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Effect of intermittent hypoxic conditioning on inflammatory biomarkers in older adults

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ABSTRACT

Ageing is associated with chronic low-grade inflammation and with a decrease in muscle mass and strength. The aim of the study was to evaluate the effect of a resistance training programme in conditions of intermittent hypoxia on inflammatory biomarkers in older people. A total of 54 older adults (aged 65–75 years), who voluntarily participated in the study, were randomly divided into three groups: the control (CON) group, the resistance training normoxia (RTN) group that performed resistance training in normoxia and resistance training hypoxia (RTH) group that trained under hypoxic conditions at a simulated altitude of 2500 m above sea level. The training programme that was carried out during 24 weeks was similar in both experimental groups and consisted of a full-body workout with elastic bands and kettlebells (three sets x 12–15 reps). Blood inflammatory parameters (CRP, VCAM-1, IL-6, IL-8 and IL-10) were analysed before and after the intervention. After the resistance training programme, a significant decrease in CRP and IL-8 levels was observed, as well as an increase in IL-10 levels, both in normoxia and hypoxia. These results show that resistance training, either in conditions of normoxia or hypoxia, is useful to deal with the chronic inflammation associated with ageing.

1. Introduction

Ageing is associated with a loss of physical and cognitive function, as well as with the development of sarcopenia (Turner, 2016; Dionyssiotis, 2019). Although mechanisms of the onset and progression of sarcopenia are complex, the disuse, the reduced anabolic signalling capacity and the chronic low-grade inflammation have been proposed as explanatory factors for the loss of strength and muscle mass in older people (Dalle et al., 2017; Wilkinson et al., 2018). Tumor necrosis factor (TNFα) is a pro-inflammatory cytokine that directly acts upon skeletal muscle to decline specific force and IL-6 has leaded to muscle atrophy via energy homeostasis (Powers et al., 2016; Tuttle et al., 2020). However, it has also been observed that muscle strength and mass might decline prior to an increase in systemic inflammation (Looijaard et al., 2021). Related to systemic inflammation, the older adults have elevated circulating levels of C-reactive protein (CRP) and pro-inflammatory cytokines that could lead to dangerous health problems (Calder et al., 2017; Tang et al., 2017). Previous studies have shown that high levels of inflammatory biomarkers (interleukin-6, CRP and TNF-α) are directly related to diseases and an increased risk of mortality (Soysal et al., 2016; Singh-Manoux et al., 2017).

Resistance training is effective at improving the health and quality of life of older adults, as well as attenuating the loss of muscle mass associated with ageing (Fragala et al., 2019; Bårdstu et al., 2020). Previous studies have shown that the skeletal muscle works as an endocrine organ that can secrete myokines in response to exercise, acting on lipid and glucose metabolism, bone formation and hypertrophy (Severinsen and Pedersen, 2020). Likewise, there is evidence that long-term physical training has a positive effect on the treatment and prevention of inflammation, reducing chronic inflammation and creating an anti-inflammatory environment (Bautmans et al., 2021; Sardeli et al., 2018). In the review by Sardeli et al. (2018), it was concluded that resistance training in older adults reduced CRP, tended to reduce IL-6 and did not change TNF-α. In the same vein, Chupel et al. (2017) concluded that a chair-based elastic strength training programme performed by older women led to an increase in the anti-inflammatory cytokine IL-10 in blood. However, other studies did not find such conclusive results on the positive mediation of exercise on inflammatory markers (Ziegler et al., 2019; Azizbeigi et al., 2015). This disparity in results could be due to the differences between the experimental

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samples (age, sex and baseline inflammatory profile) and the type of training performed (intensity, volume and duration of the intervention).

Chronic exposure to severe hypoxia is known to have negative health consequences, such as increased oxidative stress, sympathetic activation, production of systemic inflammation and increased expression of vascular adhesion molecules (Eltzschig and Carmeliet, 2011; Garvey et al., 2009; Semenza, 2007). However, previous studies have shown that moderate intermittent hypoxia, defined as short alternating exposures to hypoxia and normoxia, can have beneficial health effects (Gangwar et al., 2020). This type of hypoxia allows modulating and stabilizing the hypoxia-inducible factor (HIF)-1α, which is involved in the expression of factors related to cell survival, angiogenesis and regulation of the inflammatory response (Palazon et al., 2014). Hypoxia also induces enhanced signalling of the purine nucleoside adenosine, which has been shown to exert anti-inflammatory and tissue-protective effects (Kiers et al., 2018). Moreover, recent studies have proposed intermittent hypoxia training as a beneficial non-pharmacological intervention (Hertzog et al., 2021). Although it is true that hypoxic exposure at real altitude is not approachable for the general population, in the last years several devices, such as hypoxia tents and portable hypoxic generators have allowed easy and inexpensive access to simulated hypoxia situations. In this sense, intermittent hypoxia is receiving recent attention for its simplicity and pronounced effects.

Additionally, hypoxia conditioning has been proposed as a new therapeutic modality to deal with cardiovascular and respiratory diseases (Verges et al., 2015), as well as to mitigate the sarcopenia and loss of strength during ageing (Millet et al., 2016). Sakushima et al. (2020) have stated that the moderate hypoxia, > 10% of inspired fraction of oxygen (FiO_2), promotes skeletal muscle cell growth and hypertrophy by increasing the expression of cell differentiation (MyoD and myogenin) and muscle hypertrophy proteins (mTOR and p70s6K).

Therefore, this study was designed with the objective of evaluating the effect of a resistance training programme performed under intermittent hypoxia on inflammatory biomarkers in older people. The initial hypothesis was that resistance training would cause a decrease in systemic inflammation and that the addition of intermittent hypoxia to training would have a positive synergistic effect on the decrease in inflammatory biomarkers.

2. Material and methods

The research design and the protocol used in the research have been registered in the international registry of clinical trials of the U.S. National Institutes of Health (https://clinicaltrials.gov/), with the identifier NCT04281264.

2.1. Participants

A total of 54 older volunteers (32 women and 22 men) aged 65–75 years participated in the study. The anthropometric characteristics and control variables of the study participants are shown in Table 1. Various associations of retired people, nursing homes and senior universities were contacted to inform them about the project and recruit participants. Participants were selected after a screening visit, in which the following inclusion criteria had to be met: (1) women and men aged 65 years or older, (2) absence of participation in any other type of intervention based on physical exercise in the last 6 months, (3) not having been above 1500 m during the last 3 months, (4) no medical condition that is not compatible with resistance exercises, (5) not receiving medication that affects the immune system (e.g., steroidal and non-steroidal anti-inflammatory drugs, anti-depressants), or cancer or arthritis treatment (6) consumption of no more than two alcoholic beverages per day. Group adherence to training was set at 75% attendance. Additionally, they were asked to continue with their usual lifestyle and diet throughout the intervention, and they were allowed to continue using their usual medication. However, individuals who had unstable medical conditions or new medications within the data collection period were excluded. Details of sample dropping-out are presented in a flowchart (Fig. 1). The eligible volunteers, who finally met the inclusion criteria, were divided into three groups in a controlled and randomized design, although balancing the groups by sex: control group (CON; $n = 19$, 11 women and 8 men) that did not perform physical exercise and were instructed to continue with their normal daily activities; resistance training in normoxia group (RTN; $n = 18$, 11 women and 7 men) that trained in normoxia; and resistance training in hypoxia group (RTH; $n = 17$, 10 women and 7 men) that trained at a simulated altitude of 2500 m above sea level (asl). The research was approved by the Bioethics Committee of the university (Ref: 65/2018) and was carried out respecting the ethical principles established in the Declaration of Helsinki. The participants signed an informed consent and could leave the research at any time.

2.2. Resistance training

The intervention programme was performed by certified trainers. The design of the exercise programme was carried out following the recommendations of the American College of Sport Medicine (Nelson et al., 2007) and lasted 24 weeks, with a training frequency of 3 days per week (Monday, Wednesday and Friday). Before starting the training programme, two familiarization sessions were held with the participants to learn the technique of the exercises and to define the training load. The intensity of the exercises was controlled from the rate of perceived effort (RPE) measured with a visual analogue scale validated for strength exercises (Colado et al., 2012). This scale quantifies effort from 0 (extremely easy) to 10 points (extremely hard), and throughout the intervention the participants had to train with an effort that ranged between 6 and 8.

The duration of the training session was approximately 45 min, which included a 10-minute warm-up with body mobilization and dynamic stretching, a 30-minute main part and a 5-minute cool down with stretching exercises and relaxation. The main part of the session consisted of a circuit in which nine exercises were performed to train several body areas (pectoral, shoulders, back, arms, thighs, legs and abdominals) with a structure of three series of 12–15 repetitions per exercise, with a 1-minute rest between sets. Six exercises were performed using elastic bands (chest press, back row, biceps curl, triceps pushdown,

Table 1
Anthropometric characteristics of the participants and control variables before the intervention (mean ± SD).

Groups	Sex	Years	Weight (kg)	Height (m)	BMI	WHR	Kcal/day	%HC	%Protein	%Fat	PAR (0–7)
Control (n = 19)	8 (M) 11(F)	70.5 ± 4.0	66.1 ± 10.2	1.56 ± 0.9	26.8 ± 2.6	0.94 ± 0.7	1941.5 ± 306.1	55.7 ± 3.6	22.6 ± 1.2	21.7 ± 2.4	2.75 ± 0.85
RTN(n = 18)	7 (M) 11(F)	70.3 ± 3.3	70.9 ± 11.5	1.61 ± 0.8	27.1 ± 3.9	0.95 ± 0.8	1847.0 ± 442.6	51.4 ± 3.1	25.8 ± 2.3	22.7 ± 1.7	2.52 ± 1.46
RTH (n = 17)	7 (M) 10 (F)	68.4 ± 3.8	71.9 ± 14.9	1.64 ± 0.9	26.4 ± 3.3	0.92 ± 0.6	2004.0 ± 254.55	56.3 ± 4.1	23.6 ± 2.4	20.0 ± 1.6	2.30 ± 0.85
P	0.989	0.508	0.430	0.601	0.892	0.605	0.697	0.373	0.340	0.407	0.589

RTN: Resistance training in normoxia; RTH: Resistance training in hypoxia; M: Male; F: Female; BMI: Body mass index; WHR: Waist-hip ratio; PAR: Physical activity rating scale.

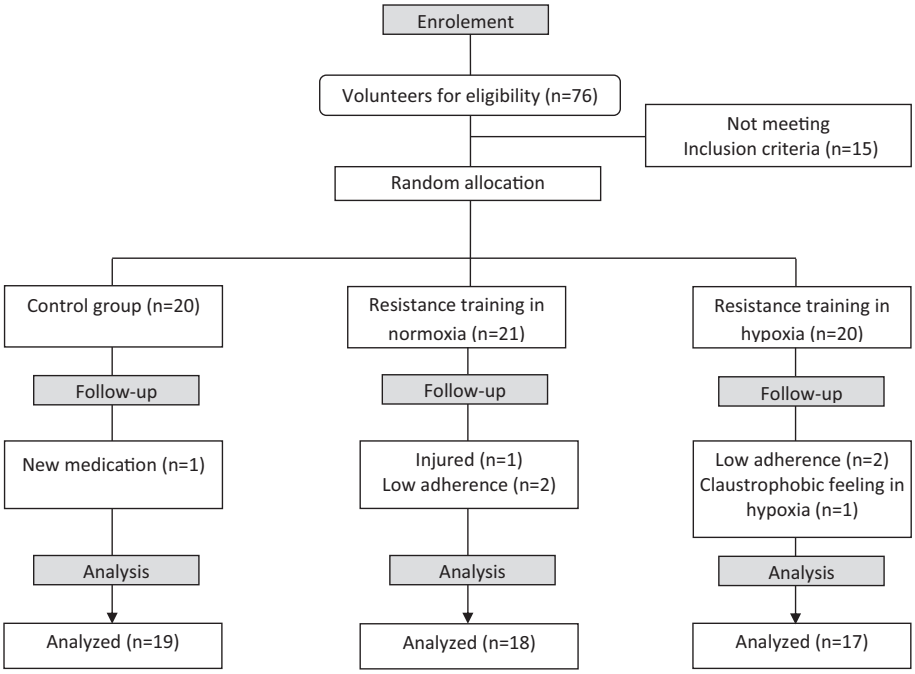

Fig. 1. Flow chart of the study.

standing lateral raise and front shoulder raise), regulating the intensity according to the colour of the band. The tension of the bands varied from low to high depending on the colour of the band (orange/green/blue) (TheraBand, Hygenic Corporation, USA). Each participant adjusted the resistance to training depending on the grip and the distance reached during the movement. The workload in all the groups was reviewed every 2 weeks throughout the training programme in order to train progressively and to encourage a change in the colour of the band until reaching blue (although always with an effort that ranged between 6 and 8 of RPE). Additionally, two other exercises were developed (squat and lying hip raise) with kettlebells, with loads that evolved from 4 kg to 10 kg. The last exercise was a front plank in isometric conditions for 15 s (and evolving up to 30 s throughout the training), which was repeated three times with a 1-minute rest between sets.

Depending on the experimental group, the trainings were performed in normoxia (RTN) or in normobaric hypoxia (RTH). The RTN group trained in a room at 459 m asl. The RTH group trained inside a hypoxia chamber (CAT 310, Colorado Altitude Training, USA) located in the training room. The hypoxic environment was produced by a hypoxic generator (CAT 12, Colorado Altitude Training, USA). It was set a FiO_2 of 16.1% to simulate an altitude of 2500 m asl. The simulated altitude was calculated according to the chart and guidelines provided by the hypoxic generator manufacturer. FiO_2 was controlled regularly with an electronic device (HANDI +, Maxtec, USA). For safety reasons, during the training session in hypoxia, a pulse oximeter was used to ensure that blood oxygen saturation (SpO_2%) did not fall below 85%.

2.3. Measurements

On their first visit to the laboratory, participants answered an adapted version of the Physical Activity Rating Questionnaire (PAR-Q) to evaluate their level of physical activity (Jackson et al., 1990), with scores between 0 (lowest level) and 7 (highest level). Likewise, the caloric intake of the participants and the percentage of the macronutrients (carbohydrate, protein and fat) were estimated using a 7-day diet inventory, which was analysed using the diet software Nutriber (Nutriber v1.1.1, Funiber, Spain).

Anthropometric measurements were taken before the intervention programme. Body mass and height was measured using a portable stadiometer (Seca 213, Germany), and body mass index (BMI) was calculated from the ratio of mass/height^2 (kg/m^2). Hip and waist circumferences were measured using a tape measure following the recommendations established by the International Society for Advances in Kineanthropometry (ISAK) (Stewart et al., 2011). Waist-hip ratio (WHR) was calculated as the waist circumference divided by the hip circumference.

Control of effort during training sessions was monitored once a week randomly between participants. SpO_2%, heart rate (HR) and RPE were measured. Measurements were made 3 min after the last exercise of the session had ended. The mean values obtained from the 24 weeks that the intervention lasted were calculated. SpO_2% was measured in duplicate using a pulse oximeter (Wristox 3100; Nonin, USA). HR was monitored using a HR monitor (Polar Z9, Finland) and beats per minute (bpm) were recorded. RPE was obtained by showing a graphical scale to participants with a category-ratio 10 scale, ranging between 1 (extremely easy) and 10 (extremely hard) (Colado et al., 2012).

2.4. Blood sampling

Blood samples were collected in the morning (between 09:00 and 10:00 h) before and after 24 weeks of intervention. The final blood extractions were carried out 3 days after the last session of the programme. The blood collection was performed after a minimum of 10 h of overnight fasting, and all the participants did not perform any physical effort in the previous 48 h. Blood samples were taken from the antecubital vein by one experienced nurse using vacutainer tubes containing gel separators for serum analytics. After 10 min of centrifugation at 1790*g* (relative centrifugal force) and room temperature, serum was extracted and injected into an micro-centrifuge tube and stored at −80 °C until determination of the serum concentrations of CRP, vascular cell adhesion molecule 1 (VCAM-1), IL-6, IL-8 and IL-10. Additionally, IL-6/IL-10 ratio was taken as a measure of the pro- and anti-inflammatory status of participants. VCAM-1 and cytokines were analysed using validated ProcartaPlex Multiplex immunoassay kits (Invitrogen, Bender Med Systems GmbH, Austria) following its standard operating procedure, which is based on Luminex technology (Bioplex 200, Bio-Rad, USA). The intra-assay coefficient of variability was <15% for VCAM-1, 6.2% for IL-6, 8.5% for IL-8 and 3.1% for IL-10. Serum CRP levels were determined

by a colorimetric sandwich ELISA kit (Proteintech, UK) with an ELISA microplate reader (SpectraMax PLUS 384, Molecular Devices, USA), following the manufacturer's instruction. The intra-assay coefficient of variability was 8.8% for CRP.

2.5. Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 23.0, Chicago, IL, USA). The Shapiro-Wilks test was applied in order to verify a normal distribution of data, and Levene's test was used to assess the homogeneity of variance. A repeated measures ANOVA was performed for each variable to explore within-group and between-group differences over time, using the baseline values as covariates. Post hoc Bonferroni tests were performed when appropriate using the SPSS syntax commands. The confidence interval (CI) for the difference between *pre-post* has been shown to provide an estimate of the absolute difference in means of variables of interest. The effect size (ES) was also calculated for all dependent variables. The magnitude of effect was classified as trivial (0.25), small (between 0.25 and 0.50), moderate (between 0.50 and 1.0) and large (> 1.0) (Rhea, 2004). The significance level was set at $p \leq 0.05$, with a confidence level of 95%. Descriptive statistics are shown as means and standard deviations (mean ± SD).

3. Results

A total of 54 older people completed the intervention, and their results were included in the analysis. There were no research-related adverse effects or injuries. However, a total of seven individuals who were evaluated at the start of the programme were not re-evaluated after 24 weeks of intervention for various reasons (Fig. 1).

The control variables of the participants are shown in Table 1. Prior to the intervention, no significant differences between groups were observed for any variable.

The SpO_2% values shown in Table 2 indicate that the environmental conditions during training sessions were different between the RTN and RTH groups. SpO_2% was significantly lower ($p = 0.001$) during the resistance training in hypoxia than in the resistance training in normoxia. No differences were observed between the experimental groups with regard to HR and RPE.

Table 3 shows the concentrations of inflammatory biomarkers before and after the intervention. In the CON group, there were no significant changes after 24 weeks. In the RTN group, significant decreases in CRP ($p = 0.001$; $ES = 1.84$), IL-8 ($p = 0.001$; $ES = 1.22$) and IL-6/IL-10 ($p = 0.028$; $ES = 0.40$) levels were observed after the training programme. In the RTH group, similar results were observed, with significant decreases in CRP ($p = 0.001$; $ES = 1.83$), IL-8 ($p = 0.004$; $ES = 1.44$) and IL-6/IL-10 ($p = 0.047$; $ES = 0.44$) levels, although a significant increase in IL-10 levels was also observed ($p = 0.042$; $ES = 0.62$). Additionally, both experimental groups, after the resistance training programme, presented with significantly lower levels of CRP ($F_{Group} = 43.08$, $p = 0.001$) and IL-8 ($F_{Group} = 11.44$, $p = 0.001$) as compared with those in the CON group, although no differences were observed between the RTN and RTH groups.

Table 2
Variables measured during resistance training sessions (mean ± SD).

Variables	RTN group	RTH group	*p*
Spo2 (%)	96.2 ± 1.8	90.0 ± 1.4	**0.001**
HR (bpm)	87.4 ± 14.7	96.5 ± 17.5	0.370
RPE	5.8 ± 0.7	5.9 ± 0.8	0.999

RTN: Resistance training in normoxia, RTH: Resistance training in hypoxia.
Spo2 (%): Blood oxygen saturation.
HR: Heart rate.
RPE: Rate of perceived effort.
Values in bold are used to highlight the significant differences.

4. Discussion

The aim of the study was to evaluate the effects of resistance training under hypoxic conditions on inflammatory biomarkers in older people. The initial hypothesis has only been partially fulfilled, since a decrease in inflammatory biomarkers (CRP and IL-8) has been observed in both groups (RTN and RTH) as compared with those in the CON group, and a significant increase in the anti-inflammatory cytokine IL-10 (only in the RTH group). However, no positive synergistic effect of hypoxia was observed in the RTH group as compared to the RTN group.

Ageing is characterized by an increase in the concentration of inflammatory biomarkers in the bloodstream (Calder et al., 2017). Serum CRP can be accurately measured and is used as a marker of chronic inflammation and tissue damage in older people (Singh and Newman, 2011). In this vein, scientific evidence suggests that moderate CRP concentrations (3–10 mg/L) could predict an increased risk of cardiovascular diseases (Dhingra et al., 2007). Consistent with these reference values, the mean baseline concentrations in our research show that the participants could suffer a low-grade inflammation, without showing classic clinical signs of inflammation but with minor CRP level elevation (Kushner et al., 2010). However, after the training programme, there was a significant decrease in CRP, both in the RTN and RTH groups, with a significant decrease as compared to the CON group. Previous studies have also shown a decrease in CRP in older adults after an 8-week training programme with kettlebells (Cheng et al., 2018) or after 12 weeks (3 days/week) of a training consisting of two sets x 8 repetitions at 70–80% of 1RM with eight resistance exercises (Stewart et al., 2007). This decline in CRP could be explained by an increase in myokines produced by muscle contraction, opposing the effect of pro-inflammatory cytokines, as well as by an increase in muscle mass that causes an increase in insulin sensitivity (Calle and Fernandez, 2010; Gonzalez-Gil and Elizondo-Montemayor, 2020). Additionally, it has been stated that the decrease in CRP after a strength training programme has been greater when participants showed CRP levels above the standard normal values at baseline (Ramel et al., 2015), which has also been observed in our research.

Regarding cytokines, after the resistance training programmes in both groups (RTN and RTH), a significant decrease in the pro-inflammatory cytokine IL-8 and the IL-6/IL-10 ratio was observed, as well as an increase in the anti-inflammatory cytokine IL-10 (reaching statistical significance only in the hypoxia group), but without significant changes in IL-6. Results similar to those of the current study were found in a group of sedentary older women who performed 8 weeks of quadriceps femoris resistance training (2–3 sets × 15 reps), decreasing their IL-8 levels and maintaining their IL-6 levels (Tucci et al., 2019). Likewise, previous studies have shown increases in IL-10 after carrying out a strength training programme of several weeks, either with elastic bands (Chupel et al., 2017) or with resistance exercises and loads ranged between 40% and 70% of 1RM (Silveira Martins et al., 2015). Chupel et al. (2018) found decreases in the IL-6/IL-10 ratio after 14 weeks of an intervention of combined strength training in older adults. Despite the different processes that modulate inflammation and the complex interrelationships that exist between cytokines, it has been stated that the myokines produced during exercise have a long-term positive anti-inflammatory effect in people (Mathur and Pedersen, 2008; Bautmans et al., 2021). In this sense, the release of IL-6 during exercise into the circulation from contracting muscle fibres seems to play an important role. IL-6 secreted by myocytes during exercise is produced by mechanisms independent of TNF-α, causing an anti-inflammatory effect opposite to the pro-inflammatory effect of the IL-6 secreted chronically by adipose tissue (Smart et al., 2011; Gleeson et al., 2011). This release of IL-6 leads to a subsequent increase in circulating levels of anti-inflammatory cytokines, such as IL-10 and IL-1 receptor antagonist (Calle and Fernandez, 2010), that could inhibit the production of IL-1β,

Table 3
Inflammatory markers before and after 24 weeks of intervention (mean ± SD).

	Control group					RTN group					RTH group					
	Pre	Post	*95%CI for difference*	*p*	ES	Pre	Post	*95%CI for difference*	*P*	ES	Pre	Post	*95%CI for difference*	*p*	ES	*Between-group differences F (p value)*
CRP (mg/L)	6.9 ± 1.3	6.5 ± 2.0	−0.4-1.2	.324	0.24	7.6 ± 4.2	**2.2 ± 0.9**††	4.5–6.3	**.001****	1.84	7.3 ± 2.0	**3.6 ± 2.2**††	2.6–4.7	**.001****	1.83	**43.083 (0.001)**
VCAM1 (ng/ml)	530 ± 223	549 ± 262	−96-59	.632	0.10	595 ± 152	665 ± 180	−153-15	.105	0.44	644 ± 267	647 ± 412	−100-92	.940	0.01	0.630 (0.537)
IL-6 (pg/ml)	2.3 ± 1.0	2.4 ± 1.2	−0.4-0.5	.999	0.01	2.5 ± 1.4	2.7 ± 1.6	−0.7-0.5	.471	0.14	2.5 ± 1.2	2.6 ± 1.4	−0.6-0.5	.612	0.08	0.161 (0.852)
IL-8 (pg/ml)	3.1 ± 2.5	2.5 ± 1.9	−0.1-1.4	.101	0.28	3.3 ± 2.6	**1.1 ± 0.5**††	1.3–3.0	**.001****	1.22	2.6 ± 1,5	**1.1 ± 0,3**††	0.4–2.4	**.004****	1.44	**11.442 (0.001)**
IL-10 (pg/ml)	1.6 ± 0.5	1.6 ± 1.3	−0.6-0.4	.897	0.01	2.0 ± 1.2	2.3 ± 0.9	−0.8-0.3	.082	0.29	1.6 ± 0.7	2.3 ± 1.5	−1.4-0.1	**.042****	0.62	1.746 (0.186)
IL-6/IL-10	1.76 ± 1.4	1,60 ± 0.7	−0.3-0.4	.447	0.07	1.53 ± 0.8	1.24 ± 0.7	−0.1-0.5	**.028***	0.40	1.86 ± 1.2	1.39 ± 1.0	−0.3-0.7	**.047***	0.44	0.783 (0.463)

RTN: Resistance training in normoxia.
RTH: Resistance training in hypoxia.
*$p < 0.05$, **$p < 0.01$. Significant differences within-group (Pre-Post).
††$p < 0.01$. Significant differences compared to control group.
Values in bold are used to highlight the significant differences.

TNF-α and IL-8 (Petersen and Pedersen, 2005).

On the other hand, the effect of resistance training on VCAM-1 was analysed, which reflects endothelial activation and can serve as a marker molecule of the chronic vascular inflammatory process (Stefanadi et al., 2010). As in previous studies (Cook et al., 2013; Olson et al., 2007), no significant changes in VCAM-1 values were observed in any of the groups after resistance training. The duration of the intervention and magnitude of the load were probably not enough to alter the values of cardiovascular adhesion molecules. However, previous studies have stated that aerobic exercise is more appropriate to modulate endothelial activation than resisted exercise among the older adults (Abd El-Kader and Al-Shreef, 2018).

Contrary to what we hypothesized, the RTH group did not show an additional anti-inflammatory effect as compared to the RTN group. However, previous studies have shown that intermittent exposure to hypoxia caused significant increases in circulating IL-10 levels (Kiers et al., 2018; Meng et al., 2018). The HIF-1α that is stabilized and not degraded under hypoxic conditions contributes to IL-10 production by B cells (Meng et al., 2018), modulating the immune and inflammatory responses. The positive effect that hypoxia seems to have could depend on the duration of the intervention, severity, and time of exposure (Gangwar et al., 2020). In our study, the intervention was carried out with a moderate level of hypoxia (with an FiO_2 of 16.1% and a mean SpO_2% value around 90%), while the investigations discussed above used an FiO_2 of 12–13.5% (Gangwar et al., 2020) with an SpO_2% of 80–85% (Kiers et al., 2018). This issue could explain the lack of significant differences in cytokines levels in the RTH group as compared with those in the RTN group. Unfortunately, this is the first study to analyze long-term adaptations of inflammatory biomarkers in older people performing resistance training in hypoxia, so we do not have previous findings to compare with. Therefore, more evidence is needed to confirm the potential anti-inflammatory effect that intermittent hypoxic conditioning could have.

The study had some limitations. Firstly, the experimental design did not have a sham group to analyze the independent effect of only hypoxia exposure. Secondly, the diet was not monitored during the intervention, as only a record of caloric intake was carried out at the beginning of the programme. On the other hand, the analysis of other molecules and cytokines (such as HIF-1α, microRNAs, IL-15, IL-2 and TNF-α), as well as the biomarkers of lipid metabolism and redox homeostasis associated with inflammatory processes, could have provided more complete information on the adaptations and physiological mechanisms involved. Finally, the evaluation of inflammatory biomarkers during detraining would have provided valuable information about the effectiveness of the long-term training programme.

In conclusion, our study shows that resistance training for older people, whether under conditions of normoxia or hypoxia, is a useful non-pharmacological tool to reduce the chronic low-grade inflammation associated to ageing. Likewise, this research provides promising data on the positive effects that intermittent hypoxic conditioning can have on inflammatory biomarkers.

CRediT authorship contribution statement

Rafael Timon: Conceptualization, Writing-Original Draft, Formal analysis, Supervision, Project administration, Funding acquisition. **Ismael Martínez-Guardado**: Methodology, Investigation, Review & Editing. **Alba Camacho-Cardeñosa**: Data Curation, Formal analysis, Visualization, Review & Editing. **Jose M. Villa-Andrada**: Methodology, Investigation. **Guillermo Olcina**: Visualization, Resources. **Marta Camacho-Cardeñosa**: Supervision, Writing - Review & Editing, Data Curation, Formal analysis.

Declaration of competing interest

The authors declare no competing interests.

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