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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.,

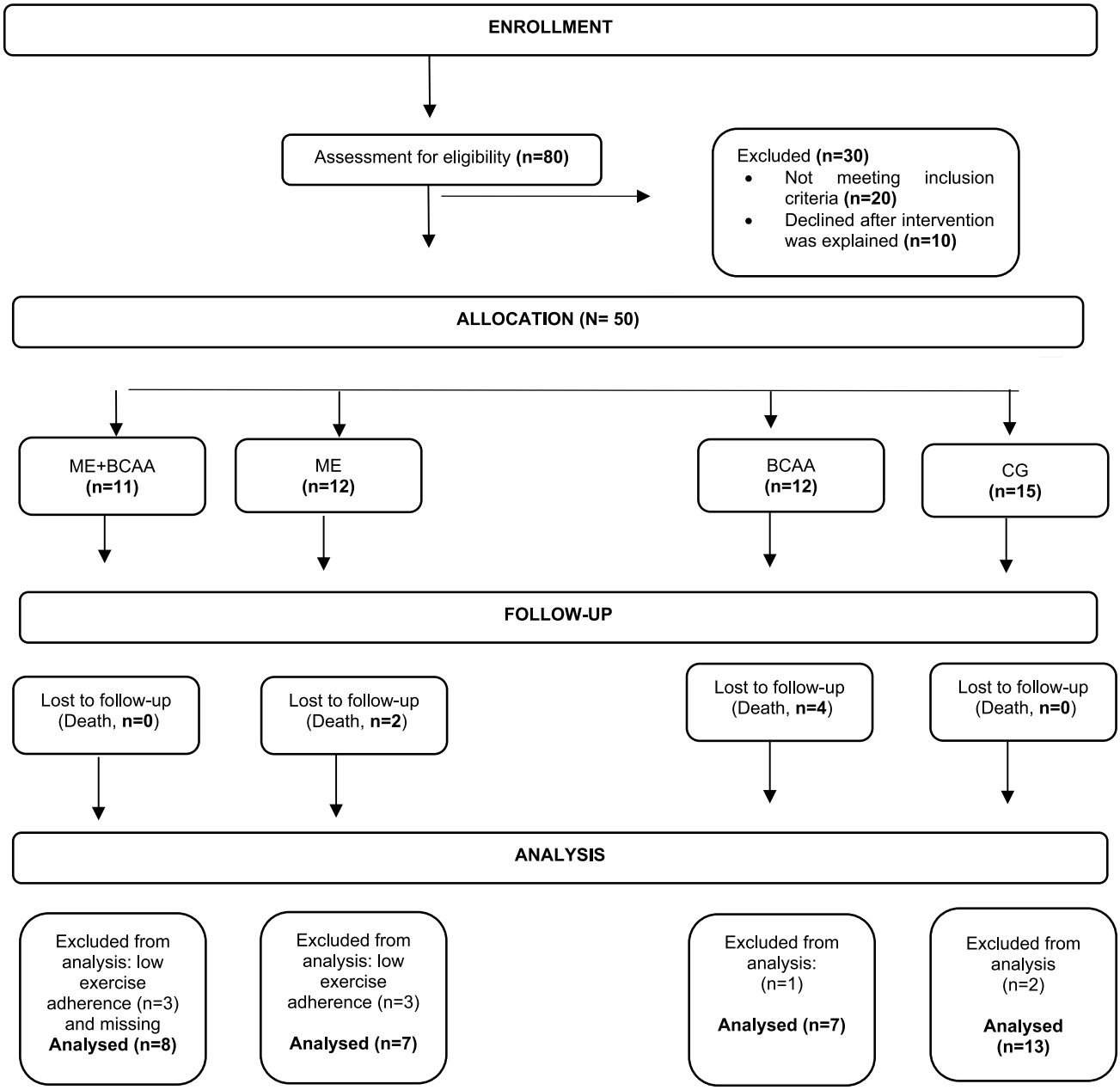

Fig. 1. CONSORT flowchart of study participants (Begg et al., 1996; Caldo-Silva et al., 2021).

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).

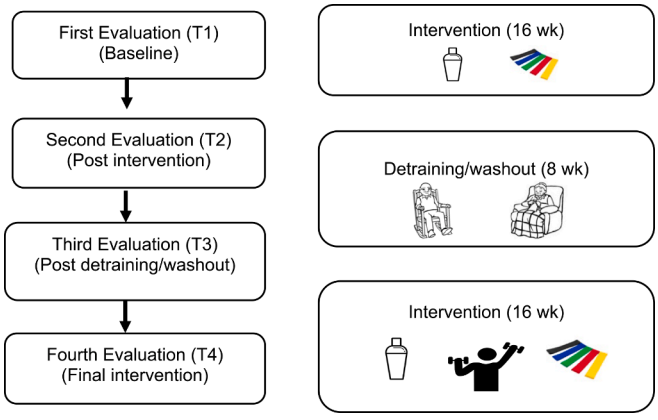

Fig. 2. Chronological order of multifactorial interventions study design. T1 to T2 (elastic-band exercise, 16 weeks), T2 to T3 (washout, 8 weeks), T3 to T4 (multicomponent exercise, 16 weeks); wk = weeks.

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%.

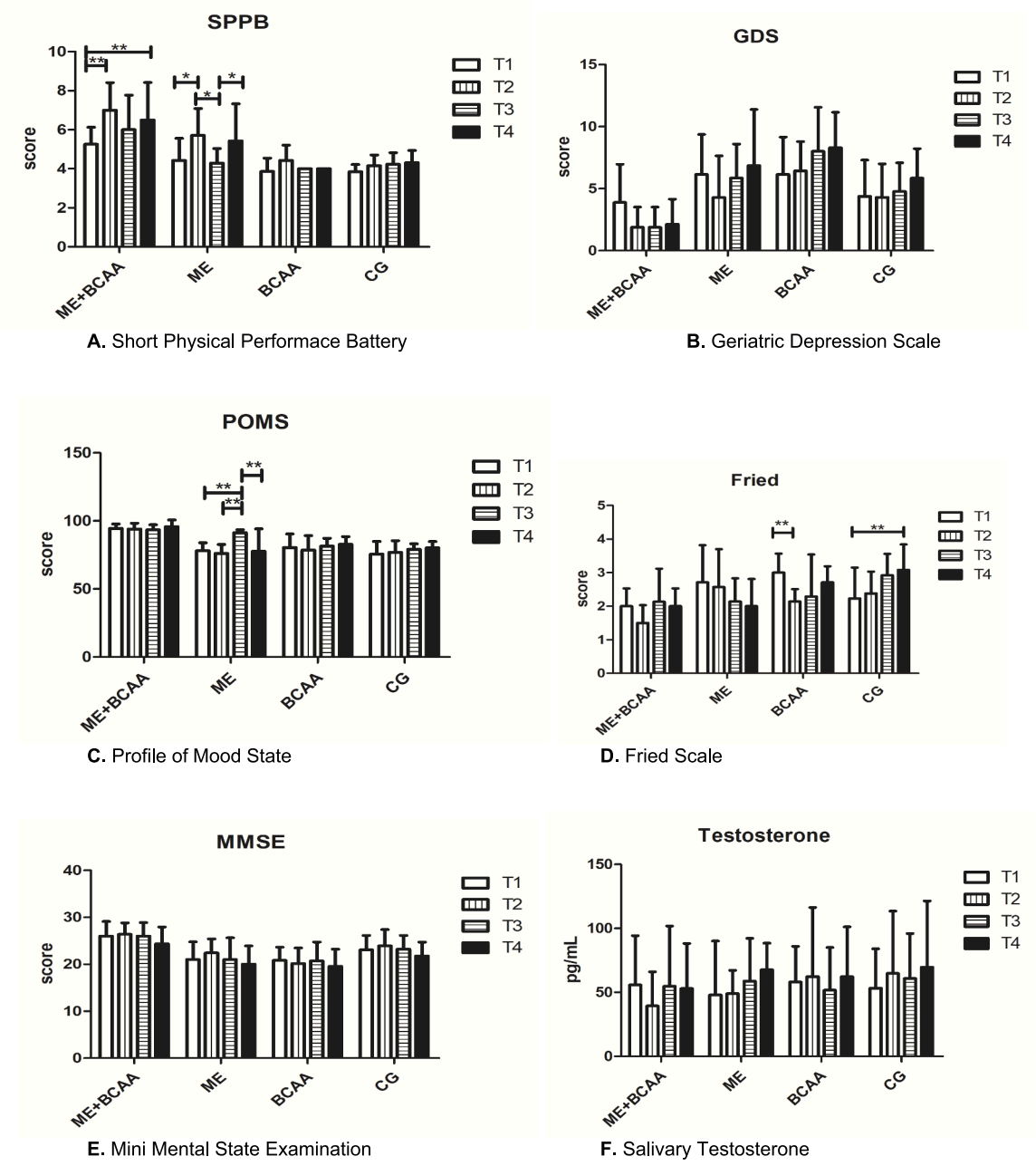

Fig. 3. Time-points assessments of pre- and post-intervention program; ME+BCAA: multicomponent exercise + BCAA supplementation; ME: multicomponent exercise only; BCAA: supplementation only; CG: control group. *Significance at $p < 0.05$; **significance at $p < 0.01$. Significance in the graph is only 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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