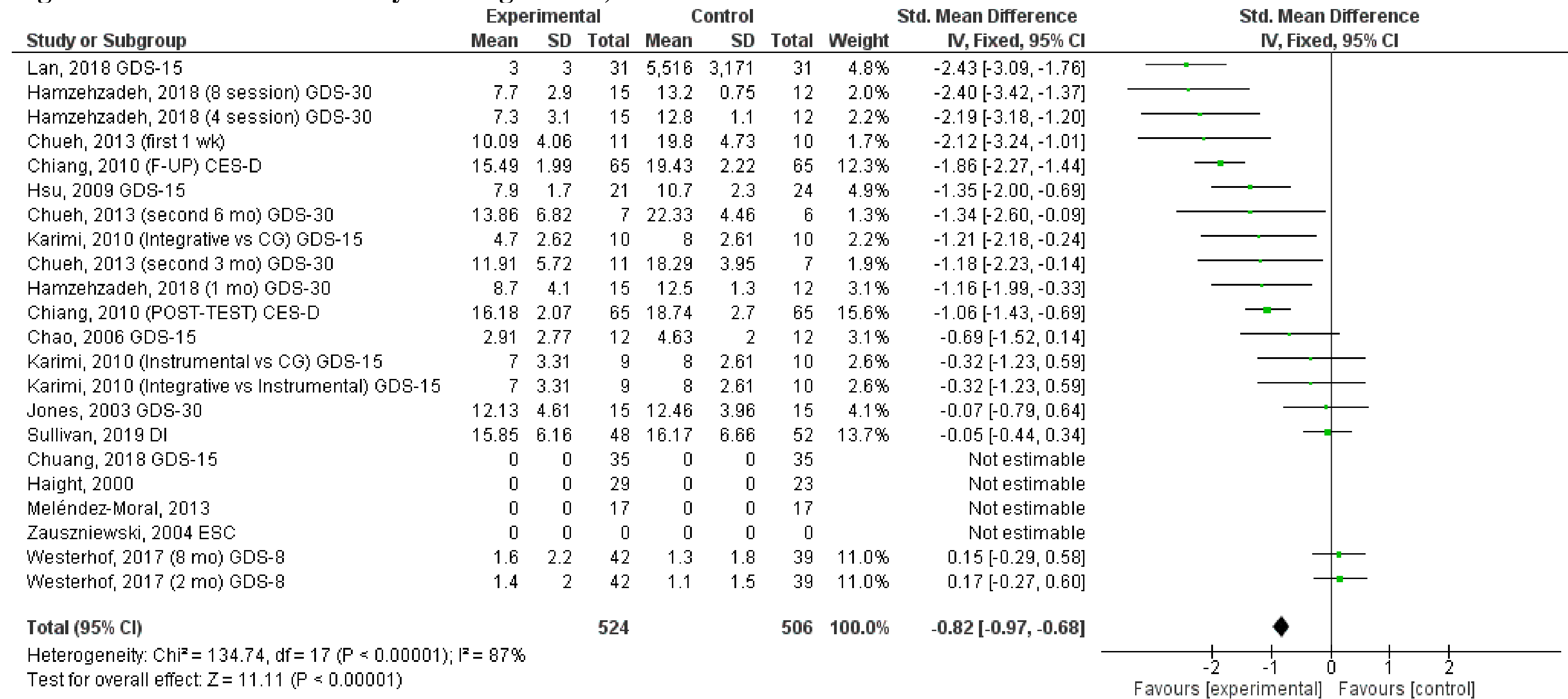
This systematic review identified seven types of effective interventions with different effect sizes. Due to the heterogeneity in sample size, eligibility criteria, differences of in participants' characteristic (such as mean age, definition of depressive symptoms), duration of intervention, number of sessions/weeks, setting, a meta-analysis could not be performed.

As reported in tables 13-14-15 and in figures 3-4-5, reminiscence, psychoeducation/rehabilitation, physical exercise interventions seem to be the most effective. Moreover, the reminiscence intervention group presented a lower risk of bias and can be administered with the cooperation of a psychologist supervised by an external expert. In conclusion the risk/benefit ratio of the assessed interventions seem to be in favor of reminiscence. Since the main objective of the systematic review was to understand which non-pharmacological approach could be suitable in the LTC setting, reminiscence was selected as a component of the lifestyle medicine intervention aimed to improve self-efficacy and motivation.

Table 13: Reminiscence & Story Sharing studies: Effect size estimation (using Review Manager 5.4)

Reminiscence & Story Sharing studies							
Study	**Experimental**			**Control**			**Std. Mean Difference**
	mean	**sd**	**n**	**mean**	**sd**	**n**	**IV, Fixed, 95% CI**
Lan, 2018 GDS-15	3	3	31	5516	3171	31	-2.43 [-3.09, -1.76]
Hamzehzadeh, 2018 (8 session) GDS-30	7.7	2.9	15	13.2	0.75	12	-2.40 [-3.42, -1.37]
Hamzehzadeh, 2018 (4 session) GDS-30	7.3	3.1	15	12.8	1.1	12	-2.19 [-3.18, -1.20]
Chueh, 2013 (first 1 wk)	10.09	4.06	11	19.8	4.73	10	-2.12 [-3.24, -1.01]
Chiang, 2010 (F-UP) CES-D	15.49	1.99	65	19.43	2.22	65	-1.86 [-2.27, -1.44]
Hsu, 2009 GDS-15	7.9	1.7	21	10.7	2.3	24	-1.35 [-2.00, -0.69]
Chueh, 2013 (second 6 mo) GDS-30	13.86	6.82	7	22.33	4.46	6	-1.34 [-2.60, -0.09]
Karimi, 2010 (Integrative vs CG) GDS-15	4.7	2.62	10	8	2.61	10	-1.21 [-2.18, -0.24]
Chueh, 2013 (second 3 mo) GDS-30	11.91	5.72	11	18.29	3.95	7	-1.18 [-2.23, -0.14]
Hamzehzadeh, 2018 (1 mo) GDS-30	8.7	4.1	15	12.5	1.3	12	-1.16 [-1.99, -0.33]
Chiang, 2010 (POST-TEST) CES-D	16.18	2.07	65	18.74	2.7	65	-1.06 [-1.43, -0.69]
Chao, 2006 GDS-15	2.91	2.77	12	4.63	2	12	-0.69 [-1.52, 0.14]
Karimi, 2010 (Instrumental vs CG) GDS-15	7	3.31	9	8	2.61	10	-0.32 [-1.23, 0.59]
Karimi, 2010 (Integr vs Instr) GDS-15	7	3.31	9	8	2.61	10	-0.32 [-1.23, 0.59]
Jones, 2003 GDS-30	12.13	4.61	15	12.46	3.96	15	-0.07 [-0.79, 0.64]
Sullivan, 2019 DI	15.85	6.16	48	16.17	6.66	52	-0.05 [-0.44, 0.34]
Chuang, 2018 GDS-15	.	.	35	.	.	35	Not estimable
Haight, 2000	.	.	29	.	.	23	Not estimable
Meléndez-Moral, 2013	.	.	17	.	.	17	Not estimable
Zauszniewski, 2004 ESC	.	.	.	.	.	.	Not estimable
Westerhof, 2017 (8 mo) GDS-8	1.6	2.2	42	1.3	1.8	39	0.15 [-0.29, 0.58]
Acronyms: CES-D Centre for Epidemiologic Studies Depression Scale; DI Depression Inventory; ESC: Emotional Symptoms Checklist; GDS Geriatric Depression Scale; SMD Standardized Mean Difference							

Figure 3: Reminiscence & Story Sharing studies, SMD



Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
Lan, 2018 GDS-15	3	3	31	5,516	3,171	31	4.8%	-2.43 [-3.09, -1.76]
Hamzehzadeh, 2018 (8 session) GDS-30	7.7	2.9	15	13.2	0.75	12	2.0%	-2.40 [-3.42, -1.37]
Hamzehzadeh, 2018 (4 session) GDS-30	7.3	3.1	15	12.8	1.1	12	2.2%	-2.19 [-3.18, -1.20]
Chueh, 2013 (first 1 wk)	10.09	4.06	11	19.8	4.73	10	1.7%	-2.12 [-3.24, -1.01]
Chiang, 2010 (F-UP) CES-D	15.49	1.99	65	19.43	2.22	65	12.3%	-1.86 [-2.27, -1.44]
Hsu, 2009 GDS-15	7.9	1.7	21	10.7	2.3	24	4.9%	-1.35 [-2.00, -0.69]
Chueh, 2013 (second 6 mo) GDS-30	13.86	6.82	7	22.33	4.46	6	1.3%	-1.34 [-2.60, -0.09]
Karimi, 2010 (Integrative vs CG) GDS-15	4.7	2.62	10	8	2.61	10	2.2%	-1.21 [-2.18, -0.24]
Chueh, 2013 (second 3 mo) GDS-30	11.91	5.72	11	18.29	3.95	7	1.9%	-1.18 [-2.23, -0.14]
Hamzehzadeh, 2018 (1 mo) GDS-30	8.7	4.1	15	12.5	1.3	12	3.1%	-1.16 [-1.99, -0.33]
Chiang, 2010 (POST-TEST) CES-D	16.18	2.07	65	18.74	2.7	65	15.6%	-1.06 [-1.43, -0.69]
Chao, 2006 GDS-15	2.91	2.77	12	4.63	2	12	3.1%	-0.69 [-1.52, 0.14]
Karimi, 2010 (Instrumental vs CG) GDS-15	7	3.31	9	8	2.61	10	2.6%	-0.32 [-1.23, 0.59]
Karimi, 2010 (Integrative vs Instrumental) GDS-15	7	3.31	9	8	2.61	10	2.6%	-0.32 [-1.23, 0.59]
Jones, 2003 GDS-30	12.13	4.61	15	12.46	3.96	15	4.1%	-0.07 [-0.79, 0.64]
Sullivan, 2019 DI	15.85	6.16	48	16.17	6.66	52	13.7%	-0.05 [-0.44, 0.34]
Chuang, 2018 GDS-15	0	0	35	0	0	35		Not estimable
Haight, 2000	0	0	29	0	0	23		Not estimable
Meléndez-Moral, 2013	0	0	17	0	0	17		Not estimable
Zauszniewski, 2004 ESC	0	0	0	0	0	0		Not estimable
Westerhof, 2017 (8 mo) GDS-8	1.6	2.2	42	1.3	1.8	39	11.0%	0.15 [-0.29, 0.58]
Westerhof, 2017 (2 mo) GDS-8	1.4	2	42	1.1	1.5	39	11.0%	0.17 [-0.27, 0.60]
Total (95% CI)			**524**			**506**	**100.0%**	**-0.82 [-0.97, -0.68]**

Heterogeneity: Chi² = 134.74, df = 17 (P < 0.00001); I² = 87%

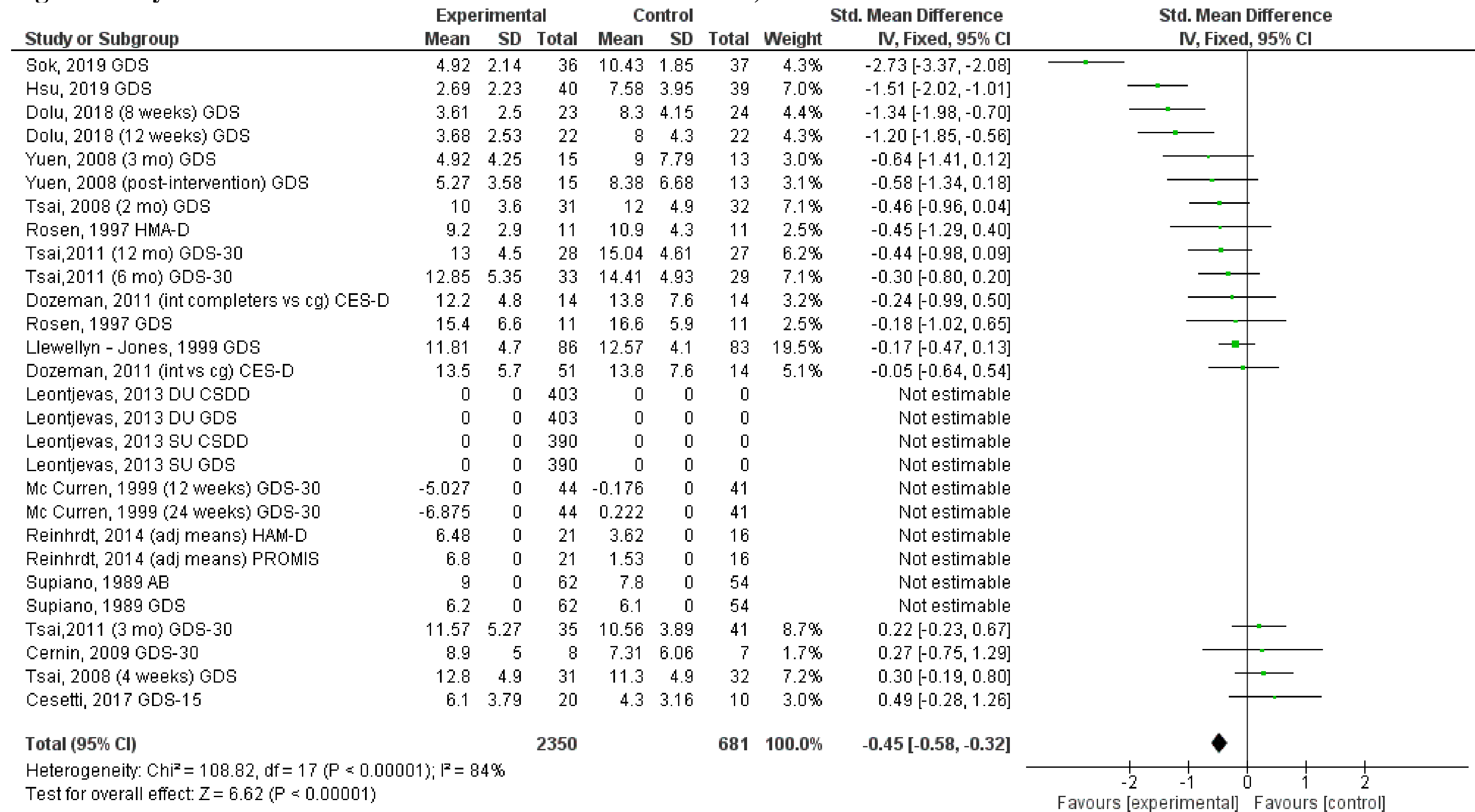
Test for overall effect: Z = 11.11 (P < 0.00001)

Table 14: Psychoeducative / Rehabilitation Interventions studies: Effect size estimation (using Review Manager 5.4)

Psychoeducative / Rehabilitation Interventions studies, SMD							
Study	**Experimental**			**Control**			**Std. Mean Difference**
	mean	**sd**	**n**	**mean**	**sd**	**n**	**IV, Fixed, 95% CI**
Sok, 2019 GDS	4.92	2.14	36	10.43	1.85	37	-2.73 [-3.37, -2.08]
Hsu, 2019 GDS	2.69	2.23	40	7.58	3.95	39	-1.51 [-2.02, -1.01]
Dolu, 2018 (8 weeks) GDS	3.61	2.5	23	8.3	4.15	24	-1.34 [-1.98, -0.70]
Dolu, 2018 (12 weeks) GDS	3.68	2.53	22	8	4.3	22	-1.20 [-1.85, -0.56]
Yuen, 2008 (3 mo) GDS	4.92	4.25	15	9	7.79	13	-0.64 [-1.41, 0.12]
Yuen, 2008 (post-intervention) GDS	5.27	3.58	15	8.38	6.68	13	-0.58 [-1.34, 0.18]
Tsai, 2008 (2 mo) GDS	10	3.6	31	12	4.9	32	-0.46 [-0.96, 0.04]
Rosen, 1997 HAM-D	9.2	2.9	11	10.9	4.3	11	-0.45 [-1.29, 0.40]
Tsai,2011 (12 mo) GDS-30	13	4.5	28	15.04	4.61	27	-0.44 [-0.98, 0.09]
Tsai,2011 (6 mo) GDS-30	12.85	5.35	33	14.41	4.93	29	-0.30 [-0.80, 0.20]
Dozeman, 2011 (int completers vs cg) CES-D	12.2	4.8	14	13.8	7.6	14	-0.24 [-0.99, 0.50]
Rosen, 1997 GDS	15.4	6.6	11	16.6	5.9	11	-0.18 [-1.02, 0.65]
Llewellyn – Jones, 1999 GDS	11.81	4.7	86	12.57	4.1	83	-0.17 [-0.47, 0.13]
Dozeman, 2011 (int vs cg) CES-D	13.5	5.7	51	13.8	7.6	14	-0.05 [-0.64, 0.54]
Leontjevas, 2013 DU CSDD	Not estimable; authors reported: effect size (0.3); 95%IC (-0.3 to 0.9); p-value 0.379						
Leontjevas, 2013 DU GDS	Not estimable; authors reported: effect size (-0.3); 95%IC (-0.7 to 0.1); p-value 0.172						
Leontjevas, 2013 SU CSDD	Not estimable; authors reported: effect size (-0.8); 95%IC (-1.4 to 0.1); p-value 0.018						
Leontjevas, 2013 SU GDS	Not estimable; authors reported: effect size (-0.1); 95%IC (-0.4 to 0.2); p-value 0.404						
Mc Curren, 1999 (12 weeks) GDS-30	-5.027	.	44	-0.176	.	41	Not estimable
Mc Curren, 1999 (24 weeks) GDS-30	-6.875	.	44	0.222	.	41	Not estimable
Reinhrdt, 2014 (adj means) HAM-D	6.48	.	21	3.62		16	Not estimable
Reinhrdt, 2014 (adj means) PROMIS	6.8	.	21	1.53	.	16	Not estimable
Supiano, 1989 AB	9	.	62	7.8	.	54	Not estimable
Supiano, 1989 GDS	6.2	.	62	6.1	.	54	Not estimable
Tsai,2011 (3 mo) GDS-30	11.57	5.27	35	10.56	3.89	41	0.22 [-0.23, 0.67]
Cernin, 2009 GDS-30	8.9	5	8	7.31	6.06	7	0.27 [-0.75, 1.29]
Tsai, 2008 (4 weeks) GDS	12.8	4.9	31	11.3	4.9	32	0.30 [-0.19, 0.80]
Cesetti, 2017 GDS-15	6.1	3.79	20	4.3	3.16	10	0.49 [-0.28, 1.26]

Acronyms: CES-D Centre for Epidemiologic Studies Depression Scale; CSDD Cornell Scale for Depression in Dementia; GDS Geriatric Depression Scale; HAM-D: Hamilton Rating Scale for Depression; PROMIS Patient Reported Outcomes Measurement Information System depression scale; SMD Standardized Mean Difference

Figure 4: Psychoeducative / Rehabilitation Interventions studies, SMD



Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
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Hsu, 2019 GDS	2.69	2.23	40	7.58	3.95	39	7.0%	-1.51 [-2.02, -1.01]
Dolu, 2018 (8 weeks) GDS	3.61	2.5	23	8.3	4.15	24	4.4%	-1.34 [-1.98, -0.70]
Dolu, 2018 (12 weeks) GDS	3.68	2.53	22	8	4.3	22	4.3%	-1.20 [-1.85, -0.56]
Yuen, 2008 (3 mo) GDS	4.92	4.25	15	9	7.79	13	3.0%	-0.64 [-1.41, 0.12]
Yuen, 2008 (post-intervention) GDS	5.27	3.58	15	8.38	6.68	13	3.1%	-0.58 [-1.34, 0.18]
Tsai, 2008 (2 mo) GDS	10	3.6	31	12	4.9	32	7.1%	-0.46 [-0.96, 0.04]
Rosen, 1997 HMA-D	9.2	2.9	11	10.9	4.3	11	2.5%	-0.45 [-1.29, 0.40]
Tsai,2011 (12 mo) GDS-30	13	4.5	28	15.04	4.61	27	6.2%	-0.44 [-0.98, 0.09]
Tsai,2011 (6 mo) GDS-30	12.85	5.35	33	14.41	4.93	29	7.1%	-0.30 [-0.80, 0.20]
Dozeman, 2011 (int completers vs cg) CES-D	12.2	4.8	14	13.8	7.6	14	3.2%	-0.24 [-0.99, 0.50]
Rosen, 1997 GDS	15.4	6.6	11	16.6	5.9	11	2.5%	-0.18 [-1.02, 0.65]
Llewellyn - Jones, 1999 GDS	11.81	4.7	86	12.57	4.1	83	19.5%	-0.17 [-0.47, 0.13]
Dozeman, 2011 (int vs cg) CES-D	13.5	5.7	51	13.8	7.6	14	5.1%	-0.05 [-0.64, 0.54]
Leontjevas, 2013 DU CSDD	0	0	403	0	0	0		Not estimable
Leontjevas, 2013 DU GDS	0	0	403	0	0	0		Not estimable
Leontjevas, 2013 SU CSDD	0	0	390	0	0	0		Not estimable
Leontjevas, 2013 SU GDS	0	0	390	0	0	0		Not estimable
Mc Curren, 1999 (12 weeks) GDS-30	-5.027	0	44	-0.176	0	41		Not estimable
Mc Curren, 1999 (24 weeks) GDS-30	-6.875	0	44	0.222	0	41		Not estimable
Reinhrdt, 2014 (adj means) HAM-D	6.48	0	21	3.62	0	16		Not estimable
Reinhrdt, 2014 (adj means) PROMIS	6.8	0	21	1.53	0	16		Not estimable
Supiano, 1989 AB	9	0	62	7.8	0	54		Not estimable
Supiano, 1989 GDS	6.2	0	62	6.1	0	54		Not estimable
Tsai,2011 (3 mo) GDS-30	11.57	5.27	35	10.56	3.89	41	8.7%	0.22 [-0.23, 0.67]
Cernin, 2009 GDS-30	8.9	5	8	7.31	6.06	7	1.7%	0.27 [-0.75, 1.29]
Tsai, 2008 (4 weeks) GDS	12.8	4.9	31	11.3	4.9	32	7.2%	0.30 [-0.19, 0.80]
Cesetti, 2017 GDS-15	6.1	3.79	20	4.3	3.16	10	3.0%	0.49 [-0.28, 1.26]
Total (95% CI)			**2350**			**681**	**100.0%**	**-0.45 [-0.58, -0.32]**

Heterogeneity: Chi² = 108.82, df = 17 (P < 0.00001); I² = 84%
Test for overall effect: Z = 6.62 (P < 0.00001)

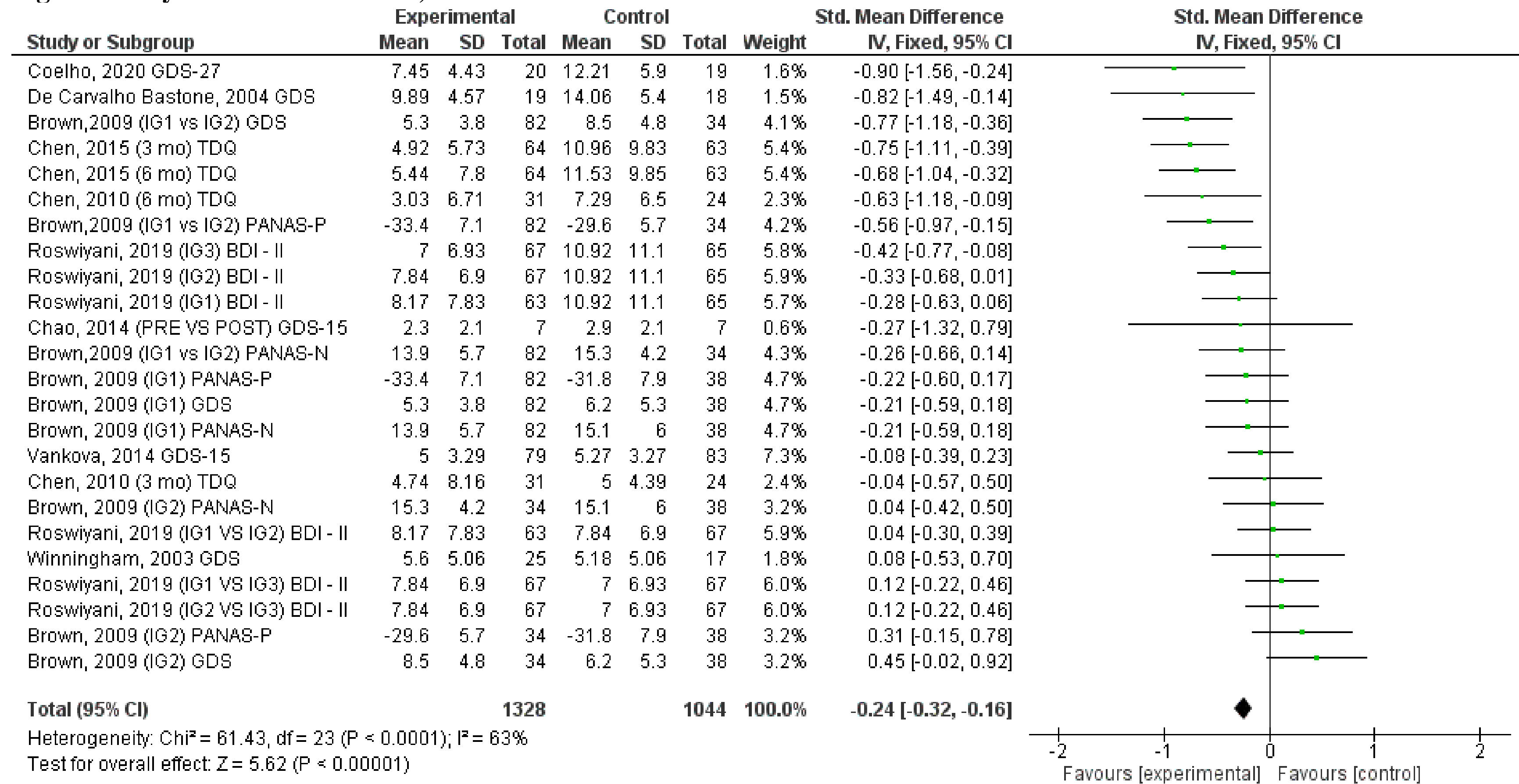
Table 15: Physical exercise studies: Effect size estimation

Physical exercise studies, SMD

Study	Experimental			Control			Std. Mean Difference
	mean	sd	n	mean	sd	n	IV, Fixed, 95% CI
Coelho, 2020 GDS-27	7.45	4.43	20	12.21	5.9	19	-0.90 [-1.56, -0.24]
De Carvalho Bastone, 2004 GDS	9.89	4.57	19	14.06	5.4	18	-0.82 [-1.49, -0.14]
Brown,2009 (IG1 vs IG2) GDS	5.3	3.8	82	8.5	4.8	34	-0.77 [-1.18, -0.36]
Chen, 2015 (3 mo) TDQ	4.92	5.73	64	10.96	9.83	63	-0.75 [-1.11, -0.39]
Chen, 2015 (6 mo) TDQ	5.44	7.8	64	11.53	9.85	63	-0.68 [-1.04, -0.32]
Chen, 2010 (6 mo) TDQ	3.03	6.71	31	7.29	6.5	24	-0.63 [-1.18, -0.09]
Brown,2009 (IG1 vs IG2) PANAS-P	-33.4	7.1	82	-29.6	5.7	34	-0.56 [-0.97, -0.15]
Roswiyani, 2019 (IG3) BDI - II	7.0	6.93	67	10.92	11.1	65	-0.42 [-0.77, -0.08]
Roswiyani, 2019 (IG2) BDI - II	7.84	6.9	67	10.92	11.1	65	-0.33 [-0.68, 0.01]
Roswiyani, 2019 (IG1) BDI - II	8.17	7.83	63	10.92	11.1	65	-0.28 [-0.63, 0.06]
Chao, 2014 (PRE VS POST) GDS-15	2.3	2.1	7	2.9	2.1	7	-0.27 [-1.32, 0.79]
Brown,2009 (IG1 vs IG2) PANAS-N	13.9	5.7	82	15.3	4.2	34	-0.26 [-0.66, 0.14]
Brown, 2009 (IG1) PANAS-P	-33.4	7.1	82	-31.8	7.9	38	-0.22 [-0.60, 0.17]
Brown, 2009 (IG1) GDS	5.3	3.8	82	6.2	5.3	38	-0.21 [-0.59, 0.18]
Brown, 2009 (IG1) PANAS-N	13.9	5.7	82	15.1	6.0	38	-0.21 [-0.59, 0.18]
Vankova, 2014 GDS-15	5.0	3.29	79	5.27	3.27	83	-0.08 [-0.39, 0.23]
Chen, 2010 (3 mo) TDQ	4.74	8.16	31	5.0	4.39	24	-0.04 [-0.57, 0.50]
Brown, 2009 (IG2) PANAS-N	15.3	4.2	34	15.1	6.0	38	0.04 [-0.42, 0.50]
Roswiyani, 2019 (IG1 VS IG2) BDI - II	8.17	7.83	63	7.84	6.9	67	0.04 [-0.30, 0.39]
Winningham, 2003 GDS	5.6	5.06	25	5.18	5.06	17	0.08 [-0.53, 0.70]
Roswiyani, 2019 (IG1 VS IG3) BDI - II	7.84	6.9	67	7.0	6.93	67	0.12 [-0.22, 0.46]
Roswiyani, 2019 (IG2 VS IG3) BDI - II	7.84	6.9	67	7.0	6.93	67	0.12 [-0.22, 0.46]
Brown, 2009 (IG2) PANAS-P	-29.6	5.7	34	-31.8	7.9	38	0.31 [-0.15, 0.78]
Brown, 2009 (IG2) GDS	8.5	4.8	34	6.2	5.3	38	0.45 [-0.02, 0.92]

Acronyms: BDI Beck Depression Inventory; GDS Geriatric Depression Scale; IG Intervention Group; PANAS Positive and Negative Affect Schedule (P Positive, N negative); SMD Standardized Mean Difference; TDQ Taiwanese Depression Questionnaire

Figure 5: Physical exercise studies, SMD



Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Fixed, 95% CI
Coelho, 2020 GDS-27	7.45	4.43	20	12.21	5.9	19	1.6%	-0.90 [-1.56, -0.24]
De Carvalho Bastone, 2004 GDS	9.89	4.57	19	14.06	5.4	18	1.5%	-0.82 [-1.49, -0.14]
Brown,2009 (IG1 vs IG2) GDS	5.3	3.8	82	8.5	4.8	34	4.1%	-0.77 [-1.18, -0.36]
Chen, 2015 (3 mo) TDQ	4.92	5.73	64	10.96	9.83	63	5.4%	-0.75 [-1.11, -0.39]
Chen, 2015 (6 mo) TDQ	5.44	7.8	64	11.53	9.85	63	5.4%	-0.68 [-1.04, -0.32]
Chen, 2010 (6 mo) TDQ	3.03	6.71	31	7.29	6.5	24	2.3%	-0.63 [-1.18, -0.09]
Brown,2009 (IG1 vs IG2) PANAS-P	-33.4	7.1	82	-29.6	5.7	34	4.2%	-0.56 [-0.97, -0.15]
Roswiyani, 2019 (IG3) BDI - II	7	6.93	67	10.92	11.1	65	5.8%	-0.42 [-0.77, -0.08]
Roswiyani, 2019 (IG2) BDI - II	7.84	6.9	67	10.92	11.1	65	5.9%	-0.33 [-0.68, 0.01]
Roswiyani, 2019 (IG1) BDI - II	8.17	7.83	63	10.92	11.1	65	5.7%	-0.28 [-0.63, 0.06]
Chao, 2014 (PRE VS POST) GDS-15	2.3	2.1	7	2.9	2.1	7	0.6%	-0.27 [-1.32, 0.79]
Brown,2009 (IG1 vs IG2) PANAS-N	13.9	5.7	82	15.3	4.2	34	4.3%	-0.26 [-0.66, 0.14]
Brown, 2009 (IG1) PANAS-P	-33.4	7.1	82	-31.8	7.9	38	4.7%	-0.22 [-0.60, 0.17]
Brown, 2009 (IG1) GDS	5.3	3.8	82	6.2	5.3	38	4.7%	-0.21 [-0.59, 0.18]
Brown, 2009 (IG1) PANAS-N	13.9	5.7	82	15.1	6	38	4.7%	-0.21 [-0.59, 0.18]
Vankova, 2014 GDS-15	5	3.29	79	5.27	3.27	83	7.3%	-0.08 [-0.39, 0.23]
Chen, 2010 (3 mo) TDQ	4.74	8.16	31	5	4.39	24	2.4%	-0.04 [-0.57, 0.50]
Brown, 2009 (IG2) PANAS-N	15.3	4.2	34	15.1	6	38	3.2%	0.04 [-0.42, 0.50]
Roswiyani, 2019 (IG1 VS IG2) BDI - II	8.17	7.83	63	7.84	6.9	67	5.9%	0.04 [-0.30, 0.39]
Winningham, 2003 GDS	5.6	5.06	25	5.18	5.06	17	1.8%	0.08 [-0.53, 0.70]
Roswiyani, 2019 (IG1 VS IG3) BDI - II	7.84	6.9	67	7	6.93	67	6.0%	0.12 [-0.22, 0.46]
Roswiyani, 2019 (IG2 VS IG3) BDI - II	7.84	6.9	67	7	6.93	67	6.0%	0.12 [-0.22, 0.46]
Brown, 2009 (IG2) PANAS-P	-29.6	5.7	34	-31.8	7.9	38	3.2%	0.31 [-0.15, 0.78]
Brown, 2009 (IG2) GDS	8.5	4.8	34	6.2	5.3	38	3.2%	0.45 [-0.02, 0.92]
Total (95% CI)			**1328**			**1044**	**100.0%**	**-0.24 [-0.32, -0.16]**

Heterogeneity: Chi² = 61.43, df = 23 (P < 0.0001); I² = 63%
Test for overall effect: Z = 5.62 (P < 0.00001)

Material and methods - Development of LSM4Long combined intervention

Study Design

In order to assess the impact of a Lifestyle Program (LP) based on Lifestyle Medicine interventions to promote longevity in elderly people admitted in a Long-Term Care setting, the Lifestyle Medicine for Longevity pragmatic randomized controlled trial (LSM4Long) has been developed.

Pragmatic randomized clinical trials (PrCTs) are conducted to answer the important question of the effectiveness of a treatment/therapy/intervention in the "real-world setting," with heterogeneous "real-world population". The main aim of a PrCT is to inform clinicians and healthcare decision-makers regarding the optimal treatment for the real-word which they work (179,180).

The pragmatic two-arm, parallel group, superiority trial has been reported according to CONSORT Statement (181). The CONSORT checklist is reported in appendix of the published article. The study was approved by the local ethics committee and written informed consent from participant was obtained.

Participants and setting

The study took place at the "Belletti Bona" nursing home, a 144-beds LTC facility located in Biella (Piedmont, Italy) managed by the not-for- profit private company Anteo Impresa Sociale.

Eligible participants have been living in a LTC facility for one or more years, were able to discern and to express informed consent, with a care intensity (CI) score ranging from 5 to 12. This score, as adopted by the Regional Piedmont Council (182) and as according to the regional law, is obtained in a multidimensional and multidisciplinary process performing a comprehensive geriatric assessment (CGA) for each resident of the LTC, which identifies medical, social and functional needs. As a result of the process an integrated/co-ordinated care plan is developed (named in Italy PAI – Piano Assistenziale Individuale) to meet those needs (183), defining the usual care and the amount of resources required by each resident. Four validated tools are used by the multidisciplinary panel (Medical Director, General

Practitioner, Psychologist, Physiotherapist, Nurses and Healthcare workers of the LTC) during the CGA to define the CI score: the functional status is assessed with Barthel index and KATZ index (activity of daily living - ADL) (184–186), SPMSQ (187), DISCO (188), and DMI (188). The CI score can range from 1 to 12 and is classified in four distinct levels. Residents with a score equal to or lower than 4 are identified as autonomous subjects necessitating minimal care, while those with a score from 9 to 12 are allocated to the level requiring continuous, intensive care. Scores within the range of 5 to 6 denote patients requiring mild care, while a score of 7 to 8 designates those in need of intermediate care.

Moreover, participants were then assessed by BIA, BINDEX to obtain measurement of body composition (such as sarcopenia, fat free mass, fat mass, hydration status and osteoporosis or osteopenia). Participants cardiac comorbidity were assessed performing an ECG.

Thus, participants were assessed a T0 and T1 (after 12 weeks of intervention) by the CGA, BIA, BINDEX.

Biomedical devices used to assess the body composition of participants are reported in table 16.

Table 16: Biomedical devices used to assess body composition in LSM4Long study

TYPE OF MEASUREMENT	METHOD OF ANALYSIS	BIOMEDICAL EQUIPMENT/DEVICE
Body composition (fat mass, lean mass, total body water, tissue hydration and skeletal muscle mass)	Bioelectric impedance analysis: body impedance measurement (bioimpedance or bioresistance) at the passage of a low- power, high-frequency (50 kHz) electrical current into extracellular liquids (extracellular water measurement).	Akern Bio-impedance meter BIA 101
Evaluation of cardiac electrical activity	Graphical recording of the electrical activity of the heart	EDAN New Smart SE-3C Electrocardiograph -1/3 channels. Medical Device Class IIB
Ultrasonographic bone mineral density (quantitative US, QUS)	Bone cortical thickness and mineral density expressed in grams/cm2. QUS is measured predominantly at tibial level, at the site specified by the instrument manufacturer.	Bindex (Quantitative high frequencies sound - UltraSonography, (QUS) bone density)

Bioelectrical Body Composition Analyzer (Akern, Florence, Italy) BIA 101 ANNIVERSARY SPORT EDITION AKERN [50-kHz BIA] was used to assess the body composition of participants.

BIA is a method for body composition assessment and it has been validated in several pathologies, this method depends on specific predictive equations for each population.(189).
BIA provides a more reliable measurement of body composition with respect to FFM and FM than does BMI5 or simpler methods such as skinfolds and height and weight (190).

This Analyzer is able to calculate:

- BMI
- FFM (% bw): Fat free mass
- FM (% bw): Fat mass
- Hydr (%): Hydratation
- SMI (kg/m^2): Skeletal Muscle index
- ASMM (kg/m^2): Appendicular Skeletal Muscle Mass
- SPA: Standardize phase angle

BMI = Body Mass Index (is measured in kg/m^2) where:

- BMI = Bw / Ht^2
- Bw= BODY WEIGHT (kg)
- Ht = height (m)

BMI variable is dependent on Body weight. BMI is also dependent on values obtained with 50-kHz BIA test of FFM, FM, Hydr SMI because those parameters are calculated as % of body weight. We also supposed that BMI is dependent on bone density measured using (BINDEX).

FFM (% bw) = FAT FREE MASS

FM (% bw) = FAT MASS

Significant changes in body composition occur with aging and are believed to be a consequence of imbalances between energy intake and energy needs associated with an increasingly sedentary life-style. Progressive increases in fat mass (FM) and progressive reductions in fat-free mass (FFM) have been noted. In adults, over-and undernutrition contribute to increased mortality and morbidity. Because weight and body mass index (BMI) alone are not an adequate guide of underlying changes in FFM and FM during menopause and aging in general, body composition should be measured (190).

Aging is frequently associated with a gradual loss of fat-free mass (FFM), which has a negative impact on health outcomes, such as morbidity, mortality and quality of life. Low FFM (also termed sarcopenia) may simultaneously occur in the presence of anormal or an elevated fat mass (FM), a condition known as sarcopenic obesity. In healthy adults FFM begins to decline at age 60 in men and 45 years in women (190), accelerated loss has been observed in men and women older than 75 years (190). Some evidences suggest that body weight and BMI increased until age 74 years and that the increase was predominantly due to higher FM. FM continued to increase in men after age 74 y, and %FM increate throughout the lifespan in men and women (190).

Kyle et al developed reference values for FFM, FM, and %FM by BIA in a large white (Western European) population for healthy subject (Switzerland) including elderly (190).

A potential method to assess the combined effect of FM and FFM is to express these variables using a ratio, FM/FFM. The NHANES III established age, sex and body mass index (BMI) specific reference values of FM/FFM in non-Hispanic white adults (191).

FFM (% bw) = FAT FREE MASS

- FFM (kg) = TBW (l) / (Hydr/100)
- FFM (%) = FFM (kg) / Bw (kg) x 100
- TBW = TOTAL BODY WATER (l)
- Hydr = HYDRATATION %
- Bw= BODY WEIGHT (kg)

As reported in Kyle et al Nutrition 17:534-541;2001 (190) FFM is dependent or related to TBW and Hydration and, we supposed, also dependent on **SMI** (50-kHz BIA) and Bone Density (BINDEX)

FM (% bw) = FAT MASS

- FM (kg) = Bw (kg) – FFM (kg)
- FM (%) = FM (kg) / Bw (kg) x 100
- Bw= BODY WEIGHT (kg)
- FFM = FAT FREE MASS (kg)

Hydr (%) = HYDRATION

Two clinical problems with nutrition in older age could be distinguished: the first one is the imbalance of body fluids, described in terms of hyper- and dehydration and hyper- or hypovolemia. The other one could be described as an imbalance of body cells and nutritional components, categorized as malnutrition, sarcopenia and cachexia with a certain overlap (192).
Dehydration is claimed to be the most common fluid disorder leading to increased morbidity and mortality as well as extended hospital stay in older patients and to emergency room visits (192).

Bio-impedance vector analysis (BIVA) has been suggested as a tool to assist in volume status assessment, BIVA allows a rapid, accurate, and non-invasive determination of body hydration status, correlates with NYHA class, and seems to demonstrate high diagnostic accuracy for the differential diagnosis of HF-induced dyspnoea (193).

The water content or hydration of fat-free body mass (FFM) is among the best known and most widely applied of the body-composition constants (194). Pace and Rathbun (195) first proposed that total body water (TBW) is a constant fraction of FFM (x – ± SD: 0.724 ± 0.021).

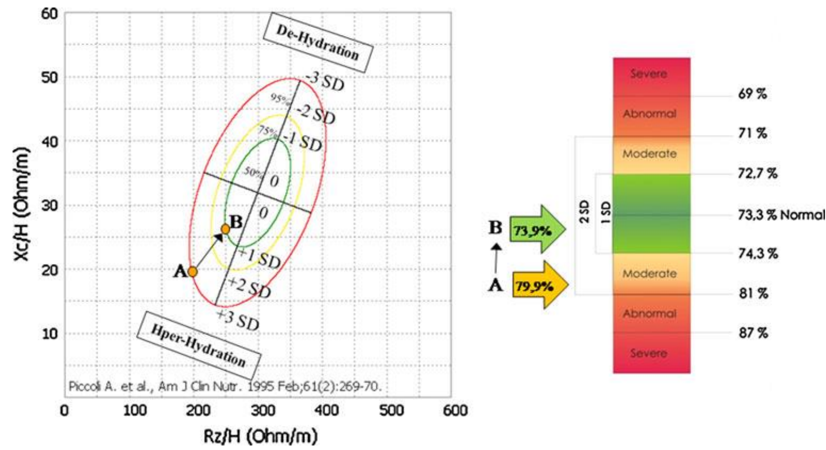
The relative stability of FFM hydration between species led to the wide use of the in vivo method, that is:
Fat = body mass - FFM = body mass – TBW/0.73 (196)

Hydration, the observed ratio of total body water to fat-free body mass, is stable at 0.73 (194).

Bioelectrical impedance vector analysis simultaneously evaluates total body by resistance and reactance (reactance is a form of opposition that electronic components exhibit to the passage of alternating current because of capacitance or inductance; in some respects, reactance is like an alternating current counter part of direct current and indicates an absolute amount of body cell mass), presenting them graphically to provide indices of total body hydration status. BIVA can also provide data regarding the ratio of extracellular water and total body water (193).
BIVA data can be visually presented as a nomogram classifying patients into 3 classes as follows: normally hydrated, hyperhydrated, or dehydrated (the latter classes can be further subdivided into mild, moderate, or severe volume abnormalities) (193).

Using BIVA (Figure 6), a forward or backward displacement of vectors parallel to the major axis of the standard deviation ellipses is associated with dehydration or fluid overloading, respectively, reaching extremes out of the poles (left panel Figure 6). Vectors above or below the minor axis (meaning upper-left or lower-right half of ellipses) are associated with more or less cell mass in soft tissues, respectively, with extremes along the minor axis. The need of a numerical value has led to the construction of a numerical scale (right panel in Fig.1) in which the normal level of hydration is set at 73.3%. Values above or below represent, respectively, dehydration or overhydration and are correlated with specific points in the BIVA nomogram (193).

Figure 6: BIVA vector and numeric scale (193)



It was reported that the phase angle, indicating the phase shift of electrical current caused by integrity and mass of cell membranes, may be a useful marker of body cell mass in geriatric in-patients. However, evaluation of body cell mass and fluids is insufficient if exclusively based on the phase angle. The very same phase angle-value may appear in a patient hyperhydrated, hydrated or dehydrated. Therefore, all BIA raw-data (resistance, reactance and phase angle) have to be considered for an interpretation to be clinically meaningful (192). Given all the above mentioned information, participants were assessed in the morning, with light clothes and without drinking the night before.

- Hydr (%) = (TBW (l) / FFM (kg)) x 100
- TBW = Total Body Water (l)
- FFM = Fat Free Mass (kg)

SMI (kg/m^2) = Skeletal Muscle Index

Aging is associated with the loss of muscle mass and strength that has been referred to as sarcopenia (197). Sarcopenia is very prevalent in persons aged 80 years and older (192). In older people the main consequence of sarcopenia is the limitation of physical performance, which increases the risk of frailty, falls, hospitalization, disability, and mortality (197). The European Working Group on Sarcopenia in Older People (EWGSOP) recommends to define sarcopenia the presence of both low muscle mass and low muscle function (strength or performance) (197): sarcopenia clinical suspicious is based on low muscle function assessed using validated tools, while a sarcopenia diagnosis is confirmed by the presence of low muscle quantity or quality (198).

Magnetic resonance imaging (MRI) and computed tomography (CT) are considered to be gold standards for non-invasive assessment of muscle quantity/mass (198).

Bioelectrical impedance analysis (BIA) has been explored for estimation of total (SSM) or ASM Appendicular Skeletal Mass Muscle (ASM) (198).

Muscle quantity can be reported as total body Skeletal Muscle Mass (SMM), as Appendicular Skeletal Muscle Mass (ASM) (199).

BIA equipment does not measure muscle mass directly, but instead derives an estimate of muscle mass based on whole-body electrical conductivity. BIA uses a conversion equation that is calibrated with a reference of DXA-measured lean mass in a specific population (198).

Age, ethnicity and other related discrepancies between those populations and patients should be considered in the clinic. In addition, BIA measurements can also be influenced by hydration status of the patient (198).

Muscle mass is correlated with body size, so SMM or ASM can be adjusted for body size in different ways, i.e. using height squared, weight or body mass index, EWGSOP make no recommendation to adjust for body size (198)

- SMI = SM / Ht^2
- SM = Skeletal Muscle Mass (kg)
- Ht = height (m)

SM is estimated by 50-kHz BIA using the Janssen Equation (200) as follow:

- $SM = [(Ht^2 / R \times 0.401) + (gender + 3.825) + (age \times -0.071)] + 5.102$
    - Ht = height (cm)
    - R = BIA resistance (Ohms)
    - Gender men = 1; women = 0
    - Age measured in years

Reference values for sarcopenia 2014 using Janssen Equation (201):

Male <8,9 kg/m2

Female < 6,4 kg/m2

<u>ASMM</u> (kg/m^2): Appendicular Skeletal Muscle Mass

EWGSOP2 recommend to calculate ASMM using the cross-validated Sergi equation (202). This equation seems to be more reliable when applied to the elderly than the one previously published in the literature.

- $ASMM (kg) = -3{,}964 + (0{,}227 * RI) + (0{,}095 * weight) + (1{,}384 * sex) + (0{,}064 * Xc)$
    - RI = Resistive Index = height^2 (m) / BIA resistance (Ohm)
    - weight in kg
    - Sex men = 1; women = 0
    - Xc= reactance (Ohm)
    - ASM / Ht^2
    - Ht = height (m)

Recently (2019) EWGSOP2 provides new sarcopenia clear cut-off points (199)

- CUT-OFF (203)
- male <7 kg/m^2
- female <5,5 kg/m^2

The ASMM is associated with disability (204) because it is involved primarily in physical activities (205).

SPA – STANDARDIZED PHASE ANGLE

Age, gender and BMI standardized phase angle, has been considered as a prognostic factor in several clinical conditions, has been considered a predictor of morbidity and mortality in several conditions such as HIV/AIDS, pancreatic, colorectal, breast and lung cancer, liver cirrhosis, dialysis, pulmonary disease, amyotrophic lateral sclerosis, geriatric and surgical patients (189,206). Malnutrition and inflammation have a strong impact on SPA in sick individuals (189).

Phase Angle (PA) is estimated using BIA by the direct ratio between resistance (R) and reactance (Xc) and can be understood as a marker of the fluid distribution between the intra and extra-cellular medium and can be considered a reliable signal of malnutrition (189).

It supposedly reflects cell membrane integrity, cell size and/or the proportion of intracellular and extracellular water. In physics, it corresponds to the time delay between the voltage waveform and the current waveform. The latter lags behind because the electrical current is partly stored in the cell membranes and tissue interfaces, which act as capacitors (207).

A low PA may suggest a decrease in the cellularity, but also could stand for impairment in the function of the cell´s membrane (e.g., cellular death or poor integrity). (189) Until 2005 there were no published reference values for PA published, some studies showed PA variations according to age and sex, from these reference values, it is possible to obtain standard phase angle (SPA), making the comparison among samples with different sex and age possible (189).

The SPA cut-off values of −1.65 stands for the fifth percentile of normal population, therefore, it can be considered as the lower limit accepted in a healthier population; a SPA cutoff value of −1.65 can be chosen to classify the patients in two groups: low PA or not (189). Low PA is a significant determining factor of a higher mortality, as higher PA suggests large quantities of intact cell membranes, while lower PhA suggests cell death or decreased cell integrity (189)(206). Low SPA patients presented an especially higher mortality rate when compared to those with $SPA<-1.65$ (RR=3.12, CI: 2.03;4.79); patients with low PA showed a relative risk 2.35 times higher for mortality ($p=0.001$) when compared to those with $SPA\geq-1.65$ (189)

Next to age, malnutrition emerged as a major determinant of PA. Moreover, sex, CRP and BMI significantly influenced PhA, whereas diagnosis showed no effect in this model. When investigating SPA, the only significant determinants were malnutrition and inflammation status (CRP: C-protein Reactive) (206) (208). Logistic regression analysis showed an association between SPA and anthropometric variables [midarm cir-cumference (MAC), triceps skinfold, and midarm musclearea (MMA)] as well as with a functional parameter (208).

Usually the PA has been calculated, in degrees, using the formula for the arc tangent of the ratio of reactance to resistance, which was transformed into SPA. Usually SPA has been SPA was obtained using the following formula reported by Barbosa-Silva et al: SPA=[(observed PA-mean PA)/SD of the PA (mean and Sd are from sex and age stratified reference values) (208).

PA values can be standardized for these parameters by calculating Z-scores using published reference values (209)

PA has been calculated by BIA as:

- PA (°) = ICM (kg) /ECM (kg)
- PA = Phase angle (°)
- ICM = INTRACELLULAR MASS (kg)
- ECM = EXTRACELLULAR MASS (kg)

The ratio of extracellular to intracellular water negatively correlates with phase angle in outpatients, this supposes that a low PA is associated with an increase in extracellular water, a decrease in intracellular water, or both. A decrease in intracellular water or cell shrinkage is considered as a catabolic and antiproliferative signal. The proportion of intracellular water to total body water is lower in diseased than healthy older adults. Many studies also report an expansion of extracellular volume relative to total body water in specific diseases, as COPD, congestive heart failure, dialysis, gastro-intestinal diseases, obesity, paraplegia, and in nursing home residents. This expansion seems to occur especially in patients who have a low fat-free mass, which is a hallmark of malnutrition. Consequently, diseases and malnutrition both seem to induce cell shrinkage and expansion of extracellular water. It is tempting to hypothesize that the link between diseases and low

PA is related to an increase in the ratio of extracellular to intracellular water or malnutrition (207)

The severity of musculo-skeletal and respiratory diseases affected the standardized PA. Severity of grade 4 in musculo-skeletal diseases corresponds to people who are wheel-chair bound, have severe joint deformities or a musculo-skeletal malignancy, while grade 1 includes patients with osteoarthritis, resolved skin cancers or infections. Thus, as the severity grade increases, there is likely a lower fat-free mass due to partial or total immobilization, which in turn is associated with a lower phase angle (207).

Reference values of body composition measurement are reported in table 17.

Table 17: Reference values of measurements of body composition assessed by BIA

Measurement of body composition	**Male**	**Female**
FFM (FAT FREE MASS)	MALE 65-74y <69,3=severe loss FFM 69,3-72,4=moderate loss FFM 72,4-80,2=normal FFM 80,2-82,8=moderate excess FFM >82,8=severe excess FFM MALE 75-84y <70=severe loss FFM 70-72=moderate loss FFM 72-78,9=normal FFM 78,9-82=moderate excess FFM >82=severe excess FFM MALE >85y <67,3=severe loss FFM 67,3-68,6=moderate loss FFM 68,6-74,1=normal FFM 74,1-80,2=moderate excess FFM >80,2=severe excess FFM	FEMALE 55-84 y <55,8= severe loss FFM 55,8-59,6= moderate loss FFM 59,6-67,2= normal FFM 67,2-70,9= moderate excess FFM >70,9= severe excess FFM >85y <54,5 severe loss FFM 54,5-57,5 moderate loss FFM 57,5-68,8 normal FFM 68,8-75,7 moderate excess FFM >75,7 severe excess FFM

FAT MASS (FM)	MALE 65-74 y <17,2=severe underfat 17,2-19,8=moderate underfat 19,8-27,6=normal 27,6-30,7=moderate overfat >30,7=severe overfat MALE 75-84y <18=severe underfat 18–21,1=moderate underfat 21,1-28,0=normal 28,0-30,3=moderate overfat >30,3=severe overfat MALE >85y <19,8=severe underfat 19,8-25,9=moderate underfat 25,9-31,4=normal 31,4-32,7=moderate overfat >32,7=severe overfat	FEMALE 55-64y <24,4=severe underfat 24,4-28,3=moderate underfat 28,3-36,0=normal 36,0-39,4=moderate overfat >39,4=severe overfat FEMALE 65-74y <27.3=severe underfat 27,3-31,4=moderate underfat 31,4-39,9=normal 39,9-42,4=moderate overfat >42.4=severe overfat FEMALE 75-84y <29,1=severe underfat 29,1-32,8=moderate underfat 32,8-40,4=normal 40,4-44,2=moderate overfat >44,2=severe overfat FEMALE >85y <24,3=severe underfat 24,3-31,2=moderate underfat 31,2-42,5=normal 42,5-45,5=moderate overfat >45,5=severe overfat
Hydration (Hydr)	<69%=severe dehydration 69-71=moderate dehydratation 71-72,7=slight dehydratation 72,7-74,3=normal hydratation 74,3-81=slight hyperhydratation 81-87=moderate hyperhydratation >87 severe hyperhydratation	
Skeletal Muscle Index in kg/m2 (SMI)	Male > 8,9 kg/m2 normal	Female > 6,4 kg/m2 normal
Appendicular Skeletal Muscle Mass (kg) ASMM	male > 7 kg/m^2 normal	female > 5,5 kg/m^2 normal
Standardized Phase Angle SPA	Acceptable > -1,65	

Description of the lifestyle medicine combined intervention

The aim of the intervention was to obtain a synergistic effect from the combination of physical exercise, healthy diet, and psychological wellbeing on functionality in older people (210). Moreover, another aim was to evaluate plasma markers of redox state in older people after a three-months-period of the lifestyle medicine program.

Physical exercise intervention

As reported above a systematic overview of the literature has been performed to retrieved the intervention needed for the nutritional and physical activity experimental arm. The intervention designed by Rondanelli 2016 (177) has been tailored to the real-world setting of the LTC Belletti Bona.

With the help of a physiotherapist (A.SD) with expertise in Lifestyle Medicine the physical exercise protocol has been translated according to Otago exercise program in a “Real-Life" High Intensity Interval Training (HIIT), that is a circuit interval training, with a supervision of a LTC staff member physiotherapist. Otago exercise program are body weight exercises based on WHO recommended level of physical activity for older adults and can be done in group (211,212). The physical exercise sessions were structured in bi-weekly 45-minute groups, requiring moderate-to-high effort to subjects (level 7–8/ 10 on a visual analogue scale). Each session was designed to burn about 190 Kcals and 170 MET per session, calculated on an average weight of 60 kg.

In order to train internal staff members, two of the four physiotherapist (M.M.. G.M) of the Belletti Bona Nursing Home attended 3 training and educational on-line meetings with the expert in Lifestyle Medicine physiotherapist who contributed to design the intervention.

The physical exercise intervention protocol is described below.

Assessment

Medical examination, performed by the medical director of LTC, to exclude contraindications to moderate and intensive exercise (e.g., severe heart failure, arthero-venous embolism…)

Exclusion of patients with:

- Severe dementia and/or with 8 or more errors in the Short Portable Mental Status Questionnaire (or similar scales)
- Several functional impairments that hamper active movements of the limbs
- Unstable cardiovascular or metabolic diseases

Exercise sessions:

Frequency: two times a week (Monday and Thursday or Tuesday and Friday)

Total duration: 45'-60'

Intensity: moderate-to-intense (self-perceived effort: 7-8/10 on a visual analogical scale)

Time: within 90' from the breakfast or lunch

General intervention structure

- 5' warm-up
- 20' resistance training exercises (lower and upper limbs)
- 10' balance reinforcement exercises
- 5' cool-down

Warm-up

- Neck (flexion/extension, rotation, lateral flexion)
- Torso (rotation, lateral flexion)
- Arms (flexion/extension)
- Legs (flexion/extension)
- Ankles (flexion/extension)

⇒ 30s for each exercise, without pauses.

Muscular strengthening exercises

Table 18: Description of muscular strengthening circuits

Circuit 1	Circuit 2	Circuit 3	Circuit 4
• Leg extension • Modified squat • Standing leg curl • Modified standing hip flexion • Calf raise • Hip abduction ⇒ 30s of exercise followed by 15s for recovery. Total time: 4m15s	• Leg extension • Modified squat • Standing leg curl • Calf raise • Hip abduction • Modified biceps curl and overhead press ⇒ 30s of exercise followed by 15s for recovery. Total time: 4m15s	• Leg extension • Modified squat • Standing leg curl • Calf raise • Modified biceps curl and overhead press ⇒ 30s of exercise followed by 15s for recovery. Total time: 3m30s	• Step up ⇒ 4m of exercise without pauses. Total time: 4m
2m of recovery between circuits, during which patients can drink a glass of water. Exercises were performed with ankle weights. The weight is increased or decreased if the patient performs more than 10 or less than 8 repetitions, respectively.			

Balance exercises

Table 19: Description of balance strengthening circuits

Circuit 1	Circuit 2
• Front step • Right one leg stance • Left one leg stance ⇒ 30s of exercise without pause. The circuit is repeated 3 times. Total time: 6m30s	• Right tandem stance • Left tandem stance ⇒ 30s of exercise without pause. Total time: 1m
2m of recovery between circuits, during which participants can drink a glass of water.	

Cool-down

- Neck (flexion/extension, rotation, lateral flexion)
- Torso (rotation, lateral flexion)
- Arms (flexion/extension)
- Legs (flexion/extension)
- Ankles (flexion/extension)

⇒ 30s for each exercise to the point of mid discomfort, without pauses.

The control group follow the standard of care.

Nutritional intervention

With the contribution of a nutritionist (A.O.) the supplements used in the Rondanelli 2016 RCT (177) has been translated in a menu provisioned by the inner kitchen of the LTC.

The diet offered four different meals (breakfast, lunch, afternoon snack, and dinner), and was designed to provide at least 30 g of proteins in each meal with high content of leucine, with the aim of stimulating protein synthesis in skeletal muscles.

Daily calcium intake was managed to reach the minimum quantity suggested by the recommended daily intake levels and phytate- and oxalate-rich food (e.g., spinach) were limited, to increase intestinal calcium absorption (213,214)

An overview of the nutritional intervention is described below.

Table 20: Overview of energy and macronutrients provision in the intervention arm.

Daily nutritional intake	Mean ± SD
Energy, kcal/day	1996 ± 11
Energy, kj/day	8351 ± 45
Proteins, g/day (%)	102,8 ± 7,1 (20,6 ± 1,4)
Leucin, mg/day	5893 ± 230
Fat, g/day (%)	63,1 ± 8,7 (28,4 ± 3,9)
Total carbohydrates, g/day (%)	268,8 ± 23,3 (50,5 ± 4,4)
Fibers, g/day	29,7 ± 4,1
Calcium, mg /day	1261 ± 223

Table 21: Reference weights for main ingredients.

Food category	Food	Weight per serving
Cereals and derivatives, tubers	Durum pasta, boiled rice	50g
	Corn flour	40g
	Potatoes gnocchi	180g
	Bread	50g
	Bread substitutes	30g
	Potatoes	150g
Fruits and vegetables	Seasonal fruit	150g
	Vegetables	200g
	Salad	80g
Meat, fish, eggs, legumes	Red or white meat	150g
	Frozen fish	200g
	Canned fish	50g
	Eggs	100g
	Frozen legumes	50-100g
	Dry legumes	30g
Milk and derivatives	Low fat milk	200ml
	Natural yoghurt	125ml
	Low fat cheese (<300 kcal/100 g)	100g
	Fat cheese (> 300 kcal/100 g)	50g
	Scratched parmesan	5g
Seasoning oil and fats	Olive oil	5ml
	Butter	5 g

Table 22: Proposed menus for the intervention group.

Week 1							
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Breakfast	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam
Lunch	Pasta with vegetables Chicken escalope Stewed kale Seasonal fruit	Risotto with mushrooms Omelette Broccoli with oil Seasonal fruit	Gnocchi with meat sauce Baked cod Fennels with oil Seasonal fruit	Grits Veal stew Lettuce Seasonal fruit	Pasta with tuna Hake with lemon Steamed carrots Seasonal fruit	Saffron risotto Chicken breast Zucchini Seasonal fruit	Vegetarian lasagna Beef meatballs with sauce Green beans with oil Sweet
Afternoon snack	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt
Dinner	Vegetable soup with rice Tuna and beans salad Carrots with rosemary Seasonal fruit	Potato cream Baked loin Green beans with oil Seasonal fruit	Lentils cream with pasta Ricotta cheese Zucchini with oil Seasonal fruit	Pumpkin cream Margherita pizza Fennels with oil Seasonal fruit	Ravioli with broth Hard-boiled eggs Mixed vegetables au gratin Seasonal fruit	Vegetable soup Fontina cheese Baked potatoes Seasonal fruit	Peas cream with pasta Blue shark with parsley Stewed kale Seasonal fruit

Week 2							
	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**
Breakfast	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam
***Lunch**	Pasta with tomatoes Omelette Fennels with oil Seasonal fruit	Pasta with cauliflowers Cod with tomatoes Zucchini with oil Seasonal fruit	Polenta Sausage with sauce Steamed fennels Seasonal fruit	Pumpkin risotto Turkey Kale Seasonal fruit	Pasta with Genovese pesto Baked blue shark Baked mixed vegetables Seasonal fruit	Saffron risotto Milanese chicken steak Peas with oil Seasonal fruit	Pasta with meat sauce Beef roast Cauliflowers with oil Seasonal fruit
Afternoon snack	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt
Dinner	Onion soup with croutons Chicken bites with lemon Seasonal fruit	Legumes soup Vegetarian parmigiana Green beans Seasonal fruit	Leeks cream with croutons Starry smooth houndwith parsley Steamed broccoli Seasonal fruit	Zucchini cream Margherita pizza Green salad Seasonal fruit	Minestrone with rice Hard-boiled eggs Carrots with rosemary Seasonal fruit	Pasta with broccoli cream Lemon cod Zucchini with herbs Seasonal fruit	Pasta and beans Ricotta cheese Green beans Seasonal fruit

Week 3							
	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**
Breakfast	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam
Lunch	Pasta with tomatoes and olives Chicken burger Zucchini with oil Seasonal fruit	Boscaiola risotto Chicken escalope with white wine Broccoli with oil Seasonal fruit	Pasta with zucchini Baked cod Stewed carrots Seasonal fruit	Polenta Calf stew Lettuce Seasonal fruit	Pasta with seafood Baby octopus with tomatoes Zucchini with herbs Seasonal fruit	Parmesan risotto Chicken with tomatoes and olives Fennels with oil Seasonal fruit	Baked pasta Pork loin Green beans with oil Seasonal fruit
Afternoon snack	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt
Dinner	Minestrone with rice Mortadella Carrots with rosemary Seasonal fruit	Vegetable cream with pasta Eggs with tomatoes Green beans Seasonal fruit	Pasta with chickpeas Turkey meatballs Cauliflowers with oil Seasonal fruit	Pumpkin cream Margherita pizza Fennels with oil Seasonal fruit	Spelt soup Tuna in oil Mixed baked vegetables Seasonal fruit	Lentil soup Ricotta cheese Broccoli with oil Seasonal fruit	Peas cream with pasta Cooked ham Stewed kale Seasonal fruit

Week 4							
	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**
Breakfast	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam	Low-fat milk Rusks Fruit jam
Lunch	Ravioli with butter and sage Omelette Mixed vegetables with oil Seasonal fruit	Pasta with pesto Cod with lemon and butter Zucchini with oil Seasonal fruit	Polenta Gorgonzola cheese Fennels au gratin Seasonal fruit	Risotto with saffron Boiled chicken Kale with herbs Seasonal fruit	Pasta with vegetables Cod with tomatoes and mozzarella Mixed cooked vegetables Seasonal fruit	Risotto with pumpkin Cooked turkey Lettuce Seasonal fruit	Gnocchi with tomatoes Roasted veal Fennels with oil Seasonal fruit
Afternoon snack	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt	Light natural yogurt
Dinner	Leek cream with croutons Chicken bites Green beans with oil Seasonal fruit	Lentil cream with pasta Vegetable parmigiana Broccoli with oil Seasonal fruit	Cereal cream Baked pork loin Lettuce Seasonal fruit	Vegetable cream Margherita pizza Seasonal fruit	Tortellini with broth Ham rolls with ricotta cheese Carrots with rosemary Seasonal fruit	Minestrone with pasta Fontina cheese Zucchini with herbs Seasonal fruit	Chickpea cream with rice Starry smooth hound with parsley Cauliflowers with oil Seasonal fruit

Psychological wellbeing intervention

As described above a systematic review was conducted in order to retrieved effective psychological intervention in fostering depression in elderly people who live in a LTC and also in improve self-efficacy, self-confidence and motivation to comply the intervention (215) [full text attached in results section]. On the basis of findings of the systematic review, Prof. Carla Gramaglia and Prof, Patrizia Zeppegno developed the structure of the psychological intervention as reported below.

Session structure

Each session, led by a psychologist, lasts for one hour and is delivered for groups of maximum 15 patients. This intervention aims to enhance overall wellbeing, leveraging music's emotional and memory-evoking capabilities.

- **Introductory session**
  - Group introduction
  - Participants share significant songs linked to their memories or life phases.
- **Weekly sessions**
  - Music listening
  - Group discussions on emotional, cognitive, and mnemonic responses evoked by the music.

In order to train the internal staff member, the psychologist (R.V.) of Belletti Bona nursing home received 2 on-line meetings with Prof Gramaglia. Due to COVID-19, access regional laws restriction in the LTC Belletti Bona, in each session the psychologist shared with Prof Gramaglia a brief summary of the discussion evoked by the music and memories linked to the significant song selected by participants.

Collection of blood sample to evaluate oxidative stress status.

In order to study the effect of HLP on ROS, blood samples were collected and processed by Physiology Laboratory of the University of East Piedmont colleagues under the supervision of Prof. Elena Grossini.

Plasma sample measurement methodology is reported in BOX 2 following the standard described in previously experiment published by the Physiology Laboratory of the University of East Piedmont team.

Blood samples were taken from participants, in the morning in fasting conditions by using BD Vacutainer tubes (sodium heparin as anticoagulant) at T1. Each sample was immediately centrifuged by a refrigerated centrifuge (Eppendorf, mod. 5702 with rotor A-4-38) for 10 min, at 3100 rpm at 4°C. The plasma obtained was divided into five tubes, which were kept at −80°C at the Physiology Laboratory of the University of East Piedmont and further processed for the quantification of markers of redox state, as specified below, and for executing the *in vitro* experiments on HUVEC.

The LSM4Long was a part of larger cohort study named Longevity Check-Up (LCU), that is not an objective of this work. At the beginning of the LCU study [January 2020], blood sample of 60 residents of the same LTC (Belletti Bona) were collected. Originally the LSM4Long trial should enrolled 54 out of 60 of the participants enrolled in LCU, but due to an outbreak of COVID-19, 32 subjects died. For this reason, new patients have been included to complete the LSM4Long study, thus by this way at T1 blood sample has been collected only from the participants to LSM4Long study.

Box 2: Plasma sample measurement methodology

GSH quantification

Plasma GSH measurement was performed by using the Glutathione Assay Kit (Cayman Chemical, Ann Arbor, MI, USA), as previously described (216–218). For the experiments, each plasma sample was deproteinated adding an equal volume of MPA solution to the sample that was centrifuged at 2000 g for 2 min. Thereafter, the supernatant was collected and 50 µl/ml of TEAM reagent was added to each sample in order to increase the pH. Fifty µl of the samples was transferred to a 96-well plate where GSH was detected following the manufacturer's instructions through a spectrophotometer (VICTOR™ X Multilabel Plate Reader), at excitation/emission wavelengths of 405-414 nm. To ensure accurate GSH quantification (as µM), a reference curve with the GSH Standard was prepared.

TBARS quantification

Plasma TBARS were determined as malonyldialdeide (MDA) release. MDA measurement was performed by using the TBARS assay Kit (Cayman Chemical), as previously performed (216,217,219). For the experiments, 100 µl of each plasma sample was added to 100 µl of sodium dodecyl sulfate (SDS) solution and 2 ml of the Color Reagent, following the manufacturer's instruction. Each sample was boiled for 1 h and then transferred on ice for 10 min in order to stop the reaction. After this time, each sample was centrifuged for 10 min at 1600 g at 4°C and then, 150 µl was transferred to a 96-well plate where MDA was detected following the manufacturer's instructions through a spectrophotometer (VICTOR™ X Multilabel Plate Reader), at excitation/emission wavelengths of 530–540 nm. In order to quantify the correct value of TBARS in each sample (expressed as MDA in µM), a reference standard curve with the TBARS Standard was prepared.

8-hydroxy 2 deoxyguanosine (8 OH-2dG) quantification

Plasma 8 OH-2dG measurement was performed by using the 8-hydroxy 2 deoxyguanosine ELISA Kit (Abcam) (216–223). For the experiments, 50 µl of each plasma sample and standard was added to each well. Fifty µl of 8-hydroxy 2 deoxyguanosine Antibody Preparation was added to each well and then, the plate was incubated at room temperature (RT) for 1 h. After 1 h, each well was washed 4 times with 300 µl of 1X Wash Buffer and then, 100 µl of Substrate Solution (tetramethyl-benzidine; TMB) was added to each well and incubated for 30 min at RT in the dark. The enzyme reaction was stopped by adding 100 µl of Stop Solution (1 M phosphoric acid) into each well and

plate was read immediately. The 8 OH-2dG was detected following the manufacturer's instructions through a spectrophotometer (VICTOR™ X Multilabel Plate Reader), using a wavelength of 450 nm. The value of each sample (ng/ml) was quantified in respect to 8-hydroxy 2 deoxyguanosine standard curve.

8 isoprostanes quantification

Plasma 8 isoprostanes (F2 isoprostanes) measurement was performed by using the 8 isoprostanes ELISA Kit (Abcam) (216–223). For the experiments, in order to isolate and purify 8 isoprostanes from each plasma sample, 1 ml of plasma (adjusted with 12 µl acetic acid to pH 4) and 1 ml of ethyl acetate were combined and centrifuged at 2000 rpm for 10 min at RT. By this way three phases were formed: the upper organic phase (ethyl acetate phase containing lipoproteins), the interphase (containing proteins) and a lower phase (aqueous phase). The organic phase was collected and transfer in a new tube. The interphase was discarded and the lower phase was transferred in a new tube and used for repeating the acetate extraction for three times. Subsequently, the organic phase was evaporated in a Speedvac. The dried residues were dissolved in 2 ml of 20% KOH solution and incubated for 1 h, at 50 °C. The 2 ml of the aqueous solution were diluted with 3 ml of H_2O and pH was adjusted using 20% formic acid. Two ml of ethyl acetate was added and centrifuged at 2000 rpm for 10 min at RT. The procedure was repeated for three times. The ethyl acetate in the upper phase was dried in a Speedvac. For the ELISA assay, the dried sample-residue was dissolved in 20 µl ethanol and 130 µl of 1X Sample Dilution Buffer. For the competitive 8 isoprostanes ELISA assay, 150 µl of sample was further diluted 1:16 with a final pH 7,4. At the end of samples preparation, 200 µl of 1X Sample Dilution Buffer was added into the blank wells, while 100 µl of 1X Sample Dilution Buffer was added into maximum binding control wells. One hundred µl of each sample and Standard were added into appropriate wells. Furthermore, 100 µl of the 1X-HRP conjugate was added in each well, except in the blank control wells. The plate was incubated at RT for 2 h. After this time, the plate was washed 3 times with 400 µl of 1X Wash Buffer and then, 200 µl of TMB was added to all of the wells and incubated for 30 min at RT. The enzyme reaction was stopped by adding 50 µl of Stop Solution (2 N sulfuric acid) and plate was read immediately. The 8 isoprostanes was detected following the manufacturer's instructions through a spectrophotometer (VICTOR™ X Multilabel Plate Reader), using a wavelength of 450 nm. The value of each sample (pg/ml) was quantified in respect to 8 isoprostanes standard curve.

25(OH) Vitamin D quantification

Plasma 25(OH) Vitamin D was measured by using the 25(OH) Vitamin D ELISA Kit (Abcam) (216–223). For the experiments, 90 µl of the Dissociation Buffer was added to each well. Ten µl of Sample Diluent was added to B0 wells (Maximum binding) and NSB (Non-Specific Binding) wells. Ten µl of plasma sample (diluted 1:10) and Standard were added to the appropriate wells with Dissociation Buffer. The plate was incubated for 5 min on a shaker at RT. After this time, 50 µl of 1X Conjugate was added to each well, 50 µl of the Conjugate Diluent was added to NSB wells and, finally, 50 µl of the supplied Antibody was added to each well, except in the NSB wells. The plate was incubated for 1 h on a shaker at RT. After 1 h, 200 µl of p-NitroPhenyl Phosphate (pNpp), the chromogenic substrate for the detection of alkaline phosphatase (AP), was added to each well and the plate incubated for 30 min on a shaker at RT. The enzyme reaction was stopped by adding 50 µl of Stop Solution (1 M phosphoric acid) into each well and plate was read immediately through a spectrophotometer (VICTOR™ X Multilabel Plate Reader), using a wavelength of 405 nm. The value of each sample (ng/ml) was quantified in respect to 25(OH) Vitamin D standard curve.

SOD activity

Total SOD activity was determined in plasma by using the Superoxide Dismutase Activity Assay Kit (Abcam) (216–223). For the experiments, 20 µl of each plasma sample (diluted 1:5) or standard were added in each well, together with 200 µl of WST working Solution and 20 µl of Enzyme Working Solution. In the Blank 1 well, 10 µl of double-distilled water (ddH_2O), 200 µl of WST working Solution and 20 µl of Enzyme Working Solution were added. In the Blank 2 well, 20 µl of each plasma sample (diluted 1:5) or standard were added in each well, together with 200 µl of WST working Solution and 20 µl of Dilution Buffer. Finally, in the Blank 3, 20 µl of ddH_2O, 200 µl of WST working Solution and 20 µl of Dilution Buffer, were added. The plate was left on a shaker for 5 min and then, 20 µl of Stop solution was added in each well. After incubation at 37°C for 20 min, the fluorescence was read by using a spectrophotometer (VICTOR™ X Multilabel Plate Reader), with a wavelength of 450 nm. In order to quantify SOD activity (%), a reference standard value prepared by using xanthine oxidase was used.

Thymosin β4

Thymosin β4 (Human TMSβ4) was measured by using the Thymosin beta 4 (Human TMSβ4) ELISA Kit (FineTest; Wuhan Fine Biotech Co.; Wuhan, China) (216–223). Before the assay the dilution of the samples was done accordingly with the provided sample diluent and plate was

washed 5 times. For the experiments, 50 µl of the standard, blank or sample were added to appropriate wells. The blank was added with 50 µl sample/dilution buffer. Then 50 µl of Biotion-labeled Antibody Working solution was added to each well immediately and incubated for 45 min at 37°C. Then the plate was washed 3 times with washing buffer. 100 µl of HRP-Streptavidin Conjugate (SABC) was added to each well and incubated for 30 min at 37°C. Again, washing of plate was carried out for 5 times with washing buffer. 90 µl of TMB Substrate was then added to each well and incubated for 10-20 min in dark at 37°C. Thereafter, 50 µl of stop solution was added to each well and the fluorescence was read by using a spectrophotometer (VICTOR™ X Multilabel Plate Reader), with a wavelength of 450 nm. Thymosin β4 was expressed as ng/ml.

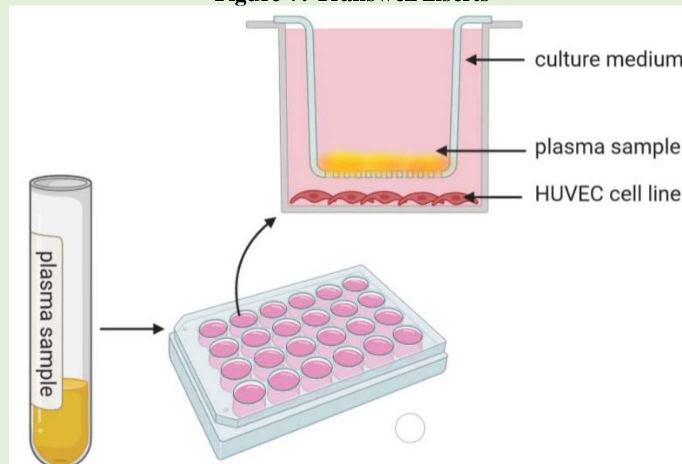
Experimental protocol

To evaluate the effects of plasma samples taken from the elderly on cell viability (MTT Assay), mitochondrial membrane potential (JC-1 Assay) and total ROS (DCFDA-Cellular ROS Detection Assay kit) on HUVEC, co-culture experiments were performed by using specific Transwell inserts (Supplementary Figure 1).

These inserts are permeable supports that permit cells to uptake and secrete molecules on both their basal and apical surfaces and thereby carry out metabolic factors in a more natural fashion.

For the experiments, plasma samples were plated in the apical compartment of the insert and left to act for 3 h, while, HUVEC were plated in the basal compartment. Experiments were performed with 10% plasma calculated in relation with total volume of each insert. Some cell samples were not treated with plasma and were used as control. After 3 h stimulation with plasma, the inserts were removed and various assays were performed in triplicate and analyzed by using a spectrophotometer.

Figure 7: Transwell inserts



Outcomes

The primary endpoint of the study was the improvement of the functionality, measured using the Barthel Index (BI) (184,185). The secondary outcomes was to investigate whether the lifestyle intervention also increased subjects' independence and reduced the risk of falls, respectively measured using Katz Activity of Daily Living (186) and Tinetti scales (224).

Regarding oxidative stress status the primary endpoint of the study was the differences between intervention and control group. As secondary outcomes, the normality distribution between T0 and T1 in participants who survived after COVID-19 outbreak.

Sample size

Sample size was calculated considering a baseline BI of 25 (standard deviation (SD) 5), to detect a 15 % increase after the intervention with a one-sided 5 % significance level and a power of 80 %, a sample size of 27 subjects per group was necessary, given an anticipated dropout rate of 10 % (225,226). The normality of distribution of continuous variables was tested by Shapiro–Wilk test.

Randomization and blinding

Patients were stratified according to CI score (two strata: CI score 5–7, and 8–12) and allocated with a balanced randomization. In detail, a researcher (AC) used a random generator software to gather two different numeric sequences, one for each stratum. Participants were recruited by a research physician (DC), who also obtained the informed consent and performed a medical examination to exclude subjects with a clinical condition for which the intervention was contraindicated. Because of the characteristics of the intervention, blinding of participants and interventionists was not possible.

Statistical analysis

The statistical analysis was conducted under the intention-to-treat approach using R version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria), and results with $p < 0.05$ were considered significant. The Shapiro–Wilk test was used to assess the normal distribution of continuous variables. The Student's T and the Mann–Whitney- U tests were

used to assess numerical continuous variables normally and non-normally distributed, respectively. Fisher's exact test was used for nominal categorical data. In addition, the association between the dependent and independent variables was assessed using the Spearman's ranks correlation test.

The LSM4Long RCT protocol was approved by the Ethical Committee of the "Azienda Ospedaliera Maggiore della Carità" University Hospital in Novara, and registered (registration number: CE 232/20). Written consents of participants was obtained before inclusion.

Cost analysis

This study was partially funded by the Italian Ministry of University and Research (MUR) program "Departments of Excellence 2023–2027", AGING Project – Department of Translational Medicine, Università del Piemonte Orientale. This is an institutional funding from Italian Ministry of University and Research (MUR). Funds of the program were to cover: materials and transport for blood sample, while biomedical devices were of proprety of University of Eastern Piedmont.

The not-for- profit private company Anteo Impresa Sociale estimate the cost of intervention regarding the nutritional, physical exercise and psychological intervention as follow.

Table 23: Food preparation costs

	COST/Resident	**N° Particiants**	**OVERALL**
Foods preparation	72.24 €	27	1,950.48 €

Table 24: Physical exercise tools costs

Tools	**N**	**Price at single unit**	**Overall cost**
Step 10-15 cm	9	29.90 €	269.10 €
Calf 1 kg	9 pairs	11.66 €	104.94 €
Calf 2 kg	9 pairs	13.48 €	121.32 €
Calf 3 kg	4 pairs	16.39 €	65.56 €
Calf 4 kg	4 pairs	15.97 €	63.88 €
Elastic bands	9 set of 5 bands	11.99 €	107.91 €

Following the intervention protocol the physiotherapist should be involved for 3 hours/weeks each group, that means 60 €/weeks (20 €/h) with an overall cost as reported in table 25

Table 25: Physiotherapist cost

	12 weeks cost	N° group each session	Overall cost
Physiotherapist	720.00 € + 4% pension contributions	3	2,246.40 €

As the psychologist should be involved 1 hour for 10 intervention each group (15 participants each group), the cost of the intervention will be as reported in table 26.

Table 26: Psychological intervention cost

	Cost for each meeting	N° intervention	COSTO TOT
Psychological intervention	20.00 € + 4% pension contributions	20 (10 each group)	416 €

Thus, the total cost of the combined lifestyle intervention should be below 2,40 €/person/day (total cost reported in table 27).

Table 27: LSM4Long total cost

Components	COSTS
Nutritional intervention	1,950.48 €
Physical exercise intervention (physiotherapist included)	3,087.02 €
Phycological intervention	416.00 €
TOTAL	**5,453.50 €**

RESULTS:

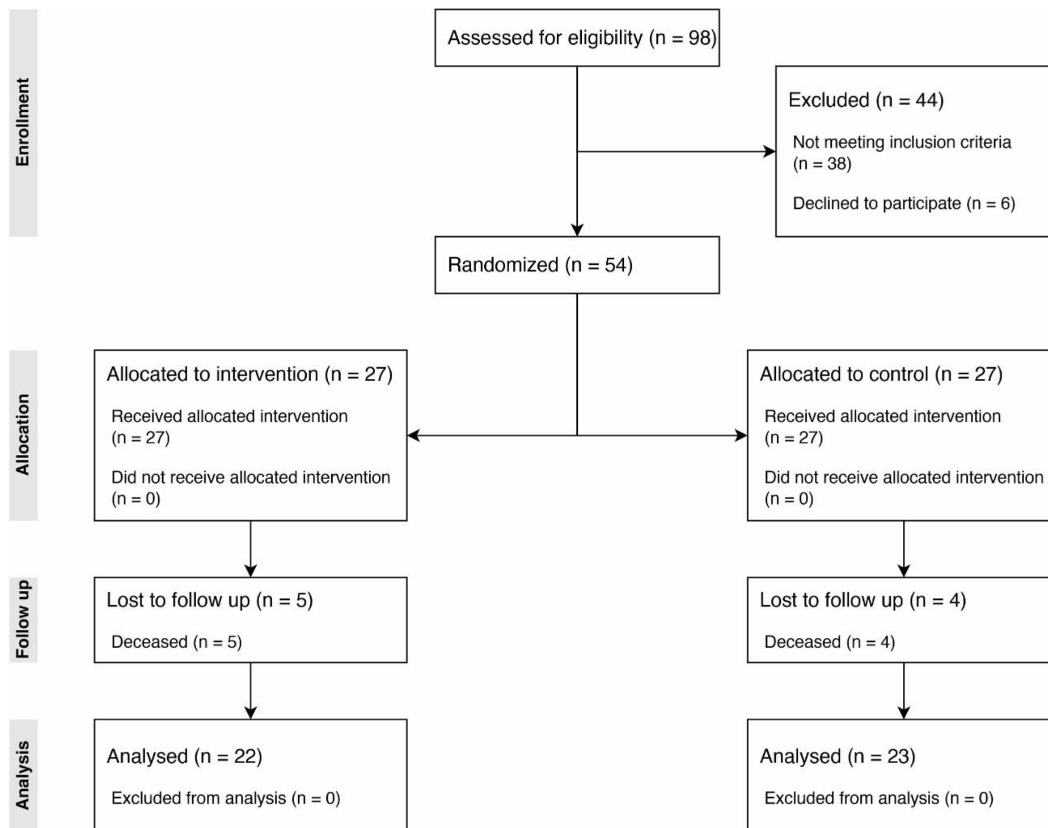
The study took place at the "Belletti Bona" nursing home, a 144-beds LTC facility located in Biella (Piedmont, Italy) managed by the not-for- profit private company Anteo Impresa Sociale.

The first attempt to conduct the study was abruptly interrupted due to a COVID-19 outbreak in the Belletti Bona: it was mandatory for ethical and health reasons to interrupt and delay the experimental program. As above reported, 32 residents enrolled in the cohort died due COVID-19.

Thus, the LSM4Long started on 15th February 2021 and lasted 3 months until 07th May 2021

Ninety-eight (n=98) patients were evaluated for inclusion, and a total of 54 subjects took part to the study (figure 8).

Figure 8: Study participants flowchart



Before the intervention, clinical records were screened to assess demographic information, current therapy, comorbidities, use of restraints, and use of tobacco and also body composition. Baseline characteristics of the two groups are shown in table 28 and 29.

Table 28: Demographic baseline characteristic of participants allocated to the Lifestyle vs Standard Care groups. **MWU: Mann–Whitney-U test; F: Fisher's exact test**

Demographic and body composition	Control (n=27)	Intervention (n=27)	p- value	Test
Age at enrollment (years, mean, SD)	83.26 (9.10)	84.85 (7.87)	0.78	MWU
Gender (female, n, %)	17 (62.96)	22 (81.48)	0.22	F
Weight (kg, SD)	59.68 (16.17)	63.36 (13.26)	0.26	MWU
Body mass index (kg/m^2, SD)	22.27 (4.83)	23.98 (5.01)	0.24	MWU
Fat-free mass ratio (mean, SD)	78.07 (9.92)	72.40 (10.91)	0.10	MWU
Hydration ratio (mean, SD)	73.40 (6.72)	74.16 (4.82)	0.94	MWU
Appendicular skeletal muscle mass (kg/m^2, SD)	6.18 (1.61)	6.20 (1.00)	0.67	MWU
Tobacco use - Never (n, %)	19 (70.4)	16 (59.3)	0.18	MWU
Ex-smoker (n, %)	4 (14.8)	2 (7.4)	-	-
Current smoker (n, %)	4 (14.8)	3 (11.1)	-	-
Unknown (n, %)	0 (0)	6 (22.2)	-	-
Cigarettes/ day (in smokers) (n, SD)	0.70 (1.96)	0.52 (1.50)	0.7	MWU
Health Comorbidities (n, SD)	5.85 (2.68)	7.11 (3.27)	0.16	MWU
Number of drugs (n, SD)	7.48 (2.85)	8.30 (3.27)	0.45	MWU
Use of restraints (daily use, n, %)	12 (44.44)	10 (37.03)	0.78	F
Food intake (mean Kcal, SD)	1615 (152)	1486 (217)	0.51	MWU

Participants were mostly normal-weight women with a mean age of 84.06 years. No significant differences were found between groups at the baseline. Regarding body composition both control and intervention residents did not show loss or excess of Fat Free Mass and no differences in sarcopenic or hydration were found between groups. In particular appendicular skeletal muscle mass mean was 6.20, thus similar to the cut-off for

sarcopenia in age related general population. Most of the participants never smoked or were former smokers, and for the majority of participants any restraints were prescribed.

Table 29: CGA baseline characteristic of participants allocated to the Lifestyle vs Standard Care groups. **MWU: Mann–Whitney-U test; F: Fisher's exact test**

CGA baseline assessment	Control (n=27)	Intervention (n=27)	p- value	Test
CI score (mean, SD)	8.30 (2.03)	7.85 (2.16)	0.39	MWU
Barthel Index – functionality (mean, SD)	19.15 (17.83)	22.00 (16.07)	0.26	MWU
Barthel Index – mobility (mean, SD)	11.70 (12.26)	14.89 (12.42)	0.25	MWU
Barthel index – total (mean, SD)	30.85 (28.44)	36.89 (27.73)	0.25	MWU
Tinetti Score – walk (mean, SD)	4.93 (4.39)	7.04 (3.63)	0.09	MWU
Tinetti score – balance (mean, SD)	4.96 (4.78)	7.44 (4.23)	0.06	MWU
Tinetti score – total (mean, SD)	9.89 (9.02)	14.48 (7.67)	0.08	MWU
CI Katz ADL* (mean, SD)	1.93 (2.20)	2.67 (2.35)	0.20	MWU
CI SPMSQ score* (mean, SD)	1.00 (0.78)	0.59 (0.57)	0.06	MWU
CI DISCO score* (mean, SD)	0.81 (0.62)	0.96 (0.64)	0.46	MWU
CI DMI score* (mean, SD)	1.70 (0.57)	1.81 (0.40)	0.52	MWU

Baseline mean BI and Katz ADL scores were 33.87, and 2.30, respectively. The mean care intensity score was 8.07 (control arm 8.30 – intervention arm 7.85), and mean energy intake was 1,551.04 Kcal. No external food supply from patients' families has been reported. Physical exercise was attended by all the subjects of the intervention group, with an average participation to 9 out of the 13 total sessions (68.98 %, SD 21.75). Supportive therapy registered an average participation to 5 out of 7 sessions (82.0 %, SD 25.02).

Table 30 shows the estimated calories consumption and food intake for the two groups.

Calories burned were estimated from MET by the assumption of 1 MET is the equivalent to a Vo2 (a measure of oxygen consumption) of 3.5mL/kg/min (resting state) and is equal to 1 calorie burned per kilogram of body weight per hour (1 kcal/kg/hour) (227).

Both groups took less calories than proposed diets (usual menu vs nutritional intervention); furthermore, the intervention group showed a lower energy and carbohydrates intake than the control group. Daily intakes were estimated from daily records of intakes with the diet diary recommended by internal protocol followed to monitoring the nutritional status of residents.

Regarding physical exercise the intervention arm burned more calories than usual care and performed physical activity at a higher METs.

Table 30: Real weekly calories consumption and food intake of control and intervention groups. **T: Student's T test; MWU: Mann–Whitney-U test; MET: metabolic equivalent of tasks**

	Control [n=27] (mean, SD)	Intervention [n=27] (mean, SD)	*p*-value	Test
Physical exercise during 45-minutes sessions				
Burned calories/week (Kcal, SD)	87.5 (32.1)	287.4 (96.3)	<0.001	T
MET/weeks (MET, SD)	64.3 (25.4)	239.2 (75.4)	<0.001	T
Healthy diet (daily intake)				
Energy intake (Kcal, SD)	1615.54 (152.31)	1456.61 (212.16)	0.01	MWU
Proteins intake (g, SD)	71.24 (10.99)	68.41 (14.06)	0.43	T
Lipids intake (g, SD)	53.70 (6.82)	51.55 (9.75)	0.37	T
Carbohydrates intake (g, SD)	224.34 (35.57)	190.44 (34.14)	0.001	T
Fibers intake (g, SD)	19.80 (6.16)	16.91 (7.12)	0.13	T
Calcium intake (mg, SD)	828.25 (211.25)	830.57 (331.43)	0.97	T
Leucine intake (mcg, SD)	3947.49 (614.16)	3974.15 (823.06)	0.89	T

Figure 9: Plot of the BIA parameters after the intervention

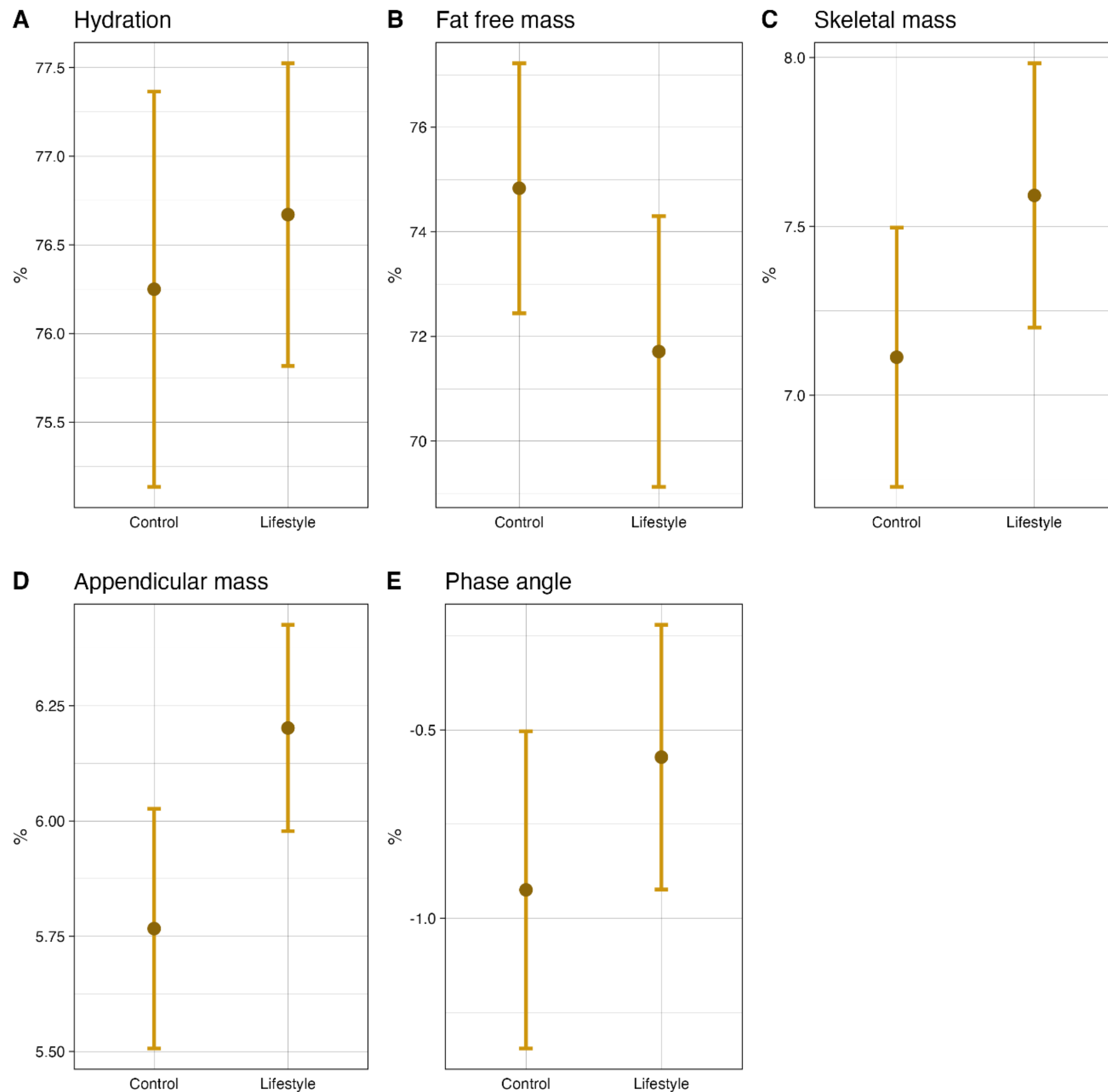


Figure 9 shows the results of body composition evaluation at the end of intervention. No significantly differences between control and intervention arm were found by BIA, but results showed that at the end of the lifestyle program the appendicular skeletal muscle mass remain quite stable (mean 6.20 kg/m^2; SD 1.00); while in the control group fell down to (mean 5.77 kg/m^2; SD 1.20). In contrast, the Fat free mass (FFM) in the intervention group fell down to 71.71%, while in the control group increased to 74.83%, with no significant differences between the two arms ($p = 0.78$). Moreover, hydration in the control group increased 76.25% and in the intervention group to 76.67% also. Finally, both the

intervention and control arm showed no significant differences in standardized phase angle (SPA control=-0,92; SPA intervention=-0,57; P-value= 0,06) [data unpublished].

Measures of functionality are shown in table 31. Overall, the intervention group showed a significant improvement in the total scores of all the measured scales. Similarly, a significant improvement was observed for the functional dimension of the BI and for both Tinetti scale dimensions (walk and balance) (figure 10). None of the participants reported adverse effects.

It is important to highlight that despite to significant improvement of Barthel Index, the intensity of care (CI or PAI) did not differ overall between control and intervention arm (CI mean control = 8.22; CI intervention = 6.78; p=0.99).

Table 31: Univariate analysis of scales after the combined intervention. **MWU: Mann–Whitney-U test**

	Control [n=27] (mean, SD)	Intervention [n=27] (mean, SD)	p- value	Test
Barthel (total)	30.04 (29.54)	44.96 (26.72)	0.02	MWU
Barthel (functional)	18.93 (18.63)	28.00 (15.72)	0.02	MWU
Barthel (mobility)	11.11 (11.94)	16.96 (11.95)	0.06	MWU
Katz ADL (total)	1.85 (2.17)	3.52 (1.93)	0.01	MWU
Tinetti (total)	9.26 (8.65)	16.04 (8.17)	0.01	MWU
Tinetti (walk)	4.59 (4.27)	7.85 (3.87)	0.01	MWU
Tinetti (balance)	4.67 (4.59)	8.19 (4.55)	0.01	MWU

Figure 10: Plot of the scales after the intervention

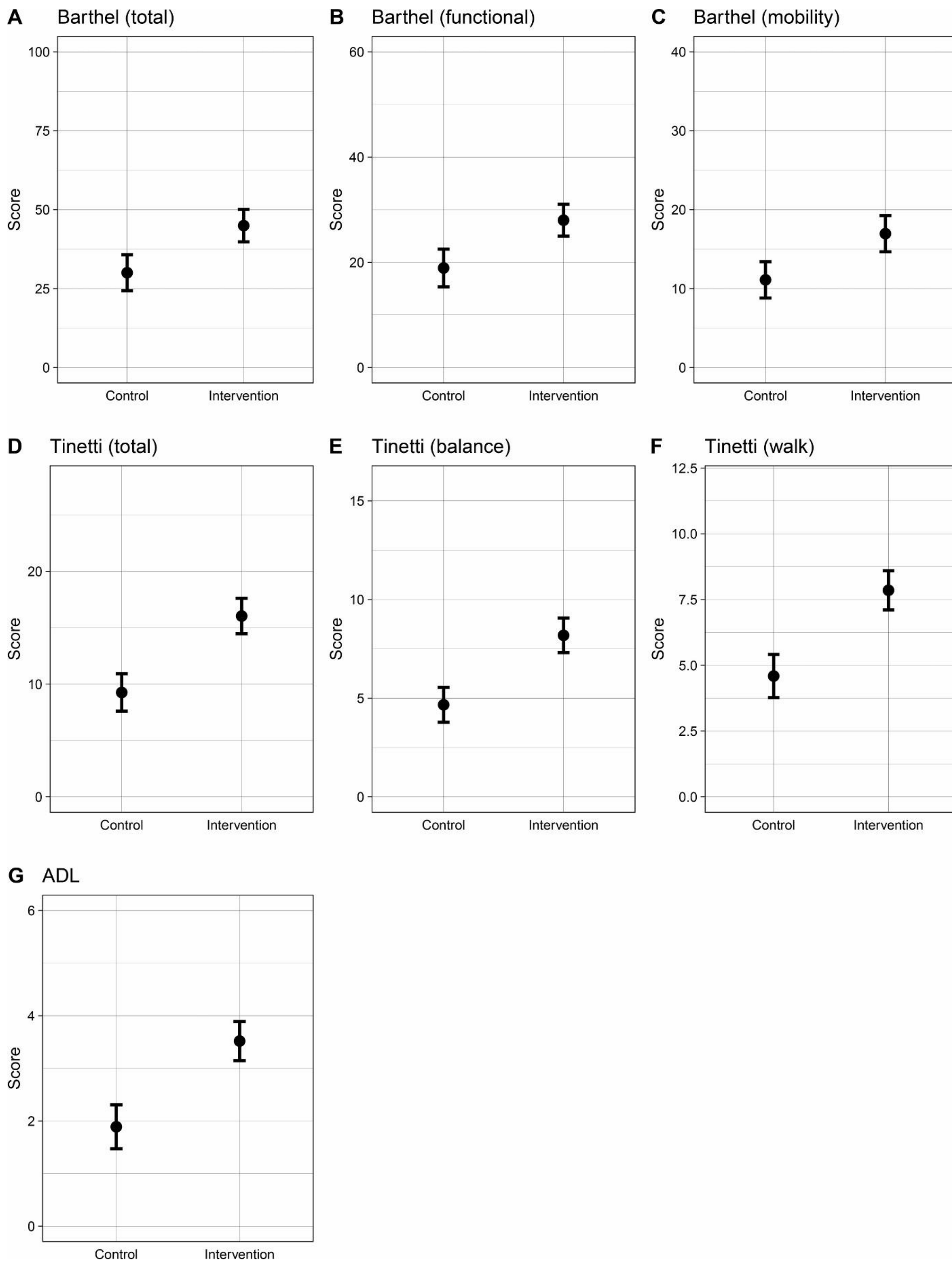
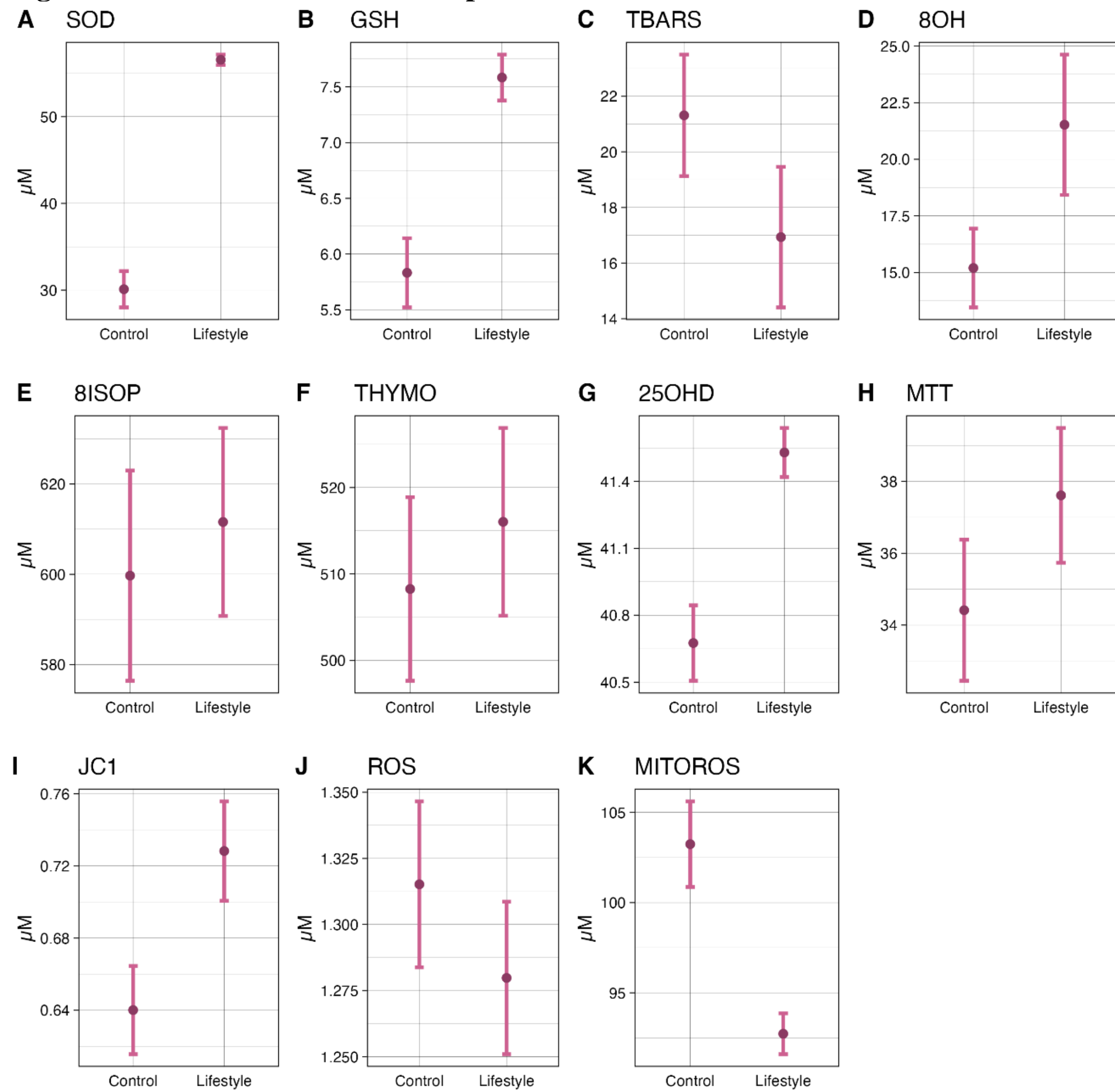


Table 32: Univariate analysis of biochemical parameters after the combined intervention. **MWU: Mann–Whitney-U test, T: Student's T test**

Parameter	Control (Mean, SD)	Intervention (Mean, SD)	p-value	Test
GSH (µM)	5.83 (1.52)	7.58 (1.01)	<0.001	MWU
TBARS (µM)	21.31 (10.70)	16.94 (12.38)	0.048	MWU
8OH-2dG (µM)	15.20 (8.53)	21.52 (14.88)	0.170	MWU
8-isoprostane (µM)	599.71 (1114.11)	611.59 (102.03)	0.706	T
Thymosine β (µM)	508.26 (52.02)	516.03 (53.19)	0.611	T
SOD (µM)	30.10 (10.25)	56.55 (2.95)	<0.001	MWU
25 OH VitD (µM)	40.68 (0.83)	42.53 (0.54)	<0.001	T
MTT (µM)	34.41 (9.62)	37.61 (9.19)	0.024	MWU
JC1 (µM)	0.64 (0.12)	0.73 (0.14)	0.021	T
ROS (µM)	1.32 (0.15)	1.28 (0.14)	0.350	MWU
MITOROS (µM)	103.24 (11.62)	92.73 (5.54)	0.0015	MWU
AOOP (µM)	281.90 (28.66)	274.50 (48.19)	0.420	MWU

The unpublished results of biochemical analyses, performed by colleagues of the Physiology laboratory of University of Eastern Piedmont, showed that after the interventions: SOD, GSH, 25OHD, and JC1 were significantly higher in the lifestyle arm compared to the control arm, while mitoROS was significantly lower in intervention group (table 32 and figure 11). Also, TBARS were significantly lower in intervention group vs control arm. No significant differences were found in Thymosin β plasma levels between groups.

Figure 11: Plot of the biochemical parameters after the intervention



As reported below, the comparison of oxidative stress biomarkers levels before and after the intervention, performed by the colleagues of Physiology laboratory of University of Eastern Piedmont, was possible only for a sub-sample (N=28, Control=13; Intervention=15) of the participants.

Results of these analyses are courtesy of Prof. Grossini and relevant findings are summarized below.

Blood sample analyses confirmed the increment found at the end of the lifestyle program in levels of SOD, GSH and the reduced levels of mitoROS. In fact, GSH plasma antioxidants levels increased in the intervention arm only at T1 (Figure 12 B), SOD activity increased at T1 only in the intervention arm, while in control group it decreased compared to what was observed at T0 (Figure 12 C, D). Moreover, they found a smaller reduction of the plasma levels of Thymosin β in the lifestyle intervention group at T1 vs T0, in comparison with control group (-62% in control group: from 1249 ± 140 ng/ml of T0 to 479 ± 42 ng/ml of T1 -37% in LSP: from 1264 ± 152 ng/ml of T0 to 797 ± 147 ng/ml of T1; $p<0.05$; Figure 12 E, F). MitoROS release was reduced at T1 vs T0, in the intervention group only. Instead, an increase of mitoROS release was found at T1 in the control group (Figure 13 A, B).

Figure 12: Plasma levels of glutathione (GSH; A, B), superoxide dismutase activity (SOD; C, D), and Thymosin beta (E, F) in intervention arm (B, D, F) versus older control arm (A, C, E). Square brackets indicate significance between groups. T0= before intervention. T1= after intervention or after 3 months from T0. A value of $p < 0.05$ is considered as statistically significant.

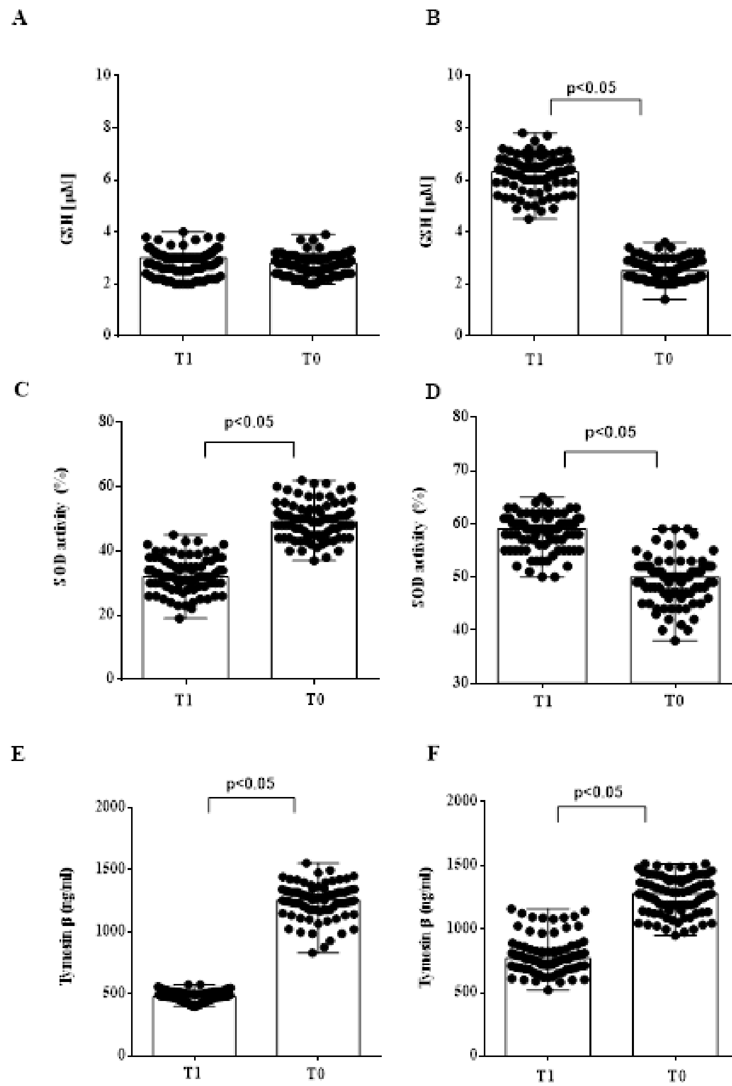
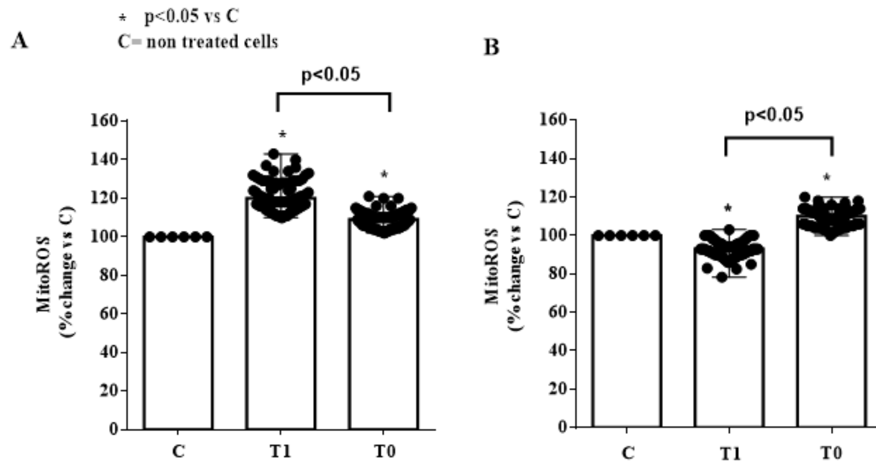


Figure 13: Effects of plasma of control residents (A) and intervention arm (B) on mitochondrial ROS (mitoROS) release in HUVEC. Square brackets indicate significance between groups. T0= = before LSP. T1= after LSP or after 3 months from T0. A value of $p < 0.05$ is considered as statistically significant.



DISCUSSION

The aim of this work was to design and implement a combined nutritional, physical exercise and psychological wellbeing intervention (lifestyle medicine intervention) in the real-world setting of a nursing home and to assess the effectiveness on ameliorated the functionality status and oxidative stress biomarkers in older residents.

A pragmatic randomized controlled trial was design using the results of an overview of systematic reviews on lifestyle interventions to improve longevity indicators (sarcopenia, hydration and osteoporosis) and a systematic review on non-pharmacological approaches to improve self-efficacy and self-confidence in elderly residents of a long-term care facility as highlighted Simning and Simons (228).

The designed intervention was tailored to the real-life of the nursing home Belletti Bona and the components of the intervention were administered by trained components of the staff members of the facility.

Both the results of the overview of systematic review and of systematic review showed that few randomized controlled trials were designed thinking about the implementation in a real-world nursing home setting. In fact, only 1 out of 150 single RCT found in the overview of systematic review met the a priori defined criteria for inclusion. Moreover, 56 out of 154 (36%) single studies were included in the systematic review. Overall, the quality of the study showed some flaws in the methodology rigor, in particular regarding the design of the studies aimed to assess the effectiveness of non-pharmacological interventions use in depressed residents.

Thus, the Lifestyle for longevity study could be considered the first randomized controlled trial aimed to assess the effectiveness of a combined lifestyle intervention (combined nutritional, physical exercise and psychological wellbeing) for elderly residents in a real LTC setting.

Participants received a structured training exercise program, ate lesser carbohydrates and more leucine without supplementation and attended a psychological support than the control arm.

At the end of 12 weeks of intervention the scores of functionality outcomes significantly increased. The Barthel Index score improved (control group mean=30.04; SD 29.54) vs intervention group mean=44.96; SD 26.72; p-value=0.02) that means a better resident functional ability in daily living activities, without an overall significant change in care index (PAI), that means without a significant reduction in overall needs of participants. The Tinetti scores showed an improvement reducing the risk of falls (control group mean=9.26; SD 8.65 vs intervention group mean=16.04; SD 8.17; p-value=0.01).

Although results from other studies are consistent with the LSM4Long findings, as mentioned above, the majority of the studies were performed in different settings (community-dwelling or home) (168,169).

Most of the RCT found in the preparatory scientific literature research achieved similar results requiring a longer follow-up period (6-12 months), whereas the LSM4Long intervention lasted less (12 weeks) (168,169). However, the intervention did not achieve all its specific objectives: no differences were found in the body composition of participants and, furthermore, failed to meet the nutritional goals. Thus, the delivery of the intervention may have been positively influenced by some unplanned factors (229) (230). First, the study started just after participants ended mandatory isolation due to the COVID-19 outbreak: all the residents respected this health policy and a structured physical exercise may have facilitated adherence to the physical activity by the resumption of a structured daily life. Moreover, the structure of the program may have been influenced by social interaction: the physical exercise sessions were carried out in groups and in the gym of the facility with the presence of the physiotherapist who could have been seen as a motivator. Moreover, physiotherapist received an educational and training meeting that may have defeat the preconception regarding physical exercise recommendations. In contrast, residents allocated to the control arm received the usual care activity (individual physical activity such as walking in the corridors of facility ward) without being required to reach a specific level of physical effort. Indeed, as acclaimed by other authors, the support from care-givers/peers and an attractive environments are well-known facilitators factors to break down physical activity barriers in older people, contributing to the overall effect of the intervention (45,46,138).

Regarding the healthy diet, residents failed to achieved the nutritional goals due to insufficient support delivered by health assistants. Although, meals were served simultaneously and without differences in both group, health assistants received less instructions and serving two different menu in the same timeframe as usual may have contribute to the incidentally decrease in food intake, included carbohydrates and proteins (47). This unplanned over-restriction of carbohydrates may have contributed to glycemic control, subsequently increasing the effectiveness of the LSM4Long intervention (231,232).

As described in the rationale chapter self-confidence, personal motivation and self-efficacy could be efforted by psychological intervention and reminiscence could foster the acceptance of oneself. Thus, the fully participation to the reminiscence intervention might arouse in residents to be more inclined to participate to the LSM4Long program (138,139).

The results of the study showed a significantly improvement in some biomarkers of oxidative stress. The observed differences in the plasma levels of antioxidants, with particular reference to the SOD activity and the differences in circulating oxidants, with particular reference to TBARS, could be explained by the synergistic effect of endurance and resistance training components of the physical exercise circuit decelerating the aging process (67,97,116–121)
The significantly lower levels of mitoROS in intervention group that could have contributed to the maintenance of skeletal muscle mass as explained in the rationale chapter that could be related to the results obtained to the functional scores (66,67).

Interestingly the results of analyses performed by the Physiology laboratory of the University of Eastern Piedmont showed a preserved levels Thymosin β (Tβ4), that as described in the rationale chapter is involved in the immunescenesce process and has been proposed as a good candidate molecule for reversing aging processes and accelerate organ regeneration in the human body (125,126,233)

As defined by Medical Research Council framework the LSM4Long should be considered a complex intervention. The combination of the three different components (exercise, diet, psychological wellbeing) with the interaction of underlying factors (residents motivation,

interventionist behavior, social context) might have led to a synergistic effect. This synergistic effect could have determinate the overall effectiveness of the intervention in 12 weeks (48,234,235).

The total cost of the combined lifestyle intervention has been estimated to be below 2,40 €/person/day, that is an incremental cost similarly found for diabetes prevention programs (236).

Results of the project should be interpreted considering other limitations.

It was not possible to blind completely participants or researcher of the study design and intervention characteristics. The intervention and control groups belonged to the same nursing home and shared bedrooms, common spaces, and healthcare professionals, potentially leading to a contamination bias, and voluntary enrollment might have introduced a selection bias (237).

The rigorous methodology has mitigated such limitations, designing a pragmatic randomized controlled trial that is the best study design to evaluate complex intervention in a single-center studies (179,180,238,239).

However, a further exploration of underlying factors is needed, to better understand the barriers that hampered a complete intervention delivery in this context.

OTHER PROJECTS:

Project 2: UPO SoGuD

Methods:

The protocol steps of UPO SoGuD Primary Prevention are: disease specific avoidable and not avoidable risk factors mapping through UpToDate, inc.; a systematic research of International and Society produced CPGs through 16 databases plus PubMed; double blind fulfilment of explicit inclusion criteria; guidelines quality assessment using AGRE(II) tool, application of a taxonomy for intervention, stakeholders and risk factors to categorize recommendations; recommendations grade alignment; assignment validation of the stakeholder category and definition of a priority rank by a pool of expert (clinicians and public health professionals) using the Delphi method.

Retrieved primary prevention recommendations are used for the development of the primary prevention model pathway needed to their synthesis into statements called "UPO Primary Prevention Best Practices", each statement is stakeholder-oriented (Clinicians, General Population and Policy Makers).

Results:

UPO SoGuD Osteoporosis: 31/103 guidelines fulfilled the inclusion criteria and 11 were included in qualitative analysis, 5/11 guidelines obtained an Agree II score >50% and were included in the sample (best Agree Score 75.00%). Overall, 86/232 (37%) primary prevention recommendations were retrieved. Clinicians, Policy Maker General Population oriented best practices were 81/86 (94%), 24/86 (28%), 49/86 (57%), respectively. 55/86 (64%) recommendations were considered of high priority by the clinicians and public health panel of experts.

The primary prevention pathway has been developed and published on the Aging Poject website (https://www.agingproject.uniupo.it/upo-so-gud/).

UPO SoGUD BPCO: 216/1298 full text were assessed for eligibility and 10 single guidelines were included; 7/10 guidelines obtained an Agree II score >50% and were included in the sample (best Agree Score 75.00%). 94/451 primary prevention

recommendations were retrieved. Clinicians, Policy Maker and General Population oriented best practices were 94 (100%), 64 (68%), 59 (65%) respectively.
The primary prevention pathway has been developed and published on the Aging Project website (https://www.agingproject.uniupo.it/upo-so-gud/).

Upo SoGUD Dementia: 100/961 full text were assessed for eligibility and 10 single guidelines were included in qualitative analysis, 4/10 guidelines obtained an Agree II score >50% and were included in the sample (best Agree Score 80.80%). Overall, 99/360 primary prevention recommendations were retrieved. Clinicians, Policy Maker and General Population oriented best practices were 99 (100%), 75 (75.7%), 80 (88.8 %) respectively.
The primary prevention pathway has been developed and published on the Aging Project website (https://www.agingproject.uniupo.it/upo-so-gud/).

UPO SoGUD Diabetes: 412/1723 full text were assessed for eligibility and 12 single guidelines were included in qualitative analysis; 5/12 guidelines obtained an Agree Score II >50% (best Agree Score 96.38%). Overall, 214/1038 primary prevention recommendations were retrieved. Clinicians, Policy Maker and General Population oriented best practices were 214 (100%), 100 (46,7 %), 92 (43.9 %) respectively.
The primary prevention pathway has been developed and published on on the Aging Project website (https://www.agingproject.uniupo.it/upo-so-gud/).

Project 3: Flu-like syndrome-COVID-19 onset

Material and Methods

In order to assess the presence of a correlation between inflammatory biomarkers and the risk of respiratory viral infection in a typical Long Term are (LTC) setting, a retrospective cohort study was designed as a part of Longevity Check Up – Long C-UP Study (LCU). The LCU Study was approved on July 27th, 2019 by the Ethical Committee of the "Azienda Ospedaliera Maggiore della Carità" University Hospital in Novara, and registered (registration number: CE 31/19 approved) and then amended in July 2020. The study was performed in the "Belletti Bona" nursing home, a beds LTC facility for elderly based in Biella, Piedmont Region Italy. 60 patients admitted to LTC unit for at least one year at moderate or high care load (Barthel Index – BI < 40) (53,54) that were able to express their consent to the study were enrolled. Patients with cognitive impairment were excluded from the study (Short Portable Mental Status Questionnaire – SPMSQ. ≥ 3 errors) (55). An ad hoc database has been developed. Demographic and clinical variables were taken: gender (male/female) age (years), total BI (TBI) (score), SPMSQ (score), flu vaccination (yes/not). The clinical outcomes data were collected from clinical records using an anonymous data abstraction form throughout all the study period from February 1st to December 31st, 2020. Those included the onset of clinical COVID-19 syndrome (yes/not), the related therapy (yes/not), the confirmation of the COVID-19 disease with real-time reverse transcription polymerase chain reaction (rRT-PCR) test (yes/not) and the patient survival status (alive/deceased). Since rRT-PCR tests were not available until April 25th 2020, a mixed approach to identify COVID-19 cases in the sample was used. First, from February to April 2020, we screened clinical records for symptoms suggestive of COVID-19. Accordingly, a clinical COVID-19 case was defined as each patient who presented at least one of the most common clinical features at the onset of illness. As it is reported in literature, these included: fever ≥ axillary temperature over 99.5°F/37.5°C, cough (dry or with expectoration), fatigue/asthenia/myalgias, dyspnea, anosmia/ageusia, blood oxygen saturation ≤92%, rhinorrhea/rhinosinusitis, and diarrhea (56–60). On April 25th 2020, the COVID-19 RT-PCR test for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal or oropharyngeal swabs was adopted in Italian nursing homes as a two-

week screening program for elderly. Therefore, until the end of the study (December 31st, 2020) a COVID-19 case was defined as each patient who was positive to the COVID-19 RT-PCR test, independently of possible associated symptoms. The possible associated symptomatic therapy included: anti-inflammatory drugs (paracetamol, dexamethasone and/or other corticosteroids), antibiotics (all the classes), respiratory assistance (drugs and/or oxygen provision) and low molecular weight heparins and/or antiaggregant drugs. Blood samples were collected (January 15th, 20th and 24th 2020) for the evaluations of oxidants/antioxidants, as thiobarbituric acid reactive substances, 8-hydroxy-2-deoxyguanosine, 8-isoprostanes, superoxide dismutase activity, glutathione and vitamin D. In vitro, human umbilical vascular endothelial cells (HUVEC) were used to examine the effects of plasma on viability, ROS release and mitochondrial membrane potential. The plasma obtained was divided into tubes that were stored at -80°C at the Physiology laboratory of the University of East Piedmont and further used for the quantification of markers of redox state. The normality of distribution of continuous variables was tested by Shapiro Wilk test. Mean/rate ± standard deviations (SD) or confident interval (CI) were given as descriptive statistics. As concerning plasma oxidants/antioxidants and the in vitro experiments, all the results obtained were examined through one-way ANOVA followed by Bonferroni post hoc tests. The Spearman rank correlation analysis was used to investigate relationship between non-normal variables. The Mann Whitney U test was used to compare non-normal numerical data among groups. A Receiver Operating Characteristics – ROC curve analysis was performed to determinate the best cut-off values for significant biomarkers in predicting COVID-19, based on univariate analysis (the associated Area Under The Curve – AUC, Sensitivity and Specificity were also calculated). The statistical analysis was performed with SPSS for Windows version 24.0. A value of $p < 0.05$ was considered statistically significant.

Results:

Through the screening of the clinical records, 37 out of 60 patients (61.67%) fulfilled criteria for clinical-COVID-19, while 36 out of 41 patients (87.80%) who have been screened with COVID-19 RT-PCR test, received a confirmation diagnosis of COVID-19 (19 elders died before the availability of the COVID-19 RT PCR test and were excluded

from the analysis). The results obtained showed that the redox state of the elderly was quite balanced; mitochondrial membrane potential of HUVEC was reduced by about 20%, only. Also, the correlation analysis evidenced the association between mitochondrial function and the patients' outcomes. Interestingly, lower levels of mitochondrial membrane potential were found in the elderly who had symptoms suggestive of COVID-19 or with a confirmed diagnosis of COVID-19. A ROC curve analysis was performed to evaluate the diagnostic ability of mitochondrial membrane potential as a predictor of the future onset of COVID-19. Moreover, results showed that a threshold mitochondrial membrane potential value ≤0.79 (%) should represent the best trade-off between sensitivity (0.76, 95CI% 0.58-0.89) and specificity (0.62, 95CI% 0.41-0.80) when detecting the patients who could develop COVID-19 (AUC ± Std. error 0.71±0.07).

Conclusion:

The results of this study highlight the importance of mitochondrial function in the predisposition to get COVID-19 infection in a population of elderly admitted to LTC unit. Overall, the results we obtained highlighted the important role played by mitochondria in aging and age-related disease. This way keeping mitochondrial health and effective mitochondrial reserves could be not only a "prolongevity" key factor but also a useful tool for resisting virus infection and for coping when the system is "stressed" (e.g., by a virus). It should be highlighted that in this study the correlation analysis showed positive relation between mitochondrial membrane potential and the levels of plasma Thymosin β4, which is an anti-inflammatory hormone that can down-regulate chemokines and cytokines, as well as increase fibrinolysis. By this way, Thymosin β4 could represent a valuable and easily detectable marker useful as a surrogate for mitochondrial function in people, particularly, in flu-like syndromes or infections like COVID-19. In this regard it is to note that Thymosin β4 has been suggested as an off-label therapy. The results obtained in this study about the redox state in a population of elderly admitted to LTC unit would strengthen the importance of mitochondrial function as biomarker and target of interventions, not only for aging in general but, even more, for the identification of patients that are most vulnerable to COVID-19 disease.

Project 4: Vaccination Strategies against Seasonal Influenza in Long Term Care Setting: a Mathematical Modelling Study

Matherial and Methods:

In order to explore the dynamics of seasonal influenza starting from real data collected from a nursing home and to simulate the occurrence of Influenza virus outbreaks in a typical Italian long-term care a stochastic transmission model should be developed. A stochastic transmission model is defined as: a type of model where the parameters, variables, and/or the change in variables can be described by probability distributions. This type of model may predict a distribution of possible health outcomes. The SEIR (Susceptible – Exposed – Infectious – Recovery) model represents one of the most adopted mathematical models to characterize the epidemic dynamics and to predict possible contagion scenarios. It is based on a series of dynamic ordinary differential equations that consider the amount of the population subject to contagion, the trend over time of individuals who recover after infection, and the individuals who unfortunately die. The aim of this study was to identify the best vaccination strategy to minimize cases (and subsequent complications) among the guests. After producing the contact matrices with surveys of both the health care workers (HCW) and the guests, the mathematical model of the disease was developed. The model consists of a classical SEIR part describing the spreading of the influenza in the general population and a stochastic agent based model that formalizes the dynamics of the disease inside the institution. After a model fit of a baseline scenario, we explored the impact of varying the HCW and guests parameters (vaccine uptake and vaccine efficacy) on the guest attack rates (AR) of the nursing home

Results:

In order to define the temporal span of observation a literature review has been performed, an ad hoc database has been developed to collected data about influenza vaccination uptake rates, influenza episodes rates of patients and Health Care Workers in a typical Long Term Care Setting from October 2019 to April 2020. The study was performed in LTC “Belletti Bona” Biella. In order to estimates the risk of being infected, the frequency of contact has been estimated surveying patients and HCW. The results obtained allowed to develop the mathematical model. The mathematical model has been developed by Prof Salinelli and

Prof. Rinaldi. The aggregate AR of influenza like illness in the nursing home was 36.4% (ward1 = 56%, ward2 = 33.3%, ward3 = 31.7%, ward4 = 34.5%). The model fit to data returned a probability of infection of the causal contact of 0.3 and of the shift change contact of 0.2. We noticed no decreasing or increasing AR trend when varying the HCW vaccine uptake and efficacy parameters, whereas the increase in both guests vaccine efficacy and uptake parameter was accompanied by a slight decrease in AR of all the wards of the LTC facility

Conclusion:

A nursing home is still an environment at high risk of influenza transmission but the shift change room and the handover situation carry no higher relative risk. Therefore, additional preventive measures in this circumstances may be unnecessary. In a closed environment such as a LTC facility, the vaccination of guests, rather than HCWs, may still represent the cornerstone of an effective preventive strategy. Finally, we think that the extensive inclusion of real life data into mathematical models is promising and may represent a starting point for further applications of this methodology.

LIST OF PUBLICATION:

Project 1: Lifestyle Medicine for Longevity (LSM4Long) trial

- Non-pharmacological approaches to depressed elderly in long-term care facilities. A systematic review of the literature (Front. Public Health, 16 July 2021 | https://doi.org/10.3389/fpubh.2021.685860) (authors: Carla Gramaglia, Eleonora Gattoni, Debora Marangon, Diego Concina, Elena Grossini, Carmela Rinaldi, Massimiliano Panella and Patrizia Zeppegno
- A systematic overview of lifestyle interventions to improve longevity indicators in elderly patients hospitalized in long-term care facilities (protocol) (authors: Gianmarco Cotrupi, Alessandra Vasile, Antonio Isabella, Maicol Andrea Rossi, Nicola Piu, Anil Babu Payedimarri, Annalisa Opizzi, Diego Concina, Marco Comba, Tommaso Testa, Massimiliano Panella, Fabrizio Faggiano) available from https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020206090.
- Longevity check-up project (authors Concina D; Conti A.; Opizzi A; Rinaldi C.; Zeppegno P.; Gramaglia C.M.; Grossini E.; Panella M.) (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2022/09/Abstract_Book_Aging_Project_UPO-1.pdf).
- Effect of diet and physical exercise on functional scales and on biochemical aging markers in institutionalized elderly: a randomized controlled trial (authors: Conti A.; Concina D.; Opizzi A.; Rinaldi C.; Zeppegno P. ; Gramaglia C.M.; Grossini E.; Panella M) (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2022/09/Abstract_Book_Aging_Project_UPO-1.pdf).
- Effects of exercise and nutritional intervention in elders admitted to a long-term care (LTC) unit (Venkatesan S-; Colombo A.; Aanastasio M; Leone E; Concina D.; Panella M.; Grossini E.) (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2022/09/Abstract_Book_Aging_Project_UPO-1.pdf).
- Effectiveness of a Combined Lifestyle Intervention for older people in LongTerm Care: A Randomized Controlled Trial (Andrea Conti, Diego Concina, Annalisa Opizzi, Agatino Sanguedolce, Carmela Rinaldi, Sophia Russotto, Elena Grossini, Carla Maria Gramaglia, Patrizia Zeppegno, Massimiliano Panella), Archives of

Gerontology and Geriatrics (2024), doi: https://doi.org/10.1016/j.archger.2024.105340

Project 2: UPO SOGUD

- UPO SoGuD Prevention Summaries of Guidelines upon Diseases Prevention Healthy Aging - Buone pratiche nella prevenzione dell'osteoporosi (authors: Diego Concina, Fabrizio Faggiano, Massimiliano Panella, Carlo Smirne, Carmela Rinaldi, Tommaso Testa) ISBN 9788831306089 available from https://www.agingproject.uniupo.it/wp-content/uploads/2021/04/UpoSoGuD-osteoporosi-professionisti_DEF.pdf).
- Healthy Aging Buone pratiche raccomandate a tutti nella prevenzione dell'osteoporosi (Authors: Carmela Rinaldi, Diego Concina, Massimiliano Panella) ISBN 9788831306072 (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2021/04/SOGUD-osteo-cittadini_DEF-1.pdf).
- UPO SoGuD Prevention Summaries of Guidelines upon Diseases Prevention - Healthy Aging Buone pratiche nella prevenzione della Broncopneumopatia Cronica Ostruttiva (BPCO) (Authros: Diego Concina, Gianmarco Cotrupi, Fabrizio Faggiano, Massimiliano Panella, Carlo Smirne, Carmela Rinaldi) ISBN 9788831306157 (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2021/10/UPOSoGud_BPCO_Professionisti.pdf)
- Healthy Aging Buone pratiche raccomandate a tutti nella prevenzione della Broncopneumopatia Cronica Ostruttiva (authors: Carmela Rinaldi, Diego Concina, Massimiliano Panella, Patrizia Salvaterra) ISBN 9788831306164 (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2021/10/SOGUD-BPCO-cittadini.pdf)
- UPO SoGuD Prevention Summaries of Guidelines upon Diseases Prevention - Healthy Aging Buone pratiche nella prevenzione del Decadimento Congnitivo (MCI) e della Demenza (Authros: Diego Concina, Gianmarco Cotrupi, Fabrizio Faggiano, Annalisa Opizzi, Massimiliano Panella, Carlo Smirne, Carmela Rinaldi, Patrizia Zeppegno) ISBN 9788831306225 (available from:

https://www.agingproject.uniupo.it/wp-content/uploads/2022/02/UPO-SoGuD-Demenza-Clinici.pdf)

- Healthy Aging Buone pratiche nella prevenzione del Decadimento Congnitivo (MCI) e della Demenza (authors: Carmela Rinaldi, Diego Concina, Massimiliano Panella, Patrizia Salvaterra) SBN 9788831306294 (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2022/02/Sogud-demenze-citt-imp.pdf)
- UPO SoGuD Prevention Summaries of Guidelines upon Diseases Prevention - Healthy Aging Buone pratiche raccomandate a tutti nella prevenzione del Diabete Mellito di Tipo 2 (DMT2) (Authros: Diego Concina, Paolo Marzullo, Massimiliano Panella, Carmela Rinaldi, Bruno Tamburini) ISBN 9788831306225 (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2023/01/Diabete-professionisti-def.pdf)
- UPO SoGuD Prevention Summaries of Guidelines upon Diseases Prevention - Healthy Aging Buone pratiche raccomandate a tutti nella prevenzione del Diabete Mellito di Tipo 2 (DMT2) (Authros: Carmela Rinaldi, Diego Concina, Massimiliano Panella, Patrizia Salvaterra) ISBN 9788831306287 (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2023/01/SOGUD-LGDiabete-cittadini-FINAL.pdf)
- Raccomandazioni per policy maker – Dalle sintesi sistematiche di UPO SoGuD (authors: Diego Concina, Andrea Conti, Carmela Rinaldi, Massimiliano Panella) ISBN 9788831306218 (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2022/04/Policymaker-web.pdf)
- UPO SoGuD Prevention - buone pratiche nella prevenzione dell'osteoporosi (authors: Carmela Rinaldi, Diego Concina, Massimiliano Panella e Gruppo di lavoro UPO SoGuD) (available from: https://www.agingproject.uniupo.it/wp-content/uploads/2022/09/Abstract_Book_Aging_Project_UPO-1.pdf).

Project 3: Flu-like syndrome-COVID-19 onset

- Association between plasma redox state/mitochondria function and a flu-like syndrome/COVID-19 in the elderly admitted to a long-term care unit (authors Elena

Grossini, Diego Concina, Sophia Russotto, Divya Garhwal, Patrizia Zeppegno, Carla Gramaglia, Seval Kul and Massimiliano Panella) Frontiers in Physiology Redox Physiology; 2021 Dec 15;12:707587; DOI: 10.3389/fphys.2021.707587

Project 4: Vaccination Strategies against Seasonal Influenza in Long Term Care Setting: a Mathematical Modelling Study

- Vaccination Strategies against Seasonal Influenza in Long Term Care Setting: Lessons from a Mathematical Modelling Study (Ratti, M.; Concina, D.; Rinaldi, M.; Salinelli, E.; Di Brisco, A.M.; Ferrante, D.; Volpe, A.; Panella, M.) Vaccines 2023, 11, 32. https://doi.org/10.3390/vaccines11010032

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