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Article

Being Physically Active for a Long-Standing Mitigates Inflammaging Progression in Older Adults during the COVID-19 Pandemic

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Abstract: **Background:** Here, we investigated the inflammaging and physical performance in older adults before and after the COVID-19 pandemic. **Methods:** Women(n=18) and men(n=7) older were evaluated before the COVID-19 pandemic(PRE), 12 months after the lockdown(POST), and 10 months after resuming exercise training(POS-TR). Physical tests [gait speed(GS) and timed-up-and-go(TUG)]; muscle strength (handgrip-HG), and serum cytokine levels were assessed. **Results:** The older women showed higher GS and TUG at POST than PRE and POST-TR, besides lower HG at POST-TR than PRE, whereas older men exhibited lower HG at POST and POST-TR than PRE. Both groups presented (1)lower IL-10 and IL-12p70 values in contrast to higher IL-6/IL-10 and IL-8/IL-10 ratios at POST than PRE; (2)higher IL-10 values and lower IL-8/IL-10 ratio at POST-TR than POST; (3)higher IL-12p70/IL-10 ratio at POST-TR than PRE and POST. Particularly older women showed: (4)lower IL-6 values at POST and POST-TR than PRE; (5)lower IL-8 and IL-10 values at POST-TR than POST; (6)and higher TNF- α /IL-10 and IFN- γ /IL-10 ratios at POST than PRE and POST-TR. Significant correlations between the variables were found in both groups. **Conclusion:** During the COVID-19 pandemic, detraining and resumption of exercise training promoted distinct alterations in physical capacity and inflammaging among older women and older men population.

Keywords: aging; older people; cytokines; gait speed; TUG; handgrip



Introduction

Among the most remarkable achievements by humanity over the last two centuries is the increase in human lifespan, which was extended mainly due to the improvement of worldwide public health policy and modern medicine[1,2]. As a consequence of this triumph, the population over 65 years, which was around 8.5-9% in 2019, in accordance with the global estimate will increase to 12% in 2030 and 17% in 2050[3]. Although the recent COVID-19 pandemic, unfortunately, caused a higher number of deaths, particularly of older adults, the estimates concerning the pace of population aging is still accelerated[4]. However, it is paramount to mention that living longer is not closely associated with living with health since it is evidenced that the expansion in lifespan is accompanied by the high prevalence of several chronic diseases and comorbidities, such as cancer, diabetes, cardiovascular, renal, lung, liver, neurological diseases, sarcopenia, frailty, and also an increased susceptibility to infectious diseases, which directly impacts the survival and well-being of older people[5,6]. Therefore, in order to minimize the burden on health systems and maintain quality of life in the aging process, the World Health Organization (WHO) has worked to stimulate public policies aimed at promoting healthy aging[7].

Regarding aging, it is a natural, dynamic, and multifactorial process characterized by a progressive decline of the majority of physiological systems[2,8]. Among these systems, the immunological is one of the most affected by aging and leads to the development of the phenomenon named immunosenescence, which affects both innate and adaptive immunity[9-11]. From 2000, when Prof. Francheschi and collaborators published the article entitled "Inflammaging: an evolutionary perspective on immunosenescence"[12], the literature has highlighted that one of the main players involved in the development and progression of immunosenescence is the phenomenon inflammaging, which translates a chronic, sterile, low-grade inflammation associated with aging. It has been reported that the increased systemic levels of some pro-inflammatory mediators, particularly the C-reactive protein (CRP), interleukins (ILs) such as IL-1, IL-6, IL-8, IL-12, the tumor necrosis factor-alpha (TNF- α), and the interferon-gamma (IFN- γ), in association with the decreased levels of IL-1Ra and IL-10, two well-known anti-inflammatory cytokines, characterizes the inflammaging[13,14]. Furthermore, it is worth mentioning that these both phenomena (immunosenescence and inflammaging) presented a virtuous circle since one can fuel the other[15] and also can be pillars to the increased risk of the occurrence of chronic and infectious diseases in older adults[16].

In order to mitigate the deleterious effect of aging, especially in the older adult population, it is consensus that some approaches related to achieving an adequate nutritional status and regular physical exercise practice are considered powerful strategies in this context[17,18]. It is broadly accepted that the adoption and maintenance of an active lifestyle, through lifelong training, can maintain performance related to endurance and strength, as also muscle mass, favoring healthy aging in different ways, which includes the regulation of systemic inflammatory status by increasing the IL-10 and decrease IL-6, IL-8, TNF- α , and CRP[15,19-21].

In opposite to the benefits of regular physical exercises in aging, a sedentary lifestyle and a decrease of strength and skeletal muscle are related to an increased risk to develop chronic diseases, comorbidities and premature mortality[22,23]. At this point, it is of utmost importance to highlight that, regarding the literature, during the confinement imposed by COVID-19 there was a significant elevation in sedentary behavior[24,25].

Although the WHO declared the end of the COVID-19 pandemic in May 2023, until now, the lockdown effects on the benefits of regular practice physical exercise performed by older adults in terms of systemic inflammatory status were scarcely assessed [26-28], thus are not fully understood. Likewise, there is a lack of information on whether resuming exercise training could impact the inflammaging state in these same physically active older women and older men. Therefore, in the present study, we investigated the effect of the interruption of the regular physical exercise imposed by the COVID-19 pandemic (detraining period) and the consequences of the return to this exercise training program on the inflammaging in a group of older adults.

Material and Methods

Participants and Study Design

This study is an interventional, multifactorial, retrospective and prospective, and convenient study, involving 25 older adults of both sexes (7 men and 18 women), aged 65-85, who had been engaged in combined exercise training for, at least, 24 months before COVID-19. Data were collected at three time points: pre-pandemic (April-May 2019), post 12-month pandemic hiatus (February-March 2021), and post 10-months of resumed exercise training (November-December 2022).

All volunteers took part in both a Primary Health Care Program for Older Adults at the Federal University of São Paulo (UNIFESP), Brazil, and the “Mané Garrincha” Sports and Education Center. As presented in Figure 1, in 2019, a pool of over 90 volunteers was enrolled to participate in a previous study[29] which aimed to study the immune/inflammatory responses, as well as physical function aspects associated with the influenza virus vaccination. However, the declaration of the COVID-19 pandemic in 2020 led us to review and expand our previous objectives. Then, in 2021, after 12 months of the COVID-19 pandemic, we invited the same volunteers to participate in this study, but only 40 volunteers agreed. It is important to clarify that, the sampling collection at this time point occurred before the volunteers were submitted to the vaccination for COVID-19. Moreover, in 2022, after 10 months of the resuming regular practice of exercise training by our volunteers, the same 40 older adults were invited to participate in a new study’s phase, but some withdrawals occurred for several reasons: (i) not return to physical exercise practice, (ii) changes of address, and (iii) fear of being contaminated during the evaluations. Based on these facts, the number of volunteers dropped again, and only 25 agreed to participate in this last study’s phase.

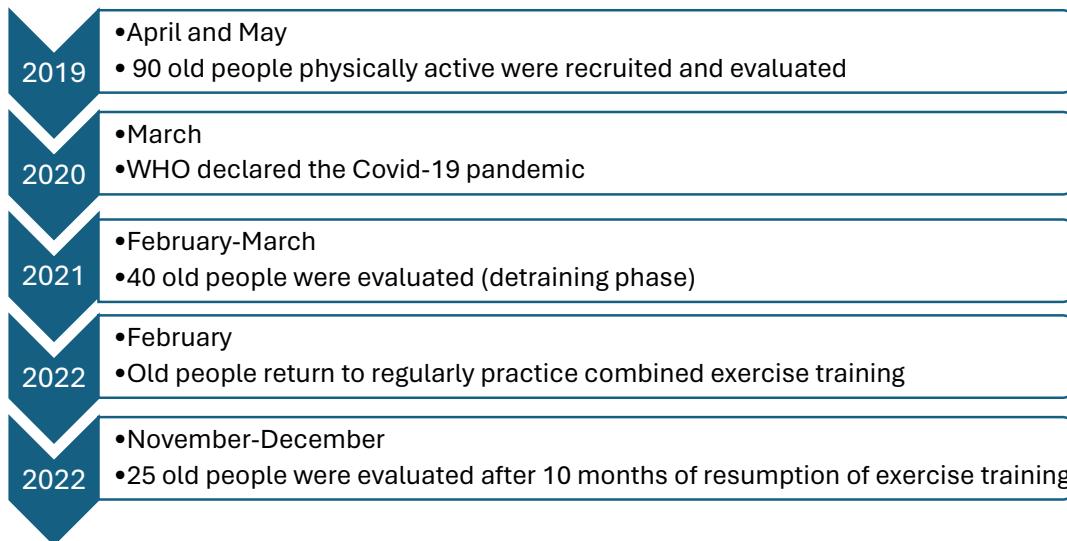


Figure 1. Timeline of study design.

Although the number of volunteers enrolled in the present study dropped from the beginning, it is important to mention that this number (n=25) was in agreement with our previous study[9,29,30], in which both immune/inflammatory and physical function aspects were assessed. Moreover, we also performed a sample calculation using the G*Power software[31] and the data concerning the inflammatory status of older adults reported by Abd El-Kader and Al-Jiffri[32], considering the Student’s t-test with effect size (0.30) at level α (0.05), the statistical power of 0.95, and also a 10% margin for losses or refusals. Based on this, a minimum of 35 individuals would be needed to carry out this study, which was reached in 2021. However, due to the refusals/withdrawals that occurred in 2022, the final population enrolled in this study (n=25) was below the minimum. Then, the study was performed with a population of convenience.

Inclusion and exclusion criteria were carefully designed and evaluate by a single physician to ensure the safety and relevance of the participant pool. The inclusion criteria were: (i) to be older than 60 years, (ii) have the autonomy to move to the exercise training center, (iii) receive medical clearance to engage in the exercise program, and (iv) perform the same exercise program and be supervised by the same physical education professional. The exclusion criteria were: (i) present diagnosis of mental and physical illness, neoplasm, renal and liver diseases, Type-1 Diabetes Mellitus, chronic infections or out-of-control cardiovascular and metabolic disease, (ii) present symptoms or diagnosis of respiratory infections, (iii) use of anti-inflammatory drugs or multivitamin/antioxidant supplements until the last 2 months before the evaluations.

All study procedures were thoroughly explained to the participants, who then provided informed consent, previously reviewed and approved by the Research Ethics Committees at UNIFESP (numbers 3,623,247 and 5,036,504) and the University of Santo Amaro—UNISA (numbers 4,350,476 and 5,318,499) and by the National Research Ethics Committee (number CAEE: 48166721.0.0000.5505), ensuring compliance with Brazilian Resolution (196/96) on research ethics with human subjects[33] and the Helsinki Declaration[34]. The study also followed international guidelines for ethics in physical exercise science research[35], and adhered to the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) guidelines[36].

Combined Exercise Training Program

The combined exercise training program, formerly described[9], was composed of aerobic and resistance exercises and was performed three times per week, lasting 60–75 minutes, for, at least, 24 months pre-pandemic, and for 10 months in 2022, during COVID-19 pandemic.

Anthropometric Data and Physical Tests

The Body Mass Index (BMI) of each volunteer was calculated by measuring height and body weight measured on digital scale and stadiometer (Personal® scale, Filizzola, São Paulo, Brazil). Physical tests were assessed through the tests: (i) Time-Up-Go (TUG) in three meters on the way there and the same distance on the way back and the results were expressed in seconds; (ii) Gait Speed (GS), on a four-meter route, starting at rest and disregarding the deceleration at the end of the route. The result were expressed in meters per second; and (iii) the muscle strength was evaluated through handgrip strength (HG) using an analog dynamometer (Jamar Hydraulic Hand Dynamometer®, Sammons Preston Rolyan, Bollingbrook, IL, USA), in which the result were shown in kilograms force. We used protocols previously described in our study[30].

Blood Sample Collection and Analysis

Fasting blood samples were collected between 8-9h a.m. in tubes without anticoagulant compound in order to obtain sera aliquots, on three different occasions: pre-pandemic (PRE, in 2019), post-pandemic (POST, in 2021), and post-training resumption (POST-TR, in 2022). Briefly, after blood coagulation, the tube was submitted to centrifugation (2000 rpm, 4°C, 10 minutes), and a minimum of 500 mL of serum was added in Eppendorf's tubes that were stored at -80°C until the cytokines analyses. The volunteers were instructed not to perform physical activities of moderate or vigorous intensity in the 24 hours prior to the collection.

Serum levels of pro- and anti-inflammatory cytokines (IL-6, IL-8, IL-10, IL-12p70, TNF- α , and IFN- γ) were determined using multiplex assay (LEGENDplex™ bead-based multiplex assays, Biolegend, San Diego, CA, USA) and analyzed with a BD Accuri™C6 Plus Flow Cytometer (BD Biosciences San Jose. CA. USA), following the data analysis with LEGENDPlex™ V8.0 software (Biolegend). The concentration of these cytokines was calculated using appropriate standard curves (following instructions from manufacturers). The linearity of multiplex analysis of all cytokines assessed here was, respectively, within the range of 0–10,000 pg/mL, which includes the range of sample determinations. All correlation coefficients of standard curves were in the range of 0.93 to 0.99, whereas intra-assay coefficients of variance were 2–4% and interassay coefficients of variance

were 7–10%. We also calculated the ratio between pro- and anti-inflammatory cytokines in order to evaluate the systemic inflammatory status[37].

Statistical Analysis

Data normality and variance homogeneity were assessed using Shapiro-Wilk and Levene's tests. Differences between parametric (anthropometrics and physical function) and non-parametric variables (cytokines) were analyzed using one-way ANOVA with Tukey's posthoc test and the Friedman test with Bonferroni's posthoc test, respectively. Spearman's rank correlation coefficient evaluated associations between variables. A significance level of 5% ($p<0.05$) was set. Analyses were performed using GraphPad Prism version 10.1.1 (GraphPad Software, Boston, Massachusetts USA).

Results

Table 1 shows the data concerning anthropometric (age, weight, height, and BMI), physical tests (GS, TUG), and muscle strength (HG) of the older groups (women and men) participants in this study. In addition to the significant chronological ageing of the all volunteers, notably the older women presented a significant worst performance not only in GS at POST compared to PRE and POST-TR but also in TUG test at POST in comparison to PRE, as well as lower HG values at POST-TR than at PRE. Older men exhibited a significant decrease in HG values at POST and POST-TR compared to PRE.

Table 1. Data (mean and standard deviation) concerning demographic and anthropometric characteristics, as well as physical function tests [assessed by gait speed—(GS) and Timed-up-and-go (TUG)] and muscle strength (assessed by handgrip—HG) of the older women and older men groups in the different occasions: before COVID-19 pandemic (PRE), after 12 months of interruption (POST, detraining phase), and also after 10 months of resuming of regular practice of combined exercise training program (POST-TR). The level of significance was established at 5% ($p<0.05$).

Variables	Volunteer Groups							
	Older women (n=18)			p-values	Older men (n=7)			p-values
	PRE	POST	POST-TR		PRE	POST	POST-TR	
Age (years)	75.2±7.0*	76.2±7.0	77.1±7.1†	*†<0.001	72.4±7.1*	73.4±7.1	75.1±7.1†	*†<0.001
Weight (kg)	60.32±14.5	59.78±14.23	59.58±14.11	0.534	70.69±9.14	67.61±10.79	70.96±8.71	0.312
Height (m)	1.53±0.07	1.53±0.07	1.53±0.07	0.955	1.68±0.08	1.68±0.08	1.68±0.08	0.915
BMI (kg/m ²)	24.32±4.21	24.14±4.46	24.29±4.16	0.694	24.82±3.29	23.59±2.05	25.30±2.98	0.401
GS (m/s)	0.97±0.21*	0.81±0.25	0.99±0.18†	*0.009 †0.015	1.03±0.15	1.06±0.18	1.02±0.19	0.914
TUG (s)	6.8±1.0*	7.3±1.2	7.1±0.8	*0.033	6.0±0.5	6.1±0.4	6.3±0.6	0.707
HG (kgf)	23.1±3.7#	21.3±4.5	20.7±3.6	#0.005	37.6±5.9*#	33.6±7.5	33.8±7.4	*0.008 #0.019

Note: *significant difference between the values obtained in PRE and POS. #significant difference between the values obtained in PRE and POS-TR. †significant difference between the values obtained in POS and POS-TR.

As presented in the up panel of Figure 2, lower levels of IL-10 (2C) and IL-12p70 (2D) were found in both groups at POST than at PRE. A significant reduction in IL-6 (2A) was verified both at POST and POST-TR compared to PRE, particularly in older women. Reduced levels of IL-8 (2B), in contrast to increased IL-10 (2C), were found in older women at POST compared to POST-TR. Lastly, IL-10 (2C) levels were significantly higher in both groups at POST-TR than at POST. Levels of TNF- α (2E) and IFN- γ (2F) were unchanged.

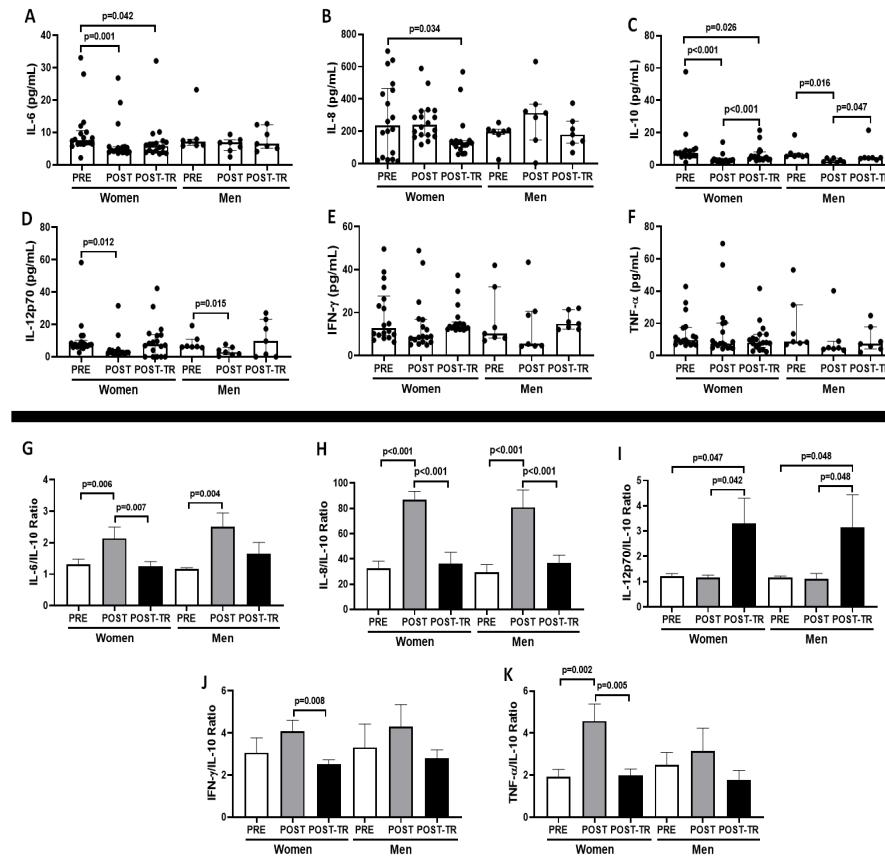


Figure 2. In the up panel is shown the comparison of the serum cytokines levels, in median and interquartile range, of IL-6 (A), IL-8 (B), IL-10 (C), IL-12p70 (D), IFN- γ (E), and TNF- α (F), as well as in the down panel the ratio, in median and standard deviation, between IL-6/IL-10 (G), IL-8/IL-10 (H), IL-12p70/IL-10 (I), IFN- γ /IL-10 (J), and TNF- α /IL-10 (K), in the volunteer groups (older women and older men) before COVID-19 pandemic (PRE), after 12 months of interruption (POST, detraining phase), and also after 10 months of resuming of regular practice of combined exercise training program (POST-TR).

The lower panel at Figure 2 shows the ratios between pro-inflammatory cytokines and IL-10. At POST, whereas both groups presented a significant increase in the ratios of IL-6/IL-10 (2G) and IL-8/IL-10 (2H), a higher TNF- α /IL-10 ratio (2K) was observed in the older women than at PRE. In contrast, at POST-TR, not only did the older women present a lower TNF- α /IL-10 ratio (2K) but also both groups showed a reduced IL-8/IL-10 ratio (2H) compared to POST. Interestingly, the IL-12p70/IL-10 ratio (2I) was higher at POST-TR than at PRE and POST, in both groups. Table S1 shows the values of cytokines and the ratios between IL-10 and the inflammatory cytokines.

Data obtained in Spearman's correlation analysis, particularly in the older women's group (Table 2), revealed positive associations between BMI and TUG, and also between IL-6, IL-10 or IL-12p70 in all time points assessed. Age correlated positively with the IL-12p70/IL-10 ratio at PRE and POST-TR, whilst GS correlated positively with IL-10 at PRE. Several other positive correlations between anthropometrics, physical function tests, and cytokines were evidenced at PRE, POST, and POST-TR, whereas negative correlations between GS and TUG at POS and POST-TR, GS and Age or IL-12p70/IL-10 ratio at PRE, GS and IL-8/IL-10 ratio at POST. And exclusively at POST, the muscle strength, assessed by HG, and IL-12p70, or the ratios IL-12p70/IL-10 and IL-8/IL-10 were found.

Table 2. In the older women group, significant associations found by using Spearman's correlation coefficient analysis between the data concerning physical function tests [(assessed by gait speed—(GS) and Timed-up-go (TUG)] and muscle strength (assessed by handgrip—HG), systemic cytokine levels, and the ratio between pro- and anti-inflammatory cytokines obtained in the different occasions: before COVID-19 pandemic (PRE), after 12 months of interruption (POST, detraining phase), and also after 10 months of resuming of regular practice of combined exercise training program (POST-TR). The level of significance was established at 5% (p<0.05).

Parameters	Older women group (n=18)							
	2019 (PRE)		2021 (POST)		2022 (POST-TR)			
	<i>rho-value</i>	<i>p-value</i>	<i>rho-value</i>	<i>p-value</i>	<i>rho-value</i>	<i>p-value</i>		
BMI X TUG	0.60	0.009	BMI X TUG	0.65	0.009	BMI X TUG	0.53	0.025
Age X GS	— 0.56	0.016	GS X TUG	— 0.63	0.012	GS X TUG	— 0.54	0.020
Age X IL-12p70/IL-10	0.58	0.012	GS X IL-8/IL-10	— 0.51	0.044	BMI X TNF- α	0.48	0.045
GS X IL-12p70/IL-10	— 0.57	0.014	TUG X IL-8	0.55	0.034	Age X IL-6	0.53	0.023
GS X IL-10	0.50	0.036	HG X IL-10	0.49	0.041	Age X IL-12p70	0.49	0.038
IL-6 X IL-10	0.99	<0.001	HG X IL-12p70	— 0.51	0.030	Age X IL-12p70/IL-10	0.48	0.044
IL-6 X IL-12p70	0.99	<0.001	HG X IL-12p70/IL-10	— 0.51	0.032	IL-6 X IL-10	0.77	<0.001
IL-6 X TNF- α	0.99	<0.001	HG X IL-8/IL-10	— 0.56	0.016	IL-6 X IL-12p70	0.58	0.011
IL-6 X IFN- γ	0.91	<0.001	IL-6 X IL-10	0.81	<0.001	IL-10 X IL-12p70	0.70	0.001
IL-10 X IL-12p70	0.99	<0.001	IL-6 X IL-12p70	0.82	<0.001			
IL-10 X TNF- α	0.99	<0.001	IL-10 X IL-12p70	0.99	<0.001			
IL-10 X IFN- γ	0.91	<0.001	IL-8 X TNF- α	0.64	0.004			
IL-12p70 X TNF- α	0.99	<0.001	IL-8 X IFN- γ	0.59	0.011			
IL-12p70 X IFN- γ	0.90	<0.001	TNF- α X IFN- γ	0.86	<0.001			

Concerning the older men group, as shown the Table 3, positive correlations between IL-10 and IL-6 or IL-8, and a negative correlation between TUG and IL-10 were found at PRE and POST-TR. The GS and IFN- γ were negatively correlated in PRE. The HG negatively correlated with the TNF- α /IL-10 ratio at POST and with TNF- α , IFN- γ , and IFN- γ /IL-10 ratio at POST-TR, in contrast to positive correlation with IL-10 at POST-TR. Other positive and negative correlations were found between anthropometrics, physical function tests, and cytokines on all occasions assessed here. Table S1 shows the values of cytokines and the ratios between IL-10 and the inflammatory cytokines.

Table 3. In the older men group, significant associations found by using Spearman's correlation coefficient analysis between the data concerning physical function tests [(assessed by gait speed—(GS) and Timed-up-go (TUG)] and muscle strength (assessed by handgrip - HG), systemic cytokine levels, and the ratio between pro- and anti-inflammatory cytokines obtained in the different occasions: before COVID-19 pandemic (PRE), after 12 months of interruption (POST, detraining phase), and also after 10 months of resuming of regular practice of combined exercise training program (POST-TR). The level of significance was established at 5% (p<0.05).

Parameters	Older men group (n=7)					
	2019 (PRE)		2021 (POST)		2022 (POST-TR)	
	Parameters	<i>rho-value</i>	Parameters	<i>rho-value</i>	Parameters	<i>rho-value</i>

	<i>rho-</i> <i>value</i>	<i>p-</i> <i>value</i>		<i>rho-</i> <i>value</i>	<i>p-</i> <i>value</i>		<i>rho-</i> <i>value</i>	<i>p-</i> <i>value</i>
GS X IFN- γ	- 0.78	0.049	Age X TUG	0.81	0.029	BMI X IL-6	0.76	0.049
TUG X IL-10	- 0.79	0.048	HG X TNF- α	- 0.79	0.048	TUG X IL-10	- 0.87	0.010
IL-6 X IL-10	0.96	0.003	IL-10 X IL-12p70	0.95	<0.001	TUG X IL-8	0.81	0.027
IL-6 X IL-12p70	0.86	0.024	IL-10 X IFN- γ	0.90	0.006	HG X TNF- α	- 0.79	0.033
IL-8 X IL-10	0.79	0.048	IL-12p70 X IFN- γ	0.93	0.002	HG X IFN- γ	- 0.83	0.022
IL-10 X TNF- α	0.86	0.024	TNF- α X IFN- γ	0.85	0.016	IL-8 X IL-10	0.80	0.032
						IL-8 X TNF- α	0.77	0.041
						IL-10 X TNF- α	0.79	0.035
						TNF- α X IFN- γ	0.85	0.015

Discussion

In general, our results showed that: (i) whether one side, the interruption of regular practice of physical exercises for one year (detraining period) can negatively impact not only physical function test performance, which could putatively impair the ability to perform some daily activities, as well as the control of systemic inflammatory status, since the occurrence of inflammaging was mainly evidenced during this period, (ii) in another side, ten months of regular engagement in a combined exercise training program showed a prominent capacity to positively benefit these parameters in the participants in this study, particularly by reverting or even mitigating the inflammaging progress, even though some physical function tests did not show the same improvements. Additionally, (iii) it is worth highlighting that, following our results, it was possible to observe that not only the interruption but also the return of the regular practice of combined exercise training differently impacted older women and older men enrolled in the present study.

Since the aging process of skeletal muscle, immune, and inflammation occurs differently in men and women[8,38], the aged people participant here was assessed separately. Besides, it is worth mentioning that the volunteers were robust[39] and very active pre-pandemic, based on the observation that they performed 600 minutes of moderate-intensity activities per week, and presented positive adaptations in physical and inflammatory aspects, as shown in our previous study[40]. Thus, even with the decrease in physical performance during the COVID-19 pandemic, the data found in the assessments of the physical characteristics, particularly BMI, handgrip, and TUG tests, suggest that volunteers maintained adequate functional ability[41].

It was reported that lockdown related to the COVID-19 pandemic increased sedentary behavior and detraining[24,25] which could putatively justify the reduction in physical performance found here. Additionally, some results of this study can reinforce already-known associations, such as the influence of age and BMI in physical tests involving body movement, and the fact that GS and TUG tests can evaluate similar muscle functions[30]. In this sense, recent studies aimed to observe whether detraining older people during the COVID-19 pandemic would nullify the physical and metabolic benefits achieved in supervised physical exercise programs prior to the pandemic. Among these studies, one was carried out with a group of thirty older men who were initially submitted to training with resistance exercises for twelve weeks followed by the same period of detraining, and a decrease in their muscular adaptations was found after the detraining period by reducing 5%, 15%, and 17% of strength and muscular power, and type II muscle fibers, respectively, in the vastus lateralis muscle[42]. In another report, in which fifty-three healthy older adults performed a resistance physical exercise program for six months, followed by one year of detraining and a sedentary lifestyle, it was found a significant loss of muscle performance in association with a remarkable decrease in the number of type II fibers, muscle fiber nuclei, and satellite cells, evidenced by the muscle biopsy[43]. As expected, our results showed differences between the groups, since after twelve months of detraining the older women group showed reductions of 37% and 7%, in GS and TUG tests, respectively, and the older men group showed a decrease around of 10% in HG. Thus, specifically during this period, whereas the older women group lost lower limb physical performance, the older men group decreased upper limb performance. Some possible explanations

for these discrepant observations could be associated with the fact that: (i) although the literature mentions that women lose muscle strength earlier than men[44] during aging, the absence of significant HG strength loss in the women's group, in the detraining phase, could be related to cultural reasons, since during the confinement period these women were possibly involved with domestic tasks, which could supposedly attenuate the reduction of muscle strength in this group[45]; besides (ii) by generally being stronger[44], the ceiling effect[46] could have occurred in the application of the physical tests in the older men, as the GS and TUG, have low correlation with classic strength tests[30].

Regarding data obtained ten months after resuming physical exercise, a remarkable observation found here was associated with the fact that the return to physical training did not "reverse" or mitigate the effect of detraining in this strength parameter on both groups. A study demonstrated that twelve weeks of regular physical training, after eight weeks of detraining, should be sufficient to recover the architecture, strength, and muscle power[42]. Likewise, notable neuromuscular adaptations could be achieved, in older adults, after ten months of regular physical training practice[47]. Despite the physical aspects of 'muscle memory'[48] were not evaluated here, the fact is that none of the volunteer groups, after 10 months of training, reached pre-pandemic HG results, even if it could be expected[49]. Regarding muscle plasticity after a long period of detraining, it is known that epigenetic factors are fundamental[50], so the significant reduction in muscle strength assessed by the HG, an average of 10% in both groups, could be supposedly associated not only with the aging process but also with behavioral, dietary, and nutritional issues related to the pandemic, recalling that none of the volunteers presented clinical symptoms related to COVID-19 during the study.

Each day, studies present different biomarkers that can be useful to determine both successful aging and vulnerabilities to frailty, as well as the physical performance and inflammaging phenomenon[2]. In this respect, it is known that cytokines present a myriad of actions and older adults who regularly practice physical exercises presented an exemplary phenotype of the best regulations of inflammaging[19]. Although the systemic levels of IL-6, IL-8, IL-12p70, and IL-10 were higher at PRE, and these findings putatively suggested that long-standing physical exercise would drive the systemic state toward a pro-inflammatory direction, the data obtained in the ratios between pro- and anti-inflammatory cytokines, in general, demonstrated an inflammatory status regulated pre-pandemic[40], a significant deregulation during detraining phase[28,51], and a "return" of regulated status with the resumption of exercise training[19]. Interestingly, a study carried out with twenty-two healthy and sedentary older men, with an average age of 62 years, showed that six weeks of high-intensity aerobic training was capable of reducing serum levels of C-reactive protein and IL-6 to values similar to those of older adult athletes who practiced competitive sports (running, swimming and cycling) for more than thirty years[27].

Based on our results, these variations in inflammatory status found in both groups can closely be related to the anti-inflammatory properties of IL-10[13], which were reduced in the detraining phase and increased with the resumption of exercise training. It is known that exercise training can improve the circulating IL-10 levels, which strengthens its role in the regulation of inflammaging[19]. During physical exercise, muscle contraction activates the transcription of several genes, mainly IL-6 that, among some actions, promote IL-10 releases, which lead to the control of inflammation in this context[17]. Particularly, our data concerning the ratios between the pro-inflammatory cytokines IL-6, IL-8, TNF- α , and IFN- γ , with IL-10, reinforces this remarkable capacity of exercise training in improving IL-10 levels, which, consequently, can favor the regulation of the systemic inflammatory state, including in older population. Likewise, the increase in the IL-12p70/IL-10 ratio in 2022 (at POST-TR), not only is interesting but also could represent a lack of systemic inflammatory control. However, the literature claims that IL-12p70 is pivotal both in increasing the immune cells' activity, especially Natural Killer (NK) cells, and its increase have been associated with successful aging[52].

Taking together, our data allows us to suppose that the interruption of regular physical exercise during the COVID-19 pandemic, in this physically active older population, led to a prominent imbalance in systemic cytokine levels that favored the progression of the inflammatory phenomenon,

as previously reported in sedentary older people and detrained individuals [20,53]. Corroborating these studies, a group of sixty-four older adults had a reduction in the functions of CD3, CD4 and CD8 T cells, as well as Natural Killer cells, eleven months after social distancing and detraining associated with COVID-19 pandemic[28]. It is paramount to point out that this finding is very important due to the fact that it has been reported that inflammaging can become aged people to be more susceptible to negative immunological outcomes, with remarkable impairment in both innate and acquired immune responses[12,54].

Regarding the correlations between physical function tests and cytokines, during the detraining period, it was found that better physical performance was associated with the best regulation of the systemic inflammatory state. Despite the older women group did not present significant alterations in the HG results, between pre-pandemic and during the detraining period, the correlations found illustrated that the stronger and faster volunteer presented advantages in the systemic inflammatory profile. Specifically to the older men group, the HG test was the most important evaluation both in the perception of strength loss during the detraining period and in the interpretation that greater muscle strength was associated with a balanced systemic inflammatory status. Indeed, the association between muscle strength and positive health outcomes has been the subject of investigation for a long time and seems to be a 'two-way street'[17]. Based on these aspects and our results, we can suggest that not only the lifestyle alterations associated with the COVID-19 pandemic could negatively influence the benefits of years of regular physical training practice, but also those physically fitter older adults might be less affected, as previously described in the literature[19,23].

Interestingly, the associations between physical performance tests and the inflammatory phenomenon found after resuming training in the group of older men can reinforce the previous suggestion that stronger and faster volunteers might generate better conditions to mitigate the development and progression of inflammaging[23,27]. However, the lack of associations between these parameters in older women can suggest that ten months of resuming physical training is not enough to promote the same regulatory effect found in older men, although it was sufficient for the ratios between pro-inflammatory (IL-6, IL-8, TNF- α) and anti-inflammatory (IL-10) cytokines to become similar to the pre-pandemic period. These results strengthen the differences between genders during aging, although the increase in BMI and age can supposedly affect this balance[40]. Therefore, these differences between the groups of volunteers during detraining and the resumption of supervised physical training, during the COVID-19 pandemic, are unprecedented and can corroborate the literature, as both in vitro and/or in vivo studies have shown that men and women are differently affected by pro-inflammatory cytokines[38,55].

It is worth mentioning that our results reinforce the perception that multiple factors are involved in the inflammaging both in men and women. For instance, it has been reported that hormonal, genetic, and epigenetic factors could favor the generation of robust anti-inflammatory responses in women[38]. Furthermore, there is an understanding that the cytokine network is pleiotropic, as it presents redundant and overlapping activities associated with chemotaxis, proliferation, development, activation, and migration of different types of cells (immune, neuronal, muscular, and vascular endothelial, among others), in addition to demonstrating positive or negative actions, particularly related to their levels and chronicity[56].

Although we were able to show interesting findings, some limitations of the study are: (a) the impossibility of completing the study with the initially eligible volunteers (n=90) due to issues related to the COVID-19 pandemic (change of address and the fear of SARS-CoV-2 contamination, as previously mentioned); (b) the discrepant number of participants in each volunteer group; (c) the absence of a comparison group comprising sedentary older individuals, encompassing both women and men; (d) insufficient information regarding the participants' nutritional habits and levels of physical exercise; (e) a lack of data from other physical function tests; and (f) the absence of data collected immediately upon the resumption of supervised physical training.

Conclusions

The findings of this study lead us to propose that: (a) the regular practice of combined exercise training can benefit and help in regulating systemic inflammatory status; (b) the period of detraining significantly impacted physical functional capacity and contributed to the progression of the inflammaging process; and also (c) older individuals, both women and men, were differently influenced by both the detraining and the resumption of exercise training.

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