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Short communication

Exercise-induced anti-inflammatory effects in overweight/obese women with polycystic ovary syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is characterized by exacerbated inflammation, which is implicated in cardiometabolic dysfunction. This study aimed to examine the potential effects of acute exercise on inflammatory responses in obese/overweight PCOS women and their controls. Participants underwent a single bout of moderate-intensity aerobic exercise (30 min at ~65% of VO_{2peak}). Blood and muscle samples were collected immediately before (PRE) and 60 min after the exercise session. Cytokines (i.e., IL-1β, IL-6, IL-4, IL-10, TNF-α) were measured both in plasma and in skeletal muscle, and proteins related to inflammatory signaling (IKKα/β and JNK) were assessed in skeletal muscle. At PRE, PCOS showed elevated muscle TNF-α (+62%, $p = 0.0012$) and plasma IL-1β (+76%, $p = 0.0010$) compared to controls. In PCOS, exercise decreased plasma and muscle TNF-α (−14%, $p = 0.0003$ and −46%, $p = 0.0003$), as well as increased plasma and muscle IL-4 (+147%, $p = 0.0018$ and +62%, $p = 0.0474$) and plasma IL-10 (+38%, $p = 0.0029$). Additionally, IKKα/β and JNK phosphorylation in skeletal muscle, which was higher in PCOS at PRE, was significantly reduced by exercise (−58%, $p < 0.0001$ and −46%, $p < 0.0001$, respectively), approaching control levels. Person's correlations between PRE values and delta changes (i.e., exercise effect) showed significant, negative associations for plasma IL-1β ($r = -0.92$, $p < 0.0001$), TNF-α ($r = -0.72$, $p = 0.0100$) and IL-6 ($r = -0.58$, $p = 0.05$), and muscle TNF-α ($r = -0.95$, $p < 0.0001$), IKKα/β ($r = -0.75$, $p = 0.005$), and JNK ($r = -0.94$, $p < 0.0001$) in PCOS. In conclusion, exercise can mitigate the inflammatory milieu in women with PCOS. The anti-inflammatory role of exercise could underlie its cardiometabolic protection in PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is characterized by menstrual irregularity, hyperandrogenemia, and polycystic ovarian morphology [1]. Women with PCOS show chronic low-grade inflammation, characterized by increased plasma levels of tumor necrosis factor-α (TNF-α) and interleukine-6 (IL-6), which have been associated with insulin resistance, a frequent disturbance in this syndrome [2].

From a mechanistic standpoint, pro-inflammatory cytokines (e.g., TNF-α) can activate c-Jun N-terminal kinase (JNK) and IκB kinase B (IKKβ), which induce serine phosphorylation of insulin receptor substrate 1 (IRS-1), thereby leading to impairment in insulin signaling [3], a mechanism already described in cultured muscle cells from obese women with PCOS [4].

Exercise can induce an anti-inflammatory effect [5], which is observed following a single exercise bout [6]. Nonetheless, the extent to which exercise can modulate inflammation appears to depend upon the disease, drug treatment, baseline inflammatory status, and body fatness [7]. Given the well-known impact of chronic inflammation on the etiology and perpetuation of several cardiometabolic risk factors [8], it becomes important to investigate whether exercise can mitigate inflammation in women with PCOS as observed in healthy individuals and in patients with other chronic diseases [9].

We examined the effects of a single bout of aerobic exercise on

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inflammatory cytokines and inflammatory signaling-related muscle proteins in women with PCOS and their controls.

2. Materials and methods

The present data are part of a larger study primarily aimed to investigate the role of acute exercise on molecular signaling associated with insulin resistance and its cross- talk with other pathways (e.g., inflammation signaling) in PCOS patients [10]. In brief, 12 overweight/obese women with PCOS diagnosed according to current guidelines [1] were recruited from the Endocrinology Division of the School of Medicine of the University of Sao Paulo (Brazil). In addition, 10 BMI-matched controls (CTRL) (age: 18–35 years, BMI: 25–40 kg/m^2) were selected to participate in this study. Baseline sample's assessments (Supplementary Table 1) included hormonal and metabolic profile, aerobic condition, physical activity levels, and body composition, as thoroughly described elsewhere [10,11]. Exclusion criteria included type 2 diabetes, smoking, and use of oral contraceptives within 6 months prior to study participation. Participants gave their informed consent before participation and the study was approved by the local ethics committee.

Subjects were instructed to refrain from alcohol consumption and vigorous physical activity for at least 48 h before the acute exercise session; subjects also consumed a standardized dinner the evening before the test [10,11]. Then, following an overnight fast (at ~8:00 am), subjects underwent a single bout of aerobic exercise (30 min at ~65% of VO_{2peak}), as previously described [10,11]. Blood and muscle samples (percutaneous biopsies from *vastus lateralis*) were collected immediately before (PRE) and 60 min after the exercise session (POST). Cytokines were measured both in plasma and muscle, and proteins related to inflammatory signaling were assessed in muscle samples.

Plasma and muscle IL-1β, IL-6, IL-4, IL-10, TNF-α levels were determined using a multiplex human panel, according to the manufacturer procedures (EMD Millipore, Milliplex®). Coefficient of variations (CVs) for IL-1β, IL-6, IL-4, IL-10, TNF-α in plasma were 7.4%, 5.1%, 8.5%, 5.4% and 8.6%, respectively, whereas in muscle were 5.5%, 6.8%, 3.6% 5.5% and 4.5%, respectively.

The expression of IKK α/β Ser 177/181 (Cell Signaling #2697), total IKK α/β (Santa Cruz Biotechnology #sc-7607), JNK Thr 183 (Santa Cruz Biotechnology #sc-135642) and total JNK (Santa Cruz Biotechnology #sc-7345) was assessed by Western blotting, following previous descriptions [10].

Data are presented as mean ± SD and relative change (pre-to-post exercise), except otherwise stated. Two-way ANOVA (group × time) with Bonferroni *post hoc* adjustment was performed to test for within- and between-group differences, using GraphPad Prism® version 6. To explore the influence of the baseline inflammatory signaling on the exercise-induced responses, *post hoc* Pearson's correlations were conducted between pre values and relative changes for all dependent variables. Significance level was set at $p \leq 0.05$.

3. Results

At PRE, PCOS showed significantly higher plasma IL-1β (Fig. 1, Panel A) than CTRL (+76%, $p = 0.0010$). After exercise, both groups showed reductions in IL-1β (PCOS: −36%, $p = 0.0002$; CTRL: −42%, $p = 0.0199$); however, plasma IL-1β remained significantly higher in PCOS vs. CTRL (+93%, $p = 0.0220$).

Plasma TNF-α (Fig. 1, Panel B) was comparable between groups at PRE ($p = 0.8276$). Both groups showed a significant reduction in TNF-α following exercise (CTRL:-12%, $p = 0.0108$; PCOS: −14%, $p = 0.0003$), although no significant between-group difference was observed ($p = 0.9999$).

Plasma IL-4 (Fig. 1, Panel C) was similar between PCOS and CTRL at PRE ($p = 0.9999$), and both groups showed increased values following exercise (CTRL: +178%, $p = 0.0171$; PCOS: +147%, $p = 0.0018$). No between-group difference was noted at POST ($p = 0.2329$).

No between-group difference was observed in plasma IL-6 (Fig. 1, Panel D) at PRE ($p = 0.9195$). Both groups showed a significant increase in IL-6 (CTRL: +122%, $p = 0.0087$; PCOS: +144%, $p < 0.0001$) at POST, with a trend towards increased levels in PCOS vs. CTRL ($p = 0.0547$).

Plasma IL-10 (Fig. 1, Panel E) was comparable between groups at PRE ($p = 0.9999$). PCOS showed an increase in IL-10 after exercise (+38%, $p = 0.0029$), whereas no changes were observed in CTRL ($p = 0.3640$). However, no between-group difference was observed at POST ($p = 0.2329$).

There were no within- or between-group differences in muscle IL-1β ($p > 0.05$, Fig. 1, Panel F).

At PRE, muscle TNF-α (Fig. 1, Panel G) was significantly higher in PCOS than in CTRL (+62%, $p = 0.0012$). Exercise reduced muscle TNF-α in PCOS (−46%, $p = 0.0003$), but not in CTRL ($p = 0.7521$). Groups did not differ at POST ($p = 0.9999$).

Muscle IL-4 (Fig. 1, Panel H) was comparable between groups at PRE ($p = 0.9999$). After exercise, only PCOS showed a significant increase in muscle IL-4 (+62%, $p = 0.0474$), but there was no between-group difference at POST ($p = 0.9999$).

Muscle IL-6 (Fig. 1, Panel I) was comparable between groups at PRE. Following exercise, significant within-group differences were observed in both groups (CTRL: +97%, $p = 0.0030$; PCOS: +60%, $p = 0.0270$), with no difference between them ($p = 0.9999$).

Muscle IL-10 (Fig. 1, Panel J) was also greater in PCOS vs. CTRL at PRE (+60%, $p = 0.0119$). Following exercise, CTRL showed a significant increase in muscle IL-10 (+50%, $p = 0.0060$), whereas no changes were seen in PCOS ($p = 0.0733$). There was no between-group difference at POST ($p = 0.1155$).

IKKα/β (Fig. 2, Panel A) and JNK phosphorylation (Fig. 2, Panel B) was increased in PCOS vs. CTRL at PRE (+109% and +89%, $p = 0.0099$ and $p < 0.0001$, respectively). Exercise diminished IKKα/β phosphorylation in both groups (CTRL: −55%, $p = 0.0377$; PCOS = −58%, $p < 0.0001$), whereas only PCOS experienced decreased JNK phosphorylation after exercise (−46%, $p < 0.0001$). The expression of IKKα/β and JNK was comparable between the groups at POST ($p = 0.4949$ and $p = 0.9999$, respectively).

Post hoc Pearson's correlations between PRE values and delta changes (i.e., exercise effect) were performed. For CTRL, we found significant, negative correlations for muscle IL-1β ($r = -0.83$, $p = 0.0031$) and IKKα/β ($r = -0.77$, $p = 0.0086$). For PCOS, we observed significant, negative correlations for plasma IL-1β ($r = -0.92$, $p < 0.0001$), TNF-α ($r = -0.72$, $p = 0.0100$) and IL-6 ($r = -0.58$, $p = 0.05$), and muscle TNF-α ($r = -0.95$, $p < 0.0001$), IKKα/β ($r = -0.75$, $p = 0.005$), and JNK ($r = -0.94$, $p < 0.0001$). No other significant associations were found.

4. Discussion

In this study, a single bout of exercise was able to elicit a general anti-inflammatory response in women with PCOS, which was evident both in plasma and in skeletal muscle.

Pro-inflammatory imbalance has been reported in PCOS [2]. In support to this, our patients showed increased levels of muscle TNF-α and plasma IL-1β, which was independent of obesity, corroborating previous evidence [12]. Considering the pathophysiologic role of exacerbated inflammation on cardiometabolic dysfunction in PCOS, interventions that elicit anti-inflammatory effects emerge as potential therapeutic tools in this condition. Exercise is one of the most effective non-pharmacological therapy to modulate inflammation. We and others have shown that exercise can mitigate inflammation in a variety of conditions, including obesity [12], type 2 diabetes [13], and autoimmune rheumatic diseases [6–8]. Interestingly, whilst these conditions are clearly distinct regarding pathophysiologic features, drug treatment, and immune function, exercise seems to reduce the inflammatory

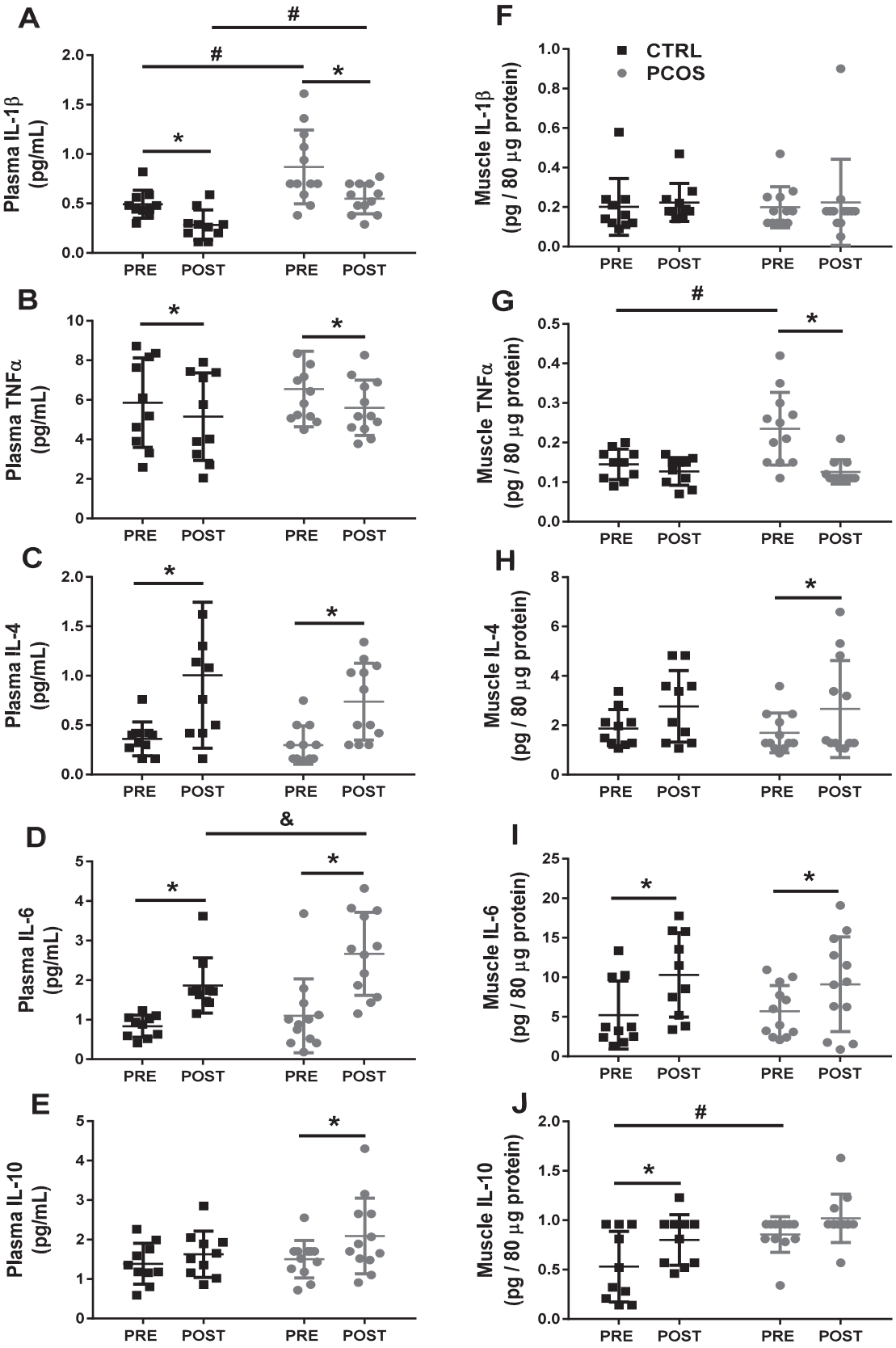

Fig. 1. Plasma and muscle cytokines (i.e., IL-1β, IL-6, IL-4, IL-10, TNF-α) in response to a single bout aerobic exercise. * Indicates significant difference vs. PRE ($p \leq 0.05$). # Indicates significant difference between CTRL vs. PCOS ($p \leq 0.05$). & Tendency towards significance between PCOS vs. CTRL. Data are expressed as mean ± SD.

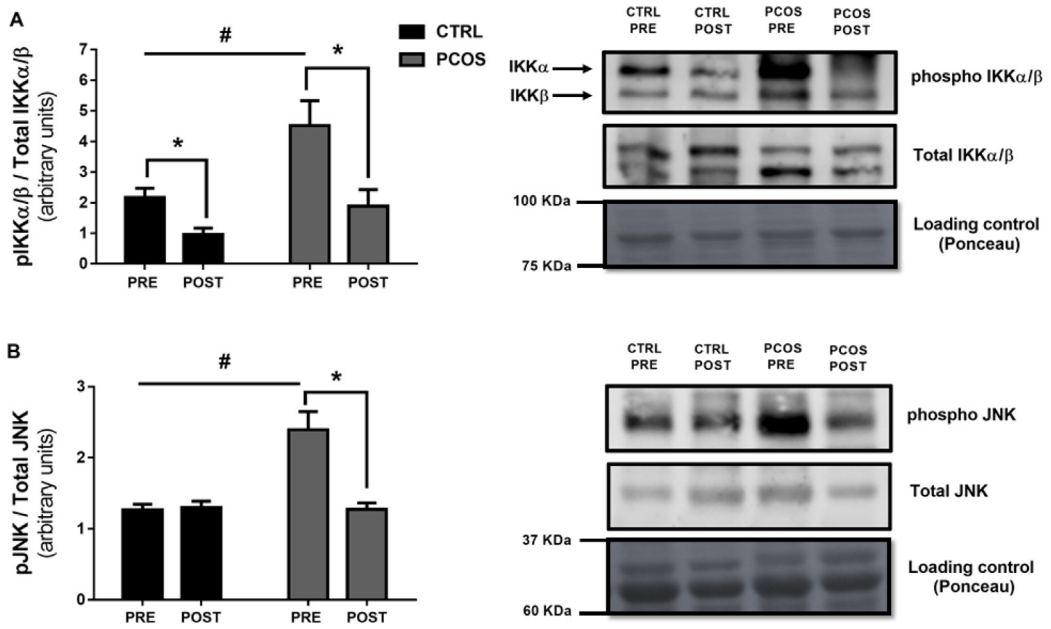

Fig. 2. Proteins related to inflammatory signaling (IKKα/β and JNK) in response to a single bout aerobic exercise. * Indicates significant difference vs. PRE ($p \leq 0.05$). # Indicates significant difference between CTRL vs. PCOS ($p \leq 0.05$). Data are expressed as mean ± SD.

milieu in all of them, although at different magnitudes. Based on the current data, this immunomodulatory response can be extended to PCOS, as evidenced by a suppression of pro-inflammatory cytokines (*i.e.*, muscle and plasma TNF-α) and a stimulation of anti-inflammatory cytokines (*i.e.*, plasma IL-4 and IL-10, and muscle IL-4).

It has been speculated that exercise could transitorily enhance muscle IL-6 secretion, which would be followed by a release of anti-inflammatory cytokines from muscle, the so-called myokines [5]. In line with this, we observed increased IL-6 levels following exercise both in CTRL and PCOS, suggesting that this exercise-mediated anti-inflammatory mechanism may be persevered in obesity, irrespective of the presence of PCOS.

IKK and JNK are involved in propagating the cellular response to inflammation and may cause insulin resistance by inhibiting insulin cascade [3]. Interestingly, exercise was capable of restoring IKK and JNK phosphorylation back to control levels, further corroborating the potent immunomodulatory role of exercise in PCOS. Considering that the skeletal muscle is the main glucose disposal site [14], and that inflammation can be detrimental to this process [3], it is plausible to speculate that resolving pro-inflammatory signaling within the muscle may help prevent insulin resistance in PCOS. However, the extent to which exercise can mitigate a pro-inflammatory pattern in patients with a more exacerbated inflammation remains to be investigated, since we observed that baseline inflammatory signaling (e.g., IKKα/β, JNK) and cytokines (e.g., TNF-α, IL-1β) were inversely associated with the ability of exercise to suppress inflammation.

With the exceptions above discussed, inflammatory markers at baseline and in response to exercise were somewhat similar between patients with and without PCOS, suggesting that obesity rather than PCOS is the major factor in inflammation. Accordingly, tackling obesity appears to be a valid therapeutic strategy to attenuate exacerbated inflammation in PCOS, ultimately mitigating insulin resistance, a condition that may be aggravated by hyperandrogenism [2,4].

In conclusion, acute aerobic exercise can mitigate the inflammatory milieu in PCOS. This anti-inflammatory response could be an important mechanism by which exercise promotes cardiometabolic protection in this syndrome. Exercise training studies should examine whether the acute effects of exercise sustain in the long run.

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Conflict of interest

The authors declare that have no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.04.006.

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