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THE EFFECTS OF DIFFERENT EXERCISE-BASED INTERVENTIONS ON FUNCTIONAL FITNESS OF OLDER ADULTS

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ABSTRACT

Ageing is a multifactorial process associated with several irreversible functional and cognitive alterations of human body and determined by genetic and environmental factors. We aimed to investigate the effects of three physical activity interventions of 40 independently living older adults before and after a 3-month training period. Thirty female (69.6 ± 5.3 years) and ten male (70.6 ± 5.4 years) participants were randomly assigned into a physical exercise group (PEG; N = 9), a concurrent physical and cognitive exercise group (PEG + COG; N = 10), a physical exercise with additional 10 g of BCAA daily supplementation group (PEG+BCAA; N = 11), and a control group (CG; N = 10). All three groups performed the same physical exercise program for 12 weeks, three days a week for 45 − 60 minutes per session. Pre- and post-measurements were performed using a standardized functional fitness assessment tool for older adults i.e. Senior Fitness Test battery, upgraded with the Four Square Step Test (FSST) and Grip Strength Test (GST). When intervention groups were pooled, we found moderate to low improvements in the Chair Stand Up, Timed Up and Go, FSST and Six-minute Walk Tests (all P < .001). However, those improvements were intervention-specific with highest improvements found in the PEG for the FSST (P = .004) and Six-minute Walk Test (P = .004); in the PEG + COG for the Timed Up and Go Test (P < .001); and in *the PEG+BCAA for body mass (P = .012) and the Chair Stand Up Test (P < .001). Although the sample size was low, our study provides further evidence of different interventional exercise-based programs that can benefit the population of independently living older adults.*

Keywords: *ageing, senior fitness test, frailty, cognition, diet, interventions.*

UČINEK RAZLIČNIH INTERVENCIJSKIH PROGRAMOV NA FUNKCIONALNO TELESNO PRIPRAVLJENOST ZDRAVIH STAREJŠIH ODRASLIH

IZVLEČEK

Staranje je pogojeno z več dejavniki, ki vodijo v funkcionalne in kognitivne spremembe, v močni soodvisnosti od genetskih in okolijskih dejavnikov. Cilj raziskave je bil preučiti učinkovitost treh 3-mesečnih gibalnih intervencij na 40 funkcionalno neodvisnih starejših preiskovancih, 30 žensk (69,6 ± 5,3 let) in 10 moških (70,6 ± 5,4 let). Preiskovanci so bili naključno razdeljeni v štiri skupine: gibalna intervencija (PEG; N = 9), gibalno-kognitivna intervencija (PEG+COG; N = 10), gibalno-prehranska intervencija z 10 gramov dodatkov BCAA dnevno (PEG+BCAA; N = 11) in kontrolna skupina (CG; N = 10). Vse tri intervencijske skupine so 12 tednov izvajale enako gibalno vadbo, tri vadbe na teden v trajanju posamezne vadbe 45-60 minut. Meritve smo opravili pred in po koncu intervencij z uporabo standardiziranega testa telesne pripravljenosti za starejše (Senior Fitness Test battery) in dodatno še testa štirih kvadratov (Four Square Step Test – FSST) ter testa silovitosti stiska pesti (Grip Strength Test – GST). Ko smo vse tri intervencijske skupine združili, smo ugotovili nizko do srednje napredovanje v testih vstajanja s stola, vstani in pojdi, FSST in hoje na 6 minut (vsi P < 0,001). Kljub temu pa smo ugotovili, da so bili napredki odvisni od specifične intervencije, saj so preiskovanci v skupini PEG najbolj napredovali v FSST (P = 0,004) in hoji na 6 minut (P = 0,004); v skupini PEG+COG v vstani in pojdi (P < 0,001); in v skupini PEG+BCAA v telesni masi (P = 0,012) in vstajanju s stola (P < 0,001). Navkljub nizkemu številu preiskovancev, smo dokazali vpliv različnih gibalnih intervencij, ki lahko pomembno prispevajo populaciji starejših, funkcionalno neodvisnih preiskovancev.

Ključne besede: *staranje, test telesne pripravljenosti za starejše, krhkost, dieta, intervencija*

INTRODUCTION

Ageing is a complex, multidimensional physiological process associated with a significant changes in the structure and function of an organism that occur as a result of time flow (Grimby & Saltin, 1983; Salthouse, 2009; Vandervoort, 2002; Verhaeghen, Steitz, Sliwinski, & Cerella, 2003; Wertz & Dronkers, 1990). These age-related changes negatively affect a broad range of tissues, major organ systems and functions (Harman, 1981; Weinert & Timiras, 2003). More specifically, aging process triggers alterations in body composition (i.e. gradual accumulation of body fat and its redistribution to central and visceral depots), the loss of muscle mass or function (sarcopenia) and muscle weakness (dynapenia) along with other alterations in metabolic, cardiovascular or skeletal (osteopenia) systems (Baumgartner, 2000; Clark & Manini, 2008; Goodpaster et al., 2006; Haramizu, Ota, Hase, & Murase, 2011; Milanovic et al., 2013). With advancing age, these alterations become more pronounced, leading to physical and cognitive functions deterioration, which eventually increase prevalence of disability and mortality risk (Atkinson HH, 2007; Kallman, Plato, & Tobin, 1990; Kokkinos, 2012; Onder et al., 2002; Power, Dalton, & Rice, 2013; Salthouse, 2009; Topinková, 2008). It is also known that functional (and cognitive) loss can be preventable and even reversible through timely detection of physical weakness and appropriate intervention (Fried, Ferrucci, Darer, Williamson, & Anderson, 2004).

Physical inactivity, a main risk factor for overall mortality, leads to severe acute deterioration of muscle mass and function that could be also irreversible if not recognized and contrasted (Pisot et al., 2016). As both lifespan and aged population are increasing (Klenk, Rapp, Büchele, Keil, & Weiland, 2007), the emerging problem should represent identification of optimal and timely pharmacological, surgical, dietary, exercise and cognitive interventions that can mitigate ageing-related changes on a number of levels (Rowe & Kahn, 1997).

The effects of medical interventions are often centered and specific (i.e. affecting only the targeted health-related issues) and often cause serious side effects (Meador, 1994; Turjanski & Lloyd, 2005). Also, along with the high costs (i.e. lifelong drug usage) they might not represent the most desirable and effective strategies. In contrast, various exercise / physical activity interventions, when individually and professionally programed, have been proven to produce beneficial effects on physical and cognitive functions as well (Hallage et al., 2010; Hanson et al., 2009; Karavirta et al., 2011); even for population at-risk and / or patients (Flansbjer, Miller, Downham, & Lexell, 2008; Heyn, Abreu, & Ottenbacher, 2004; Morris, Dodd, & Morris, 2004). Accordingly, it is well established that regular physical activity is an efficient strategy for successful ageing (Kokkinos, 2012; Nelson et al., 2007; Taylor et al., 2004). Physical activity (PA) engagement increases life expectancy and improves the quality of life (Blair et al., 1989; Nelson et al., 2007; Paffenbarger, Hyde, Wing, & Hsieh, 1986). Further, it has been shown that PA reduces all-cause mortality (Blair et al., 1989; Kampert, Blair, Barlow, & Kohl, 1996; Nelson et al., 2007; Paffenbarger et al., 1986) by 22% even if practiced with a low dose (< 150 min/week) of moderate-to-vigorous intensity (Hupin et al., 2015). However, additional benefits might be expected when the amount of PA progressively increases regarding both the intensity and the volume of exercise (Chodzko-Zajko et al., 2009). Moreover, PA effects are positively correlated with a higher level of individual fitness (Kampert et al., 1996), where the percentage of functional fitness decline (e.g. lower and upper body muscle strength, lower and upper body flexibility, aerobic endurance, and motor agility / dynamic balance) is generally consistent with age-related declines in physical performance (Rikli & Jones, 1999).

Therefore, interventions including both endurance and strength activities (Nelson et al., 2007) alone or along with dietary manipulation and / or cognitive interventions might be plausible strategies that might counteract the aforementioned negative changes and risks, making older adults' life more comfortable (Ball, Berch, & Helmers, 2002; Buchman et al., 2012; Harman, 1981; Marusic et al., 2016; Pišot et al., 2015). Accordingly, there is ample evidence suggesting that the combination of proper dieting (Fiatarone et al., 1994; Kim et al., 2012; Messier et al., 2004) and PA could show greater benefits on functional fitness than either intervention alone.

The current Recommended Dietary Allowance for the minimum protein intake for adults, including older adults population, is 0.8 g protein / kg BM / day (WHO, 2007). However, recent research results suggest that the recommended protein intake does not promote optimal health or protect older adults from age-related body changes (Morley et al., 2010; Paddon-Jones & van Loon, 2012; Volpi et al., 2003). Based on new evidence, the PROT-AGE Study Group recommends an average daily intake in the range of 1.0 to 1.2 g / kg BM / d for healthy older adults, 1.2 to 1.5 g / kg BM / d for those who have acute or chronic diseases and 2.0 g / kg BM / d for people with severe illness or injury or with recognizable malnutrition (Bauer et al., 2013).

The consumption of higher protein diet, at least 1.2 to 1.6 g / kg BM / day of high-quality protein with concentrated source of essential amino acids, including branched amino acids (BCAA) leucine, could prevent age-related sarcopenia, the loss of muscle mass and strength. Including ~30 g of protein per meal seems a successful strategy to achieve optimal health outcomes in adults (Phillips, Chevalier & Leidy, 2016).

Exercise greatly increases energy expenditure and promotes oxidation of BCAAs (Rennie, 1996). BCAAs are regulators of protein metabolism and are key metabolic precursors for glutamine and alanine synthesis (Choudry, Karinch & Souba, 2006). These properties have suggested that BCAAs may have interesting and clinically-relevant metabolic effects. The effects of BCAA supplementation before and after exercise has beneficial effects for decreasing exercise-induced muscle damage and are responsible for the direct stimulation of muscle protein synthesis and the suppression of exercise-induced protein breakdown (Coombes & McNaughton 2000; Fujita & Volpi, 2006; MacLean, Graham & Saltin, 1994; Nosaka, 2003). In addition, amino acids, particularly BCAAs, may be used clinically to attenuate diet-induced muscle atrophy (Layman, 2003) and prevent sarcopenia in older adults (Koopman et al., 2006; Volpi et al., 2003; Volpi et al., 2007).

Various cognitive training approaches revealed considerable beneficial effects in improving specifically targeted cognitive abilities (i.e. memory, reasoning and processing speed) (Ball et al., 2002; Edwards et al., 2005; Saczynski, Willis, & Schaie, 2002), with some evidence of positive transfer to non-specifically trained cognitive functions and activities of daily living (Ball et al., 2002; Marušič et al., 2016; Willis, Tennstedt, Marsiske, & et al., 2006). For example, the study by Edwards et al. (2005) provides evidence that speed processing training has the potential to enhance everyday functions that help to maintain independence and quality of life, particularly when the training is targeted toward individuals who most need it. In a five-year study, Willis et al. (2006) showed that cognitive training resulted in lower functional decline in self-reported instrumental activities of daily living, which was evident up to 5 years after the initiation of the intervention. Furthermore, a multidimensional intervention which combined diet, exercise, cognitive training and vascular risk monitoring, showed 25 % better results in cognitive functions as compared to the controls (Ngandu et al., 2015). Regarding the aforementioned positive influence of cognitive and PA interventions and their training specificity, their combined effects might have greater effects on instrumental activities of daily living or even functional fitness. Moreover, Theill et al. (2013) investigated the effects of a simultaneously performed motor-cognitive training compared to a single cognitive training and to controls. They concluded that the combined motor-cognitive training presents a promising concept to improve cognitive and motor-cognitive dual-task performance, offering greater potential on daily functioning, which usually involves the recruitment of multiple abilities and resources rather than a single one.

Thus, the effects of physical training alone or in combination with dietary interventions, as well as cognitive training, on physical and cognitive functions are well documented, while there are almost no experimental studies that directly compare the effects of these interventions or its combination between each other and / or controls. Furthermore, the aforementioned studies mainly include community-dwelling older adults where outcome measures were specific cognitive abilities and daily activities assessed by participants' self-reports and / or with only a few physical performance tests. Thus, the influences of combined interventions in healthy, independent older adults in respect to functional fitness are unknown. In addition, according to our knowledge, this type of interventional study is one of the first performed among Slovenian older adults.

Therefore, the aim of this study was to compare the effects of three different 3-month interventions on functional fitness of healthy older adults chosen among the population of independently living Slovenians. We hypothesized that all three intervention groups (physical exercise only; combined physical and cognitive exercise; combined physical exercise and diet supplement) will have significant effects on physical fitness in comparison to the control group (CG).

METHODS

Participants

After the initial screening of 195 older adults, we recruited 40 individuals of which 30 were females (69.2 ± 5.3 years) and ten males (70.6 ± 5.4 years). The participants were randomly assigned to one of three intervention groups: i) a physical exercise group (PEG), ii) a concurrent physical and cognitive exercise group (PEG+COG), iii) a physical exercise with additional branched-chain amino acids (BCAA) daily supplementation group (PEG+BCAA) or iv) in a control group (CG). The final sample of the study included nine participants in PEG (age: 68.7 ± 5.3 years, height: 165.5 ± 6.9 cm, body mass: 71.5 ± 13.7 kg), ten in PEG+COG (age: 70.6 ± 5.4 years, height: 162.3 ± 6.7 cm, body mass: 68.8 ± 15.1 kg), eleven in PEG+BCAA (age: 69.9 ± 6.8 years, height: 166.2 ± 8.1 cm, body mass: 73.2 ± 9.1 kg) and ten in CG (age: 68.9 ± 3.4 years, height: 161.8 ± 5.1 cm, body mass: 64.4 ± 8.4 kg). The participants were healthy volunteers without serious cardiovascular or musculoskeletal diseases. The inclusion criteria were: i) at least 65 years old, ii) independently living at home (i.e. performing everyday activities without mobility aids), iii) residents of the city of Ljubljana (where the measurements were performed) and iv) feeling healthy and able to walk 2 km without stopping and using walking aids. All the participants provided a written informed consent to participate in the study according to Helsinki – Tokyo Declaration. The study obtained ethical approval from the National Medical Ethics Committee of the Republic of Slovenia.

Interventions

Each intervention lasted for 12 weeks, 3 times per week (altogether 34 sessions), with individual sessions' duration of 45 to 60 minutes. Each training session was performed at the same time of the day (starting at 10 a. m.). Thus, the training sessions consisted of three parts explained in details in Table 1 (on the left side). The volume and intensity in the second (main) part of the session was controlled by the ratio of the working and resting training time. At the beginning of the intervention (weeks 1 to 3) the working time for each exercise was 20 seconds with 40 seconds rest (work / rest ratio was 1:2); from week 4 to week 8 the ratio was 1:1 (30 s : 30 s) and at the end of the intervention (from week 9 to week 12) the ratio was 2:1 favouring the working time.

PEG and PEG+BCAA intervention consisted of the same physical activity program. The participants in the PEG+BCAA group were receiving orally 10 g of BCAA supplement immediately after each training session, three times per week and at the same time on the days without training.

Table 1: Approximate duration and examples of physical and cognitive exercises and dietary supplements.

PEG	PEG + COG	PEG + BCAA
Part I (10 − 15min):		
Dynamic warm up: a combination of stepping on site or walking forward / sideways with (additional) active flexibility stretching and rhythmic exercises were used (e.g. marching with rising and lowering the arms, high knee walking, side walking with crossover steps on clapping etc.).	Physical exercises: the same as in PEG; cognitive exercises performed concurrently with physical exercises: e.g.: list of words to remember "what to bring from the supermarket": eggs, milk, cheese, butter, cream, tomatoes, bread or "which ingredients are needed for the Savory Garlic Marinated Steaks": balsamic vinegar, soy sauce, garlic, honey, olive oil, black pepper etc.	Physical exercises: the same as in PEG;
Part II (30 − 40min):		
Eight strength and power exercises (main part) based on circuit training principle for trunk, upper- and lower--body muscles (e.g. squats, lunges, calf rises, push-ups and planks on a chair, crunches and side crunches).	Physical exercises: the same as in PEG; cognitive exercises performed concurrently with physical exercises: e.g.: naming cities, starting with the last letter of the previous city: LjubljanA, AncarA, AthenS, SydneY etc.; counting backwards from 100: in a step of 1 (99, 98, 97 etc.); in a step of 17 (83, 66, 49 etc.).	Physical exercises: the same as in PEG.
Part III (10-15min):		
Flexibility and relaxation exercise, with focus on breathing technique in order to restore body functions on its initial level was used.	Physical exercises: the same as in PEG; Cognitive exercises performed concurrently with physical exercises: List all the words from the Part I.	Physical exercises: the same as in PEG.
Post exercise		
/	/	10 g BCAA

Comparing with PEG and PEG+BCAA intervention program, PEG+COG intervention consisted of similar physical exercises, while cognitive tasks were added. During the warm up exercises, each participant in PEG+COG received a list of words that he / she needed to remember and report at the end of each session, see Table 1. (mid-side). The lists of words were different for each participant and were changing each session. The main part consisted of the same physical exercises as for the PEG with additional cognitive tasks targeting on working and short-term memory, attention, mental rotation and visual-spatial perception. Examples of cognitive tasks were naming animals on a certain letter, counting backwards, listing all flavours that remind you of summer etc. while performing physical exercises. Finally, during the flexibility and relaxation exercises, the participants in PEG+COG were asked to disclose previously delivered list of words.

Participants in the CG had only pre- and post-measurement screening with no specific interventions in-between and were asked to maintain their usual daily activities.

Measurements

All groups were tested before (pre) and after (post) the interventions. On the testing day, the participants completed all Senior Fitness Test items (Rikli & Jones, 1999; 2001) in order to determine subjects' lower and upper body strength, flexibility, agility, aerobic endurance and dynamic standing balance. The Senior Fitness Test consists of six assessment items (i.e. the Chair Stand Test, Arm Curl Test, Chair Sit and Reach Test, Back Scratch Test, Timed Up and Go Test and Six-Minute Walk Test). The Chair Stand Test assesses lower body strength. Each subject completed two practice repetitions and one 30-second test trial. The recorded score was the total number of stands executed correctly within 30 seconds. The Arm Curl Test assesses upper body strength. Each subject completed two practice repetitions and one 30-second test trial sitting on a chair. Women used 2.5 kg, while men 3.5 kg barbells. The score was the total number of arm flexions and extensions through the full range of motion in 30-seconds. The Chair Sit and Reach Test assesses lower body flexibility. Each subject completed two practice trials and two test trials. The score was the longest distance achieved between the extended fingers and the tip of the toe. The Back Scratch Test assesses upper body flexibility. Each subject completed two practice trials and two test trials. The score was the shortest distance achieved between the extended middle fingers. The Timed Up and Go Test assesses agility and dynamic balance. Each subject completed one practice trial and two test trials. The score was the shortest time to rise from a seated position, walk 8 feet, turn around the cone, and return to the seated position. The Six-Minute Walk Test assesses aerobic endurance. The score was the total distance walked in six minutes around the two cones 15 m apart.

Additional to the Senior Fitness Test battery, participants also performed the Four Square Step Test / FSST (Dite & Temple, 2002) and the Grip Strength Test. The FSST involves stepping over 4 canes that are laid on the ground at 90° angles to each other (like a "plus" sign). The canes were 90 cm in length as first described by Dite and Temple (2002). Subjects were asked to stand in 1 square facing forward with their shoes on. They then rotated clockwise around the "plus sign" by moving forward, to the right, backward, to the left. The patients then reversed their path and moved in a counterclockwise direction. The instructions were as follows: "Try to complete the sequence as fast as possible without touching the sticks. Both feet must make contact with the floor in each square. If possible, face forward during the entire sequence." (Dite & Temple, 2002). Each subject had 1 practice trial and 2 timed trials; with all subjects completing the testing within 5 minutes. If a patient touched the cane, lost his / her balance, or did not place both feet in the square, he / she was asked to repeat the trial. The best score achieved was used for further analysis. Maximal grip strength was measured bilaterally with a portable Jamar Hydraulic Hand Dynamometer (Sammons Preston, Rolyan, Bolingbrook, IL, USA). In accordance with American Society of Hand Therapy recommendations, subjects were seated with their shoulders in 0° abduction and neutral rotation, their elbows in 90° of flexion, and their forearms in neutral pronation / supination. The average of three and two maximal repetitions was used for further analysis.

Habitual dietary intake was assessed by three-day food record and the participants were asked to continue habitual diet during the study adding 10 g of BCAA each day at 11 AM.

Statistical Analysis

All data are presented as means ± standard deviations. Data were analysed using SPSS software (version 20.0). After confirming normality and homogeneity of distribution using Shapiro-Wilk and Leven test, a 1-way analysis of variance (ANOVA) was used to compare baseline values between the groups. A two-way, repeated measures ANOVA was performed to determine changes within groups over time (pre to post) and between groups. Firstly, we compared the pooled interventions group (PEG) vs. CG (2x2 repeated measure ANOVA), and also each intervention group separately vs. CG (2x4 repeated measures ANOVA). Individual group changes from pre- to post period were assessed using the Paired-sample Student's *t*-test (two-tailed). The level of significance was set at 0.05. When significant changes were confirmed, the effect size was calculated as the mean change found in a variable divided by the standard deviation at baseline of that variable; an effect size of 0.10 – 0.19 was considered very small, 0.20 – 0.49 small, 0.50 – 0.79 moderate, 0.80 – 1.19 large, 1.20 – 1.99 very large and 2.00 or greater was considered a huge effect (Sawilowsky, 2009).

RESULTS

Initially, 67 participants were randomly divided in 4 groups; however, 40 of them passed > 90 % of sessions adherence with both pre- and post-testing. A vast majority of drop-out was a consequence of holidays, trips and large daily travel distances to admit sessions. There was no injury occurrence during this study. The average adherence to interventions was more than 80 %.

The participants followed their habitual diet and the estimated dietary intake was not different between the beginning and the end of the study. The mean daily protein intake was 0.92 ± 0.3 g / kg BM / day.

There were no significant differences in pre-tests between PEG and CG, neither in all four groups nor individually. The results from the 2 x 2 repeated measures ANOVA indicated a significant main effect of time for chair stand ($F[1,38] = 17.4$; $p < .001$; $\eta^2 = .31$), the timed up and go ($F[1,38] = 8.49$; $p = .006$; $\eta^2 = .183$), and the six-minute walk ($F[1,38] = 11.46$; $p = .002$; $\eta^2 = 0.232$) tests, while the arm curl ($F[1,38] = 4.07$; $p = .051$; $\eta^2 = .090$) and the four square step test ($F[1,38] = 3.55$; $p = .067$; $\eta^2 = .085$) were near-significant. There were significant time x group interaction effects for the timed up and go ($F[1,38] = 6.93$; $p = .012$; $\eta^2 = .154$) and the four square step test ($F[1,38] = 5.76$; $p = .021$; $\eta^2 = .132$). Furthermore, in Table 2, paired sample *t*-tests indicated that participants in PEG performed better at the post-test compared to the pre-test in lower body strength, agility, dynamic standing balance, and aerobic endurance, while the CG did not improve in any test performed.

Results of the 4x2 repeated measures ANOVA confirmed main time effect for body mass ($F[3,38] = 5.06$; $p = .031$; $\eta^2 = .123$), the chair stand ($F[3,38] = 34.34$; $p < .001$; $\eta^2 = .488$), the arm curl ($F[3,38] = 7.64$; $p = .009$; $\eta^2 = .177$), the timed up and go ($F[3,38] = 23.24$; $p < .001$, $\eta^2 = .392$), the four square step ($F[3,38] = 12.0$; $p < .001$; $\eta^2 = .251$) and the 6-minute walk ($F[3,38] = 20.1$; $p < 0.001$; $\eta^2 = .359$), while the back stretch ($F[3,38] = 3.30$; $p = .078$; $\eta^2 = .084$) and the grip strength ($F[3,38] = 3.36$; $p = .075$; $\eta^2 = .085$) were near-significant. There were significant time x group interaction effects for the timed up and go ($F [3,38] = 3.36$;, $p = .029$; $\eta^2 = .219$), while in the four square test ($F[3,38] = 2.31$; $p = .093$; $\eta^2 = .161$) was almost significant.

The paired sample *t*-tests indicated that the participants in PEG improved in back stretch for 100 % ($p = .005$), the timed up and go for 8.3 % ($p = .022$), the four square step for 13 % ($p = .004$), and the 6-minute walk for 9.6 % ($p = .004$), while for the chair stand up the improvement was near-significant ($p = .052$). The PEG+COG improved in chair stand up for 26.1 % ($p = .017$), the timed up and go for 15.6 % ($p < .001$) and the four square step for 8.8 % ($p = .014$). Moreover, PEG+BCAA improved significantly in almost all functional tests performed; the chair stand up for 35.3 % ($p < .001$), the arm curl for 16.6 % ($p = .023$), timed up and go for 15.8 % ($p = .028$), the four square step for 16.6 % ($p = .043$), the 6-minute walk for 10.9 % ($p = .004$), and had lower body mass for 1.8 % ($p = .012$) at post-test (Table 3).

Table 2: Pre- to post-comparison between the pooled interventions groups (PEG) and control group (CG).

	PEG (n=30)			CG (n=10)		
Tests	Pre	Post	P (*d*)	Pre	Post	P (*d*)
Body Mass (kg)	71.1±12.5	69.8±12.1	NA (-)	64.4±8.41	64.1±12.5	NA (-)
Body Mass Index (kg/m^2)	26.17 ± 3.83	25.65 ± 3.63	NA (-)	24.64 ± 3.45	23.95 ± 4.07	NA (-)
Chair Stand Up (reps)	17.2±4.52	21.7±4.94	<0.001 (**.99**)	18.1±3.44	19.6±4.78	.110 (-)
Arm Curl (reps)	19.1±3.91	20.9±4.19	NA (-)	18.9±3.84	19.7±3.19	NA (-)
Sit and Reach (cm)	3.11±10.8	1.16±10.4	NA (-)	7.31±15.1	6.31±11.9	NA (-)
Back Stretch (cm)	-4.31±9.64	-2.91±10.7	NA (-)	.511±7.71	1.71±8.21	NA (-)
Timed Up and Go (s)	5.02±1.22	4.33±0.70	<.001 (**.57**)	4.65±0.53	4.62±0.46	.790 (-)
Grip Strength (kg)	31.8±8.84	30.9±8.87	NA (-)	30.1±4.94	29.1±4.79	NA (-)
4-square step (s)	6.66±1.93	5.81±1.34	<.001 (**.45**)	6.41±1.54	6.51±1.38	.778 (-)
6-min walk (m)	537±67.8	578±66.1	<.001 (**.61**)	595±81.8	621±75.3	.286 (-)

NA – Not Applicable as time or time x group interaction effects were not confirmed.
P – Significance level of Paired-sample t-test.
d – Effect size.

Table 3: Pre- to post-comparison between physical group (PEG), physical-cognitive group (PEG+COG), physical-dietary group (PEG+BCAA), and control group (CG).

		PEG (n=9)		PEG+COG (n=10)		PEG+BCAA (n=11)		CG (n=11)	
			P (*d*)		P (*d*)		P (*d*)		P (*d*)
Body mass (kg)	pre	71.6 ±13.7		68.8 ±15.1		73.2 ±9.17		64.4 ±8.41	
	post	69.6 ±13.5	.068 (.14)	67.7 ±13.8	.109 (-)	71.9 ±9.79	.012 (.14)	64.0 ±12.5	.831 (-)
Body Mass Index	pre	25.98 ±3.65		26.01 ±5.14		26.48 ±2.79		24.64 ±3.46	
	post	25.24 ±3.41	NA	25.61 ±4.63	NA	26.02 ±3.06	NA	23.95 ±4.07	NA
Chair stand up (reps)	pre	20.0 ±4.12		17.2 ±2.48		14.9 ±5.24		18.1 ±3.44	
	post	23.6 ±5.38	.052 (.86)	21.7 ±4.49	.017 (1.81)	20.2 ±4.89	<.001 (1.01)	19.6 ±4.78	.110 (.21)
Arm curl (reps)	pre	20.7 ±3.35		19.2 ±3.52		17.4 ±4.36		18.9 ±3.84	
	post	22.9 ±4.85	.126 (.66)	19.7 ±2.83	.740 (.14)	20.4 ±4.43	.023 (.67)	19.7 ±3.19	.235 (-)
Sit and reach (cm)	pre	9.11 ±13.12		1.60 ±9.43		-0.45 ±8.75		7.30 ±15.1	
	post	6.44 ±8.80	NA	0.90 ±12.0	NA	-2.90 ±9.13	NA	6.30 ±11.9	NA
Back stretch (cm)	pre	-3.11 ±9.14		-4.00 ±10.5		-5.55 ±9.99		0.50 ±7.70	
	post	.00 ±8.06	NA	-4.40 ±13.8	NA	-3.90 ±10.0	NA	1.70 ±8.20	NA
Timed up and go (s)	pre	4.42 ±0.24		5.17 ±0.64		5.38 ±1.85		4.65 ±0.52	
	post	4.05 ±0.40	.022 (1.48)	4.36 ±0.41	<.001 (1.26)	4.53 ±1.00	.028 (.46)	4.62 ±0.46	.790 (-)
Grip strength (kg)	pre	34.6 ±8.58		32.7 ±10.2		28.7 ±7.53		30.0 ±4.94	
	post	33.88 ±8.78	NA	32.1 ±8.22	NA	27.5 ±9.15	NA	29.1 ±4.79	NA
4 square step (s)	pre	6.23 ±1.13		6.80 ± 1.01		6.90 ±2.93		6.41 ±1.54	
	post	5.41 ±1.08	.004 (.72)	6.20 ±0.78	.014 (.59)	5.75 ±1.86	.043 (.39)	6.51 ±1.38	.778 (-)
6 min walk (m)	pre	557 ±55.3		542 ±53.6		517 ±86.6		596 ±81.8	
	post	611 ±57.0	.004 (.97)	555 ±58.8	.343 (.25)	574 ±73.6	.004 (.66)	621 ±75.4	.286 (.31)

NA – Not Applicable as time or time x group interaction effects were not confirmed.
P – Significance level of Paired-sample t-test.
d – Effect size.

DISSCUSSION

Individual intervention groups have small sample sizes, therefore, we compared cumulative effects of all three interventions, by pairing all intervention groups together and contrasting vs. the CG. Thus, the results showed significant time effects for Chair Stand Up, the Timed Up and Go, and the Six-Minute Walk tests in interventions group, while the CG did not improve in any test. Furthermore, certain meaningful interactions were seen in respect to the timed up and go as well for the four square test, suggesting that the applied physical training program influenced mostly agility and dynamic standing balance, which was expected in relation to physical intervention program design. The effects were large (the chair stand up), moderate (the timed up and go and the 6 min walk) and small (the 4 square step). When all groups were compared between each other individually, there was significant improvement observed in physical fitness tests after three months of interventions compared to the baseline in all intervention groups, except in CG, where body mass decreased only in PEG+BCAA.

Our findings are consistent with findings from the literature (Chang et al., 2004). Although we cannot compare our interventions directly regarding the training design, their results are similar with ours, which suggests that older people could benefit from various supervised physical exercise programs. However, the intensity and the volume of exercise is important as demonstrated by Cyarto et al. (2008) when comparing the effects of home-based resistance training, group-based resistance training, and group-based walking on functional ability in older adults. After 20 weeks of training with only two weekly sessions, they showed that both groups of resistance training experienced improvements in strength, lower-body flexibility, and agility / dynamic balance while there were no observed improvements in the walking group. Therefore, frequency of two sessions per week used in the aforementioned study was far below those generally suggested (i.e. 30 minutes in duration and up to five weekly sessions) in order to promote and maintain health (Nelson, et al., 2007, Haskell, et al., 2007).

Taken together, the results of some systematic reviews and meta-analysis (Cermak, de Groot, Saris, & van Loon, 2012; Cruz-Jentoft et al., 2014; Finger et al., 2015; Nowson & O'Connell, 2015) which assessed the effect of combined intervention with protein supplementation and resistance exercise on muscle mass and the function in aged population, show the inconsistency of studies to prove the effectiveness of protein supplementation. Overall, the studies show the capacity of such intervention for electing gains in fat-free mass, a limited evidence for improvement in muscle strength, but no significant improvement to increase muscle function in older adults. The variation among studies regarding the supplementation protocols, protein sources, and amounts used are making it difficult to reach firm conclusions on this question. It was proposed that older adults need higher levels of protein intake due to their lower responsiveness to the anabolic stimulus (Baum, Kim, & Wolfe, 2016). In fact, larger experiments that have demonstrated a significant functional benefit from combined intervention with resistance exercise and protein intake exceeded the range from inadequate intakes to optimal protein intakes (≥1.2 g/kg BM/day) and lasting for few months (Chalé et al., 2012; Tieland et al., 2012).

Although protein supplementation failed to point out consistent results, new evidence shows that branched chain amino acids (BCAA), such as leucine, at a daily amount of either 2.5 g or 2.8 g in combination with resistance exercise may affect muscle protein synthesis, muscle recovery following illness, and muscle mass (Bauer et al., 2013). Katsanos et al. (2006) demonstrate that the attenuated response of muscle protein synthesis in older adults, following the ingestion of small amounts of amino acids, can be reversed by the ingestion of additional amount of leucine. These data may explain the role of BCAA, especially leucine in reversing the lack of response following the protein-based supplementation.

In our study, the PEG+BCAA group was supplemented with 10g BCAA immediately after the exercise. However, even not significantly different, this intervention group experienced greater improvements in leg strength compered to others. For example, leg strength improved most (35.3 %) in the PEG+BCAA group, while the PEG and PEG+COG groups improved less i.e. 26.1 % and 17.7 %, respectively, which is consistent with previous findings (Verdijk et al., 2009). In the study by Verdijk et al. (2009), 26 healthy older man, aged between 70 and 74 years were randomly assigned to a progressive, 12-week resistance-type exercise training program with (protein group) or without (placebo group) protein provided before and immediately after each exercise session (3 sessions per week, 20 g protein per session). Although they trained with the resistance intensity of 65 % to 75 %, both training groups showed improvements of 25 % to 30 %, similar to our results.

It is difficult to compare our results to other studies due to different exercise protocols and supplementation used. A few studies that show improvement in functional outcome in older adults included the participants who generally have an inadequate protein intake of 0.8 g / kg BM / day (Kim et al., 2012; Tieland et al., 2012). Similarly, our participants had lower dietary protein intake (0.92 g / kg BM / day) than recommended (WHO, 2007) and, therefore, may benefit from the combined intervention.

Regarding aerobic endurance, only the PEG and PEG+BCAA improved for 9.6 % and 10.9 %, respectively. Bearing in mind that the PEG+COG had a more demanding cognitive aspect of exercise rather than physical, our results suggest that more than 30 minutes of moderate intensity PA is needed to induce positive alterations in aerobic endurance in older population; and / or two exercise models should be performed separately (i.e. not combined in the same task). Furthermore, future studies should include measurements of various cognitive-motor tasks. In 375 elderly community residents, Oswald and colleagues (2006) found significant training-related effects after five years of combined interventions. In detail, they showed that when compared with a non-treatment group, both physical and cognitive status can be preserved on a higher level, as well as emotional status of involved participants (as reflected through fewer depressive symptoms). Our PEG+COG group was included in the so-called broad approach that targets multiple domains of cognitive processes, which might be more effective than a specific one that includes only a sole or limited-set targeted cognitive functions (Holtzer et al., 2006). Recent studies revealed that cognitive-based protocols can positively influence mobility-related outcomes in different population of older adults, namely, community-dwelling older adults (Smith-Ray et al., 2014), patients with Parkinson's disease (Milman et al., 2014) and older adults during a prolonged bed rest (Marusic et al., 2015; Marusic et al., in press). Therefore, we can further speculate that lack of improvements in the PEG+COG group could also originate from inadequate measurement tests which were not sensitive enough to detect cognitive-motor related improvements.

CONCLUSION

Our research findings indicate that physical activity, alone or combined with cognitive and nutritional interventions could enhance functional fitness in older adults. More than 30 minutes of moderate-intensity physical activity, three times per week is needed for an improvement in aerobic endurance, while same volume of specific training program is enough to induce positive alterations in dynamic balance and agility. Thus, when nutritional supplementation is added to physical exercise, results regarding functional strength task and body mass were more pronounced.

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