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MULTICOMPONENT EXERCISE AND BRANCHED CHAIN AMINO ACIDS SUPPLEMENTATION IN PRE-FRAIL AND FRAIL OLDER PERSONS

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EXERCÍCIO MULTICOMPONENTE E SUPLEMENTAÇÃO DE AMINOÁCIDOS DE CADEIA RAMIFICADA EM IDOSOS PRÉ-FRÁGEIS E FRÁGEIS

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“ Não se esqueça de olhar em 6 direções todos os dias...

Para frente*: para saber onde você está indo e planejar com antecedência,*

Para trás: *para lembrar de onde você veio e evitar os erros do passado,*

Para baixo*: para se certificar de que você não está pisando em outras pessoas e causando sua ruína ao longo do caminho,*

Para os lados: *para ver quem está lá para apoioá-lo, e ver quem precisa do seu apoio,*

Para cima*: para lembrar que Deus está no controle e que cuida de tudo e de todos,*

Para dentro: *para você lembrar de o quanto precisa melhorar”*

Autor desconhecido

“Life is not easy for any of us. But what of that? We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained”

“Cada pessoa deve trabalhar para o seu aperfeiçoamento e, ao mesmo tempo, participar da responsabilidade coletiva por toda a humanidade.”

Marie Curie

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SUMMARY

Frailty is a multifactorial syndrome that triggers a series of restrictive factors associated with ageing, causing many cumulative disorders in different biological systems. Proposed by Linda Fried in 2001, some pillars of this syndrome correspond to weight loss, physical inactivity, reduced muscle strength, slow walking and fatigue, resulting in the frailty phenotype that encompasses the 5 dimensions mentioned above and allows dividing this syndrome into different categories (frail, pre-frail and robust). Physical exercise has been shown as a non-pharmacological treatment in the beneficial modulation of several factors associated with frailty.

To provide an additional contribution to the quality of life of older persons, the objectives of this project were to study the effect of exercise alone, exercise plus BCAAs supplementation and supplementation alone in their ability to prevent/attenuate frailty by looking at different predisposing factors for sarcopenia and cognitive impairment. We designed this project in a systematic review of the literature and three peered reviewed publications. This project was approved by the Ethical Committee of Faculty of Sport Sciences and Physical Education, University of Coimbra (CE/FCDEFUC/00282018), and received the signed consent forms from the institution's directors, the participants and their legal representatives before testing and intervention.

Participants (n = 35; age = 83 ± 3 years-old) were selected through a non-probabilistic trial (plus controlled sampling) based on the geographical area of Coimbra, Portugal, living in public and private residential care homes (RCH) and were divided into four groups: Multicomponent Exercise (ME, n = 7); Multicomponent Exercise plus branch-chained amino acids (BCAA) supplementation (ME+BCAA, n = 8); BCAA supplementation only (BCAA, n = 7); and Control Group (CG, n = 13). Evaluations were made at four moments: T1, baseline; T2, after 16 weeks of intervention; T3, after 8 weeks of washout; and T4, after 16 weeks of retraining, totalizing 40 weeks of intervention. The evaluations included: Frailty Assessment (Fried et al, 2001), cognitive impairment (Mini Mental State Examination), depression (Geriatric Depression Scale), mood states (Profile of Mood State questionnaire), comorbidities (Charlson comorbidity

index), body mass index, nutritional assessment (Mini Nutritional Assessment questionnaire and Diet analysis), physical function (short physical performance battery for older persons of Guralnik et al., 1994), blood biomarkers (inflammatory cytokines interleukin-10, Tumour Necrosis Factor alpha, myeloperoxidase activity, albumin) and salivary testosterone.

Our systematic review of the literature highlighted that physical multicomponent exercise could contribute in a very positive way to decrease/revert frailty and alter the biochemical profile in elderly populations. Frailty and pre-frailty were closely linked to pro-inflammatory parameters, mainly CRP, IL-6, TNF- α, and to metabolic and hormone markers. Focusing on the impact on functional capacity, our first and second intervention studies showed that short- and long-term exercise programs, independently of beeing multicomponent or elastic band strength based, were effective in improving functional capacity in institutionalised older persons and prevented an increase in frailty as compared to a control group without exercise. BCAAs supplement alone had no impact on physical test performance but in a short time period (16 week) contributed to diminish frailty and combined with an exercise program may have the potential to reduce the effect of a detraining period on functional capacity. The combination of exercise and BCAAs did not present advantages on the exercise only program to improve exercise performance. In the second study, salivary testosterone levels correlated with handgrip strength, which may be a useful indicator of susceptibility to frailty. No effect was found for mood states, cognition, and depression, but there is a tendency for depression to decrease in exercising groups and to increase in non-exercising groups. The last intervention study showed that exercise training, regardless of BCAA supplementation, triggered slight alterations in the inflammatory status, cognitive, and physical profiles of older persons, which, taken together, could provide sufficient independence for their daily activities. Unlike other studies, our results showed that supplementation with BCAA did not induce substantial changes in health-related parameters at older ages.

However, this was the first study on the long-term effects of physical exercise and BCAA supplementation on biomarkers, especially albumin, in frail and pre-frail elderly of the institutionalized Portuguese population. The results on frailty scores showed a reduction in physical frailty in the intervention groups, but

an increase in the control group, concluding that a hybrid intervention (involving physical exercise and complementary nutritional support) plays a relevant role in the treatment of the frailty syndrome. Further research is needed to explore the best practices, the feasibility of implementation, the physical exercise programs, as well as strategies to augment compliance and long-term behaviour maintenance. It is possible that the heterogeneity and limited sample size restricted the statistical relevance of our results.

Keywords: *Frail Older Persons, Multicomponent Exercise, Branched Chain Amino Acid Supplementation, Inflammation*

RESUMO

A fragilidade é uma síndrome multifatorial que desencadeia uma série de fatores restritivos associados ao envelhecimento, causando muitos transtornos cumulativos em diferentes sistemas biológicos. Proposta por Linda Fried em 2001, alguns pilares dessa síndrome correspondem à perda de peso, inatividade física, redução da força muscular, caminhada lenta e fadiga, resultando no fenótipo de fragilidade que engloba as 5 dimensões mencionadas acima e permite dividir essa síndrome em diferentes categorias (frágil, pré-frágil e robusta). O exercício físico tem sido mostrado como um tratamento não farmacológico na modulação benéfica de vários fatores associados à fragilidade.

De modo a contribuir adicionalmente para a qualidade de vida das pessoas idosas, os objetivos deste projecto foram estudar o efeito do exercício isolado, exercício mais suplementação com aminoácidos de cadeia ramificada (BCAAs) e suplementação apenas na sua capacidade de prevenir/atenuar a fragilidade, olhando para diferentes fatores de predisposição para sarcopenia e comprometimento cognitivo.

Esta tese doutoral foi escrita em modelo de artigo e como um primeiro estudo foi: revisão sistemática da literatura e três publicações revistas por pares. Este projecto foi aprovado pelo Comitê de Ética da Faculdade de Ciências do Esporte e Educação Física da Universidade de Coimbra (CE/FCDEFUC/00282018), e recebeu os formulários de consentimento assinados pelos diretores das instituições, pelos participantes e seus representantes legais antes dos testes e intervenção.

Os participantes (n = 35; idade = 83 ± 3 anos) nesta intervenção foram selecionados por meio de ensaio não probabilístico (mais amostragem controlada) com base na área geográfica de Coimbra, Portugal, residentes em casas de cuidados residenciais públicos e privados e divididos em quatro grupos: Exercício Multicomponente (ME, n = 7); Exercício multicomponente mais suplementação com BCAA (ME+BCAA, n = 8); suplementação com BCAA (BCAA, n = 7); e Grupo de Controle (CG, n = 13). As avaliações foram feitas em quatro momentos: T1, linha de base; T2, após 16 semanas de intervenção; T3, depois de 8 semanas de destreino; e T4, após 16 semanas de retreinamento, totalizando 40 semanas de intervenção. As avaliações incluíram: Avaliação da

fragilidade (Fried et al, 2001), prejuízo cognitivo (Mini Exame do Estado Mental), depressão (Escala de Depressão Geriátrica), estados de humor (Questionário Perfil do Estado de Humor), índice de comorbidades Charlson, índice de massa corporal, avaliação nutricional (Mini Avaliação Nutricional e Análise dietética), função física (bateria de desempenho físico para idosos de Guralnik et al., 1994), biomarcadores sanguíneos (citocinas inflamatórias: interleucina-10, Fator de Necrose tumoral alfa, mieloperoxidase, albumina) e testosterona salivar.

Nossa revisão sistemática destacou que o exercício físico multicomponente poderia contribuir de forma muito positiva para diminuir/reverter a fragilidade e alterar o perfil bioquímico em populações idosas. A fragilidade e a pré-fragilidade estavam intimamente ligadas aos parâmetros pró-inflamatórios, principalmente CRP, IL-6, TNF- α, e a marcadores metabólicos e hormonais. Com foco no impacto da capacidade funcional, nosso primeiro e segundo estudo de intervenção mostraram que o programa de exercícios de curto e longo prazo, independentemente de ser multicomponente ou de força com banda elástica, foi eficaz na melhoria da capacidade funcional em idosos institucionalizados, impedindo um aumento da fragilidade em relação a um grupo controle sem exercício. A suplementação com BCAAs não teve impacto no desempenho físico, mas num curto período (16 semanas) contribuiu para diminuir a fragilidade, e combinado com o programa de exercício pode ter o potencial de reduzir o efeito de um período de destreino na capacidade funcional. A combinação de exercício com BCAAs não apresentou vantagem em relação ao programa de apenas exercício, na melhoria do desempenho físico.

No terceiro estudo, os níveis de testosterona salivar correlacionaram-se com a força da preensão, o que pode ser um indicador útil de suscetibilidade à fragilidade. Não foi encontrado efeito para estados de humor, cognição e depressão, mas há uma tendência para a depressão diminuir nos grupos de exercício e aumentar nos grupos de não-exercício.

O último estudo de intervenção mostrou que o exercício físico, independentemente da suplementação com BCAA, desencadeou pequenas alterações no estado inflamatório, cognitivo e físico das pessoas idosas, o que, em conjunto, poderia proporcionar independência suficiente para suas atividades diárias. Ao contrário de outros estudos, nossos resultados mostraram

que a suplementação com BCAA não induziu mudanças substanciais nos parâmetros relacionados com a saúde em idades mais avançadas.

Este foi o primeiro estudo sobre os efeitos a longo prazo do exercício físico e da suplementação com BCAA em biomarcadores, especialmente na albumina em idosos frágeis e pré-frágeis da população portuguesa institucionalizada. Os resultados dos escores de fragilidade mostraram redução da fragilidade física nos grupos de intervenção, mas um aumento dos escores no grupo controle, concluindo que uma intervenção híbrida (envolvendo exercício físico e apoio nutricional complementar) desempenha papel relevante no tratamento da síndrome da fragilidade. Mais pesquisas são necessárias para explorar as melhores práticas, a viabilidade da implementação, os programas de exercício físico, bem como as estratégias para melhorar o comportamento a longo prazo. É possível que a heterogeneidade e o tamanho limitado da amostra restringissem a relevância estatística de nossos resultados.

Palavras-chave: *Idosos frágeis, Exercício multicomponente, Suplementação de Aminoácidos de cadeia ramificada, Inflamação*

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LIST OF ABBREVIATIONS

4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1
5-HT	5-hydroxytryptamine
5-HTTP	5-Hydroxytryptophan
ACTN3	α-actinina-3
AD	Alzheimer Disease
ATP	Adenosine Triphosphate
ACSM	American College of Sports Medicine
BCAA	Branched Chain Amino Acids
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body Mass Index
BS	BCAA Supplementation
BPM	Beat per Minute
CAF	C-terminal Agrin Fragment
CI	Cognitive Impairment
CF	Cognitive Frailty
CNS	Central Nervous System
CCI	Charlson Comorbidity Index
CES-D	Center of Epidemiologic Studies on Depression
CHS	Care Health Support
CONSORT	Consolidated Standards of Reporting Trials
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
DRI	Dietary Recommended Intake
eIF4G	Eukaryotic translation initiation factor 4 G
ES	Effect Size
FS	Frailty Syndrome
GDS	Geriatric Depression Scale
IU	International unit
IFN-γ	Interferon Gamma
IgA	Immunoglobulin-A
IgG	Immunoglobulin-G

IGF-1	Insulin-like growth factor 1
IL-2	Interleukin 2
IL-10	Interleukin 10
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
Mesh	Medical Subjects Headings
MIP	Multifactorial Intervention Program
MMSE	Mini Mental State Examination
MNA	Mini Nutritional Assessment
MSE	Muscle Strength Exercise
mRNA	messenger Ribonucleic Acid
mTOR	mammalian Target of Rapamycin
NH	Nursing Homes
p70S6K	Proteins Ribosomal Protein Kinase S6 de 70 kDA
PA	Physical Activity
PF	Physical Frailty
PICOS	Patients, Intervention, Comparison, Outcomes
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSE	Perceived Stress Effort
RDA	Recommendary Daily Allowance
rPE	Rating of Perceived Exertion
SNP	Single Nucleotide Polymorphism
TNF-α	Tumour Necrosis Factor alpha
WK	Week
WHO	World Health Organization

CONTENTS

CHAPTER I

GENERAL INTRODUCTION

1.1- Background

Older frail persons have a greater intrinsic vulnerability to biological and environmental stressors, increasing the risk for physical decline and adverse health related outcomes, such as disability, comorbidity and lower cognitive function when compared to robust older persons (Clegg et al., 2013). Cognitive frailty (CF) refers to the heterogeneous syndrome that is characterized by concomitant physical frailty (PF) and potentially reversible cognitive impairment (CI) so excluding established Alzheimer disease and other dementias. The phenotype of PF is characterized by muscle loss, negative energy balance, sedentarism, low gait speed and fatigue (Fried et al., 2001). CI and PF often present in the same population (Kelaiditi et al., 2013).

The interconnection between physical fitness and cognition is of high scientific interest because sarcopenia is part of CF (Chang, Hsu, Wu, Huang, & Han, 2016). Several predisposing factors underlying sarcopenia (i.e. oxidative stress, inflammation) are also associated with CI which could explain the common etiology (Jensen, et al., 2015). Studies from our group have shown that strength and multicomponent exercise programs were able to decrease IL-6 and TNF-α plasma levels and the inflammatory indexes TNF-α/IL-10, IL-1β/IL-1ra and IL-6/IL-10, improve cognition and functional fitness (Chupel et al., 2017; Furtado et al., 2020). Studies examining the effects of exercise and protein or BCAAs consumption in frail older persons (Ikeda et al., 2016; Bjorkman et al., 2013) showed that the combination of exercise and daily BCAAs or protein supplementation increased muscle strength and muscle mass. BCAAs participate directly and indirectly in a variety of essential biochemical tasks in the Central Nervous System (CNS), for example protein synthesis, energy production, the synthesis of 5-hydroxytryptamine (5-HT), dopamine, and noradrenaline (Fernstrom, 2013). The ingestion of BCAAs causes a a rapid elevation of their plasma concentrations and increases their uptake into the brain (Anish, 2005).

In the pro-inflammatory state of muscle cells, observed in extreme conditions, BCAAs have been described as a strong nutritional stimulus able to increase protein translation initiation and decrease proteolysis (Neishabouri et al., 2015). In frail older persons there are only a few studies using exercise and BCAAs, and even fewer studies examining the cognitive and immune impact of

exercise in this population. To our knowledge, no one study has investigated if the well-known effects of exercise on physical frailty in seniors could be enhanced by supplementation with BCAAs and prevent/reduce cognitive frailty, or if they could be mediated by biochemicals markers involved in inflammation, oxidative stress and neurogenesis. The impact of the detraining/physical inactivity on the body, namely in the older persons functional capacity could be counteracted by exercise and supplementation and constitute a helpful treatment to avoid disabilities. We hypothesize that this could be mediated by several biochemical markers involved in inflammation, oxidative stress and neurogenesis. The combined ingestion of BCAA with physical exercise could result in muscle strengthening which is critical for the prevention of sarcopenia and PF in older persons living in residential care homes.

1.2- General and Specific Objectives

To provide an additional contribution to the quality of life of older persons, the objectives of this project were to study the effect of exercise alone, exercise plus BCAAs supplementation and supplementation alone in their ability to prevent/attenuate frailty by looking at different predisposing factors for sarcopenia and cognitive impairment. To check if the combination of physical exercise with BCAA supplementation contributes to the prevention of the evolution of physical and cognitive impairment, to the modulation of immune biomarkers, skeletal muscle strength and psychological factors in older persons living in residential care homes in the city of Coimbra.

Towards these objectives, four studies were performed:

1- The first study aimed to identify the gaps in this area of research by performing systematic review of the literature related to frailty, exercise programs and their effects on biochemical profiles.

2- The second study aimed to explore the isolated and combined effects of a 16-weeks BCAAs supplementation and muscle strength exercise program on the functional-fitness of pre-frail an frail octogenarians persons living in residential care homes.

3- The third study aimed to explore the isolated and combined effects of a 40 weeks intervention with BCAAs supplementation and exercises programs on

physical and cognitive frailty, mood states and mental health in pre-frail an frail older persons living in residential care homes.

4- The fourth study aimed to explore the isolated and combined effects of a 40-week intervention with BCAAs supplementation and exercises programs on physical and cognitive frailty and biochemical profile (inflammation, oxidative stress, and neurogenesis) of pre-frail a frail older persons living in residential care homes.

1.3- Study Relevance

This study will try to compensate the lack of information on the effects of BCAAs supplementation with exercises programs, on PF, CI, and contribute to the prevention of frailty and to the quality of life of pre-frail and frail older persons living in residential care homes, by creating clear guidelines for adapted physical activity programs, with the additional help of the supplementation. Meanwhile, a small quantity of studies have investigated the neuroendocrine, immunological, and psychological (mood and cognitive status) profiles and their possible associations with the PF variables (Yao, Li, & Leng, 2011) in older populations, since the integrated action of the aforementioned biomarkers modulated by different exercise programs may reveal aspects not yet studied in samples of pre-frail and frail older persons. This research is also justified by the need to analyse the chronic responses (long-term) to multicomponent exercise (muscular strength, endurance, flexibility and balance), combined with or without BCAA supplementation.

1.4- Organization of PhD Thesis Document

Based on the Scandinavian model, this doctoral thesis is composed by scientific articles prepared as part of the research in the doctoral study. These scientific articles are complemented by a common introduction, methodology, discussion and conclusions.

The first chapter is a general Introduction that includes objectives, studies aims and state of the art.

The second chapter, Material and Methods, describes the study design and methodologies used in different phases of both cross-sectional and intervention studies. It also includes the characterization of the study participants.

In chapter III (Study 1), a systematic review approach was used to verify which biochemical markers were being investigated in frail older persons taking into account the effect of multicomponent exercise.

In chapter IV (Study 2), an exploratory study was conducted to understand the isolated and combined effects of a 16-weeks muscle strength exercise program (MSE) and branched chain amino acids (BCAA) supplementation on functional physical fitness in octagenarians living in residential home care.

In chapter V (Study 3), an intervention study looks at the combination of exercise and supplementation with BCAA as a tool for the treatment of PF, as BCAA was able to provide an additional contribution to skeletal muscle strength and improve mood states of pre-frail and frail older persons.

Chapter VI (Study 4), describes a longitudinal intervention aimed at investigating the independent and combined effects of a multicomponent exercise program (ME) plus BCAAs suplementation on the modulation of several biomarkers, skeletal muscle strength, cognitive impairment and physical frailty.

Chapter VII includes a general discussion and conclusion, and chapter VIII includes the references from chapters I,II and VII.

1.5- State of the Art

Ageing is a natural, progressive and irreversible process characterized by morphological, psychological, functional, biochemical and nutritional changes, (World Health Organization Regional Office for Europe, 2005), with WHO describing the quality of life associated with ageing as *"A broad and subjective concept that includes in a complex way physical health, psychological state, degree of independence, social relations, personal beliefs and convictions and their relation with important aspects of the environment".* WHO also describes active ageing as the improvement of the opportunities for health, participation and safety to promote the well-being of the population along the ageing process.

It is a multidisciplinary theme aimed at a common goal, increasing life expectancy and quality of life, the main basis relying on three points: promotion of mental health, perfect integration in society and reduction of risk of illness or disability. Ageing alone leads to a higher incidence of chronic deteriorating diseases that have negative influences on the quality of life of the older persons,

resulting in falls, fractures, hospitalization, high rates of comorbidities and general incapacity (Bandeen-Roche et al., 2006; Fried et al., 2001).

1.5.1- Physical Frailty and Cognitive Impairment

The first study that coined the term “frailty” appeared in the 80’s (Woodhouse et al., 1988), defining older persons considered frail, as aged over 65 years old and depending on others to perform their daily life activities, living in dwelling-communities (Gillick, 1989). More recently, a study defined frail older persons as debilitated persons that cannot take care of themselves (Lally & Crome, 2007). Meanwhile, the (FS) frailty syndrome defined by Fried and colleagues (2001), is a construct of 5 factors responsible for the presence of the syndrome, the frailty phenotype identifying a group at risk for health. This phenotype facilitated diagnosis and contained the following criteria: non-intentional weight loss of over 4,5 kg or 5% body mass in the last year, reduction of hand grip force, decrease in walking speed, exhaustion (evaluated by self-reported fatigue) and low levels of physical activity (Fried et al., 2001).

In the context of clinical diagnosis, individuals with three or more of these criteria are considered frail, the ones with one or two criteria are classified as pre-frail and individuals that do not present any of the above alterations are considered robust or non-frail.

The frailty syndrome represents a physiological precursor and etiological factor of a state of incapacity. Considering the exhaustive usage of the frailty concept, there is still not a consensus about the exact definition that will make it measurable, facilitating quantitative studies on the characteristics of frailty in older persons, so that preventive actions could be implemented (Marzetti et al., 2019).

Rockwood and colleagues (2011) include the cognitive deficit in the frailty syndrome, saying that cognition is one of the systems that contributes to the accumulation of this deficit. Frailty and muscle mass reduction, seem to be associated with poor physical performance and decline in cognitive performance (Rivière et al., 2002).

The slow gait speed test has a strong association with cognitive decline, in counterpart, the faster speed gait was associated with enhanced cognitive performance and tasks that required attention. In addition, weak gait velocity and

grip strength in frail older persons were associated with lower Mini Mental State Examination (MMSE) test evaluation scores (Guedes et al., 2014; Fried et al, 2001). Cognitive Frailty (CF) was used as a clinical description to indicate a specific state of cognitive susceptibility in mild cognitive impairment (MCI) (Panza et al., 2006) and corresponds to the value between 19-24 points in the MMSE test.

Cognitive-debilitated older persons syndrome (CDS) is one of the most problematic (Clegg et al., 2011), and is characterized by a multifactorial clinical syndrome, characterized by a decline in biological reserves, a low tolerance to stressors resulting in the reduction of many physiological systems (Fried et al., 2001; Viana et al., 2013). According to Kelaiditi and colleagues (Kelaiditi et al., 2013), cognitive frailty is a clinical manifestation characterized by simultaneous PF and cognitive decline, although excluding the diagnosis of Alzheimer's disease (AD) and other dementias. Reduced grip strength was associated with cogntitive decline (Alfaro-Acha, 2006). Difficulties in locomotion and posture were also related to cognitive decline, and the relative risk of cognitive decline with ageing was attenuated with the pratice of physical exercise (Jedrziewski, 2007). Resistance exercise was also able to improve inflammatory balance and physical performance, concurrently with an increase in the cognitive profile of women with mild cognitive impairment (Chupel et al., 2017).

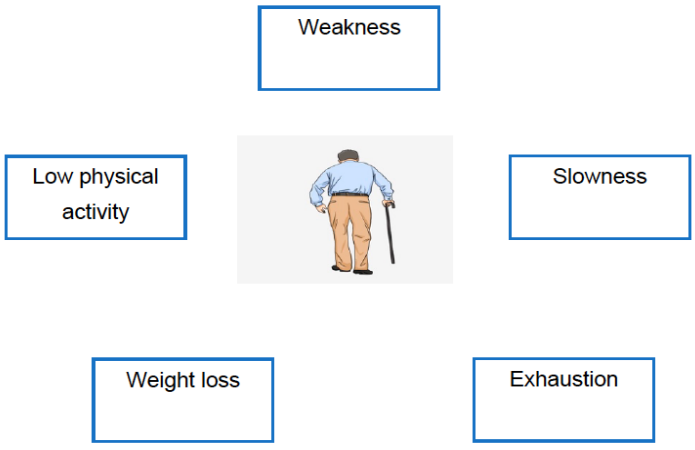

Figure 1.1 Frail phenotype (Fried et al., 2001)

1.5.2- Impact of Exercise on Frail Older Persons

The results of a meta-analysis showed that training interventions involving multidirectional movements, weight transfers, and elements of functional strength training, improved functional capacity, and also decreased falls by 52% in older persons (Sherrington et al., 2017). Strength exercise has been proposed as one of the most effective methodologies to improve performance in common tasks of the daily life in older persons, focusing on the optimization of neuromuscular function to obtain better benefits (Cadore et al., 2014b). A systematic review concluded that structured physical training has positive impacts on frailty and should be used for management of the syndrome (Theou et al., 2011).

Exercise improves physical function and quality of life, reduces premature death from cardiovascular disease, cancer, and chronic lower respiratory tract diseases, and is a possible key to longevity (Kujala, 2018). Multicomponent programs involve aerobic and strength exercises combined with other physical valences such as balance and flexibility (Baker et al, 2007), and are considered as the type of training more beneficial to optimize functional capacity in frail older persons (Villareal et al., 2011) and to prevent functional and physical incapacity (Cadore et al., 2014 a,b). Multicomponent exercise programs have also resulted in major improvements in functionality capacity, which is strategic for maintaining the skills and independence to perform the basic tasks of daily living (Casas-Herrero et al., 2019).

Scientific literature on the effects of multicomponent exercises in older persons is still very scarce, especially when looking at the cognitive, emotional and psychological states (Justine & Hamid, 2010).

The outcome of physical exercise as a systematic and planned tool in the risk reduction of cognitive decline is still not clear, however in this context, the most beneficial type of exercise also seems to be the multicomponent exercise (Furtado et al., 2020a).

1.5.3- Exercise and Biochemical Markers

The word *“inflamm-aging”* is used to describe a state of chronic low grade inflammation related to ageing that is implicated on several chronic metabolic and degenerative related disorders (Franceschi et al., 2000). Inflammation is associated with anorexia and protein degradation creating nutritional disfunction

and muscle weakness. The frailty syndrome (FS) has a direct relationship with sarcopenia, neuroendocrine and immune disfunction (Fried et al., 2004), and has also been related to low cognitive performance (Panza et al., 2011).

Research on the impact of sarcopenia in frailty, showing that multiple physiological, metabolic and cognitive systems beginning to fall (Weening-Dijksterhuis et al., 2011; Morley et al., 2013) is available, with the complex mechanisms of ageing being influenced by genetic, environmental and epigenetic factors.

Several genes that can potentially be associated with frailty index, show molecular variations that can include polymorphisms, for the inflammatory pathway (IL-18 SNP rs360722, IL-12 SNPs rs9852519 e rs4679868, SELP SNP rs6131, TNF SNP rs1800629A), for muscle loss (insulin signalling pathway IGF2 820G, A rs680), and for muscle biogenesis (ACTN3 R577X ACVR18 SNP rs2854464 MSTB SNP rs1805086 AMPD1).

The above-mentioned genes are involved in the inflammatory pathway and provide further support for the involvement of the immunological processes in frailty (Mekli et al., 2015), together with oxidative stress, mitochondrial losses, DNA damage and cellular ageing. The increase in IL-6 and C-reactive protein (CRP) levels, have also been associated with an increase in mortality among the older persons (Puzianowska-Kuźnicka et al., 2016). The reduction of IL-2, IgA, IgG and increase of IL-6 and IL-1β levels are consequences of the immune dysfunction associated with the frailty syndrome (Senchina & Kohut, 2007).

In addition, elevated levels of cortisol in frail older persons have been associated with augmented catabolism, decreased muscle mass, anorexia, weight loss and reduced energy (Langmann et al., 2017; Attaix et al., 2005). According to our systematic review (see study one, chapter 3) the main biomarkers related to the risks of frailty studied (Al Saedi et al., 2019), included serum metabolic markers (Haemoglobin, Albumin, product oxidation, antioxidants, HbA), hormones (DHEA, testosterone, PTH, IGF-1), inflammatory markers, (CRP, IL-6, TNF-α) and Vitamin D. Frailty and pre-frailty were closely linked to pro-inflammatory parameters, mainly CRP, IL-6, TNF-α and to metabolic and hormone markers. A lack of uniformity between studies was highlighted.

1.5.3.1- Functional Relevance of the Biochemical Markers under Study

Several studies on the physiopathology of frailty and sarcopenia demonstrated an imbalance in cytokine levels as major drivers of ageing and associated states (Franceschi et al., 2018, Picca et al., 2020). Meanwhile, the inclusion of pro-inflammatory markers in a clinical diagnostic of frailty, could be an instrument to identify this specific condition.

A main inflammatory profile consisting of greater concentrations of C-reactive protein and lower concentrations of myeloperoxidase (MPO) and Interleukin (IL- 8) with gender-specific signatures has been recognised in the background of PF (Marzetti et al., 2019). Also, based on the results found by Giovannini and colleagues (2010) MPO could be an inflammatory marker with a directly associated risk of death in a population of frail octogenarians and nonagenarians.

Myeloperoxidase is an enzyme largely released by activated neutrophils, characterised by potent pro-oxidative/pro-inflammatory properties, which are generally sensible for significant patient mortality and morbidity (Loria et al., 2008; Davies & Hawkins, 2020).

Serum albumin concentration allows for the assessment of the health status of an individual, and low albumin concentrations are associated with increased mortality, after correction for age, Body Mass Index (BMI), gender and several chronic comorbidities (Akirov, Masri-Iraqi, Atamna, & Shimon, 2017).

Lower albumin concentrations have been correlated with lower gait speed and lower handgrip strength, both linked to sarcopenia and physical frailty (van Atteveld et al., 2019). Malnutrition therefore looks to be an important factor in the frailty syndrome.

The sedentary lifestyle is one of the most important contributors to age-related illness, whereas regular physical exercises chronically slow down the ageing immune/inflammatory dysfunctions (Duggal et al., 2019). In this sense, reduction of systemic levels of interleukin-10 (IL-10), a classical anti-inflammatory cytokine, with elevation on Tumor Necrosis Factor-alpha (TNF-α) levels are associated with ageing. Physical exercise stimulates the release of cytokines, such as IL-6 and IL-10, in response to contracting skeletal muscles, which are responsible not only for tissue restoration and energy metabolism, but also for the adjustment of the systemic inflammatory status (Pedersen & Febbraio, 2012).

Because of their possible associations with frailty, in this study we decided to evaluate the markers TNF-α, IL-10, MPO and Albumin.

1.5.4- Exercise and Supplementation in Older Persons

Several studies have shown that the combination of exercise and nutrition improves fitness and strength, cerebral blood flow, body composition, insulin sensitivity, vascular risk profile and decreases blood pressure (Burckhardt, Dawson-Hughes, & Weaver, 2010). Although many nutrients have been tested, such as vitamin D, omega 3 and others, except for amino acids and protein, their effectiveness on frailty seems to be very limited (Landi et al., 2016).

Protein supplementation is a common and a well-defined intervention that can prevent and treat frailty symptoms. In this regard, one recent review suggested that a daily dose of 30 g of protein supplements could inhibit frailty in older persons (Hernández Morante, Martínez, & Morillas-Ruiz, 2019).

According to Liao and colleagues (2018) protein supplementation together with physical exercise seems to be effective in promoting muscle mass and strength increase, improving the functional capacity and frailty index, of frail older persons.

A central factor for the lower muscle mass observed in older persons could be the low intake of proteins in their diet (Deer & Volpi, 2015), also considered an essential element of frailty (Rockwood et al., 2011; Cruz-Jentoft et al., 2010; Kirkwood, 2005).

In frail older persons the recommended ingestion of proteins in the diet is 1,5 g/kg/day, distributed throughout the main meals and can be combined with calcium (1000-1200 mg/day) and vitamin D (over 800 IU/day), to diminish the risk of falls (Wolfe, Miller, & Miller, 2008; Bischoff-Ferrari et al., 2009). Diet can influence the frailty state, since unvoluntary weight loss is one of the criteria of the frailty phenotype, and protein sources in the diet can help prevent and treat the frailty syndrome (Aquilani et al., 2008 and 2014; Beasley Shikany, & Thomson, 2013).

Protein also represents an important macronutrient to bone health, avoiding osteoporosis, stimulating bone anabolism through IGF-1, and a strong component in the construction of the organic matrix of the skeletal muscle system (Bonjour, 2016). The adequate ingestion of nutrients, especially proteins and

amino acids, seem to have positive effects in the protein synthesis. A diet rich in protein provides a pool of optimal amino acids, creating the ideal environment for the maintenance of bone and muscle structure (Hoffman & Falvo, 2004).

Rondanelli and colleagues (2011) reported that supplementation with amino acids in older persons could improve physical capacity, muscular strength, health status and depressive symptoms.

In complementarity with protein intakes, the literature supports the positive effects of BCAA supplementation. In community dwelling older persons, the ingestion of a diet with BCAA improved depression (Gariballa & Forster, 2007), the perception of fatigue and performance in a mental task, possibly due to the synthesis of 5-HTTP, a natural amino acid with a similar action as tryptophan, which is a precursor of the serotonin neurotransmitter, providing a feeling of well-being (Fernstrom, 2013). BCAAs may act by direct and indirect means, to increase serotonin synthesis in the brain. The concentration of tryptophan in patients treated with BCAAs was higher than in patients treated with placebo (Rondanelli et al., 2011) and was associated with mood enhancement that could be more serotonergic than that due to other neurotransmitter pathways, including through norepinephrine.

Branched Chain Amino Acids, particularly Leucine showed significant results in relation to muscle hypertrophy in older persons and in their functional capacity, and a myriad of studies have shown its potential benefits in different populations (Blomstrand et al., 1997; Neishabouri, Hutson, & Davoodi, 2015). The branched chain amino acids, Leucine, Isoleucine and Valine, ingested before or after exercising can stimulate protein synthesis and decrease damage related to muscle injuries, suggesting that it could also increase the release of hormones like growth hormone and testosterone (Blomstrand et al., 2006; Rondanelli et al., 2011).

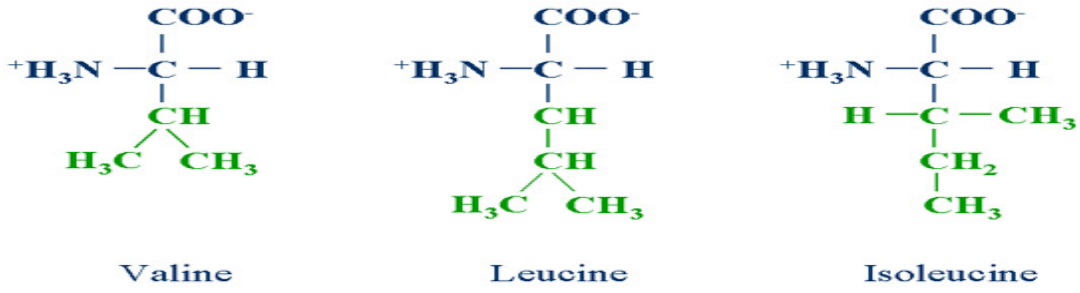

Figure 1.2- Molecular Design BCAA (Valine, Leucine and Isoleucine)

The supplementation with BCAAs, mainly Leucine, activates a cascade of protein phosphorylation in the protein synthesis pathway, promoting the muscle proteins synthesis through (mTOR), that stimulates the three ribosomal proteins: kinase S6 of 70 kDA (p70S6K), 4E-BP1, and 4G (eIF4G) (Yang et al., 2010).

Thus, the role of Leucine together with strength training as important regulators of muscle protein biosynthesis stands out. Through insulin dependent and independent pathways, Leucine and strength training are potent activators of mTOR, a protein that is involved in increasing the rate of mRNA translation of myofibrillar proteins (Millward, Layman, Tomé, & Schaafsma, 2008; Aguirre, Van Loon, & Baar, 2013).

BCAA supplementation as been shown to increase lean mass, strength and physical capacity in older persons, and seems to be quite effective in increasing protein intake, without altering the sensation of satiety (Ferrando et al., 2010).

Therefore, the use of BCAAs in older persons seems to be very promising in order to increase their quality of life, including in those with more depressive tendencies (Fujita & Volpi, 2006).

Leucine-enriched food supplements are used to increase lean mass, anabolism and are often consumed under conditions of poor nutrition, physical inactivity and even frailty (Borack & Volpi, 2016). Enhanced rates of skeletal muscle synthesis, were obtained with older persons supplementing for two weeks with 12g Leucine per day and normal diet (Casperson et al., 2012). Supplementation with 7.9g of Leucine during 16 week increased lean body mass and strength in older persons (Børsheim et al., 2008). Aged muscle may be less sensitive to the stimulatory effect of amino acids, especially Leucine, at low physiologic concentrations of 1.7g, but this impairment can be overcome by the provision of a larger amount of 2.8g of Leucine (Fujita & Volpi, 2006). In a study by Paddon-Jones, the minimum dose of Leucine needed to stimulate muscle growth and strength in older persons was at least 2.8 g (Paddon-Jones, 2009).

In malnourished haemodialyzed older persons, 6 months of 12 g/day BCAAs supplementation increased lean body mass and plasma albumin concentrations (Hiroshige et al., 2001). In patients with cirrhosis of the liver, increases in albumin blood level were obtained with varied doses of BCAAs (6 to 15 g per days) and durations (from 2 weeks to 1 year) (Ruiz-Margáin et al., 2018).

On the contrary, two weeks of 6.98g of BCAAs did not invert the catabolic state (lean body mass, albumin concentration, nitrogen balance) in acute hospitalized older persons (Bonnefoy et al., 2010). Many factors can influence the reaction to amino acid supplementation, and health status is an important one. Sufficient regular protein intake (about 0.9 g/kg/ per day) combined with a normal meal pattern (i.e. providing protein three times daily) allowed for substantial gains in muscle mass and strength with resistance exercise training in older persons (Koopman, 2011).

A study (Ispoglou et al., 2016) evaluated, in sedentary older persons, the effect of a daily amino acids supplementation during 12 weeks, containing 132mg BCAA/kg BW or 90mg BCAA/kg BW, compared to a placebo. The ratio of Leucine: Isoleucine: Valine, was in the proportion of 2:1:1 in both BCAA supplements. Lean body mass significaly increase only with 0.132g of BCAA per kg of BW and functional performance increase in the two BCAA groups. Twice-weekly during 3 month of a combined program with physical exercise (strength, aerobic, balance) and supplementation containing 1560 mg of BCAAs (Leucine: Isoleucine: Valine, in the proportion of 2:1:1), taken 10 minute before training, improved the physical capacity (lower limb strength and dynamic balance) of frail and pre-frail older persons (Ikeda et al, 2016). For Brestensky and colleagues (2015) the best ratio for BCAAs administration is Leucine, Isoleucine and Valine in the proportions of 2:1:1.

Taking into account the BCAA and Leucine studies, aimed at increasing muscle synthesis in older persons, when coupled with an exercise program, a minimum of 200 mg of BCAA/kg/BW/day supplementation for long periods seems to be necessary to achieve this goal. The ratio of Leucine: Isoleucine: Valine, in the BCAA supplement should be 2:1:1 and a daily a minimum of 2.8 g of Leucine should be provided (Jackman et al., 2010). To avoid an excessive satiety effect of the proteins it is recommended to take the supplement in the morning (between 09:00 and 11:30 AM) (Negro et al, 2019).

1.6 Conclusion

Physical lethargy is a factor that contributes to the starting of mass and muscle function deterioration in older persons and is also related to frailty (Lehmann et al., 2018). It also has a strong relationship with the increase in the

occurrence of falls in persons over 50 years (Pereira et al., 2014). The reduction or impossibility of the older person to move may cause a greater prevalence of hospitalization time and immobility, resulting in the reduction of muscle and bone mass and may generate a greater risk of developing osteoporosis and sarcopenia (Langmann et al., 2017).

Sirven & Rapp (2017) studied older persons over 65 years and concluded that frailty represented a progressive condition and had a growing effect in the expenses related to health of about 750 euros for pre frail individuals and 1500€ for frail ones. Another study conducted in Germany concluded that those numbers increased to 5000 euros a year for frail adults (Bock et al., 2016). Precarious health, incapacity and dependence should not be inevitable effects of ageing. The adoption of healthier lifestyles, including physical exercise and sedentarism avoidance, proved to be effective in the management of frailty in older persons, thus improving their physical independence, (Izquierdo et al., 2016).

Till now, there is no treatment to cure frailty, so, efforts should concentrate in preventive actions. However, it is possible to reverse or alleviate the negative effects of frailty (Sadjapong et al., 2020). Some of the interventions described as effective are physical exercise and nutritional supplementation (Hernández Morante, Martínez, & Morillas-Ruiz, 2019). Physical exercise programs have been shown to be a fundamental non-pharmacological tool in the prevention and treatment of frailty, preserving physical and cognitive abilities. However, little is known about the hypothetical restructuring of functional reserves in individuals who need long-term care (Apóstolo et al., 2019).

So, actions in primary education for the prevention of frailty, will provide awareness and motivate older persons to keep their functional integrity with the main aim of preserving autonomy and independence. In this sense treatments to improve neuromuscular, cardiorespiratory function, functional capacity and quality of life of frail older persons might be facilitated by the supplementation with BCAA and multicomponent exercise programs.

CHAPTER II

MATERIAL AND METHODS

This chapter describes the methodology used in the study and includes the initial general caracterization of the sample.

2.1- Preliminary Procedures Actions and Ethics

This PhD project is inserted in the thematic line of CIDAF - Research Centre for Sport and Physical Activity Research Centre in Physical Activity; Ageing: Is It Ever Too Late to Be Active and Healthy/Fit? It is a prospective, naturalistic, controlled clinical trial (treatment vs care) study. We selected subjects living in public and private residential care homes (RCH) or frequenting day centres in the geographical area of the Portuguese Centre region of Coimbra. The participating RCH were: Cáritas Diocesana de Coimbra, Centro Social de São João, Fundação Sophia and Associação Recreativa Cultural Social de Cioga do Monte.

In their premises, all institutions had an adequate physical space to perform the exercise sessions and the necessary support of caregivers to assist in the displacement of the older persons during the exercise sessions. All subjects volunteered to participate in the exercise classes or the supplementation programs. Consent forms were signed by the institution's directors, the participants, and their legal representatives before testing and intervention. This study was approved by the Ethical Committee of the Faculty of Sport Sciences and Physical Education, University of Coimbra (reference number: CE/FCDEFUC/00282018), respecting the Portuguese Resolution (Braga, 2013) (Art.°4th; Law no. 12/2005, 1st series) on ethics in human research and the Helsinki's Declaration. This study was also registered with clinicaltrials.gov register NCT04376463.

2.2- Inclusion and Exclusion Criteria

The participants were selected using a non-probably convenience sampling based on the geographical area of the Portuguese Centre region of Coimbra and living in residential care homes or frequenting their day care. The eligible criteria for the participants in this study were, at the time of first screening: (i) Participants had to be 75 years old or more; (ii) physically frail and pre-frail according to the Fried protocol (Fried et al, 2001); (iii) clinically stable with their drug therapy updated; (iv) being able to perform the Time Up and Go test in ≤50s that indicates mobility independence (Guralnik et al., 1994); (v) not participating in other

structured physical exercise programs (vi) not presenting any type of health condition or use medication that might prevent the functional self-sufficiency test performance (such as severe cardiopathy, hypertension, uncontrolled asthmatic bronchitis or severe musculoskeletal conditions); (vii) not presenting mental disorders, attention impairment or hearing/visual impairment that could prevent the evaluations and activities proposed, according to the institutional medical staff; (viii) not presenting morbid obesity (BMI ≥ 40). If the participant presented a clinical condition or comorbidity, it had to be stable and allow for participation in the exercise sessions, as decided by the medical team and staff nurses. At the end of the recruitment process, 80 older persons entered the enrollment phase.

2.3- Sample Size Estimation and Participant Allocation

Estimation of sample size was performed by comparison of frail outcomes between pre-and post-exercise interventions, based on previous studies, that reported high effect size (ES) for the frailty composed score (De Labra et al., 2015). Assuming a minimum power of 0.80 as realistically expected and moderate ES (0.5) the recommended sample size for this study were 19 participants per group. Further assuming a dropout rate of 25 to 35% (Picorelli et al., 2014) the sample size was increased to 22 per group, to a total of 50 participants, allocated into four groups. Since the desired sample number was not reached, the sample calculation was carried out in the "post hoc" method to stipulate the power value (beta) achieved in the study. Level of significance was set at 0.05, and 35 subjects were computed at final sample in a 4x4 repeated measures ANOVA test. The G*Power software was used for this purpose. The power β of the study was 0.81.

From the 80 participants initially screened, 50 eligible participants were allocated in their respective intervention groups. However, for the specific reasons highlighted in Figure 2.1, only 35 (14 men and 21 women) participants (age = 83 ± 3 years-old) completed the 40 weeks multifactorial intervention (MIP), divided in the following groups: Multicomponent Exercise (ME n = 7), Multicomponent Exercise + Branched Chain Amino Acid (ME+BCAA n = 8), Branched Chain Amino Acid (BCAA n = 7), and the no-regular exercise/no-supplementation Control Group (CG n = 13). All the procedures were performed

according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Begg et al., 1996).

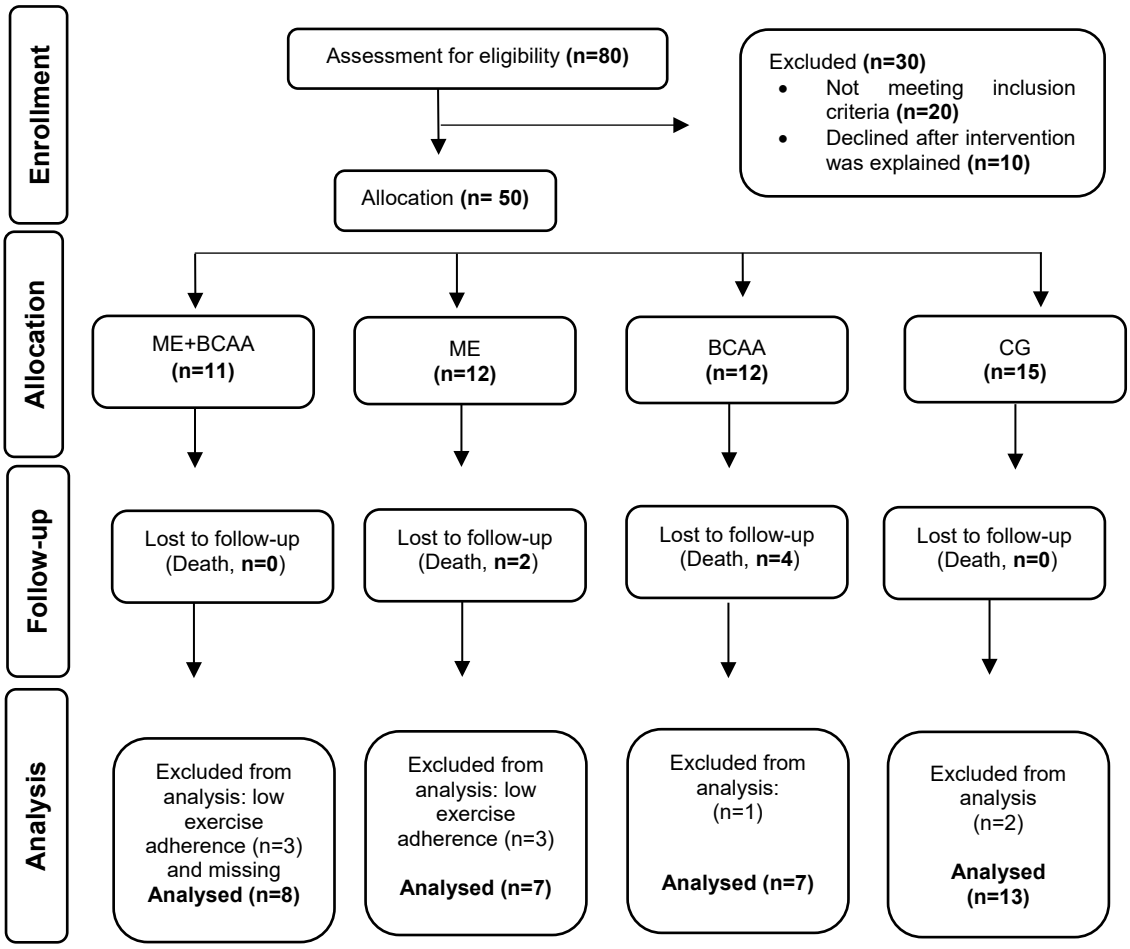

Figure 2.1- CONSORT Flowchart of study participants.

2.4- Experimental Design

This research project is a prospective, naturalistic, controlled clinical trial (treatment vs care as usual), with four groups (ME+BCAA n=8), (Multicomponent Exercise n=7), (BCAA n=7), (Control n=13). In the first phase, a baseline data collection (T1) was done followed by 16 weeks of Multifactorial Intervention Protocol (MIP). The second phase consisted of a second data collection (T2) followed by an 8-week washout phase. Phase 3 consisted of a third data collection, followed by the resumption of the MIP for a period of 16 weeks. The

last data collection took place after the 16 weeks of intervention (T4) (Figure 2.2). Totalizing 40 weeks of intervention.

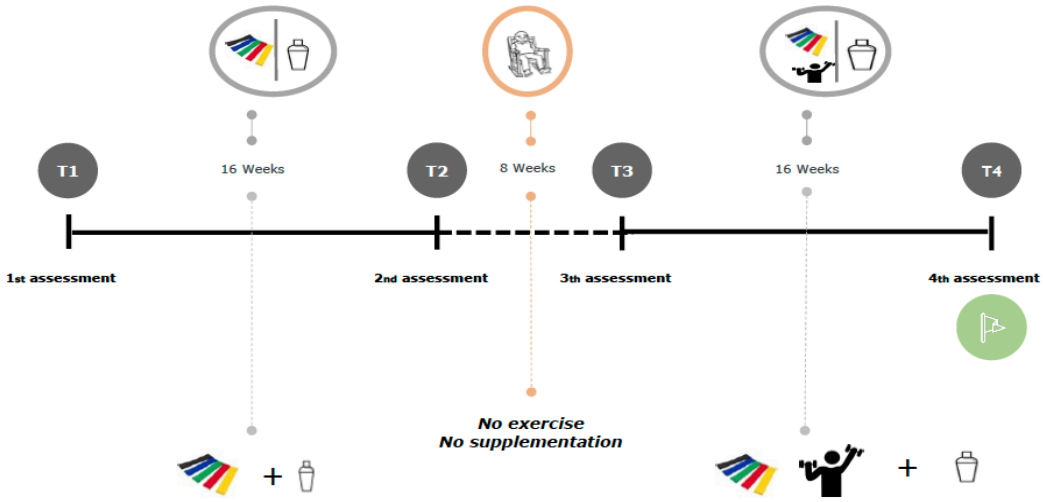

Figure 2.2- Chronological order of multifactorial interventions study design. T1 to T2 (elastic-band exercise, 16 weeks, T2 to T3 (washout, 8 weeks), T3 to T4 (multicomponent exercise, 16 weeks).

2.5- Outcomes Measures

All assessements were performed in the morning, between 10 and 11:45 am. One session was used to apply a short test battery to measure biosocial, global health status, cognitive profile, nutritional, physical fitness and physical frailty status. In the second consecutive day, biological material (saliva and blood samples) were collected and stored at -80ºC until further analysis.

2.5.1- Frailty Assessment

The phenotype of Fried's physical frailty index was used (Fried et al, 2001). Weight loss was assessed by a self-report of unintentional weight loss of 4 kg or more in the last 6 months. Self-reported exhaustion was evaluated by a negative concordance of question number (7- "I felt that everything I did was an effort") and (20-"I could not get going") of the Center of Epidemiologic Studies for Depression (CES-D) scale (Gonçalves, Fagulha, Ferreira, & Reis, 2014). Hand-grip strength was assessed in kilograms by a hand-held (HGT) dynamometer

(Lafayette 78,010, Sagamore, United States). The best result of the two trials was used for scoring purposes. Participants who were unable to perform the HGT and those in the lowest 20% were categorized as positive (Syddall, Cooper, Martin, Briggs, & Sayer, 2003). The cut-off reference values for HGT of ≥29 kg for male and ≥17 kg for female were adopted. Slowness was measured by the "15 feet (4.6 m) walking test". Based on the cut-off values of Fried's study population, times of ≥7 s for males and ≥6 s for females were adopted for positive scores of slowness. The best time of the two trials was used for the final scoring. Low physical activity (PA) levels were assessed by the International PA Questionnaire short version (IPAQ-SV) (Campaniço & Sardinha, 2016).

There are three levels of PA suggested for classification: Inactive, minimally active, and highly active. Participants classified as inactive had a positive score for this PF component. A positive evaluation in one or two criteria classified the participants as pre-frail, in three or more criteria as frail, and as non-frail when none of the five PF indicators was present. The prevalence of PF was calculated to generate a frailty total score, as well as the presence of each of the five criteria of the Fried's model (0 to 5 points). In this study, participants classified as frail (3 or more points) and pre-frail (2 points) were included.

2.5.2- Cognitive Impairment

The Portuguese version of the Mini Mental State Examination (MMSE) was used (Morgado et al., 2009). The MMSE is a 30-point scale instrument that evaluates five domains of cognition: Orientation, immediate recall, attention and calculation, delayed recall, and language. It is generally used to track dementia. It is also used to estimate the severity of cognitive loss at a specific time (Folstein, 1975).This scale classifies individuals by progressive cognitive skills: (0–9 points) severe cognitive impairment; (10–18 points) moderate cognitive impairment; (19–24 points) mild cognitive impairment; and (25–30 points) normal cognitive profile (Pezzotti et al., 2014).

2.5.3- Assessment of Mood State and Depressive Symptoms

The Geriatric Depression Scale (GDS), adapted to the Portuguese population by Apóstolo and Colleagues, (2011), was used to access the state of depression in the participants (Yesavage et al., 1982). The GDS evaluation

consists of 15 yes-no questions, which permits the classification of the psychological condition related to depression and its symptoms. Total GDS scores within 0 to 5 points range indicate normal psychological condition (no symptoms of depression), whereas, 6 to 10 points indicate mild depressive symptoms, and 11 to 15 points indicate symptoms of serious depression. The Profile of Mood State questionnaire (POMS) (McNair, 1971) was used to evaluate the participants mood state using the validated version for the Portuguese population (Viana, Almeida, & Santos, 2001).

The POMS questionnaire consists of 22 Likert-type questions, divided by six dimensions with scales from 0 to 4. The final score consists of the sum of all negative dimensions (Tension-Anxiety, Depression-Melancholia, Hostility-Anger, Fatigue-Inertia, Confusion) subtracting the positive dimensions (Vigour). Lower values denote a better mood state.

2.5.4- Clinic and Health Status

The Charlson comorbidity index (CCI) was used to classify comorbid conditions and was calculated based on the registry of each individual comorbidities combined with age and gender, to account for a final score (Charlson et al., 1994). The anthropometric assessment included body mass (kg) and stature (m).

Body mass was determined using a portable scale (Seca®, model 770, Berlin, Germany) with a precision of 0.1 kg, whereas stature was determined using a portable stadiometer (Seca Body meter®, model 208, Berlin, Germany) with a precision of 0.1 cm. Body Mass Index (BMI) was calculated according to the formula (BMI = body mass/stature^2). For older persons BMI values of <18.49 denote underweight, values between 18.5-24.99 denote normal weight, values between 25.0-29.99 denote overweight, and values $\geq$30 denote obesity (WHO, 2004). The standardized procedures described in previous studies were followed (Lohman et al., 1992).

To classify the CCI values, the median was determined among all present values. Median value was 5 (five), the classification as “Lower Comorbidity Score” and “High Comorbidity Score” represented by results lower or higher than 5, respectively. Since the CCI does not have a standard classification and the values are distributed in a continuous fashion (with higher values being those

more likely to have comorbidities), an attempt to classify through the median division allowed us to observe the frequency of cases with more or less comorbidities.

2.5.5- Nutritional Assessment

Daily diet at the RCH was prescribed by a registered nutritionist and was provided for all the participants without any change or interference of the research team. On the basis of the information provided, the diet was analysed using specific tools (photographic quantification of portions, food table) for the Portuguese population (Torres et al., 2016; Goios et al., 2016; Marques et al., 1996; INSA, 2006 and 2016). Due to the relationship between the frailty status and severe decrease of muscle mass (or sarcopenia) which had already been demonstrated in several studies, the objective of this nutritional assessment was to characterize the protein consumption of the participants. In addition, the Mini Nutritional Assessment (MNA) questionnaire (Vellas et al.,2006; Guigoz et al., 2006) adapted for the Portuguese population by Loureiro (2008), was applied. This consists of 18 questions that present a maximum score of 30 points and classifies the participants as malnourished (≤17 points), at risk of malnutrition (17 < MNA < 23.5 points), and as having a normal nutritional status (MNA > 23.5 points).

2.5.6- Physical Function

The short physical performance battery (SPPB) was applied to evaluate the physical function of participants and is a test based on the performance of lower limb function designed for elderly participants (Guralnik et al., 1994). It consists of three parts: (i) Static Balance Test, (ii) Walking Speed Test; and (iii) Chair Standing Test. The SPPB is scored from 0 to 4, with a score of 0 representing inability to carry out the test, and 4 the best performance. The static balance tests consist of 3 tests: Test 1 is with feet in side-by-side, Test 2 is with the heel of one foot placed next to the first toe of the other foot (semi-tandem), Test 3 is with one foot in front of the other (tandem). The score for the two first tests is 1 point if the subject maintains the position for 10 seconds and 0 if he is unsuccessful. In the third test, if the participant reaches the 10 seconds, the score is 2 points, if he reaches between 3-9 seconds, 1 point is attributed and if under, 0 points are

attributed, with a total score for the balance tests of 4. The Walking Speed Test is a timed 3-m walk at the participants' usual speed. The score attributed to the walking speed test is: 4 points if the time is less than 3.62 seconds; 3 points if the time is between 3.62-4.65 seconds; 2 points if the time is between 4.66-6.52 seconds; and 1 point if the time is longer than 6.52 seconds. For the Five-Times-Sit-to-Stand-Test (5TSS test), participants were asked to stand up and sit down five times as quickly as possible, without stopping in the middle. In addition, the participant should be encouraged to keep his arms crossed over his chest. The instructor must count the time with a stopwatch and must count each position out loud so that the participant remains oriented. The test is stopped when the participant reaches the orthostatic position at the 5th repetition (Guralnik et al., 1994).

This test assesses the functional strength of the lower limbs, transition movements, balance, and risk of falling. The calculation is made with the following score: if the time is more than 60 seconds, 0 points are assigned; if the time is 16.70 seconds or more, 1 point is assigned; if time is between 13.70-16 seconds, 2 points are assigned; if the time is between 11.20-16.69 seconds, 3 points are assigned and if the time is less than 11.19 seconds, 4 points are assigned.

Table 2.1- SPPB Score

SPPB Score Total	
Incapable	0 to 3 points
Low performance	4 to 6 points
Moderate performance	7 to 9 points
Good performance	10 to 12 points

2.5.7- Blood Biomarkers Analysis

Non-fasting blood collection was done in the morning (between 10:00 a.m. and 11:00 a.m.). The collection was done after a period of 4 days from the last intervention session (physical exercise/supplementation with BCAA or both), at the 4 four time-points of the study assessment. Blood samples were collected by venepuncture, after 15 min of individual rest in an isolated and quiet room. The participants were asked to avoid alcohol and caffeine intake on the previous day of blood collection, and to maintain their sleep habits during the previous night. All participants were instructed not to engage in extreme physical efforts in the

24 hours prior to the collection. Determination of blood counts was done immediately after blood sample collection, using an automated haematology analyser Coulter Act Diff (Beckman Coulter, USA).

The remaining blood was separated after centrifugation at 3000 rpm at 4°C during 15 min, plasma and serum samples were aliquoted into Eppendorf tubes and stored at −80 °C until used for the determination of interleukin-10 (IL-10), Tumour Necrosis Factor alpha (TNF-α), myeloperoxidase (MPO), and total albumin concentrations. The ELISA (Thermo Fisher, Gloucester, UK) intra-assay coefficients of variability were 4.1% for IL-10 and 3.0% for TNF-α and MPO 22.8%. The Albumin levels were determined by colorimetry (assay kit A11a01664 Horiba-abx, Montpellier, France) using an automated equipment (Horiba Medical Pentra C200, Kyoto, Japan).

2.5.8- Salivary Testosterone

Non-fasting saliva samples were collected by passive drool, always at the same time in the morning (between 10:00 a.m. and 11:00 a.m.) to minimize any circadian effect (Papacosta & Nassis, 2011). The salivary samples were collected with the participant with the head and trunk lowered to facilitate the collection, for a period of 3 minutes. They were stored in a polypropylene tube to avoid contamination and retention of samples. The volumes measured, the flow rate calculated, the samples centrifuged and stored and frozen at -20º C until used for the determination of salivary testosterone levels. Prior to the saliva collection subjects were asked to rinse their mouth with water to remove any food residues, 10 minutes before sample collection, and to avoid the ingestion of: alcohol for 12 hours, dairy products for 20 minutes, foods with high sugar or acidity, or high caffeine content immediately before sample collection.

All participants were instructed not to engage in extreme physical efforts in the 24 hours prior to the collection. Salivary testosterone (ST) levels were measured by competitive ELISA (kit #1-2402, Salimetrics, United Kingdom). The intra-assay coefficients of variability were 2.19%.

2.5.9- Characterization of the Multifactorial Intervention Program (MIP)

2.5.9.1- Oral Supplementation in BCAA´s

The BCAAs power mixture was composed of L-leucine (Leu), L-isoleucine (Ile), and L-valine (Val) in the proportion of 2:1:1 (My Protein®, Cheshire, UK), accounting for 20 kcal per portion, comprising 5 grams (g) of supplement: 1.85 g Leu, 0.93 g Ile, and 0.93 g Val. The unflavoured supplement was used as to not induce ingestion preferences for specific flavours. The BCAAs were diluted in 200 mL of water and given immediately after the exercise sessions to the participants in the ME + BCAAs and BCAAs groups (Ispoglou et al., 2016). The supplement dose was fixed at 0.21 g total BCAAs/kg/session, with individual portion sachets, administered in the morning, between 09:00 and 11:30 a.m. (Negro et al, 2019).

We opted to exclude maltodextrin or the carnosine-based placebo here, since the carbohydrate ingestion could mask the effort perception and cognitive indexes in our older persons volunteers, compared to the amino acid supplementation (Honka et al., 2016). In addition, carnosine, as well as other β-alanine derivatives, were shown to affect cognitive functions, including the perception of wellness, mood, and depression indexes (Solis et al., 2015). Therefore, we decided to split BCAA-supplemented (ME + BCAAs and BCAAs) and BCAAs-absent groups (ME and CG) according to the proximity between the residential care homes (RCH), where the ME programs were effectively applied. No communication was reported between volunteers from the BCAA-supplemented and no-BCAA supplemented groups in our study.

2.5.9.2- Washout Period (Oral BCAA´s)

In this phase, the participants endured a cessation period of 8 weeks, when suplementation of the ME+BCAA and BCAA groups was suspended in order to verify whether the supposed benefits of BCAA supplementation were maintained or lost (Ikeda et al., 2016).

2.5.9.3- Exercise Intervention (Phase 1)

The exercise program was divided in two interventions of 16 weeks each, separated by an 8-week detraining (washout) period. Exercise sessions were offered twice a week, with an interval of 36 hours for adequate physiological recovery and rest. The exercise protocol respected the guidelines for exercise

prescription for older persons and the guidelines for exercise periodization by the American College of Sports Medicine (ACSM) (Chodzko-Zajko et al., 2009; De Souto Barreto et al., 2016). The program started with an adaptation period of 2 weeks, in which 7 different exercises were performed using elastic bands [TheraBand®, Hygenic Corporation, Akron, USA]. The participants were closely supervised for two initial sessions aiming at equipment familiarization and adjustments to the RPE OMNI scale (Colado et al., 2018). During these familiarization sessions, the participants learnt the correct technique of the exercises, and selected the proper color, length, and grip width of the elastic bands. The exercise intensity was indirectly calculated using the Karvonen's formula to predict target heart rate (HR), with HR_{max} being calculated by an adjusted formula for older persons (Tanaka et al., 2001).

$$HR = [(HR_{max} - \text{resting HR}) \times \%\text{Intensity}] + \text{resting HR}$$

After the adaptation period, the exercise program was progressively intensified with increments in both the number of exercises (from 8 to 10 exercises during the rest of the exercise intervention) and the proposed physical effort, imposed by different intensity colour bands, according to the OMNI table (Colado et al., 2018). The elastic-band exercises applied in the Phase 1 period are shown in Table 2.2. For safety reasons, the exercise programs were also monitored using heart rate monitors (Polar M200; Polar Electro Oy, Kempele, Finland) randomly distributed among participants. Additionally, intensity was measured through the specific rating perceived exertion (RPE) scales for each exercise program (Borg, 1982). The RPE used is an arbitrary scale ranging from 0 to 10 points, with identical intervals and with reference to the quality of effort: (0) Nothing at all; (1) very weak; (2) weak; (3) moderate; (4) somewhat strong; (5–6) strong; (7–9) very strong; (10) very, very strong (almost maximal).

Table 2.2 Elastic-Band Muscle Strenght (EMS)

Warm-up				5 minutes	PSE 1-3	**Progression**	**Weeks**	**Intensity (colour)**
Exercises (8-10)	**Sets**	**Repetitions**	**Cadence**	**Interval**	**PSE**	2x10	2	Yellow
Front squat	2-3	10-20	2:3	30-45 seconds	4 to 6	3x20	2	Yellow
Chair unilateral hip flexion	2-3	10-20	2:3	30-45 seconds	4 to 6	3x10	2	Red
Chair Bench over row (with flexion)	2-3	10-20	2:3	30-45 seconds	4 to 6	3x20	2	Red
Chest Press (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6	3x10	2	Green
Standing (or chair) reverse fly	2-3	10-20	2:3	30-45 seconds	4 to 6	3x20	2	Green
Shoulder Press/twist arm front position	2-3	10-20	2:3	30-45 seconds	4 to 6	3x15	2	Blue
Chair (or stand) frontal total raiser	2-3	10-20	2:3	30-45 seconds	4 to 6	3-4x10-15	2	Blue
Biceps arm curl (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6			
Chair Overhead triceps extension	2-3	10-20	2:3	30-45 seconds	4 to 6			
Cooling down				5 minutes	PSE 1-2			

Note: PSE- Perception Subjective Effort

2.5.9.4- Washout Period (ME Detraining)

In this phase, the participants endured a detraining period of 8 weeks, when the ME programs were suspended. The aim was to check if the physiological and psychological adaptations acquired during the first phase of ME were maintained or if an 8-week interruption was able to revert the effects seen after the 16weeks of intervention (Sakugawa et al., 2019; Lim et al., 2014).

2.5.9.5- Exercise Retraining Protocol of Multicomponent Exercise (ME)

The phase 3 (exercise retraining) protocol was also based on the resistant TheraBand (TheraBand®, Hygenic Corporation, Akron, OH, USA) elastic bands (Table 2.2), but included walking, steps, and balance exercises (sometimes with dumbbells and ankle/wrist weights) to compose a multicomponent exercise program for an identical 16-week period (twice a week, on alternate days, also totalizing 32 sessions). The multicomponent program (Table 2.3) was described by Furtado et al. (2019). The phase 3 program aimed to reproduce most of the daily activities of the older persons in this study (Baker et al., 2007; Tarazona-Santabalbina et al., 2016).

Table 2.3- Protocol of Multicomponent Exercise (ME).

Exercises (8-10)	**Sets**	**Repetitions**	**Cadence**	**Interval**	**PSE**
Front squat	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair unilateral hip flexion	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair Bench over row (with flexion)	2-3	10-20	2:3	30-45 seconds	4 to 6
Chest Press (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6
Standing (or chair) reverse fly	2-3	10-20	2:3	30-45 seconds	4 to 6
Shoulder Press/twist arm front position	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair (or stand) frontal total raiser	2-3	10-20	2:3	30-45 seconds	4 to 6
Biceps arm curl (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair Overhead triceps extension	2-3	10-20	2:3	30-45 seconds	4 to 6
Circuit training					
Walk	3 minutes				4 to 6
Balance/agility exercise	3 minutes				4 to 6

Note: PSE- Perception Subjective Effort

2.5.10- Statistical Analysis

Statistical analyses were performed using SPSS (version 26; SPSS Inc, Chicago, IL). All descriptive statistics (see studies 2, 3, 4) are shown as means ± standard deviations. Normality of data was verified by the Shapiro-Wilk Test. A Wilcoxon Test was used to compare each SPPB test measure and final score, and statistical significance was set $p < 0,05$.

To compare the mean changes over time between groups, repeated measures ANOVA (4x4 group vs. time) were performed. Bonferroni´s post hoc analysis was performed for paired comparisons of means when significant interactions were found in the dependent variables.

All the variables were checked for the normally residual distribution and values were logarithmically transformed when appropriate. One-way Analysis of Variance ANOVA was used to determine baseline differences between the four groups in all the parameters. Effects of time, group, and time x group interactions were assessed through repeated measures ANOVA and Bonferroni post-hoc for multiple comparisons. Additionally, univariate analysis was performed using the paired *t*-test for comparisons during the first phase of interventions (T1 vs. T2).

2.5.11- Participants Characterization and Socio Demografic Data

The following individual and sociodemographic data were assessed: i) Chronological age was assessed by subtracting the date at testing by the date of birth and analysed as a continuous variable. ii) stature, iii) body mass, iv) Body Mass Index, v) MNA nutritional score, vi) CCI score, vii) Time in residential care (years), viii) Level of education classified according to the Portuguese educational system and analysed as a continuous variable (Fernandes, 2007); xix) physical frailty, x) cognition and xi) SPPB scores. The total ingestion of protein (g/kg/day) for the different groups at baseline was: ME+BCAA 1.42±0.28; ME 1.83±.044; BCAA 1.476±0.22; CG 1.595±0.23 ($p= 0.159$).

Table 2.4- Baseline levels: Frailty index, cognitive score and physical test.

Characteristics	M ± SD	%	Men (n=14)	Women (n=21)
Chronological age (years)	83±3		81.28±5.84	84.76±5.25
Height (m)	1.56±0.10		1.65±0.59	1.50±0.73
Weight (Kg)	70.24±11.86		77.71±8.47	65.25±11.27
Body Mass Index (kg/m^2)	28.66±4.51		28.42±3.74	28.83±5.05
MNA (score, 0-30)	24.17±2.6		24.89±2.2	23.69±2.74
CCI (score)	5.08±1.12			
Low CCI (≤ 5 point)	n=21	78.6%	n=11	n=10
High CCI (≥ 5 points)	n=14	21.4%	n=3	n=11
Polypharmacy (day)	7.21±1.61		5.42±4.08	4.85±4.84
Time in residential care (years)	4.45±0.60		3.65±1.42	4.52±1.01
Schooling time (years)	4.0±0		4.0±0	4.0±0
Frailty (0-5 points)	Pre-frail (n=5) 1±00	14.3%	n=2	n=3
	Frail (n=30) 2.67±0.71	85.7%	n=12	n=18
Cognition (MMSE score 0-30 points)	Mild Cognitive Impairment n=21 20.38±2.13	60%	21.00±2.44 n=9	19.92±1.83 n=12
	Normal Cognition n=14 26.64±1.39	40%	26.20±1.64 n=5	26.89±1.26 n=9
Cognitively Impaired and Physically Frail	n=20	57.1%	n=8	n=12
Partially Frail	n=12	33.3%	n=5	n=7
Robust	n=3	8.6%	n=1	n=2
SPPB (0-12 points)	Very poor capacity (n=6)	17.1%	n=2	n=4
	Low capacity (n=28)	80%	n=11	n=17
	Moderate capacity (n=1)	2.9%	n=1	n=0

Notes: M, mean; SD, Standard deviation; MNA- Mini Nutritional Assessment; CCI- Charlson Comorbidity Index; MMSE- Mini Mental State Examination; SPPB- Short Physical Performance Battery.

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CHAPTER III

Evidence-Based Effects of Multicomponent Exercise Training on Biomarkers Related to Frailty in Older Persons: A Systematic Review

STUDY #1

Caldo-Silva, A., Furtado, G. E., Bachi, A. L. L., Barros, M. P., Zanchi, N. E., Massart, A., A., Teixeira, A. M. (2021). Evidence-Based Effects of Multicomponent Exercise Training on Biomarkers Related to Frailty in Older Persons: A Systematic Review. Journal of Physical Education and Sport July. 2021. DOI:10.7752/jpes.2021.s3282

ABSTRACT

Background: The effects of multicomponent exercise training on biomarkers in frailty are not yet fully explored in the scientific literature. **Objective:** Based on the existing studies in the scientific literature, our first endpoint in this systematic review was to present the current knowledge on the influence of multicomponent exercise training in older persons presenting with frailty. In addition, our second endpoint was to identify not only the most evaluated and more responsive biochemical markers, as well as how they respond to multicomponent exercise training. **Methods:** The electronic databases PubMed, SciELO, LILACS, Science Direct, and databases were accessed. The search was conducted in the English language using MeSH keywords in order to verify which biochemical markers were investigated in studies that associated frail older people and multicomponent physical exercise. This systematic review was registered under the reference CRD42018089912. **Results:** Six randomized controlled trial studies meet our inclusion criteria and were used to perform this review. In response to our endpoints, these studies reported that multicomponent exercise training was able to decrease the symptoms of frailty. In addition, this type of exercise training was associated with alteration in several biomarkers, namely C-reactive protein (CRP), interleukin (IL-6), Tumour Necrosis Factor (TNF-α), insulin-like growth factor (IGF-1), haemoglobin, albumin, oxidative stress oxidation products, antioxidants, HbA, Vitamin D, and hormone levels of DHEA, Testosterone, PTH. **Conclusion**: Multicomponent exercise training is a powerful tool to prevent or even revert frailty development in older persons, particularly, by its remarkable action in several metabolic, hormone and pro-inflammatory markers.

Keywords: Physical Exercise Protocol, Older Persons, Review, Cytokines, Hormones.

3.2- Introduction

The Frailty Syndrome (FS) can be defined as a loss of physical and functional capacity with multifactorial traits in which the loss of muscle mass is highlighted (Marzetti et al., 2019; Fried et al., 2001). It has been purposed that these losses could be closely associated with a sedentary lifestyle and the lack of previous interventions (physical exercise program) (Milanović et al., 2013; Booth et al., 2012). In fact, these factors are also associated with the increase in number and risk of falls in older frail persons (Leitão et al., 2015).

There are a handful of studies that aimed to investigate whether exercise training could putatively reverse frailty (frail to pre-frail or to non-frail), or, at least, achieve a lower degree of frailty in older people (Manãs et al., 2019). Regarding this, it was demonstrated that physical exercise programs were able to reduce frailty by improving functional capacity, muscle strength, speed, and agility. However, these benefits did not seem to ellicit alterations in several systemic biomarkers (Ferreira et al., 2018). Although these biomarkers were unchanged, there is a consensus in the literature that physical exercise training has the potential to provide protection against frailty in advanced aged persons (Higueras-Fresnillo et al., 2018), and that most of these benefits, at least in part, are related to the anti-inflammatory and metabolic effects of exercise (Aguirre & Villareal, 2015; Gleeson et al., 2011; Petersen & Pedersen, 2005).

It is paramount to mention that, among several exercise training protocols, multicomponent exercise training programs, focused on the development of physical capacity, balance, and muscle strength, could promote healthy ageing (Leitão et al., 2015), by its inclusion of, at least, 3 different physical modalities in the same exercise session (Chodzko-Zajko et al., 2009; Baker et al, 2007). There is growing evidence that the elderly subjects engaged in a multicomponent exercise training program presented, in a general way, a better health status (Cadore et al. 2019; Arrieta et al., 2019; Silva et al., 2017; Makizako et al., 2012; Hopps et al., 2011; Theou et al., 2011). Multicomponent programs involve aerobic exercise and strength exercise combined with other physical valences such as balance and flexibility (Baker et al, 2007), and are considered as the type of training more beneficial to optimize functional capacity of frail older persons (Villareal et al., 2011) and to prevent functional and physical incapacity (Cadore et al., 2014).

It is broadly accepted that more studies regarding how to prevent and manage frailty are mandatory and could illuminate the way on how to maintain functional independence and health throughout ageing. Therefore, in this systematic review, we aimed to discuss, the relevance of multicomponent exercise training and health-related biomarkers on frailty in older persons.

3.3- Methods

This study follow a pre-determined SR protocol registrated in the PROSPERO database, under the number CRD42018089912, carried out taking into account different guidelines, utilized to stratify, evaluate and select the scientific reports included in this Systematic Review (SR).

In order to guarantee the rigor, accuracy, and replicability in this SR, the following steps were followed: i) definition of systematic search terms through operationalization and concepts description; ii) pilot systematic search of articles in order to verify the search accuracy in each previously selected database.

3.3.1- Search Strategy for Identifying Studies

We performed a systematic search for English studies published on the following databases: PubMed, SciELO, LILACS and Science Direct. Scientific reports were accessed between July 2018 and January 2020 in order to identify original studies, published between 2001 and 2020, presenting multicomponent exercise training, volunteers over 60 years of age, of both sexes, who were living or not, in residential care homes. In the searching criteria for inclusion, the following MeSH terms were used: “multicomponent exercise”, or “physical exercise”, or “multi-modal exercise training”, or “exercise therapy”, or “combined exercise training”, or “circuit-based exercises”, or “circuit training”, or “muscle strength exercises”, or “muscle strength training”, or “resistance training”, or “physical fitness programs”, or “concurrent training”, or “home-based exercise” AND elderly or older subjects, or older populations AND biomarkers or biochemicals, or immune system, or hallmarks AND Fried frailty criteria (Fried et al., 2001).

3.3.2- Articles Evaluation

Search strategies followed the PRISMA checklist (Panic et al., 2013). In addition, the CONSORT checklist was used, because it provides guidelines for writing and evaluating RCT studies (Begg et al., 1996), allowing to identify possible errors or methodological weaknesses. In order to avoid the risk of bias, the evaluation of the articles was carried out by 2 different researchers (blinded), with help of a third researcher if no consensus on the score of one of the articles was reached (Donato et al., 2019).

The following eligibility criteria were adopted: (1) in relation to the population: over 60 years of age individuals who presenting frailty (defined as frail or pre-frail through the Fried criteria; (2) outcome measures: quantitative variables obtained by biochemicals analysis; (3) intervention: multicomponent exercise training protocol; (4) randomized controlled trial or clinical trials investigating the effect of physical exercise on physical frailty and biomarkers.
The exclusion criteria used were non-original studies, reviews or meta-analysis, non-randomized controlled trials (nRCT).

3.4- Results

A total of 130 articles were identified through database searching (PubMed=25, SciELO=15, and Science Direct=90). The screening of database LILACS did not produce any records. As the formerly mentioned, the titles, abstracts, and full text of the selected articles were gradually screened for eligibility by two independent researchers.

By applying the first study selection criteria, 45 studies were excluded, and 85 studies proceeded to the screening phase. At this stage, after reading the abstracts, 43 studies were excluded. From the 42 remained articles, 10 studies were included after the eligibility phase. After that, 4 were excluded because they did not use Fried´s frailty criteria or due to study protocols incompatibilities. A total of 6 articles met the inclusion criteria and were used to perform this SR (see Flowchart of figure 3.1).

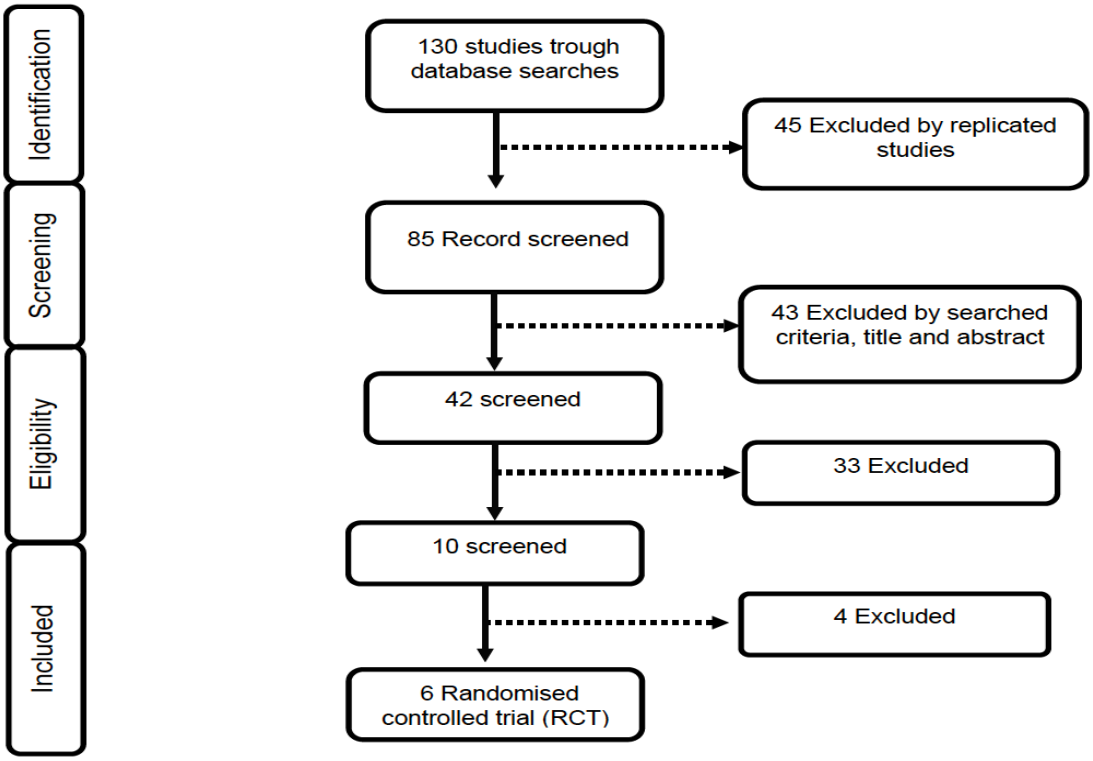

Fig.3.1 Prisma Flowchart of the process of literature search and extraction of studies meeting the inclusion criteria.

3.4.1- Sample Study Characteristics

The six studies included in this SR, respresent a total of 336 frail men and women, aged ≥70 years and living in residential care homes (Tarazona-Santabalbina, 2016, Arrieta et al., 2018, Furtado et al 2019; 2020, Sadjapong et al., 2020, Ferreira et al., 2018).

3.4.2- Charcteristics of Exercise Programs

All the studies included in this SR presented interventions with multicomponent exercise training programs, which seem to be predominantly used in frail older people (Silva et al., 2017). Interestingly, among the 42 articles selected in the first evaluation phase, 10 (24%) studies used multicomponent exercise training programs, which confirms their frequent use in frail older populations.

3.4.3- Main Results of the Selected Studies

Regarding multicomponent exercise training program in frail older persons, the study performed by Tarazona-Santabalbina and collaborators (2016), demonstrated that this program was able to revert frailty, improving

physical function, anthropometric parameters, cognitive and emotional domains; Sadjapong and collaborators (2020), showed reversing frailty to pre-frailty status, with improved physical performance, especially balance; Furtado and colleagues (2019, 2020), observed decreased frailty, with benefits in physical activity levels (gait speed and muscle strength) and functional disability; Ferreira and colleagues (2018), also found reduced frailty by improvement in functional capacity, muscle strength, speed, and agility; Arrieta and collaborators (2018), evidentiated improvement in physical fitness. For more details, see table 3.1

Regarding biomarkers (Table 3.2), Tarazona-Santabalbina and collaborators (2016), used BDNF; Arrieta and collaborators (2018), used myostatin; Ferreira and colleagues (2018) used glucose, insulin, total cholesterol, triglycerides, vitamin D3, and CRP; Furtado and colleagues (2019, 2020), used cortisol, testosterone, dehydroepiandrosterone, testosterone/cortisol ratio, sIgA, sLys, IL-1β, and IL-6, TNF-α, IFN-γ, IL-10, CRP, Sadjapong and collaborators (2020), used IL-6 and CRP.

Table 3.1- Summary of Study and Participant Characteristics

Author (year)	Main Goals	Characteristics of participants	Biomarkers	Comparison	Central outcomes
Arrieta et al (2018) SPAIN	to determine the association of serum myostatin concentration with body composition, physical fitness, physical activity level, frailty.	Women 86.2±6.8 years men 82.0±6.3 years n=88	Myostatin	6-months exercise, twice a week, involving strength, balance, stretching exercises, and walking recommendations	higher serum levels of myostatin were found to be associated with better physical fitness. however, the use of this protein as a biomarker for physical fitness, rather than frailty, merits further study.
Tarazona-Santabalbina et al (2016) SPAIN	to multicomponent exercise program (mep) performed by frail older and improve functionality, cognitive, emotional, and social networking, biomarkers	Men and women 79.5 years n= 100	BDNF	24 weeks a combined program of endurance, strength, coordination, balance, and flexibility exercises. 65 min session, 5 days/week	The mep reversed frailty and improved functional measurements, cognitive, emotional, and social networking determinations. it also leads to a decrease significant improvement in frailty biomarkers
Ferreira et al (2018) BRAZIL	To verify the effects of exercise training on biochemical, inflammatory, and anthropometric indices and functional performance in institutionalized frail elderly	Men and women 73.3 ± 6.4 years n=71	Glycaemia, Insulin TC, TR HDL, LDL Vitamin D3, CRP IL6, IL10 IL1a, IL1RAcP	12-weeks physical exercise program strength, coordination, balance, and flexibility	Improving muscle strength, speed, agility, and biochemical variables, with reversal of the frailty condition.but, no effects in anthropometric and inflammatory parameters were noted.
Furtado et al (2019) PORTUGAL	To analyse the effect of two different 28-weeks chair exercise programs (multimodal and muscles strengthening with elastic bands on physical frailty, functional disabilities and steroid hormones in institutionalized pre-frail and frail women	Institutionalized dwelling women n=60 81±7.84 years	Cortisol Testosterone Dehydroepiandrosterone Testosterone/Cortisol ratio	28-weeks chair exercise programs multimodal and muscles strengthening with elastic bands	Both exercise interventions used in this study produced significant benefits in order to diminish the physical frail condition, decreased functional disability and also, stimulated satisfactory hormonal responses.
Furtado et al (2020) PORTUGAL	to analyse the effects of 28-weeks of two different exercise protocols on the functional fitness and immune profiles of institutionalized pre-frail and frail women with mild cognitive impairment.	Institutionalized pre-frail and frail women with mild cognitive Impairment 81±7.84 years n=60	sIgA, sLys, IL-1β, and IL-6; TNF-α, IFN-γ IL-10 CRP	28-weeks chair exercise programs multimodal and muscles strengthening with elastic bands	The evidence regarding the use of systematic and moderate long-term exercise as therapy for promoting a better balance between pro- and anti-inflammatory environments and a decrease in the inflammatory index for the cme group were the most promising results from this study and reduces levels frailty.
Sadjapong et al (2020) THAILAND	this study aimed to investigate the effectiveness of a multicomponent exercise program (mcep) on frailty, physical performance (handgrip strength, berg balance scale (bbs), timed up and go test (TUG), and vo2max), blood biomarkers (interleukin-6 (IL-6) and c-reactive protein (CRP) in frail older adults.	Community-Dwelling older adults n=64 77.78 ± 7.24 years	IL-6 CRP	12-weeks the multicomponent exercise program (mcep), including aerobic training, resistance training, and balance training was tailored to participant ability by gradually increasing the intensity from moderate to high. it was of 60 min duration and took place over 3 days per week	The combined center- and home-based mcep were effective in reversing frailty to pre-frailty and improving physical performance especially balance in the older population

Table 3.2- Identification of the biological systems involved.

Author	Biochemical/molecular mechanisms studied	Confirmation of hypothesis
Arrieta et al (2018)	Muscle Tissue	↑ myostatin concentration (muscle activity)
Tarazona-Santabalbina et al (2016)	Nervous System	↑ BDNF serum levels group multicomponent
Ferreira et al (2018)	Lipid profile and cytokines pro inflammatory	↓ fraily score
Furtado et al (2019)	Hormonal	Testosterone = DHEA.
Furtado et al (2020)	Immune system, pro/anti inflammatory	↓ inflammation in frail older adults
Sadjapong et al (2020)	Pro/anti inflammatory	↓ inflammation in frail older adults

3.5- Discussion

We only found 6 studies that reached our eligibility criteria, evidiencing a lack of studies looking the mediating effects of multicomponent exercise training effects on biomarkers in frail in older persons, in other to better understand frailty prevention and development, and the consequences on aging well being.

Among the 42 articles selected in the first evaluation phase, 10 (24%) studies used the multicomponent exercise training programs, which confirms their increasing use in frail older populations. Multicomponent exercise training programs (ME) seem to be predominantly used in frail older people (Silva et al., 2017), and may be considered an ideal intervention for this population (Pillatt et al., 2019). The combination of multicomponent strength, aerobic, and balance exercises have demonstrated remarkable positive effects on health outcomes as compared to their prescription in isolation (Baker et al., 2007). Furthermore, multicomponent exercise training has also been shown to be advantageous and most attractive for older participants due to the fact that they are similar to the activities of daily living (Angulo et al., 2020). Our SR confirms the potential of ME with all the studies showing improvements: tree reverted frailty, two diminished

frailty and one improved physical fitness. They are also in accordance with other studies using ME in frail older subjects (Cadore et al., 2019; Arrieta et al., 2019; Silva et al., 2017; Makizako et al., 2012; Hopps et al., 2011; Theou et al., 2011).

Regarding the performance frequency of the multicomponent exercise programs described in the studies included in this SR, a variation of 2-5 times per week with a total duration between 3 to 8 months was found. Moreover, there seems to be a consensus that the inclusion of resistance, gait, and balance training in the multicomponent exercise training programs is the corollary strategy for improving the frailty hallmarks, as well as for reducing falls risk in frail older persons. In addition to maintain functional capacity during ageing, ME combines several actions, such as cognitive training that also enhances cognitive performance and, thus, prevents cognitive impairment (Tarazona-Santabalbina et al. 2016). Results from other studies using different exercise protocols than ME (Cadore et al., 2019; Theou et al., 2011; Arrieta et al., 2019; Makizako et al., 2012; Justine et al., 2010) also confirm this.

Corroborating these pieces of information, this SR showed that the preferential and the most responsive biomarkers assessed in the selected studies were IL-6, CRP, and TNF-α (see Table 2), with IL-6, CRP and TNF-α being evaluated in 3 of the 6 studies. In this respect, it is noteworthy to point out that multicomponent exercise training showed the capacity to impose significant alterations in these biomarkers, especially in the studies of Furtado and collaborators (2020) and Sadjapong and collaborators (2020) indicating that ME contributed for decrease of inflammation levels in frail older persons. Taking into account other studies performed with different populations of young and elderly subjects, there is solid evidence that the regular practice of physical exercise decreases systemic levels of CRP, IL-6, and TNF-α, reinforcing the findings above cited (Hopps et al., 2011; Nicklas et al., 2008; Petersen & Pedersen, 2005; Petersen et al., 2007).

Recently Petrella and collaborators (2021) reported that the multicomponent exercise training program is an inexpensive intervention that could be replicated in care for the avoidance and treatment of frailty, mainly acting in the pro and anti-inflammatory pathways, but also in improving the anabolism and other biomarkers identified such as: Glycaemia, Insulin, Total Cholesterol, TR-Triglycerides; HDL- High-Density Lipoprotein; LDL- Low-Density Lipoprotein;

CRP C-reactive protein; Vitamin D3; IL-6, IL-10, IL-1a, IL1RAcP, Myostatin, Cortisol, Testosterone, Dehydroepiandrosterone, Testosterone/Cortisol ratio. Tarazona-Santabalbina and collaborators (2016), Arrieta and collaborators (2018), Ferreira and colleagues (2018) Furtado and colleagues (2019, 2020), Sadjapong and collaborators (2020). In agreement with the literature, immune/inflammatory dysregulation is considered the core of frailty, by its interrelating with neuroendocrine dysregulation and neuromuscular (Ng et al., 2018).

Thus, among the well-accepted benefits of physical exercise training, such as maintaining the health and functionality of older people by increasing muscle strength, improving balance, and avoiding falls, this nonpharmacological intervention has also shown the capacity to decrease the levels of pro-inflammatory biomarkers (Seguin & Nelson, 2003; Furtado et al., 2020), which can impact positively in the prevention and/or reversion of frailty development in older persons.

Despite the precise mechanisms by which physical exercise promotes healthier ageing still not being fully understood (Sadjapong et al., 2020), as described in the studies included here, a relevant part of that could be explained by a favourable improvement in muscle mass and a reduction in adipose tissue leading to a decrease in pro-inflammatory levels and a better inflammatory balance (Cartee et al., 2016; Gonzalez-Gil et al., 2020).

Other point that should be considered is that most of the studies subjects included in our SR were institutionalized-dwelling older subjects, who usually are at higher risk than elderly subjects who do not live in residential home care (Soriano, DeCherrie, & Thomas, 2007), and despite this disadvantageous situation the impacts of ME were significant, highlighting the usefulness of this practise for maintaining the quality of life, especially in residential care homes.

Although there are convincing signs that multicomponent exercise training in frail older persons is an effective intervention for improving biomarkers of frailty, further studies are necessary to increase our understanding of the mediating effects of exercise on immune and hormonal pathways involved in frailty.

3.5.1- Study limitations, Suggestion for Future Studies

The fact that only 6 studies reached our inclusion criteria could be considered a limitation of this study, but it is important to clarify that the criteria used to perform this SR were designed to identify those studies with a consistent frailty phenotype evaluation, allowing for a better comparation between studies. Nevertheless, some other limitations should be taken into account, like different follow-up times, differences in physical exercise programs (sequence, time, progression, intensity), and differences in previous levels of physical fitness, which could lead to some potential bias. Future research should focus on a better definition of the physical activity “dose” capable of improving the biochemical profile in older persons and its association with frailty, in order to promote healthier ageing with more years and better quality of life. In addition, the study of the impact of nutritional supplementation in combination with multicomponent exercise training could also be important in the context of frailty.

3.5.2- Practical Applications

Since a constant rise in the number of older persons in Europe is estimated, the development of studies associating the triad: frailty, inflammaging, and physical exercise training, especially multicomponent exercise training, are mandatory in order to increase our knowledge on how to improve functional fitness, health and quality of life, in order for older populations to be able to maintain the autonomy and independence for longer years.

3.6- Conclusion

This SR showed that multicomponent exercise training designed to addressee frailty in older persons, was able not only to improve several parameters associated with physical function and biochemical profile but also benefited cognitive, emotional, and social functions. In addition, frailty and pre-frailty were closely associated with increased pro-inflammatory parameters, mainly CRP, IL-6, TNF-α, and with the disruption of metabolic and hormone markers. Lastly, we confirmed the growing interest in the application of multicomponent exercise training programs for the prevention and management of frailty in this population.

Contributors

Adriana Caldo organized acquisition of data and writing of the paper; Rafael Nogueira, helped in the acquisition of data. Alain Massart supported the interpretation of data and reviewed the paper critically. Marcelo Barros e André Bachi helped in the discussion. Guilherme Furtado, José Pedro Ferreira and Ana Maria Teixeira reviewed the paper critically and coordinated the research study protocol.

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CHAPTER IV

Impact of 16 weeks of Exercise and Protein Supplementation on Functional-Physical Fitness of Dwelling-Institutionalised Elders

STUDY #2

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ABSTRACT

Background: Ageing is a natural, progressive and irreversible process characterized by morphological, psychological, functional, biochemical and nutritional changes. Physical inactivity (PI) is a factor that contributes to the starting of mass and muscle function decline in the elderly, often related to sarcopenia and physical frailty. Combined to protein nutritional intervention, exercise appears as an effective way to prevent muscle mass and physical fitness decline. In addition, the elderly population has difficulty in maintaining adequate protein nutrition and are the least involved in systematic exercise programs. This exploratory study was conducted to understand the isolated and combined effects of a 16-weeks of Branched Chain Amino Acids (BCAA) supplementation (BS) and Muscle Strength Exercise program (MSE) on functional-fitness performance in octogenarians. **Methods**: The sample consisted of 18 participants aged 82.97±8.05 years old, institutionalized in social care centres. They were divided into two groups: group 1 (MSE+BS, n=10); group 2 (BS, n= 8). Group 1 performed an elastic band strength exercise program carried out during 16-weeks together with BCAA supplementation consisting of ingesting 0.21g/kg/day of unflavoured powder diluted into 200mls of water, immediately after exercise. Group 1 did only the BCAA supplementation. To evaluate the functional capacity of the elderly, the short battery of tests SPPB was used in the initial and final intervention evaluation. **Results:** After 16 weeks, group 1 (MSE+BS) showed a significant increase in all the SPPB tests performance, particularly in the test consisting in rising from a chair and seating down for 5 times. The SB group showed only a short decrease in the time taken to perform the 3 meters walk test ($p < .05$). **Conclusion:** Our study revealed that exercise plus supplementation with BCAAs was able to improve physical fitness function, while BCAA supplementation alone had limited effects. Satisfactory results in physical function could be explained by the added effects of exercise and BCAA supplementation on the protein synthesis effect.

Keywords: Older adults, Physical Exercise, Protein Supplementation, Functional Physical

4.2- Introduction

Ageing leads to muscle loss and to increased risk of falls and loss of independence. Resistance exercise can be a useful intervention to prevent sarcopenia and frailty. However, muscle protein synthesis in older adults is decreased (Drummond & Rasmussen, 2008). Ageing is a natural, progressive and irreversible process characterized by morphological, psychological, functional, biochemical and nutritional changes (World Report on Ageing And Health, 2015). Physical inactivity (PI) is a factor that contributes to the starting of mass and muscle function decline in the elderly often related to sarcopenia and physical frailty (Lehmann, Baar, & de Keizer, 2018). Combined to protein nutritional intervention, exercise appears as an effective way to prevent muscle mass and physical fitness decline (Hernández Morante, Martínez, & Morillas-Ruiz, 2019).

However, the elderly population has difficulty in maintaining adequate protein nutrition and in addition, they are the least involved in systematic exercise programs (Landers-Ramos & Dondero, 2019). Regarding nutritional supplementation, branched-chain amino acids (BCAA) act as a major energy substrate in the muscle along with the increased contribution of fat as an energy source when the exhaustion of carbohydrates during long periods of exercise occurs (Kim et al, 2013). Some European references on the amount of protein intake in the elderly RDA population suggest ingesting 0.8g/kg/ (WHO/FAO/UNU Expert Consultation, 2007) while a study by a European PROT-AGE group recommends about 1.0 to 1.2g/proteins kg/day (Bauer et al., 2013).

In this way, a multicomponent exercise program, which develops different physical abilities, can also be beneficial in maintaining the functionality and capacity of the elderly (Baker et al, 2007). Recently, researchers stated that programs including BCAA supplementation present a more effective mean of increasing muscle strength than other types of protein supplementation (Rondanelli et al., 2011).

This exploratory study was conducted to understand the isolated and combined effects of 16-weeks BCAA supplementation (BS) and elastic-band muscle strength exercise program (MSE) on functional-fitness performance in octogenarians elders.

4.3- Methods

4.3.1- Study design

This study was conducted according to guidelines in the Declaration of Helsinki for procedures involving human subjects and was approved by the University of Coimbra, Faculty of Sport Science and Physical Education Ethical Committee (reference number: CEFCDEF/0028/2018) respecting the Portuguese Resolution (Art. 4th; Law nº. 12/2005, 1st series) on ethics in human research (Braga, 2013).

4.3.2- Participants

The sample consisted of 18 participants institutionalized in social care centres. They were divided into two groups: group 1 (MSE+BS, n=10); group 2 (BS, n= 8). Group 1 performed an elastic band exercise Muscle Strength Exercise program, carried out during 16-weeks together with BCAA supplementation consisting of ingesting 0.21g/kg/day (Ispoglou et al., 2016) of unflavoured aminoacids powder, immediately after exercise. Group 2 did only the BCAA supplementation and no exercise program was performed.

Table 4.1- Characteristics of Participants

	MSE+BS Group 1	**BS Group 2**
	Baseline	Baseline
Chronological age (years)	82.80±6.80	83.13±9.30
Body mass index (BMI)	28.39±4.91	25.99±2.96
Charlson Comorbidity Index (CCI)	5.00±1.05	5.00±1.60

4.3.3- Interventions

4.3.4- Muscle Strength Exercise (MSE)

A progressive program of exercises performed with a determined number of exercises (8-10), sets (2-3); repetitions (10-15), a cadence of repetitions execution in 2 seconds concentric and 3 seconds eccentric (2:3) (Skovdal Rathleff, Thorborg, & Bandholm, 2013) and a passive rest in the seated position between sets (30-45 seconds), following a bi-set protocol method was created. The first three levels of the TheraBand® System were used. Level one (yellow colour) elastic-bands were used during the first 4 weeks, progressing to a different colour every four weeks. Intensity was measured through the OMINI

PES scale (Robertson et al., 2003), that consists of a subjective effort scale ranging from 0 to 10 points. The goal is to keep the intensity of the exercise activities between 1 to 6 in the PES levels. It is expected that the relationship with the real effort would be 55-80% of maximum HR and muscle intensity was evaluated by OMINI using Colado and colleagues approach (Colado et al., 2014). The session was divided into three parts: 5 minutes of warm-up (PSE 1 to 3, HRmax = 45-55%), 35 minutes of muscle-strength elastic-band exercises in PSE 4 to 6 (HRmax = 56-75%) and finally, 5 minutes of cooling-down (PSE 1 to 2, HRmax = 45-50%). A low to moderate intensity effort around 50-75% of HRmax values was warranted.

Table 4.2- Protocol Muscle Strength Exercise

Warm-up				5 minutes	PSE 1-3
Exercises (8-10)	Sets	Repetitions	Cadence	Interval	PSE
Front squat	2-3	10-15	2:3	30-45 seconds	4 to 6
Chair unilateral hip flexion	2-3	10-15	2:3	30-45 seconds	4 to 6
Chair Bench over row (with flexion)	2-3	10-15	2:3	30-45 seconds	4 to 6
Chest Press (stand and/or chair)	2-3	10-15	2:3	30-45 seconds	4 to 6
Standing (or chair) reverse fly	2-3	10-15	2:3	30-45 seconds	4 to 6
Shoulder Press/twist arm front position	2-3	10-15	2:3	30-45 seconds	4 to 6
Chair (or stand) frontal total raiser	2-3	10-15	2:3	30-45 seconds	4 to 6
Biceps arm curl (stand and/or chair)	2-3	10-15	2:3	30-45 seconds	4 to 6
Chair Overhead triceps extension	2-3	10-15	2:3	30-45 seconds	4 to 6
Cooling down				5 minutes	PSE 1-2

4.3.5- Nutritional supplement (BCAA)

The BCAA supplement was composed by L-Leucine, L-Isoleucine and L-Valine in the proportion of 2:1:1 respectively [product of MYPROTEIN®, UK]. All compounds were packed in individual sachets and administered to the MSE+BS and BS groups. The supplement was supplied through the company with about 95% purity and trade certificated in the EU. The subjects ingested 1 sachet (0.21g/kg/session) (Ispoglou et al., 2016) prepared after finishing the exercise

session around 11h am. The contents of each sachet were mixed with 200mL water and consumed. The package contents have about 58 kcal.

4.3.6- Short Physical Performance Battery

The Short Physical Performance Battery (SPPB) is used for the assessment of physical functioning and disability the evaluation of older persons in both clinical and research settings. It is composed of gait speed, chair stand, and balance tests. This SPPB battery has 3 subdimensions: i) static balance (composed of 3 tests), ii) muscular strength of the lower limbs, which consist of getting up and seating in a chair, 5 times, with arms crossed at the chest, and iii) 3-meter test, that measures the time to travel the 3-meter course, at the persons habitual speed (Guralnik et al., 1994). The static balance evaluation is done using 3 tests: Test 1 – stay still for 10 seconds with the feet parallel to each other, the score for each balance test is 1 point if the determined time has been completed (10 seconds) and 0 if you were unable to perform the test; Test 2 - stay still for 10 seconds with the heel of one foot placed next to the first toe of the other foot, the score is 1 point if the determined time has been completed (10 seconds) and 0 if not able to perform the test; Test 3 - stay still for 10 seconds with one foot in front of the other, the score is 2 points, if the time measured was between 3-9 seconds 1 point is attributed, if lower 0 points. The total score for the balance tests is 4. For the 3-meter walk test, participants were instructed to walk at their normal pace the 3 meters distance. Two attempts are performed, and the shortest time assigned. The score attributed to the walk test is: If the time is less than 3.62 seconds, 4 points are assigned; if the time is between 3.62-4.65 seconds, 3 points are assigned; if the time is between 4.66-6.52 seconds, 2 points are assigned and if the time is longer than 6.52 seconds one point is assigned. For the 5x seat and stand Chair Test, the participant was instructed to cross his arms over his chest and get up and sit on the chair five times as quickly as possible and timed to the final standing position. Score calculation is made as follows: if the time is more than 60 seconds, 0 points are assigned; if the time is between 16.70 seconds or more, 1 point is assigned; if the time is between 13.70-16 seconds, 2 points are assigned; if the time is between 11.20-16.69 seconds, 3 points are assigned and if the time is less than 11.19 seconds, 4 points are assigned. Total

score of the SPPB is calculated as the sum between the tests described above (table 4.3):

Table 4.3- Short Physical Performance Battery

SPPB Score Total	
Incapable	0 to 3 points
Low performance	4 to 6 points
Moderate performance	7 to 9 points
Good performance	10 to 12 points

4.4- Statistical Analysis

Statistical analyses were performed using SPSS (version 26; SPSS Inc, Chicago, IL). Descriptive statistics (Table 4) are shown as means ± standard deviations. Normality of data was verified by the Shapiro-Wilk Test. A Wilcoxon Test was used to compare each SPPB test measure and final score, and statistical significance was set $p < 0,05$.

4.5- Results

After 16 weeks, group 1 (MSE+BS) showed a significant increase in the performance of all the SPPB tests, particularly in the 5x sit and stand test. Group 2 (BS) showed only a short decrease in the time taken to perform the 3 meters walk test ($p < .05$) (see table 4.4). According to the nutritional recommendations of 1.2 g/kg BM of protein intake. Adherence of the subjects to your supplementation program was 100%. No changes in body mass index were found for both groups.

Table 4.4- Results Short Physical Performance Battery

	MSE+BS Group 1				BS Group 2			
	Baseline	**Post**	**Z**	***p***	**Baseline**	**Post**	**Z**	***p***
Balance	2.60 ± 0.52	2.90 ± 0.57	-1.732	0.083	1.75 ± 0.71	2.13 ± 0.35	-1,342	0,180
Seat in a chair	23.64 ± 5.99	15.96 ± 1.14	-2.803	0.005	29.96 ± 10,23	26.53 ± 9.50	-1,540	0,123
Chair (pts)	1.00 ± 0.00	2.30 ± 0.483	-2.919	0.004	1.13 ± 0,35	1.50 ± 0.535	-1,732	0,083
3m	6.77 ± 1.85	5.88 ± 0.78	-0.764	0,445	20.72 ± 13.63	9,81 ± 5,85	-2,521	0,012
3m (pts)	2.10 ± 0.57	2.20 ± 0.42	-1.000	0,317	1.00 ± 0.00	1.63 ± 0.518	-2,236	0,025
Total Score	5.80 ± 1.14	7.40 ± 1.17	-2.724	0,006	3.87 ± 1.13	5,25 ± 1,04	-2,428	0,015

4.6- Discussion

Institutionalized elderly people have greater difficulty in maintaining a balanced nutritional status, so a specific nutritional intervention may be

necessary to prevent the onset and development of the frailty syndrome (Hernández Morante et al., 2019). Food rich in proteins is necessary, especially in the elderly population, as it helps building a consistent muscle mass, an important factor for preventing sarcopenia and frailty.

A combination intervention of muscle strength exercise and BCAA supplementation was able to improve the SPBB specific strength test. The sit and stand movements are considered fundamental for mobility and functional independence, since this movements are part of several activities of daily living. The functional action of getting up from a chair, which requires the muscular strength and power of the lower limbs, although seemingly a simple ability, is a functional action that can demand a lot from the elderly (Marzetti et al., 2018). According to Dirks and colleagues (Dirks et al., 2017) protein supplementation was necessary in the frail elderly population in order to increase muscle mass after resistance exercises.

4.6.1- Study Limitation

Although the sample size was small, significant improvements in physical functional fitness, were still found after the exercise plus supplementation intervention.

4.7- Conclusion

Our study revealed that exercise plus BCAA supplementation was able to improve physical fitness function, while BCAA supplementation alone had limited effects. Satisfactory results in physical function could be explained by the added effects of exercise and BCAA supplementation on the protein synthesis effect.

4.7.1- Practical Applications

Our results support the importance of the implementation of specific physical exercise plus supplementation programs designed especially for frail elderly populations. Protein and/or branched chain amino acids supplementation could be important in order to achieve the nutrition recommendations for the elderly in social care institutions.

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CHAPTER V

Effect of a 40-weeks multicomponent exercise program and branched chain amino acids supplementation on functional fitness and mental health in frail older persons

STUDY #3

Caldo-Silva, A., Furtado, G. E., Chupel, M. U., Letieri, Valente, P. A., Farhang, M., Barros, M. P. Bachi, A. L. L., Marzetti, E., Teixeira, A. Massart, A., (2021). Effect of a 40-weeks multicomponent exercise program and branched chain amino acids supplementation on functional fitness and mental health in frail older persons Experimental Gerontology. https://doi.org/10.1016/j.exger.2021.111592

ABSTRACT

Background: The ageing process implies several physiological and psychological changes that hence affect the general health, mood states, and quality of life of older persons. Exercise and adequate nutrition are renowned non-pharmacological strategies that significantly delay and alleviate the adverse consequences of the ageing process. This study aimed to evaluate the effects of branched-chain amino acid (BCAA) supplementation and a multicomponent exercise program (ME) on physical frailty and mood states of older persons. **Methods:** 35 participants (women and men; 83±3 years old) from residential care homes were submitted to a 40-week exercise-washout-retraining intervention (16 wk of exercise and/or suplementation, 8 wk of washout, and 16 wk of exercise and/or resuplementing), with or without BCAA supplementation. The experimental groups were: (i) ME plus BCAA supplementation (ME+BCAA); (ii) ME; (iii) BCAA supplementation (BCAA), and (iv) control group (CG). Fried's phenotype was used to assess frailty prevalence. Geriatric Depression Scale (GDS), Profile of Mood State (POMS), Mini Mental State Examination (MMSE), were used to access mental health and cognition. The Short Physical Performance Battery (SPPB) was used to access functional capacity. Salivary testosterone levels (ST) were also determined. **Results:** Exercise was effective in improving functional capacity and prevented the increase in frailty that occurred in the non-exercising CG, where the frailty scores increased over time ($p<0.01$). BCAAs supplement alone had no impact on functional fitness, but in a short time period (16 weeks) contributed to diminish frailty and combined with exercise may have the potential to reduce the effect of a detraining period on functional capacity. Salivary testosterone levels correlated with hand-grip strength and may be a useful indicator of susceptibility to frailty. No effects were found for mood states, cognition and depression. **Conclusion:** This study shows that a long-term exercise program, independently of being multicomponent or elastic band-based, was effective in improving functional capacity and prevented an increase in frailty in frail and pre-frail older persons living in residential care homes.

Keywords: Multicomponent Exercise, Testosterone, Protein Supplementation, Frailty, Longitudinal study.

5.2- Introduction

Ageing is a natural degenerative process, which dramatically increases the risk of many diseases in older populations (Franceschi et al., 2018). The sedentary lifestyle, per se, is one of the most important contributors to age-related illness, whereas regular exercises – based on hormesis principles – could chronically revert the ageing dysfunctions (Hayes, 2007).

The physical frailty syndrome (PFS) is defined as an ageing-related multifactorial clinical condition marked by a progressive decline of multiple physiological domains that compromise the individual capacity to withstand stress (Fried et al., 2001). PFS can be assessed by evaluating 5 factors: lean mass loss, diminished handgrip strength, reported fatigue/exhaustion, reduction of walking speed, and low physical activity levels (Angulo et al., 2020). Physical exercises also provide protection against frailty and cognitive impairment in advanced aged people (Bherer et al., 2013). Interventions that might induce a positive impact on muscle mass in older persons could also represent a supporting treatment for improving mental health. In fact, there is a growing body of evidence that support the involvement of shared pathophysiological pathways that link sarcopenia and common mental disorders (Pasco et al., 2015).

Ageing is characterized by reduced synthesis of hormones, including growth hormone, estrogen, dehydroepiandrosterone (DHEAS), thyroid hormone and testosterone (Perrini et al., 2005). Serum levels of these hormones are important indicators of the overall degeneration processes occurring in physiological systems during ageing, Lower testosterone levels are associated with the decrease of muscle mass and strength (an essential cause of sarcopenia), which, therefore, may contribute to the progress of frailty in older persons (Srinivas-Shankar et al., 2010).

Older persons malnutrition is a concern in health systems around the world, since it carries a high risk of ageing comorbidities and increased health costs (Roberts et al., 2019). Indeed, nutritional supplementation with vitamins, antioxidants, and protein components (including isolated amino acids) have already demonstrated positive results against frailty, cognitive impairment, sarcopenia and other age-related disorders (Gómez-Gómez & Zapico 2019). Supplementation with BCAAs, especially in association with regular exercise, was demonstrated to improve muscle strength and cognitive functions in older

persons, thus comprising a safe and low-cost strategy to circumvent the harmful effects of ageing (Ko et al., 2020).

Regarding the decline of body muscle mass in PFS, Branched-Chain Amino Acids (BCAAs), especially L-Leucine, are considered efficient nutrients to induce positive adaptative muscle responses, upon the stimulus provided by physical exercise (Yanai, 2015). Accordingly, BCAAs supplementation has been shown to mitigate the loss of muscle mass, stimulate anabolic responses and elicit an effective muscle restricting, especially in older persons (Fujita & Volpi, 2006).

An adequate protein intake is essential for efficient muscle protein turnover, but also, to maintain physical function in older persons (Rondanelli et al., 2011). In general, amino acid supplementation represents a suitable strategy to attenuate and/or manage some specific age-related pathologies, such as chronic inflammation myopathies, and muscle catabolic state (Dato et al., 2018).

Moreover, interventions that might induce muscle mass increase in older persons were also shown to represent a supporting treatment for concomitant mental health improvement (Gariballa & Alessa 2020). Therefore, strategies that improve the physical and mental aspects of older persons, such as exercise programmes designed for this population, could improve the well-being and mitigate the adverse effects of family abandonment, depression and other psychosomatic disorders (Monteiro-Junior et al., 2017; Portugal et al., 2013).

The positive impact of assisted and regular (moderate) exercise programs on physiology and cognition prompted us to ask some questions on the putative coadjutant role of BCAA in the process. Can BCAA supplementation and multicomponent exercises promote better physical and mental function in older persons? Therefore, the aim of this study is to evaluate the effect of a long-term multifactorial exercise program and/or BCAAs supplementation (including detraining/washout period) on functional capacity, depression, mood state, cognition, and testosterone levels (here as a biomarker of sarcopenia) in frail older persons living in residential care homes.

5.3- Methods

5.3.1- Preliminary Procedures and Ethics

All subjects volunteered to participate in the exercise and/or the supplementation interventions. Consent forms were signed by the Residential

Care Homes (RCH) directors, the participants and their legal representatives before testing and intervention. This study was approved by the Ethical Committee of Faculty of Sport Sciences and Physical Education, University of Coimbra (reference number: CE/FCDEFUC/00282018), respecting the Portuguese Resolution (Art.°4th; Law no. 12/2005, 1st series) on ethics in human research and the Helsinki's Declaration (Braga, 2013). This study was properly registered with clinicaltrials.gov register NCT04376463 and is a complementary part of the recently published article (Caldo-Silva et al., 2021).

5.3.2- Participants Eligibility

The eligible criteria for the participants at the time of first screening in this study were: (i) Participants had to be 70 years old or more; (ii) physically frail or pre-frail, but not presenting morbid obesity (BMI ≥ 40); (iii) clinically stable (physically and mentally) and with their drug therapy updated; (iv) being able to perform the Time Up and Go test in ≤50 s (longer durations indicate severe mobility dependence) (Guralnik et al., 1994) (v) not participating in other structured regular physical exercise programs; (vi) not reporting any type of health condition or use of specific medication that might prevent the functional self-sufficiency test performance or attention impairment; (vii) not reporting chronic mental disorders or hearing/visual impairment that could interfere with the evaluations and activities proposed, according to the RCH medical staff. At the end of the recruitment process, 80 older persons from different RCH entered the enrolment phase.

5.3.3- Participants Allocation

All the participants were selected through a non-probabilistic trial (plus controlled sampling) based on the geographical area of Coimbra, Portugal, living in public and private RCH or frequenting day centres in the local community. From the 80 participants initially screened, 50 eligible participants were allocated to their respective intervention groups. However, for the specific reasons highlighted in Figure 1, only 35 participants (age = 83±3 years-old) completed the 40 weeks multifactorial intervention, who were divided in the following groups: Multicomponent exercises (ME, n = 7), Multicomponent exercises plus BCAA supplementation (ME+BCAA, n = 8), BCAA supplementation (BCAA, n = 7), and

the no-regular exercise/no-supplementation control group (CG, n = 13). The procedures were performed according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Begg et al., 1996).

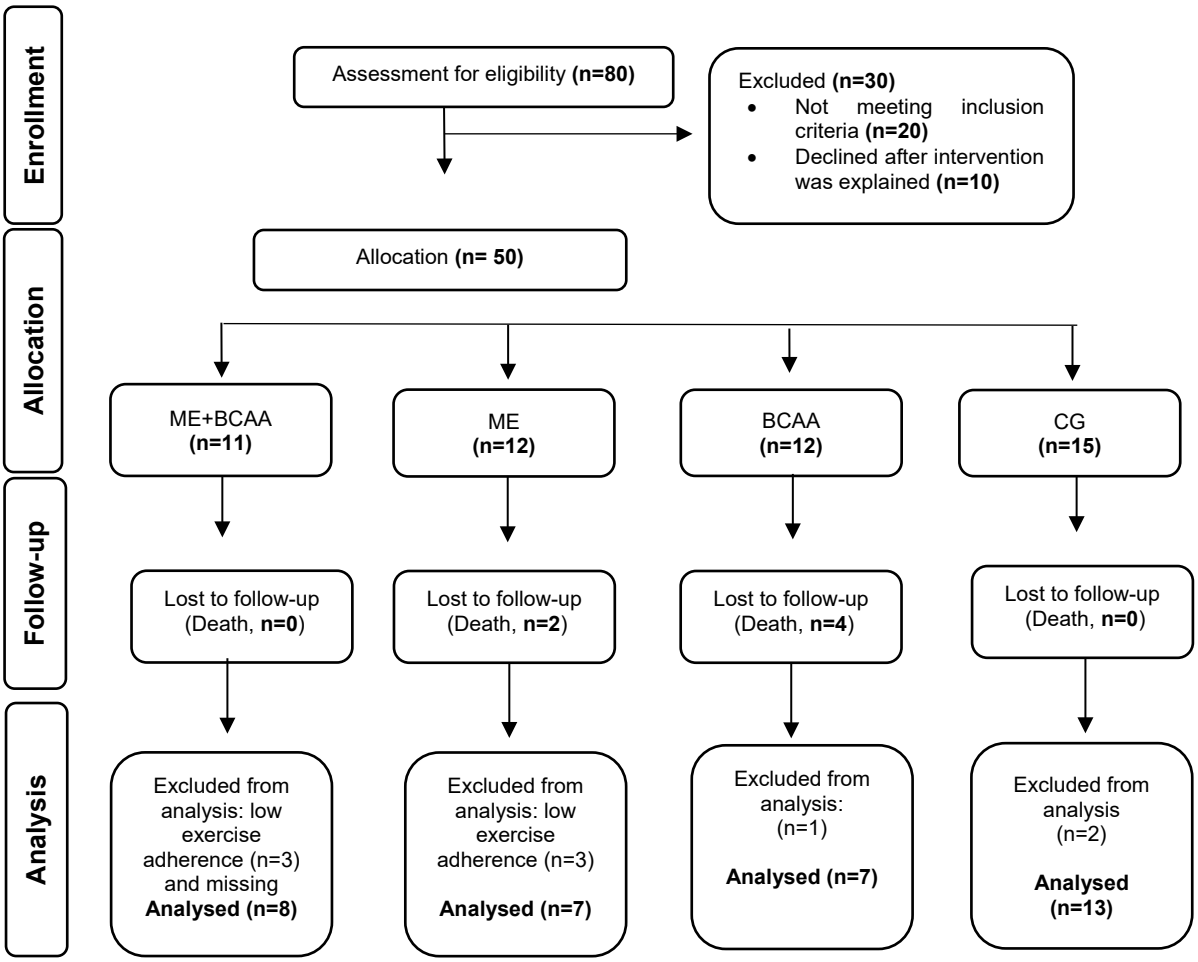

Figure 5.1- CONSORT Flowchart of study participants.

5.3.4- Experimental Design

This study is a four-phase prospective, naturalistic, controlled clinical trial with four arms of a multifactorial intervention program (MIP) experimental design, composed of regular exercises and supplementation interventions (ME+BCAA, ME, BCAA, and CG). In the first phase, a baseline data collection (T1) was done followed by 16 weeks of intervention and a second data collection (T2). This sencond phase was followed by 8 weeks of both an exercise and supplementation washout phase. Phase 3 consisted of a third data collection (at

the end of the washout period), followed by the resumption of the exercise/supplementation intervention for an additional period of 16 weeks. Finally, the last data collection took place after 16 weeks of the second intervention (T4) (Figure 2).

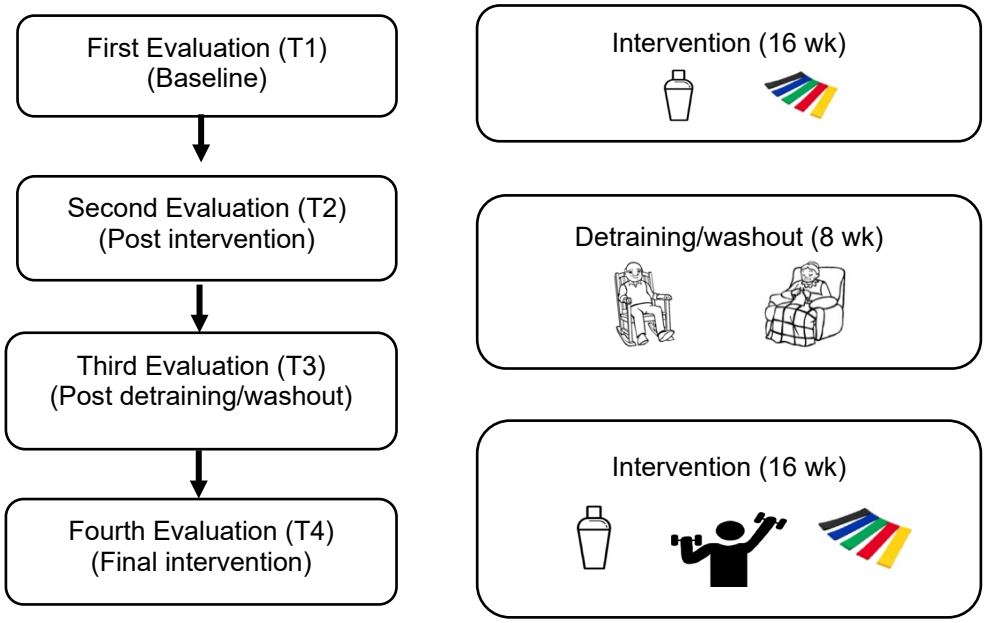

Figure 5.2- Chronological order of multifactorial interventions study design. T1 to T2 (elastic-band exercise, 16 weeks, T2 to T3 (washout, 8 weeks), T3 to T4 (multicomponent exercise, 16 weeks).

5.4- Outcomes Measures

All the assessments were performed in the morning, between 10:00 am and 11:45 am. One session was used to apply a short test battery to measure biosocial, global health status, cognition profile, nutritional, physical, and physical frailty status and to collect saliva samples.

5.4.1- Physical Frailty Criteria

The phenotype of Fried's physical frailty index was used (Fried et al., 2001). Weight loss was assessed by a self-report of unintentional weight loss of 4 kg or more in the last 6 months.

Self-reported exhaustion was evaluated by a negative concordance of questions number (7- *"I felt that everything I did was an effort"*) and (20-*"I could*

not get going") of the Center of Epidemiologic Studies for Depression (CESD) scale (Gonçalves et al., 2014).

Hand-grip strength (HGS) was assessed (in kg) using a hand-held dynamometer (Lafayette 78,010, Sagamore, United States). The best result of the two trials was used for scoring purposes. Participants who were unable to perform the handgrip strength test and those in the lowest 20% tier were categorized as positive for low HGS (Syddall et al., 2003). The cut-off reference values for HGS of ≥29 kg for male and ≥17 kg for female were adopted.

Slowness was measured by the "15 feet (4.6 m) walking test". Based on the cut-off values of Fried's study population, the times of ≥7 s for males and ≥6 s for females were adopted for positive scores of slowness. The best time of the two trials was used for the final scoring.

Low physical activity (PA) levels were assessed by the International PA Questionnaire short version (IPAQ-SV) (Campaniço, 2016). There are three levels of PA suggested for classification: Inactive, minimally active, and highly active. Participants classified as inactive had a positive score for this physical frailty component. A positive evaluation in one or two criteria classified the participants as pre-frail, in three or more criteria as frail, and as non-frail when the subject did not score in any of the five physical frailty indicators. A frailty total score was calculated, and the physical frailty prevalence accessed.

5.4.2- Nutritional Assessment

Daily diet at the RCH was prescribed by a registered nutritionist and was provided for all the participants without any change or interference of the research staff. On the basis of the information provided, the diet was analysed using specific tools (photographic quantification of portions, food table) for the Portuguese population (Torres et al., 2016; Goios, 2016; INSA, 2006 and 2016). Due to the relationship between the frailty status and severe decrease of muscle mass (or sarcopenia), the objective of this nutritional assessment was to characterize the protein consumption of the participants. In addition, the Mini Nutritional Assessment (MNA) questionnaire was applied (Vellas et al., 1999; Loureiro, 2008). The MNA evaluation contains 18 questions accounting for a maximum score of 30 points, and classifies the participants as malnourished (≤17

points), at risk of malnutrition (17 < MNA < 23.5 points), and as having a normal nutritional status (MNA > 23.5 points).

5.4.3- Physical Function

The Short Physical Performance Battery (SPPB) was applied to evaluate the physical function of the participants. It is a test battery based on the performance of lower limb function designed for older persons. It consists of three assessments: (i) the Balance Test, (ii) the Walking Speed Test; and (iii) the Chair Standing Test. The SPPB is scored from 0 to 4, with a score of 0 representing inability to carry out the test, and 4 the best performance. For balance, the participants were asked to maintain their feet side-by-side, in semi-tandem and tandem positions for 10 seconds each. For gait, a 3-m walk at the participants' usual speed was timed. For the chair stand test, participants were asked to stand up and sit down five times as quick as possible (Guralnik et al., 1994).

5.4.4- Clinical and Health Status

The Charlson Comorbidity Index (CCI) was used to classify comorbid conditions based on personal scores combined with age and gender to achieve a single index (Charlson et al., 1994). Anthropometric assessment, including body mass and stature, was performed based on the standardised procedures described elsewhere (Lohman et al., 1992). This assessment was determined using a portable scale (Seca®, model 770, Germany) with a precision of 0.1 kg; stature was determined using a portable stadiometer (Seca Body meter®, model 208, Germany) with a precision of 0.1 cm. Body mass index (BMI) was calculated according to the formula (BMI= body mass/stature^2).

5.4.5- Assessment of Mood State and Depressive Symptoms

The Geriatric Depression Scale (GDS), adapted to the Portuguese population by Apóstolo (2011), was used to access the level of depression in the participants (Yesavage et al., 1982). The GDS evaluation consists of 15 yes/no questions, which allows the classification of the psychological condition related to depression and its symptoms. Total GDS scores within the [0 – 5] points range indicate normal psychological condition (no symptoms of depression), whereas,

6 to 10 points indicate mild depressive symptoms, and 11 to 15 points indicate symptoms of serious depression.

The Profile of Mood State questionnaire (POMS) (McNair, 1971) was used to evaluate the participants mood state, using the validated version for the Portuguese population (Viana, Almeida, & Santos, 2001). The POMS questionnaire consists of 22 Likert-type questions, divided in six dimensions with scales from 0 to 4. The final score consists of a sum of all negative dimensions (Tension-Anxiety, Depression-Melancholia, Hostility-Anger, Fatigue-Inertia, Confusion) subtracting the positive dimensions (Vigour).

5.4.6- Global Cognition- Mini Mental State Examination

The Portuguese version of the Mini Mental State Examination (MMSE) was used (Morgado et al., 2009). The MMSE is a 30-point scale instrument that evaluates five domains of cognition: orientation, immediate recall, attention-calculation, delayed recall, and language. It is generally used to track dementia. It is also used to estimate the severity of cognitive loss at a specific time (Folstein, 1975). This scale classifies individuals by progressive cognitive skills: (0–9 points) severe cognitive impairment; (10–18 points) moderate cognitive impairment; (19–24 points) mild cognitive impairment; and (25–30 points) normal cognitive profile (Pezzotti et al., 2014).

5.4.7- Salivary Testosterone

Non-fasting saliva samples were collected by passive drool, with the participant with the head and trunk lowered for a period of 3 minutes to facilitate the collection, always at the same time in the morning (between 10:00 a.m. and 11:00 a.m.) to minimize the circadian effect of the markers under study (Papacosta and Nassis, 2011). Before the saliva collection (approximately 20 minutes), subjects were asked to rinse their mouth with water to remove any food residues. Participants were instructed to avoid the ingestion of alcohol for 12 hours, dairy products for 20 minutes, foods with high sugar or acidity, or high caffeine content immediately before sample collection. All participants were also instructed not to engage in extreme physical efforts 24 hours before the collection. Saliva samples were stored in a polypropylene tube to avoid contamination and retention of samples and then centrifuged, stored and frozen

at -20º C for further analysis. Salivary testosterone (ST) concentration was determined by competitive ELISA (Salimetrics, UK) according to the manufacturer instructions. The intra-assay coefficient of variability was 2.19%.

5.4.8- Full Characterization (MIP)

The physical exercise program and BCAA supplementation protocol were previously published by (Caldo-Silva et al., 2021).

5.4.8.1- BCAA Supplementation

The BCAA power mixture, a 5 g portion accounting for 20 kcal, was composed of L-leucine (Leu), L-isoleucine (Ile), and L-valine (Val) in the proportion of 2:1:1 (MyProtein®, Cheshire, UK). We opted for an unflavored supplement in order to avoid any ingestion preferences for specific flavors between participants. The BCCAs portion were diluted in 200 mL of water and given immediately after the exercise sessions to the participants of the ME + BCAA and BCAA groups (Ispoglou et al., 2016). The supplement dose was fixed at 0.21 g total BCAA/kg/session, as individual portion sachets, administered in the morning, between 09:00 and 11:30 a.m. (Negro et al, 2019).

5.4.8.2- Elastic-Band Exercise Intervention (Phase 1)

The exercise program was divided in two interventions of 16 weeks each, separated by an 8-week detraining (washout) period. Exercise sessions were offered twice a week, with an interval of 36 hours for adequate physiological recovery and rest. The exercise protocol respected the guidelines for exercise prescription for older persons and the guidelines for exercise periodization by the American College of Sports Medicine (Nelson et al., 2007; de Souto Barreto et al., 2016). The program started with an adaptation period of 2 weeks, in which seven different exercises were performed using elastic bands (TheraBand®, Hygenic Corporation, Akron, OH, USA). The participants were closely supervised for two initial sessions aiming for equipment familiarization and adjustments to the Rating Perceived Exertion scale (RPE OMNI) (Colado et al., 2018). During these familiarization sessions, the participants learned the correct technique of the exercises, and selected the proper colour, length, and grip width of the elastic bands. The exercise intensity was indirectly calculated using the Karvonen’s

formula to predict the target heart rate (HR), with HR_{max} being calculated by an adjusted formula for older persons (Tanaka et al., 2001).

$$HR = [(HR_{max} - \text{resting HR}) \times \%\text{Intensity}] + \text{resting HR}$$

After the adaptation period, the exercise program was progressively intensified by increments in both the number of exercises (from 8 to 10 exercises during the rest of the exercise intervention) and the proposed physical effort, imposed by different intensity color bands, according to the OMNI scale (Colado et al., 2018). The elastic-band exercises applied in the Phase 1 period are shown in Table 1. For safety reasons, the exercise programs were also monitored using heart rate monitors (Polar M200; Polar Electro Oy, Kempele, Finland), and calculated by the Karvonen´s formula (HR = 220 – age).

Additionally, intensity was measured through the specific rating perceived exertion (RPE) scales for each exercise program (Borg, 1982). The RPE used is an arbitrary scale ranging from 0 to 10 points, with identical intervals and with reference to the quality of effort: (0) Nothing at all; (1) very weak; (2) weak; (3) moderate; (4) somewhat strong; (5–6) strong; (7–9) very strong; (10) very, very strong (almost maximal).

Table 5.1- Example of Elastic-Band Exercises Session Applied in Phase 1

Warm-up				5 minutes	PSE 1-3	**Progression**	**Weeks**	**Intensity (colour)**
Exercises (8-10)	**Sets**	**Repetitions**	**Cadence**	**Interval**	**PSE**	2x10	2	Yellow
Front squat	2-3	10-20	2:3	30-45 seconds	4 to 6	3x20	2	Yellow
Chair unilateral hip flexion	2-3	10-20	2:3	30-45 seconds	4 to 6	3x10	2	Red
Chair Bench over row (with flexion)	2-3	10-20	2:3	30-45 seconds	4 to 6	3x20	2	Red
Chest Press (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6	3x10	2	Green
Standing (or chair) reverse fly	2-3	10-20	2:3	30-45 seconds	4 to 6	3x20	2	Green
Shoulder Press/twist arm position	2-3	10-20	2:3	30-45 seconds	4 to 6	3x15	2	Blue
Chair (or stand) frontal total raiser	2-3	10-20	2:3	30-45 seconds	4 to 6	3-4x10-15	2	Blue
Biceps arm curl (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6			
Chair Overhead triceps extension	2-3	10-20	2:3	30-45 seconds	4 to 6			
Cooling down				5 minutes	PSE 1-2			

Note: PSE- Perception Subjective Effort

5.4.8.3- Washout ME and BCAA period (Phase 2)

After 16 weeks of Phase 1, the participants endured a detraining period of 8 weeks, when the ME programs and BCAA suplemmentation were suspended. The aim was to check if the physiological adaptations acquired during the first

phase of ME were maintained (Sakugawa et al., 2019) or if a 8-weeks interruption was able to revert the possible effects on mood state.

5.4.8.4- Multicomponent Exercise (Retraining Protocol- Phase 3)

The phase 3 (exercise retraining) protocol was also based on the resistant TheraBand elastic bands (Table 1), but included walking, steps, and balance exercises (sometimes with dumbbells and ankle/wrist weights) to compose a multicomponent exercise program for an identical 16 week-period (twice a week, alternate days, also totaling 32 sessions). BCAA supplementation was restored as described in Phase 1. The multicomponent program (Table 5.2) was properly described by Furtado and colleagues (Furtado et al., 2019). The phase 3 program aimed to reproduce most of the daily activities of the participants in this study (Baker et al., 2007).

Table 5.2- Multicomponent Exercise Program (ME)

Exercises (8-10)	Sets	Repetitions	Cadence	Interval	PSE
Front squat	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair unilateral hip flexion	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair Bench over row (with flexion)	2-3	10-20	2:3	30-45 seconds	4 to 6
Chest Press (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6
Standing (or chair) reverse fly	2-3	10-20	2:3	30-45 seconds	4 to 6
Shoulder Press/twist arm front position	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair (or stand) frontal total raiser	2-3	10-20	2:3	30-45 seconds	4 to 6
Biceps arm curl (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair Overhead triceps extension	2-3	10-20	2:3	30-45 seconds	4 to 6
Circuit training					
Walking around the room	2-3	3 minutes		30-45 seconds	4 to 6
Balance/ agility exercise	2-3	3 minutes		30-45 seconds	4 to 6

Note: PSE- Perception Subjective Effort

5.5- Statistical Analysis

The Shapiro-Wilk test was used to verify the normality distribution of the data and log transformed when this was not present. Descriptive values are expressed as mean ± standard deviation. To compare the mean changes over time between groups, repeated measures ANOVA (4x4 group vs. time) were performed. Bonferroni´s post hoc analysis was performed for paired comparisons of means when significant interactions were found in the dependent variables (ST, SPPB, POMS, MMSE, GDS and Fried). The Pearson correlation was used to access the correlation between salivary testosterone levels and HGS. The level of significance was set at $p<0.05$. All statistical analysis was done using IBM SPSS Statistics version 23.0 (Armonk, NY: IBM Corp, USA).

5.6- Results

The selected group of participants in this study closely represent the typical population living in the residential care homes of central Portugal: octogenarian people, at risk of malnutrition, a certain extent of physical disability and the presence of mild cognitive impairment (Madeira et al., 2016). Characterization of our sample at baseline (see table 3) revealed that 85,7% of the participants were physically frail, 14,3% pre-frail, 60% had mild cognitive impairment, while 57% were both physically and cognitively frail.

Table 5.3- Baseline Levels Characterization of all participants

Characteristics	**All sample (n=35, 100%)**	**%**	**Men (n=14, 39%)**	**%**	**Women (n=21, 61%)**	**%**
Age (years, M±SD)	83±3		81±6		85±5	
Height (m)	1.56±0.10		1.65±0.59		1.50±0.73	
Weight (Kg)	70.2±11.9		77.7±8.5		65.2±11.3	
Body Mass Index (kg/m^2)	28.7±4.5		28.4±3.7		28.8±5.1	
MNA (score, 0-30 points)	24.2±2.6		24.9±2.2		23.7±2.7	
CCI (score, 0-10 points)	5.08±1.12		4.57±1.22		5.42±0.92	
Low CCI (≤ 5 points)	n=21	78.6%	n=11	41.17%	n=10	37.43%
High CCI (≥ 5 points)	n=14	21.4%	n=3	4.59%	n=11	16.81%
Polypharmacy (day, M±SD)	7.2±1.6		5.4±4.1		4.9±4.8	
Time in residential care (years, M±SD)	4.5±0.6		3.7±1.4		4.5±1.0	
Schooling time (years, M±SD)	4.0±0		4.0±0		4.0±0	
Physical Frailty index (n; M±SD)	2.42±0.88		2.36±0.84		2.48±0.98	
Frail (3-5 points)	(30) 2.67±0.7	85.7%	n=12	34.28%	n=18	51.42%
Pre-Frail (1-2 points)	(5) 1±00	14.3%	n=2	5.72%	n=3	8.58%
Robust (0 points)	0	0	0	0%	0	0%
MCI by MMSE (n; M±SD)	22.88±3.61		22.85±3.34		22.90±3.87	
MCI (19-24 points)	(21) 20.4±2.1	60%	(9) 21.0±2.44	24%	(12) 19.9±1.83	36%
NC (25-30 points)	(14) 26.6±1.4	40%	(5) 26.2±1.6	16%	(9) 26.9±1.3	24%
Both MCI and PF	n=20	57.1%	n=8	22.84%	n=12	34.26%
SPPB (0-12 points)						
(0-3) Very poor functional status	n=6	17.1%	n=2	5.70%	n=4	11.40%
(4-6) Low functional status	n=28	80%	n=11	31.43	n=17	48.57%
(7-9) Moderate functional status	n=1	2.9%	n=1	2.9%	n=0	0%
(10-12) Good functional status	n=0	0%	n=0	0%	n=0	0%

Notes: M = mean; SD = Standard deviation; MNA = Mini Nutritional Assessment; CCI = Charlson Comorbidity Index; MMSE = Mini Mental State Examination; SPPB = Short Physical Performance Battery; MCI = Mild Cognitively Impaired; Normal cognition; Physically Frail = PF

Drop-out from the study was mainly caused by the unexpected relocation of participants to other institutions, low adherence to the exercise protocol and death.

There were no adverse effects resulting from the interventions (exercise or supplementation), except for one case of diarrhea after the first supplementation,

from which the participant rapidly recovered and continued with the protocol as normal.

There was a significant effect of time (F(df: 3, 9)=9.925, p=0.000) but not for time*group interaction (p>0.05) for changes observed in SPPB scores. Even at baseline, SPPB differences emerged between ME+BCAA and BCAA (p=0.007) and between ME+BCAA and CG (p=0.002). The increase in the SPPB score observed for ME+BCAA over the first period of intervention (p<0.01) was maintained during the washout period and remained until T4. The ME group also showed higher SPPB scores between T1 and T2 (p=0.02), but a decrease was observed between T2 and T3 (p=0.04), returning to the T2 levels after the reintervention period (between T3 and T4, p=0.03). No significant differences were found for the BCAA and CG groups over time (p>0.05).

Regarding the Fried frailty score, there was no effect of time alone (p>0.05). However, an effect for the interaction time*group was observed (F(df: 7.088, 73.248)=3.862, p=0.001). No differences emerged between groups at baseline, T2 and T3 (p>0.05). Nevertheless, after the reintervention period (T4), controls were different from the ME+BCAA group (p<0.01) and ME (p=0.01). Within groups comparison using the Bonferroni adjustment, the Fried frailty score only decreased in the BCAA supplemented group between T1 and T2 (p<0.01), while it increased between T1 and T4 for the CG (p<0.01).

Although no time*group interaction (p>0.05) was observed in the GDS score, the reported data showed a significant effect of time (F(df: 3, 93)=3.054, p=0.03) . Despite no differences at baseline or even within groups over time were observed for all interventions, changes between groups emerged in the follow-ups. Bonferroni comparisons showed that scores for GDS in the ME+BCAA and BCAA groups were significantly different at T2 (p=0.01) which was sustained throughout the further evaluations (p<0.05). After washout (at T3), the GDS score for ME+BCAA group was also different compared to ME (p=0.03), while no difference emerged between the CG and the other groups, for all evaluations (p>0.05).

Significant effects of time (F(df: 2.188, 67,826)=5.026, p=0.008) and time*group interaction (F(df: 6.564, 67,826)=3.005, p=0.01) were found for POMS scores. At baseline (T1), the ME+BCAA group presented significant higher POMS scores in comparison with all other groups (p<0.05). Within

comparisons using Bonferroni adjustment showed, however, that those changes were observed only in the ME between T1 and T3, T2 and T3, and T3 and T4 ($p<0.01$ for all comparisons). Despite small variations in all other groups, no significant changes emerged over time ($p>0.05$).

There was an effect of time ($F(df: 2.184, 67.705)=6.457$, $p=0.002$) but not for time*group interaction ($p>0.05$) on MMSE indexes. At baseline, the average score of the cognitive index MMSE in the ME+BCAA group was significantly higher than those from the ME ($p=0.029$) and BCAA ($p=0.023$) groups. The differences in MMSE scores remained between ME+BCAA and BCAA groups at T2 ($p<0.01$) and T3 ($p=0.04$). However, no differences between interventions were observed for this parameter overtime ($p>0.05$).

There were no effects of time ($F(df: 3, 57)=1.712$, $p=0.175$) or time*group interaction ($F(df: 9, 57)=1.383$, $p=0.217$) for testosterone (ST) in all groups. A similar unresponsive pattern was also observed for HGT between interventions: no significant effects of time ($p=0.145$) or time*group interaction ($p=0.066$). However, correlations between ST levels and HGT emerged in all-time points, with correlation indexes of $r = 0.414$, $r = 0.389$, $r = 0.394$, and $r = 0.385$ for T1, T2, T3 and T4, respectively ($p<0.05$).

Table 5.4- Statistical Analysis T1-T4 Handgrip Test and Salivary Testosterone

		Time points of evaluation				Effect	F	*p*
	Groups	**T1**	**T2**	**T3**	**T4**			
		M ± SD	**M ± SD**	**M ± SD**	**M ± SD**			
Handgrip Test (HGT) (kgf)	**ME+BCAA**	15.7±2.4	16.5±1.6	16.5±1.7	16.7±2.3			
	ME	13.4±6.6	14.3±5.0	12.2±5.4	14.3±4.9	Time	1.841	.145
	BCAA	18.4±7.9	17.1±7.5	16.3±7.9	16.2±8.2	Time*group	1.870	.066
	CG	16.1±5.3	15.3±5.2	15.0±5.2	14.5±4.5			
Testosterone (pg/mL)	**ME+BCAA**	55.7±38.6	39.3±26.7	54.6±47.2	52.9±35.3			
	ME	47.9±42.1	48.9±18.3	58.6±33.6	67.6±20.9	Time	1.712	.175
	BCAA	58.0±27.9	62.3±54.1	51.7±33.4	62.2±39.0	Time*group	1.383	.217
	CG	53.2±30.8	64.7±48.8	60.8±35.1	69.5±52.0			

Notes: M±SD = mean (standard and deviation); ME = multicomponent exercise; BCAA = branched-chain amino acids; *T1 to T2 (elastic-band exercise, 16 weeks, 8 weeks), T2 to T3 (washout) T3 to T4 (multicomponent exercise, 16 weeks).

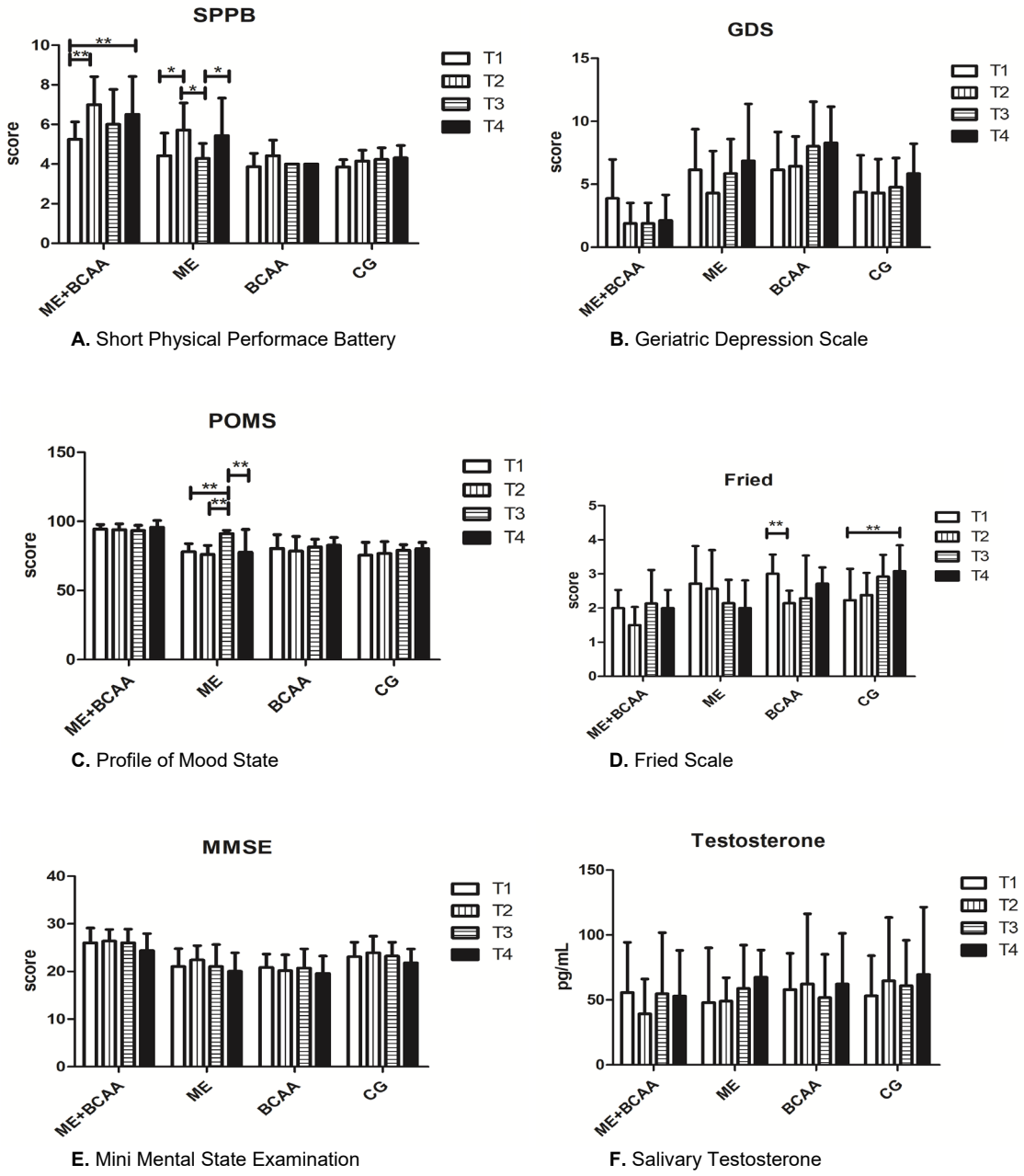

Figure 5.3- Time-points assessments of pre- and post-intervention program; ME+BCAA: multicomponent exercise + BCAA supplementation; ME: multicomponent exercise only; BCAA: supplementation only; CG: control group. * Significance at p<0.05; ** significance at P<0.01. Significance in the graph is only represented for within-group comparisons. Figure **A-**Short Physical Performance Battery (SPPB); **B-** Geriatric Depression Scale (GDS); **C-** Profile of Mood State (POMS); **D-** Fried scale; **E-** Mini-Mental State Examination (MMSE); **F-** Salivary Testosterone (ST).

5.7- Discussion

Despite our tentative to match the groups at baseline, because of the loss of subjects, logistic issues (many selected HCR) and low adherence in some of

the groups, the ME+BCAA group presented a better initial profile than the other groups, especially regarding SPPB, POMS and MMSE scores. It also presented the lowest level of initial frailty. However, this group also showed positive achievements along the study, with a significant improvement in physical performance, a tendency for a decrease in depression and frailty, and the maintenance of the mood state, cognition and testosterone levels. In comparison with the control group – which showed significant increases in frailty and a tendency of depression along the study – the ME+BCAA group did not present any deterioration with time, highlighting the potential of this combined treatment for the prevention of physical and mental health deterioration in older persons. In fact, considering that the total Fried score is supported by parameters related to the level of physical activity, gait efficiency and perception of fatigue, all these aspects were positively affected by the practice of physical exercise. As protein intake also is required to induce positive benefits in muscle mass (Tieland et al., 2012) it seems clear that BCAA supplementation could affect the total Fried score in the older persons.

Regarding the physical performance evolution in the exercise only (ME) group, the positive influence of the exercise is clear with significant improvements in the two moments after the exercise periods (T2 and T4), and a significative decrease in the washout period, demonstrating the efficacity of the two exercise programs and how important is exercise regularity for older persons. Because no effect on SPPB was found in the BCAA alone group, exercise is probably the unique influencer on this parameter in the ME+BCAA group. It is possible that the BCAA supplement may have contributed to an attenuation of the detraining effect, since the ME group presented a significant SPPB decrease in the washout period, but the ME+BCAA group did not. Strength exercise has been proposed as one of the most effective methodologies and with better results in the common tasks of the daily life of older persons, focusing on the optimization of neuromuscular function to obtain better benefits (Cadore et al., 2014).

Multicomponent exercise programs have been demonstrated to result in major improvements in functionality capacity, which is key for maintaining independence and the ability to perform basic activities of daily living (Casas-Herrero et al., 2019, Angulo et al., 2020).

Our study did not confirm any superiority of the multicomponent exercise on the elastic band only exercise, both programs presenting significant improvements on exercise performance, as it is recurrently presented for exercise in older persons (Gine-Garriga et al., 2014). However, Ikeda et al. (2016) showed that a combined program of physical exercise (strength, aerobic, balance), and BCAA supplementation, twice a week, for 3 months, achieved better results than exercises performed alone. Our study failed to show any effect of the BCAAs supplementation alone on exercise performance. In contrast, one study (Ispoglou et al., 2016) that evaluated in sedentary older persons the effect of a daily BCAAs supplementation during 12 weeks, and compared it to a placebo, obtained significative functional performance increases. More recently short time effects of BCAAs supplementation alone on exercise performance in older persons have been identified (Ko et al., 2020). Differences in the frequency and dosage of the BCAA administration may have, in part, contributed for such contrasting results. However, a recent meta-analysis review showed no effect of protein supplementation on SPPB scores in frail older persons (Oktaviana et al., 2019). Similar results were found by Amasene and co-workers, who demonstrated that protein supplementation did not enhance functional status of post-hospitalized older persons more than exercise *per se* (Amasene et al., 2019).

To our knowledge, this is probably the first time that the combination of BCAAs and exercise was studied in frail older persons living in RCH during a detraining period. The overall observed tendency of BCAA to decrease the loss of exercise performance under these circumstances may be an interesting topic for future studies.

In our study, frailty in the ME group tended to diminish along time, which possibly highlights the positive influence of exercise. BCAAs supplementation significantly improved frailty but only in the first 16 weeks of treatment. The same tendency was observed in the ME+BCAA group, reinforcing the role of BCAAs supplementation. Since the control group was the only one to show a significant deterioration in the frail condition, we suggest that exercise and BCAA, or a combination of both, could contribute to the prevention of the deterioration of this parameter in older persons in RCH. There are a handful of studies that aimed to investigate whether exercise training could putatively reverse frailty (frail to pre-

frail or non-frail), or, at least, to achieve a lower degree of frailty in older persons (Manãs et al., 2019). In this respect, studies demonstrated that a physical exercise program was able to reduce frailty by improving the functional capacity, muscle strength, speed, and agility of older persons (Ferreira et al., 2018). There is a consensus in the literature that physical exercise training has the potential to provide protection against frailty in advanced aged people (Higueras-Fresnillo et al., 2018), and most of these benefits of physical exercise, at least in part, are related to the improvement of several immune inflammatory parameters (Aguirre & Villareal, 2015; Gleeson et al., 2011; Petersen & Pedersen, 2005).

BCAA supplementation has been verified to increase plasma and muscular BCAA concentrations, increasing substrate availability for protein synthesis. An increase in amino acid transport post resistance training (with a concomitant increase in plasma and muscles) may enhance protein synthesis (Sharp & Pearson, 2010).

The supplementation with BCAA, mainly because of its L-Leucine content, activates a cascade of protein phosphorylation that culminates in muscle protein synthesis through mTOR, with subsequent stimulation of three key ribosomal proteins: kinase S6 of 70 kDA (p70S6K), 4E-BP1, and 4G (eIF4G) (Apró & Blomstrand, 2010). Through insulin dependent and independent pathways, L-Leucine and strength training are potent activators of mTOR, a protein that is involved in increasing the rate of mRNA translation of myofibrillar proteins (Millward et al., 2008; Aguirre, Van Loon, & Baar, 2013).

The use of BCAA in the older persons seems to be very promising in order to increase their quality of life, including those with higher depressive tendencies (Fujita & Volpi, 2006). In community dwelling older persons, the ingestion of a BCAA-rich diet diminished depression symptoms (Gariballa & Forster, 2007), the perception of fatigue and improved performance in a mental task, possibly due to the synthesis of 5-HTTP, a natural amino acid with a similar action as tryptophan (precursor of the serotonin neurotransmitter, associated with a well-being feeling) (Fernstrom, 2005, 2013).

BCAA amino acids may act by direct and indirect means, to increase serotonin synthesis in the brain (Rondanelli et al., 2011). Other mechanisms could include the direct action of BCAA, particularly L-Leucine, in the Central Nervous System (CNS) improving the availability of insulin in the brain, and the

use of amino acids to produce energy and synthesize proteins in the CNS (Aquilani et al., 2008). Whereas no significant changes were observed for the GDS scale between different interventions in this study, it seems reasonable to hypothesize that the applied interventions could modulate differences between groups over time, since a tendency to reduce depression was seen in the exercising groups, with a possible positive effect of exercise but not of BCAAs on depression levels. The positive effect of exercise on depression in older persons is well recognize (Rondanelli et al., 2011; Arent et al., 2000). Differences in frequency and dosage of BCAAs administration may in part have contributed to the difference of results.

An increase in POMS scores were only seen for the ME group at (T3), which probably reflected a decrease in vigour after the washout period. Overall, it is possible to conclude that mood state; cognition; and testosterone remained stable in all groups along with the study, with no effects of the treatments on those parameters. In contrast, for mood state and cognition, other studies have shown positive effects of exercise in older persons (Furtado et al 2020, Monteiro-Junior et al., 2017; Sarid et al., 2010; Smolarek et al., 2016).

Although not very strong, the correlation between ST levels and HGS is also particularly interesting, since in older men and women, low testosterone levels were each independently associated with an increased progressive frailty status (Wu et al., 2010), and are in agreement with data published by other groups (Hsu et al., 2018). Although ST did not vary between groups, as was expected, it is possible that the individual variability and the low sample number may have disguised the mediating effects of exercise on testosterone levels. Also, it is important to highlight that there is no scientific consensus about the testosterone response to exercise, especially regarding older persons. Further investigation to assess the improvements that testosterone could provide in functional fitness and frailty in older persons (Hsu et al., 2018) is warranted.

5.7.1- Study Limitation and Direction for Future Research

The small sample size can undoubtedly limit the power of our results here. Unfortunately, despite the efforts of the research team and the nursing home staff, it was not possible to eliminate the dropout rate observed. However, our results reflect real-world data mimicking what actually happens in RCH, with

participants presenting several disabilities and comorbidities, and all the difficulties associated with older persons motivation to accomplish the proposed goals. The execution of a controlled study over 40 weeks with such population is also subjected to other unforeseen limitations (e.g. death or change of RHC). We suggest that the use of other methods of exercise training, such as the use of playful activities (dance and music sessions) might increase the adherence levels.

5.7.2- Practical Applications

Our results support the importance of the implementation of specific physical exercise programs designed especially for frail older persons. It is crucial for public health to identify the main factors associated with physical frailty for the development of new methods for complementary therapies, such as the use of nutritional tools combined with long-term exercise interventions.

5.8- Conclusion

This study shows that a long-term exercise program, independently of being multicomponent or elastic band-based, was effective in improving functional capacity in institutionalised older persons, as in slowing/preventing the progression of frailty, when compared to a control group without exercise. BCAA supplementation alone had no impact on functional fitness. However, in a short period of time (16 week), BCAA supplementation contributed to diminish frailty. Moreover, the combination of BCAA and exercise demonstrated the potential in reducing the effects of a detraining period on functional capacity. Overall, the intervention periods had no significant effect on the mood state, depression scores, cognitive function, or salivary testosterone levels, but there was a tendency of diminished depression scores in the exercising groups (compared to the increasing tendency in the non-exercising ones). Further research is needed to define the best practices, the feasibility of implementation, the best supplementation strategies and the suitable physical exercise programs for this special population, in order to augment compliance and long-term behaviour maintenance.

Contributors

Caldo drafted the paper. Furtado and Valente helped with data acquisition. Chupel and Letieri statically analysed the data. Teixeira, Massart and Marzetti developed the study the proposal, revised the manuscript critically and suggested additional statistical analyses. Teixeira coordinated the research study and, together with Massart, Marcelo Barros and Andre Bachi revised the manuscript critically. All the authors approved the final version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflict of Interest: The authors declare that there are no conflicts of interest.

Ethical standards: This study was approved by the University of Coimbra, Faculty of Sport Sciences and Physical Education Ethical Committee (reference number: CE/FCDEFUC/00282018), clinicaltrials.gov register NCT04376463

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CHAPTER VI

Effect of Training-Detraining Phases of Multicomponent Exercises and BCAA Supplementation on Inflammatory Markers and Albumin Levels in Older Persons

STUDY #4

Caldo-Silva, A. Furtado, G. Uba-Chupel, M. Bachi, A. Barros, Neves, R. Marzetti, E. Massart, A. Teixeira, AM. (2021). Effect of Training-Detraining Phases of Multicomponent Exercises and BCAA Supplementation on Inflammatory Markers and Albumin Levels in Older Persons. Published March 28 Nutrients ID-1147227.

ABSTRACT

Background: Nowadays, it is accepted that the regular practice of exercise and Branched Chain Amino Acids supplementation (BCAAs) can benefit the immune responses in older persons, prevent the occurrence of physical frailty (PF), cognitive decline, and aging-related comorbidities. However, the impact of the combination of these non-pharmacological interventions in albumin and the inflammatory markers in is not fully understood. Therefore, we investigated the effect of a 40-weeks multifactorial intervention (multicomponent exercise (ME), associated or not with BCAAs on plasma levels of inflammatory markers and albumin of frail older persons (≥ 75 years old) living in residential care homes (RCH). **Methods:** This study consisted of a prospective, naturalistic, controlled clinical trial with 4 arms of multifactorial and experimental (interventions-wahshout-interventions) design. The final groups of the interventions consisted in ME+BCAA (n=8), ME (n=7), BCAA (n=7) and control group (n=13). Lower limb muscle-strength, cognitive profile, and PF tests e were concomitantly evaluated with serum levels of albumin, anti- and pro-inflammatory cytokines (IL-10, TNF-α, and the TNF-α/IL-10 ratio), and myeloperoxidase concentration (MPO) were evaluated at four different time-points: baseline (T1), after 16-weeks of multifactorial intervention (T2) followed by a subsequent 8-weeks of a washout phase (T3) and finally, the study participants were assessed after an additional 16 weeks of intervention (T4). No significant variations were observed over time for TNF-α/IL-10 ($p = 0.703$) or MPO ($p=0.323$) plasma levels. **Conclusions:** Overall, the study showed that ME plus BCAAs, triggered slight, but positive, alterations in the inflammatory and physical function of the RCH participants, that could culminate in independence and higher quality of life for this population.

Keywords: Inflammaging, Cognitive Impairment, Cytokines, Protein Intake, Physical Frailty.

6.2- Introduction

Ageing is characterized as a natural degenerative process strongly linked to diminished immune efficiency, and also to enhanced inflammatory responses, and thus, to higher risks of infections in older persons (Aiello et al., 2019). The sedentary lifestyle, per se, is one of the most important contributors to age-related illness, whereas regular physical exercises (rPE)—based on hormesis principles—could chronically slow down the aging immune/inflammatory dysfunctions (Duggal et al., 2019). In this sense, reduction of systemic levels of interleukin-10 (IL-10), a classical anti-inflammatory cytokine, with elevation on Tumor Necrosis Factor – alpha (TNF-α) levels are associated with aging (Amirato et al., 2021). Although the participation in rPE programs does not stop the progression of aging (Chodzko-Zajko et al., 2009) staying in moderate rPE programs can help making the aging process more rewarding, with lower incidence of premature chronic diseases (Bauman et al., 2016). In addition to the comorbidities outcomes, both aging and the sedentary behavior may speed up the loss of mobility and functional autonomy (Sherrington et al., 2016) reducing the quality of life (Cavalcante et al., 2015), and also increasing the susceptibility to physical frailty (PF) and cognitive decline (Covinsky, Eng, Lui, Sands, & Yaffe, 2003).

The age-related PF syndrome is defined by loss of muscle mass (and sarcopenia), by low physical activity levels, and often accompanied by low protein intake (Fried et al, 2001). Cognitive decline, in turn, is characterized by confusion and progressive loss of memory and neuromotor skills (Rodakowski, Saghafi, Butters, & Skidmore, 2015). However, these two outcomes reveal biological and phenotypic similarities, which is the reason leading to the current scientific interest in investigating populations affected by these disorders (Ruan et al., 2015). In this sense, rPE could also provide protection against both PF and cognitive decline in very old people (Higueras-Fresnillo et al., 2018), with most of these benefits related, at least in part, to changes that occur in the immune system (Gleeson et al., 2011). Recent findings have shown that multicomponent exercise (ME) interventions, those that include different types of endurance, muscle strength, and balance exercises in the same session, appear to have a superior effect on cognitively and physically frail older persons (Tarazona-Santabalbina et al., 2016;Theou et al., 2011).

Participation of older persons in rPE ameliorates not only antigen recognition, but also immune responsiveness in general, as some evidence has shown that increased levels of physical activity using exercise routines can even extend the protection provided by the influenza vaccine in older persons (Woods et al., 2009) as well as a regulation of systemic inflammatory status (Paixão et al., 2021). Apart from the modulating effects of rPE, nutritional habits also play an important role in determining immune and inflammatory efficiency, especially in older persons (Duggal et al., 2019). In fact, malnutrition in older population is a serious concern for health systems around the world, since it increases the risk of comorbidities occurrence with subsequent higher health care costs (Cavalcante et al., 2015; Covinsky et al., 2003). Indeed, nutritional supplementation with vitamins, antioxidants, and protein components (including isolated amino acids) have already demonstrated positive results against PF, cognitive impairment, sarcopenia and other age-related disorders (Abizanda et al., 2016).

Supplementation with BCAA, in the absence of branched-chain aminotransferase (BCAT) activity in the liver implies that a dietary supply of BCCAs would ensure an almost intact passage through the liver directly to the muscle tissue, which seems to be advantagous to restrain sarcopenia and frailty (Goates, Du, Braunschweig, & Arensberg, 2016). Supplementation with BCAA, especially in association with regular exercises, was demonstrated to improve muscle strength and cognitive functions in the older population, which are safe and low-cost strategies to circumvent the general limitations imposed by the aging process (Artaza-Artabe et al., 2016; Ikeda et al., 2016, 2019).

Among several pro/anti-inflammatory biomarkers used in the context of exercise and nutrition sciences (Giannopoulou et al., 2005) myeloperoxidase (MPO) stands out as a valid marker largely released by activated neutrophils, with potent pro-oxidative/pro-inflammatory actions (Loria et al., 2008). MPO activity also appears as a biomarker that was strongly associated with frailty and risk of mortality in a study conducted in a large community-dwelling frail octogenarians and nonagenarians (Giovannini et al., 2010). Recently, a similar intervention demonstrated the slight reduction of serum MPO activity triggered by the combination of Taurine and ME in older persons (Uba-Chupel et al., 2021). Instead, albumin concentrations are currently used for the assessment of the

nutritional status of an individual, and low albumin concentrations have been associated with increased mortality after correlation for age, body mass index (BMI), gender, and several chronic comorbidities (Alcorta et al., 2018). In this sense, multifatorial interventions programs (MIP, exercise plus protein suplmentattion) that target to maintain (or even increase) albuminemia in older persons could characterize an important strategy to diminish the harmful effects of aging and its comorbidities (Abizanda et al., 2016).

Therefore, the aim of this work was to evaluate the effect of a 40-week MIP on plasma/serum pro- and anti-inflammatory markers of the immune system in older persons living in residential care homes (RCH). Furthermore, we hypothesized that ME plus BCAAs may have an impact on the systemic albumin levels, inflammatory variables, cognitive profile, and physical function of the participants.

6.3- Methods

6.3.1- Preliminary Procedures and Ethics

This is a prospective, naturalistic, controlled clinical trial (treatment vs care). All subjects volunteered to participate in the exercise classes or the supplementation programs. Consent forms were signed by the institution's directors, the participants and their legal representatives before testing and intervention. This study was approved by the Ethical Committee of Faculty of Sport Sciences and Physical Education, University of Coimbra (reference number: CE/FCDEFUC/00282018), respecting the Portuguese Resolution (Art.°4th; Law no. 12/2005, 1st series) on ethics in human research and the Helsinki's Declaration. This study was properly registered with clinicaltrials.gov register NCT04376463.

6.3.2- Participants Elegibility

Study participants were selected through a non-probabilistic trial (plus controlled sampling) living in public and private RCH. The eligible criteria for the participants in this study were, at the time of first screening: (i) Participants had to be 70 years old or more; (ii) physically frail and pre-frail; (iii) clinically stable with their drug therapy updated; (iv) being able to perform the Time Up and Go test in ≤50 s that indicate severe mobility independence (Guralnik et al., 1994) (v)

not participating in other structured rPE; (vi) not presenting any type of health condition or use medication that might prevent the functional self-sufficiency test performance or attention impairment (such as severe cardiopathy, hypertension, uncontrolled asthmatic bronchitis or severe musculoskeletal conditions); (vii) not presenting mental disorders or hearing/visual impairment that could prevent the evaluations and activities proposed, according to the institutional medical staff; (viii) not presenting morbid obesity (BMI ≥ 40). At the end of the recruitment process, 80 older persons entered the enrollment phase.

6.3.3- Partipants Allocation

All the participants were selected through a non-probabilistic trial (plus controlled sampling) based on the geographical area of Coimbra, Portugal, living in public and private residential care homes (RCH) or frequenting day centres in the local community. From the 80 participants initially screened, 50 eligible participants were allocated in their respective intervention groups. However, for the specific reasons highlighted in Figure 1, only 35 participants (age = 83 ± 3 years-old) completed the 40 weeks multifactorial intervention, divided in the following groups: ME (*n* = 7), ME + BCAA (*n* = 8), BCAA (*n* = 7), and the no-regular exercise/no-supplementation control group (CG, *n* = 13). All the procedures were performed according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Begg et, 1996).

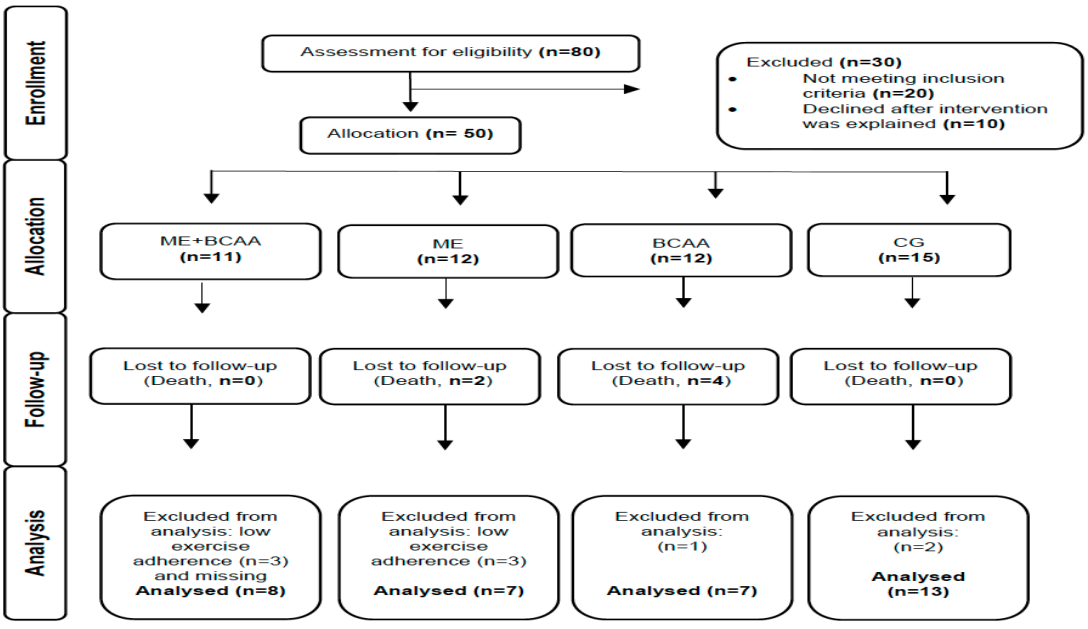

Figure 6.1. Consort Flowchart of Study Participants

6.3.4- Experimental Design

This study is a four-phase prospective, naturalistic, controlled clinical trial with four arms of MIP experimental design (ME + BCAAs, BCAAs, ME, and CG). In the first phase, a baseline data collection (T1) was done followed by 16 weeks of MIP. The second phase consisted of a second data collection (T2) followed by an 8 week washout phase. Phase 3 consisted of a third data collection, followed by the resumption of the MIP for a period of 16 weeks. The last data collection took place after the 16 weeks of intervention (T4) (Figure 6.2).

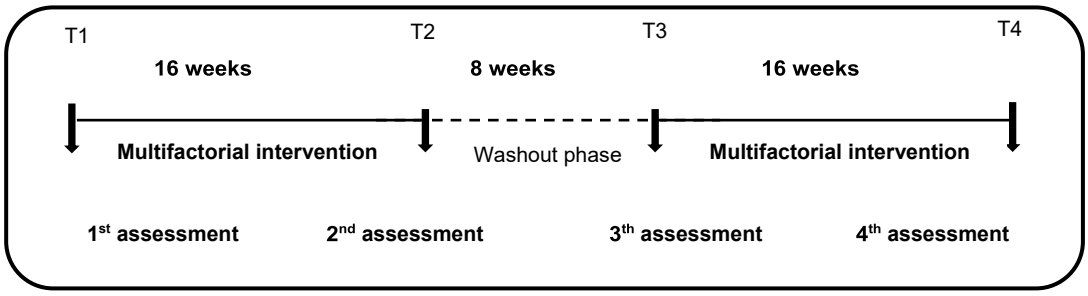

Figure 6.2- Chronological order of multifactorial interventions study design. T1 to T2 (elastic-band exercise, 16 weeks, 8 weeks), T2 to T3 (washout), T3 to T4 (multicomponent exercise, 16 weeks).

6.4- Outcomes Measures

All the assessments were performed in the morning, between 10 and 11:45 a.m. One session was used to apply a short test battery to measure biosocial, global health status, cognition profile, nutritional, physical, and physical frailty status. In the second consecutive day, blood samples were collected and stored at −80 °C until further analysis.

6.4.1- Physical Frailty Index

The phenotype of Fried's physical frailty index was used (Fried et al, 2001). Weight loss was assessed by a self-report of unintentional weight loss of 4 kg or more in the last 6 months. Self-reported exhaustion was evaluated by a negative concordance of question number 7 and 20 of the Center of Epidemiologic Studies for Depression scale (Gonçalves, Fagulha, Ferreira, & Reis, 2014). Hand-grip strength was assessed in kilograms by a hand-held (HGT) dynamometer (Lafayette 78010, Sagamore, United States). The best result of the two trials was used for scoring purposes. Participants who were unable to perform the HGT and

those in the lowest 20% were categorized as positive (Syddall, Cooper, Martin, Briggs, & Sayer, 2003). The cutoff reference values for HGT of ≥29 kg for male and ≥17 kg for female were adopted. Slowness was measured by the “15 feet (4.6 m) walking test”. Based on the cutoff values of Fried´s study population, the times of ≥7 s for males and ≥6 s for females were adopted for positive scores of slowness. The best time of the two trials was used for the final scoring. Low physical activity (PA) levels were assessed by the International PA Questionnaire short version (IPAQ-SV) (Campaniço, 2016). There are three levels of PA suggested for classification: Inactive, minimally active, and highly active. Participants classified as inactive had a positive score for this PF component. A positive evaluation in one or two criteria classified the participants as pre-frail, in three or more criteria as frail, and as non-frail when the subject scored none of the five PF indicators. The prevalence of PF was calculated to generate a frailty total score, as well as the presence of each of the five criteria of the Fried’s model (0 to 5 points). In this study, participants classified as frail (3 or more points) and pre-frail (2 points) were included.

6.4.2- Nutritional Assessment

Daily diet at the RCH was prescribed by a registered nutritionist and was provided for all the participants without any change or interference of the research staff. On the basis of the information provided, the diet was analyzed using specific tools (photographic quantification of portions, food table) for the Portuguese population (Torres et al., 2016; Goios, 2016; INSA, 2006). Due to the relationship between the frailty status and severe decrease of muscle mass (or sarcopenia) which had already been demonstrated in several studies, the objective of this nutritional assessment was to characterize the protein consumption of the participants. In addition, the Mini Nutritional Assessment (MNA) questionnaire was applied (Vellas et al., 1999; Loureiro, 2008). This consists of 18 questions that present a maximum score of 30 points, and classifies the participants as malnourished (≤17 points), at risk of malnutrition (17 < MNA < 23.5 points), and as having a normal nutritional status (MNA > 23.5 points).

6.4.3- Lower Limb Muscle-Strength Test

The Five-Times-Sit-to-Stand-Test 5TSS-Test was applied. This test assesses the functional strength of the lower limbs, transition movements, balance and risk of falling. The participant is instructed to stand as quickly as possible 5 times, without stopping in the middle. In addition, the participant should be encouraged to keep his arms crossed over his chest. The instructor must count the time with a stopwatch and must count each position out loud so that the participant remains oriented. The test is stoped when the participant reaches the orthostatic position at the 5th repetition (Guralnik, 1994).

6.4.4- Clinical and Health Status

The Charlson comorbidity index (CCI) was calculated based on the registry of individual comorbidities combined with age and gender, to account for a final score (Charlson et al., 1994). The anthropometric assessment included body mass (kg) and stature (m). Body mass was determined using a portable scale (Seca®, model 770,Berlin , Germany) with a precision of 0.1 kg, whereas stature was determined using a portable stadiometer (Seca Body meter®, model 208, Berlin, Germany) with a precision of 0.1 cm. Body mass index (BMI) was calculated according to the formula (BMI = body mass/stature^2). The standardized procedures described in previous studies were followed (Lohman et al., 1992).

6.4.5- Cognitive Profile

The Portuguese version of Mini Mental State Examination (MMSE) was used (Morgado et al., 2009). The MMSE is a 30-point scale instrument that evalueted five domains of cognition: orientation, immediate recall, attention and calculation, delayed recall, and language This scale classifies individuals by progressive cognitive skills: (0–9 pts.) severe cognitive impairment; (10–18 pts.) moderate cognitive impairment; (19–24 pts.) mild cognitive impairment; and (25–30 pts.) normal cognitive profile (Folstein, 1975).

6.4.6- Biochemical Analysis

Non-fasting blood collection was done in the morning (between 10:00 a.m. and 11:00 a.m.). Blood samples were collected by venipuncture, after 15 min of

individual rest in an isolated and quiet room, at the four time-points of the study assessment. The participants were asked to avoid alcohol and caffeine intake on the previous day of blood collection, and also to maintain their sleep habits during the previous night. After centrifugation at 3000 rpm at 4 °C during 15 min, plasma and serum samples were aliquoted into Eppendorf tubes and stored at −80 °C until used for the determination of interleukin-10 (IL-10), tumour necrosis factor alpha (TNF-α), myeloperoxidase activity (MPO), and total albumin concentrations. The ELISA (Thermo Fisher, Gloucester, UK) intra-assay coefficients of variability were 4.1% for IL-10 and 3.0% for TNF-α.

6.4.7- Full Characterization of the MIP

6.4.7.1- Oral BCAA's

The BCAAs power mixture was composed of L-leucine (Leu), L-isoleucine (Ile), and L-valine (Val) in the proportion of 2:1:1 (MyProtein®, Cheshire, UK), accounting for 20 kcal per portion, comprising 5 grams (g) of supplement: 1.85 g Leu, 0.93 g Ile, and 0.93 g Val. The unflavored supplement was used as to not induce ingestion preferences for specific flavors. The BCCAs were diluted in 200 mL of water and given immediately after the exercise sessions to the participants in the ME + BCAAs and BCAAs groups (Ispoglou et al., 2016). The supplement dose was fixed at 0.21 g total BCAAs/kg/session, with individual portion sachets, administered in the morning, between 09:00 and 11:30 a.m. (Negro et al, 2019). We opted to exclude maltodextrin or the carnosine-based placebo here, since the carbohydrate ingestion could mask the effort perception and cognitive indexes in our older persons volunteers, compared to the amino acid supplementation (Honka et al., 2016). In addtion, carnosine, as well as other β-alanine derivatives, were shown to affect cognitive functions, including the perception of wellness, mood, and depression indexes (Solis et al., 2015). Therefore, we decided to split BCAA-supplemented (ME + BCAAs and BCAAs) and BCAAs-absent groups (ME and CG) according to the proximity between the residential care homes (RCH), where the ME programs were effectively applied. No communication was reported between volunteers from the BCAA-supplemented and no-BCAA supplemented groups in our study.

6.4.7.2- Washout Period (Oral BCAA´s)

In this phase, the participants endured a cessation period of 8 weeks, when suplementation of the ME+BCAA and BCAA groups was suspended in order to verify whether the supposed benefits of BCAA supplementation were maintained or lost (Ikeda et al., 2016).

6.4.8- Exercise Intervention (phase 1)

The exercise program was divided in two interventions of 16 weeks each, separated by an 8-week detraining (washout) period. Exercise sessions were offered twice a week, with an interval of 36 hours for adequate physiological recovery and rest. The exercise protocol respected the guidelines for exercise prescription for older persons and the guidelines for exercise periodization by the American College of Sports Medicine (ACSM) (Nelson et al., 2007; de Souto Barreto et al., 2016). The program started with an adaptation period of 2 weeks, in which seven different exercises were performed using elastic bands (TheraBand®, Hygenic Corporation, Akron, OH, USA). The participants were closely supervised for two initial sessions aiming for equipment familiarization and adjustments to the Rating Perceived Exertion (RPE OMNI) scale (Colado et al., 2018). During these familiarization sessions, the participants learned the correct technique of the exercises, and selected the proper color, length, and grip width of the elastic bands. The exercise intensity was indirectly calculated using the Karvonen's formula to predict the target heart rate (HR), with HR_{max} being calculated by an adjusted formula for older persons (Tanaka et al., 2001).

$$HR = [(HR_{max} - \text{resting HR}) \times \%\text{Intensity}] + \text{resting HR}$$

After the adaptation period, the exercise program was progressively intensified by increments in both the number of exercises (from 8 to 10 exercises during the rest of the exercise intervention) and the proposed physical effort, imposed by different intensity color bands, according to the OMNI table (Colado et al., 2018). The elastic-band exercises applied in the Phase 1 period are shown in Table 6.1. For safety reasons, the exercise programs were also monitored using heart rate monitors (Polar M200; Polar Electro Oy, Kempele, Finland). Additionally, intensity was measured through the specific rating perceived

exertion (RPE) scales for each exercise program (Borg, 1982). The RPE used is an arbitrary scale ranging from 0 to 10 points, with identical intervals and with reference to the quality of effort: (0) Nothing at all; (1) very weak; (2) weak; (3) moderate; (4) somewhat strong; (5–6) strong; (7–9) very strong; (10) very, very strong (almost maximal).

Table 6.1 Example of elastic-band exercises session applied in phase 1

Warm-up	5 minutes	PSE 1-3				**Progression**	**Weeks**	**Intensity (colour)**
Exercises (8-10)	**Sets**	**Repetitions**	**Cadence**	**Interval**	**PSE**	2x10	2	Yellow
Front squat	2-3	10-20	2:3	30-45 seconds	4 to 6	3x20	2	Yellow
Chair unilateral hip flexion	2-3	10-20	2:3	30-45 seconds	4 to 6	3x10	2	Red
Chair Bench over row (with flexion)	2-3	10-20	2:3	30-45 seconds	4 to 6	3x20	2	Red
Chest Press (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6	3x10	2	Green
Standing (or chair) reverse fly	2-3	10-20	2:3	30-45 seconds	4 to 6	3x20	2	Green
Shoulder Press/twist arm position	2-3	10-20	2:3	30-45 seconds	4 to 6	3x15	2	Blue
Chair (or stand) frontal total raiser	2-3	10-20	2:3	30-45 seconds	4 to 6	3-4x10-15	2	Blue
Biceps arm curl (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6			
Chair Overhead triceps extension	2-3	10-20	2:3	30-45 seconds	4 to 6			
Cooling down		5 minutes	PSE 1-2					

Notes: PSE- Perception Subjective Effort

6.4.8.1- Washout (ME detraining)

In this phase, the participants endured a detraining period of 8 weeks, when the ME programs were suspended. The aim was to check if the physiological adaptations acquired during the first phase of ME were maintained (Sakugawa et al., 2019) or if a 8-weeks nterruption was able to revert the possible effects on immune changes.

6.4.9- Exercise Retraining Protocol

The phase 3 (exercise retraining) protocol was also based on the resistant TheraBand elastic bands (Table 6.2), but included walking, steps, and balance exercises (sometimes with dumbbells and ankle/wrist weights) to compose a multicomponent exercise program for an identical 16 week-period (twice a week, alternate days, also totalizing 32 sessions). The multicomponent program (Table 6.3) was described by Furtado and colleagues (Furtado et al., 2019). The phase 3 program aimed to reproduce most of the daily activities of the older persons participants in this study (Baker et al., 2007).

Table 6.2- Multicomponent Exercise Program (ME)

Exercises (8-10)	Sets	Repetitions	Cadence	Interval	PSE
Front squat	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair unilateral hip flexion	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair Bench over row (with flexion)	2-3	10-20	2:3	30-45 seconds	4 to 6
Chest Press (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6
Standing (or chair) reverse fly	2-3	10-20	2:3	30-45 seconds	4 to 6
Shoulder Press/twist arm front position	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair (or stand) frontal total raiser	2-3	10-20	2:3	30-45 seconds	4 to 6
Biceps arm curl (stand and/or chair)	2-3	10-20	2:3	30-45 seconds	4 to 6
Chair Overhead triceps extension	2-3	10-20	2:3	30-45 seconds	4 to 6
Circuit training					
Walking around the room	2-3	3 minutes		30-45 seconds	4 to 6
Balance/ agility exercise	2-3	3 minutes		30-45 seconds	4 to 6

Notes: PSE- Perception Subjective Effort

6.5- Statistical Analysis

The descriptive statistics for each group, at baseline and follow-up evaluations, were reported as mean plus standard deviation (M±SD), except when mentioned otherwise. All variables were checked for normally residual distribution and values were logarithmic transformed when appropriate. One-way Analysis of Variance (ANOVA) were used to determine baseline differences between the four groups in all parameters. Effects of time, group, and time x group interactions were assessed through repeated measures ANOVA and Bonferroni post-hoc for multiple comparisons. Additionally, univariate analysis was performed using paired t-test for within comparisons during the first phase of interventions (T1 vs T2). All statistical analysis were performed by using the SPSS 21 (SPSS Inc., Chicago, USA), and level of significance was set at $p < 0.05$.

6.6- Results

The dynamics of the MIP groups and drop-outs are presented in detail in Figure 6.2. From the 50 (100%) participants initially selected, only 35 participants (70%) completed the intervention. This is an expected experimental loss, as reported by several previous studies (Rivera-Torres, Fahey, & Rivera, 2019). None of the dropouts left the intervention due to injuries or adverse responses. Reported deaths were due to acute events triggered by chronic clinical conditions. Table 6.3 shows the characterization of participants by MIP groups at the baseline, including nutritional, cognitive, frailty, anthropometric, and body composition status. No statistically significant differences in all the variables appeared, expect for time in residential care and nutritional status assessed by MNA ($p < 0.05$). However, all the groups were within the well-nourished category.

Table 6.3- Characterization of participants by intervention groups

	ME+BCAA (n=8)	ME (n=7)	BCAA (n=7)	CG (n=13)	p *value*
	M±SD	M±SD	M±SD	M±SD	
Age (years)	80±6.1	86.7±4	84.2±5.8	83.1±5.4	0.139
Time in residential care (years)	3.6±1	4.7±1.4	4.5±1.1	5±1	0.06
MNA (0-30 pts)	25.5±2.2	24±2.7	21.7±2.8	24.7±1.8	0.02
BMI (kg/m^2)	28.53±5.1	28.7±5.6	25.8±3.1	30.2±3.7	0.23
Stature (cm)	158±0.05	150±0.06	161±0.12	155±011	0.16
Comorbidity index (0-10 pts)	4.87±1.12	5.28±0.95	5.42±1.1	4.92±1.2	0.71
Schooling time (years)	4±0	4±0	4±0	4±0	0.99
Cognitive profile (0-30 pts)	26.00(3.11)	21.00(3.78)	20.85(2.79)	21.69(2.89)	0.00
Physical Frailty index (0-5 pts)	2.00(0.53)	2.71(1.1)	3.00(0.57)	2.16(0.71)	0.40
Daily Individual Protein (gr/Kg/day)	1.42 ± 0.28	1.83 ± 0.44	1.48 ± 0.22	1.60 ± 0.23	0.159
BCAAs (per person/gr/week)	30.3 ± 6.0	n.d.[1]	28.4 ± 5.0	n.d.[1]	--

Notes: BMI = body mass index; MNA = Mini nutritional assessment; M±SD = mean (standard and deviation); pts = points; Kg/m^2 = kilograms; cm = centimeters; One-way ANOVA was used to compare groups, except for Comorbidity index (Fisher Exact Test).

6.6.1- Biochemicals Analysis

Table 6.4 shows the results for IL-10; TNF-α and TNF-α/IL-10 ratio; MPO; Albumin; 5TSS-Test; Fried (score) and MMSE. Concerning the IL-10 levels, a classical anti-inflammatory cytokine, not only no effects of time ($p = 0.690$), or time versus experimental groups were found (CG, BCAA, ME, and ME+BCAA), [$F(df:9, 51) = 1.567$, $p=150$], but also Bonferroni post hoc comparisons did not result in significant variations between time vs. groups ($p > 0.05$). Regarding TNF-α levels, although we did not observe any interference of time on this pro-inflammatory cytokine levels ($p > 0.05$, Table 5), repeated ANOVA analyses revealed significant interactions between time vs. groups: $F(df: 6.758, 47.303) = 2.524$, $p = 0.029$. In addition, Bonferroni post hoc comparisons showed not only higher TNF-α values in ME+BCAA group between T2 and T3 ($p = 0.01$) but also a significant decrease was observed between T3 and T4 within the same experimental group (ME+BCAA, $p<0.01$). TNF-α values were unchanged in all other experimental groups. Regarding the TNF-α/IL-10 ratios, no significant variations were observed over time ($p = 0.703$), or within interactions (time vs. group, $p = 0.638$).

Concerning myeloperoxidase activity (MPO), table 6.4 shows that they were not influenced by time (T1, T2, T3, and T4), excepting a slight tendency regarding interactions (time vs. group): [$F(df: 9, 48) = 2.010$, $p = 0.059$]. Particularly, the Bonferroni post hoc comparisons showed that the BCAA group presented higher MPO activity after re-supplementation (T4) than the values found in the T2 time-

point (after the first 16-weeks of the supplementation period, p = 0.026). No significant alterations in MPO activity were observed in other comparisons between groups.

6.6.2- Five-Times-Sit-to-Stand-Test (5TSS-Test)

Table 6.4 shows that there was no effect of time (p = 0.841) or interactions (time vs. group, p = 0.846) on the time elapsed to perform the 5TSS-Test. However, post hoc adjustments showed that the ME+BCAA and BCAA groups presented a significant reduction of the time elapsed to perform this test at time-points T2, T3, and T4 (p = 0.009, p = 0.014, and p = 0.024, respectively).

6.6.3- Cognitive Assessment

In relation to the results obtained in the cognitive assessment (Table 6.5), it is worth mentioning that, at baseline (T1 time-point), 65,7% of the participants (n = 23) scored below the 24-point threshold of the cognitive MMSE test, indicating that a significant fraction of participants was within the mild/moderate cognitive impairment classification. In addition, at the same time-point (T1), significant differences were found for the cognitive score between the ME+BCAA group and the other groups (p < 0.05). An effect of time [F(df: 3, 93) = 4.262, p = 0.007], but not interaction (time vs. group, p = 0.296), was observed in MMSE results. The cognitive MMSE scores increased in the control group between T1 and T2 time-points but decreased subsequently in T3 and T4 time-points (p = 0.008). No significant alterations were observed in other groups. At baseline 45,7% of the participants were classified as frail and 54,3% as pre-frail. At the end of the intervention.

Table 6.4- Statistical analysis comparison of 4-time points moments of multifactorial intervention for biochemical, cognitive profile, physical frailty index and functional fitness test.

Biomarker/ Variables	Groups	Time points of evaluation				Effect	F	Overall *p*
		T1*	T2*	T3*	T4*			
		M ± SD	M ± SD	M ± SD	M ± SD			
IL-10 (µg/mL)	ME+BCAA	10.36(6.96)	12.0(6.53)	15.99(7.98)	11.52(7.56)			
	ME	8.68(7.68)	12.25(12.35)	4.16(3.39)	10.53(5.82)	Time	.491	.690
	BCAA	7.71(2.54)	9.24(4.15)	13.83(6.94)	9.85(10.89)	Time*group	1.567	.150
	CG	16.10(7.4)	12.21(2.81)	12.74(7.36)	20.45(5.42)			
TNF-α (pg/mL)	ME+BCAA	62.44(53.65)	71.42(38.06)	112.86(62.51)	57.37(31.18)			
	ME	41.78(54.08)	45.83(21.07)	24.92(15.60)	54.05(29.19)	Time	1.552	.210
	BCAA	32.65(15.74)	37.18(26.91)	62.93(35.77)	60.02(55.42)	Time*group	**2.524**	**.015**
	CG	44.46(41.72)	44.81(37.16)	41.78(37.86)	57.01(44.15)			
TNF-α/IL-10 ratio (pg/mL)	ME+BCAA	6.24(4.46)	7.47(4.09)	6.96(1.63)	6.10(3.25)			
	ME	4.43(1.99)	9.06(10.46)	8.64(7.36)	5.70(3.27)	Time	.472	.703
	BCAA	5.44(3.39)	3.85(1.84)	5.45(1.54)	11.19(9.77)	Time*group	.777	.638
	CG	4.10(1.27)	5.37(1.56)	4.56(1.80)	4.41(0.38)			
MPO (µg/mL)	ME+BCAA	5653.91(1106.71)	5871.97(1159.09)	4843.50(1221.63)	5196.53(591.62)			
	ME	5935.71(1315.33)	5252.76(1084.06)	4685.42(1043.31)	4512.34(794.61)	Time	1.191	.323
	BCAA	5139.04(909.07)	4069.64(1009.10)	5416.47(1539.50)	5575.80(1181.43)	Time*group	2.010	.059
	CG	4623.56(699.03)	4593.56(1310.34)	4655.42(815.10)	4327.39(863.95)			
Albumin (g/dl)	ME+BCAA	3.60(0.39)	3.63(0.61)	3.82(0.54)	3.75(0.63)			
	ME	3.73(0.61)	4.12(0.74)	3.57(0.43)	4.13(0.22)	Time	**3.841**	**.013**
	BCAA	3.77(0.39)	3.61(0.40)	1.56(2.15)	2.83(1.60)	Time*group	1.446	.185
	CG	3.75(0.72)	3.60(0.35)	2.59(1.85)	2.96(1.69)			
5TSS-Test (seconds)	ME+BCAA	21.87(3.64)	18.71(3.59)	20.66(4.98)	17.54(4.4)			
	ME	26.69(12.98)	28.02(11.28)	26.08(10.46)	27.56(12.24)	Time	.165	.841
	BCAA	36.54(14.14)	36.24(13.39)	36.74(11.89)	35.76(17.28)	Time*group	.436	.846
	CG	24.58(8.99)	24.76(9.0)	23.66(9.30)	25.17(9.75)			
Physical Frailty (index)	ME+BCAA	2.00(0.53)	1.50(0.53)	2.12(0.99)	2.00(0.53)			
	ME	2.71(1.1)	2.57(1.13)	2.14(0.69)	2.00(0.81)	Time	2.702	.05
	BCAA	3.00(0.57)	2.14(0.37)	2.28(1.25)	2.71(0.48)	Time*group	**3.799**	**.00**
	CG	2.16(0.71)	2.25(0.75)	2.66(0.49)	3.16(0.71)			
MMSE (0-30 points)	ME+BCAA	26.00(3.11)	26.37(2.44)	26.00(2.87)	24.37(3.58)			
	ME	21.00(3.78)	22.42(2.99)	21.00(4.65)	20.00(3.91)	Time	**4.262**	**.13**
	BCAA	20.85(2.79)	19.42(4.07)	20.71(4.02)	19.57(3.64)	Time*group	1.214	.305
	CG	21.69(2.89)	23.92(3.47)	23.23(3.83)	21.76(2.94)			

Notes: M±SD = mean (standard and deviation); ME = multicomponent exercise; BCAA = branched-chain amino acids; IL = interleukin; TNF-α = Tumour Necrosis Factor-alpha; MPO = myeloperoxidase; MMSE = Mini Mental State Exam; 5TSS-Test = Five-Times-Sit-to-Stand-Test; *T1 to T2 (elastic-band exercise, 16 weeks, 8 weeks), T2 to T3 (washout) T3 to T4 (multicomponent exercise, 16 weeks).

6.7- Discussion

This study evaluated the effects of exercise and BCAAs on biomarkers of immunity, total albumin, and the cognitive profile of institutionalized older persons. The main findings were that ME showed more proemint result, particullary with BCAA in the improve cognitive profile and muscle strength-related albumin levels in plasma and diminish the frailty status. Moreover, exercise induced slight changes on the pro-inflammatory marker TNF-α.

Albumin levels tend to decrease with age, and this effect seems to imply an increased risk of complications and higher rate of mortality, morbidity, and disabilities such as sarcopenia and frailty (Vandewoude, Alish, Sauer, & Hegazi, 2012). Despite the key participation of albumin on pH balance and ionic homeostasis in blood, most of the free fatty acid (and some other lipids) transport

in bloodstream is also performed by serum albumin (Pilgeram, 2010). Not surprisingly, age-related impaired albuminemia and elevated serum anion gap is known to be associated with hypertension, low cardiorespiratory fitness, and decreased renal function, common morbidities of advanced aged people (Ahn et al., 2014). Therefore, interventions that aim to sustain (or even increase) albuminemia in older persons could represent an important strategy to mitigate the harmful effects of aging and its comorbidities. In this respect, some studies have already shown that BCAAs apparently increases albumin levels in older persons suffering from malnutrition (Hiroshige et al., 2001).

Our results showed that the serum albumin levels were efficiently sustained or even augmented, in exercising participants (both ME and ME + BCAAs groups) during the first 16 weeks of intervention (phase 1). However, the withdrawal of BCAAs during the washout period (phase 2) quickly decreased those albumin levels, especially in the BCAAs group. The prominent effect of exercise on albumin levels was evident since its levels in both ME and ME + BCAAs groups were fully restored after the phase 3 period (T3 to T4 time-points), whereas only partial recoveries were observed in albumin levels in the BCAAs group at the same time-point. Low serum albumin levels were shown to be the most relevant biomarkers associated with poor physical strength in the older persons (Barbalho et al., 2020).

It is broadly accepted that the regular practice of exercise training imposes metabolic, endocrine/physiological, immune, and cognitive adaptations that, among many benefits, can increase skeletal muscle mass and strength, thus, circumventing the deleterious effects of sarcopenia in older person (Pedrero-Chamizo et al., 2020).

The chronic exercise-mediated adjustments on insulin/glucagon balance, thyroid, and steroid hormones, such as testosterone, cortisol, and estrogens, can also be involved in the enhancement of hepatic and protein muscle metabolism (proteolysis, proteogenesis, and protein turnover), with clear consequences on the circulating amino acid levels (e.g., glutamine and alanine), blood pH and electrolyte balance (hydric/ionic homeostasis), and renal functions (Starling, Ades, & Poehlman, 1999).

However, it was reported that the putative effect of amino acid/protein supplementation in older women could be masked by sufficient daily protein

intake, as we attested in all institutionalized participants in this study (Zhu et al., 2015). Thus, the proper mechanism behind this effect still needs to be fully understood for this special population. In fact, to our knowledge, this is the first study to show the potential of physical exercise associated or not with BCAAs supplementation to maintain serum albumin levels in older persons living in RCH. Contrarily to the albumin results, the monitored inflammatory markers (IL-10, TNF-α, and MPO) did not show significant alterations over time. Apparently, we can putatively suggest, that the physical exercise intensities reached in the sessions, as well as the BCAAs supplementation effect compared to the daily protein intake in this population, were not sufficient to induce a significant impact on the inflammatory status in the participants in this study. Other interventions with older persons have been able to show a strong anti-inflammatory effect of exercise training, but it seems that these results were observed for intervention periods longer than 16 weeks (Ispoglou et al., 2016;Negro et al, 2019).

Interestingly, even though an increase in the levels of the pro-inflammatory cytokine TNF-α was observed in the ME + BCAAs group from T1 to T2 and T3, this finding was accompanied by a proportional increase of the anti-inflammatory cytokine IL-10, since the TNF-α/IL-10 ratio was not different in this group over time. Moreover, at the end of the intervention, TNF-α levels significantly decreased in this group. In accordance with the literature, IL-10 is a key anti-inflammatory cytokine that acts by inhibiting systemic inflammation mediated by TNF-α (Saraiva & O'Garra, 2010).

Concomitantly, BCAAs alone did not induce alterations in both IL-10 and TNF-α levels. These results differ slightly from what is observed in the literature regarding this type of intervention on inflammatory status (Ohno et al., 2008). Based on the literature, there is a close interaction between the inflammatory status and aging, and in this respect, it is widely accepted that older persons, especially sedentary people, present a chronic, systemic, sterile low-grade inflammation associated with aging, a phenomenon named inflammaging (Franceschi et al., 2007). It is highlighted that inflammaging plays an important role in the loss of lean mass, which leads to sarcopenia and frailty, as well as increases the risk of the development of diseases and comorbidities, such as cognitive decline, atherosclerosis, insulin resistance (Liu, Wang, & Jiang, 2017).

Despite the fact that literature defines the ability to induce an anti-inflammatory change as a hallmark of physical exercise, in general, our results did not corroborate this fact. It is paramount to mention that some factors could putatively influence the lack of significant results in the inflammatory analysis. Firstly, the occurrence of inflammaging and pathophysiological disturbances in our participants could be crucial for the response magnitude observed during the interventions here. Second, the low level of physical activity of our participants before the interventions could mitigate the benefits that would be achieved with the physical exercise sessions and, consequently, limit physiological adaptation. These factors, associated with polypharmacy, a high rate of comorbidities, and the small sample size that finished the study, may determine the lack of significant effects observed.

There is a consensus in the literature that physical exercise sessions stimulate the release of cytokines, such as IL-6, IL-10, and TNF-α, in response to contracting skeletal muscles, which are responsible not only for tissue restoration and energy metabolism, but also for the adjustment of the systemic inflammatory status (Pedersen & Febbraio, 2012; Windsor et al., 2018). As appealing as these effects are, physical exercise training also improves human antioxidant defenses as observed in several studies which may also justify the use of exercise interventions to counteract the progression of oxidative-related diseases (Simioni et al., 2018).

There are solid pieces of evidence that the loss of muscle strength and power in the lower limbs, which is characterized by a decline of up to 50% in overall muscle strength from the age of 30 to 80 years (Sakugawa et al., 2019) Furtado et al., 2019) is associated with an increased incidence of falls.

Particularly, physical exercise training improves body composition, muscle strength, metabolic parameters, bone health, and functionality as well as reduces the risk of mortality, chronic diseases, cognitive deterioration, falls, and depression (Beard, Officer, & Cassels, 2016). Here, we observed that only the ME + BCAAs group presented an improved physical performance in the 5TSS test. Neither ME or BCAAs alone were sufficient to mediate improvements in lower body strength. Only the combination of exercise and supplementation did so. This result was achieved probably due to multiple factors, from physiological to cognitive positive effects that were not directly assessed by the applied

methodology here. According to the literature, the 5TSS test is an important performance test that invokes physical skills and abilities that could have been particularly developed during phase 3 of this study. The phase 3 of our study included walking activities, steps, and balance exercises, which mimic the participants' regular daily life activities.

It is important to point out that strength exercise training has been proposed as one of the most effective methodologies, presenting best results in bringing back safety in performing the common tasks of daily life, focusing on the optimization of neuromuscular function for better benefits (Cadore et al., 2014). Multicomponent programs combine aerobic and strength exercises, including other physical skills, such as balance and flexibility (Baker et al., 2007), in order to optimize the functional capacity of frail older persons (Villareal et al., 2011) as well as to maintain their independence to perform basic activities of daily living (Casas-Herrero et al., 2019). Concerning supplementation, it was reported that branched-chain amino acids, particularly L-leucine, showed significant results in inducing hypertrophy in older persons and improving their functional capacity (Ahn et al., 2014; Hiroshige et al., 2001).

Taking into account that cognitive impairment is one of the main factors that cause morbidity and high health costs worldwide (Alzheimer's Association, 2020), our results show that physical exercise training, in association or not with BCAAs, was able to maintain the cognitive scores of the participants and could have important practical applications. Considering the population enrolled here (pre-frail and frail octogenarians) and the trend for the natural decline of their cognitive functions, the maintenance of those cognitive scores by exercise is, per se, a remarkable achievement. The literature supports the positive effect of BCAAs in older persons, to improve their mood state (Gariballa & Forster, 2007), the perception of fatigue, and their performance in a mental task (Fernstrom, 2013), which are abilities that were not evaluated here. Leucine is important since it activates the mammalian target of rapamycin complex 1 (mTORC1) and the downstream phosphorylation of p70S6 kinase and 4E (eIF4E)-binding protein 1 (4E-BP1) and related signalling pathways (Neishabouri, Hutson, & Davoodi, 2015). The aging muscle is less responsive to lower doses of amino acids when compared to the young muscle and may require higher quantities of protein to acutely stimulate equivalent muscle protein synthesis (Ko et al, 2020).

Nevertheless, the dose and duration of BCAAs proposed here did not affect the cognition scores in our participants.

6.7.1- Study Limitation and Perspectives for Futures Researchers

The entire study was conducted with human octogenarians and, given the difficulty to control several influencing factors in this type of population, this study had the additional merit of causing a minimal impact on their daily routines at the residential care homes. In addition, our results here represent real-world data reflecting the reality at residential care homes. We screened participants with disabilities and comorbidities that, although expecting high rate of dropouts and low motivational issues, we could accomplish the proposed goals with a reasonable number of participants. The execution of a controlled study over 40 weeks with such a particular population also introduces other limitations. We suggest that the use of other methods of exercise training, such as the use of playful activities (dance and music sessions) might elevate the adherence of this population to the program.

6.8- Conclusion

This study showed that multicomponent exercise training, with minor effect of BCAAs, triggered alterations in the inflammatory status and physical profiles of older persons, while helping maintain cognitive levels. Taken together, the achieved results, could help increase autonomy and efficiency in the performance of daily activities. Unlike other studies, our results showed that supplementation with BCAA did not induce substantial changes in health-related parameters at older ages. It is possible that the heterogeneity and limited sample size might have limited the statistical relevance of our results. Despite a slight and transient variation over time observed in some inflammatory and cognitive parameters, it is possible that the results here were influenced by the comorbidity status of each group.

Author Contributions: A.C.-S. drafted the paper; G.E.F. worked on the methodology of the study aspects of RCT; R.N. helped with data acquisition; M.U.C. statically analyzed the data; A.M.T., A.M., and E.M. developed the study proposal, revised the manuscript critically, and suggested additional statistical analyses; A.M.T. coordinated the research study and, together with M.P.d.B. and A.L.L.B., revised the manuscript critically. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted according to the guidelines of the and approved by the University of Coimbra, Faculty of Sport Sciences and Physical Education Ethical Committee (reference number: CE/FCDEFUC/00282018), https://clinicaltrials.gov/ct2/show/NCT04376463 (accessed on 05 May 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. Data supporting the reported results is the property of CIDAF, Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal.

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CHAPTER VII

FINAL CONSIDERATIONS: GENERAL DISCUSSION AND CONCLUSION

7.1- Discussion

This chapter will be dedicated to discussing the main results found in the studies carried out, as well as the conclusion of this doctoral thesis. Initially, some considerations are important regarding the study sample.

This is a non-randomized, prospective, controlled intervention, performed in independent groups, 40 weeks long and with and without BCAA supplementation and a physical exercise program.

The sample consisted of individuals over 80 years of age (83±3 years old). It is important to note that this sample has older ages than similar studies that involved exercise in older persons, and that evaluated similar parameters (Ikeda et al., 2016). Consequently, they have worse levels of physical fitness (Fernández-García et al., 2020), higher incidence of frailty (Chevalier, Saoud, Gray-Donald, & Morais, 2008), than several studies present in the literature. As usual in interventions with older persons, the sample in this study has several comorbidities and makes use of a large number of medications.

Although no adverse effects of BCAA supplementation were related, only one participant reported brief intestinal discomfort in the first week of supplementation, during which she underwent medical evaluation and returned to daily activities and continued the intervention protocol.

The first study of this thesis (Study 1), presents the results of a systematic review, which involved the analysis of studies with multicomponent exercise interventions in older persons, and the effects on the levels of physical frailty proposed by Fried and colleagues (Fried et al., 2001). In the systematic review, the biomarkers CRP, IL-6, and TNF-α, emerged as the main indicators of inflammation associated with frailty. We concluded that multicomponent exercise could contribute in a very positive way to decrease/reverse the frailty and change the profile of these biomarkers, since frailty and pre-frailty are closely linked to pro-inflammatory parameters. Intervention research indicates that regular moderate exercise improves strength, function, and older people. There is also a growing understanding of the potential role of exercise programs as a non-pharmacological treatment to improve inflammation in the frail older person setting, which may be particularly useful for this specific population and as an adjunctive treatment.

The second study of this thesis (Study 2), aimed to understand the isolated and combined effects of 16 weeks of Branched Chain Amino Acid supplementation (BCAA) (BS) and muscle strength exercise program (MSE) on the performance of functional fitness in octogenarians. Our results demonstrated that after 16 weeks, group 1 (MSE + BS) showed a significant increase in the performance in all SPPB tests, particularly in the sit and stand test 5 times and revealingly that exercise plus BCAA supplementation was able to improve physical fitness function, while BCAA supplementation alone had limited effects. We concluded that the results were satisfactory and could be explained by the additional effects of exercise and BCAA supplementation on the effect of protein synthesis. In fact, other studies involving BCAA supplementation in older persons have already shown the beneficial effects of these amino acids on physical fitness, whether with exercise (Takeuchi et al., 2018) or even in younger older persons (~ 66 years old) without intervention with exercise (Ko et al., 2020).

Our results follow the same direction as other evidence in the literature, however, the trend of improvement in physical function was observed only in the older persons who associated supplementation with physical exercise.

The third study in this thesis (Study 3) shows that a long-term exercise program, independently of being multicomponent or strength elastic band-based, was effective in improving functional capacity in Institutionalised older persons, and in slowing/preventing the progression of frailty, when compared to a control group without exercise. BCAA supplementation alone had no impact on functional fitness. However, in a short period (16 weeks), BCAA supplementation contributed to diminishing frailty. Moreover, the combination of BCAA and exercise demonstrated the potential in reducing the effects of a detraining period on functional capacity. Overall, the intervention periods had no significant effect on the mood state, depression scores, cognitive function, or salivary testosterone levels, but there was a tendency of diminished depression scores in the exercising groups (compared to the increasing tendency in the non-exercising ones). Further research is needed to define the best practices, the feasibility of implementation, the best supplementation strategies, and the suitable physical exercise programs for this special population, to augment compliance and long-term behaviour maintenance.

The fourth study of this thesis (Study 4) presents the results of an investigation of 40 weeks of intervention (16 weeks of exercises with elastic bands, with and without BCAA supplementation, a period of detraining/withdrawal of interventions of 8 weeks, followed by a 16-week retraining period with multicomponent exercise) on various biochemical markers (such as IL-10, TNF-α, IL-10 / TNF-α, ratio, serum albumin and MPO).

This study started from the premise that ageing affects the immune system, increasing chronic inflammation, which, consequently, is related to the appearance of several diseases that are more prominent in older persons. Despite the complex relationship between genetic and environmental factors, it is known that a change in lifestyle (such as increased physical activity and better nutritional balance), can promote health improvements related to the quality of life, ultimately determining functional status autonomy of older persons.

Currently, it is recognized that the significant associations between serum albumin and the functional capacity of older persons have implications for the prevention of physical disability in ageing. Some studies suggest that low levels of albumin are markers of nutritional risk and quite useful in assessing the functional decline in older persons (Aung et al., 2011). Our study showed that physical exercise, even as an isolated intervention, was able to maintain serum albumin levels in older persons.

Albumin is an abundant protein in the blood, also considered a biomarker of health status, and in older persons, low levels have been reported to have an influence on decreased performance and low functional capacity, as well as deterioration of muscle strength (Schalk, et al., 2005).

Despite the difficulties in diagnosing malnutrition in older persons, one of its most feared consequences is the change in body composition, weight loss, reductions in BMI and mainly decreased muscle mass, that are indirect markers of malnutrition. Albumin levels may be more reliable alternatives in assessing nutritional risk, especially in older persons (Cabrerizo et al., 2015).

As an indicator of oxidative stress, this investigation also analysed the effects of the interventions with exercise and supplementation on the concentrations of myeloperoxidase (MPO) over time. MPO is an enzyme expressed by neutrophils and is the most toxic enzyme found in the azurophilic granules of this cell, that can also contain phospholipase A2, elastase, defensins,

lysozyme, cathepsin G, proteinase 3 and proteoglycans (Strzepa et al., 2017). MPO is a marker of oxidative stress and was previously proposed as a useful indicator of risk and a diagnostic tool for acute coronary syndromes (Loria et al., 2008).

Our results showed that isolated interventions (ME and BCAA groups), but not the combined intervention (ME+BCAA), tended to decrease MPO levels over time in our participants. This trend was not observed in the controls (where the MPO concentration remained unchanged over time), nor in the group that combined exercise + supplementation with BCAA. In the light of these results, it is difficult to definitively establish the reasons that led to fluctuations in the MPO levels between the groups, but some reasons may explain these effects, increased expression in neutrophils and monocytes which can lead to an increase in the inflammatory environment compared to studies in the literature. First, previous evidence in community older persons has shown that 12 weeks of explosive resistance exercise significantly reduces MPO levels (Beltrán et al., 2014), as well as the same endurance training period in people at cardiovascular risk (Richter et al., 2005).

It must be considered, however, that both samples are significantly younger compared to the older persons in this investigation, whereas the type of training, intensity achieved, duration of interventions, and even the level of comorbidity (supposedly higher in this one), are also different. Although our study points the same direction for the reduction of MPO with exercise, this effect was not observed in those individuals who associated exercise and supplementation (ME+BCAA). In a recent study involving supplementation with taurine, however, it was shown that the isolated intake of this amino acid is able to reduce the concentrations of MPO in institutionalized older persons, even with a large number of comorbidities (Chupel et al., 2021). However, it is considered that the type of supplementation is different from BCAA, since taurine reacts with hypochlorous acid (HOCl-), a by-product of the oxidative action of MPO, which may, on the other hand, influence the lower expression of this enzyme and consequent decrease in its concentration (Marcinkiewicz & Kontny, 2014).

Other immune parameters evaluated in this investigation involved the concentration of some pro and anti-inflammatory cytokines. In general, it can be said that there were no major changes regarding whether the intervention was

exercise, supplementation, or the combination of both treatments, in the modulation of the cytokines IL-10 and TNF-α.

The excess of some pro-inflammatory cytokines, especially CRP, TNF-α and IL-6, may contribute to an increased risk of loss of skeletal muscle mass and strength, resulting in sarcopenia and frailty (Wang, Maxwell, & Yu, 2019).

In addition, at the end of the intervention (T4), TNF-α levels decreased significantly in the group (ME+BCAA). According to the literature, IL-10 is an anti-inflammatory cytokine and acts by inhibiting the systemic action mediated by TNF-α. Normally, exercise-induced changes in the levels of cytokines such as IL-6 and TNF-α could increase age-related metabolic diseases such as insulin resistance, sarcopenia, and obesity (Pedersen & Saltin 2015).

Regarding the cognitive aspects, the overview of our results shows that there were no major cognitive changes, whether those that could be mediated by the practice of physical exercise, supplementation with BCAA, or association of both interventions, in the mean values of MMSE of the sample. In fact, it was observed that all interventions resulted in the maintenance of cognition over time, which seems to indicate that the practice of physical exercise (associated with or not with BCAA supplementation), as well as supplementation alone, can assist in executive functions that are usually impaired with advancing age.

The cognitive aspects are researched in literature with exercise in older persons, and - in general, there seems to be an important effect of increasing the level of physical activity and the improvement of cognitive functions (Barha et al., 2017). Specifically, activities involving combined and / or multicomponent training have already shown positive effects on the cognition of institutionalized older persons with a mean age similar to our investigation (Chupel et al., 2018), but the most striking effect seems to emerge from the combination of nutritional supplementation (in the aforementioned case, with the amino acid taurine) added to the practice of physical exercise. Part of the most striking effects of the contribution of physical exercise to the improvement of the cognitive aspect is attributed to the improvement in immunity (Chupel et al., 2017) and the increase in BDNF levels (Marinus et al., 2019).

The administration of BCAA contributes to the cognitive recovery of patients with head trauma (Aquilani et al., 2005) but it does not seem to produce beneficial effects on cognition tested in an experimental model (Tournissac et al., 2018).

A recent review points out that BCAA can be (hypothetically) an important component in the treatment of neurodegenerative processes - such as the case of Alzheimer's disease, mainly due to the beneficial effect of the increase in valine in reducing the risk associated with this disease (Polis & Samson, 2020). Our results with supplementation showed, however, that although there was no significant improvement in the cognitive aspects measured through the MMSE, maintenance of these values was observed even in the long term. This effect, by itself, suggests the idea that supplementation with BCAA may contribute to the treatment of cognitive impairment, since it is expected that there will be a decline in these values over time - especially in older persons (Rocca et al., 2011).

However, systematic studies in BCAA supplementation in older persons and its effects on cognition are necessary.

It is observed that lower physical capacity in frail older persons, mood and pro-inflammatory cytokines generate a vicious circle related to physical frailty and cognitive impairment, but physical exercise was able to maintain cognition in the groups that performed physical exercise (ME+BCAA; ME).

Physical activity/exercise is known for its ability to reduce / delay physical dependence, as well as for its protective effect on multiple physical disabilities and metabolic diseases, and by itself explaining the positive impact identified in the studies analysed, promoting healthy ageing, and reducing the risk of frailty.

Multicomponent exercise training programs seem to be predominantly used in frail older persons (Silva et al., 2017), being a beneficial intervention for frail older persons and ideal for this population (Pillatt et al., 2019).

Regarding exercise, our results corroborate the recent findings that physical exercise, is an excellent ally for the treatment of frail older persons (Angulo et al., 2020), Based on our data, we can suggest that the mechanism behind the observed improvement in frailty scores in our participants (Exercise or BCAA supplementation) was mediated by specific changes in specific domains of the Fried scale. In fact, considering that Fried’s total score is supported by parameters related to the level of physical activity, gait efficiency and perception of fatigue, all these aspects were positively affected by the practice of physical exercise. Therefore, it can be said that the beginning of a physical activity program in older persons (who generally do not perform supervised body

movements), tends to induce biological / physiological adaptations that will affect the final score on the Fried scale.

As protein intake is also necessary to induce positive benefits in muscle mass (Tieland et al., 2012) and some components of BCAA contribute to this, like L-Leucine (Amasene et al., 2019) it seems clear that BCAA supplementation can affect Fried´s total score in older persons.

Ingestion of a Leucine/essential amino acid nutrient solution fast and effectively activates the mammalian target of rapamycin (mTOR) signalling pathway and protein synthesis in human skeletal muscle (Drummond & Rasmussen, 2008). Additionally, mTOR signalling and muscle protein synthesis are enhanced when leucine nutrients are ingested following resistance exercise. The addition of leucine to regular meals may improve the facility of eating to stimulate protein synthesis in older muscle.

Frailty, morbidity and mortality are the consequences demonstrated by the response of some cytokines such as CRP, IL-6, TNF and IL-1αβ (Frank et al., 2015).

The excess of some pro-inflammatory cytokine production, especially CRP, TNF-*α* and IL-6, increases the risk of loss of skeletal muscle mass, strength, sarcopenia and frailty (Wang et al., 2019).

Regarding the period of detraining and washout (withdrawal of supplementation) we can conclude that this phase, (called phase 2), corroborates a certain scientific principle (principle of continuity). There is a time between a training session and another one that must be respected so that the adaptations achieved are not lost. Therefore, the maintenance and evolution of the conditioning acquired over time (16 weeks) and the premeditated interruption of this period (8 weeks) should be done to avoid the dreaded stagnation or even detraining.

The interruption of exercise encouraged negative effects in older population, showing that the deterioration in effects started after three months and were persistent over the twelve months (Modaberi et al. 2021), identifying that detraining periods of more than 8 weeks began to affect older participants, even in the most successful exercise programs.

Therefore, it seems clear that long-term exercise training can be a useful treatment to reduce inflammation during the ageing progression. Studies have

shown that training interruption resulted in significant loss of upper and lower limb muscle strength and decreased levels of aerobic capacity (Ratel et al., 2012) and that with changes related to strength, detraining may also affect metabolic biomarkers.

Since increased metabolic disorders are highly prevalent in older persons, mainly in those with obesity, it is important to understand the impacts of detraining in the older persons. Despite evidence showing the benefits of physical exercise for the older population, there is restricted data regarding the unfavorable effects that detraining could pose to the inflammatory biomarkers of this group.

Our research group concluded that physical exercise, with and without BCAA supplementation, triggers small changes in the immune, cognitive, and physical profiles of older persons. Unlike other studies in the literature, our results show that supplementation with BCAA does not cause major impacts on health-related parameters at older ages, the limited number and heterogeneity of our sample may explain the absence of more significant results in this context.

The cognition assessed through the (MMSE) practically remained unchanged in the groups. The results of albumin were interesting, since the groups ME+BCAA and ME were able to maintain their levels, which shows a strong effect of the performance on this marker. In contrast, TNF-α increased in the same period, which demonstrates that the pro-inflammatory state returns during this period of interruption.

Although not very strong, the correlation between ST levels and HGS is also particularly interesting, since in older men and women, low testosterone levels were each independently associated with an increased progressive frailty status (Wu et al., 2010), and agree with data published by other groups (Hsu et al., 2018). Although ST did not vary between groups, as was expected, it is possible that the individual variability and the low sample number may have disguised the mediating effects of exercise on testosterone levels. Also, it is important to highlight that there is no scientific consensus about the testosterone response to exercise, especially among older persons. This deserves further investigation to assess the improvements that testosterone could provide in functional fitness and frailty in older persons (Hsu et al., 2018).

Despite a small and transient change over time seen in some immunological and cognitive parameters, the results appear to be influenced by the state of

comorbidity of each group. The main finding of this study was the result obtained for the albumin concentrations in the ME + BCAA group, as already mentioned, it seems to be the first study showing that the combination can maintain serum albumin levels in older persons.

7.2- Assumption and Limitations

The non-randomization of the sample was probably one of the main factors that made the groups heterogeneous for some variables and the N was relatively low, which may have decreased the power of the results. Another concern of this study was the period of protein intake. The research team's option was for it to be performed within ten minutes after the exercise. The number of participants (n=15) that we lost during the study and the difference between the intervention and control groups.

The design, experimental application and data processing of this study was developed considering the existence of certain assumptions and some study limitations, which are important to identify. The premises in which the problem was presented are based on the following assumptions: i) given the reproducibility of the theory of physical frailty and its application in countless longitudinal studies, the same will occur in the Portuguese population; ii) the same pattern of associations between the FP and the different general health parameters associated with the older persons; iii) the hypothetical positive effect of physical exercise as a complementary tool in attenuating the frail state, may also have an effect on affecting the additional global health outcomes explored.

In relation to the limitations of the study, the following questions are: i) low adherence of participants to different physical exercise programs; ii) the lack of authorization by the directors of the institutions to send the participants to the FCDEF-UC laboratory for anthropometric assessment iii) the need to carry out exercise programs, which reduced the number of institutions that participated in this study, iv) prejudice towards nutritional supplementation (relative to amino acids), v) difficulty in collecting blood samples from older persons.

7.3- Main Conclusions

So far, this has been the first study to observe the effects of long-term physical exercise and BCAA supplementation on biomarkers, especially Albumin

in the frail and pre-frail older persons of the institutionalized Portuguese population, our results of frailty final score showed a decrease in physical frailty in intervention groups but with an increase in the control group, concluding that the intervention hybrid fulfilled its role in frailty syndrome.

7.4- Directions for Future Studies

Physical exercise can be seen as a non-pharmacological drug with easy access, and seen as medicine, as this treatment can be combined with other therapies, such as supplementation with proteins or amino acids, to increase the benefits associated with the hybridization of the intervention.

Another aspect appears in the frailty construct, nutritional frailty (Tanaka et al., 2018; Morley, 2020) that can be explored, through tools such as physical exercise and nutritional supplementation. Health status among older persons can also be influenced by being isolated, a poor financial situation, immobility, and decreased ability to perform their daily life activities. Some psychosocial factors, including loneliness, sleep disorders, dementia and depression can also have a negative impact on the nutritional intake of older persons.

It is strongly recommended that institutions for the elderly adopt physical exercise programs as they are an effective strategy to promote physical health in this population. BCAA supplementation is justified if it is associated with a physical exercise program. Physical and nutritional frailty may be related.

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ANNEX I

CONSENT FORMS

TERMO DE CONSENTIMENTO DIRIGIDO À INSTIUIÇÃO

Obrigado por ter demonstrado interesse neste projecto. Por favor leia cuidadosamente esta folha informativa antes de decidir participar. Desde já agradecemos a sua adesão, no entanto não existirá qualquer tipo de desvantagem se a sua decisão for contraria e agradecemos de qualquer modo, o facto de ter ponderado a sua participação. Em qualquer altura poderá abandonar este projecto sem qualquer desvantagem. Este projecto de estudo insere-se no âmbito das Ciências do Desporto e tem por objectivo verificar se em diferentes programas de exercício físico moderado e suplementação de aminoácidos de cadeia ramificada (BCAA) são capazes de modificar o "ambiente" hormonal e imunitário em idosos e se correlacionam com variáveis cognitivas, imunes, podendo o exercício funcionar como um factor protector contra as doenças crônicas próprias do envelhecimento.

Ao integrar este projecto, será pedido aos participantes que autorizem a recolha de amostras de sangue venoso, saliva, medidas corporais, o preenchimento de vários questionários destinados a avaliar o seu nível de estresse, a realização de vários testes de cognição, testes de avaliação funcional/condição física e dados biográficos. Todos os dados recolhidos serão confidenciais e só a equipa de avaliação terá acesso a eles. Os resultados deste projecto poderão ser publicados, mas jamais permitirão a identificação de qualquer elemento. Se for o seu desejo os responsáveis pelo projecto prontificam-se a disponibilizar os resultados obtidos. Os dados recolhidos serão armazenados em segurança e só os que foram mencionados poderão ter acesso a eles. No final de todas as informações recolhidas serão destruídas, excepto aquelas que por política de investigação tenham implicações relativamente às conclusões deste projecto, que serão armazenadas em segurança. Se tiver dúvidas, acerca do projecto agora ou no futuro não hesite em colocá-las aos responsáveis do projecto.

O investigador responsável: Professora Doutora Ana Maria Teixeira

e-mail: ateixeira@fcdef.uc.pt

A Faculdade de Ciências do Desporto e Educação Física da Universidade de Coimbra (FCDEF-UC), através do Centro de Investigação do Desporto e Actividade Física (CIDAF), reconhecido pela Fundação para Ciência e Tecnologia, (FCT), conduz uma linha de investigação na área de envelhecimento ativo e saudável. O projecto intitula-se **"Exercício Multicomponente e Suplementação de Aminoácidos de Cadeia Ramificada em Idosos Pré-Frágeis e Frágeis"** está sob a coordenação dos Professores Doutores Ana Maria Miranda Botelho Teixeira e Alain Massart, para que os objectivos desta investigação sejam alcançados buscamos a vossa colaboração para a constituição da amostra do estudo que será constituída por participantes voluntários, homens e mulheres com idade acima dos 60 anos.

O desenvolvimento do projecto pressupões as seguintes premissas:
ter o seu início em Abril de 2018, duração total de 40 semanas. Caso seja necessário será protocolado um acordo de cooperação entre os representantes de ambas as instituições; as atividades do projecto serão desenvolvidas nas instalações da instituição aderente sendo os dados recolhidos por professores e investigadores do projecto; os dados serão recolhidos em dia e horários previamente combinados preservando ao máximo as rotinas dos sujeitos voluntários e da instituição aderente; todas as etapas e ações relacionadas com o projecto serão comunicadas à direção da instituição assegurando a transparência em todo o processo; será elaborado um "Termo de Consentimento informado dirigido aos participantes" para participação voluntária no programa.
Os objectivos gerais da presente investigação são:

Caracterizar a população em função da fragilidade e perfil cognitivo, examinar os efeitos do exercício na capacidade física funcional e nas habilidades cognitivas dos participantes.

Analisar os efeitos da suplementação com aminoácidos de cadeia ramificada (combinado ou não com o exercício) em idosos e sua implicação nos

aspectos da fragilidade física e cognitiva, dos sistemas neuroendócrinos, imunológicos e na capacidade funcional dos participantes.

Programa de Exercícios

Os idosos que farão parte da investigação participarão de um programa de exercícios com bandas elásticas na cadeira, realizados durante 40 semanas, 2 vezes por semana, em intensidade variante entre leve/moderada (50-60%FCM), controlada através da utilização da percepção subjetiva de esforço (Escala de Borg) e cardiofrequencímetro. O programa de exercícios será dividido em 3 fases:

Fase 1 - Chair based exercise (16 semanas) - Os exercícios são realizados em formato "*chair- based exercise",* que consistem na realização do programa de movimentos corporais com base na cadeira, possuindo boa amplitude de movimentos, segurança adequada ao exercício, e sustentação que permite descanso entre as execuções com maior exigência muscular. Serão utilizadas bandas elásticas de diversas graduações, cuja disponibilidade será assegurada pelos responsáveis do projecto.

Fase 2 - Destreino, *Washout* (8 semanas) - Neste período não serão realizadas nenhum exercício/atividade física com os utentes, pois iremos avaliar o efeito que o destreino (falta de exercício) resulta na capacidade funcional do mesmo.

Fase 3 - Exercício multicomponente (16 semanas) - são exercícios de (força+equilíbrio+aeróbico) na mesma sessão de exercício. Visto na literatura científica como o programa de exercícios mais adequado e completo para população estudada.

A sessão de exercício envolve uma aula de 60 minutos, dividida em:

5 minutos de aquecimento;

25 minutos de treinamento de força, e movimentação geral de membros inferiores e superiores;

10 minutos de exercício de equilíbrio;

10 minutos de exercício aeróbio (caminhada);

5 minutos exercício de relaxamento e volta à calma;

Protocolos de avaliação

Os participantes serão examinados nas seguintes dimensões: a) Avaliação do Perfil Cognitivo, b) Análise de biomarcadores sanguíneos e salivares, c) Aplicação de testes de Aptidão física, avaliação antropométrica e da composição corporal, d) aplicação de questionário biossocial, e) Escala de Fragilidade (Fried et al., 2001). Será feita uma colheita de sangue venosos por técnico creditado para o efeito com o objectivo de determinar os biomarcadores sanguíneos.

Resultados esperados

Após a caracterização da população de idosos em função do perfil, espera-se a confirmação da resposta imunológica e neuroendócrina positiva e da melhoria dos níveis de bem-estar psicológico e autonomia funcional causada pela mediação do exercício físico. Acredita-se ainda que as respostas positivas estarão associadas à melhoria da integridade das capacidades físicas e cognitivas associadas à prática de exercício físico. É esperada ainda a melhoria na força e resistência muscular, flexibilidade e coordenação motora oriunda do aumento da atividade física diária.

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CONSENTIMENTO INFORMADO, ESCLARECIDO E LIVRE PARA PARTICIPAÇÃO EM ESTUDOS DE INVESTIGAÇÃO
(de acordo com a Declaração de Helsinque e a Convenção de Oviedo)

A Faculdade de Ciências do Desporto e Educação Física da Universidade de Coimbra, no âmbito do doutoramento em Ciências do Desporto da Mestre Adriana Caldo, vai desenvolver um projecto intitulado: **"Exercício Multicomponente e Suplementação de Aminoácidos de Cadeia Ramificada em Idosos Pré-Frágeis e Frágeis"** para o qual gostaríamos de contar com a sua colaboração. Este projecto é orientado pelos Prof. Doutores. Ana Maria Teixeira e Alain Massart que estarão à sua disposição para qualquer esclarecimento.

Este projecto tem como objectivo determinar os efeitos combinados do exercício físico e da suplementação com aminoácidos de cadeia ramificada em idosos institucionalizados. Neste estudo iremos implementar um programa de exercício adaptado à sua idade e capacidade física, aliado a toma de um suplemento nutricional proteico de modo a melhorar entre outras, a sua mobilidade e força muscular. O estudo envolve o preenchimento de vários questionários, a execução de alguns testes físicos e a recolha de sangue e saliva de modo a perceber se a intervenção efetuada tem benefícios ao nível da sua saúde física e na sua qualidade de vida. Para que possa dar o seu consentimento com a máxima sinceridade e liberdade, queremos garantir que a investigação segue os termos da Resolução 196/96 do Conselho Nacional de Saúde, sendo garantido: a) o sigilo da privacidade do participante quanto aos dados de identificação e resultados obtidos; b) que as informações sobre o estudo serão fornecidas pelo investigador para que possa decidir livremente sobre a sua participação na investigação; c) a liberdade de recusar a participação ou retirar o consentimento, a qualquer momento. Para que possamos recolher as

informações necessárias para o desenvolvimento da investigação, pedimos a sua colaboração, manifestando a sua aceitação em participar neste estudo.

Assim, na expectativa de contar com a sua colaboração, agradecemos a sua atenção e colocamo-nos à sua disposição para esclarecer quaisquer dúvidas. Por favor, leia com atenção a seguinte informação. Se achar que algo está incorreto ou que não está claro, não hesite em solicitar mais informações. Se concorda com a proposta que lhe foi feita, queira assinar este documento.

Adriana Caldo	Contacto: (aluna Doutoramento)
Ana Maria Teixeira, PhD	Contacto:
Alain Massart, PhD	Contacto:

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Declaro ter lido e compreendido este documento, bem como as informações verbais que me foram fornecidas pela/s pessoa/s que acima assinaram. Foi-me garantida a possibilidade de, em qualquer altura, recusar participar neste estudo sem qualquer tipo de consequências. Desta forma, aceito participar neste estudo e permito a utilização dos dados que de forma voluntária forneço, confiando em que apenas serão utilizados para esta investigação e nas garantias de confidencialidade e anonimato que me são dadas pela investigadora.

Nome:_____

Data:____/____/____

Assinatura:

_____

Impressão digital

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TERMO DE CONSENTIMENTO PARA RECOLHA DE AMOSTRA DO PARTICIPANTE

Nome:
Data: ____/____/_____ **Hora:**

Eu,_____

Declaro que estou ciente da realização da recolha de material biológico (SANGUE E SALIVA), para análise laboratorial. Declaro que estou ciente da possibilidade de proceder uma segunda recolha de material biológico, após o total das sessões de exercícios físicos.

Assinatura do Participante ou Responsável

_____

( ) Não autorizo a recolha do meu material biológico para essa finalidade.

Caso o paciente ou responsável estejam impossibilitados de assinar o termo, justificar o motivo

_____
_____
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AUTORIZAÇÃO PARA UTILIZAÇÃO DE IMAGENS

Declaro, para os devidos efeitos legais, que autorizo a utilização das fotografias e imagens captadas durante o projecto de investigação científica com o tema **"Exercício Multicomponente e Suplementação de Aminoácidos de Cadeia Ramificada em Idosos Pré-Frágeis e Frágeis"**

Mas declaro expressamente, que as referidas imagens e fotografias poderão ser utilizadas no âmbito de qualquer iniciativa ou ação de publicidade promovida pela Faculdade de Ciências do Desporto e Educação Física da Universidade de Coimbra, renunciando desde já a quaisquer direitos ou compensação que desta utilização possa eventualmente resultar.

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As imagens captadas em vídeo poderão, de igual modo, ser utilizadas para qualquer fim publicitário ou promocional, decorrente da ação da Instituição. Por ser verdade, e por nada haver a obstar, esta declaração vai ser assinada por mim.

_____

Assinatura ou Impressão digital

ETHICAL COMMITEE AND SUPPLEMENTATION ANALYSIS

Gmail **Adriana Caldo <dricaldo@gmail.com>**

Deliberação do Conselho Científico

1 mensagem

Conselho Científico <cc@fcdef.uc.pt> 28 de fevereiro de 2018 16:45
Para: Adriana Caldo <dricaldo@gmail.com>, uc2016164960@student.uc.pt
Cc: Ana Botelho Teixeira <ateixeira@fcdef.uc.pt>, Alain Massart <alainmassart@fcdef.uc.pt>

Caro/a Estudante

Para conhecimento e devidos efeitos, cumpre-nos dar conhecimento do deliberado pelo Conselho Científico na reunião realizada no dia 19/01/2018, vertido no ponto 13 das respetivas deliberações que a seguir se transcreve:

" Aceitou, por maioria, o Projeto de Doutoramento em Ciências do Desporto da seguinte estudante:

Adriana Caldo Silva
Ramo do Doutoramento: Atividade Física e Saúde
Título do Projeto: Efeito de um programa de exercício físico multicomponente e suplementação de aminoácidos de cadeia ramificada em parâmetros de saúde de idosos institucionalizados
Orientadores (aprovados por unanimidade): Professora Doutora Ana Maria Miranda Botelho Teixeira e Prof. Doutor Alain Guy Marie Massart
Os orientadores propostos foram aprovados por unanimidade. "

--
Com os melhores cumprimentos | Best regards,

Armando Beirão

Gabinete Técnico de Apoio ao Director | Support Management Staff

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· U C ·

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To Whom It May Concern

The project entitled *"Efeito de um programa de exercício físico multicomponente com e sem suplementação de aminoácidos de cadeia ramificada em parâmetros de saúde de idosos institucionalizados"* (ref. CE/FCDEF-UC/00282018) of Mestre Adriana Caldo Silva was approved by the Ethics Commitee of the Faculty of Sport Sciences and Physical Education of the University of Coimbra on the 24th May 2019.

Coimbra, 01/08/2019,

The President of the Ethics Commitee,

(Prof. Doutor Carlos Gonçalves)

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Item	Specification	Results
Description	White crystals or crystalline powder	Conforms
L-Leucine Content	46.0% - 54.0%	47.0 %
L-Isoleucine Content	22.0% - 27.0%	23.5 %
L-Valine Content	22.0% - 27.0%	23.8 %
Lecithin	0.3% - 1.0%	0.8 %
Bulk Density	0.20 g/ml - 0.50 g/ml	0.46 g/ml
Residue on ignition	≤0.4%	0.06 %
Loss on drying	≤1.0%	0.11 %
Chloride (Cl), %	≤0.05	Conforms
Iron (Fe),ppm	≤30	Conforms
Sulfate (SO4)	≤0.03	Conforms
Heavy metals	≤10.0 ppm	<10.0 ppm
Lead (Pb)	≤3.0 ppm	<3.0 ppm
Arsenic(As)	≤1 ppm	<1.0 ppm
Cadmium (Cd)	≤1 ppm	<1.0 ppm
Mercury (Hg)	≤0.1 ppm	<0.1 ppm
Total Plate Count	≤1000 cfu/g	150 cfu/g
Yeasts and Molds	≤100 cfu/g	<10 cfu/g
Escherichia coli	Negative	Conforms
Salmonella	Negative	Conforms
Staphy lococcus aureus	Negative	Conforms

Certificate of Analysis

Product : BCAA2: 1: 1 Instant

Product no.:

Quality:

Production date:

Expiry date:

Batch no:

Quantity: 400 kg

ClinicalTrials.gov PRS DRAFT Receipt (Working Version)
Last Update: 05/04/2020 14:49

ClinicalTrials.gov ID: NCT04376463

Study Identification

Unique Protocol ID:	UC
Brief Title:	Effects of a Multicomponent Exercise and Supplementation of BCAA´s in Immunity System Dwelling Elderly
Official Title:	Effects of a Multicomponent Exercise and Supplementation of Branched Chain Aminoacids Health Parameters in the Dwelling Elderly
Secondary IDs:	

Study Status

Record Verification:	May 2020
Overall Status:	Active, not recruiting
Study Start:	April 13, 2019 [Actual]
Primary Completion:	September 14, 2019 [Actual]
Study Completion:	December 15, 2020 [Anticipated]

Sponsor/Collaborators

Sponsor:	University of Coimbra
Responsible Party:	Principal Investigator Investigator: Adriana Caldo [acaldo] Official Title: Researcher, principal investigator Affiliation: University of Coimbra
Collaborators:	University of Coimbra

ANNEX II

QUESTIONNAIRES AND PHYSICAL TESTS

Caro participante (ler):

Estes questionários destinam-se à realização de um trabalho de investigação para verificar a saúde relacionada à condição física em pessoas da vossa idade. Trata-se de um conjunto de testes que envolve a **recolha de informação confidencial** pelo que nunca no decorrer deste trabalho será divulgada a identificação dos indivíduos neles intervenientes. Ao responder às questões faça- o de uma forma sincera e, por favor, não deixes qualquer questão por responder, pois disso dependerá o rigor científico deste trabalho.

MUITO OBRIGADA!!

Questionário Biossocial

1	Nome completo:
2	Qual a sua idade?
3	Sexo 1. Masculino 2. Feminino
4	Estado civil: 1. Solteiro 2. Casado/união de fato 3.Viúvo 4. Separado/divorciado
5	Escolaridade: 1. Nunca frequentou a escola 2. Não completou Primário 3. Primário 4. Preparatório 5. Secundário 6. Ensino profissional 7. Universitário
6	Naturalidade (Concelho):
7	Residência (Concelho):
8	Onde vive atualmente? 1. Casa própria 2. Lar. 3. Casa dos filhos. 4. Casa dos parentes
9	Pratica exercício físico/ginástica de manutenção/ginásio? 1. Sim 2. Não
10	Qual a frequência semanal? 1. Uma vez 2.Duas vezes 3. Três vezes 4. Quatro vezes ou mais

Índice de Comorbilidade de Charlson (ICC)

(Mourão, 2008; Charlson et al., 1987)

INSTRUÇÕES: Marcar com uma cruz caso seja acometido por uma ou mais destas doenças ou condições

	1	Enfarte do Miocárdio
	2	Insuficiência Cardíaca
	3	Doença Arterial Periférica
	4	Doença Cerebrovascular (AVC)
	5	Demência
	6	Doença Respiratória Crónica
	7	Doença do Tecido Conjuntivo
	8	Úlcera Gastroduodenal
	9	Hepatopatia Crónica Leve
	10	Diabetes
	11	Hemiplegia
	12	Insuficiência Renal Crónica Moderada/Severa
	13	Diabetes com Lesão em Órgãos Alvo
	14	Tumor ou Neoplasia Sólida
	15	Leucemia
	16	Linfoma
	17	Hepatopatia Cronica Moderada/Severa
	18	Tumor ou Neoplasia
	19	Sida definida
	20	_____

Mini Avaliação Nutricional (MNA)

(Guigoz, 2006; Vellas et al.,1999; Loureiro, 2008)

I	**TRIAGEM**
A	**Nos últimos três meses houve diminuição da ingesta alimentar devido a perda de apetite, problemas digestivos ou dificuldade para mastigar ou deglutir?** 0 = diminuição grave da ingesta. 1 = diminuição moderada da ingesta 2 = sem diminuição da ingesta
	Perda de peso nos últimos 3 meses 0 = superior a três quilos 1 = não sabe informar 2 = entre um e três quilos 3 = sem perda de peso
C	**Mobilidade** 0 = restrito ao leito ou à cadeira de rodas 1 = deambula mas não é capaz de sair de casa 2 = normal
D	**Passou por algum *stress* psicológico ou doença aguda nos últimos três meses?** 0 = sim 2 = não
E	**Problemas neuropsicológicos** 0 = demência ou depressão graves 1 = demência ligeira 2 = sem problemas psicológicos

F	**Índice de Massa Corporal (IMC)** 0 = IMC < 19 1 = 19 ≤ IMC < 21 2 = 21 ≤ IMC < 23 3 = IMC ≥ 23
	Pontuação da Triagem (subtotal, máximo de 14 pontos) 12-14 pontos: estado nutricional normal 8-11 pontos: sob risco de desnutrição 0-7 pontos: desnutrido
	AVALIAÇÃO GLOBAL
G	**O utente vive na sua própria casa (não em instituição geriátrica ou hospital)** 1 = sim 0 = não
H	**Utiliza mais de três medicamentos diferentes por dia?** 0 = sim 1 = não
I	**Lesões de pele ou escaras?** 0 = sim 1 = não
J	**Quantas refeições faz por dia?** 0 = uma refeição 1 = duas refeições 2 = três refeições
K	**O utente consome:** pelo menos uma porção diária de leite ou derivados (leite, queijo, iogurte)? duas ou mais porções semanais de leguminosas ou ovos? carne, peixe ou aves todos os dias? 0.0 = nenhuma ou uma resposta «sim» 0.5 = duas respostas «sim» 1.0 = três respostas
L	**O utente consome duas ou mais porções diárias de fruta ou produtos hortícolas?** 0 = não 1 = sim
M	**Quantos copos de líquidos (água, sumo, café, chá, leite) o utente consome por dia?** 0.0 = menos de três copos 0.5 = três a cinco copos 1.0 = mais de cinco copos
N	**Modo de se alimentar** 0 = não é capaz de se alimentar sozinho 1 = alimenta-se sozinho, porém com dificuldade 2 = alimenta-se sozinho sem dificuldade
O	**O utente acredita ter algum problema nutricional?** 0 = acredita estar desnutrido 1 = não sabe dizer 2 = acredita não ter um
	Em comparação com outras pessoas da mesma idade, como considera o utente a sua própria saúde? 0.0 = pior 0.5 = não sabe 1.0 = igual 2.0 = melhor
Q	**Perímetro braquial (PB) em cm** 0.0 = PB < 21 0.5 = 21 ≤ PB ≤ 22 1.0 = PB > 22
R	**Perímetro da perna (PP) em cm** 0 = PP < 31 1 = PP ≥ 31
	Avaliação do Estado Nutricional **de 24 a 30 pontos _____ estado nutricional normal** **de 17 a 23,5 pontos_____ sob risco de desnutrição** **menos de 17 pontos _____ desnutrido**

Escala de Depressão Geriátrica (GDS)

(Yesevage 1982; Apóstolo 2011)

Questão		Resposta	Pontuação	Resposta	Pontuação
1	Está satisfeito (a) com a sua vida?	SIM ( )	0	NÃO ( )	1
2	Interrompeu muitas de suas atividades?	SIM ( )	1	NÃO ( )	0
3	Acha sua vida vazia?	SIM ( )	1	NÃO ( )	0
4	Aborrece-se com frequência?	SIM ( )	1	NÃO ( )	0
5	Sente-se bem com a vida na maior parte do tempo?	SIM ( )	0	NÃO ( )	1
6	Teme que algo ruim lhe aconteça?	SIM ( )	1	NÃO ( )	0
7	Sente-se alegre a maior parte do tempo?	SIM ( )	0	NÃO ( )	1
8	Sente-se desamparado com frequência?	SIM ( )	1	NÃO ( )	0
9	Prefere ficar em casa a sair e fazer coisas novas?	SIM ( )	1	NÃO ( )	0
10	Acha que tem mais problemas de memória que as outras pessoas?	SIM ( )	1	NÃO ( )	0
11	Acha que é maravilhoso estar vivo (a)?	SIM ( )	0	NÃO ( )	1
12	Sente-se inútil?	SIM ( )	1	NÃO ( )	0

13	Sente-se cheio (a) de energia?	SIM ( )	0	NÃO ( )	1
14	Sente-se sem esperança?	SIM ( )	1	NÃO ( )	0
15	Acha que os outros têm mais sorte que você?	SIM ( )	1	NÃO ( )	0
TOTAL					

INTERPRETAÇÃO

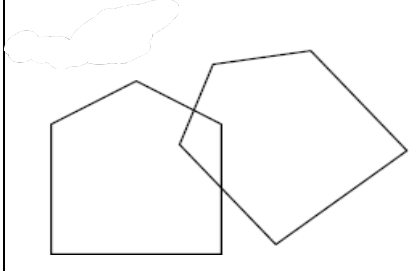
0 a 5 pontos: indica quadro psicológico normal.

6 a 10 pontos: indica quadro de depressão l

11 a 15: Quadro de depressão severa

Mini Exame do Estado Mental (MEEM)

(Folstein et al. 1975; Morgado et al. 2009; Guerreiro et al., 1994)

I	**Orientação (Um ponto por cada resposta certa)**
1	Em que ano estamos?
2	Em que mês estamos?
3	Em que dia do mês estamos?
4	Em que dia da semana estamos?
5	Em que estação do ano estamos?
6	Em que país estamos?
7	Em que distrito vive?
8	Em que terra vive?
9	Em que casa estamos?
10	Em que andar estamos
II	**Retenção (contar um ponto por cada palavra corretamente repetida)**
11	"Vou dizer três palavras; queria que as repetisse, mas só depois que eu as dizer todas, procure sabê-las de cor": (PÊRA – GATO – BOLA) Pontos:_____
III	**Atenção e cálculo (um ponto por cada resposta correta. Se der uma errada mas depois continuar a subtrair, consideram-se as seguintes como corretas. Pára ao fim de 5 respostas)**
12	"Agora peço-lhe que me diga quantos são 30 menos 3 e depois ao número encontrado voltar a tirar 3 e repete assim até eu dizer para parar" 30___27___24___21___18___15___ Pontos:_____
IV	**Evocação (um ponto por cada resposta correta)**
13	Veja se consegue dizer as 3 palavras que pedi há pouco para decorar" (Pêra – Gato – Bola) Pontos:
V	**Linguagem (um ponto por cada resposta correta)**
14	"Como se chama isto?" Mostrar os objetos: Relógio e lápis Pontos:
15	"Repita a frase que eu vou dizer: "O RATO ROEU A ROLHA" Pontos:
16	"Quando eu lhe der esta folha, pegue nela com a mão direita, dobre-a ao meio e coloque-a sobre a mesa", (ou "sobre a cama", se for o caso); dar a folha, segurando com as duas mãos. a) Pega com a mão direita; b) Dobra ao meio; c) Coloca onde deve Pontos
17	"Leia o que está neste cartão e faça o que lá diz". Mostrar um cartão com a frase bem legível, "FECHE OS OLHOS"; sendo analfabeto lê-se a frase. *Feche os olhos* Pontos
18	"Escreva uma frase inteira aqui". Deve ter sujeito e verbo e fazer sentido; os erros gramaticais não prejudicam a pontuação. Frase: Pontos
VI	**Habilidade construtiva (um ponto pela cópia correta do desenho**
19	Deve copiar um desenho. Dois pentágonos parcialmente sobrepostos; cada um deve ficar com 5 lados, dois dos quais intersectados. Não valorizar tremor ou rotação.  Pontos:_____
	Pontuação Máxima 30 ----------Pontuação total MMSE:

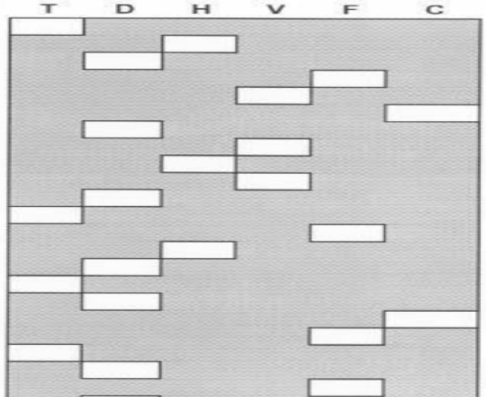
Perfil do Estado de Humor (POMS)

(McNair et al., 1971; Adaptado Viana et al., 2001)

		Nada	Um pouco	Moderadamente	Bastante	Muitíssimo
		0	1	2	3	4
1	Tenso					
2	Irritado					
3	Imprestável					
4	Esgotado					
5	Animado					
6	Confuso					
7	Triste					
8	Activo					
9	Mal-humorado					
10	Enérgico					
11	Sem valor					
12	Inquieto					
13	Fatigado					
14	Aborrecido					
15	Desencorajado					
16	Nervoso					
17	Só					
18	Baralhado					
19	Exausto					
20	Ansioso					
21	Deprimido					
22	Sem energia					

Não escreva nos espaços abaixo. Só para uso interno.

T D H V F C



Desempenho funcional

Eu gostaria que você se sentasse nesta cadeira com as costas e os braços relaxados. Quando eu digo 'JÁ', por favor, levantasse e ande num ritmo seguro e confortável até à marca no chão (aproximadamente 3 m de distância), volte para a cadeira e sente-se novamente.

0-10 Segundos b) 11-20 Segundos c) Mais de 20 segundos

Avaliação da Fragilidade

(Fried, 2001; Furtado, Teixeira & Ferreira, 2014)

Instruções: Um aplicador externo conduz os testes, respeitando a forma de avaliação de cada dimensão. Todavia, é valido recorrer aos arquivos médicos, caso alguma questão suscite dúvidas no seu preenchimento. Cada dimensão deverá passar pelo ajustamento estatístico para pçosterior calculo do traço de fragilidade. Ver apendice do estudo original.

1	**Lentidão da marcha: Ao sinal o participante deve percorrer a distância de a distância de 4,6 metros em velocidade confortável num terreno plano, utilizando técnica de caminhada.** Executar 3 tentativas para posterior cálculo da média dos valores, que deverão ser registrados em segundos: 1ª_____ 2ª_____ 3ª_____ Média _____	**2**	Perda de peso não intencional: **Você perdeu 4 quilos ou mais de peso corporal de forma não intencional neste último ano? (ou valor acima de 5% do peso corporal, segundo registro médico). Duas opções de resposta:** ( ) 1. Sim ( ) 2. Não
3	**Força muscular:** utilizando o teste de força de preensão manual, mensurar e registar a força no membro superior dominante. Executar 3 tentativas para posterior cálculo da média dos valores, que deverão ser registrados em quilos: Lado Direito: 1_____,2_____, 3_____ Lado Esquerdo: 1_____, 2_____, 3_____ FPM (total)_____	**4**	**Exaustão (perceção subjetiva):** Fazer as 2 perguntas abaixo (7 e 20 CES-D) ao participante e verificar a concordância negativa ou positiva entre elas: **Sentia que tudo do que fazia era um esforço: Sim_____ Não_____** **Sente falta de energia:** ( ) Sim ( ) Não
5	Avaliação dos níveis de atividade física: **Aplicar o questionário IPAQ versão curta, que se encontra abaixo. Ler atentamente as questões antes de iniciar a aplicação.**		

Questionário Internacional de Atividade Física (IPAQ)

(Craig et al., 2001; Campaniço & Sardinha, 2016)

Estamos interessados em saber os tipos de atividades físicas que faz na sua vida quotidiana. As perguntas que lhe irei fazer são sobre o tempo que gastou a ser fisicamente ativo nos últimos 7 dias. Por favor, responda a cada pergunta, mesmo que não se considere uma

pessoa ativa. Por favor, pense sobre as atividades que faz no trabalho, em casa, a ir de um lugar para outro, e no seu tempo livre para o exercício, lazer ou desporto. Pense em todas as atividades vigorosas que fez nos últimos 7 dias. Atividades físicas vigorosas referem-se a atividades de esforço físico elevado e que o fazem respirar com mais dificuldade do que o normal. Pense apenas nas atividades físicas que por pelo menos 10 minutos.

1	**Durante os últimos 7 dias, em quantos dias fez atividades físicas vigorosas, como levantamento de pesos, cavar, aeróbica, ou andar de bicicleta?**
	_____ dias por semana _____Não fez atividades físicas vigorosas. Passar para a questão 3
2	**Quanto tempo gastou fazendo atividades físicas vigorosas naqueles dias?**

Pense em todas as atividades **moderadas** que fez nos últimos 7 dias. Atividades moderadas referem-se a atividades de esforço físico moderado e que o fazem respirar com um pouco mais de dificuldade do que o normal. Pense apenas nas atividades físicas que fez por pelo menos 10 minutos.

3	**Durante os últimos 7 dias, em quantos dias fez atividades físicas moderadas, como o transporte de cargas leves, ciclismo a um ritmo regular, ténis? Não incluem caminhar.**
	_____ dias por semana _____Não fez atividades físicas moderadas. Passar para a questão 5
4	**Quanto tempo gastou fazendo atividades físicas moderadas naqueles dias?**
	_____horas por dia _____minutos por dia não sabe

Pense sobre o tempo que gastou **caminhando** nos últimos 7 dias. Isto inclui no trabalho e em casa, andar de um lugar para outro, e qualquer outro passeio que tenha feito exclusivamente para a recreação, desporto, lazer ou exercício.

5	**Durante os últimos 7 dias, em quantos dias caminhou por pelo menos 10 minutos de cada vez?**
	____ dias por semana ____ Não caminhou - Passar para a questão 7
6	**Quanto tempo gastou caminhando naqueles dias?**
	_____horas por dia _____minutos por dia _____Não sabe/Não tem a certeza

A última questão é sobre o tempo que gastou **sentado** em dias de semana durante os últimos 7 dias. Incluem o tempo gasto no trabalho/escola, em casa, e durante o tempo de lazer. Inclui o tempo gasto sentado à mesa, visitando amigos, lendo ou estando sentado ou deitado a ver televisão.

7	**Durante os últimos 7 dias, quanto tempo passou sentado em dias da semana?**
	_____ horas por dia _____minutos por dia _____Não sabe/Não tem a certeza

Short Physical Performance Battery (SPPB)

(Guralnik et al., 1994)

Equilíbrio (se a pessoa não terminar um dos testes, indiquem o tempo que permaneceu em cada posição)

Pés juntos -_____ 

Completou tempo 10"____1 ponto
Não completou _____0 ponto

Pontuação total SPPB

Pontuação total teste equilíbrio_____
Pontuação teste velocidade da marcha_____
Pontuação teste da cadeira_____
Pontuação total: _____

1- 0 a 3 pontos_____Incapaz
2- 4 a 6 pontos_____Baixo desempenho
3- 7 a 9 pontos_____Moderado desempenho
4- 10 a 12 pontos_____Bom desempenho

O calcanhar de um pé colocado ao lado do dedo grande do outro pé
Completou_____1 ponto
Não completou____0 ponto

Um pé à frente do outro 10”

Completou tempo_____2 pontos
Completou de 3 “-9” _____ 1 ponto
Menos de 3” _____0 ponto
Não fez _____0 ponto

Pontuação total teste equilíbrio_____

Classificação geral

Teste de Velocidade da marcha (3,00 m)

Se o tempo for menor 3,62”_____4 pontos
Se o tempo for 3,62”-4,65”_____ 3 pontos
Se o tempo for 4,66”-6,52” _____2 pontos
Se o tempo for maior 6,52”maior _____1 ponto

Ajuda técnica: Não usou ( ) Usou ( ) Indique qual_____

Pontuação teste de velocidade_____

Sentar e levantar da cadeira (5x sem parar)
Instruções: Manter os braços cruzados ao peito
Levantou sem ajuda e com segurança ( ) Sim, ( )Não
Levantou sem ajudar com os braços ( ) Sim, ( ) Não

Tempo maior que 60_____ 0 ponto
Tempo entre 16,70” ou mais_____1 ponto
Tempo entre 13,70”-16,70_____2 pontos
Tempo entre 11,20”-13,69_____3 pontos
Tempo menor 11,19”_____4 pontos
Pontuação total teste de sentar e levantar_____

Força de preensão

Força muscular: Utilizando o teste de força de preensão manual, mensurar e registar a força no membro superior dominante, executar 3 tentativas, que deverão ser registrados em kgf:

Lado dominante: Direito: 1ª 2ª 3 ª Esquerdo: 1ª 2ª 3ª

Lentidão da marcha: Ao sinal o participante deve percorrer a distância de a distância de 4,6 metros em velocidade confortável num terreno plano, utilizando técnica de caminhada. Executar 3 tentativas para posterior cálculo da média dos valores, que deverão ser registrados em segundos:

1ª 2ª 3ª Média____

Desempenho funcional: Eu gostaria que você se sentasse nesta cadeira com as costas e os braços relaxados. Quando eu digo 'JÁ', por favor, levanta-se e ande num ritmo seguro e confortável até à marca no chão (aproximadamente 3 m de distância), volte para a cadeira e sente-se novamente.

a) 0-10 Segundos b) 11-20 Segundos c) Mais de 20 segundos

APPENDIX I

PUBLISHED ARTICLES AND ACCEPTED FOR PUBLICATION

- **Caldo-Silva, A.,** Furtado, G.E., Rodrigues, R., Letieri, R., Santos Neves, R., Vieira-Pedrosa, A., Valente, P., Zanchi, N., Massart, A., Teixeira, A.M. (2020) **The Independent and Combined Effects of a 16-week Exercise and BCAA Supplementation on Frailty and Mood States of Older Women.** European Congress of Sport Science (ECSS), Sevilla - Spain. October.

- **Caldo-Silva, A;** Furtado, G; Neves, R; Rodrigues, R; Vieira-Pedrosa, A; Zanchi, N; Massart, A; Teixeira, AM. (2020). **Impact of 16 weeks of Exercise and Protein Supplementation on Functional-Physical Fitness of Dwelling-Institutionalised Elders.** AgeingCongress, Leiria – Portugal, Thomson Reuters.

- **Caldo-Silva, A.** et al., 2021. Effect of Training-Detraining Phases of Multicomponent **Exercises and BCAA Supplementation on Inflammatory Markers and Albumin Levels in Frail Older Persons. Nutrients**, *13*(4), 1106; **https://doi.org/10.3390/nu13041106**

- **Caldo-Silva, A.** et al., 2021. **Effect of a 40-weeks multicomponent exercise program and branched chain amino acids supplementation on functional fitness and mental health in frail older persons.** Experimental Gerontology https://doi.org/10.1016/j.exger.2021.111592

APPENDIX II

MAIN ACTIVITIES CARRIED OUT DURING THE COURSE

- **Caldo, A.** Rodrigues, R et al. 2020. **Atividade Física, ingestão proteica e Imunidade em Idosos Fisicamente Frágeis.** Book Chapter, Temas Emergentes em Atividade Física, Saúde e Bem-Estar: Realidades de Brasil e Portugal.

GENERAL INFORMATION AND BIOGRAPHY

Adriana Caldo Silva was born in 1981, Ribeirão Preto-SP, graduated in Physical Education from UNAERP in 2003; and a master's degree from the University of São Paulo-USP, Faculty of Medicine of Ribeirão Preto 2016 title “Effects of non-functional overreaching on the mTOR pathway in liver tissue in mice”, obtained a degree equivalence from FCDEF in 2018. She took 5 isolated courses, three for degree equivalence. Large professional experience in exercise programs. Currently, research focused effects exercise program in older persons and skills in nutrition with emphases in frailty conditions.

CONGRESS, SYMPOSIUM AND SCIENTIFIC MEETING

- **Caldo-Silva, A.,** Furtado, G.E., Rodrigues, R., Letieri, R., Santos Neves, R., Vieira-Pedrosa, A., Valente, P., Zanchi, N., Massart, A., Teixeira, A.M. **The Independent and Combined Effects of a 16-week Exercise and BCAA Supplementation on Frailty and Mood States of Older Women.** European Congress of Sport Science (ECSS), Sevilla - Spain. October.

PARTICIPATION IN SHORT COURSES, LECTURE AND SEMINARS

- Ageing@Coimbra (2017) 5º Congresso Regional sobre o Envelhecimento Ativo e Saudável. Convento São Francisco, novembro.

- Ageing@Coimbra (2018) 6º Congresso Regional sobre o Envelhecimento Ativo e Saudável. Convento São Francisco, novembro.

- Ageing@Coimbra (2019) 7º Congresso Regional sobre o Envelhecimento Ativo e Saudável. Convento São Francisco, novembro.

- Curso FFUL/SPCAL em Ciências em Animais de Laboratório (Teórico), em andamento.

- Curso de Formação de Formadores - Agosto 90h (2020).

- 18 Fórum Internacional do Desporto - Março de 2018.

- 19 Fórum Internacional do Desporto - Março de 2019.

- World Health Summit Regional Meeting – 2018 Coimbra, Portugal.

ELABORATION AND PARTICIPATION IN SCIENTIFIC ARTICLES

- **Caldo-Silva, A** et al., 2021. Effect of a 40-weeks multicomponent exercise program and branched chain amino acids supplementation on functional fitness and mental health in frail older persons Experimental Gerontology **https://doi.org/10.1016/j.exger.2021.111592**

- **Caldo-Silva, A** et al., 2021. Evidence-Based Effects of Multicomponent Exercise on Several Health-Related Markers in Frail Older Persons: A Systematic Review. Journal of Physical Education and Sport July. 2021. DOI:10.7752/jpes.2021.s3282

- **Caldo-Silva, A** et al., 2021. Effect of Training-Detraining Phases of Multicomponent Exercises and BCAA Supplementation on Inflammatory Markers and Albumin Levels in Frail Older Persons. *Nutrients, 13*(4), 1106; **https://doi.org/10.3390/nu13041106**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THE INDEPENDENT AND COMBINED EFFECTS OF A 16-WEEK EXERCISE AND BCAA SUPPLEMENTATION ON FRAILTY AND MOOD STATES OF OLDER WOMEN

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INTRODUCTION:
The frailty syndrome (FS) is characterized by a multifactorial clinical syndrome influenced by environmental and biological factors. The progressive losses of functions that occur in the body and consequently physical function decrease are hallmarks of this negative process (1). Some studies have shown that regular exercise has positive effects on several factors correlated with frailty (i.e. physical, psychological) in elderly persons (2). Current findings shown that protein supplementation together with exercise seems to be more effective in decreasing frailty, in promoting muscular mass and strength, in improving functional capacity as well as psychological health (2). Although many nutrients have been tested, (BCAA) showed more promising results. The aim of this study was to analyse the isolated and combined effects of BCAA supplementation and exercise on physical frailty status and mood states in pre-frail institutionalized older women
METHODS:
The sample consisted of 35 female participants (age = 83±3.21) from four social care institutions. A multicomponent exercise (ME) and BCAA was carried out during 16-weeks, following the four-arm experimental design: group 1 (ME+BCAA, n= 8); group 2 (ME, n= 8); group 3 (BCAA, n=6) and control group non-exercising (CGne, n=13). To assess the five criteria of the frailty, we used Fried's scale (1) that assesses: a) gait speed of 4,6 meters; b) non-Intentional weight loss; c) hand grip strength; d) Subjective perception of exhaustion and e) low levels of physical activity. This protocol classifies individuals as frail, pre-frail or robust. The (POMS) and the (GDS) scale were also applied. In addition, the SPPB was aplied. All the tests were applied before and 16-weeks after the exercise program. The (CGne) did not alter their usual routine. Comparative (T-test and Wilcoxon) and effect size (Cohens' d) statistical analysis was performed.

E-poster not debated

RESULTS:
After 16 weeks, both BCAA+ME and BCAA groups showed improvements in SPPB performance and decreased frailty ($p<.05$) with a moderate size effect ($d < 0.30$). In the BCAA+ME a moderate effect size ($d=0.44$) was also found towards more positive mood states.
CONCLUSION:
Our study revealed that exercise plus BCAA supplementation was able to improve physical and psychological health, corroborating previous findings that found satisfactory results in physical function explained by the protein synthesis effect. Moreover, the trend for the improvement of mood states may be related to the effect of BCAAs in increasing serotonin levels modulated by exercise (2).
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Original Article

Evidence-based effects of multicomponent exercise training on biomarkers related to frailty in older persons: a systematic review

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Abstract:
Introduction: Frailty is a complex syndrome that can is characterized by the loss of physical functional capacity. The effects of multicomponent exercise training on frailty and correlated biomarkers are not yet fully explored in the scientific literature. **Objective:** Based on the available literature data, our first endpoint in this systematic review was to present the current knowledge on the influence of multicomponent exercise training in older persons diagnosed with the frailty syndrome. In addition, a second endpoint was to identify not only the most evaluated and responsive biochemical markers in this population, but also how they responded to multicomponent exercise training. **Methods:** The electronic databases PubMed, SciELO, LILACS, and Science Direct, were accessed. The search was conducted in the English language using MeSH keywords in order to verify which biochemical markers were investigated in the studies that associated frail older persons and multicomponent physical exercise. This systematic review was registered under the reference CRD42018089912. **Results:** Six randomized controlled trial studies met our inclusion criteria and were used to perform this review. In response to our endpoints, these studies reported that multicomponent exercise training was able to decrease or reverse frailty by improving functional fitness, muscle strength, speed and agility. In addition, this type of exercise training was associated with alteration in several biomarkers, namely C-reactive protein (CRP), interleukin (IL-6), Tumour Necrosis Factor (TNF-α), insulin-like growth factor (IGF-1), haemoglobin (HbA), albumin, oxidation products, antioxidants, Vitamin D, and serum/plasma/saliva hormone levels of dehydroepiandrosterone (DHEA), testosterone and parathyroid hormone (PTH). **Conclusion:** Multicomponent exercise training is a powerful tool to prevent or even revert frailty development in older persons, namely through its mediating action in several metabolic, hormone and pro-inflammatory markers.
Keywords: Physical function, RCT, Muscle Strength, Inflammatory markers, Hormones.

Introduction

The Frailty Syndrome (FS) can be defined as a loss of physical and functional capacity with multifactorial traits in which the loss of muscle mass is highlighted (Marzetti et al., 2019) The physical frailty syndrome (PFS) is defined as an ageing-related multifactorial clinical condition marked by a progressive decline of multiple physiological domains that compromise the individual capacity to withstand stress (Fried et al., 2001) and can be assessed by evaluating 5 factors: lean mass loss, diminished handgrip strength, reported fatigue/exhaustion, reduction of walking speed, and low physical activity levels. It has been proposed that these losses could be closely associated with a sedentary lifestyle and the lack of previous beneficial interventions, such as physical exercise programs (Milanović et al., 2013; Booth et al., 2012). In fact, these factors are also associated with the increasing number (and risk) of falls in frail older persons (Leitão et al., 2015). There are a handful of studies that aimed to investigate whether exercise training could putatively reverse frailty (frail to pre-frail or pre-frail to robust), or, at least, achieve a lower degree of frailty in older persons (Manãs et al., 2019). A systematic review concluded that structured physical training has positive impacts on frailty and should be used for management of the syndrome (Theou et al., 2011). Therefore, it can be said that the beginning of a physical

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activity program in an older person (which usually does not perform supervised body movements), tends to induce biological/physiological adaptations that will definitely affect the final score of the Fried scale.

It is paramount to mention that, among several exercise training protocols, multicomponent exercise training programs that focus on at least 3 different physical modalities in the same exercise session (Chodzko-Zajko et al., 2009; Baker et al, 2007) including aerobic, strength, balance and flexibility exercises, could promote healthy ageing (Leitão et al., 2015). The results of a meta-analysis showed that training interventions involving multidirectional movements, weight transfers, and elements of functional strength training, improved functional capacity, and also decreased falls by 52% in older persons (Sherrington et al., 2017). Multicomponent exercise programs have also resulted in major improvements in functional capacity, which is strategic for maintaining the skills and independence to perform the basic tasks of daily living (Casas-Herrero et al., 2019). There is growing evidence that the older persons engaged in a multicomponent exercise training program present, in a general way, a better health status as a long-term result (Cadore et al. 2019; Arrieta et al., 2019; Silva et al., 2017; Makizako et al., 2012; Hopps et al., 2011; Theou et al., 2011). Multicomponent programs are considered, by some authors, as the most beneficial type of training to optimize functional capacity of frail older persons and to prevent functional and physical incapacity (Cadore et al., 2014; Villareal et al., 2011), but to our knowledge there are no systematic reviews to clarify the impact of this kind of exercise program in frail older persons.

A main inflammatory profile consisting of greater concentrations of C-reactive protein and lower concentrations Interleukin 8 (IL- 8) with gender-specific signatures has been recognised in the background of physical frailty, together with decreased systemic levels of interleukin-10 (IL-10), a typical anti-inflammatory cytokine, and increases in Tumor Necrosis Factor-alpha (TNF-α) levels are linked with ageing (Marzetti et al., 2019). For many authors, physical exercise training has the potential to provide protection against frailty in advanced aged persons, and that most of these benefits, at least in part, are related to the cognitive, anti-inflammatory and metabolic effects of exercise (Aguirre & Villareal, 2015; Gleeson et al., 2011; Petersen & Pedersen, 2005). Physical exercise stimulates the release of cytokines, such as IL-6, IL-10 and IL-1ra, in response to contracting skeletal muscles, which are responsible not only for tissue restoration and energy metabolism, but also for the adjustment of the systemic inflammatory status (Pedersen & Febbraio, 2012). However, the benefits of physical exercise programs in reducing frailty by improving functional capacity, muscle strength, speed, and agility, did not seem to elicit significant alterations in several systemic biomarkers in some studies (Ferreira et al., 2018). A review on the effect of multicomponent exercise on biochemical markers related to health in frail older persons is needed to better clarify the role of these biomarkers and help understand on how to detect, prevent and manage frailty.

Materials & methods

This study followed a pre-determined Systematic Review (SR) protocol redistricted in the PROSPERO database, under the number CRD42018089912, carried out by taking into account different guidelines, utilized to stratify, evaluate and select the scientific reports included in this SR.

In order to guarantee the rigor, accuracy, and replicability in this SR, the following steps were followed: (i) definition of systematic search terms through operationalization and concepts description; and (ii) pilot systematic search of articles in order to verify the search accuracy in each previously selected database.

Search Strategy

To formulate our research question and choose our MeSH search Terms we used the PICO (population, intervention, comparison, outcomes) methodology (Doig et al., 2003). We performed a systematic search for studies published in English on the following databases: PubMed, SciELO, LILACS and Science Direct. Scientific reports were accessed between July 2018 and January 2020 in order to identify original studies, published between 2001 and 2020, presenting multicomponent exercise training, volunteers over 60 years of age, of both sexes, who were living or not, in residential care homes. The following MeSH (Medical Subject Headings) terms were used: “multicomponent exercise”, or “physical exercise”, or “multi-modal exercise training”, or “exercise therapy”, or “combined exercise training”, or “circuit-based exercises”, or “circuit training”, or “muscle strength exercises”, or “muscle strength training”, or “resistance training”, or “physical fitness programs”, or “concurrent training”, or “home-based exercise” AND “elderly” or “older subjects”, or “older populations” AND “biomarkers” or “biochemicals”, or “immune system”, or “hallmarks” AND “Fried frailty criteria”.

Quality of assessment:

Selection of the article’s strategies followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist (Panic et al., 2013). In addition, the CONSORT checklist was used, since it provides guidelines for evaluating the quality of the selected studies (Begg et al., 1996), allowing to identify possible errors or methodological weaknesses. In order to avoid the risk of bias, the evaluation of the articles was carried out by 2 different researchers (blinded), with help of a third researcher if no consensus on the scores of the articles was reached (Donato et al., 2019).

The following eligibility criteria were adopted: (i) regarding the population: individuals over 60 years of age presenting frailty (defined as frail or pre-frail, by the Fried criteria (Fried et al., 2001)); and (ii) randomized controlled trials or clinical trials investigating the effect of multicomponent exercise training protocols on physical frailty and biomarkers. The exclusion criteria used were: non-original studies, reviews or meta-analysis, non-randomized controlled trials (nRCT).

Results

A total of 130 articles were identified through database searching (PubMed=25, SciELO=15, and Science Direct=90). The screening of database LILACS did not render any record.

By applying the first study selection criteria, 45 studies were excluded, and 85 studies proceeded to the screening phase. At this stage, after reading the abstracts, 43 studies were further excluded. From the 42 remaining articles, 10 studies were included after the eligibility phase. After that, 4 were excluded because they did not use Fried´s frailty criteria or due to incompatibilities on study protocols. A total of 6 articles met the inclusion criteria and were used to perform this SR (see Flowchart of figure 1).

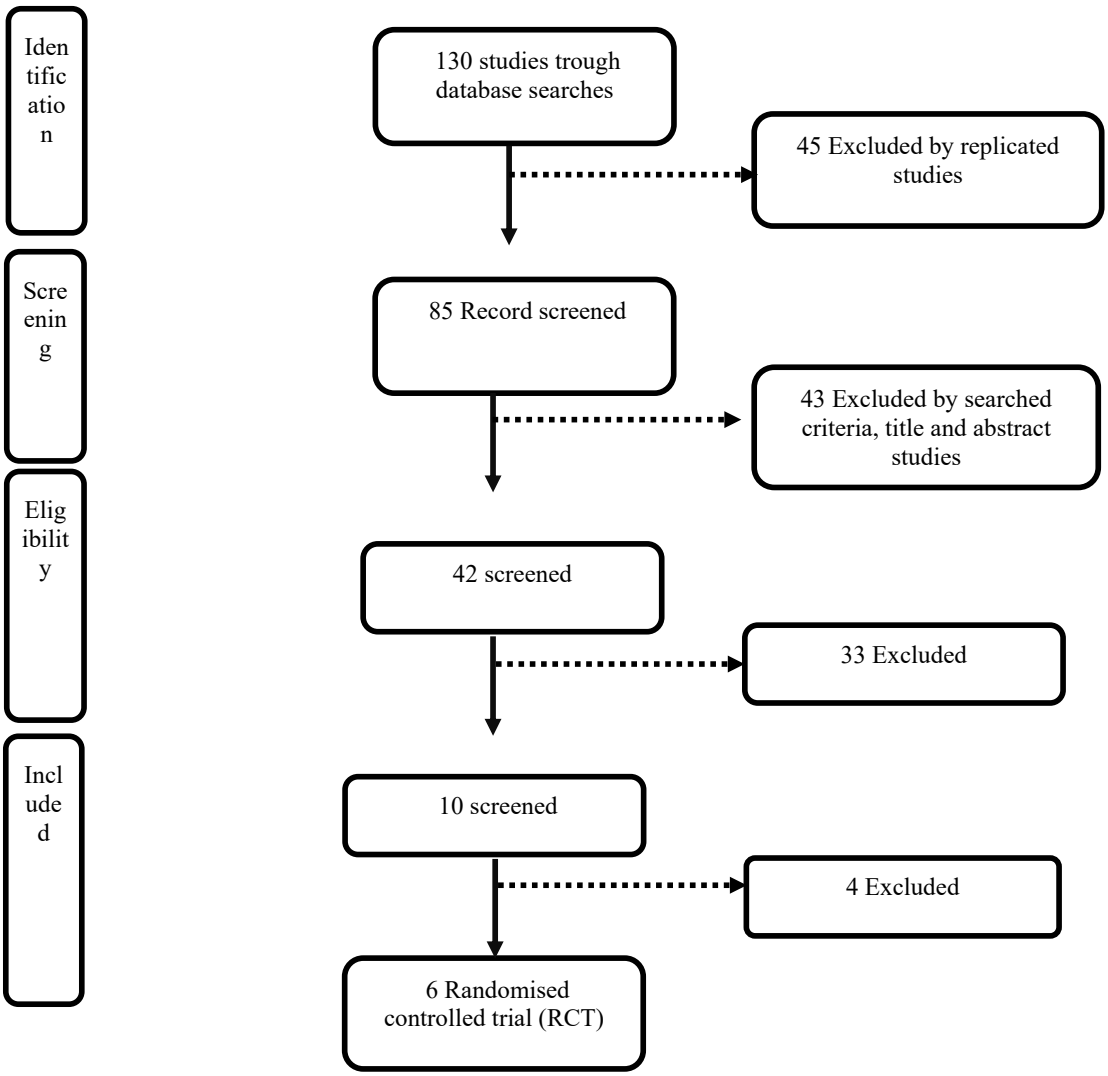

Figure- 1 Prisma Flowchart of the process of literature search and extraction of studies meeting the inclusion criteria.

Sample Study Characteristics

The six studies included in this SR, represent a total of 336 frail men and women, aged ≥70 years and living in residential care homes. Supervised exercise interventions were used, with in some cases adition of non-supervised exercises. Tarazona-Santabalbina, 2016, used a 24 weeks combined program of endurance, strength, coordination, balance, and flexibility exercises; Arrieta et al., 2018, a 6 months multicomponent exercise protocol. Furtado et al 2019; 2020, a 28 weeks multi-modal exercise; Sadjapong et al., 2020, a 12 weeks

multicomponent exercises; Ferreira et al., 2018, a 12-weeks physical exercise program strength, coordination, balance, and flexibility.

Main Results of the Selected Studies

Regarding multicomponent exercise training programs in frail older persons, the study performed by Tarazona-Santabalbina and collaborators (2016), demonstrated that their program was able to revert frailty, improving physical function, anthropometric parameters, cognitive and emotional domains. Sadjapong and collaborators (2020) showed the reverse of frailty to pre-frailty status, with improved physical performance, especially balance. Furtado and colleagues (2019, 2020) observed decreased frailty, with benefits in physical activity levels (gait speed and muscle strength) and diminished functional disability. Ferreira and colleagues (2018) also found reduced frailty through improvements in functional capacity, muscle strength, speed, and agility. Arrieta and collaborators (2018), evidenced improvement in physical fitness. Full descriptions of these studies are presented in table 1.

Concerning biomarkers (Table 2), Tarazona-Santabalbina and collaborators (2016), monitored an increase in the levels of brain-derived neurotrophic factor (BDNF). Arrieta and collaborators (2018), found an increase in myostatin levels. Ferreira and colleagues (2018) monitored glucose, insulin, total cholesterol, triglycerides, vitamin D3, and C-reactive protein (CRP; pro-inflammatory marker) and found improvements in biochemical variables but no effects in inflammatory parameters. Furtado and colleagues (2019, 2020), focused on cortisol, testosterone, dehydroepiandrosterone, testosterone/cortisol ratio, salivary immunoglobulin-A (IgA), salivary lysozyme levels (Lys), pro-inflammatory interleukines-1 beta and 6 (respectively, IL-1β, IL-6) and Tumour Necrosis Factor-alpha (TNF-α), anti-inflammatory interleukine-10 (IL-10), interferon-gamma (IFN-γ), and CRP, with improvement in hormonal response and better balance between pro- and anti-inflammatory environments. Sadjapong and collaborators (2020), measured IL-6 and CRP and obtained decreases in these inflammation markers.

Table 1- Summary of Study and Participant Characteristics

Author (year)	Main Goals	Characteristics of participants	Biomarkers	Comparison	Results on Biomarkers	Results on frailty outcomes
Arrieta et al (2018) SPAIN	to determine the association of serum myostatin concentration with body composition, physical fitness, physical activity level, frailty.	Women 86.2±6.8 years men 82.0±6.3 years n=88	Myostatin	6-months exercise, twice a week, involving strength, balance, stretching exercises, and walking recommendations	higher serum levels of myostatin were found to be associated with better physical fitness. however, the use of this protein as a biomarker for physical fitness, rather than frailty, merits further study.	Decrease frailty
Tarazona-Santabalbina et al (2016) SPAIN	to multicomponent exercise program (mep) performed by frail older and improve functionality, cognitive, emotional, and social networking, biomarkers	Men and women 79.5 years n= 100	BDNF	24 weeks a combined program of endurance, strength, coordination, balance, and flexibility exercises. 65 min session, 5 days/week	Improved functional measurements, cognitive, emotional, and social networking determinations. it also leads to a decrease significant improvement in frailty biomarkers	The men group reversed frailty
Ferreira et al (2018) BRAZIL	To verify the effects of exercise training on biochemical, inflammatory, and anthropometric indices and functional performance in institutionalized frail elderly	Men and women 73.3 ± 6.4 years n=71	Glycaemia, Insulin TC, TR HDL, LDL Vitamin D3, CRP IL6, IL10 IL1a, IL1RAcP	12-weeks physical exercise program strength, coordination, balance, and flexibility	Improving muscle strength, speed, agility, and biochemical variables, with but, no effects in anthropometric and inflammatory parameters were noted.	Reversal of the frailty condition
Furtado et al (2019)	To analyse the effect of two different 28-weeks chair exercise programs	Institutionalized dwelling women n=60 81±7.84 years	Cortisol Testosterone Dehydroepiandrosterone Testosterone/Cortisol	28-weeks chair exercise programs multimodal and muscles	Both exercise interventions used in this study produced significant benefits	Diminish the physical frail condition

PORTUGAL	(multimodal and muscles strengthening with elastic bands on physical frailty, functional disabilities and steroid hormones in institutionalized pre-frail and frail women		ratio	strengthening with elastic bands	in order, decreased functional disability and also, stimulated satisfactory hormonal responses.	
Furtado et al (2020) PORTUGAL	to analyse the effects of 28-weeks of two different exercise protocols on the functional fitness and immune profiles of institutionalized pre-frail and frail women with mild cognitive impairment.	Institutionalized pre-frail and frail women with mild cognitive Impairment 81±7.84 years n=60	sIgA, sLys, IL-1β, and IL-6; TNF-α, IFN-γ IL-10 CRP	28-weeks chair exercise programs multimodal and muscles strengthening with elastic bands	The evidence regarding the use of systematic and moderate long-term exercise as therapy for promoting a better balance between pro- and anti-inflammatory environments and a decrease in the inflammatory index for the cme group were the most promising results from this study..	Reduces levels frailty

Table 2- Identification of the biological systems involved.

Author	Biochemical/molecular mechanisms studied	Confirmation of hypothesis
Arrieta et al (2018)	Muscle Tissue	Increases in myostatin concentration (muscle activity)
Tarazona-Santabalbina et al (2016)	Nervous System	Increase in the BDNF serum levels group multicomponent
Ferreira et al (2018)	Lipid profile and cytokines pro inflammatory	Improvements in biochemical variable but no effect in inflammatory parameters
Furtado et al (2019)	Neuroendocrine Cortisol, Testosterone, Dehydroepiandrosterone, Testosterone/ Cortisol Ratio	Stimulated satisfactory hormonal responses.
Furtado et al (2020)	Immune system, pro/anti inflammatory	Decreased inflammation in frail older adults.
Sadjapong et al (2020)	Immune system pro/anti inflammatory	Decreases inflammation in frail older adults

Discussion

Unfortunately, we could only find 6 studies that reached our eligibility criteria, evidencing a lack of studies targeting biomarkers as the mediatiors of multicomponent exercise training in frail older persons. Those biomarkers are crucial to better understand frailty prevention, development and progression, and its consequences on ageing well being.

Among the 42 articles selected in the first evaluation phase, 10 (24%) studies used multicomponent exercise training programs, which confirms their increasing use in frail older persons. Multicomponent exercise training programs (ME) seem to be predominantly used in frail older persons (Silva et al., 2017), and may be

considered an ideal intervention for this specific population (Pillatt et al., 2019). The combination of multicomponent strength, aerobic, and balance exercises demonstrated remarkable positive effects on health outcomes as compared to their isolated prescription (Baker et al., 2007). Furthermore, multicomponent exercise training has also been shown to be advantageous and most attractive for older persons as they are similar to their daily activities (Angulo et al., 2020). Our SR confirms the potential of ME in preventing or revert frailty in older persons: three (3) studies showed reverted frailty, two (2) diminished frailty and one (1) improved physical fitness. Moreover, these studies are also in accordance with other robust studies using ME in frail older persons (Cadore et al., 2019; Arrieta et al., 2019; Silva et al., 2017; Makizako et al., 2012; Hopps et al., 2011; Theou et al., 2011).

Regarding the frequency of the multicomponent exercise programs described in this SR, a variation of 2-5 times per week with a total duration between 3 to 8 months was found, with the ability to attenuate frailty and positive impact on biomarkers. Moreover, there seems to be a consensus that the inclusion of resistance, gait, and balance training in the multicomponent exercise training programs is the corollary strategy for improving frailty hallmarks, as well as for reducing falls risk in frail older persons. In addition to maintain functional capacity during ageing, ME combines several actions, such as cognitive training that also enhances cognitive performance and, thus, prevents cognitive impairment (Tarazona-Santabalbina et al. 2016). Results from other studies using different exercise protocols also confirm this (Cadore et al., 2019; Theou et al., 2011; Arrieta et al., 2019; Makizako et al., 2012; Justine et al., 2010).

This SR showed that the preferential and the most responsive biomarkers assessed in the selected studies were IL-6, CRP and TNF-α (see Table 2), as those biomarkers were evaluated in 3 of the 6 studies. In this respect, it is noteworthy to point out that multicomponent exercise training showed the capacity to induce significant alterations in these biomarkers, especially in the studies of Furtado and collaborators (2020) and Sadjapong and collaborators (2020), which indicated that ME contributed to lower inflammation levels in frail older persons. Taking other studies with young and older persons into account, there is solid evidence that the regular practice of physical exercise decreases systemic levels of CRP, IL-6, and TNF-α, reinforcing the findings discussed above (Hopps et al., 2011; Nicklas et al., 2008; Petersen & Pedersen, 2005; Petersen et al., 2007).

Recently Petrella and collaborators (2021) reported that the multicomponent exercise training program is an inexpensive intervention that could be replicated in care centers, for the avoidance and treatment of frailty, mainly acting in the pro and anti-inflammatory pathways, but also optimizing anabolic processes (based biomarkers). Among the studied biomarkers of frailty, it is worth mentioning: Glycaemia, Insulin, Total Cholesterol, Triglycerides, High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), CRP, Vitamin D3, cytokines IL-6, IL-10, IL-1a, IL-1RAcP, Myostatin, Cortisol, Testosterone, Dehydroepiandrosterone, and Testosterone/Cortisol ratio [Tarazona-Santabalbina and collaborators (2016); Arrieta and collaborators (2018); Ferreira and colleagues (2018); Furtado and colleagues (2019, 2020); and Sadjapong and collaborators (2020)]. In agreement with the literature, immune/inflammatory dysfunction is considered to be at the core of frailty, due to its direct crosstalk with neuroendocrine and neuromuscular impairments (Ng et al., 2018).

Thus, among the well-accepted benefits of physical exercise training – such as maintaining the health and functionality in older people by increasing muscle strength, improving balance, and avoiding falls – this nonpharmacological intervention has also demonstrated the capacity to decrease the levels of pro-inflammatory biomarkers (Seguin & Nelson, 2003; Furtado et al., 2020), which can cause a positive impact in older persons by preventing and/or reversing frailty development.

Despite the precise mechanisms by which physical exercise promotes healthier ageing are still not fully understood (Sadjapong et al., 2020), a relevant contribution for that, could be associated to the favourable improvement in muscle mass and reduction in adipose tissue, which has been linked to lower pro-inflammatory levels and, thus, a better inflammatory balance (Cartee et al., 2016; Gonzalez-Gil et al., 2020).

Moreover, most of the subjects studied here were institutionalized-dwelling older, who usually are at higher risk of frailty compared to those not living in residential home care centers (Soriano, DeCherrie, & Thomas, 2007). Although in apparent disadvantage, the impacts of ME were still significant for this specific population, highlighting the usefulness of this practice in maintaining the quality of life, especially in residential care homes.

Although there are convincing signs that multicomponent exercise training in frail older persons is an effective intervention for controlling frailty, further studies are necessary to increase our understanding of the mediating effects of exercise on immune and hormonal pathways involved in frailty.

Study limitations, Suggestion for Future Studies

The fact that only 6 studies matched our inclusion criteria could be considered a limitation of this SR, but it is important to clarify that the criteria used here were designed to identify those studies with a consistent frailty phenotype evaluation, allowing a better comparison between studies. Nevertheless, some other limitations should be taken into account, like different follow-up times, differences in physical exercise programs (sequence, duration, progression, intensity), and differences in previous levels of physical fitness, which could lead to some potential bias. Future research should focus on a better definition of the physical activity "dose" capable of

improving the biochemical profile in older persons and its association with frailty, in order to promote healthier ageing with more years and better quality of life. In addition, the study of the impact of nutritional supplementation in combination with multicomponent exercise training could also be important in the context of frailty.

Practical applications

Since a progressive rise in the number of older persons in Europe is still predicted for the next decades, accurate studies associating the triad frailty-inflammaging-physical exercise training (especially multicomponent exercise programs) are reasonably necessary to increase our knowledge on how to improve functional fitness, health and quality of life of older people, assuring their autonomy and independence for longer years.

Conclusion

This SR showed that multicomponent exercise training, designed to address frailty in older persons, was able not only to improve several parameters associated with physical function and metabolic balance, but also benefited cognitive, emotional, and social functions. Our SR confirms the potential of ME programs of 2-5 times per week with a total duration between 3 to 8 months, in preventing or reverting frailty in older persons, associated with positive impacts on biomarkers, especially those associated with decreased pro-inflammatory parameters, mainly CRP, IL-6, TNF-α, and with better regulation of metabolic and hormone pathways. The number of studies is still scarce and more looking at the mediating effect multicomponent exercise training programs on biomarkers are needed, in other to better understand the aetiology of the frailty syndrome and improve the prevention and management of frailty in older populations.

Conflicts of interest – No conflicts of interest to declare.

Contributors

Adriana Caldo organized acquisition of data and writing of the paper, Guilherme Furtado helped in the acquisition of data. Nelo Zanchi, Alain Massart and Guilherme Furtado supported the interpretation of data and reviewed the paper critically. Marcelo P. Barros and André L.L. Bachi helped in the discussion as well as reviewed the paper critically. Alain Massart and Ana Maria Teixeira reviewed the paper critically and coordinated the research study protocol.

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Impact of 16 weeks of Exercise and Protein Supplementation on Functional-Physical Fitness of Dwelling-Institutionalised Elders

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Keywords: Older adults, Physical Exercise, Protein Supplementation, Functional Physical.

ABSTRACT

Background: Ageing is a natural, progressive and irreversible process characterized by morphological, psychological, functional, biochemical and nutritional changes. Physical inactivity (PI) is a factor that contributes to the starting of mass and muscle function decline in the elderly, often related to sarcopenia and physical frailty. Combined to protein nutritional intervention, exercise appears as an effective way to prevent muscle mass and physical fitness decline. In addition, the elderly population has difficulty in maintaining adequate protein nutrition and are the least involved in systematic exercise programs. This exploratory study was conducted to understand the isolated and combined effects of a 16-weeks

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of branched chain aminoacids (BCAA) supplementation (BS) and muscle strength exercise program (MSE) on functional-fitness performance in octogenarians.

Methods: The sample consisted of 18 participants aged 82.97±8.05 years old, institutionalized in social care centres. They were divided into two groups: group 1 (MSE+BS, n=10); group 2 (BS, n= 8). Group 1 performed an elastic band strength exercise program carried out during 16-weeks together with BCAA supplementation consisting of ingesting 0.21g/kg/day of unflavoured powder diluted into 200mls of water, immediately after exercise. Group 1 did only the BCAA supplementation. To evaluate the functional capacity of the elderly, the short battery of tests SPPB was used in the initial and final intervention evaluation.

Results: After 16 weeks, group 1 (MSE+BS) showed a significant increase in all the SPPB tests performance, particularly in the test consisting in rising from a chair and seating down for 5 times. The SB group showed only a short decrease in the time taken to perform the 3 meters walk test ($p < .05$).

Conclusion: Our study revealed that exercise plus supplementation with BCAAs was able to improve physical fitness function, while BCAA supplementation alone had limited effects. Satisfactory results in physical function could be explained by the added effects of exercise and BCAA supplementation on the protein synthesis effect.

INTRODUCTION

Ageing leads to muscle loss and to increased risk of falls and loss of independence. Resistance exercise can be a useful intervention to prevent sarcopenia and frailty. However, muscle protein synthesis in older adults is decreased (Drummond & Rasmussen, 2008). Ageing is a natural, progressive and irreversible process characterized by morphological, psychological, functional, biochemical and nutritional changes (World Report on Ageing And Health, 2015). Physical inactivity (PI) is a factor that contributes to the starting of mass and muscle function decline in the elderly often related to sarcopenia and physical frailty (Lehmann, Baar, & de Keizer, 2018). Combined to protein nutritional intervention, exercise appears as an effective way to prevent muscle mass and physical fitness decline (Hernández Morante, Martínez, & Morillas-Ruiz, 2019).

However, the elderly population has difficulty in maintaining adequate protein nutrition and in addition, they are the least involved in systematic exercise programs (Landers-Ramos & Dondero, 2019). Regarding

nutritional supplementation, branched-chain amino acids (BCAA) act as a major energy substrate in the muscle along with the increased contribution of fat as an energy source when the exhaustion of carbohydrates during long periods of exercise occurs (Kim et al, 2013). Some European references on the amount of protein intake in the elderly RDA population suggest ingesting 0.8g/kg/ (WHO/FAO/UNU Expert Consultation, 2007) while a study by a European PROT-AGE group recommends about 1.0 to 1.2g/proteins kg/day (Bauer et al., 2013).

In this way, a multicomponent exercise program, which develops different physical abilities, can also be beneficial in maintaining the functionality and capacity of the elderly (Baker et al, 2007). Recently, researchers stated that programs including BCAA supplementation present a more effective mean of increasing muscle strength than other types of protein supplementation (Rondanelli et al., 2011).

This exploratory study was conducted to understand the isolated and combined effects of 16-weeks BCAA supplementation (BS) and elastic-band muscle strength exercise program (MSE) on functional-fitness performance in octogenarians elders.

METHODS

STUDY DESIGN

This study was conducted according to guidelines in the Declaration of Helsinki for procedures involving human subjects and was approved by the University of Coimbra, Faculty of Sport Science and Physical Education Ethical Committee (reference number: CEFCDEF/0028/2018) respecting the Portuguese Resolution (Art. 4th; Law n.°. 12/2005, 1st series) on ethics in human research (Braga, 2013).

PARTICIPANTS

The sample consisted of 18 participants institutionalized in social care centres. They were divided into two groups: group 1 (MSE+BS, n=10); group 2 (BS, n= 8). Group 1 performed an elastic band exercise Muscle Strength Exercise program, carried out during 16-weeks together with BCAA supplementation consisting of ingesting 0.21g/kg/day (Ispoglou et al., 2016) of unflavoured aminoacids powder, immediately after exercise. Group 2 did only the BCAA supplementation and no exercise program was performed.

Table 1: Characteristics of participants

	MSE+BS Group 1	BS Group 2
	Baseline	Baseline
Chronological age (years)	82.80±6.80	83.13±9.30
Body mass index (BMI)	28.39±4.91	25.99±2.96
Charlson Comorbidity Index (CCI)	5,00±1.05	5.00±1.60

INTERVENTIONS

MUSCLE STRENGTH EXERCISE (MSE)

A progressive program of exercises performed with a determined number of exercises (8-10), sets (2-3); repetitions (10-15), a cadence of repetitions execution in 2 seconds concentric and 3 seconds eccentric (2:3) (Skovdal Rathleff, Thorborg, & Bandholm, 2013) and a passive rest in the seated position between sets (30-45 seconds), following a bi-set protocol method was created. The first three levels of the Thera Band® System were used. Level one (yellow colour) elastic-bands were used during the first 4 weeks, progressing to a different colour every four weeks. Intensity was measured through the OMINI PES scale (Robertson et al., 2003), that consists of a subjective effort scale ranging from 0 to 10 points. The goal is to keep the intensity of the exercise activities between 1 to 6 in the PES levels. It is expected that the relationship with the real effort would be 55-80% of maximum HR and muscle intensity was evaluated by OMINI using Colado and colleagues approach (Colado et al., 2014). The session was divided into three parts: 5 minutes of warm-up (PSE 1 to 3, HRmax = 45-55%), 35 minutes of muscle-strength elastic-band exercises in PSE 4 to 6 (HRmax = 56-75%) and finally, 5 minutes of cooling-down (PSE 1 to 2, HRmax = 45-50%). A low to moderate intensity effort around 50-75% of HRmax values was warranted.

Table 2: Protocol Muscle Strength Exercise

Warm-up	5 minutes	PSE 1-3			
Exercises (8-10)	Sets	Repetitions	Cadence	Interval	PSE
Front squat	2-3	10-15	2:3	30-45 seconds	4 to 6

Warm-up	5 minutes	PSE 1-3			
Exercises (8-10)	Sets	Repetitions	Cadence	Interval	PSE
Chair unilateral hip flexion	2-3	10-15	2:3	30-45 seconds	4 to 6
Chair Bench over row (with flexion)	2-3	10-15	2:3	30-45 seconds	4 to 6
Chest Press (stand and/or chair)	2-3	10-15	2:3	30-45 seconds	4 to 6
Standing (or chair) reverse fly	2-3	10-15	2:3	30-45 seconds	4 to 6
Shoulder Press/twist arm front position	2-3	10-15	2:3	30-45 seconds	4 to 6
Chair (or stand) frontal total raiser	2-3	10-15	2:3	30-45 seconds	4 to 6
Biceps arm curl (stand and/or chair)	2-3	10-15	2:3	30-45 seconds	4 to 6
Chair Overhead triceps extension	2-3	10-15	2:3	30-45 seconds	4 to 6
Cooling down	5 minutes	PSE 1-2			

NUTRITIONAL SUPPLEMENT (BCAA)

The BCAA supplement was composed by L-Leucine, L-Isoleucine and L-Valine in the proportion of 2:1:1 respectively [product of MYPROTEIN®, UK]. All compounds were packed in individual sachets and administered to the MSE+BS and BS groups. The supplement was supplied through the company with about 95% purity and trade certificated in the EU. The subjects ingested 1 sachet (0.21g/kg/session) (Ispoglou et al., 2016) prepared after finishing the exercise session around 11h am. The contents of each sachet were mixed with 200mL water and consumed. The package contents have about 58 kcal.

PHYSICAL PERFORMANCE ASSESSMENT

SHORT PHYSICAL PERFORMANCE BATTERY

The Short Physical Performance Battery (SPPB) is used for the assessment of physical functioning and disability the evaluation of older persons in both clinical and research settings. It is composed of gait speed, chair stand, and balance tests. This SPPB battery has 3 subdimensions: i) static balance (composed of 3 tests), ii) muscular strength of the lower limbs,

A combination intervention of muscle strength exercise and BCAA supplementation was able to improve the SPBB specific strength test. The sit and stand movements are considered fundamental for mobility and functional independence, since this movements are part of several activities of daily living. The functional action of getting up from a chair, which requires the muscular strength and power of the lower limbs, although seemingly a simple ability, is a functional action that can demand a lot from the elderly (Marzetti et al., 2018). According to Dirks and colleagues (Dirks et al., 2017) protein supplementation was necessary in the frail elderly population in order to increase muscle mass after resistance exercises.

CONCLUSION

Our study revealed that exercise plus BCAA supplementation was able to improve physical fitness function, while BCAA supplementation alone had limited effects. Satisfactory results in physical function could be explained by the added effects of exercise and BCAA supplementation on the protein synthesis effect.

STUDY LIMITATION

Although the sample size was small, significant improvements in physical functional fitness, were still found after the exercise plus supplementation intervention.

PRATICAL APPLICATIONS

Our results support the importance of the implementation of specific physical exercise plus supplementation programs designed especially for frail elderly populations. Protein and/or branched chain amino acids supplementation could be important in order to achieve the nutrition recommendations for the elderly in social care institutions.

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Review

Effect of a 40-weeks multicomponent exercise program and branched chain amino acids supplementation on functional fitness and mental health in frail older persons

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ABSTRACT

Background: The ageing process implies several physiological and psychological changes that hence affect the general health, mood states, and quality of life of older persons. Exercise and adequate nutrition are renowned non-pharmacological strategies that significantly delay and alleviate the adverse consequences of the ageing process. This study aimed to evaluate the effects of branched-chain amino acid (BCAA) supplementation and a multicomponent exercise program (ME) on the physical frailty and mood states of older persons.

Methods: 35 participants (women and men; 83 ± 3 years old) from residential care homes were submitted to a 40-week exercise-washout-retraining intervention (16 weeks of the elastic band based exercise and/or supplementation, 8 weeks of washout, and 16 weeks of multicomponent exercise and/or resupplementing), with or without BCAA supplementation. The experimental groups were: (i) ME plus BCAA supplementation (ME+BCAA); (ii) ME; (iii) BCAA supplementation (BCAA), and (iv) control group (CG). Fried's phenotype was used to assess frailty prevalence. Geriatric Depression Scale (GDS), Profile of Mood State (POMS), Mini-Mental State Examination (MMSE), were used to access mental health and cognition. The Short Physical Performance Battery (SPPB) was used to access functional capacity. Salivary testosterone levels (ST) were also determined to access the anabolic effects of the intervention.

Results: Exercise was effective in improving functional capacity and prevented the increase in frailty that occurred in the non-exercising CG, where the frailty scores increased over time ($p < 0.01$). BCAAs supplement alone had no impact on functional fitness, but in a short time (16 weeks) contributed to diminishing frailty and combined with exercise may have the potential to reduce the effect of a detraining period on functional capacity.

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Salivary testosterone levels correlated with handgrip strength and could be a useful indicator of susceptibility to frailty. No effects were found for mood states, cognition, and depression.
Conclusion: This study showed that a long-term exercise program, independent of being multicomponent or strength elastic band-based, was effective in improving functional capacity and prevented an increase in frailty in frail and pre-frail older persons living in residential care homes.

1. Introduction

Ageing is a natural degenerative process, which dramatically increases the risk of many diseases in older populations (Franceschi et al., 2018). The sedentary lifestyle, per se, is one of the most important contributors to age-related illness, whereas regular exercises – based on hormesis principles – could chronically revert the ageing dysfunctions (Hayes, 2007).

The physical frailty syndrome (PFS) is defined as an ageing-related multifactorial clinical condition marked by a progressive decline of multiple physiological domains that compromise the individual capacity to withstand stress (Fried et al., 2001). PFS can be assessed by evaluating 5 factors: lean mass loss, diminished handgrip strength, reported fatigue/exhaustion, reduction of walking speed, and low physical activity levels (Angulo et al., 2020). Physical exercises also protect against frailty and cognitive impairment in advanced aged people (Bherer et al., 2013). Interventions that might induce a positive impact on muscle mass in older persons could also represent a supporting treatment for improving mental health. In fact, there is a growing body of evidence that supports the involvement of shared pathophysiological pathways that link sarcopenia and common mental disorders (Pasco et al., 2015).

Ageing is characterized by reduced synthesis of hormones, including growth hormone, estrogen, dehydroepiandrosterone (DHEAS), thyroid hormone, and testosterone (Perrini et al., 2005). Serum levels of these hormones are important indicators of the overall degeneration processes occurring in physiological systems during ageing, Lower testosterone levels are associated with the decrease of muscle mass and strength (an essential cause of sarcopenia), which, therefore, may contribute to the progress of frailty in older persons (Srinivas-Shankar et al., 2010).

Older person's malnutrition is a concern in health systems around the world since it carries a high risk of ageing comorbidities, and increased health costs (Roberts et al., 2019). Indeed, nutritional supplementation with vitamins, antioxidants, and protein components (including isolated amino acids) has already demonstrated positive results against frailty, cognitive impairment, sarcopenia, and other age-related disorders (Gómez-Gómez and Zapico, 2019). Supplementation with BCAAs, especially in association with regular exercise, was shown to improve muscle strength and cognitive functions in older persons, thus comprising a safe and low-cost strategy to circumvent the negative effects of ageing (Ko et al., 2020).

Regarding the decline of body muscle mass in PFS, branched-chain amino acids (BCAAs), especially L-leucine, are considered efficient nutrients to induce positive adaptive muscle responses, upon the stimulus provided by physical exercise (Yanai, 2015). Accordingly, BCAAs supplementation has been shown to mitigate the loss of muscle mass, stimulate anabolic responses, and elicit an effective muscle protein synthesis, probably due to the direct effect of leucine on the initiation of mRNA translation, which is still present in older age (Fujita and Volpi, 2006).

An adequate protein intake is essential for efficient muscle protein turnover, but also, to maintain physical function in older persons (Rondanelli et al., 2011). In general, amino acid supplementation represents a suitable strategy to attenuate and/or manage some specific age-related pathologies, such as chronic inflammation myopathies, and muscle catabolic state (Dato et al., 2019). Moreover, interventions that might induce muscle mass increase in older persons were also shown to represent a supporting treatment for concomitant mental health improvement (Gariballa and Alessa, 2020). Therefore, strategies that improve the physical and mental aspects of older persons, such as exercise programs designed for this population, could improve the well-being and mitigate the adverse effects of family abandonment, depression, and other psychosomatic disorders (Monteiro-Junior et al., 2017; Portugal et al., 2013).

The positive impact of assisted and regular (moderate) exercise programs on physiology and cognition prompted us to ask some questions on the putative coadjutant role of BCAA in the process. Can BCAA supplementation and multicomponent exercises promote better physical and mental function in older persons? Therefore, this study aims to evaluate the effect of a long-term multifactorial exercise program and/or BCAAs supplementation (including detraining/washout period) on functional capacity, depression, mood state, cognition, and testosterone levels (here as a biomarker of sarcopenia) in frail older persons living in residential care homes.

2. Methods

2.1. Preliminary procedures and ethics

All subjects volunteered to participate in the exercise and/or the supplementation interventions. Consent forms were signed by the Residential Care Homes (RCH) directors, the participants, and their legal representatives before testing and intervention. This study was approved by the Ethical Committee of Faculty of Sport Sciences and Physical Education, University of Coimbra (reference number: CE/FCDEFUC/00282018), respecting the Portuguese Resolution (Art.°4th; Law no. 12/2005, 1st series) on ethics in human research and the Helsinki's Declaration (Braga, 2013). This study was properly registered with clinicaltrials.gov register NCT04376463 and is a complementary part of the recently published article (Caldo-Silva et al., 2021).

Participants eligibility and allocation have been described in detail by Caldo-Silva et al. (2021). Briefly, participants had to be 70 years old or more, physically frail or pre-frail without morbid obesity, clinically stable with their drug therapy updated, being able to perform the Time Up and Go test in $\leq$50 s (longer durations indicate severe mobility dependence) (Guralnik et al., 1994), not participating in other structured regular physical exercise programs, not reporting any type of health condition or use of specific medication that might prevent the functional self-sufficiency test performance or attention impairment, not reporting chronic mental disorders or hearing/visual impairment that could interfere with the evaluations and activities proposed.

At the end of the recruitment process, 80 older persons from different RCH entered the enrolment phase. From the 80 participants initially screened, 50 eligible participants were allocated to their respective intervention groups. However, at the end only 35 participants (age = 83 $\pm$ 3 years-old) completed the 40 weeks multifactorial intervention, and were divided into the following groups: Multicomponent exercise (ME, n = 7), Multicomponent exercise plus BCAA supplementation (ME+BCAA, n = 8), BCAA supplementation (BCAA, n = 7), and the no-regular exercise/no-supplementation control group (CG, n = 13). The procedures were performed according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Begg et al., 1996).

2.2. Experimental design

This study is a four-phase prospective, naturalistic, controlled clinical trial with four arms of a multifactorial intervention program (MIP)

experimental design, composed of regular exercises (16 weeks of a strength based exercise program + 8 weeks of a washout period + 16 weeks of a multicomponent exercise program) and supplementation interventions (ME+BCAA, ME, BCAA, and CG). In the first phase, a baseline data collection (T1) was done followed by 16 weeks of intervention and a second data collection (T2). This second phase was followed by 8 weeks of both an exercise and supplementation washout phase. Phase 3 consisted of a third data collection (at the end of the washout period), followed by the resumption of the exercise/supplementation intervention for an additional period of 16 weeks. Finally, the last data collection took place after 16 weeks of the second exercise/supplementation intervention (T4) (Fig. 1).

2.3. Outcomes measures

All the assessments were performed in the morning, between 10:00 am and 11:45 am. One session was used to apply a short test battery to measure biosocial, global health status, cognition profile, nutritional, physical, and physical frailty status and to collect saliva samples.

2.4. Physical frailty criteria

The phenotype of Fried's physical frailty index was used (Fried et al., 2001). Weight loss was assessed by a self-report of unintentional weight loss of 4 kg or more in the last 6 months.

Self-reported exhaustion was evaluated by a negative concordance of questions number (7-"*I felt that everything I did was an effort*") and (20-"*I could not get going*") of the Center of Epidemiologic Studies for Depression (CESD) scale (Gonçalves et al., 2014).

Hand-grip strength (HGS) was assessed (in kg) using a hand-held dynamometer (Lafayette 78,010, Sagamore, United States). The best result of the two trials was used for scoring purposes. Participants who were unable to perform the handgrip strength test and those in the lowest 20% tier were categorized as positive for low HGS (Syddall et al.,

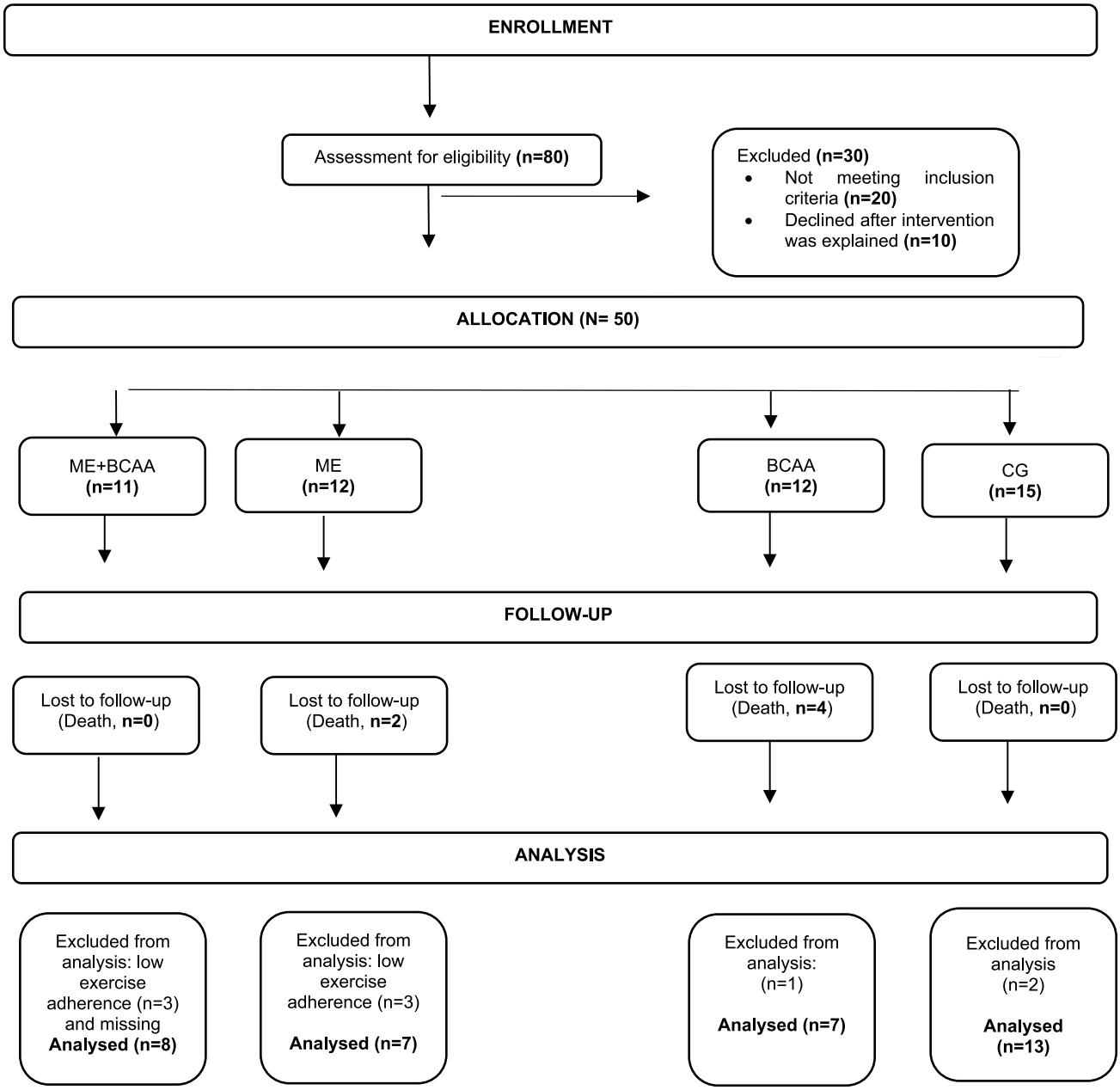

Fig. 1. CONSORT flowchart of study participants (Begg et al., 1996; Caldo-Silva et al., 2021).

2003). The cut-off reference values for HGS of ≥29 kg for males and ≥17 kg for females were adopted.

Slowness was measured by the "15 feet (4.6 m) walking test". Based on the cut-off values of Fried's study population, the times of ≥7 s for males and ≥6 s for females were adopted for positive scores of slowness. The best time of the two trials was used for the final scoring.

Low physical activity (PA) levels were assessed by the International PA Questionnaire short version (IPAQ-SV) (Campaniço and Sardinha, 2016). There are three levels of PA suggested for classification: Inactive, minimally active, and highly active. Participants classified as inactive had a positive score for this physical frailty component. A positive evaluation in one or two criteria classified the participants as pre-frail, in three or more criteria as frail, and as non-frail when the subject did not score in any of the five physical frailty indicators. A frailty total score was calculated, and the physical frailty prevalence was accessed (Fig. 2).

2.5. Nutritional assessment

Daily diet at the RCH was prescribed by a registered nutritionist and was provided for all the participants without any change or interference from the research staff. Based on the information provided, the diet was analysed using specific tools (photographic quantification of portions, food table) for the Portuguese population (Torres et al., 2016; Goios, 2016; INSA, 2006 and 2016). Due to the relationship between the frailty status and severe decrease of muscle mass (or sarcopenia), the objective of this nutritional assessment was to characterize the protein consumption of the participants. In addition, the Mini Nutritional Assessment (MNA) questionnaire was applied (Vellas et al., 1999; Loureiro, 2008).

2.6. Physical function

The Short Physical Performance Battery (SPPB) was applied to evaluate the physical function of the participants. It is a test battery based on the performance of lower limb function designed for older persons. It consists of three assessments: (i) the Balance Test, (ii) the Walking Speed Test; and (iii) the Chair Standing Test. The SPPB is scored from 0 to 12 (with each of the tasks of the SPPB scoring from 0 to 4), with a score of 0 representing inability to carry out the tests, and 12 the best performance. Very poor capacity 0–3, low capacity 4–6, moderate capacity 7–9, good capacity 10–12. For balance, the participants were asked to maintain their feet side-by-side, in semi-tandem and tandem positions for 10 s each. For gait, a 3-m walk at the participants' usual speed was timed. For the chair stand test, participants were asked to stand up and sit down five times as quickly as possible (Guralnik et al., 1994).

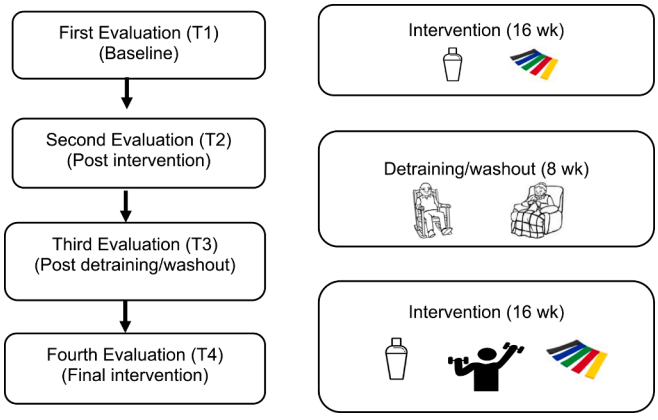

Fig. 2. Chronological order of multifactorial interventions study design. T1 to T2 (elastic-band exercise, 16 weeks), T2 to T3 (washout, 8 weeks), T3 to T4 (multicomponent exercise, 16 weeks); wk = weeks.

2.7. Clinical and health status

The Charlson Comorbidity Index (CCI) was used to classify comorbid conditions based on personal scores combined with age and gender to achieve a single index (Charlson et al., 1994). Anthropometric assessment, including body mass and stature, was performed based on the standardised procedures described elsewhere (Lohman et al., 1992). This assessment was determined using a portable scale (Seca®, model 770, Germany) with a precision of 0.1 kg; stature was determined using a portable stadiometer (Seca Body meter®, model 208, Germany) with a precision of 0.1 cm. Body mass index (BMI) was calculated according to the formula (BMI = body mass / stature^2).

2.8. Assessment of mood state and depressive symptoms

The Geriatric Depression Scale (GDS), adapted to the Portuguese population by Apóstolo and Reis (2011), was used to access the level of depression in the participants (Yesavage et al., 1982). The GDS evaluation consists of 15 yes/no questions, which allows the classification of the psychological condition related to depression and its symptoms. Total GDS scores within the [0–5] points range indicate the normal psychological condition (no symptoms of depression), whereas, 6 to 10 points indicate mild depressive symptoms, and 11 to 15 points indicate symptoms of serious depression (Fig. 3).

The Profile of Mood State questionnaire (POMS) (McNair et al., 1971) was used to evaluate the participants' mood state, using the validated version for the Portuguese population (Viana et al., 2001). The POMS questionnaire consists of 22 Likert-type questions, divided in six dimensions with scales from 0 to 4. The final score consists of a sum of all negative dimensions (Tension-Anxiety, Depression-Melancholia, Hostility-Anger, Fatigue-Inertia, Confusion) subtracting the positive dimensions (Vigour).

2.9. Global cognition - Mini-Mental State Examination

The Portuguese version of the Mini-Mental State Examination (MMSE) was used (Morgado et al., 2009). The MMSE is a 30-point scale instrument that evaluates five domains of cognition: orientation, immediate recall, attention-calculation, delayed recall, and language. It is generally used to track dementia and to estimate the severity of cognitive loss at a specific time (Folstein et al., 1975). This scale classifies individuals by progressive cognitive skills: (0–9 points) severe cognitive impairment; (10–18 points) moderate cognitive impairment; (19–24 points) mild cognitive impairment; and (25–30 points) normal cognitive profile (Pezzotti et al., 2008).

2.10. Salivary testosterone

Non-fasting saliva samples were collected by passive drool, with the participant with the head and trunk lowered for 3 min to facilitate the collection, always at the same time in the morning (between 10:00 a.m. and 11:00 a.m.) to minimize the circadian effect of the marker under study (Papacosta and Nassis, 2011). Before the saliva collection (approximately 20 min), subjects were asked to rinse their mouth with water to remove any food residues. Participants were instructed to avoid alcohol ingestion for 12 h, dairy products for 20 min, foods with high sugar or acidity, or high caffeine content immediately before sample collection. All participants were also instructed not to engage in extreme physical efforts 24 h before the collection. Saliva samples were stored in polypropylene tubes to avoid contamination and retention of samples and then centrifuged, stored, and frozen at −20 °C until further analysis. Salivary testosterone (ST) concentration was determined by competitive ELISA (Salimetrics, UK) according to the manufacturer's instructions. The intra-assay coefficient of variability was 2.19%.

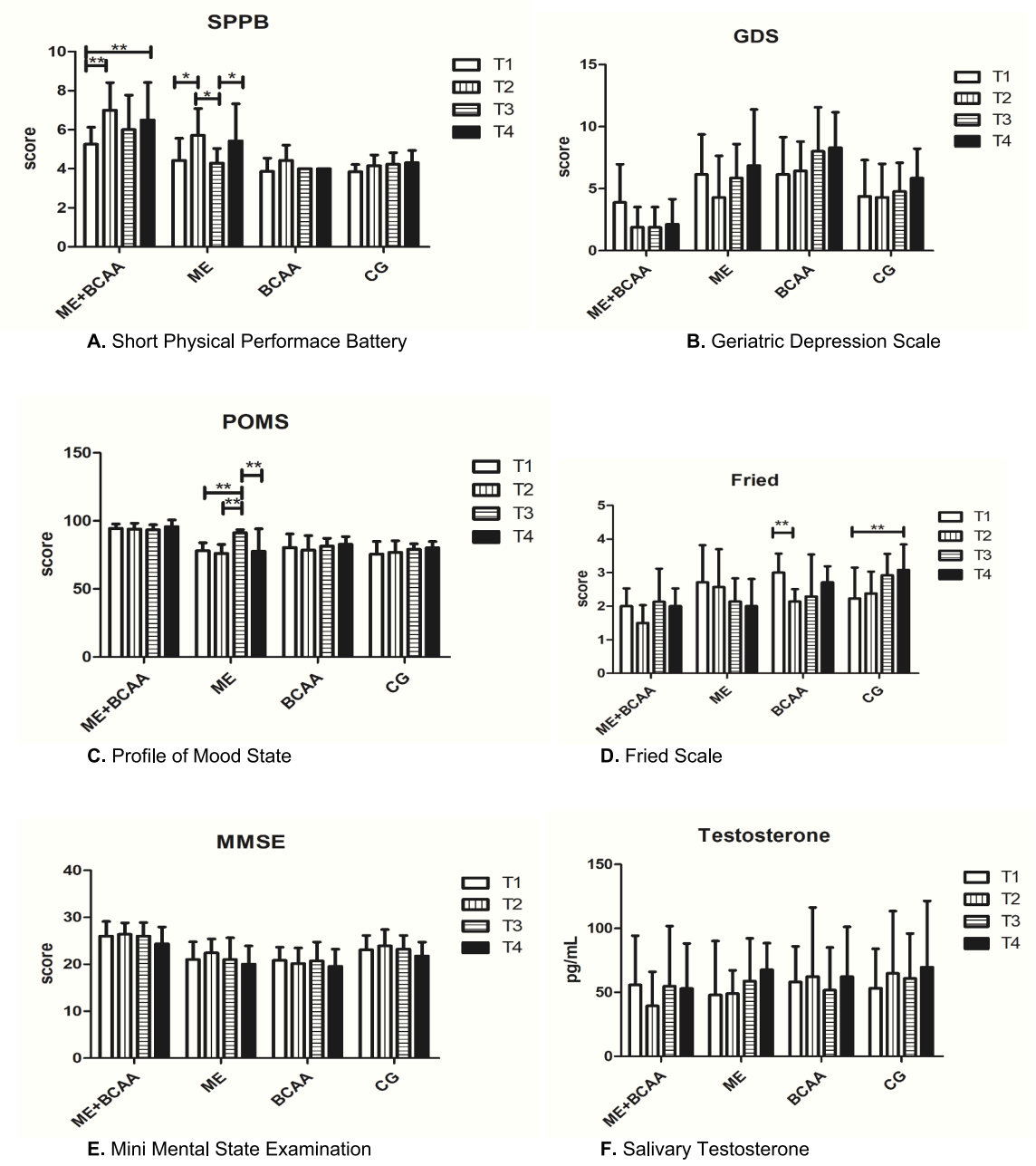

Fig. 3. Time-points assessments of pre- and post-intervention program; ME+BCAA: multicomponent exercise + BCAA supplementation; ME: multicomponent exercise only; BCAA: supplementation only; CG: control group. *Significance at $p < 0.05$; **significance at $p < 0.01$. Significance in the graph is only represented for within-group comparisons. Fig. A-Short Physical Performance Battery (SPPB); B-Geriatric Depression Scale (GDS); C-Profile of Mood State (POMS); D-Fried scale; E-Mini-Mental State Examination (MMSE); F-Salivary testosterone (ST).

2.11. Full characterization of the Multicomponent Intervention Protocol (MIP)

The detailed physical exercise program and BCAA supplementation protocol were previously published by Caldo-Silva et al. (2021). Briefly, a 5 g portion of BCAA mixture, accounting for 20 kcal, composed of L-leucine (Leu), L-isoleucine (Ile), and L-valine (Val) in the proportion of 2:1:1 (MyProtein®, Cheshire, UK) was used. The BCAA portions were diluted in 200 mL of water and given immediately after the exercise sessions to the participants in the ME + BCAA and BCAA groups (Ispoglou et al., 2016), between 09:00 and 11:30 a.m. (Negro et al., 2019). The supplement dose was fixed at 0.21 g total BCAA/kg/session.

2.11.1. Elastic-band exercise intervention (Phase 1)

The exercise program was divided into two interventions of 16 weeks each, separated by an 8-week detraining (washout) period. Exercise sessions were offered twice a week, with an interval of 36 h for adequate physiological recovery and rest. The exercise protocol respected the guidelines for exercise prescription for older persons and the guidelines for exercise periodization by the American College of Sports Medicine (Nelson et al., 2007; de Souto Barreto et al., 2016). The program started with an adaptation period of 2 weeks, in which seven different exercises were performed using elastic bands (TheraBand®, Hygenic Corporation, Akron, OH, USA). The participants were closely supervised for two initial sessions aiming at equipment familiarization and adjustments to the Rating Perceived Exertion scale (RPE OMNI) (Colado et al., 2018). During these familiarization sessions, the participants learned the

correct technique of the exercises and selected the proper color, length, and grip width of the elastic bands. The exercise intensity was indirectly calculated using Karvonen's formula to predict the target heart rate (HR), with HR_{max} calculated with an adjusted formula for older persons (Tanaka et al., 2001).

$$HR = [(HR_{max} - \text{resting HR}) \times \%\text{Intensity}] + \text{resting HR}$$

After the adaptation period, the exercise program was progressively intensified by increments in both the number of exercises (from 8 to 10 exercises) and the proposed physical effort, imposed by different intensity color bands, according to the OMNI scale (Colado et al., 2018). The elastic-band exercises applied in the Phase 1 period are shown in the Supplementary material Table S1. For safety reasons, the exercise programs were also monitored using heart rate monitors (Polar M200; Polar Electro Oy, Kempele, Finland).

Additionally, the intensity was measured through the specific rating perceived exertion (RPE) scales for each exercise program (Borg, 1982).

2.11.2. Washout ME and BCAA period (Phase 2)

After 16 weeks of Phase 1, the participants endured a detraining period of 8 weeks, when the ME programs and BCAA supplementation were suspended. The aim was to check if the physiological adaptations acquired during the first phase of ME were maintained (Sakugawa et al., 2019) or if an 8-weeks interruption was able to revert the possible effects on mood state.

2.11.3. Multicomponent exercise (retraining protocol - Phase 3)

The phase 3 (exercise retraining) protocol was also based on the resistant elastic band exercises but included walking, steps, and balance exercises (sometimes with dumbbells and ankle/wrist weights) to compose a multicomponent exercise program for an identical 16 week-period. Participants attended the program twice a week, on alternate days, also totaling 32 sessions (see Supplementary Table S1). BCAA supplementation was restored as described in Phase 1. The multicomponent program (Supplementary Table S2) was properly described by Furtado and colleagues (Furtado et al., 2019). The phase 3 program aimed to reproduce most of the daily activities of the participants in this study (Baker et al., 2007).

2.12. Statistical analysis

The Shapiro-Wilk test was used to verify the normality distribution of the data and log-transformed when this was not present. Descriptive values are expressed as mean ± standard deviation. To compare the mean changes over time between groups, repeated measures ANOVA (4 × 4 group vs. time) were performed. Bonferroni's post hoc analysis was performed for paired comparisons of means when significant interactions were found in the dependent variables (ST, SPPB, POMS, MMSE, GDS, and Fried). The Pearson correlation was used to access the correlation between salivary testosterone levels and HGS. The level of significance was set at $p < 0.05$. All statistical analyses were done using IBM SPSS Statistics version 23.0 (Armonk, NY: IBM Corp, USA).

3. Results

The selected group of participants in this study closely represents the typical population living in the residential care homes of central Portugal: octogenarian people, at risk of malnutrition, a certain extent of physical disability, and the presence of mild cognitive impairment (Madeira et al., 2016). Characterization of our sample at baseline (see Table 1) revealed that 85,7% of the participants were physically frail, 14,3% pre-frail, 60% had mild cognitive impairment, while 57% were both physically and cognitively frail.

Drop-out from the study was mainly caused by the unexpected relocation of participants to other institutions, low adherence to the exercise protocol, and death. In addition, no adverse effects were resulting from the interventions (exercise or supplementation), except for one case of diarrhea after the first supplementation, from which the participant rapidly recovered and continued with the protocol as normal.

There was a significant effect of time ($F(df: 3, 9) = 9.925$, $p = 0.000$) but not for time * group interaction ($p > 0.05$) for changes observed in SPPB scores. Even at baseline, SPPB differences emerged between ME+BCAA and BCAA ($p = 0.007$) and between ME+BCAA and CG ($p = 0.002$). The increase in the SPPB score observed for ME+BCAA over the first period of intervention ($p < 0.01$) was maintained during the washout period and remained until T4. The ME group also showed higher SPPB scores between T1 and T2 ($p = 0.02$), but a decrease was

Table 1
Baseline characterization of the all participants.

Characteristics	All sample (n = 35, 100%)	%	Men (n = 14, 39%)	%	Women (n = 21, 61%)	%
Age (years, M ± SD)	83 ± 3		81 ± 6		85 ± 5	
Height (m)	1.56 ± 0.10		1.65 ± 0.59		1.50 ± 0.73	
Weight (kg)	70.2 ± 11.9		77.7 ± 8.5		65.2 ± 11.3	
Body mass index (kg/m^2)	28.7 ± 4.5		28.4 ± 3.7		28.8 ± 5.1	
MNA (score, 0–30 points)	24.2 ± 2.6		24.9 ± 2.2		23.7 ± 2.7	
CCI (score, 0–10 points)	5.08 ± 1.12		4.57 ± 1.22		5.42 ± 0.92	
Low CCI (≤5 points)	n = 21	78.6%	n = 11	41.17%	n = 10	37.43%
High CCI (≥5 points)	n = 14	21.4%	n = 3	4.59%	n = 11	16.81%
Polypharmacy (days, M ± SD)	7.2 ± 1.6		5.4 ± 4.1		4.9 ± 4.8	
Time in residential care (years, M ± SD)	4.5 ± 0.6		3.7 ± 1.4		4.5 ± 1.0	
Schooling time (years, M ± SD)	4.0 ± 0		4.0 ± 0		4.0 ± 0	
Physical frailty index (n; M ± SD)	2.42 ± 0.88		2.36 ± 0.84		2.48 ± 0.98	
Frail (3–5 points)	(30) 2.67 ± 0.7	85.7%	n = 12	34.28%	n = 18	51.42%
Pre-frail (1–2 points)	(5) 1 ± 00	14.3%	n = 2	5.72%	n = 3	8.58%
Robust (0 points)	0	0	0	0%	0	0%
MCI by MMSE (n; M ± SD)	22.88 ± 3.61		22.85 ± 3.34		22.90 ± 3.87	
MCI (19–24 points)	(21) 20.4 ± 2.1	60%	(9) 21.0 ± 2.44	24%	(12) 19.9 ± 1.83	36%
NC (25–30 points)	(14) 26.6 ± 1.4	40%	(5) 26.2 ± 1.6	16%	(9) 26.9 ± 1.3	24%
Both MCI and PF	n = 20	57.1%	n = 8	22.84%	n = 12	34.26%
SPPB (0–12 points)						
(0–3) Very poor functional status	n = 6	17.1%	n = 2	5.70%	n = 4	11.40%
(4–6) Low functional status	n = 28	80%	n = 11	31.43	n = 17	48.57%
(7–9) Moderate functional status	n = 1	2.9%	n = 1	2.9%	n = 0	0%
(10–12) Good functional status	n = 0	0%	n = 0	0%	n = 0	0%

Notes: M = mean; SD = standard deviation; MNA = Mini Nutritional Assessment; CCI = Charlson Comorbidity Index; MMSE = Mini Mental State Examination; SPPB = Short Physical Performance Battery; MCI = mild cognitively impaired; normal cognition; physically frail = PF.

observed between T2 and T3 ($p = 0.04$), returning to the T2 levels after the 2nd intervention period (between T3 and T4, $p = 0.03$). No significant differences were found for the BCAA and CG groups over time ($p > 0.05$). Variables related to SPPB did not differ between genders ($p > 0.05$).

Regarding the Fried frailty score, there was no effect of time alone ($p > 0.05$). However, an effect for the interaction time * group was observed (F[df: 7.088, 73.248] = 3.862, $p = 0.001$). No differences emerged between groups at baseline, T2 and T3 ($p > 0.05$). Nevertheless, after the last intervention period (T4), controls were different from the ME+BCAA group ($p < 0.01$) and ME ($p = 0.01$). Within groups comparison using the Bonferroni adjustment showed that the Fried frailty score only decreased in the BCAA supplemented group between T1 and T2 ($p < 0.01$), while it increased between T1 and T4 for the CG ($p < 0.01$).

Although no time * group interaction ($p > 0.05$) was observed in the GDS score, the reported data showed a significant effect of time (F[df: 3, 93] = 3.054, $p = 0.03$). Despite no differences observed at baseline, or even within groups overtime for all interventions, changes between groups emerged in the follow-ups. Bonferroni comparisons showed that scores for GDS in the ME+BCAA and BCAA groups were significantly different at T2 ($p = 0.01$). Those differences were sustained throughout the next evaluations ($p < 0.05$). After washout (at T3), the GDS score for the ME+BCAA group was also different compared to ME ($p = 0.03$), while no differences emerged between the CG and the other groups, for all evaluations ($p > 0.05$).

Significant effects of time (F[df: 2.188, 67,826] = 5.026, $p = 0.008$) and time * group interaction (F[df: 6.564, 67,826] = 3.005, $p = 0.01$) were found for POMS scores. At baseline (T1), the ME+BCAA group presented significantly higher POMS scores in comparison with all other groups ($p < 0.05$). Within comparisons using the Bonferroni adjustment showed, however, that those changes were observed only in the ME between T1 and T3, T2 and T3, and T3 and T4 ($p < 0.01$ for all comparisons). Despite small variations in all other groups, no significant changes emerged over time ($p > 0.05$).

There was an effect of time (F[df: 2.184, 67.705] = 6.457, $p = 0.002$) but not for time * group interaction ($p > 0.05$) on MMSE indexes. At baseline, the average score of the cognitive MMSE index in the ME+BCAA group was significantly higher than those from the ME ($p = 0.029$) and BCAA ($p = 0.023$) groups. The differences in MMSE scores remained between ME+BCAA and BCAA groups at T2 ($p < 0.01$) and T3 ($p = 0.04$). However, no differences between interventions were observed for this parameter over time ($p > 0.05$).

There were no effects of time (F[df: 3, 57] = 1.712, $p = 0.175$) or time * group interaction (F[df: 9, 57] = 1.383, $p = 0.217$) for testosterone (ST) in all groups. A similar unresponsive pattern was also observed for HGT between interventions: no significant effects of time ($p = 0.145$) or time * group interaction ($p = 0.066$). However, correlations between ST levels and HGT emerged in all-time points, with correlation indexes of $r = 0.414$, $r = 0.389$, $r = 0.394$, and $r = 0.385$ for T1, T2, T3 and T4, respectively ($p < 0.05$) (Table 2).

4. Discussion

Physical performance was enhanced in both exercising groups, independently of BCAA supplementation. Regarding the physical performance evolution in the exercise-only (ME) group, the positive influence of exercise is clear with significant improvements in the two moments after the exercise periods (T2 and T4), while a significant decrease was seen during the washout period. Although the ME+BCAA group presented a better initial profile than the other groups, especially regarding SPPB, significant functional fitness increases were also obtained for this group. This demonstrates the efficacy of the two exercise programs and how important regular exercise is for older persons functional fitness. Because no effect on SPPB was found for the BCAA only group, exercise was probably the main influencer on this parameter in the ME+BCAA group. It is possible that the BCAA supplement, when administered with exercise, may have contributed to an attenuation of the detraining effect since no significant decrease in SPPB was found in this group, while the exercise-only group presented a significant SPPB decrease during the washout period. According to Perera et al. (2006) in similar populations, changes in SPPB scores between 0.27 and 0.55 are considered as having small meaningfulness. In the two periods of training, the ME group successively presented an increase of 1.3 and 1.1 points, the ME+BCAA group an increase of 1.75 and 0.5 points, the BCAA group an increase of 0.57 and 0 points, while the CG only increased 0.076 and 0.077 points. Taking this into account, a small positive effect of BCAA supplementation after the first 16 weeks was also found. Regarding the detraining period, the ME group registered a decrease of 1.42 points, the ME+BCAA group a decrease of 1 point, the BCAA group a decrease of 0.42, and the CG control no decreases. These results confirm the potential of BCAA supplementation to attenuate the SPPB score during a washout period.

Strength exercise has been proposed as one of the most effective strategies and with better results in the common tasks of the daily life of older persons, focusing on the optimization of neuromuscular function to obtain better benefits (Cadore et al., 2014). Multicomponent exercise programs have been demonstrated to result in major improvements in functional capacity, which is key for maintaining independence and the ability to perform basic activities of daily living (Casas-Herrero et al., 2019; Angulo et al., 2020).

Our study did not confirm any superiority of the multicomponent exercise over the elastic band exercise only, since both programs induced significant improvements on exercise performance in older persons (Giné-Garriga et al., 2014). Ikeda et al. (2016) showed that a combined physical exercise (strength, aerobic, balance) program with BCAA supplementation, twice a week, for 3 months, achieved better results than the exercise program alone. Our study failed to show any

Table 2
Statistical analysis T1-T4 Handgrip Test and testosterone.

	Groups	Time points of evaluation				Effect	F	p
		T1[a]	T2	T3	T4			
		M ± SD	M ± SD	M ± SD	M ± SD			
Handgrip Test (HGT) (kgf)	ME + BCAA	15.7 ± 2.4	16.5 ± 1.6	16.5 ± 1.7	16.7 ± 2.3			
	ME	13.4 ± 6.6	14.3 ± 5.0	12.2 ± 5.4	14.3 ± 4.9	Time	1.841	0.145
	BCAA	18.4 ± 7.9	17.1 ± 7.5	16.3 ± 7.9	16.2 ± 8.2	Time * group	1.870	0.066
	CG	16.1 ± 5.3	15.3 ± 5.2	15.0 ± 5.2	14.5 ± 4.5			
Testosterone (pg/mL)	ME + BCAA	55.7 ± 38.6	39.3 ± 26.7	54.6 ± 47.2	52.9 ± 35.3			
	ME	47.9 ± 42.1	48.9 ± 18.3	58.6 ± 33.6	67.6 ± 20.9	Time	1.712	0.175
	BCAA	58.0 ± 27.9	62.3 ± 54.1	51.7 ± 33.4	62.2 ± 39.0	Time * group	1.383	0.217
	CG	53.2 ± 30.8	64.7 ± 48.8	60.8 ± 35.1	69.5 ± 52.0			

Notes: M ± SD = mean (standard and deviation); ME = multicomponent exercise; BCAA = branched-chain amino acids.
[a] T1 to T2 (elastic-band exercise, 16 weeks, 8 weeks), T2 to T3 (washout) T3 to T4 (multicomponent exercise, 16 weeks).

effect of the BCAAs supplementation alone on exercise performance. In contrast, one study that evaluated the effect of daily BCAAs supplementation during 12 weeks, in sedentary older persons, showed an increased functional performance (Ispoglou et al., 2016). More recently, short time effects of BCAAs supplementation alone on the exercise performance of older persons have been identified (Ko et al., 2020). Differences in the frequency and dosage of the BCAA administration might have, in part, contributed for such contrasting results. However, a recent meta-analysis showed no effect of protein supplementation on SPPB scores in frail older persons (Oktaviana et al., 2019). Similar results demonstrated that protein supplementation did not enhance the functional status of post-hospitalized older persons more than exercise per se (Amasene et al., 2019).

To our knowledge, this is probably the first time that the combination of BCAAs and exercise was studied in frail older persons living in RCH involving a detraining period. The overall observed trend of BCAA to attenuate loss of exercise performance under these circumstances may be an interesting topic for future studies.

In our study, frailty in the ME group tended to diminish throughout time, which highlights the positive influence of exercise. BCAAs supplementation significantly improved frailty but only in the first 16 weeks of treatment. The same trend was observed in the ME+BCAA group, reinforcing the role of BCAAs supplementation in combination with exercise. Since the control group was the only one to show a significant deterioration in the frail condition, we suggest that exercise and BCAA, or a combination of both, could contribute to attenuate this condition in older persons in institutionalized.

Several studies aimed to investigate whether exercise training could putatively reverse frailty (frail to pre-frail or non-frail), or, at least, achieve a lower degree of frailty in older persons (Mañas et al., 2019). In this respect, a physical exercise program was able to reduce frailty by improving the functional capacity, muscle strength, speed, and agility of older persons (Ferreira et al., 2018). There is a consensus in the literature that physical exercise training has the potential to protect against early frailty condition in advanced aged persons (Higueras-Fresnillo et al., 2018), and most of these benefits are related to the improvement of several immune-inflammatory parameters (Aguirre and Villareal, 2015; Gleeson et al., 2011; Petersen and Pedersen, 2005).

BCAA supplementation has been shown to increase plasma and muscular BCAA concentrations, increasing substrate availability for protein synthesis. An increase in amino acid transport post resistance training (with a concomitant increase in plasma and muscles) may enhance protein synthesis (Sharp and Pearson, 2010). The supplementation with BCAA, mainly because of its L-leucine content, activates a cascade of protein phosphorylation that culminates in muscle protein synthesis through mTOR, with subsequent stimulation of three key ribosomal proteins: kinase S6 of 70 kDa (p70S6K), 4E-BP1, and 4G (elF4G) (Apró and Blomstrand, 2010). Through insulin-dependent and independent pathways, L-Leucine and strength training are potent activators of mTOR, a protein that is involved in increasing the rate of mRNA translation of myofibrillar proteins (Millward et al., 2008; Aguirre et al., 2013).

BCAA amino acids may act by direct and indirect means, to increase serotonin synthesis in the brain (Rondanelli et al., 2011). Other mechanisms could include the direct action of BCAA, particularly L-leucine, in the central nervous system (CNS) improving the availability of insulin in the brain, and the use of amino acids to produce energy and synthesize proteins in the CNS (Aquilani et al., 2008). However, no significant changes were observed for the GDS scale between different interventions in this study. The positive effect of exercise on depression in older persons is well recognized (Rondanelli et al., 2011; Arent et al., 2000), but differences in frequency and dosage of BCAAs administration, or even the type of exercise, might have contributed to the difference in results. An increase in POMS scores was only seen for the ME group at T3, which probably reflected a decrease in vigour after the washout period. Overall, we observed that mood state, cognition, and testosterone remained stable in all groups over time, with no effects of the treatments on those parameters. In contrast, for mood states and cognition, other studies have shown positive effects of exercise in older persons (Furtado et al., 2020; Monteiro-Junior et al., 2017; Sarid et al., 2010; Smolarek et al., 2016).

Although not very strong, the correlation between ST levels and HGS is also particularly interesting, since in older men and women low testosterone levels were each independently associated with an increased progressive frailty status (Wu et al., 2010), and agree with previous data (Hsu et al., 2018). Although ST did not differ between groups (an increase in the exercising groups could be expected), it is possible that the individual variability and the low sample size might have hidden the mediating effects of exercise on testosterone levels. Additionally, it is important to highlight that there is no scientific consensus about the testosterone response to exercise, especially regarding older persons. Further investigation to assess the improvements that testosterone could provide in functional fitness and frailty in older persons is warranted.

Despite our effort to match the groups at baseline, because of the loss of subjects, logistic issues (many selected HCR), and low adherence in some of the groups, the ME+BCAA group presented a better initial profile than the other groups, especially regarding SPPB, POMS and MMSE scores. It also presented the lowest level of initial frailty.

The small sample size may have restricted the power of our observed results, and is a study limitation Unfortunately, despite the efforts of the research team and the nursing home staff, it was not possible to eliminate the dropout rate. However, our results reflect real-world data mimicking what happens in RCH, with participants presenting several disabilities and comorbidities, and all the difficulties associated with older persons' motivation to accomplish the proposed goals. The execution of a controlled study over 40 weeks with such a population is also subjected to other unforeseen limitations (e.g. death or change of RHC). We suggest that the use of other methods of exercise training, such as the use of playful activities (dance and music sessions) might increase the adherence levels.

Considering that the total Fried score is supported by parameters related to the levels of physical activity, gait efficiency, and perception of fatigue, all these aspects were positively affected by the practice of physical exercise. As protein intake is also required to induce positive benefits in muscle mass (Tieland et al., 2012) further studies are needed to analyse the biological mechanisms behind the exercise and BCAA supplementation effects on total Fried score in older persons.

Our results support the importance of the implementation of specific physical exercise programs designed especially for frail older persons. It is crucial for public health to identify the main factors associated with physical frailty for the development of new methods for complementary therapies, such as the use of nutritional tools combined with long-term exercise interventions.

5. Conclusion

This study showed that the long-term exercise programs, independently of being multicomponent or elastic band-based, were effective in improving functional capacity, and in slowing/preventing the progression of frailty in older persons living in RCH, when compared to a control group without exercise. BCAA supplementation alone had no impact on functional fitness. However, in a short period (16 weeks), BCAA supplementation contributed to diminishing frailty. Moreover, the combination of BCAA and exercise demonstrated the potential in reducing the effects of a detraining period on functional capacity. Overall, the intervention periods had no significant effect on the mood state, depression scores, cognitive function, or salivary testosterone levels, but there was a tendency of diminished depression scores in the exercising groups (compared to the increasing tendency in the non-exercising ones). Further research is needed to define the best practices, the feasibility of implementation, the best supplementation

strategies, and the suitable physical exercise programs for this special population, to augment compliance and long-term behavior maintenance.

CRediT authorship contribution statement

Caldo drafted the paper. Valente helped with data acquisition. Chupel and Letieri statically analysed the data. Furtado, Teixeira, Massart and Marzetti developed the study proposal, revised the manuscript critically and suggested additional statistical analyses. Teixeira and Massart coordinated the research study and, together with Massart, Marcelo Barros and Andre Bachi revised the manuscript critically. All the authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Ethical standards

This study was approved by the University of Coimbra, Faculty of Sport Sciences and Physical Education Ethical Committee (reference number: CE/FCDEFUC/00282018), clinicaltrials.gov register NCT04376463.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2021.111592.

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Effect of Training-Detraining Phases of Multicomponent Exercises and BCAA Supplementation on Inflammatory Markers and Albumin Levels in Frail Older Persons

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Abstract: Nowadays, it is accepted that the regular practice of exercise and branched-chain amino acids supplementation (BCAAs) can benefit the immune responses in older persons, prevent the occurrence of physical frailty (PF), cognitive decline, and aging-related comorbidities. However, the impact of their combination (as non-pharmacological interventions) in albumin and the inflammatory markers is not fully understood. Therefore, we investigated the effect of a 40-week multifactorial intervention [MIP, multicomponent exercise (ME) associated or not with BCAAs] on plasma levels of inflammatory markers and albumin in frail older persons (≥75 years old) living at residential care homes (RCH). This study consisted of a prospective, naturalistic, controlled clinical trial with four arms of multifactorial and experimental (interventions-wahshout-interventions) design. The intervention groups were ME + BCAAs (n = 8), ME (n = 7), BCAAs (n = 7), and control group (n = 13). Lower limb muscle-strength, cognitive profile, and PF tests were concomitantly evaluated with plasma levels of albumin, anti- and pro-inflammatory cytokines [Interleukin-10 (IL-10) and Tumor Necrosis Factor-alpha (TNF-α) respectively], TNF-α/IL-10 ratio, and myeloperoxidase (MPO) activity at four different time-points: Baseline (T1), after 16 weeks of multifactorial intervention (T2), then after a subsequent 8 weeks washout period (T3) and finally, after an additional 16 weeks of multifactorial intervention (T4). Improvement of cognitive profile and muscle strength-related albumin levels, as well as reduction in the TNF-α levels were found particularly in ME plus BCAAs group. No significant variations were observed over time for TNF-α/IL-10 ratio or MPO activity. Overall, the study showed that MIP triggered slight alterations in the inflammatory and physical function of the frail older participants, which could provide independence and higher quality of life for this population.

Keywords: inflammaging; cognitive impairment; cytokines; protein intake; physical frailty

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1. Introduction

Aging is characterized as a natural degenerative process strongly linked to diminished immune efficiency, and also to enhanced inflammatory responses, and thus, to higher risks of infections in older persons [1]. The sedentary lifestyle, per se, is one of the most important contributors to age-related illness, whereas regular physical exercises (rPE)—based on hormesis principles—could chronically slow down the aging immune/inflammatory dysfunctions [2]. In this sense, reduction of systemic levels of interleukin-10 (IL-10), a classical anti-inflammatory cytokine, with elevation on Tumor Necrosis Factor-alpha (TNF-α) levels are associated with aging [3]. Although the participation in rPE programs does not stop the progression of aging [4], staying in moderate rPE programs can help making the aging process more rewarding, with lower incidence of premature chronic diseases [5]. In addition to the comorbidities outcomes, both aging and the sedentary behavior may speed up the loss of mobility and functional autonomy [6], reducing the quality of life [7], and also increasing the susceptibility to physical frailty (PF) and cognitive decline [8].

The age-related PF syndrome is defined by loss of muscle mass (and sarcopenia), by low physical activity levels, and often accompanied by low protein intake [9]. Cognitive decline, in turn, is characterized by confusion and progressive loss of memory and neuromotor skills [10]. However, these two outcomes reveal biological and phenotypic similarities, which is the reason leading to the current scientific interest in investigating populations affected by these disorders [11]. In this sense, rPE could also provide protection against both PF and cognitive decline in very old people [12], with most of these benefits related, at least in part, to changes that occur in the immune system [13]. Recent findings have shown that multicomponent exercise (ME) interventions, those that include different types of endurance, muscle strength, and balance exercises in the same session, appear to have a superior effect on cognitively and physically frail older persons [14,15].

Participation of older persons in rPE ameliorates not only antigen recognition, but also immune responsiveness in general, as some evidence has shown that increased levels of physical activity using exercise routines can even extend the protection provided by the influenza vaccine in older persons [16], as well as a regulation of systemic inflammatory status [17]. Apart from the modulating effects of rPE, nutritional habits also play an important role in determining immune and inflammatory efficiency, especially in older persons [2]. In fact, malnutrition in older population is a serious concern for health systems around the world, since it increases the risk of comorbidities occurrence with subsequent higher health care costs [7,8]. Indeed, nutritional supplementation with vitamins, antioxidants, and protein components (including isolated amino acids) have already demonstrated positive results against PF, cognitive impairment, sarcopenia and other age-related disorders [18].

Supplementation with BCAA, in the absence of branched-chain aminotransferase (BCAT) activity in the liver implies that a dietary supply of BCCAs would ensure an almost intact passage through the liver directly to the muscle tissue, which seems to be advantagous to restrain sarcopenia and frailty [19]. Supplementation with BCAA, especially in association with regular exercises, was demonstrated to improve muscle strength and cognitive functions in the older population, which are safe and low-cost strategies to circumvent the general limitations imposed by the aging process [20–22].

Among several pro/anti-inflammatory biomarkers used in the context of exercise and nutrition sciences [23], myeloperoxidase (MPO) stands out as a valid marker largely released by activated neutrophils, with potent pro-oxidative/pro-inflammatory actions 24 [24]. MPO activity also appears as a biomarker that was strongly associated with frailty and risk of mortality in a study conducted in a large community-dwelling frail octogenarians and nonagenarians [25]. Recently, a similar intervention demonstrated the slight reduction of serum MPO activity triggered by the combination of Taurine and ME in older persons [26]. Instead, albumin concentrations are currently used for the assessment of the nutritional status of an individual, and low albumin concentrations have been associated with increased mortality after correlation for age, body mass index (BMI), gender, and several chronic comorbidities [27]. In this sense, multifatorial interventions programs

(MIP, exercise plus protein suplmentattion) that target to maintain (or even increase) albuminemia in older persons could characterize an important strategy to diminish the harmful effects of aging and its comorbidities [28].

Therefore, the aim of this work was to evaluate the effect of a 40-week MIPon plasma/serum pro- and anti-inflammatory markers of the immune system in older persons living in residential care homes (RCH). Furthermore, we hypothesized that ME plus BCAAs may have an impact on the systemic albumin levels, inflammatory variables, cognitive profile, and physical function of the participants.

2. Materials and Methods

2.1. Preliminary Procedures and Ethics

This is a prospective, naturalistic, controlled clinical trial (treatment vs care). All subjects volunteered to participate in the exercise classes or the supplementation programs. Consent forms were signed by the institution's directors, the participants and their legal representatives before testing and intervention. This study was approved by the Ethical Committee of Faculty of Sport Sciences and Physical Education, University of Coimbra (reference number: CE/FCDEFUC/00282018), respecting the Portuguese Resolution (Art.°4th; Law no. 12/2005, 1st series) on ethics in human research and the Helsinki's Declaration. This study was properly registered with clinicaltrials.gov register NCT04376463.

2.2. Participants Elegibility

Study participants were selected through a non-probabilistic trial (plus controlled sampling) living in public and private RCH. The eligible criteria for the participants in this study were, at the time of first screening: (i) Participants had to be 70 years old or more; (ii) physically frail and pre-frail; (iii) clinically stable with their drug therapy updated; (iv) being able to perform the Time Up and Go test in $\leq$50 s that indicate severe mobility independence [29]; (v) not participating in other structured rPE; (vi) not presenting any type of health condition or use medication that might prevent the functional self-sufficiency test performance or attention impairment (such as severe cardiopathy, hypertension, uncontrolled asthmatic bronchitis or severe musculoskeletal conditions); (vii) not presenting mental disorders or hearing/visual impairment that could prevent the evaluations and activities proposed, according to the institutional medical staff; (viii) not presenting morbid obesity (BMI $\geq$ 40). At the end of the recruitment process, 80 older persons entered the enrollment phase.

2.3. Participants Allocation

All the participants were selected through a non-probabilistic trial (plus controlled sampling) based on the geographical area of Coimbra, Portugal, living in public and private residential care homes (RCH) or frequenting day centres in the local community. From the 80 participants initially screened, 50 eligible participants were allocated in their respective intervention groups. However, for the specific reasons highlighted in Figure 1, only 35 participants (age = 83 $\pm$ 3 years-old) completed the 40 weeks multifactorial intervention, divided in the following groups: ME (n = 7), ME + BCAA (n = 8), BCAA (n = 7), and the no-regular exercise/no-supplementation control group (CG, n = 13). All the procedures were performed according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines [30].

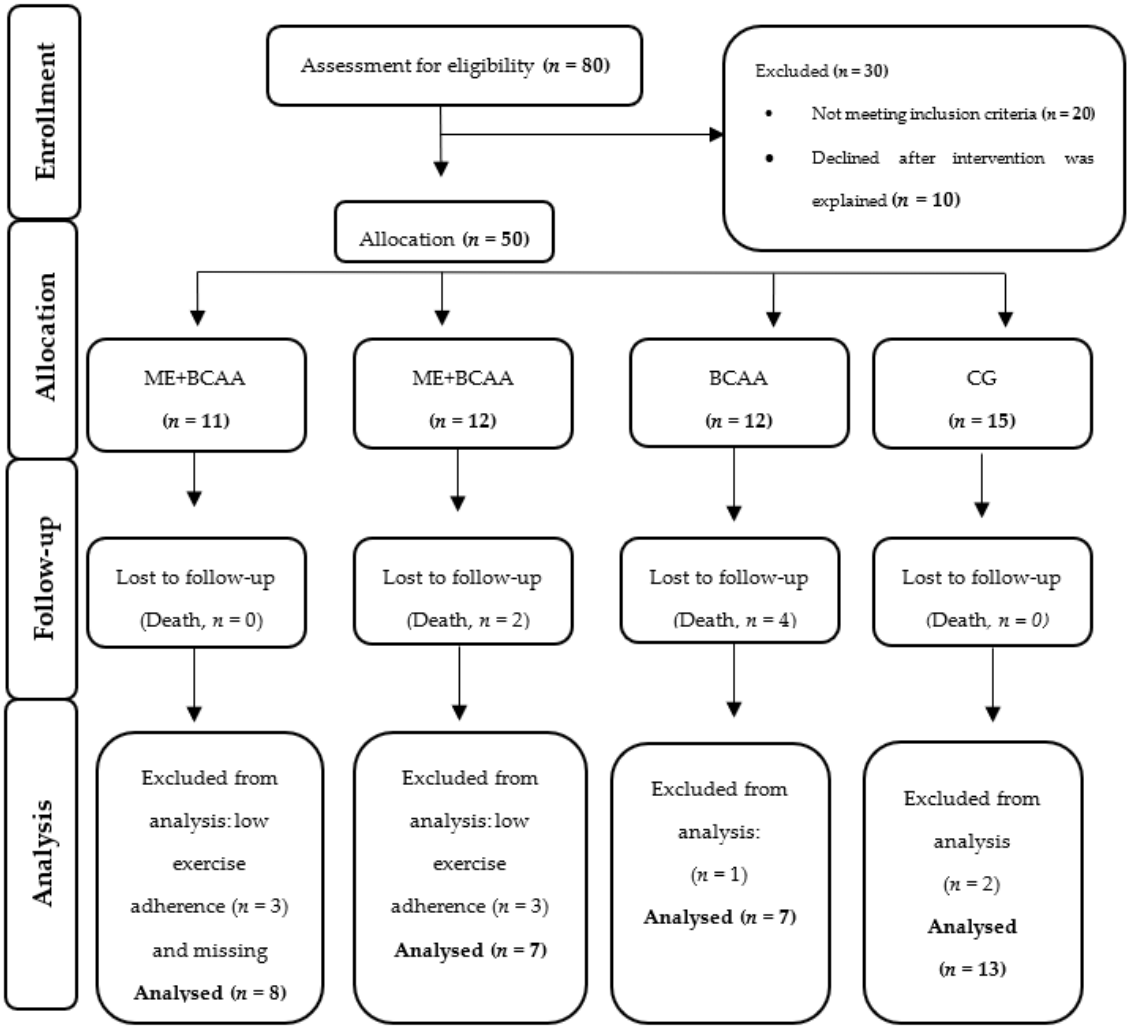

Figure 1. CONSORT Flowchart of study participants [30]. ME + BCAA, Multicomponent Exercise + Branched Chain Amino Acid; ME, Multicomponent Exercise; BCAA, Branched Chain Amino Acid; CG, Control Group.

2.4. Experimental Design

This study is a four-phase prospective, naturalistic, controlled clinical trial with four arms of MIP experimental design (ME + BCAAs, BCAAs, ME, and CG). In the first phase, a baseline data collection (T1) was done followed by 16 weeks of MIP. The second phase consisted of a second data collection (T2) followed by an 8 week washout phase. Phase 3 consisted of a third data collection, followed by the resumption of the MIP for a period of 16 weeks. The last data collection took place after the 16 weeks of intervention (T4) (Figure 2).

3. Outcome Measures

All the assessments were performed in the morning, between 10 and 11:45 a.m. One session was used to apply a short test battery to measure biosocial, global health status, cognition profile, nutritional, physical, and physical frailty status. In the second consecutive day, blood samples were collected and stored at −80 °C until further analysis.

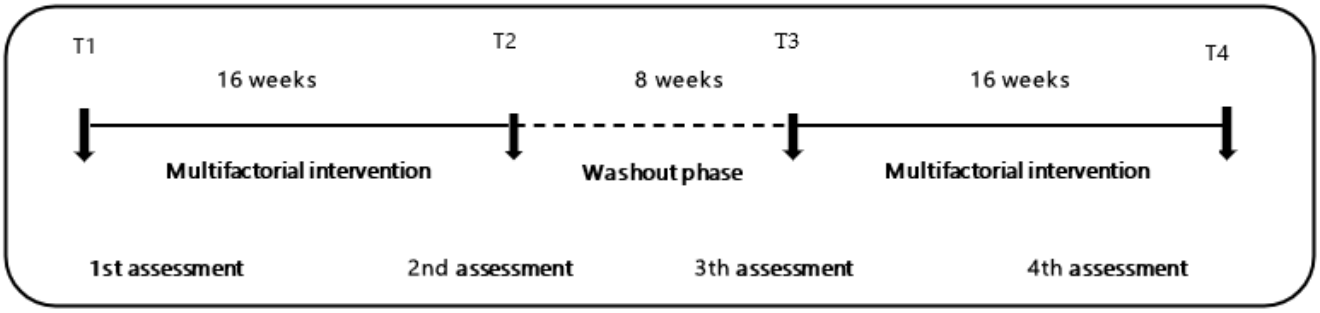

Figure 2. Chronological order of multifactorial interventions study design. T1 to T2 (elastic-band exercise, 16 weeks, 8 weeks), T2 to T3 (wash-out), T3 to T4 (multicomponent exercise, 16 weeks).

3.1. Physical Frailty Index

The phenotype of Fried's physical frailty index was used [9]. Weight loss was assessed by a self-report of unintentional weight loss of 4 kg or more in the last 6 months. Self-reported exhaustion was evaluated by a negative concordance of question number 7 and 20 of the Center of Epidemiologic Studies for Depression scale [31]. Hand-grip strength was assessed in kilograms by a hand-held (HGT) dynamometer (Lafayette 78,010, Sagamore, United States). The best result of the two trials was used for scoring purposes. Participants who were unable to perform the HGT and those in the lowest 20% were categorized as positive [32]. The cutoff reference values for HGT of $\geq$29 kg for male and $\geq$17 kg for female were adopted. Slowness was measured by the "15 feet (4.6 m) walking test". Based on the cutoff values of Fried's study population, the times of $\geq$7 s for males and $\geq$6 s for females were adopted for positive scores of slowness. The best time of the two trials was used for the final scoring. Low physical activity (PA) levels were assessed by the International PA Questionnaire short version (IPAQ-SV) [33]. There are three levels of PA suggested for classification: Inactive, minimally active, and highly active. Participants classified as inactive had a positive score for this PF component. A positive evaluation in one or two criteria classified the participants as pre-frail, in three or more criteria as frail, and as non-frail when the subject scored none of the five PF indicators. The prevalence of PF was calculated to generate a frailty total score, as well as the presence of each of the five criteria of the Fried's model (0 to 5 points). In this study, participants classified as frail (3 or more points) and pre-frail (2 points) were included.

3.2. Nutritional Assessment

Daily diet at the RCH was prescribed by a registered nutritionist and was provided for all the participants without any change or interference of the research staff. On the basis of the information provided, the diet was analyzed using specific tools (photographic quantification of portions, food table) for the Portuguese population [34–36]. Due to the relationship between the frailty status and severe decrease of muscle mass (or sarcopenia) which had already been demonstrated in several studies, the objective of this nutritional assessment was to characterize the protein consumption of the participants. In addition, the Mini Nutritional Assessment (MNA) questionnaire was applied [37,38]. This consists of 18 questions that present a maximum score of 30 points, and classifies the participants as malnourished ($\leq$17 points), at risk of malnutrition (17 < MNA < 23.5 points), and as having a normal nutritional status (MNA > 23.5 points).

3.3. Lower Limb Muscle-Strength Test

The Five-Times-Sit-to-Stand-Test (5TSS test) was applied. This test assesses the functional strength of the lower limbs, transition movements, balance, and risk of falling. The participant is instructed to stand as quickly as possible five times, without stopping in the middle. In addition, the participant should be encouraged to keep his arms crossed over his chest. The instructor must count the time with a stopwatch and must count each

position out loud so that the participant remains oriented. The test is stopped when the participant reaches the orthostatic position at the 5th repetition [29].

3.4. Clinical and Health Status

The Charlson comorbidity index (CCI) was calculated based on the registry of individual comorbidities combined with age and gender, to account for a final score [39]. The anthropometric assessment included body mass (kg) and stature (m). Body mass was determined using a portable scale (Seca®, model 770,Berlin, Germany) with a precision of 0.1 kg, whereas stature was determined using a portable stadiometer (Seca Body meter®, model 208, Berlin, Germany) with a precision of 0.1 cm. Body mass index (BMI) was calculated according to the formula (BMI = body mass/stature^2). The standardized procedures described in previous studies were followed [40].

3.5. Cognitive Profile

The Portuguese version of the Mini Mental State Examination (MMSE) was used [41]. The MMSE is a 30-point scale instrument that evaluates five domains of cognition: Orientation, immediate recall, attention and calculation, delayed recall, and language. This scale classifies individuals by progressive cognitive skills: (0–9 points) severe cognitive impairment; (10–18 points) moderate cognitive impairment; (19–24 points) mild cognitive impairment; and (25–30 points) normal cognitive profile [42].

3.6. Biochemical Analysis

Non-fasting blood collection was done in the morning (between 10:00 a.m. and 11:00 a.m.). Blood samples were collected by venipuncture, after 15 min of individual rest in an isolated and quiet room, at the four time-points of the study assessment. The participants were asked to avoid alcohol and caffeine intake on the previous day of blood collection, and also to maintain their sleep habits during the previous night. After centrifugation at 3000 rpm at 4 °C during 15 min, plasma and serum samples were aliquoted into Eppendorf tubes and stored at −80 °C until used for the determination of interleukin-10 (IL-10), tumour necrosis factor alpha (TNF-α), myeloperoxidase activity (MPO), and total albumin concentrations. The ELISA (Thermo Fisher, Gloucester, UK) intra-assay coefficients of variability were 4.1% for IL-10 and 3.0% for TNF-α.

3.7. Full Characterization of the MIP

3.7.1. Oral BCAAs

The BCAAs power mixture was composed of L-leucine (Leu), L-isoleucine (Ile), and L-valine (Val) in the proportion of 2:1:1 (MyProtein®, Cheshire, UK), accounting for 20 kcal per portion, comprising 5 grams (g) of supplement: 1.85 g Leu, 0.93 g Ile, and 0.93 g Val. The unflavored supplement was used as to not induce ingestion preferences for specific flavors. The BCCAs were diluted in 200 mL of water and given immediately after the exercise sessions to the participants in the ME + BCAAs and BCAAs groups [43]. The supplement dose was fixed at 0.21 g total BCAAs/kg/session, with individual portion sachets, administered in the morning, between 09:00 and 11:30 a.m. [44]. We opted to exclude maltodextrin or the carnosine-based placebo here, since the carbohydrate ingestion could mask the effort perception and cognitive indexes in our older persons volunteers, compared to the amino acid supplementation [45]. In addtion, carnosine, as well as other β-alanine derivatives, were shown to affect cognitive functions, including the perception of wellness, mood, and depression indexes [46]. Therefore, we decided to split BCAA-supplemented (ME + BCAAs and BCAAs) and BCAAs-absent groups (ME and CG) according to the proximity between the residential care homes (RCH), where the ME programs were effectively applied. No communication was reported between volunteers from the BCAA-supplemented and no-BCAA supplemented groups in our study.

3.7.2. Washout Period (Oral BCAAs)

In this phase, the participants endured a cessation period of 8 weeks, when supplementation of the ME + BCAAs and BCAAs groups was suspended in order to verify whether the supposed benefits of BCAAs were maintained or lost [21].

3.7.3. Exercise Intervention (Phase 1)

The exercise program was divided in two interventions of 16 weeks each, separated by an 8-week detraining (washout) period. Exercise sessions were offered twice a week, with an interval of 36 h for adequate physiological recovery and rest. The exercise protocol respected the guidelines of exercise prescription for older persons and the guidelines of exercise periodization by the American College of Sports Medicine (ACSM) [47,48]. The program started with an adaptation period of 2 weeks, in which seven different exercises were performed using elastic bands (TheraBand®, Hygenic Corporation, Akron, OH, USA). The participants were closely supervised for two initial sessions aiming for equipment familiarization and adjustments to the Rating Perceived Exertion (RPE OMNI) scale [49]. During these familiarization sessions, the participants learned the correct technique of the exercises, and selected the proper color, length, and grip width of the elastic bands. The exercise intensity was indirectly calculated using the Karvonen's formula to predict the target heart rate (HR), with HR_{max} being calculated by an adjusted formula for older persons [50].

$$HR = ((HR_{max} - \text{resting HR}) \times \%\text{Intensity}) + \text{resting HR} \quad (1)$$

After the adaptation period, the exercise program was progressively intensified by increments in both the number of exercises (from 8 to 10 exercises during the rest of the exercise intervention) and the proposed physical effort, imposed by different intensity color bands, according to the OMNI table [49]. The elastic-band exercises applied in the Phase 1 period are shown in Table 1. For safety reasons, the exercise programs were also monitored using heart rate monitors (Polar M200; Polar Electro Oy, Kempele, Finland). Additionally, intensity was measured through the specific rating perceived exertion (RPE) scales for each exercise program [51]. The RPE used is an arbitrary scale ranging from 0 to 10 points, with identical intervals and with reference to the quality of effort: (0) Nothing at all; (1) very weak; (2) weak; (3) moderate; (4) somewhat strong; (5–6) strong; (7–9) very strong; (10) very, very strong (almost maximal).

Table 1. Example of elastic-band exercise sessions applied in phase 1.

Warm-Up				**5 min**	**PSE 1–3**	**Progression**	**Weeks**	**Intensity (Color)**
Exercises (8–10)	**Sets**	**Repetitions**	**Cadence**	**Interval**	**PSE**	2 × 10	2	Yellow
Front squat	2–3	10–20	2:3	30–45 s	4 to 6	3 × 20	2	Yellow
Chair unilateral hip flexion	2–3	10–20	2:3	30–45 s	4 to 6	3 × 10	2	Red
Chair Bench over row (with flexion)	2–3	10–20	2:3	30–45 s	4 to 6	3 × 20	2	Red
Chest Press (stand and/or chair)	2–3	10–20	2:3	30–45 s	4 to 6	3 × 10	2	Green
Standing (or chair) reverse fly	2–3	10–20	2:3	30–45 s	4 to 6	3 × 20	2	Green
Shoulder Press/twist arm position	2–3	10–20	2:3	30–45 s	4 to 6	3 × 15	2	Blue
Chair (or stand) frontal total raiser	2–3	10–20	2:3	30–45 s	4 to 6	3–4 × 10^{-15}	2	Blue
Biceps arm curl (stand and/or chair)	2–3	10–20	2:3	30–45 s	4 to 6			
Chair Overhead triceps extension	2–3	10–20	2:3	30–45 s	4 to 6			
Cooling down				5 min	PSE 1–2			

Notes: PSE—Perception subjective effort.

3.7.4. Washout (ME Detraining)

In this phase, the participants endured a detraining period of 8 weeks, when the ME programs were suspended. The aim was to check if the physiological adaptations acquired during the first phase of ME were maintained or if an 8-week interruption was able to revert the possible effects on immune changes [52].

3.7.5. Exercise Retraining Protocol

The phase 3 (exercise retraining) protocol was also based on the resistant TheraBand (TheraBand®, Hygenic Corporation, Akron, OH, USA) elastic bands (Table 2), but included walking, steps, and balance exercises (sometimes with dumbbells and ankle/wrist weights) to compose a multicomponent exercise program for an identical 16-week period (twice a week, on alternate days, also totalizing 32 sessions). The multicomponent program (Table 2) was described by Furtado et al. [53]. The phase 3 program aimed to reproduce most of the daily activities of the older persons in this study [54].

Table 2. Example of multicomponent exercise sessions applied in phase 2.

Exercises (8–10)	Sets	Repetitions	Cadence	Interval	PSE
Front squat	2–3	10–20	2:3	30–45 s	4 to 6
Chair unilateral hip flexion	2–3	10–20	2:3	30–45 s	4 to 6
Chair Bench over row (with flexion)	2–3	10–20	2:3	30–45 s	4 to 6
Chest Press (stand and/or chair)	2–3	10–20	2:3	30–45 s	4 to 6
Standing (or chair) reverse fly	2–3	10–20	2:3	30–45 s	4 to 6
Shoulder Press/twist arm front position	2–3	10–20	2:3	30–45 s	4 to 6
Chair (or stand) frontal total raiser	2–3	10–20	2:3	30–45 s	4 to 6
Biceps arm curl (stand and/or chair)	2–3	10–20	2:3	30–45 s	4 to 6
Chair Overhead triceps extension	2–3	10–20	2:3	30–45 s	4 to 6
Circuit Training					
Walking around the room	2–3	3 min		30–45 s	4 to 6
Balance/agility exercise	2–3	3 min		30–45 s	4 to 6

Notes: PSE—Perception subjective effort.

3.8. Statistical Analysis

The descriptive statistics for each group, at the baseline and follow-up evaluations, were reported as the mean plus standard deviation (M ± SD), except when mentioned otherwise. All the variables were checked for the normally residual distribution and values were logarithmically transformed when appropriate. One-way Analysis of Variance ANOVA was used to determine baseline differences between the four groups in all the parameters. Effects of time, group, and time x group interactions were assessed through repeated measures ANOVA and Bonferroni post-hoc for multiple comparisons. Additionally, univariate analysis was performed using the paired *t*-test for comparisons during the first phase of interventions (T1 vs. T2). All statistical analyses were performed using the SPSS 21 (SPSS Inc., Chicago, IL, USA), and the level of significance was set at $p < 0.05$.

4. Results

The dynamics of the MIP groups and drop-outs are presented in detail in Figure 1. From the 50 (100%) participants initially selected, only 35 participants (70%) completed the intervention. This is an expected experimental loss, as reported by several previous studies [55]. None of the dropouts left the intervention due to injuries or adverse responses. Reported deaths were due to acute events triggered by chronic clinical conditions. Table 3 shows the characterization of participants by MIP groups at the baseline, including nutritional, cognitive, frailty, anthropometric, and body composition status. No statistically significant differences in all the variables appeared, expect for time in residential care and nutritional status assessed by MNA ($p < 0.05$). However, all the groups were within the well-nourished category.

Table 3. Characterization of participants by intervention groups at baseline.

Variables	ME + BCAA (n = 8)	ME (n = 7)	BCAA (n = 7)	CG (n = 13)	p-Value
	M ± SD	M ± SD	M ± SD	M ± SD	
Age (years)	80 ± 6.1	86.7 ± 4	84.2 ± 5.8	83.1 ± 5.4	0.139
Time in residential care (years)	3.6 ± 1	4.7 ± 1.4	4.5 ± 1.1	5 ± 1	0.06
MNA (0–30 pts)	25.5 ± 2.2	24 ± 2.7	21.7 ± 2.8	24.7 ± 1.8	0.02
BMI (kg/m^2)	28.53 ± 5.1	28.7 ± 5.6	25.8 ± 3.1	30.2 ± 3.7	0.23
Stature (cm)	158 ± 0.05	150 ± 0.06	161 ± 0.12	155 ± 011	0.16
Comorbidity index (0–10 pts)	4.87 ± 1.12	5.28 ± 0.95	5.42 ± 1.1	4.92 ± 1.2	0.71
Schooling time (years)	4 ± 0	4 ± 0	4 ± 0	4 ± 0	0.99
Cognitive profile (0–30 pts)	26.00 (3.11)	21.00 (3.78)	20.85 (2.79)	21.69 (2.89)	0.00
Physical Frailty index (0–5 pts)	2.00 (0.53)	2.71 (1.1)	3.00 (0.57)	2.16 (0.71)	0.40
Daily Individual Protein (gr/kg/day)	1.42 ± 0.28	1.83 ± 0.44	1.48 ± 0.22	1.60 ± 0.23	0.159
BCAAs (per person/gr/week)	30.3 ± 6.0	n.d.	28.4 ± 5.0	n.d.	

Notes: BMI: Body mass index; MNA: Mini nutritional assessment; M ± SD: Mean (standard and deviation); pts: Points; Kg/m^2: Kilograms; cm: Centimeters; One-way ANOVA was used to compare groups, except for the Comorbidity index (Fisher Exact Test). BCAA Branched Chain Amino Acids.

4.1. Biochemical Analysis

Table 4 shows the results for IL-10, TNF-α and TNF-α/IL-10 ratio, MPO, albumin, 5TSS-Test, as well as Fried (score) and MMSE. Concerning the IL-10 levels, a classical anti-inflammatory cytokine, not only no effects of time (p = 0.690) or time vs. experimental groups were found (CG, BCAAs, ME, and ME + BCAAs), F(degrees of freedom-df:9, 51) = 1.567, p = 150), but also Bonferroni post-hoc comparisons did not result in significant variations between time vs. groups ($p > 0.05$). Regarding the TNF-α levels, although we did not observe any interference of time on these pro-inflammatory cytokine levels ($p > 0.05$, Table 4, repeated ANOVA analyses revealed significant interactions between time vs. groups: F(df: 6.758, 47.303) = 2.524, p = 0.029. In addition, Bonferroni post-hoc comparisons showed not only higher TNF-α values in the ME + BCAAs group between T2 and T3 (p = 0.01), but also a significant decrease of TNF-α was observed between T3 and T4 within the same experimental group (ME + BCAAs, $p < 0.01$). The TNF-α values were unchanged in all other experimental groups. Regarding the TNF-α/IL-10 ratios, no significant variations were observed over time (p = 0.703) or within the interactions (time vs. group, p = 0.638).

Concerning MPO activity, Table 4 shows that this biomarker was not influenced by time (T1, T2, T3, and T4), except for a slight tendency regarding interactions (time vs. group): F(df: 9, 48) = 2.010, p = 0.059. Particularly, the Bonferroni post-hoc comparisons showed that the BCAAs group presented higher MPO activity after re-supplementation (T4) than the values found in the T2 time-point (after the first 16 weeks of the supplementation period, p = 0.026). No significant alterations in the MPO activity were observed in other comparisons between groups.

In terms of serum albumin (Table 4), a statistically significant difference in the effect of time was found (F(df: 1949; 46,784) = 3.841, p = 0.02), but no other (time vs. group) significant difference was detected between the albumin levels (p = 0.219). The pair-wise comparison using Bonferroni post-hoc showed a decrease of albumin levels in the BCAAs group in the T3 time-point (after the washout period, p = 0.04) as compared to the values found in T1, whereas no other significant variations were observed in the other groups ($p > 0.05$).

4.2. Five-Times-Sit-to-Stand-Test (5TSS test)

Table 4 shows no effect of time (p = 0.841) or interactions (time vs. group, p = 0.846) on the time elapsed to perform the 5TSS test. However, post-hoc adjustments showed that the ME + BCAAs and BCAAs groups presented a significant reduction of the time elapsed to perform this test at time-points T2, T3, and T4 (p = 0.009, p = 0.014, and p = 0.024, respectively).

Table 4. Statistical analysis comparison of four time-points moments of multifactorial intervention for biochemical, cognitive profile, physical frailty index, and functional fitness test.

Biomarker/Variables	Groups	Time-Points of Evaluation T1	T2	T3	T4	Effect	F	Overall *p*
		M ± SD	M ± SD	M ± SD	M ± SD			
IL-10 (μg/mL)	ME + BCAA	10.36 (6.96)	12.0 (6.53)	15.99 (7.98)	11.52 (7.56)	Time	0.491	0.690
	ME	8.68 (7.68)	12.25 (12.35)	4.16 (3.39)	10.53 (5.82)	Time*group	1.567	0.150
	BCAA	7.71 (2.54)	9.24 (4.15)	13.83 (6.94)	9.85 (10.89)			
	CG	16.10 (7.4)	12.21 (2.81)	12.74 (7.36)	20.45 (5.42)			
TNF-α (pg/mL)	ME + BCAA	62.44 (53.65)	71.42 (38.06)	112.86 (62.51)	57.37 (31.18)	Time	1.552	0.210
	ME	41.78 (54.08)	45.83 (21.07)	24.92 (15.60)	54.05 (29.19)	Time*group	2.524	0.015
	BCAA	32.65 (15.74)	37.18 (26.91)	62.93 (35.77)	60.02 (55.42)			
	CG	44.46 (41.72)	44.81 (37.16)	41.78 (37.86)	57.01 (44.15)			
TNF-α/IL-10 ratio (pg/mL)	ME + BCAA	6.24 (4.46)	7.47 (4.09)	6.96 (1.63)	6.10 (3.25)	Time	0.472	0.703
	ME	4.43 (1.99)	9.06 (10.46)	8.64 (7.36)	5.70 (3.27)	Time*group	0.777	0.638
	BCAA	5.44 (3.39)	3.85 (1.84)	5.45 (1.54)	11.19 (9.77)			
	CG	4.10 (1.27)	5.37 (1.56)	4.56 (1.80)	4.41 (0.38)			
MPO (μg/mL)	ME + BCAA	5653.91 (1106.71)	5871.97 (1159.09)	4843.50 (1221.63)	5196.53 (591.62)	Time	1.191	0.323
	ME	5935.71 (1315.33)	5252.76 (1084.06)	4685.42 (1043.31)	4512.34 (794.61)	Time*group	2.010	0.059
	BCAA	5139.04 (909.07)	4069.64 (1009.10)	5416.47 (1539.50)	5575.80 (1181.43)			
	CG	4623.56 (699.03)	4593.56 (1310.34)	4655.42 (815.10)	4327.39 (863.95)			
Albumin (g/dL)	ME + BCAA	3.60 (0.39)	3.63 (0.61)	3.82 (0.54)	3.75 (0.63)	Time	3.841	0.013
	ME	3.73 (0.61)	4.12 (0.74)	3.57 (0.43)	4.13 (0.22)	Time*group	1.446	0.185
	BCAA	3.77 (0.39)	3.61 (0.40)	1.56 (2.15)	2.83 (1.60)			
	CG	3.75 (0.72)	3.60 (0.35)	2.59 (1.85)	2.96 (1.69)			
5TSS test (s)	ME + BCAA	21.87 (3.64)	18.71 (3.59)	20.66 (4.98)	17.54 (4.4)	Time	0.165	0.841
	ME	26.69 (12.98)	28.02 (11.28)	26.08 (10.46)	27.56 (12.24)	Time*group	0.436	0.846
	BCAA	36.54 (14.14)	36.24 (13.39)	36.74 (11.89)	35.76 (17.28)			
	CG	24.58 (8.99)	24.76 (9.0)	23.66 (9.30)	25.17 (9.75)			
Physical Frailty (index)	ME + BCAA	2.00 (0.53)	1.50 (0.53)	2.12 (0.99)	2.00 (0.53)	Time	2.702	0.05
	ME	2.71 (1.1)	2.57 (1.13)	2.14 (0.69)	2.00 (0.81)	Time*group	**3.799**	**0.00**
	BCAA	3.00 (0.57)	2.14 (0.37)	2.28 (1.25)	2.71 (0.48)			
	CG	2.16 (0.71)	2.25 (0.75)	2.66 (0.49)	3.16 (0.71)			
MMSE (0–30 points)	ME + BCAA	26.00 (3.11)	26.37 (2.44)	26.00 (2.87)	24.37 (3.58)	Time	**4.262**	**0.13**
	ME	21.00 (3.78)	22.42 (2.99)	21.00 (4.65)	20.00 (3.91)	Time*group	1.214	0.305
	BCAA	20.85 (2.79)	19.42 (4.07)	20.71 (4.02)	19.57 (3.64)			
	CG	21.69 (2.89)	23.92 (3.47)	23.23 (3.83)	21.76 (2.94)			

Notes: M ± SD: Mean (standard and deviation); ME: Multicomponent exercise; BCAA: Branched-chain amino acids; IL: Interleukin; TNF-α: Tumor Necrosis Factor-alpha; MPO: Myeloperoxidase; MMSE: Mini Mental State Exam; 5TSS test: Five-Times-Sit-to-Stand-Test; T1 to T2 (elastic-band exercise, 16 weeks, 8 weeks), T2 to T3 (wash-out), T3 to T4 (multicomponent exercise, 16 weeks). * time versus group interactions. Statistically significant differences are denoted in bold.

4.3. Cognitive Assessment

The results obtained in the cognitive profile (Table 4), show that, at baseline (T1), 65.7% of the participants (n = 23) scored below the 24-point threshold in the MMSE test, indicating that a significant fraction of participants was within the mild/moderate cognitive impairment classification. In addition, at the same time-point (T1), significant differences were found for the cognitive score between the ME + BCAAs group and the other groups ($p < 0.05$). An effect of time (F(df: 3, 93) = 4.262, p = 0.007), but not interaction (time vs. group, p = 0.296), was observed for the MMSE results. The cognitive MMSE scores increased in the control group between T1 and T2 but decreased subsequently in T3 and T4 (p = 0.008). No significant alterations were observed in the other groups. At baseline, 45.7% of the participants were classified as frail and 54.3% as pre-frail.

5. Discussion

This study evaluated the effects of exercise and BCAAs on biomarkers of immunity, total albumin, and the cognitive profile of institutionalized older persons. The main findings were that ME showed more proemint result, particullary with BCAA in the improve cogni-

tive profile and muscle strength-related albumin levels in plasma and diminish the frailty status. Moreover, exercise induced slight changes on the pro-inflammatory marker TNF-α.

Albumin levels tend to decrease with age, and this effect seems to imply an increased risk of complications and higher rate of mortality, morbidity, and disabilities such as sarcopenia and frailty [56]. Despite the key participation of albumin on the pH balance and ionic homeostasis in blood, most of the free fatty acid (and some other lipids) transport in the bloodstream is also performed by serum albumin [57]. Not surprisingly, the age-related impaired albuminemia and elevated serum anion gap are known to be associated with hypertension, low cardiorespiratory fitness, and decreased renal function, which are common morbidities of advanced aged people [58]. Therefore, interventions that aim to sustain (or even increase) albuminemia in older persons could represent an important strategy to mitigate the harmful effects of aging and its comorbidities. In this respect, some studies have already shown that BCAAs apparently increases albumin levels in older persons suffering from malnutrition [59].

Our results showed that the serum albumin levels were efficiently sustained or even augmented, in exercising participants (both ME and ME + BCAAs groups) during the first 16 weeks of intervention (phase 1). However, the withdrawal of BCAAs during the washout period (phase 2) quickly decreased those albumin levels, especially in the BCAAs group. The prominent effect of exercise on albumin levels was evident since its levels in both ME and ME + BCAAs groups were fully restored after the phase 3 period (T3 to T4 time-points), whereas only partial recoveries were observed in albumin levels in the BCAAs group at the same time-point. Low serum albumin levels were shown to be the most relevant biomarkers associated with poor physical strength in the older persons [60].

It is broadly accepted that the regular practice of exercise training imposes metabolic, endocrine/physiological, immune, and cognitive adaptations that, among many benefits, can increase skeletal muscle mass and strength, thus, circumventing the deleterious effects of sarcopenia in older persons [61].

The chronic exercise-mediated adjustments on insulin/glucagon balance, thyroid, and steroid hormones, such as testosterone, cortisol, and estrogens, can also be involved in the enhancement of hepatic and protein muscle metabolism (proteolysis, proteogenesis, and protein turnover), with clear consequences on the circulating amino acid levels (e.g., glutamine and alanine), blood pH and electrolyte balance (hydric/ionic homeostasis), and renal functions [62].

However, it was reported that the putative effect of amino acid/protein supplementation in older women could be masked by sufficient daily protein intake, as we attested in all institutionalized participants in this study [63]. Thus, the proper mechanism behind this effect still needs to be fully understood for this special population. In fact, to our knowledge, this is the first study to show the potential of physical exercise associated or not with BCAAs supplementation to maintain serum albumin levels in older persons living in RCH.

Contrarily to the albumin results, the monitored inflammatory markers (IL-10, TNF-α, and MPO) did not show significant alterations over time. Apparently, we can putatively suggest, that the physical exercise intensities reached in the sessions, as well as the BCAAs supplementation effect compared to the daily protein intake in this population, were not sufficient to induce a significant impact on the inflammatory status in the participants in this study. Other interventions with older persons have been able to show a strong anti-inflammatory effect of exercise training, but it seems that these results were observed for intervention periods longer than 16 weeks [43,44].

Interestingly, even though an increase in the levels of the pro-inflammatory cytokine TNF-α was observed in the ME + BCAAs group from T1 to T2 and T3, this finding was accompanied by a proportional increase of the anti-inflammatory cytokine IL-10, since the TNF-α/IL-10 ratio was not different in this group over time. Moreover, at the end of the intervention, TNF-α levels significantly decreased in this group. In accordance with

the literature, IL-10 is a key anti-inflammatory cytokine that acts by inhibiting systemic inflammation mediated by TNF-α [64].

Concomitantly, BCAAs alone did not induce alterations in both IL-10 and TNF-α levels. These results differ slightly from what is observed in the literature regarding this type of intervention on inflammatory status [65]. Based on the literature, there is a close interaction between the inflammatory status and aging, and in this respect, it is widely accepted that older persons, especially sedentary people, present a chronic, systemic, sterile low-grade inflammation associated with aging, a phenomenon named inflammaging [66]. It is highlighted that inflammaging plays an important role in the loss of lean mass, which leads to sarcopenia and frailty, as well as increases the risk of the development of diseases and comorbidities, such as cognitive decline, atherosclerosis, insulin resistance, etc. [67].

Despite the fact that literature defines the ability to induce an anti-inflammatory change as a hallmark of physical exercise, in general, our results did not corroborate this fact. It is paramount to mention that some factors could putatively influence the lack of significant results in the inflammatory analysis. Firstly, the occurrence of inflammaging and pathophysiological disturbances in our participants could be crucial for the response magnitude observed during the interventions here. Second, the low level of physical activity of our participants before the interventions could mitigate the benefits that would be achieved with the physical exercise sessions and, consequently, limit physiological adaptation. These factors, associated with polypharmacy, a high rate of comorbidities, and the small sample size that finished the study, may determine the lack of significant effects observed.

There is a consensus in the literature that physical exercise sessions stimulate the release of cytokines, such as IL-6, IL-10, and TNF-α, in response to contracting skeletal muscles, which are responsible not only for tissue restoration and energy metabolism, but also for the adjustment of the systemic inflammatory status [68].As appealing as these effects are, physical exercise training also improves human antioxidant defenses as observed in several studies which may also justify the use of exercise interventions to counteract the progression of oxidative-related diseases [69].

There are solid pieces of evidence that the loss of muscle strength and power in the lower limbs, which is characterized by a decline of up to 50% in overall muscle strength from the age of 30 to 80 years [52,53] is associated with an increased incidence of falls.

Particularly, physical exercise training improves body composition, muscle strength, metabolic parameters, bone health, and functionality as well as reduces the risk of mortality, chronic diseases, cognitive deterioration, falls, and depression [70]. Here, we observed that only the ME + BCAAs group presented an improved physical performance in the 5TSS test. Neither ME or BCAAs alone were sufficient to mediate improvements in lower body strength. Only the combination of exercise and supplementation did so. This result was achieved probably due to multiple factors, from physiological to cognitive positive effects that were not directly assessed by the applied methodology here. According to the literature, the 5TSS test is an important performance test that invokes physical skills and abilities that could have been particularly developed during phase 3 of this study. The phase 3 of our study included walking activities, steps, and balance exercises, which mimic the participants' regular daily life activities.

It is important to point out that strength exercise training has been proposed as one of the most effective methodologies, presenting best results in bringing back safety in per-forming the common tasks of daily life, focusing on the optimization of neuromuscular function for better benefits [71].

Multicomponent programs combine aerobic and strength exercises, including other physical skills, such as balance and flexibility [54], in order to optimize the functional capacity of frail older persons [72], as well as to maintain their independence to perform basic activities of daily living [73]. Concerning supplementation, it was reported that branched-chain amino acids, particularly L-leucine, showed significant results in inducing hypertrophy in older persons and improving their functional capacity [58,59].

Taking into account that cognitive impairment is one of the main factors that cause morbidity and high health costs worldwide [74], our results show that physical exercise training, in association or not with BCAAs, was able to maintain the cognitive scores of the participants and could have important practical applications. Considering the population enrolled here (pre-frail and frail octogenarians) and the trend for the natural decline of their cognitive functions, the maintenance of those cognitive scores by exercise is, per se, a remarkable achievement. The literature supports the positive effect of BCAAs in older persons, to improve their mood state [75], the perception of fatigue, and their performance in a mental task [76], which are abilities that were not evaluated here. Leucine is important since it activates the mammalian target of rapamycin complex 1 (mTORC1) and the downstream phosphorylation of p70S6 kinase and 4E (eIF4E)-binding protein 1 (4E-BP1) and related signaling pathways [77]. The aging muscle is less responsive to lower doses of amino acids when compared to the young muscle and may require higher quantities of protein to acutely stimulate equivalent muscle protein synthesis [78]. Nevertheless, the dose and duration of BCAAs proposed here did not affect the cognition scores in our participants.

Study Limitation and Perspectives for Future Researchers

The entire study was conducted with human octogenarians and, given the difficulty to control several influencing factors in this type of population, this study had the additional merit of causing a minimal impact on their daily routines at the residential care homes. In addition, our results here represent real-world data reflecting the reality at residential care homes. We screened participants with disabilities and comorbidities that, although expecting high rate of dropouts and low motivational issues, we could accomplish the proposed goals with a reasonable number of participants. The execution of a controlled study over 40 weeks with such a particular population also introduces other limitations. We suggest that the use of other methods of exercise training, such as the use of playful activities (dance and music sessions) might elevate the adherence of this population to the program.

6. Conclusions

This study showed that multicompetent exercise training, with minor effect of BCAAs, triggered alterations in the inflammatory status and physical profiles of older persons, while helping maintain cognitive levels. Taken together, the achieved results, could help increase autonomy and efficiency in the performance of daily activities. Unlike other studies, our results showed that supplementation with BCAA did not induce substantial changes in health-related parameters at older ages. It is possible that the heterogeneity and limited sample size might have limited the statistical relevance of our results. Despite a slight and transient variation over time observed in some inflammatory and cognitive parameters, it is possible that the results here were influenced by the comorbidity status of each group.

Author Contributions: A.C.-S. drafted the paper; G.E.F. worked on the methodology of the study aspects of RCT; R.N. helped with data acquisition; M.U.C. statically analyzed the data; A.M.T., A.M. and E.M. developed the study proposal, revised the manuscript critically, and suggested additional statistical analyses; A.M.T. coordinated the research study and, together with M.P.d.B. and A.L.L.B., revised the manuscript critically. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. Data supporting the reported results is the property of CIDAF, Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal.

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