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Lifelong exercise practice and immunosenescence: Master athletes cytokine response to acute exercise

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ABSTRACT

The study aimed to analyze the effects of aging and lifelong training on the main pro- and anti-inflammatory cytokines, and the impact of acute exercise on the expression of these cytokines. Thirty-nine participants were allocated into 3 groups: young (31.8 ± 3.00 yrs.), middle-aged (54.2 ± 5.9 yrs.) and master athletes (53.1 ± 8.8 yrs.) and performed a maximal incremental test on a cycle ergometer. Blood samples were obtained before (Pre), 10 min post-exercise (Post) and 1 h post-exercise (Post 1 h). Mean VO_{2max} was similar for master athletes and youngers and higher compared to the middle-aged group. Resting values of the IL-1ra, IL-1β, IL-4, and IL-8 were higher in master athletes compared to the young and middle-aged groups ($P < 0.01$), while the highest values of IL-10 and IL-17 were observed for the youngers (29.49 ± 18.00 pg/mL and 66.24 ± 23.23 pg/mL, respectively) with the middle-aged group showing the lowest values (2.13 ± 1.40 pg/mL). Acute exercise effects (Post) were observed for IL-1β in the master athletes group, IL-6 in the young group and IL-4 for both groups ($P < 0.05$). No Post effects were observed for the middle-age group for all cytokines. The TNF-α/IL-10 ratio was higher in all moments for the middle-aged ($P < 0.05$). In conclusion, lifelong training helps to maintain the balance of pro- and anti-inflammatory cytokines, together with IL-10 levels close to those found in young adults.

1. Introduction

A prolonged pro-inflammatory state called “inflammaging” characteristic of many chronic diseases is often present in older populations [1–3]. Intracellular signaling leading to inflammation is controlled by extracellular molecular regulators, including members of the cytokine families that mediate both immune cell recruitment and complex intracellular signaling control mechanisms that characterize inflammation [4]. Thus, aging may result in chronic low-grade inflammation, that is defined by increased concentration in pro-inflammatory mediators, such as tumor necrosis factor-alfa (TNF-α), interleukin (IL)-6, IL-1β, Interferon-gamma (IFN-γ) and increased numbers of white blood cells [5,6]. Studies have shown that aging is associated with the development of a low-grade pro-inflammatory state of a vast majority of individuals and that such condition is a significant risk factor for numerous diseases, physical and cognitive disability, fragility and death [7–9]. In the other hand, physical exercise has been shown to exert an anti-inflammatory effect, suggesting that physical exercise training could be an efficient counter-measure to either prevent or delay the onset of some chronic diseases associated with this low-grade inflammatory status [10–15]. The anti-inflammatory effect of physical exercise training can be mediated not only through a reduction of visceral fat mass (with a subsequent decrease in the production and release of pro-inflammatory adipokines) but also through the induction of an anti-inflammatory environment, with each exercise series [16–18].

In this context, master athletes with a lifelong practice of regular exercise training are an exciting group that could be used as a unique model to study aging in the context of optimized behavior regarding active, healthy aging [19,20]. Although the studies already published on master athletes and the beneficial effects of physical exercise training on aging is growing [19,21–24], the consequences of lifelong training on aging are not clear, in large part due to the difficulty in dissociating natural aging effects from those brought by a lifespan

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practice of regular exercise.

This study aimed to investigate the effects of aging and lifelong training on VO_{2max}, basic hematological parameters and pro- and anti-inflammatory cytokine balance by comparing master athletes, a group of age-matched healthy middle-aged group and a healthy non-trained young group, submitted to an incremental cycling exercise. We hypothesize that lifelong training has a positive effect on the balance of pro- and anti-inflammatory cytokines and this is relevant for the preservation of health and quality of life.

2. Materials and methods

2.1. Participants

A group of 20 master athletes (53.15 ± 8.8 yrs.), and two groups of non-athletes, 9 young adults (31.7 ± 3.0 yrs.) and 10 middle-aged adults (54.2 ± 5.9 yrs.) were recruited for this study. All participants were healthy individuals without any illness, determined via a questionnaire. The participants of young and middle-aged groups had not participated in regular physical training for the past 20 years. A self-reported survey was used for master athletes, to access their sports history. In this study, master athletes were defined as individuals aged over 40 years old and who had a minimum of 20 years of regular participation in competitive sports and remained conditioned by over 20 years of training throughout life. Athletes who trained or competed sporadically or older competitors who had resumed their physical training after long periods of physical inactivity were not eligible for this study. The master athletes group was composed of athletes currently participating in national and international competitions from three sports: athletics, swimming, and judo. On average, master athletes had experience in training of 24.6 ± 1.83 years. Each year, the athletes trained 10.27 ± 0.24 months and approximately 5 h per week (5.45 ± 0.42 h) in each month trained. All participants provided their written, informed consent to participate in the study, which was approved by the Ethics and Human Subjects Review Board at the Faculty of Sports Science and Physical Education, University of Coimbra.

2.2. Anthropometric measures

Each participant's height and body mass were determined using a stadiometer Harpenden, model 98.603 (Holtain Limited, Crosswell, UK) and a calibrated digital scale Seca model 770 (Seca, Hamburg, Germany), respectively and the Body Mass Index (BMI) calculated.

2.3. VO_{2max} exercise testing

The responses to an incremental exercise test were determined using a continuous progressive exercise test on a cycle ergometer (Lode, Groningen, Netherlands). Participants started cycling with a load of 75 W for a 3 min warm-up stage followed by 25 W increments every 3 min until volitional exhaustion. The participants cycled at a constant rate between 80 and 85 rpm. Breath-by-breath measurements of O_2 and CO_2 were recorded continuously throughout the test, by using a gas analyzer with a cycle to cycle recording system (Quark CPET, COSMED, Rome, Italy). Participants breathed continuously through a facemask. The O_2 and CO_2 analyzers were calibrated with known gases by the manufacturer's guidelines (COSMED, Rome, Italy). The adopted criterion to define the VO_{2max} attainted was proposed previously [25]. Heart rate was monitored throughout the test by short-wave telemetry (COSMED, Rome, Italy).

2.4. Blood collection and cytokine assays

Venous blood samples (16 mL) were obtained from the antecubital vein by venipuncture collected before exercise (Pre), 10 min post-exercise (Post), and 1 h post-exercise (Post 1 h). The blood was collected into tubes containing EDTA and immediately analyzed for leukocytes, erythrocytes, granulocytes, lymphocytes, monocytes, platelets, mean platelet volume (MPV), hemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), hematocrit (Ht), and mean corpuscular hemoglobin (MCH), with a full cell count, using a blood analyzer (Beckman Coulter, Inc., Miami, Florida). All blood cells counts were corrected for changes in plasma volume (Pv) [26]. The remaining blood sample was centrifuged at 4 °C for 10 min at 1500 g. After centrifugation, plasma was stored at −80 °C until use for the cytokine levels assays.

Plasma levels of IL-1ra, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-15, IL-17 and TNF-α, were determined using Duo set enzyme-linked immunosorbent assay (ELISA) kits (Quantikine, R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. Serum C-reactive (CRP) protein levels were determined using Horiba Medical Pentra C200 analyzer (Kyoto, Japan) following the manufacturer's instructions. The TNF-α/IL-10 ratio was calculated to assess the inflammatory balance [27].

2.5. Statistical analysis

Statistical analyses were performed using SPSS statistical package software version 23 for Mac (Chicago, IL, USA). Assumptions of homogeneity were checked. Effects of age (middle-aged vs. young), training (master athletes vs. young) and age * training interaction (master athletes vs. middle-aged) were tested using two-way ANOVA, with Bonferroni multiple comparison *post hoc* testing. The effects of exercise over time (i.e., Pre, Post and post 1 h) on hematological parameters, leukocyte counts, and cytokine levels were tested by two-way repeated measures ANOVA for normally distributed data or by Friedman's test for data that were not normally distributed. Significant effects for the ANOVA or Friedman tests were analyzed further using Bonferroni correction or Dunn's test for multiple comparisons, respectively. Statistical significance was accepted at $P < 0.05$. Data are presented as mean + SD. Correlations between test time and changes in cytokines were assessed using the Spearman's rank correlation coefficient.

3. Results

3.1. Differences in anthropometric profile, VO_{2max} and hematological parameters at baseline

There were no significant differences in stature, body weight and BMI between groups (Table 1).

VO_{2max} was similar for the young and master athletes groups and higher when compared to the middle-aged group ($P < 0.001$; Table 1). The time to complete the exercise test was similar between master athletes and young individuals, with both groups showing higher values than the middle-aged group ($P < 0.05$; Table 1). Significant differences were found for HR_{max}, with the young individuals presenting the highest values than middle-aged and master athletes ($P < 0.001$; Table 1). No differences were observed for Lac_{max} between groups (Table 1).

The young group showed higher values for lymphocyte and granulocyte counts when compared to the other groups at baseline ($P < 0.05$; Table 2). No differences between groups were found for all the other hematological parameters at baseline ($P > 0.05$; Table 2).

3.2. Differences in cytokine profile at baseline

TNF-α levels were undetectable for 4 participants in young, 2 participants in middle-aged and 5 participants in master athletes group. No differences were found for IL-6 and TNF-α between groups at baseline (Fig. 1G and I, respectively). Resting values of the IL-1ra, IL-4, IL-8 and IL-1β were higher in master athletes compared to the young and

Table 1
Participant characteristics.

	Young (N = 9)	Middle-aged (N = 10)	Masters athletes (N = 20)
Age (years)	31.8 ± 3.00[a]	54.2 ± 5.9	53.1 ± 8.8
Stature (cm)	173.4 ± 9.16	168.6 ± 8.5	170.7 ± 5.3
Body mass (kg)	65.2 ± 11.2	70.5 ± 12.9	74.9 ± 15.1
BMI (kg m^{-2})	21.8 ± 2.0	24.3 ± 3.2	25.1 ± 4.6
Test time (min)	18.2 ± 7.4	10.3 ± 3.5[a]	16.8 ± 5.5
Lac_{max} (mmol L^{-1})	11.2 ± 3.1	9.2 ± 2.1	9.1 ± 2.1
HR_{max} (bpm)	187.8 ± 4.1[a]	166.3 ± 6.0	166.6 ± 8.7
VO_{2max} (ml min^{-1})	3142.8 ± 732.8	2076.0 ± 355.5[a]	2916.5 ± 661.1
VO_{2max} (ml kg min^{-1})	46.82 ± 6.05	29.29 ± 4.14[a]	40.33 ± 11.15

Abbreviations: BMI = Body Mass Index; VO_{2max} = Maximal Oxygen Consumption; Lac_{max} = Maximal lactate concentration obtained in the end of the test. HR_{max} = Maximal heart rate obtained in the end of the test. [a]P < 0.05 compared to other groups.

middle-aged groups (P < 0.01; Fig. 1A, E, F, H, respectively). The concentration of IL-1ra in master athletes and young groups were 10-fold higher to IL-1β; meanwhile, this proportion was lower (7-fold) in the middle-aged group.

The IL-10 and IL-17 concentrations were different between groups at baseline, with the young group presenting the highest values, followed by master and middle-aged (P < 0.01; Fig. 1B, D, respectively). Although no differences for IL-15 at Pre were found between groups a tendency for increased levels appeared with the master athletes showing a difference in mean values for IL-15 higher than 20 pg/ml when compared to the other groups at Pre (Fig. 1C).

3.3. Acute responses for hematological parameters

The values of erythrocytes, hemoglobin, hematocrit, and packed cell volume are presented in Table 2. There were no differences between the pre- and post-exercise values for hematocrit and hemoglobin. The values of erythrocytes after exercise increased in all groups (P < 0.05). The erythrocytes' indices (MCV, MCH, MCHC) also changed, decreasing in Post for all groups (P < 0.01). All values were restored to baseline in post 1 h (Table 2).

Exercise induced a significant leukocytosis in all groups (P < 0.01). This increase was also observed for lymphocytes in master athletes and middle-aged groups (P < 0.001). Acute exercise also increased monocytes count in middle-aged, and granulocytes count in master athletes (P < 0.01; Table 2). Platelets and relative indices (MPV) increased after exercise in all groups (P < 0.01; Table 2). These values returned to baseline at post 1 h.

3.4. Acute responses in cytokine profile

Acute exercise effects (Post) were observed for IL-1β in the master athletes group, IL-6 in the young group and IL-4 for both groups (P < 0.05; Fig. 1H, G and E, respectively). No Post effects were observed for the middle-age group for all cytokines. As, IL-6, IL-8, and IL-10 decreased at Post 1 h in comparison to Post in the young and master athletes groups (P < 0.05; Fig. 1G, F and B, respectively). Also, IL-6, IL-8 and IL-10 decreased for the master athletes only between Pre and Post 1 h (P < 0.05; Fig. 1G, F and B, respectively). IL-4 concentration decreased at 1 h Post for the master athletes when compared to Post (P < 0.0001; Fig. 1E).

IL-15 concentration was higher for master athletes in Post and 1 h-Post when compared to the Young at Post and to Young and Middle-age groups at Post and 1 h-Post (P < 0.05, Fig. 1C). IL-17 showed higher values for the Young group at 1 h-Post when compared to the master athletes (P < 0.05, Fig. 1C).

The level of IL-10 was lower in the middle-aged group compared to the master athletes group at all time points (P < 0.01; Fig. 1B). Age accounted for 38% (P < 0.001) to the differences observed in IL-10. Training by itself accounted for 27% of the variation in IL-10 (P < 0.01) and the interaction age * training status accounted for 32% (P < 0.001) of the IL-10 differences seen between the groups. Post-exercise levels of IL-10 and IL-1β was positively correlated with the duration of the incremental test to exhaustion (r = 0.470, P < 0.01;

Table 2
Hematological parameters, leucocytes count and erythrocytes' indices.

	Pre	Post	1 h
Erythrocytes ($\times 10^6/\mu L$)			
Young	5.04 ± 0.39	5.46 ± 0.50*	5.00 ± 0.40&
Middle-aged	4.90 ± 0.50	5.16 ± 0.50*	4.84 ± 0.47&
Masters athletes	5.16 ± 0.37	5.34 ± 0.43*	5.10 ± 0.38&
Hemoglobin (g/dL)			
Young	14.48 ± 1.19	12.74 ± 3.31	14.62 ± 1.47
Middle-aged	13.82 ± 1.51	13.30 ± 1.59	13.63 ± 1.49
Masters athletes	14.87 ± 1.14	14.51 ± 1.47	15.16 ± 1.37&
Hematocrit (%)			
Young	44.78 ± 3.35	42.63 ± 5.00	45.21 ± 4.55
Middle-aged	43.27 ± 3.97	41.64 ± 4.35	42.98 ± 4.20
Masters athletes	45.41 ± 2.57	44.10 ± 3.65	46.52 ± 3.27&
Leucocytes ($\times 10^3/\mu L$)			
Young	6.28 ± 1.10	10.53 ± 1.46*	6.27 ± 0.83&
Middle-aged	6.84 ± 1.22	9.12 ± 1.98*	6.57 ± 1.73&
Masters athletes	7.55 ± 1.90	9.73 ± 3.17*	7.36 ± 2.09&
Lymphocytes ($\times 10^3/\mu L$)			
Young	3.31 ± 0.66[a]	4.02 ± 0.41	2.39 ± 0.39&
Middle-aged	2.31 ± 0.46	3.64 ± 0.98*	2.21 ± 0.46&
Masters athletes	2.35 ± 0.69	3.42 ± 1.40*	1.97 ± 0.74&
Monocytes ($\times 10^3/\mu L$)			
Young	0.36 ± 0.17	0.51 ± 0.18	0.38 ± 0.22
Middle-aged	0.38 ± 0.17	0.62 ± 0.18*	0.36 ± 0.10&
Masters athletes	0.47 ± 0.19	0.59 ± 0.19	0.40 ± 0.18&
Granulocytes ($\times 10^3/\mu L$)			
Young	5.93 ± 1.11[a]	5.33 ± 0.51	6.82 ± 1.02&
Middle-aged	4.16 ± 0.98	4.87 ± 1.20	4.01 ± 1.30&
Masters athletes	4.60 ± 1.37	5.39 ± 2.02*	4.87 ± 1.71
Platelets ($\times 10^3/\mu L$)			
Young	255.7 ± 68.3	326.3 ± 79.5*	261.6 ± 62.3&
Middle-aged	248.3 ± 97.4	317.5 ± 87.3	267.4 ± 65.2&
Masters athletes	239.8 ± 79.2	285.0 ± 85.6*	255.2 ± 84.4&
MCV (fL)			
Young	88.97 ± 2.89	77.93 ± 6.02*	90.28 ± 3.76&
Middle-aged	88.34 ± 2.74	80.75 ± 4.20*	88.85 ± 4.82&
Masters athletes	88.86 ± 4.09	83.78 ± 8.18*	92.27 ± 7.44&
MCH (pg)			
Young	28.74 ± 1.31	25.22 ± 2.02*	29.25 ± 1.66&
Middle-aged	28.14 ± 0.74	25.76 ± 1.49*	28.17 ± 1.82&
Masters athletes	28.72 ± 1.43	27.04 ± 2.71*	29.50 ± 2.19&
MCHC (g/dL)			
Young	32.32 ± 0.92	28.23 ± 2.18*	33.15 ± 2.30&
Middle-aged	31.88 ± 0.97	29.20 ± 1.71*	31.88 ± 1.79&
Masters athletes	32.50 ± 0.86	30.66 ± 2.23*	33.67 ± 1.81&
MPV (fL)			
Young	8.31 ± 0.57	7.54 ± 0.72*	8.36 ± 1.00
Middle-aged	8.31 ± 0.70	7.67 ± 0.73*	8.13 ± 1.02
Masters athletes	8.66 ± 1.05	8.04 ± 0.99*	8.25 ± 1.33

Young (N = 9); Middle-aged (N = 10); Masters athletes (N = 20). Pre (before exercise), Post (into 10 min post-exercise), and 1 h (1 h post-exercise). *P < 0.05 Pre vs Post. &P < 0.05 Pre vs 1 h. [a]P < 0.05 compared to other groups. Abbreviations: MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; MPV = Mean Platelet Volume.

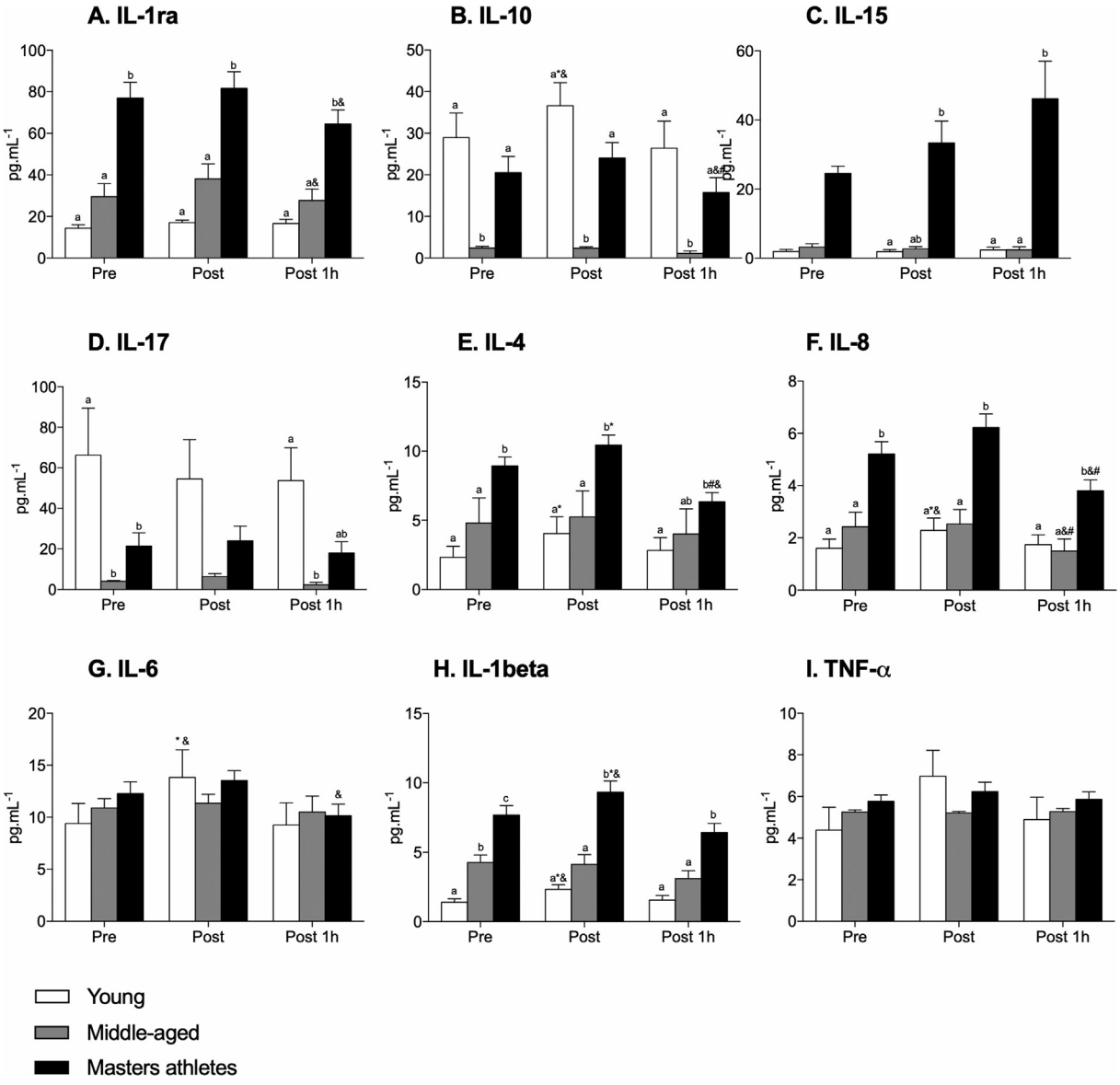

Fig. 1. Plasma cytokines levels for (A) IL-1ra, (B) IL-10, (C) IL-15, (D) IL-17, (E) IL-4, (F) IL-8, (G) IL-6, (H) IL-1β, (I) TNF-α and in response to maximal exercise. Young (N = 9); Middle-aged (N = 10); Master athletes (N = 20). Pre (before exercise), Post (into 10 min post-exercise) and Post 1 h (1 h post-exercise). $^{*}P < 0.05$ Pre vs Post; $^{\#}P < 0.05$ Pre vs 1 h; $^{\&}P < 0.05$ Post vs 1 h. Different letters mean significant difference between groups.

r = 0.436, P < 0.05; respectively). Also, we found a positive correlation between IL and 10 levels and VO_{2max} (0.366, P < 0.05).

TNF-α/IL-10 ratio was higher at all time points for the middle-aged group when compared to master athletes and young groups (P < 0.001; Fig. 2). TNF-α/IL-10 ratio also increased in response to exercise in the middle-aged group in Post 1 h (P < 0.05; Fig. 2). For the TNF-α/IL-10 ratio, age accounted for 50% of the variation of this index. The interaction age * training accounted for 60% of the changes in the TNF-α/IL-10 ratio (Fig. 2).

Serum concentration in CRP was undetectable for 1 participant in young, 1 participant in middle-aged and 5 participants in master athletes group. CRP levels showed no significant differences between groups at baseline and after exercise (Fig. 3).

4. Discussion

The objective of this study was to analyze the effects of aging and lifelong training on the main pro- and anti-inflammatory cytokines, as well as the impact of acute exercise training on the expression of these cytokines. In this study, we compared VO_{2max}, basic hematological parameters and pro- and anti-inflammatory cytokines in master athletes, their age-matched non-trained counterparts and young individuals. Master athletes had similar VO_{2max} values to those of healthy young adults. The levels of pro- and anti-inflammatory cytokines were different according to training history and age. At rest, the levels of pro-inflammatory IL-8 and IL-1β and anti-inflammatory IL-1ra and IL-4 cytokines were higher in the master athletes group compared to the young one. The IL-6 and TNF-α plasma concentrations were not different between young and middle-aged individuals. Interestingly, aging seemed to have a most pronounced effect on IL-10 and IL-17 levels.

VO_{2max} is strongly and positively related to endurance performance in highly trained runners of varied ages, where reductions in endurance performance with age are strongly associated with corresponding declines in VO_{2max} [20]. The mechanisms responsible for the decrease in VO_{2max} with aging may be related to failure to maintain regular training, namely by decreases in exercise intensity, duration, and frequency of training sessions. Within the physiological context, regarding the mechanisms responsible for the reduction in VO_{2max} with age, it

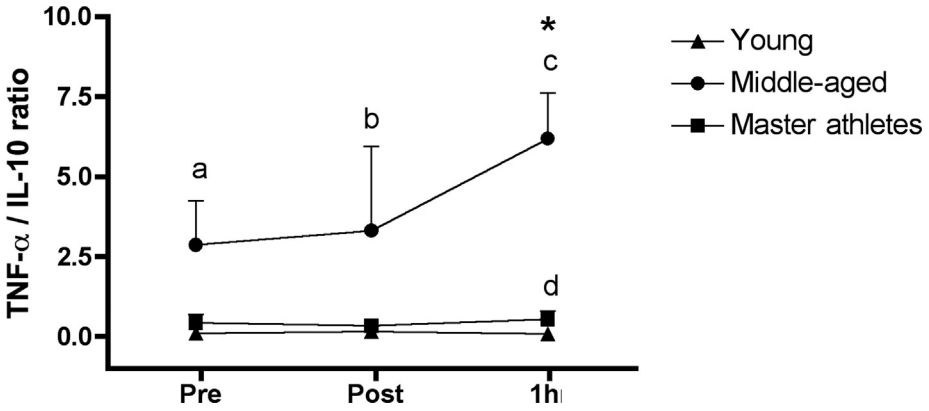

Fig. 2. TNF-α/IL-10 ratio in response to maximal exercise. Young (N = 5); Middle-aged (N = 8); Master athletes (N = 15). Pre (before exercise), Post (into 10 min post-exercise) and Post 1 h (1 h post-exercise). $^{*}P < 0.05$ Pre vs Post; $^{\#}P < 0.05$ Pre vs 1 h; $^{\&}P < 0.05$ Post vs 1 h. Letters mean significant difference between other groups.

appears that decreases in both maximal cardiac output and maximal arterio-venous O_2 difference may play a role [20]. However, the age-dependent reduction in VO_{2max} with aging seems to be attenuated in individuals practicing regular exercise training as showed by our group [15]. Our results confirm previous research that found similar VO_{2max} values between master athletes and healthy young adults, contrary to those values referenced in other studies with middle-aged sedentary individuals [19,20,23]. The training history reported by master athletes could contribute to explain how their VO_{2max} values were similar to those shown by the healthy young adults.

Regular endurance training may play a role in lowering some markers of systemic inflammation, and in regulating important metabolic and physiological muscle parameters that change with aging [19]. In this study, IL-6 levels were not altered by aging or training that may reflect the prevalent role of health status over the chronological age of individuals [28]. IL-6 can activate molecular signaling cascades leading to an inflammatory response mediated by the IKK/NFKB pathway. IL-6 triggers the activation of IKB kinase (IKK) enzyme complex and subsequently the translocation of the protein complex NF-KB into the nucleus. This results in the transcription of target genes for inflammatory immune reaction including cytokines like IL-6, TNF-α, and IL-15. However, the transient rise in IL-6 also appears to be responsible for the production of anti-inflammatory mediators like IL-10, IL-1ra, and cortisol [29].

Furthermore, elevated levels of IL-6 from skeletal muscle stimulate an anti-inflammatory signaling cascade that inhibits the secretion of pro-inflammatory cytokines like TNF-α and IL-1β, suppresses the secretion of CRP (a general and unspecific marker for systemic inflammation) from liver [16], downregulates monocyte TLR expression, and finally inhibits the IKK/NFKB pathway [30,31]. A decreased plasma IL-6 concentration in response to chronic exercise appears to characterize a normal training adaptation [32]. Additionally, other studies have shown that acute as well as chronic exercise training can reduce the activation of this inflammatory signaling pathway [31].

Although the molecular mechanisms associated with inflammaging and the loss of skeletal muscle mass are not yet understood entirely, studies indicate that low-grade inflammation contributes to the induction of sarcopenia because it affects the positive protein turnover in the skeletal muscle [7]. In particular, elevated levels of TNF-α, IL-6, IL-1, and CRP favors the catabolism process and inhibit anabolism in the skeletal muscle through the activation of the NF-kB and ubiquitin-proteasome pathways. This sustained catabolic process culminates with muscular atrophy, a decline in muscle function, making the individual more susceptible to falls and the development of metabolic diseases such as obesity and type 2 diabetes [33].

On the other hand, master athletes had higher resting values of IL-1β, IL-4, IL-8 and IL-1ra in comparison with young and middle-aged untrained individuals. Some studies have shown that exercise training can promote the increase of IL-1β [34], and high levels of IL-1β are expected after muscle damage [35]. We believe that regular training may result in a specific accumulation of muscle damage, and this may explain the higher levels of IL-1β (resting values) in master athletes. As such, master athletes presented the most important rise in IL-1β concentration after exercise compared to the other groups. Probably, there is a residual effect of training in increasing this pro-inflammatory cytokine, but this seems to be compensated by the increase in IL-1ra secretion that inhibits the pro-inflammatory actions of IL-1β. In particular, IL-1ra prevents inflammatory processes by blocking signal

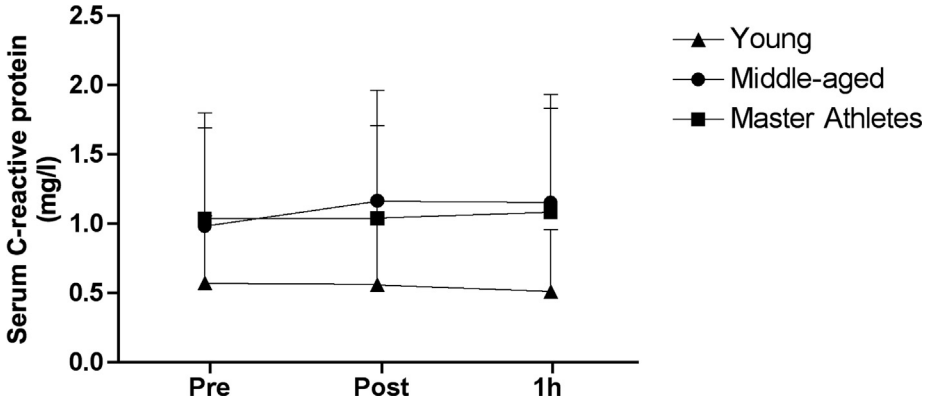

Fig. 3. Serum C-reactive protein levels in response to maximal exercise. Young (N = 8); Middle-aged (N = 9); Master athletes (N = 15). Pre (before exercise), Post (into 10 min post-exercise) and Post 1 h (1 h post-exercise).

transduction of the pro-inflammatory cytokine IL-1 and also creates an anti-inflammatory balance to the pro-inflammatory cytokine IL-1β [16]. The balance between IL and 1β and IL-1ra in local tissues influences the possible development of inflammatory disease and resultant structural damage. IL-1ra must be produced abundantly to block the effects of IL-1β. It was proposed that levels of IL-1ra 100-fold greater than IL-1β levels are necessary to inhibit the biologic impact of IL-1β on target cells [36]. Considering that the IL-1ra levels observed at baseline were 10-fold higher than those of IL-1β for both the master athletes and young groups, and approximately 7-fold higher in the middle-aged group, we suggest that training, more than age, may regulate the balance between pro- and anti-inflammatory cytokines.

The increased levels of IL-4 and IL-15 seen in the master atletes group seem to represent an adaptation to training. In fact, IL-4 and IL-15 are expressed in human skeletal muscle and seem to accumulate within the muscle with regular training [40,41]. IL-15 inhibits cell death (i.e., anti-apoptotic) and promotes the production of many immune cells including natural killer (NK) cells, neutrophils, eosinophils, mast cells, monocytes, and B lymphocytes [42]. In addition to its well-characterized effects on innate immunity, IL-15 has been proposed to modulate skeletal muscle and adipose tissue mass [43], as well as insulin sensitivity [44]. It is possible that high levels or prolonged expression of IL-15 in master athletes could be one factor responsible for the better body composition and immune responses observed in master athletes.

Aging seems to have a more pronounced effect on IL-10 and IL-17 concentration because the middle-aged participants had very low levels of IL-10 when compared to young participants and master athletes. IL-10 has a crucial role in preventing inflammatory and autoimmune pathologies [37]. The primary function of IL-10 seems to be the reduction of inflammatory-induced tissue damage, by decreasing the expression of MHC molecules, the intercellular adhesion molecule 1 (ICAM1) and co-stimulatory CD80 and CD86 molecules in antigen-presenting cells [16]. Likewise, IL-10 interferes or completely inhibits the expression of various pro-inflammatory cytokines [38,39]. Exercise-induced IL-10 secretion has also been associated with increased numbers of circulating regulatory T cells [16]. Our group showed that master athletes generally maintain the number and markers of activation of regulatory T cells as adaptive responses to lifelong training [15]. Both Treg and Th17 cells share common developmental pathways. TGF-β induces the Treg specific transcription factor FoxP3, essential for the induction and maintenance of induced Treg cells. However, in the presence of IL-6, generation of Treg cells is inhibited and Th17 are induced [51]. Production of IL-17 by Th17 cells also requires IL-6 and IL-1β, cytokines that increase with exercise. Although IL-17 has been defined has a potent pro-inflammatory cytokine and implicated in autoimmune diseases like psoriasis and rheumatoid arthritis, it is essential in controlling the clearance of specific types of pathogens (like certain types of fungi) that require a massive inflammatoty response. It is possible that exercise may help maintain IL-17 levels in the master athletes closer to those observed in the younger population.

Leukocytosis is the most consistent hematological change observed in response to prolonged exhaustive exercise [45,46]. In our study, we found significant increases in leukocyte counts after exercise training (Table 2). Changes in leukocyte count should be interpreted with caution since it may reflect only the redistribution of leukocytes from tissues to circulation, possibly to enhance immune surveillance or due to tissue trauma/injury associated with prolonged strenuous exercise [47]. It is not necessarily a harmful immunological disorder [48]. Another change in response to exercise training came from lymphocytes (Table 2). The lymphocytes response to intense and short exercise training is biphasic. The initial mobilization after exercise (lymphocytosis) is followed by a reduction in the number of circulating lymphocytes (lymphopenia) during the exercise recovery period. The initial increase in blood lymphocytes is thought to reflect both the mobilization of cells from the pool of peripheral blood lymphocytes as well as from the lymphoid organs [49]. Besides the increase observed in monocytes for the middle-aged group and also in granulocytes (mainly neutrophilia) for the master athletes group, other studies were unable to confirm an exercise-induced inflammatory response or tissue damage [50].

Furthermore, the pro-inflammatory cytokines did not follow this increase in monocytes (Fig. 1). It is possible that the duration of the exercise test was not enough to elicit significant variations in cytokine secretion, or that post-exercise monitoring was too short to detect these changes. Also, levels of CRP, a nonspecific marker of chronic low-grade inflammation, were similar for athletes and controls and did not change in response to the incremental test. In future studies it will be essential to evaluate variables of the inflammatory process in athletes of the same sporting modality, allowing to minimize possible differences related to the specificity of each type of training. Also, the analysis at different moments after exercise and in a range higher than 10 min and 1 h with the aim of analyzing the behavior of the cytokines at different points may offer relevant information regarding the peak concentration of some cytokines.

In summary, our results demonstrated that aerobic capacity and anti-inflammatory status of master athletes were similar to those found in young adults. Aging had the most pronounced effects in lowering IL-10 levels and increasing TNF-α/IL-10 ratio. Lifelong training showed a beneficial effect on the pro- and anti-inflammatory cytokine balance which may play a role in attenuating the age-related immune suppression and reduce the risk of age-related chronic diseases.

Compliance with ethical standards

Before participation, written informed consent was obtained from all participants. All procedures were approved by the by the Ethics and Human Subjects Review Board at the Faculty of Sports Science and Physical Education, University of Coimbra. The authors have contributed sufficiently to the manuscript and, therefore, share collective responsibility and accountability for the paper. All authors declare no conflicts of interest.

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