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The mediating effect of different exercise programs on the immune profile of frail older women with cognitive impairment

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Abstract

Background: Frail individuals experience an accelerated immunosenescence, and exercise has been identified as a therapy to promote a better inflammatory environment. **Objective:** To analyze the effects of 28-weeks of different exercise protocols on immune profiles of institutionalized (pre)frail women with cognitive impairment (CI). **Methods:** Participants residing in care homes ($n=60$, 81 ± 7.84 years) and were randomized into three groups: chair elastic band muscle-strength (CSE, $n=21$; 81 ± 4.79), chair multimodal (CME, $n=20$; 80 ± 8.19) and a control non-exercise (CGne, $n=19$; 80 ± 10.01). Both CME and CSE performed a progressive circuit-training exercises. The controls did not change their usual lifestyle. The Fried's phenotype and Mini Mental State Examination was used to identify frail subgroups with cognitive impairment. Data for anti and pro-inflammatory markers and physical fitness were analyzed pre and post-interventions. **Results:** After interventions, a significant effect of time and time by group for sIgA and time by group for IL-10 levels were found ($p > 0.05$). Within-group analysis showed a significant moderate decrease in the TNF- α to IL-10 ratio for the CME group and an increase in the controls ($p > 0.05$). The controls showed a negative trend towards a decrease in physical fitness and slight reduction in pro-inflammatory IL-6 and IL-1 β concentrations in the CME participants, while in the controls a trend for an increase in levels of these markers was seen. **Conclusions:** The evidence regarding the use of systematic and moderate long-term exercise as therapy in promoting better balance of the pro- and anti-inflammatory environment were the most promising results of this study.

Key-words: Frail older adults, muscle-strength, multimodal, exercise, cytokines, inflammation, upper respiratory tract infectior

1. Introduction

Older frail adults have a greater intrinsic vulnerability to biological and environmental stressors, increasing the risk for physical decline and adverse health related characteristics, such as disability, comorbidity and lower cognitive function when compared to robust older adults [1]. The phenotype of physical frailty (PF) is characterized by muscle loss, negative energy balance, physical inactivity, low gait speed and fatigue [2]. More recently, the frailty syndrome has been reported to modulate the risk of numerous types of dementias and cognitive impairment (CI) [3], with cognitive impairment and physical frailty often present in the same population [4]. The novel age-related concept of Cognitive Frailty is a form of pathological brain ageing and is characterized by concurrent physical frailty and potentially reversible cognitive impairment [5]. Causes for CI include cardiometabolic diseases, chronic kidney disease, insulin insensitivity, sleep disorders, chronic inflammation, immunosenescence, and obesity [6] and appear to be potentiated by low levels of physical activity and inadequate nutritional intake [7]. Some of the predisposing factors underlying sarcopenia (e.g. oxidative stress, inflammation, etc.) are also associated with CI, which explains the common etiology [8].

Immunosenescence is also a part of the ageing process and is associated with an increased risk for autoimmune disorders, tumours, infectious disease and inflammatory and hormonal deregulation [9]. The mucosal immune system, including the upper respiratory tract, is considered a first barrier to the colonisation by pathogenic agents, reducing the incidence of upper respiratory tract infections (URTI) in humans. During ageing, there is a decrease in salivary immunoglobulin-A (sIgA) secretion, an important anti-bacterial protein involved in the mechanisms of defence against pathogens that penetrate the mucosal epithelium, [10]. The mucosal immune function is less impaired in highly conditioned versus non-active older adults and in reduced incidence of URTI [11]. To the best of our knowledge, very few studies have looked at the effect of regular moderate exercise on salivary levels of lysozyme (sLys) and interferon gamma (IFN- γ) in older populations [12]. In addition to the mucosal immunity markers, several cytokines that interact in anti- and pro-inflammatory processes triggered by aging and affecting physical health have been associated with the risk of developing neurodegenerative diseases (i.e. Alzheimer's disease) in older adults [13,14].

Exercise could be a significant factor in ameliorating the deleterious effects of chronic biological and psychological stress, and can positively affect many immune parameters [15]. However, the type, intensity, duration and frequency of exercise that should be performed in order to effectively improve the immune response in very old persons needs to be defined more clearly [12]. A literature review found that regular exercise has a positive impact on immune status in frail individuals,

especially on TNF- α [16]. Several studies have also shown the exercise effect on the TNF- α /IL-10 ratio and its potential in promoting an anti-inflammatory environment in humans [17]. A systematic review also showed that multimodal exercise programs seem to be particularly effective in improving general physical fitness, while there is little evidence that muscle strength exercises can achieve the same improvements in frail older adults [18]. However, the muscle-strengthening elastic-band system has been offered as a successful method to promote significant beneficial effects on cognition status, physical fitness and immune profile [19]. Thus, the aim of this study was to analyse the effects of 28-weeks of two different chair-based group exercise protocols on functional fitness and immune profile in institutionalized cognitive frail older women.

2. Material and Methods

2.1 Study design

This randomized controlled study consisted of three arms, with controlled trial chair-exercise based interventions, that included a subset of the participants of the previous published study protocol [20]. Participants or their legal tutors signed an informed consent term. This study was approved by the Ethics and Human Subjects Review Board of the Faculty of Sport Sciences and Physical Education, University of Coimbra, Portugal, code number (CE/ FCDEF-UC/000202013), in respect of Portuguese Resolution on ethics in research [21], respecting the guidelines for human research of the Helsinki declaration for human research [22].

2.2 Participants recruitment

Participants were institutionalized-dwelling pre-frail and frail women living in centres of health and social support, aged 75 years and above with updated medical therapy and who were clinically stable. The exclusion criteria were: not completing the dynamic balance test in 30 seconds; an involvement in other exercise programs; not having controlled and updated drug therapy; the presence of any type of health condition that could prevent testing of autonomy, such as severe cardiomyopathy, uncontrolled cardiorespiratory illness and any musculoskeletal conditions that might prevent testing (e.g. recent fractures); the use of medication that could significantly impair attention and disturb psychomotor activity (e.g. anxiolytics or anti-depressants); morbid obesity; the presence of mental, hearing and vision impairment and use of hormone therapy.

2.3 Sample size and allocation

The estimated required sample size was 19 participants per group (alpha type I error rate was adjusted at 0.05 and power (type II error rate) at 0.80 using G* Power (version 3.1.9.2), taking into account the comparison of frailty outcomes between exercise treatments in previous studies [23]. Further anticipating a dropout rate of 30% [24], the initial sample calculation was increased to 28 per group, randomly allocated into three arms using a computer generated algorithm. After follow-up, the final data of 60 participants (81 ± 7.84 years old), distributed into three groups were analysed: muscle-strength (CSE, $n=21$; 81 ± 4.79), multimodal (CME, $n=20$; 80 ± 8.19) and a control non-exercise (CGne, $n=19$; 80 ± 10.01). The allocation to treatment followed CONSORT Consolidated Standards of Reporting Trials (*see figure 1*).

- Insert figure 1 about here, please -

2.4 Assessment Procedures

All the tests were applied by an independent assistant who established contact with the participants without reference to the exercise program. The instructor of the exercise sessions did not take part in the data collection procedures. Precaution was taken to avoid interaction of exercise programs between individuals of the two groups by staggering the classes' schedules. The data collection was done in the morning period (09:00 to 11:30 am). The quality of data assessment was made and reported for each of the physical markers through internal consistency reliability scores (ICR).

2.4.1 Physical Frailty screen

The Fried PF protocol was used [2]. Shrinking was assessed by self-report of unintentional weight loss of four kilograms or more in the last six months; Self-reported exhaustion was evaluated by concordance of two questions (7 and 20) of the Center for Epidemiology-Depression (CES-D) scale; Weakness was analyzed using the handgrip strength test, adjusted for gender and body mass index (ICR = .88). Participants who were unable to perform the HGT and those in the lowest 20% were categorized as positive; Slowness was measured by the 4.6 meters walking test, adjusted for gender and height (ICR = .80) and times of ≥ 7 seconds for males and ≥ 6 seconds for females were adopted for positive scores of slowness; levels of PA were assessed by the International Questionnaire short form (ICR = .78) and participants were classified as inactive, minimally active and highly active

with those participants classified as inactive scoring positively for the PF condition. The prevalence of PF was calculated to generate a PF total score. Positive scores in one or two criteria classified the participants as pre-frail, in three or more as frail and as non-frail when none of the five PF independent components were present, establishing a categorical classification [2].

2.4.2 Cognitive status screen

The Mini Mental State Examination (MMSE) assesses five areas of cognition: orientation, immediate recall, attention and calculation, delayed recall, and language [25]. The MMSE was used to classify participants by cognitive profile as a category variable, according to the following cut off values: severe cognitive impairment - 1 to 9 points; moderate cognitive impairment - 10 to 18 points; mild cognitive impairment - 19 to 24 points and normal cognitive status - 25 to 30 points [26]. For this study, our interest was to select a sample of physical frail and pre-frail individuals with mild cognitive impairment.

2.4.3 Immune and biochemical markers determination

Non-fasting blood and saliva samples collection were done in the morning (between 10:00h – 12:00h) in order to minimize the circadian effect seen in some of the markers under study [10]. Saliva samples were collected by passive drool, allowing the saliva to collect on the floor of the mouth for three minutes, then leaning forward and dribbling into an high quality polypropylene tube. Ten minutes prior to the saliva collection, the participants were asked to rinse their mouth with water to remove food residues. Sample volumes were determined by weighing the collection tubes before and after saliva collection. Samples were then stored at -20°C until determination of the saliva markers. Salivary concentrations of sIgA, sLys, IL-1 β and IL-6 were analyzed by ELISA according to the manufacturer instructions [Salimetrics®, USA]. The sensitivity and assay range were reported by the manufacturer as 2.5 ug/dL and 2.50 – 100 ug/dL for sIgA; 5 ug/dL and 0–300 ug/dL for sLys; <0.037 ug/dL and 3.13–200 ug/dL for IL-1 β ; 0.07 ug/dL and 0–100 ug/dL for IL-6, respectively. The interassay coefficient of variation was less than 10% for all salivary biological analysis [27].

Blood samples were collected by venipuncture by a registered nurse, and were allocated into serum and EDTA tubes. Determination of blood counts was done immediately after blood collection, using an automated haematology analyser (Coulter AcT Diff, Beckman Coulter, USA). The remaining blood samples were centrifuged, serum and plasma aliquoted into several Eppendorf tubes and kept frozen at -80°C until further use. The plasma levels of pro-and anti- inflammatory cytokines, such as TNF- α , IFN- γ and IL-10 were analyzed by ELISA (Invitrogen®, CA), according to the manufacturer instructions. The sensitivity and assay range were described by the manufacturer as 2.3 pg/mL and 7.8–

500 pg/dL for TNF- α ; 4 pg/mL and 4–500 pg/dL for IFN- γ ; <3 pg/mL and 15.36–600 pg/mL for IL-10, respectively. The intra-assay coefficient of variability was 5.2% for IL-10, 4.1% for TNF- α , and 0.6% for IFN- γ [28]. Serum CRP was determined using the Horiba Medical Pentra C200 (Kyoto, Japan). The sensitivity and assay range were described by the manufacturer as <4 mg/mL and 4–50 mg/mL, respectively [29].

2.4.4 Functional Fitness status

The Senior Fitness Test battery developed by Rikli and Jones (2013) was used [30]. Lower body strength was determined with the ‘30 second’s chair-and-stand test’ (30s-CS), measuring the number of total full chair-stands that can be completed in 30 seconds with the arms folded across the chest. Upper body strength was assessed with the ‘30 seconds arm-curl test’ (30s-AC) that measures the total number of bicep curls that can be completed in 30 seconds, seated in a chair and holding a hand weight of 5 lbs (2.27 kg) for women. Aerobic resistance was measured through the “2-minutes step test” (2m-ST), that consisted of the number of full steps the subject completed in two minutes performing a stationary gait, with one hand resting on the wall and raising each knee to a midway point between the kneecap and iliac crest. For all the Functional Fitness tests the best score of the two trials performed was used for final scoring purposes

2.4.5 General health status

The Mini Nutritional Assessment (MNA, $k = .74$) consists of 18 questions [31]. The maximum score is 30 points (pts), and classifies subjects as well-nourished (24 to 30 pts), at risk of malnutrition (17 to 23.5 pts) or as malnourished (<17 points) [32]. The Charlson Comorbidity Index (CCI, ICR = .94) was used to classify comorbid conditions based on the medical registry of each individual, and this score can be combined/adjusted to age and gender to form a single index (0-10 points) and used as a continuous variable [33]. Systolic and diastolic blood pressure were recorded using a digital device of OMRON model M1 Plus - HEM-4011C-E® [34]. Daily medication use was assessed through question number six of the MNA, that asks whether the participant takes more than 3 prescription drugs per day and this was confirmed by medical records over one year. In addition, the incidence (%) of the most frequently used drugs were reported and classified according to the European pharmacy classification system [35]. To reduce potential confounding effects, all participants were encouraged to maintain unchanged diets, drugs treatment, and routines across the 28-weeks of the intervention.

2.4.6 Anthropometric assessment

Information about chronological age was collected. For anthropometric assessment, the guidelines described in previous study were followed [36]. Body mass was determined using a portable scale (Seca®, model 770, Germany) with a precision of 0.1 kilograms (ICR = .89). Stature was determined using a portable stadiometer (Seca Body meter®, model 208, Germany) with a precision of 0.1 centimetres (ICR = .87). Body mass index (BMI) was calculated according the formula ($BMI = \text{weight}/\text{height}^2$). Standardized procedures were followed [37].

2.4.7 Intervention programmes

The chair-based exercise programmes were conducted by exercise specialists. For safety reasons, in both exercises programs intensity was indirectly calculated using the Karvonen's formula to predict target heart rate (HR), with $HR_{\max}$ being calculated using a specific formula for older populations [$HR_{\max} = 207 \text{ beats per minute (bpm)} - 0.7 \times \text{chronological age}$] and was monitored using heart rate monitors Polar, RCX5® model [38]. The classes were offered for 28-weeks with two-three sessions per week on non-consecutive days (separated by 48 hours). The percentage of exercise frequency was calculated individually through the total sum of class participation entries recorded in a checklist. A total of 74 (100%) sessions were offered and the values of engagement in different moments were reported as a percentage. Based on previous reports, an adherence to the exercise program up to 65% was established as a minimum for each participant to be included in the final data analysis [39]. The CG_{ne} group did not participate in any physical exercise interventions but was encouraged to maintain their normal routine, which included a monthly agenda of artistic and cultural activities.

2.4.8 Chair Multimodal exercises (CME)

The chair CME program was performed with a predetermined number of exercises (7-9), repetitions (8-16), sets (2-4), rhythm of execution (1:2) and active rest in a seated position between sets (20-30 seconds) and with passive rest between circuits (60-120 seconds). Each session was conducted using a functional circuit training protocol. During the first 12 weeks, the body weight exercises were executed in the seat and reach position. In the last 12 weeks, exercise intensity increase was induced by the inclusion of more difficult and complex exercises and challenge sequences, increasing walking time and placing obstacles along the walking route (cones, floor markers, arcs) to work handedness, changes in direction, static/dynamic balance and coordination. A low to moderate intensity effort around 50-75% of $HR_{\max}$ values was attained. Additionally, intensity was measured

through the modified BORG PES, that consists of an arbitrary scale ranging from 0 to 10 points 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) . Each exercise session was divided into three parts: 5 minutes of warm-up exercises for general body mobilization (PSE = 1-3, HRmax = 50-55%); 35 minutes of body weight exercises aimed to improve walking proficiency in a sitting and standing position (PSE 3-5, HRmax = 56-85%) and finally 5 minutes of cooling down (PSE 1-2, HRmax = 45-50%).

2.4.9 Chair Muscle-Strength exercises (CSE)

To create a progressive chair CSE program, exercises were performed with a predetermined number of exercises (8-10), sets (2-3); repetitions (10-15), a cadence of repetitions execution in 2 seconds concentric and 3 seconds eccentric (2:2) and a passive rest in the seated position between sets (30-45 seconds), following a circuit training protocol method. The first three levels of the Thera Band® System were used. Level one (yellow colour) elastic-bands were used during the first 12 weeks. In the remaining 16 weeks, the increase in the exercise intensity was introduced by changing to the elastic-bands level 2 and 3 (red and green colour respectively). Intensity was measured through the OMNI PES, that comprises a subjective effort scale ranging from 0 to 10 points, with identical intervals and with reference to the quality of effort: (0) extremely easy; (1-2) easy; (3-5) somewhat easy, (6-7) somewhat-hard; (8) hard; (9-10) extremely-hard [40]. The goal was to keep the intensity of the exercise activities between 1 to 6 in PES levels. It was expected that the relationship with the real effort would be 55-80% of maximum heart-rate. 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%).

3. Statistical procedures

Descriptive data were presented as mean (and standard deviations) or frequencies. For the baseline scores, comparisons between groups were analysed by one-way ANOVA. Repeated measures ANOVA was used to analyse the effect of time and time by group interactions on the frailty total score, functional fitness and immune profile changes over time, followed by Bonferroni *post hoc* tests, reporting the confidence interval (CI:95%) for the statistically significant results. Since there was no normality distribution in TNF- α /IL-10 ratio, pre- and post- interventions comparisons were made using the Kruskal-Wallis test. The percent of change after the interventions was calculated ([post

value/pre value]-1). The magnitude of the global effect size (ES) was calculated by exercise-modulation treatment. For continuous variables, ES were computed using Cohen's (d). The effect size was considered trivial ($d \leq .20$), small ($.21 < d < .50$), moderate ($.51 < d < .79$) and large ($d > .80$) [41]. The IBM SPSS Statistics package 24.0 and in R 3.3.1 software's for all statistical procedures were used. The level of significance adopted was $p\text{-value} = 0.05$.

4. Results

From the 119 potential participants initially recruited, a total of 29% ($n=70$) were excluded for not meeting the inclusion criteria or declining to participate in the study spontaneously. In the follow-up phase, 6 participants of CME training, 10 of CSE and 9 of CG_{ne} withdrew their willingness to participate before or immediately after the beginning of the study for various reasons (*see figure 1*). A total of 24 drop-outs were registered during the intervention period because of low exercise engagement or discontinued participation. None of the drop-outs left the intervention because of adverse responses to the intervention. A total of 60 participants were examined at completion of the 28 weeks exercise program. Characteristics of experimental and control groups at baseline are presented in Table 1. The statistical analysis showed that no significant differences between the three groups were found, demonstrating that the three groups have similar characteristics in relation to diet, physical attributes, cognitive profile, medication use and diseases ($p>0.05$).

4.1 Immune markers

Table 2 shows the changes in inflammatory markers and physical fitness after the 28 weeks intervention programs. The results showed a significant effect of time [$F(2,44) = 6.681$; $p = 0.014$] and time by group for sIgA changes over the interventions [$F(1,44) = 4.661$; $p = 0.015$]. Bonferroni corrections applied *post hoc* showed a moderate decrease in sIgA levels in the CSE group ($ES = 0.68$, $\Delta\% = -26$; $p < 0.01$, $CI_{95\%}$: 0.08 to 0.313), while the others remained unchanged over time ($p > 0.05$). No effects of time or time *by* group were observed for Lys levels ($p > 0.05$), despite an observed decrease with a small effect size in both CME and CSE groups ($ES = 0.33$, $\Delta\% = -36$ and $ES = 0.44$, $\Delta\% = -18$, respectively). No effects of time or time *by* group were observed for changes in IL-6 and IL-1 β ($p > 0.05$), despite a small decrease in the CME group ($ES = 0.23$, $\Delta\% = -130$) and increase in the GC_{ne} group ($ES = 0.31$, $\Delta\% = +88$) in IL-1 β levels. Significant interactions were observed for time *by* group in IL-10 levels [$F(2,45) = 4.514$; $p = 0.01$], where concentrations increased in CME ($ES = 0.39$; $\Delta\% = +62$; $p = 0.023$; $CI_{95\%}$: -0.247 to -0.019) and CSE ($ES = 0.36$; $\Delta\% = +46$; $p = 0.029$; $CI_{95\%}$: -241 to -013) groups. However, no significant change was observed in controls ($p = 0.147$).

No effect of time or time *by* group were observed for TNF- α levels ($p>0.05$), despite a trend for changes over time [$F(1,41) = 3.280$, $p=0.07$]. There was an effect of time for IFN- γ changes [$F(1, 42) = 6.117$, $p=0.01$], but no significant interactions with group ($p>0.05$). No differences were observed for time and time *by* group interaction in CRP levels ($p>0.05$). Despite a trend for these to be increased in the CG_{ne} group ($p = 0.06$). Regarding the TNF- α /IL-10 ratio, univariate analysis showed no significant differences between groups at baseline and after 28 weeks ($p > 0.05$). However, within-group analysis using Wilcoxon-signed rank test showed a significant moderate decrease in this ratio for CME ($z = -2.551$; $p = 0.01$; $ES = 0.33$; $\Delta\% = -43$), and an increase in controls ($z = -2.312$, $p = 0.02$; $ES = 0.40$; $\Delta\% = +74$).

Insert table 2 about here, please

4.2 Functional Fitness Status

Analyzing the physical fitness parameters after the 28-weeks intervention programs, the results showed that there was only an effect of time on CSR-M [$F(1; 52) = 5.259$, $p=0.02$], but no interactions of time with group (Table 3). Both CME and CSE groups showed increased scores with large effect size ($ES = 0.40$; $\Delta\% = -12$ and $ES = 0.49$; $\Delta\% = -16$, respectively). A significant effect of time on 30s-AC was shown [$F(1;52) = 16.045$, $p < 0.01$]. *Post hoc* analysis showed a decrease in both CME and CSE on 30s-AC with a large and moderate effect-size, respectively ($ES = 1.05$; $\Delta\% = + 51$; $p = 0.000$ and $ES = 0.60$; $\Delta\% = + 42$; $p = 0.021$). There was a significant effect of time [$F(1;52) = 22.675$, $p < 0.01$] and time *by* group interactions [$F(2;52) = 5.556$; $p = 0.006$] on the 30s-CS test, and Bonferroni analysis showed an increase for CSE after exercise intervention ($p < 0.01$). Both CSE and CME demonstrated a large magnitude of effect after interventions ($ES = 1.05$, $\Delta\% = +37$ and $ES = 1.51$; $\Delta\% = 82$, respectively). A significant effect of time [$F(1;52) = 21.739$, $p < 0.01$] and time *by* group [$F(2;52) = 8.474$, $p < 0.01$] on the 2M-ST was shown after the intervention program. *Post hoc* analysis showed an increase in both CME and CSE on the 2M-ST ($p < 0.01$), with moderate ($ES = 0.70$, $\Delta\% = +30$) and large effects ($ES = 1.04$; $\Delta\% = +51$), respectively. The CG_{ne} remained unchanged over time ($p>0.05$).

Insert table 3 about here, please

4.3 Exercise effort and engagement

The final scores of minimum, maximum and mean HR achieved at the end by the CME group was 65.75 (± 9.96), 97.56 (± 12.40) and 77.27 (± 8.21) bpm, respectively. This corresponded to 57% to 70% of the predicted HRmax. The final mean of PES ranged from moderate (3.14 ± 0.98) to somewhat strong (4.92 ± 1.01). For the CSE group, the scores of HR achieved at the end were 69.08 (± 14.77), 96.24 (± 14.13) and 84.48 (± 11.52) bpm. This corresponded to 48% to 74% of the predicted HRmax. The final mean of PES oscillated from somewhat easy (3.98 ± 1.99) to somewhat strong (6.02 ± 2.22), according to the participants self-perception. Regarding adherence, the CSE participants attended an average of 58 sessions (78% of attendance), out of 74 sessions offered in total. In the CME, the participants had a 72% adherence, corresponding to 54 sessions attended.

4. Discussion

The goals of this study were to assess the impact of two different structured exercise programs, CSE and CME, in older frail women with mild cognitive impairment, on immune profile and physical fitness. The assumption was that including regular exercise as a co-adjuvant therapy would be effective in reducing chronic inflammation. The main results of this study were that both CSE and CME programs were able to increase physical fitness and simultaneously increased anti-inflammatory cytokine concentrations promoting a better inflammatory balance in institutionalized older frail women after 28-weeks of systematic exercise. However, CSE and CME programs promoted different responses for some of the analyzed variables, even though both similar intensity of effort and adherence to the exercise programs were achieved by both groups.

4.1 Immune Markers

It is generally understood that immune function declines with age, and, compared with young people, older people are less resistant to pathogenic microorganisms [42]. In the case of frail individuals, the susceptibility to increased episodes of the URTI appears to be greater [43]. Several exercise intervention have examined markers of URTI, including salivary antimicrobial proteins, such as sIgA [44–46]. However, results showed a slight conflict in the interpretation of results, especially in short-term programs (up to 12 weeks) [42]. In the results of Akimoto and colleagues, regular moderate exercise seemed to enhance mucosal immune function (sIgA) in elderly subjects after 12-weeks of combined training [47]. But, this study reveals another important question in the comparison with our results, although the exercise program used shows some similarity with the CME used in this

study: this sample has a younger age group living in the community and for this reason the authors may have observed better results. In the other study which aimed to investigate the effects of aerobic conditioning in a very similar population, sIgA concentration was unchanged, suggesting that alterations in sIgA levels in response to exercise may also be related to age [48]. In another recent study, no change was observed in sIgA after 28 weeks of Yoga, however, a trend towards an moderate increase in both sIgA (ES = 0.20, $\Delta\%$ = 26) and sLys (ES = 0.48, $\Delta\%$ = 87) secretion rates were found, while in the control group a trend towards a decrease in the sIgA secretion rate was found. In our study, unlike these studies cited above, there was a trend to reduced sIgA, except for the CSE group, which is in line with the results of the previous studies using strength training [49].

Unlike data on sIgA, there is little data available regarding the changes in sLys concentrations with acute or chronic exercise in this type of population. A recent study described that sLys secretion increased after moderate intensity exercise and increased further after high intensity exercise, which implies that sLys levels may be related with exercise intensity [10]. Despite the lack of research involving modulating effects of exercise on sLys, the studies showed that sLys and sIgA and others salivary markers, such as alpha-amylase, seem to demonstrated a similar comportment during and after physical exercise, because they share the same control and activation is regulated by the autonomic nervous system [44].

The subjacent mechanism evolving inflammation process is one such potential pathophysiological change which may be closely linked with the physical frailty condition [50]. However, it is consensual that chronic subclinical inflammation contributes to impaired physical functioning in older adults [51]. The concept of a beneficial ‘anti-inflammatory exercise-effects’ in older populations who participate in regular programs seems to justify the possible beneficial increase that occurred in IL-10 in both CSE and CME programs, while in the CG_{ne} there was a slight decrease, taking into account the magnitude of effect size. In the other hand, TNF- α and IL-6 are important pro-inflammatory markers and have previously been found to be elevated in cases of frail individuals in comparison to healthy age-matched controls [52]. In the present study, the increase in IL-10 levels may partly justify the behaviour of inflammatory markers observed, since this marker influences the reduction of mRNA levels of TNF- α , IL-1 β , and IL-6 in skeletal muscle of older adults [53]. In similar research with obese frail older patients, a combined exercise program (strength plus muscular resistance training) led to a significant decrease in IL-6 and TNF- α mRNA, despite weight loss having no effect [54]. In the other intervention study, significant decreases in TNF α and mRNA protein expression were reported after resistance training in frail older men and women after 12-weeks [54].

Our results showed, after 28-weeks of systematic exercise, a slight reduction in pro-inflammatory IL-6 and IL-1 β concentrations in the CME participants, while in the controls a trend for an increase in levels of these markers was seen. Changes in TNF- α levels appear to be inconclusive, but when analysing the TNF- α to IL-10 ratio, we observed the effect of exercise on the pro-inflammatory balance, an immune physiological phenomenon recently observed by our research group in a study with a very similar population [19]. The mechanism explaining this balance is associated with the attenuated-effect triggered by IL-10 on TNF- α receptors expression, stimulating release of their extracellular domains into systemic circulation. Additionally, IL-10 also downregulates cell-mediated immune responses and cytotoxic inflammatory responses, and for this reason, the imbalance in these cytokines appears to be associated with coronary heart disease [55].

Similar to TNF- α , a several cross-sectional studies associated high levels of CRP to abnormal cardiovascular risk and stroke. Results from these studies are consistent in showing that low general physical function (gait speed, muscle mass, muscle strength and self-reported functional ability) are associated with high inflammatory marker concentrations [53]. Therefore, low concentrations of these inflammatory markers are essential for the maintenance of better neuromuscular performance. In this study, the results showed no significant alterations in CRP levels, despite for CGne to show a trend for a moderate increase (ES = 0.47; $\Delta\%$ = 87). Unlike our study, in other two similar study conducted with postmenopausal women, a 14 and 20 weeks of aerobic exercise intervention decreased CRP by 15% and 25%, respectively [56]. Comparable to the present study, the exercise-mediated effect on the increase of anti-inflammatory makers (IL-10 and IFN- γ) was the plausible explanation for this results. Similar to the increase in IL-10, the concurrent small increase of IFN- γ in both the CME and CSE exercise groups (and unchanged in the controls) seems to be beneficial, since this biomarker is considered as an anti-inflammatory cytokine in low concentrations. Regular moderate exercise (as was the case in this study) and not intense exercise has beneficial effects on health by way of increasing satisfactory plasma IFN- γ levels [57].

The magnitude of exercise-induced immune variations in CME and CSE was not substantially different, while age, sex, presence of frailty and exercise intensity were the same between groups. Evidence in the literature showing that both exercise programs can ameliorate the immune profile [58]. However, in the recent study aimed to investigate the effects of 16-weeks of regular aerobic and strength-based exercise programs on CRP serum levels evolving women and men aged >64 years was found that decreases in CRP concentrations were associated with strength gains and adiposity loss [59]. In the present study, CSE kept levels stable, while CME slightly reduced CRP levels (in the control group it increased after 28-weeks). Another study compared the effect of 12 months of exercise

(strength, aerobic and multicomponent) on an elderly population, and although the methodology used was very robust (multicenter randomized, controlled trial), the researchers concluded that there were no substantial differences between exercise types. In addition, it was possible to verify only trends to decrease some scores of cytokine such as TNF- α , IL-1, IL-6, IL-8 and adiponectin for all groups [60]. Therefore, further research is needed to determine if one type of exercise exerts different and substantial effects that justify their use, over another type of intervention, for an elderly population.

4.2 Physical Fitness status

Analysing the physical fitness data, we observed that the large and beneficial improvements achieved by exercise groups were quite similar after the 28-weeks programs, despite the fact that the design characteristics of CSE and CME exercise programs were quite specific and different. According to the American College of Sport Medicine (ACSM), CME are categorized as a group class where the instructor uses aerobic exercises interspersed with endurance, strength, balance and other types of exercises [23]. The main purposes of CME program is to encourage older people to work on muscle strength and endurance, integrating other physical fitness domains, such as time reaction, gait performance, static balance and improved stability in dynamic balance [61]. Although ACSM classified the evidence level of CME as C or moderate (on a scale ranging from A to D) [62], most current studies have demonstrated a general health benefit of CME in older patients with physical and cognitive frailty conditions when compared to other programs or controls, especially to increase cognitive functions, decrease falls incidence and improve physical fitness related to daily tasks autonomously [63].

The most probable explanation for the results similarities between the CME and CSE groups, was the progressive increase in difficulty (effort) of the muscle resistance body weight exercise components over time (e.g. chair muscle up and seat to chair muscle up and stand), demonstrating beneficial effects on muscle strength after 28 weeks. A complete and comprehensive review indicated that longer-term (over 16 weeks) multimodal exercise interventions with shorter-duration sessions (30 to 45 minutes) might be a better option for these populations, especially for the prevention of adverse health consequences [16]. In this sense the results of our study are in agreement with the current literature. In this sense, the results of our study are in agreement with the current literature. Unlike the multimodal exercise programs, the strength exercise programs are classified as evidence level A (strong), and its benefits are also well researched and scientifically proven. Current evidence indicates that supervised and controlled trials of CSE represent an effective intervention in physical frailty treatment.

Unlike the traditional strength exercise program, which opts for the use of free weights and machines, our program used a workout with gradual increases in elastic bands resistances. Recent studies have demonstrated the effectiveness of exercise interventions with elastic bands in improving muscle strength and functional capacity in older adults [63,64]. In addition to the fundamental characteristics such as ergonomics, practicality, and gradual control of the effort (Andersen et al., 2010), some studies with older participants have proven that some physiological adaptations (including changes in immunological parameters) inherent to the traditional strength exercises, can also be achieved using the gradual system of elastics bands [65], some studies with older participants have proven that some physiological adaptations (including changes in immunological parameters) inherent to the traditional SE, can also be achieved using the gradual system of elastics bands [19,66].

One other hypothesis, that could explain the substantial physical fitness improvements in this sample, is that gains are most evident in older people with low levels of physical functioning, as was the case in this sample. The control group, as expected, did not shown any improvement in physical fitness, on the contrary a decreased performance in almost all of the physical fitness tests used in this study was seen, emphasizing the importance of being and staying active across the life span. Lack of exercise/physical activity is a well-known risk factor for the loss of independence in older adults.

4.3 limitations and future directions

Despite the interesting results, this study presented some limitations. Firstly, we must interpret the results carefully, since the degree of comorbidity and the use of drugs may influence the variation of biochemical markers. In our study sample, for example, there was a high prevalence of hypertension (78%), dyslipidemia and metabolic syndrome (64%), heart failure (37%), rheumatic or connective tissue disease (48%) and diagnosed mental disorders, such as depression, anxiety and chronic stress (28%). Some of these diseases are treated with beta-blockers that may have interfered with autonomous nervous system functioning [67]. Statins, the commonly prescribed drug to reduce cardiovascular disease risk has been linked to skeletal muscle myopathy and attenuates increases in cardiorespiratory fitness and skeletal muscle mitochondrial content when combined with exercise [68]; the pragmatic treatment for rheumatic disorders included medication for pain and inflammation (methotrexate and leflunomide) or biologic drugs (infliximab, adalimumab, etanercept, golimumab and certolizumab), that while improving pain, mood disorders, and fatigue, also act as inhibitors of IL-6 and TNF- α [67]. Notwithstanding this, effects on IL-6 and TNF- α were not seen in controls despite this group having equal medication use and morbidity (see table) as the two exercise groups.

Secondly, we opted for a naturalistic controlled trial, due to the very poor physical functional condition of the sample, that would have made moving to an exercise center very difficult. However,

it is essential to mention that participants of this study started the exercise programs under the same conditions. In addition, this study shows that group exercises in a care home are a feasible, safe and cost-effective option for improved muscle strength and immune system profiles possibly reducing risk of falls and further morbidity.

5. Conclusion

Our study describes important evidence regarding the use of systematic and moderate long-term physical exercise as co-adjutant therapy in achieving a better balance/environment between the pro- and anti-inflammatory markers. Also, these findings revealed the expected capability of multimodal or elastic-band strength exercise programs to substantially increase physical fitness and promote independence in cognitive frail older women. The chair-based exercise method in groups does not appear to have negatively influenced the results. As using a chair reduces the risk for adverse events, this approach should be most advocated for frail older people in care homes.

Consent for Publication

Our research group allowed the data publication because the study participants were guaranteed that their identities would be preserved.

Conflicts of interest

The authors declare no conflict of interest, financial or otherwise.

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