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Exercise-Induced Change in Plasma IL-12p70 Is Linked to Migraine Prevention and Anxiolytic Effects in Treatment-Naïve Women: A Randomized Controlled Trial

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Keywords
Migraine · Chronic pain · Physical activity · Cytokines · Anxiety

Abstract
Objective: To conduct a randomized controlled trial to evaluate the effect of a 12-week aerobic exercise program for migraine prevention, plasma cytokines concentrations (TNF-α, interleukin [IL]-1β, IL-6, IL-8, IL-10, and IL-12p70), and anxiety in women with migraine. ***Methods:*** Women with episodic migraine (ICHD-II), aged between 20 and 50 years, who had never taken any prophylactic medication, and were physically inactive in the past 12 months were recruited from the university's hospital and a tertiary headache clinic between March 2012 and March 2015. Migraine attacks were recorded in headache diaries, cytokines were quantified by flow cytometry, and anxiety was assessed by the 7-item General Anxiety Disorder (GAD-7) scale. Blood sampling and psychometric interviews were undertaken on headache-free days. ***Results:*** Twenty participants ([mean ± SD] age 33.8 ± 10.5; BMI 26 ± 5.2) were randomly assigned and received intervention ("trained": $n = 10$) or entered on a waitlist ("inactive": $n = 10$). There were no differences between groups regarding patients' characteristics and baseline data. Days with migraine ($p = 0.001$), IL-12p70 levels ($p = 0.036$), and GAD-7 score ($p = 0.034$) were significantly reduced in the trained group after the intervention period, but there were no significant changes in these variables in the inactive group. There was no change in the levels of the other cytokines in either group. There were positive correlations between a reduction in IL-12p70 level and a reduction in the number of days with migraine ($R^2 = 0.19$, $p = 0.045$), and GAD-7 score ($R^2 = 0.53$, $p < 0.001$). ***Conclusion:*** The clinical and psychological therapeutic effects of aerobic exercise in treatment-naïve women with migraine may involve the downregulation of IL-12p70.

Trial registration: #NCT01972607.

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Introduction

Extensive literature indicates that regular moderate aerobic exercise modulates the immune system, affecting mental [1] and physical health [2–4]. A consistent line of evidence suggests an intricate exercise-dependent regulation between the stress neuroendocrine system and type 1/type 2 cytokine balance, with repercussions on pathogenic processes depending on anti- or proinflammatory actions of these cytokines [1, 2, 5]. Endurance exercise stimulates interleukin (IL)-10 production, orchestrated by massive production of IL-6 (also known as "myokines"), which is thought to promote anti-inflammatory actions [1, 2]. On the other hand, endurance athletes exhibit a blunted secretion of the proinflammatory cytokine IL-12p70 after a marathon race [6]. Less is known regarding the balance of these cytokines with chronic moderate exercise (i.e., exercise training) in clinical populations, and its influence on clinical outcomes.

In chronic pain disorders such as migraine, aerobic exercise has shown to be an effective therapeutic approach to manage clinical symptoms and comorbidities [7–11]. Migraine is a common neurologic disorder and a leading cause of disability in the world, especially among young and middle-aged women [12, 13]. Besides headache, migraine disability is considerably increased by psychiatric symptoms/comorbidities, especially anxiety disorders [14–16]. Both during attacks or on headache-free days, migraine patients exhibit aberrant cytokine production, suggesting that immune dysregulation contributes to the pathogenesis of the disease [17–23]. There is a dominant type 1 proinflammatory cytokine profile in migraine patients. Consensus about a cytokine pattern specific to migraine has yet to be determined.

Recently, our group found a skewed plasma IL-12p70/IL-10 balance, coupled with higher anxiety scores, in women with migraine compared to non-headache controls, suggesting a T helper 1 (Th1)-dominant proinflammatory profile in this condition [24]. Higher plasma IL-12p70 (proinflammatory) concentrations were strongly negatively correlated with IL-10 (a Th2 cytokine) concentrations, in line with the well-known mutual negative regulation by these cytokines [25]. IL-12p70 was positively associated with both anxiety symptoms and migraine diagnosis, indicating a potential role for this cytokine in the overlap between migraine and anxiety disorders [14–16].

With this background, we wondered whether increased plasma IL-12p70 and reduced IL-10 in migraine patients could be changed by regular moderate aerobic exercise, and, if so, whether there would be any correlation with clinical and anxiety-related psychological outcomes. Therefore, we hypothesized that an aerobic exercise training program might prevent migraine, reduce IL-12p70, and increase IL-10, and reduce anxiety scores, and that there would be correlations between changes in these variables.

Methods

Trial Design

This is a randomized controlled trial, designed to compare the effects of an intervention involving aerobic exercise on clinical, psychological, and immune outcomes in women with episodic migraine. Participants were randomly allocated to receive the exercise program (designated "trained" group) or entered on a waitlist (designated "inactive" group). We used simple randomization with an allocation ratio of 1:1. Two headache-trained neurologists (R.T.R., M.F.P.P.) conducted a physical and neurological examination of all participants. As participants were screened for inclusion in the study, they were instructed to fill a headache diary and were followed up for 4 weeks for checking baseline headache status. Headache diaries were checked every 4 weeks until the end of the study. At baseline and after the intervention period, participants underwent a psychometric interview, blood sampling, and a maximal cardiopulmonary exercise test. Blood sampling was scheduled within the follicular menstrual period, and on headache-free days (interictal period). Figure 1 illustrates the study's design.

All procedures in this study complied with the Declaration of Helsinki on Human Research, and was approved by the UNIFESP Research Ethics Committee, registered under #081511. This study is registered in www.ClinicalTrials.gov under #NCT01972607. All participants gave written informed consent. The study complies with the CONSORT statement on data reporting for studies with nonpharmacological treatments [26].

Participants

Participants were recruited from the cohort of a previous study [24], who were screened in the Hospital São Paulo's Headache Unit and a tertiary headache clinic, and then invited to participate in an exercise program. Inclusion criteria were: episodic migraine (with and/or without aura) defined according to the ICHD-II criteria [27], aged between 20 and 50 years, having never been on any prophylactic medication for migraine or other diseases, and being physically inactive (defined as engaging in leisure-time physical activity ≤1 times/week in the last 12 months). Exclusion criteria were: unwillingness to continue the protocol, a clinical history of cardiovascular, metabolic, or musculoskeletal disease, or other types of headache, and taking any dietary supplementation or engaging in other behavioral interventions (e.g., mind-body practices).

Interventions/Measures

The exercise program consisted of 30 min of walking on a treadmill (plus 5-min warm-up and cool-down periods), performed 3 times per week at an exercise intensity corresponding to the ventilatory threshold, as determined by a gold-standard maximal cardiopulmonary exercise test (described below). All sessions

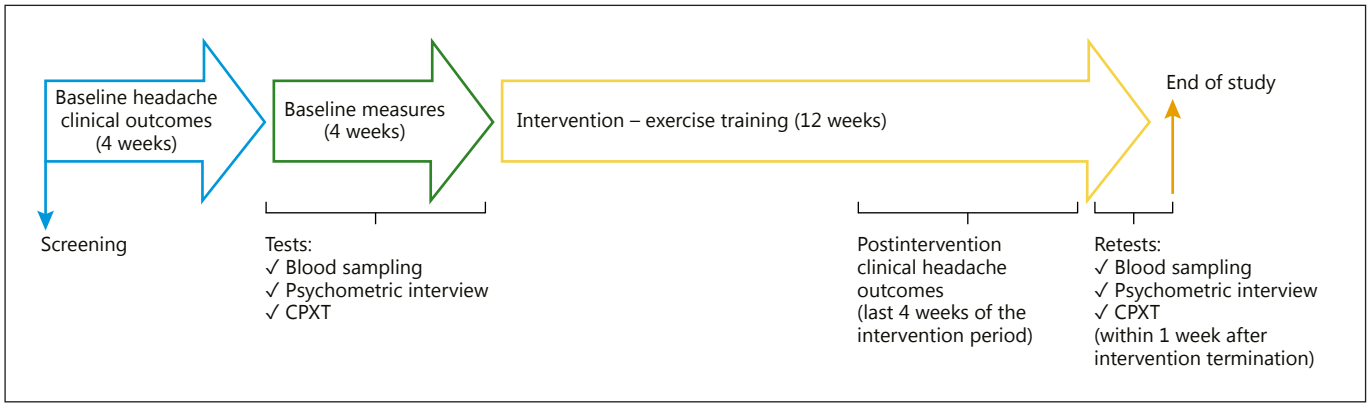

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Fig. 1. Study design. CPXT, maximal cardiopulmonary exercise test.

were supervised by 2 experienced exercise physiologists (A.B.O. and M.T.M.).

Psychometric interviews were conducted in the morning by a certified clinical exercise physiologist (A.B.O.) at the Psychobiology Department of the Federal University of São Paulo. Anxiety was assessed by a 7-item General Anxiety Disorder (GAD-7) scale. The GAD-7 scale screens typical anxiety-related indicators experienced in the previous 2 weeks, and has been validated and translated into Brazilian Portuguese [28].

Blood samples were collected by Psychobiology Department nurses (by venipuncture of the antecubital vein) into cooled EDTA vacutainers (BD Vacutainer®, Franklin Lakes, NJ, USA), and centrifuged immediately for 10 min at 3,400 *g* and 4 °C. Plasma was separated, aliquoted into 1-mL vials, and stored at –80 °C until analysis. Plasma cytokines (TNF-α, IL-1β, IL-6, IL-8, IL-10, and IL-12p70) assays were performed on duplicate by a biologist (A.L.L.B.), using a BD CBA human inflammatory cytokines kit (BD Bioscience, San José, CA, USA), following the manufacturer's instructions. Data were obtained on the BD FACS Accuri flow cytometer and analyzed by FCAP Array software (BD Bioscience). Postintervention blood sampling was scheduled for at least 48 h after the last exercise session.

Maximal cardiopulmonary exercise tests were performed in the morning at the Center for Studies in Psychobiology and Exercise by independent cardiologists and exercise physiologists. Measurements of respiratory gas exchange, obtained breath-by-breath by an open-circuit computerized spirometry system (Quark CPET, COSMED, Rome, Italy), were used for determining the peak oxygen uptake ($VO_{2\,peak}$) and the ventilatory threshold. Oliveira et al. [24] describe $VO_{2\,peak}$ and ventilatory threshold determination criteria. All exercise tests-retests were scheduled for within a week after blood sampling.

Outcomes

The primary outcome of this study was any change in the number of days with migraine. The number days with migraine in the last 4 weeks of the intervention period was set as postintervention clinical data (Fig. 1). The secondary outcome was any change in plasma cytokine concentrations and GAD-7 score, both assessed within a week after the intervention period (Fig. 1).

Sample Size, Randomization, and Blinding

No a priori sample size calculation was performed, so this constituted a sample of convenience. Randomization of participants was performed by online software (www.randomizer.org), with even and odd numbers being attributed to the "trained" and "inactive" allocations, respectively. Participants were randomized (by A.B.O.) at the blood sampling/psychometric interview. There was no randomization of neurologists, nurses, and assessors of maximal cardiopulmonary exercise tests, but clinical visits and measures were scheduled without previous knowledge of their work shifts, and the assessors were not informed about the allocation of the participants. Participants were instructed to report/comment on any exercise-related issue only to the exercise supervisors. Vials of aliquoted blood samples were randomly numbered by a laboratory staff member independent of this study, so the biologist (A.L.L.B.) responsible for the assays had no information about their origin.

Statistical Analysis

The assumption of normality for variables was tested by the Shapiro-Wilk test. Variables with normal distribution were tested with the Student *t* test for baseline analyses, and the paired-samples *t* test for longitudinal analyses. Variables with nonnormal distribution were tested with the Mann-Whitney U test for baseline analyses and the Wilcoxon signed-rank test for longitudinal analyses. Effect sizes for each test were calculated accordingly. The Pearson and Spearman correlations tests were used for variables with normal and nonnormal distribution, respectively. $p < 0.05$ was considered statistically significant. SPSS software (for Windows, v20.0, IBM, Armonk, NY, USA) was used for computing statistical tests, and graphs were generated by GraphPad Prism® software (v5.0, San Diego, CA, USA).

Results

The study recruitment period was from March 2012 to March 2015, and the study ended at the completion of the research chronogram. Twenty participants ([mean ± SD]

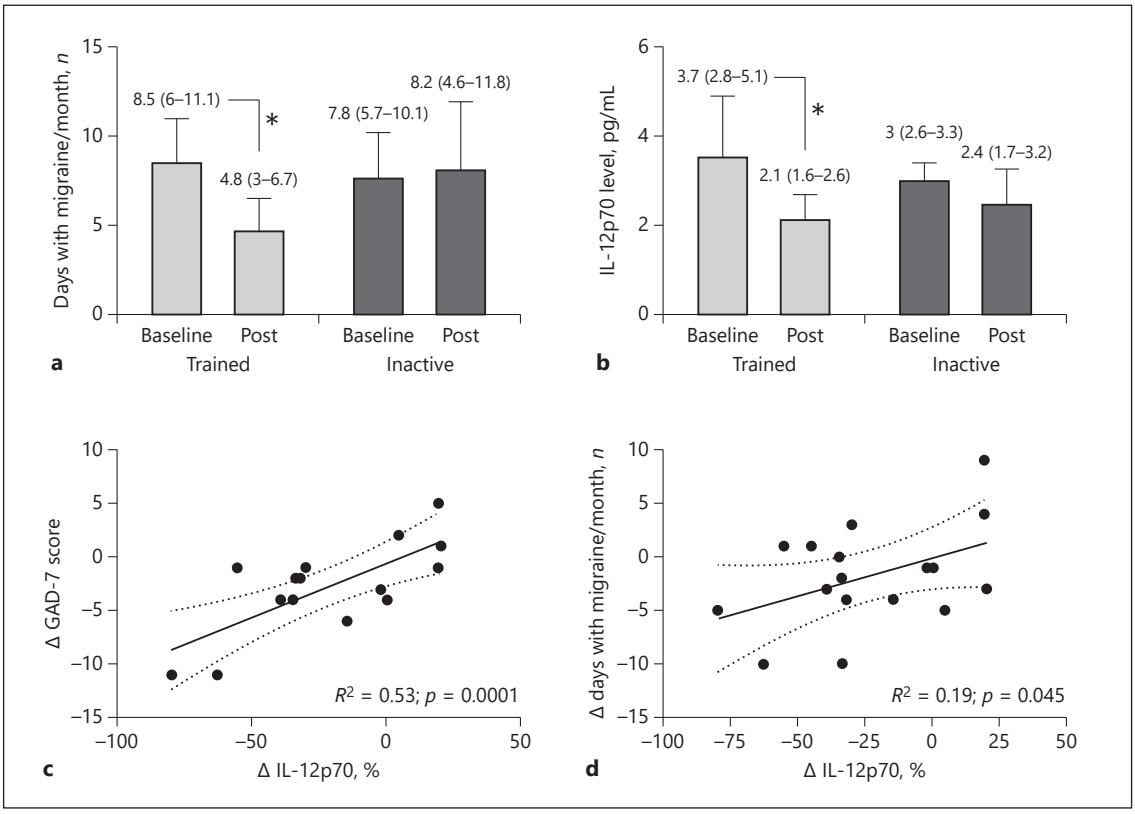

Fig. 2. Primary (**a**) and secondary (**b**) outcomes had changed significantly after the intervention period. Data are presented as mean (95% CI). * $p > 0.01$, paired-samples t test. **c**, **d** Correlations, expressed as 95% CI. Post, at postintervention.

Table 1. Participants' characteristics

	Inactive (n = 10)	Trained (n = 10)
Age, years	33.3±9	34.1±12.1
Weight, kg	65.5±18.5	68.9±13
Height, m	1.57±0.06	1.62±0.04
BMI	26.2±6.7	25.9±3.8
$VO_{2\ peak}$, mL/kg/min	30.3±5.6	31±6
Years living with migraine, n	16.4±9.1	16.6±13.6
Days with headache/month, n	7.6±3.2	8.5±3.6
GAD-7 score	7.0±4.7	7.8±5.3
IL-12p70, pg/mL	2.9±0.5	3.5±1.7

Values are expressed as mean ± SD.

age 33.8 ± 10.5; BMI 26 ± 5.2) were randomly assigned and received treatment. Two participants from the trained group and 1 participant from the inactive group were excluded from the cytokine analyses, as their postintervention IL-12p70 level was below the detection limit. We could not perform statistics comparing IL-10 levels between and within groups, as these were below the detection limit in most of the patients (65%). No adverse effects were reported by any participant with regard to the exercise testing and training protocol. On average, participants performed at around 50–55% of $VO_{2\ peak}$. Participants' baseline data are shown in Table 1. There were no statistically significant differences between groups for any baseline measurements.

The compliance to the exercise program in the trained group was around 67%. Figure 2(a, b) shows the primary and secondary outcomes. There was no significant change in the number of days with migraine in the inactive group (mean [95% CI of difference] = 0.4 [–3.6 to 4.5]; $p = 0.8$; $r = 0.08$), but there was a significant reduction in the trained group (–3.8 [–5.6 to 9 –1.9]; $p = 0.001$; $r = 0.82$). The plasma IL-12p70 concentration in the trained group showed a significant reduction (–1.6 [–3.5 to 0.2]; $p = 0.036$; $r = 0.6$), but no significant change was found in the

inactive group (–0.4 [–1.0 to 0.2]; $p = 0.18$; $r = 0.14$). There was no significant change in the levels of other cytokines for both groups after the intervention period (not shown). GAD-7 scores were significantly reduced in the trained group (–3.4 [–6.5 to –0.3]; $p = 0.034$; $r = 0.61$), but no significant change was found in the inactive group (–2.4 [–7.7 to 2.8]; $p = 0.3$; $r = 0.15$).

In the whole cohort, the Pearson correlation test showed a significant positive correlation between changes (i.e., delta values) in the number of days with migraine and the GAD-7 score (Fig. 2c), and the percentage of reduction in IL-12p70 level (Fig. 2d). Split-groups correlation analyses showed an even stronger correlation between changes in GAD-7 score and IL-12p70 level (as a percentage of reduction) in the trained group ($R^2 = 0.72$; $p = 0.004$), and no correlation in the inactive group, while correlations between the number of days with migraine and IL-12p70 level disappeared for both groups.

Discussion

Here, corroborating other studies [7–11], we found that moderate aerobic exercise promoted a preventive effect on migraine attacks and also reduced anxiety symptoms. Our findings for patients under no other prophylactic treatment are particularly relevant, considering that there is a lack of specific prophylactic drugs for migraine and several side effects from current migraine pharmacotherapy (e.g., antidepressants, anticonvulsants, etc.) [29]. In addition, we report for the first time a reduction in the plasma IL-12p70 level in migraine patients after aerobic exercise training, and this correlated with psychological outcomes.

With regard to the interpretation of these results, Il-12p70 has been shown to promote hyperalgesia in rodents [30], and has been linked to an anxiety phenotype in a rodent strain [31] used as preclinical model of post-traumatic stress syndrome [32]. These animal data, together with our previous clinical findings linking IL-12p70 with migraine and anxiety [24], underscore a potential role for this cytokine in mediating the overlap between migraine and anxiety disorders [14–16].

As a major stimulus of physiological stress, exercise modulates the balance of type 1 and type 2 cytokines, mostly by the secretion of glucocorticoids, epinephrine, and norepinephrine [33]. These neurohormones are indeed, in this order, the most effective suppressors of the IL-12p70 production by immune cells [25, 34]. However, exercise-dependent IL-12 changes largely depend on several factors such as exercise intensity (moderate vs. exhaustive), the subject's training status (sedentary vs. athletic), exercise modality (aerobic vs. resistance), and the tissue location of IL-12 production (plasma vs. skeletal muscle) [3, 4, 6]. In a study on sedentary, healthy women, the plasma IL-12p70 concentration remained unaltered after a bout of moderate exercise (45 min at 55% of $VO_{2\,max}$), but increased 24 h after intense exercise (60 min at 70% of $VO_{2\,max}$) [35]. In male athletes, IL-12p70 is blunted (undetectable) after exhaustive exercise such as running a marathon, as there is a pronounced reduction of circulating Th1 cells, which is considered an immunodepressant effect of strenuous exercise [6, 36]. However, there are only scanty data regarding the chronic effects of moderate exercise on IL-12p70. In this regard, our data put forth the idea that regular moderate aerobic exercise may promote an attenuation of IL-12p70 production in previously physically inactive women with migraine. It is possible that even moderate exercise might have constituted a physiological workload capable of promoting immunodepressant effects on IL-12p70 in these patients.

Interestingly, it is known from clinical literature that migraine attacks are attenuated, or there may even be migraine remission in conditions associated with cortisol/catecholamine overproduction, such as pregnancy [37, 38]. Migraine attacks progressively drop during pregnancy, reaching the highest remission rates in the 3rd semester [37, 38], which coincides with the suppression of IL-12 production [34]. Migraine is also closely associated with heightened perceived stress [39]. Migraine patients exhibit an abnormal neuroendocrine response to mental stress [40], a reduced lymphocyte β-adrenergic receptor sensitivity [41], and the nocturnal cortisol curve is disrupted in the chronic form of the disease [42]. These data indicate a hypofunction of the neuroendocrine response to stress, which could be partly responsible for the greater peripheral IL-12p70 production in this population [24], and may also represent a neuroendocrine-immune mechanism through which moderate exercise can promote a therapeutic effect on migraine. Corticosteroids still represent an effective alternative to treat resistant migraine attacks in emergency care settings [43]; these data concur with translational data showing the antinociceptive effect of corticosteroids on IL-12p70-induced hyperalgesia in rodents [30].

Finally, IL-12p70 is the bioactive heterodimer formed by the subunits p35 and p40 [44]. IL-12p70 is also down-regulated upon IL-12p40 formation, and after a marathon race, blunted IL-12p70 production reflects increased IL-12p40 production [6]. In this sense, it would be in-

sightful to investigate whether changes in IL-12p70 after exercise training in migraine patients are paralleled by changes in IL-12p40. In fact, IL-12p40 has been shown to promote antinociception in animal models of neuropathic pain [45]. Thus, the involvement of the IL-12 family of cytokines in migraine is worthy of further investigation.

Limitations and Strengths

It is worth mentioning study limitations when drawing conclusions from our findings. A small female cohort, a per-protocol analysis, and the lack of parallel groups controlling for disease and intervention (i.e., including healthy women) underpower the generalizability of the results in this study. Accordingly, although the *t* tests showed a medium-to-large effect size for outcomes, the paired *t* tests are poorly controlled for type 1 error rates related to repeated measures, and the post hoc power calculations show that the effects of the intervention on the outcomes were underpowered ($\beta = 0.02, 0.21$, and 0.33 for days with migraine, IL-12p70, and GAD-7 score, respectively). This study did not include follow-up analyses, so we cannot estimate the long-term efficacy of aerobic exercise for migraine prevention. Furthermore, although we tried to blind as many assessors as possible, one of the researchers responsible for exercise training supervision and psychometric interviews was not blinded to the allocation of the participants. This could have led to unbalanced care and attention, biasing the response to the anxiety questionnaire. Lastly, correlational data do not establish causality, so spurious correlations cannot be ruled out.

In spite of these drawbacks, this study has methodological strengths, such as the neurologist-based diagnosis of migraine, the randomized design, and the supervised exercise prescribed based on gold-standard cardiopulmonary parameters.

In summary, this study suggests a new immunomechanism by which regular moderate aerobic exercise promotes a clinical benefit in women with migraine, that involves the downregulation of IL-12p70. The observed reduction in the IL-12p70 level in these women after aerobic exercise training represents a new finding in the literature, and it certainly merits further investigation.

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Disclosure Statement

The authors declare there is no conflict of interest in this study.

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