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Circulating myokines IL-6, IL-15 and FGF21 response to training is altered by exercise type but not by menopause in women with obesity

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Circulating myokines IL-6, IL-15 and FGF21 response to training is altered by exercise type but not by menopause in women with obesity

Running head title: Myokines, exercise type and menopause

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ABSTRACT

Objective: To examine the effects of a time-matched endurance versus concurrent training on circulating IL-6, IL-13, IL-15, IL-15Ra, FGF21 levels in postmenopausal women with obesity, and to determine these myokines response to endurance training pre- and postmenopause.

Methods: Thirty-five sedentary postmenopausal women with obesity were randomly divided into endurance training (EN1, N=10), concurrent training (CON, N=13) or no training group (CT, N=12). Additionally, twelve sedentary premenopausal women with obesity were added to an endurance training group (EN2, N=12). Participants took part in a 12-week supervised intervention, performing 3 sessions/week of 60 min/session. Before and after the interventions, body composition and fitness were assessed, and blood samples obtained to measure serum myokines levels.

Results: Total fat mass decreased in all exercised groups (CON,-5.2%; EN1,-5.3%; EN2,-5.6%). In postmenopausal women, serum IL-6, IL-15 and IL-15Ra decreased after training ($P<0.01$), finding a pronounced reduction in IL-6 (-42% vs. -16%) and IL-15 (-50% vs. -31%) when comparing EN1 to CON ($P<0.05$). Serum FGF21 was only reduced in the EN1 (-27%; $P=0.012$). While EN1 and EN2 comparison, reported differences for IL-15Rα concentration (-28% vs. -40%; $P=0.023$). Finally, in EN2, the delta change of fat mass and IL-6, IL-15 and IL-15Rα were associated ($r=0.605$; $r=0.546$; $r=0.515$; $P<0.05$). IL-13 showed undetected concentrations.

Conclusions: Circulating IL-6, IL-15 and FGF21 response to training is altered by exercise type but not by menopause in women with obesity. Endurance training promotes a higher reduction of these myokines, potentially activating their intricate immune and fat mass regulation roles in postmenopausal women with obesity.

Keywords: myokines, inflammation, endurance and concurrent training, menopause, body composition, physical activity.

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1. INTRODUCTION

In postmenopausal women with obesity, the increased inflammatory state accentuates the lipid and glucose metabolism alteration caused by menopause, leading to comorbidities such as cardiovascular diseases, stroke, type 2 diabetes or breast cancer.[1] Although the chronic presence in the circulation of pro-inflammatory cytokines can damage several tissues,[2] the acute release from skeletal muscle of interleukin (IL)-6, IL-13, IL-15 and its alpha receptor (IL-15Rα), and fibroblast growth factor 21 (FGF21) can reduce inflammation, promote fat oxidation and improve insulin sensitivity in skeletal muscle and adipose tissue.[3-7] Therefore, determining myokines response to different exercise types would contribute to understand how exercise-induced myokines help to restore lipid and glucose metabolism by reducing low-grade systemic inflammation in a population of pre and postmenopausal women with overweight and obesity.

During menopause, adipose tissue became the primary source of estrogens and androgens production via precursors conversion, which altered glucose and lipid metabolism, increasing visceral fat mass accumulation.[8, 9] When these metabolic changes are accompanied by physical inactivity, the risk of obesity and the development of comorbidities, such as breast cancer, increased due to an augmented inflammatory state among other factors.[1, 10] When the inflammatory state persist, adipocytes and immune cells release pro-inflammatory cytokines (e.g., IL-6, IL-13 or IL-15) into the circulation to promote damaged tissue reparation and toxic agent neutralization caused by physical inactivity and visceral fat accumulation.[11, 12] The chronic presence in the circulation of these pro-inflammatory cytokines, called low-grade systemic inflammation, contributes to the inflammatory spiral that damages several tissues,[2] elevating the risk of non-communicable diseases.[13] Exercise training can restore some of these metabolic disturbances experimented during menopause by promoting anti-inflammatory effects,[14, 15] but the mechanisms by which exercise facilitates muscle-adipose tissue crosstalk communication resulting in a reduction of fat mass are not completely understood in humans.

Myokines are among the main candidates that may enable muscle-adipose tissue crosstalk communication and prevent low-grade systemic inflammation. The acute release of some of these myokines stimulates fatty acid oxidation in skeletal muscle

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(e.g., IL-6 and IL-15) and liver (e.g., FGF21), lipolysis in skeletal muscle (e.g., IL-6 and IL-15), a reduction in lipid accumulation in adipose tissue (IL-15 and IL-15Rα), fat browning (e.g., FGF21) and improve glucose uptake and oxidation in skeletal muscle, liver and adipose tissue (e.g., IL-6, IL-13, IL-15 and FGF21).[3-7] All these effects allow establishing a muscle-adipose tissue-immune system by which exercise attenuate inflammatory state and reduce fat mass accumulation. However, although repeated doses of exercise may promote myokines-induced health benefits on inflammation, glucose and lipid metabolism,[16] there is scarce of evidence regarding which exercise type facilitates the higher expression and release of these myokines, particularly in postmenopausal women with obesity were combination of endurance and resistance training are recommended,[17] and if the metabolic disturbances that occur during menopause alter myokines' response to exercise.

Therefore, the primary purpose of the study was to examine the effects of a time-matched endurance and concurrent training on circulating myokines (IL-6, IL-13, IL-15, IL-15Rα and FGF21) levels in postmenopausal women with obesity, and secondary to determine the myokines' response to endurance training between pre- and postmenopausal women. We hypothesized that endurance training would promote a higher reduction in fat mass loss,[15] which would lead to a higher decrease of circulating myokines concentrations (IL-6, IL-13, IL-15, IL-15Rα and FGF21), particularly in postmenopausal women, owing to the accentuated inflammatory state of this population.

2. MATERIAL AND METHODS

A full description of participants' characteristics, study designs and procedures, from which part of the postmenopausal women data are drawn, has been previously published.[15]

2.1. Participants

Forty-eight patients were recruited from the Department of Nutrition and Endocrinology at La Paz Hospital and the Department of Nutrition at Ramón y Cajal Hospital (Madrid, Spain). All participants underwent a health screening. Initially, thirty-six women aged from 50 to 65 yeas old were included in the study if they were postmenopausal (>1 year since last menstrual cycle), with overweight or obesity (body mass index, >25kg/m^2)

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and had low levels of physical activity (<150 minutes/week of physical activity). Participants showed a >1.66% of Gail 5-years risk score of breast cancer. Then, the same inclusion criteria were used to recruit twelve premenopausal women aged from 40 to 50 years old, who had regular menstrual cycle and no alteration in sex hormones. Pre- and postmenopausal women who took any medication that could alter skeletal muscle metabolism, were diagnosed with any immune, neuromuscular or cardio-metabolic disease (apart from overweight and obesity) or experimented any limitation to participate in an exercise program were excluded from this study.

Before study enrolment, all procedures were explained to participants who then provided written informed consent. Ethical approval was obtained from the Ethics Committee of Investigation from University of Alcalá (CEI 2013/047/20140528 and CEI/HU/2016/29) in accordance with the Declaration of Helsinki.

2.2. Experimental design

Postmenopausal women were divided using block randomization (1:1:1 order) into three different groups, endurance training, concurrent training and control group. Premenopausal women were included in a parallel endurance training group, to compare pre and postmenopausal women adaptation to endurance exercise, particularly the anti-inflammatory effect of this type of exercise. Before a familiarization week in which participants were required to learn the adequate technique for each exercise and exercise testing procedures, pre and postmenopausal women were enrolled in a 12-weeks training program according to their group assignment. They reported to the laboratory before and after the 12-week intervention, and in all these visits, body composition and fitness were assessed and blood samples obtained to analyze myokines concentrations, glucose and lipid profiles. After drop-outs, thirty-five sedentary postmenopausal women and twelve premenopausal completed the intervention.

2.3. Experimental protocol

After the familiarization week, all women came in fasted state (8-10 h) to the laboratory, where body composition was determined by electrical bioimpedance (Tanita BC-418, Tanita Corporation of America Inc. IL, USA) as previously described.[16] Blood samples were obtained from the forearm vein and fitness tests were assessment. Women completed a submaximal cardiorespiratory test on a cycloergometer to calculate maximal oxygen consumption ($\dot{V}O_{2max}$) and maximal strength tests were obtained using

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an adapted and progressive one repetition maximum test (1RM) protocol, as previously described.[15] Participants were asked to maintain their regular diet intake (3-day food records) and physical activity habits (IPAQ).[18] The same procedure was performed at the end of the 12-week intervention.

After the first visit, participants start the 12-weeks intervention where postmenopausal women performed endurance training (EN1, N=10), concurrent training (CON, N=13) or no training (C, N=12); while premenopausal women performed endurance training (EN2, N=12). Participants allocated in the EN1, EN2 or CON groups carried out a 12-week training program performing 3 sessions per week of endurance (EN1 and EN2) or endurance plus resistance exercise (CON) in a time-matched manner. A detailed explanation of the training protocols are showed elsewhere.[15] Briefly, endurance training consisted of 60 min of exercise on a treadmill, cycloergometer and elliptical machine performed at moderate intensity (55-75% heart rate reserve (HRR)), while concurrent training consisted of a combination of endurance (20 min) and resistance exercise (40 min). Resistance training involved 6 exercises performing 3 sets of 8-12 repetitions at 65% of one-repetition maximum (1RM) with 60-90 s of rest between sets on each exercise. Participants' physical fitness was evaluated every 3 weeks to ensure progressive training overload.

2.3.1. Blood analysis

Before and after the 12-weeks intervention, blood samples were obtained from the forearm vein and drawn into prepared tubes for serum and plasma separation (BD, Oxford, UK). Then, samples were centrifuged, aliquoted and stored at -80ºC until analysis. Blood glucose and lipid profiles (total cholesterol, low-density (LDL) and high-density lipoproteins (HDL), triglycerides, fasting glucose and HbA_{1c} were measured using Advia 2400 (Siemens Healthineers, Erlangen, Germany) and Abbott Aeroset Automated Instrumen Analysed (Abbott Laboratories, Abbott Park, IL, USA). Fasting insulin was obtained by immune chemiluminescence (Ommunite 200, Diagnostic Products, Corporation, Los Angeles, CA, USA) and HOMA-IR was calculated using the homeostatic model.

High-sensitivity enzyme-linked immunosorbent assay (ELISA) kits were utilized to determine serum concentrations of myokines. IL-6, IL-13, IL-15 and FGF-21 were measured using the appropriate Human Quantikine ELISA kit (R&D, Systems, MN, USA), while IL-15Rα was measured using Human IL-15 receptor subunit alpha ELISA

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kit (Wuhan EIAab Science, Wuhan, China). In all cases, the intra- and inter-assay CVs were < 6% and < 9%, respectively.

2.4. Statistical Analysis

The sample size calculation revealed that 36 participants were sufficient for the primary purpose of the study (type of exercise) to show an effect size of 0.45 ($\alpha = 0.05$; $1 - \beta = 0.80$) (v3.1, G*power, Dusseldorf University, Germany), for that reason 39 participants were initially recruited and after drop-outs 35 participants completed the intervention. Data collected were analyzed using the SPSS package (v. 26.0, SPSS Inc., Chicago, IL, USA). Shapiro-Wilks test was used to test the normality of the data ($P<0.05$) and non-normally distributed variables were logarithmically transformed. At baseline, groups were compared using one-way analysis of variance (ANOVA) and Chi-squared test. Then, two-way repeated-measures ANOVA was used to compare changes among exercise types (endurance training, concurrent training and control group) across time (pre- and post-training intervention) in response to 12-weeks of exercise training. Besides, the same ANOVA was performed to study the effect of menopausal status (pre- vs. postmenopausal women) in response to endurance training across time. In both cases, Mauchly was conducted to test sphericity. Holm-Bonferroni was the *post hoc* test performed when significant differences were found. Linear regression analysis was used to determine the relationship of the circulating levels of myokines with body composition, glucose and lipid metabolism variables. Values are reported as mean ± standard deviation (SD), unless otherwise stated. Statistical significance was set at $P <0.05$.

3. RESULTS

At baseline, no statistically significant differences among groups were found among baseline characteristics of participants except for age (Table 1). Premenopausal women allocated in the EN2 training group reported being younger than their postmenopausal counterpart, as expected ($P = 0.001$).

3.1. Cardiorespiratory fitness and exercise adherence

No significant differences in peak power output (W_{peak}) or $\dot{V}O_{2max}$ were found among exercised groups. Similar improvement of W_{peak} (18%, 15% and 17%) and $\dot{V}O_{2max}$

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(12%, 13%, 14%) were found in CON, EN1 and EN2, respectively. Exercise adherence was similar among groups (>85%, P>0.05).

3.2. Body composition and metabolic health markers

Body composition and metabolic health markers response to the 12-weeks intervention are presented in table 2. All exercise groups reported a statistically significant reduction of body mass and fat mass after the intervention (P_{time}= 0.01). Moreover, fat mass was significantly decreased in CON (-5.2%) and EN1 (-5.3%) compared to control group ($P_{interaction}$= 0.007), but no differences were found between EN1 and EN2 (-5.6%, $P_{interaction}$= 0.645).

The glucose profile analysis showed that Hb_{A1c} was statistically significant reduced in EN1 compared to CON and control groups ($P_{interaction}$ = 0.027). While fasting glucose and HOMA-IR reported a statistically significant reduction in EN2 compared to EN1 ($P_{interaction}$= 0.019 and $P_{interaction}$= 0.044, respectively).

The lipid profile analysis showed pre- vs. post-intervention statistically significant reduction in EN2 for total cholesterol, LDL and triglycerides (P_{time}< 0.05), while EN1 also showed a statistically significant reduction for triglycerides (P_{time}= 0.003).

Sex hormones data have been previously published.[15]

3.3. Myokines

Myokines IL-6, IL-15, IL-15Ra and FGF-21 response to the 12-weeks intervention are illustrated in figure 1. Compared to the control group, a statistically significant decrease in circulating IL-6 concentrations was found in the exercised groups ($P_{interaction}$< 0.001). Moreover, IL-6 concentrations were statistically reduced in EN1 compared to CON (-42% vs. -16%; P= 0.001). When IL-6 concentrations were compared between pre- and postmenopausal women (EN1 vs. EN2), no statistical differences were noted (-42% vs. -38%).

Circulating IL-15 and IL-15Rα concentrations revealed a similar pattern of response. Compared to the control group, a statistically significant decrease in circulating IL-15 and IL-15Rα concentrations was found in the exercised groups ($P_{interaction}$< 0.001). The reduction of IL-15 levels was statistically significant in the EN1 compared to the CON group (-31% vs. -50%; P= 0.005), but a similar reduction was found between EN2 and EN1 group (-51% vs. -50%). In IL-15Rα, the exercised groups of postmenopausal women also showed a statistically significant reduction compared to the control group

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($P_{interaction}$= 0.001), but no statistical differences were noted between EN1 and CON groups (-26% vs. -28%). When comparing pre- vs. postmenopausal women, menopause by time interaction was found ($P_{interaction}$= 0.023), revealing a higher reduction in EN2 compared to EN1 group (-40% vs. -28%, P= 0.01).

Furthermore, circulating FGF-21 concentrations showed a statistically significant decrease in EN1 (-23%) compared to control (P= 0.001) and CON groups (-3%; P= 0.007). Moreover, a similar reduction in EN2 was found compared to EN1 (-23% vs.-23%).

Finally, very low (<0.3 pg/ml) or undetected concentrations of IL-13 were found in the four study groups. For that reason, circulating IL-13 concentrations was not presented in figure 1.

3.4. Association between myokines with body composition and metabolic markers.

In the EN2, the delta change (post- minus pre-intervention values) of fat mass was correlated to the delta change of IL-6 (r= 0.605, P= 0.037), IL-15 (r= 0.546, P= 0.047) and IL-15Rα (r= 0.515, P= 0.001). Moreover, the delta change of HOMA-IR was inversely correlated to IL-15Rα (r= -0.345, P= 0.039) and FGF-21 delta changes (r= -0.775, P< 0.001). Finally, delta changes of FGF-21 and triglycerides were inversely correlated (r= -0.431, P= 0.041). None of these association were found in the EN1 group, but in the CON group, delta changes of fat-free mass was inversely correlated to IL-6 (r= -0.927, P< 0.001), IL-15 (r= -0.675, P= 0.011) and IL-15Rα delta changes (r= -0.654, P= 0.015).

4. DISCUSSION

The present study showed that exercise type, but not menopause except for IL-15Rα, alters circulating myokines (IL-6, IL-15 and FGF-21) response of women with obesity to a 12-weeks training intervention. In postmenopausal women, endurance training was most effective than concurrent training to reduce the circulating expression of IL-6, IL-15 and FGF-21. When comparing endurance training groups of pre- and postmenopausal women, although pre-intervention levels of IL-6, IL-15 and IL-15Rα were higher in postmenopausal women, no between-groups differences were found after the training intervention except for except for IL-15Rα. Interestingly, both endurance training groups caused a similar reduction of fat mass (~5.5%) but, only in the

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premenopausal women group, fat mass reductions were associated with changes in myokines concentrations. Therefore, endurance training is more effective type of exercise than concurrent training to restore regular baseline levels of myokines in postmenopausal women with overweight and obesity. However, despite the metabolic changes that occur during menopause, particularly in fat mass, a similar reduction in circulating IL-6, IL-15 and FGF-21 was found in pre and postmenopausal women.

Muscle contraction is the central stimuli to promote skeletal muscle expression and production of myokines. The endocrine effects of myokines depend on their acute circulating presence. In contrast, the chronic presence in blood of some myokines (i.e., IL-6 or IL-15) may impair myokines' beneficial effect and even cause detrimental effects,[16] potentially by reducing tissue permeability to myokines and activating inflammatory pathways. Postmenopausal women with obesity can show systemic low-grade inflammation that may explain the elevated concentrations of myokines observed pre-intervention (figure 1), compared to non-obese populations.[16] Prolonged exercise training may restore circulating levels of some myokines at baseline,[19-21] but there is controversy in the literature.[21-24] This result can be attributed to the different exercise types used but, although some studies have evaluated the acute effect of a single session of different exercise types,[25] the effects of a long-term intervention using different types of training remained to be investigated.

In the present study, 12-week of endurance and concurrent training caused differences in the circulating concentrations of four myokines (IL-6, IL-15, IL-15Rα and FGF-21) implicated in glucose and lipid metabolism.[3-7] We have observed that circulating IL-6 concentrations decreased by 16% and 42% in response to concurrent and endurance training. These results are consistent with previous evidence in a similar population of overweight older adults [26] and obese adults,[19] in which 40 and 12 weeks of endurance training reported a decrease of this myokine. Interestingly, in Bruun et al.,[19] the reduction in the circulating presence of IL-6 was accompanied by a decrease in mRNA expression and a reduction of body mass. In contrast, resistance training seems to cause an inverse effect, increasing IL-6 levels after training in pre- and postmenopausal women,[21] while in response to concurrent training, no differences were found.[22]

In lean and obese adults, circulating concentrations of IL-15 and IL-15Rα are reduced in response to regular physical activity [16, 27] and after 12 weeks of structured endurance training.[20] Interestingly, in another study, protein expression of IL-15, but no the plasma

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or mRNA, was upregulated in skeletal muscle.[28] In contrast, resistance training seems to not altered circulating IL-15 concentration on postmenopausal women [23] or even increase its concentrations in pre- and postmenopausal women.[21] Overall, these studies are in accordance with the higher reduction of serum IL-15 after endurance (-50%) compared to concurrent training (-31%) found here. On the other hand, circulating IL-15Rα has been less studied. Although to date no studies have investigated this myokine response to a long-term training program, regular physical activity seems to reduce circulating IL-15Rα concentrations in adults with obesity,[16] which support the reduction of serum IL-15Rα found here after 12-week of concurrent (-26%) and endurance training (-28%). Since IL-15Rα respond to acute doses of resistance exercise [29], the lack of differences found between types of exercise may be due to a muscle mass preservation mechanism involving IL-15Rα in postmenopausal women with overweight and obesity.

Circulating FGF21 concentration was only reduced in response to endurance training (-23%). Previous studies have reported that endurance training decreases serum FGF21 levels, being cardiorespiratory fitness and visceral fat mass negatively associated with this myokine/hepatokine.[30] Thus, the stress induced by exercise can be responsible for the regulation of the circulating levels of FGF21,[31] however, in the present study, concurrent training did not alter FGF21 concentrations. Previously, 8 weeks of resistance training performed in a circuit mode reported a significant increase of circulating FGF21 levels in healthy postmenopausal women.[24] While, after 12-week of concurrent training (45 min endurance and 20 min resistance training) performed 5 times a week, serum FGF21 was significantly decreased in postmenopausal women with obesity.[32] In contrast to these studies, we found that postmenopausal women with obesity did not experiment a reduction in circulating FGF21 after concurrent training. This result can be attributed to the distribution of endurance and resistance training performed here (20 and 40 min, respectively) in contrast to Yang et al. (45 and 20 min, respectively).[32] Therefore, when resistance exercise predominates over endurance exercise in a concurrent training program, the regulation of the circulating levels of FGF-21 is not altered, which suggest that the stress induced by exercise is not the only factor able to modulate this myokine/hepatokine, at least in postmenopausal women with overweight or obesity. In fact, the higher elevation of insulin produced by resistance training can be responsible of the lack of alteration observed in the concurrent training group.[33]

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Overall, the opposing effects caused by endurance (decrease) and resistance training (increase) on circulating myokines concentrations seem to be the reason behind the lower reduction of circulating myokines levels, except for IL-15Rα, found in this study. Thus, endurance training may be a more effective strategy to promote circulating restoration of myokines levels at baseline in a population of postmenopausal women with overweight or obesity.

Obesity is a critical health problem in postmenopausal women that can lead to comorbidities such as breast cancer, particularly in inactive populations.[1] The metabolic changes that occurred during menopause (e.g., reduction of resting metabolic rate) tend to promote an accumulation of visceral fat mass in this population, an increase of inflammatory state and a reduction in muscle mass.[34-36] Exercise training is an effective non-pharmacological strategy to reverse these effects. We have found a statistically significant decrease of fat mass after 12-weeks of training (CON, -5.2%; EN1, -5.3%; EN2, -5.6%). Myokines are one of the leading candidates which may enable the muscle-adipose tissue crosstalk communication.[37, 38] affecting the link between obesity and its comorbidities. Here, although a similar fat mass reduction was noted between exercised groups, endurance training was the exercise type that facilitated a higher decrease in circulating myokines concentrations. However, only in premenopausal women, an association between changes in fat mass and myokines (IL-6, IL-15, IL-15Ra) was observed. Moreover, despite pre-intervention concentrations of IL-6, IL-15 and IL-15Rα were higher in post vs. premenopausal women ($P < 0.05$), after 12-weeks of endurance training, a similar decrease of these myokines was noted, except for IL-15Rα. Taken together, these results may indicate that the metabolic disruption occurring in adipose tissue metabolism during menopause may alter the muscle-adipose tissue crosstalk communication established by myokines, but not affecting myokines' effect on exercise-induced fat mass loss.

Postmenopausal women have shown an accentuated inflammatory state characterized by an increased systemic inflammation as well as an augmented presence, exhausted and senescent of T-cells.[39] Since immune cells (e.g., lymphocytes) can also produce IL-6 and IL-15 to promote pro- and anti-inflammatory responses,[40] the chronically elevated presence of these myokines in blood may respond to the predominance of immune cells on IL-6 and IL-15 regulation in physically inactive postmenopausal women with obesity.[16] In contrast, when these women were involved in a 12-weeks of endurance

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training intervention, myokines restore regular baseline concentration, potentially indicating a switch in the role and tissue responsible for IL-6 and IL-15 availability. Thus, when the adequate exercise stimuli (potentially endurance training) is performed, skeletal muscle produces and releases IL-6 and IL-15, reducing inflammation and the disruption caused by the inflammatory state on fat mass accumulation.[41]
Supporting this idea, in response to concurrent training an attenuated reduction of circulating myokines was found, an effect that was inversely associated with fat-free mass in IL-6 ($r = -0.927$, $P < 0.001$), IL-15 ($r = -0.675$, $P = 0.011$) and IL-15Rα ($r = -0.654$, $P = 0.015$). Since these myokines respond to resistance exercise and are related to muscle mass increase and maintenance,[29, 42] these results may be attributed to a resistance exercise-induced inflammation which facilitate muscle cell repair and the adaptions to this type of exercise. However, further studies are needed to explore the potential relationship of these myokines on muscle mass maintenance in postmenopausal women, particularly analyzing their local expressions (mRNA and protein) in skeletal muscle.

Finally, this study shows two main limitations. First, the small and unequal sample size caused by the drop-out experimented during the trial. Second, the comparison between pre- and postmenopausal women was limited to endurance training. Despite these limitations, we believe that the study provides new information regarding how exercise training modulates inflammatory markers and myokines in postmenopausal women with obesity.

To summarize, in postmenopausal women with obesity, 12-weeks of endurance training stimulates a higher reduction of IL-6, IL-15 and FGF-21 than concurrent training. Although endurance exercise promotes a similar decreased of myokines and fat mass in pre and postmenopausal women, only in premenopausal women changes in myokines and fat mass were associated. This data, together with the higher pre-intervention levels of cytokines found in postmenopausal compared to premenopausal women, may indicate that endurance exercise is an effective training strategy to reduce systemic low-grade inflammation caused by fat mass accumulation and menopause.
Therefore, in postmenopausal women with obesity, training programs can be initiated with endurance training in order to elicit a reduction in systemic low-grade inflammation while promoting fat mass loss. When cytokines and growth factors restore

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healthy levels at baseline, other types of exercise can be performance in this population focusing on improving glucose and lipid metabolism and body mass remodeling.

AUTHORS' CONTRIBUTIONS

APL, PGE and DV conceived and designed the experiment. APL and PGE collected the data. APL, PGE, BPK, NGH and DV analysed and interpreted the data. APL drafted the manuscript. APL and PGE prepared figures and tables. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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FIGURE LEGEND

Figure 1. Changes in the myokines IL-6 (A), IL-15 (B), IL-15Rα (C) and FGF-21 (D) after 12 weeks of intervention.

* $P < 0.05$ compared pre- vs post-intervention; # $P < 0.05$ compared to C; † $P < 0.05$ compared to CON; § $P < 0.05$ compared to EN1. Abbreviations: C, control group; CON, concurrent training group; EN1, endurance training group of postmenopausal women; EN2, endurance training group of postmenopausal women; IL-6, interleukin-6; IL-15, interleukin-15; IL15-Rα, interleukin-15 receptor alpha; FGF-21, fibroblast growth factor 21.

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Table 1. Participants' characteristics at baseline

	Control (N = 12)	CON training (N = 13)	EN1 training (N = 10)	EN2 training (N = 12)	*P* value
Age (yr)	56.9 ± 5.8*	58.7 ± 2.9*	56.7 ± 3.7*	43.1 ± 2.8	0.001
Age at menopause (yr)	48.5 ± 5.1	49.7 ± 3.4	49.3 ± 3.7	-	0.790
Height (cm)	159 ± 5	160 ± 5	161 ± 6	159 ± 7	0.989
Body mass (kg)	93.0 ± 13.9	86.4 ± 11.5	84.7 ± 9.1	93.2 ± 7.0	0.193
Ethnicity					0.567
White, N (%)	10 (83)	12 (92)	9 (92)	12 (100)	
Other, N (%)	2 (17)	1 (8)	1 (8)	0 (0)	
Education					0.194
High School, N (%)	3 (25)	1 (8)	4 (40)	3 (25)	
College/Trade; N (%)	9 (75)	11 (85)	4 (40)	7 (58)	
Graduate degree; N (%)	0 (0)	1 (8)	2 (20)	2 (17)	
Blood pressure					
Systolic (mmHg)	127 ± 16	121 ± 12	126 ± 28	130 ± 18	0.375
Diastolic (mmHg)	85 ± 11	84 ± 6	82 ± 11	78 ± 6	0.209
Total physical activity (min/week)	5624 ± 5265	6104 ± 5875	6032 ± 5733	5648 ± 5050	0.677
Dietary habits					
Energy intake (kcal/day)	1859 ± 238	1809 ± 292	1683 ± 282	1899 ± 189	0.249
Carbohydrates (%)	45 ± 6	46 ± 12	41 ± 10	44 ± 10	0.670
Fats (%)	36 ± 4	36 ± 11	36 ± 7	36 ± 9	0.994
Proteins (%)	19 ± 3	18 ± 4	22 ± 4	20 ± 3	0.170

Values are presented as mean ± SD. Abbreviations: CON, concurrent training; EN1, endurance training in postmenopausal women; EN2, endurance training in premenopausal women. * P < 0.05 compared to EN2 training.

Table 2. Body composition and metabolic health markers response by exercise type and menopause after the 12-weeks intervention.

	Control (N = 12)		**CON** (N = 13)			**EN1** (N = 10)			**EN2** (N = 12)			**Exercise type**			**Pre- vs Postmenopause**		
	Pre	Post	Pre	Post	Dif.	Pre	Post	Dif.	Pre	Post	Dif.	**T**	**C**	**T x C**	**T**	**C**	**T x C**
Body composition																	

n																	
Body mass (kg)	93.0 ± 13.9	93.1 ± 14.5	86.4 ± 11.5	85.0 ± 11.5	*	84.7 ± 9.1	83.0 ± 8.9	*#	93.2 ± 7.0	90.4 ± 8.4	*§	**0.002**	0.148	**0.0133**	**0.001**	**0.038**	**0.000**
BMI (kg/m^2)	34.9 ± 6.4	36.9 ± 6.6	33.8 ± 5.3	33.3 ± 5.5		32.9 ± 4.2	32.2 ± 4.2		37.0 ± 2.8	35.8 ± 3.0	*	0.105	0.192	0.4588	**0.031**	**0.014**	0.234
FM (kg)	44.1 ± 10.0	44.0 ± 9.5	38.0 ± 8.1	36.6 ± 8.5	*#	37.4 ± 7.6	35.4 ± 7.6	*#	41.5 ± 4.1	39.2 ± 3.7	*	**0.001**	0.109	**0.0007**	**0.000**	0.081	0.645
FFM (kg)	49.3 ± 4.6	49.8 ± 4.6	47.9 ± 3.6	48.5 ± 4.0	*	47.3 ± 2.1	47.6 ± 1.6		49.5 ± 3.4	49.9 ± 3.7		**0.004**	0.469	0.7200	**0.042**	0.087	0.155
Glucose profile																	
Glucose (mg/dl)	93.8 ± 11.1	95.3 ± 8.2	95.5 ± 7.8	96.2 ± 8.2		98.1 ± 9.1	101 ± 9.3		103 ± 11.7	97.7 ± 9.2	*§	0.278	0.372	0.8855	0.432	0.743	**0.019**
Insulin (µU/ml)	115.9 ± 8	142 ± 9.9	133.8 ± 4	111.3 ± 5.8		143.5 ± 1	133.5 ± 11.		133.1 ± 3	111.4 ± 3.5	*	0.076	0.414	0.6922	**0.026**	0.718	0.269

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	.6		.6			0.95	4		.85							
HbA1C	5.6±0.34	5.5±0.3	5.6±0.33	5.5±0.3	*§	5.7±0.43	5.7±0.3	#†	5.4±0.63	5.4±0.5		**0.009**	0.652	**0.027**	0.501	0.182	0.120
HOMA-IR	.2±2.9	3.7±2.7	.3±1.2	2.8±1.6		.5±2.7	3.5±3.2		.4±1.3	2.8±1.0	*§	0.177	0.545	0.633	0.085	0.755	**0.044**
Lipid profile																	
Cholesterol (mg/dl)	190±34	193±42	217±42	215±38		215±27	208±21		199±16	190±18	*	0.648	0.149	0.716	**0.022**	**0.027**	0.599
HDL (mg/dl)	50.8±11.6	48.3±8.3	52.8±13.3	53.1±12.5		60.6±14.6	64.1±16.4		49.7±4.6	50.0±5.4		0.667	0.200	0.152	0.254	**0.002**	0.066
LDL (mg/dl)	108±31	118±37	138±39	137±34		129±24	123±16		123±12	114±11	*	0.863	0.137	0.430	**0.003**	0.213	0.520
Triglycerides (mg/dl)	155±74	124±43	154±78	130±44	*	129±53	102±26		142±30	114±21	*	**0.003**	0.417	0.861	**0.002**	0.250	0.738
Inflammation																	
CRP (mg/l)	4.0±	5.1±4.	3.1±	2.9±3.		5.5±	4.5±6.		6.0±	5.4±4.	*	0.686	0.532	0.096	0.099	0.751	0.401

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	3 6 . 4		4 3 . 7		8 1 . 5		4 1 . 5

Values are presented as mean ± SD.

Abbreviations: BMI, Body mass index; C, condition or group effect; CON, concurrent training; Dif., differences among or between groups; EN1, endurance training in postmenopausal women; EN2, endurance training in premenopausal women; FFM, Fat-free mass; FM, Fat mass; HbA1c, glycosylated hemoglobin type A1c; HDL, high-density lipoprotein; HOMA-IR, Homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; PRE, pre-intervention; POST, post-intervention; T, time effect; T x C, time x condition effect.

* $P < 0.05$ comparing PRE vs POST.

# $P < 0.05$ compared to Control.

† $P < 0.05$ compared to CON.

§ $P < 0.05$ compared to EN1.

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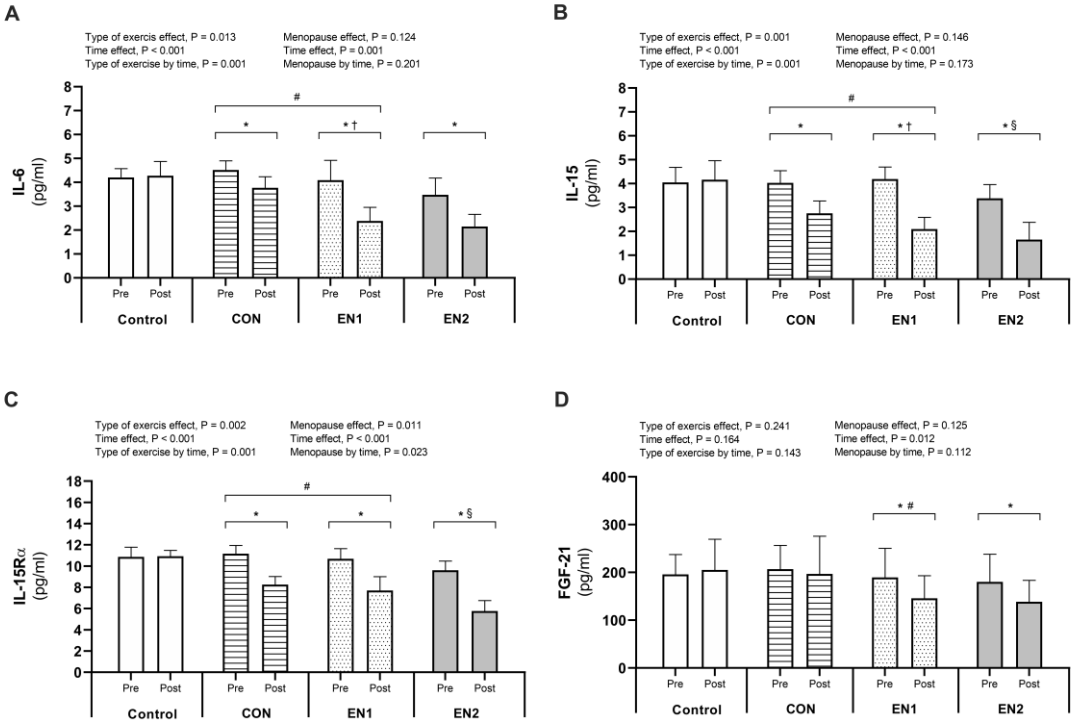

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