

# Unbalanced plasma TNF- $\alpha$ and IL-12/IL-10 profile in women with migraine is associated with psychological and physiological outcomes

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## ABSTRACT

Increased plasma pro-inflammatory and decreased anti-inflammatory cytokines have been implicated in physiological and behavioural aspects of mood- and pain-related disorders, including migraine. In this case-control study, we assessed mood scores, cardiorespiratory fitness ( $VO_{2Peak}$ ), and plasma concentrations of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-10, and IL-12p70 interictally in women with episodic migraine with/without aura (ICHD-II), taking no preventive medicine, and in healthy women recruited from São Paulo Hospital and local community, respectively. Thirty-seven participants (mean  $\pm$  SD age = 34  $\pm$  10 and BMI = 26.5  $\pm$  4.9) were assessed. Groups (Control,  $n$  = 17; Migraine,  $n$  = 20) showed no differences in age, BMI, and  $VO_{2Peak}$ . Migraine patients showed higher tension ( $p$  = 0.019) and anxiety scores ( $p$  = 0.046), TNF- $\alpha$  ( $p$  < 0.01), and IL-12p70 ( $p$  = 0.01), while IL-6 ( $p$  < 0.01), IL-8 ( $p$  < 0.01), and IL-10 ( $p$  < 0.01) were decreased compared to control group. Multiple linear regression models showed that migraine was positively associated with TNF- $\alpha$  and IL-12p70, and negatively associated with IL-6, IL-8, and IL-10. Anxiety scores were positively associated with IL-12p70, and  $VO_{2Peak}$  was negatively associated with TNF- $\alpha$ . In conclusion, an exaggeratedly skewed cytokine profile, in particular the TNF- $\alpha$  and 12p70/IL-10 balance may be related to migraine pathomechanisms, and its psychiatric comorbidities and functional capacity. Additional studies are needed to confirm these results.

## 1. Introduction

Migraine constitutes a very disabling neurological disorder affecting around 10% of worldwide population (Woldeamanuel and Cowan, 2017). Among women between 25 and 49 years, migraine was recently ranked the third more disabling disorder in the world in terms of days lived with disability (Vos et al., 2016). Although recurrent pain is the most stereotyped feature, migraine carries several psychiatric comorbidities (Mercante et al., 2011; Peres et al., 2017; Victor et al., 2010) and is associated with several inflammatory and allergic diseases (Cámara-lemarroy et al., 2016; Davey et al., 2002; Loewendorf et al., 2016; Martin et al., 2016, 2014). Additionally, migraine patients often complain of fatigue (Peres et al., 2002), have lower physical activity levels (Bond et al., 2015; Stronks et al., 2004), while poor cardiorespiratory fitness increases the risk for migraine 3.7 fold (Hagen et al., 2016), underscoring a deterioration of physiological and psychological functioning in this population.

The pathophysiology of migraines involves complex and multifactorial factors (Noseda and Burstein, 2013). Mounting evidence point to abnormal cytokine production (Boćkowski et al., 2010; Duarte et al., 2014; Kemper et al., 2001; Perini et al., 2005; Sarchielli et al., 2006; Uzar et al., 2011). Although there is still no established “signature” of cytokine production in migraine, during headache-free days (interictal period), most studies have shown increased peripheral circulation concentrations of pro-inflammatory interleukin (IL)-1 $\beta$ , IL-6 (Uzar et al., 2011), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Kemper et al., 2001), and the pro-inflammatory chemokine IL-8 (Duarte et al., 2014). On the other hand, IL-10, an anti-inflammatory cytokine, has been found either similar (Perini et al., 2005) or reduced (Boćkowski et al., 2010; Uzar et al., 2011) compared to healthy subjects. During migraine attacks (ictal period), IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  are further increased (Perini et al., 2005; Sarchielli et al., 2006), the anti-inflammatory cytokines IL-4 and IL-5 are decreased (Martelletti et al., 1997; Sarchielli et al., 2006), while IL-10 increases compared with the interictal period (Fidan

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et al., 2006; Munno et al., 2001; Perini et al., 2005). Moreover, data from translational models indicates the participation of pro-inflammatory cytokines (*i.e.*, IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) in some step of the pathomechanisms related to migraines, such as sterile neurogenic inflammation (Theoharides et al., 2005) and cortical spreading depression (Jander et al., 2001; Karatas et al., 2013).

Apart from their recognizable modulatory effects on nociception, an unbalanced cytokine profile with elevated pro-inflammatory and lower anti-inflammatory cytokines are linked to depressive-like and anxiogenic behavior in rodents, and are believed to play a role in mood and anxiety disorders (Eyre et al., 2013; Hou et al., 2017a; Patel, 2013). Nevertheless, there is scarce data exploiting the relationship between mood/anxiety symptomatology and cytokines in migraine patients, and to date no study has investigated the interactions between cytokines, mood/anxiety, and functional capacity parameters (*i.e.*, cardiorespiratory fitness) in this population.

Of particular interest, the fluctuating levels of IL-10 observed in migraine patients could be related to the head pain and higher psychiatric comorbidities/symptomatology observed in this condition. This cytokine is a potent inhibitor of pro-inflammatory cytokines, promotes anti-nociception (Bao et al., 2014; Tai et al., 2009; Vale et al., 2003), and protects against depressive-like/anxiogenic behavior (Eyre et al., 2013). Additionally, IL-10 is a T helper 2 (Th2) cytokine, reciprocally and negatively regulated by TNF- $\alpha$  and IL-12 (Elenkov et al., 2000). To date there is no study investigating IL-12 in migraine patients. IL-12, or IL-12p70, the bioactive heterodimer formed by the subunits p35 and p40 (Gee et al., 2009), is a pro-inflammatory cytokine produced primarily by antigen presenting cells and plays an essential role in cell-mediated immunity by stimulating the differentiation of naïve T<sub>0</sub> cells towards the T helper 1 (Th1) phenotype (Elenkov et al., 2000). At neurobehavioral level, IL-12p70 also mediates mechanical hyperalgesia in rodents (Verri et al., 2005), and has been implicated in preclinical studies on anxiogenic behavior (Elenkov et al., 2008; Goswami et al., 2012), suggesting that this cytokine may also participate as a pain mediator and could also be associated with anxiety symptoms.

As such, we thought it would be plausible to investigate the balance between pro- and anti-inflammatory cytokines, in particular the balance between IL-12p70/IL-10 in migraine patients. Therefore, the purposes of the present study are to compare the plasma concentrations of pro/anti-inflammatory cytokines, especially the IL-12p70/IL-10 balance between healthy individuals and migraine patients, and to test whether there would be an association between these cytokines and migraines, mood/anxiety scores, and functional capacity.

## 2. Methods

### 2.1. Study design/participants

This is a case-control study designed to compare cytokines levels, mood profile, and cardiorespiratory fitness between migraine and non-headache women. We recruited participants from the Headache Unit of the São Paulo Hospital and from the local community through electronic and press media advertisements. Participants were recruited from March 2012 to March 2015. The inclusion criteria were women aged between 20 and 50 years, sedentary for at least 12 months (defined as  $\leq 1$  day/week of leisure-time physical activity participation), without history of recurrent headaches, or suffering from any headache in the last 3 months, and women with episodic migraine with aura (MwA) and/or migraine without aura (MwoA), according to the 2nd Edition of the International Classification of Headache Disorders (Headache Classification Subcommittee of the International Headache Society, 2004). The exclusion criteria were disagreement with the research terms and/or refusal to sign informed consent, patients under any prophylactic treatment (either pharmacological or non-pharmacological) for migraines the previous 6 months (with the exception of acute abortive medication for migraine attacks), currently taking any

other prescribed medication, use of tobacco, alcohol or abuse drugs, use of any dietary supplement, pregnancy, history of cardiovascular, pulmonary, metabolic, rheumatic, musculoskeletal or other neurological disease (including other headaches subtypes).

### 2.2. Procedures and measures

All participants had a physical and neurological examination by headache-trained neurologists (RTR, MFPP) before inclusion in study and gave signed informed consent. After screened for inclusion in the study, participants were given a headache diary and were followed up for 4 weeks for checking headaches diagnosis. After this period, participants underwent the psychometric interview, blood sampling, and maximal cardiopulmonary exercise test, all scheduled within the early phase of the follicular menstrual period (day 1–7), and at the interictal period for migraine patients. Cytokine analyses and maximal cardiopulmonary tests were performed blindly for participants' assignment. The principal author labelled all blood sample vials with random numbering and supervised all maximal cardiopulmonary tests to assure the concealment of participants' assignment from the assessors. The study protocol complied with the guidelines of the revised Declaration of Helsinki on proper human research conduct, and was approved by the Research Ethics Committee of the Sao Paulo Federal University (UNIFESP), registered under #08152011.

#### 2.2.1. Psychometric data/blood sampling

Psychometric interviews were conducted by a certified clinical exercise physiologist (ABO), between 9:00 AM and 10:00 AM at the Psychobiology Department of the Federal University of São Paulo. Participants were instructed to breakfast regularly, but to abstain from coffee. Anxiety was assessed by the 7-item general anxiety disorder scale (GAD-7). GAD-7 screens typical anxiety-related indicators experienced the previous two weeks. Total scores were used in the analyses. The Profile of Mood State (POMS) questionnaire was adopted to assess other affective domains beside anxiety, namely, depression, anger-hostility, vigor, fatigue, and confusion. Both questionnaires have been validated and translated into Brazilian Portuguese (Peluso, 2003; Sousa et al., 2015).

After questionnaires filling, participants were conducted to the blood sampling room. Blood samples were obtained by venipuncture from the antecubital vein of the forearm by an experienced nurse, collected in ice-chilled EDTA tubes (BD Vacutainer®, Franking Lakes, NY, USA), immediately centrifuged at 3.400 rpm at 4 °C for 10 min. The plasma was separated and aliquoted in 2 mL-Eppendorf vials and stored at  $-80$  °C until assay.

#### 2.2.2. Plasma cytokine assays

Plasma samples were transported in dry ice to the Pharmacology Department of the Federal University of Sao Paulo for analyses. Plasma concentrations of IL-1b, IL-6, IL-8, IL-10, IL-12p70 and TNF- $\alpha$  (pg/mL) were measured on duplicate using BD CBA Human Inflammatory Cytokines kit (BD Bioscience®, San Jose, CA, USA), performed by a biologist (ALLB), following the manufacture's instruction. Data were obtained on the BD FACS Accuri Flow Cytometer and analysed by FCAP Array™ Software (BD Bioscience®).

#### 2.2.3. Cardiorespiratory fitness assessment

Participants underwent a maximal cardiopulmonary exercise test on treadmill (Centurion 300, MICROMED, Brasília, DF, Brazil) with ramp protocol for determination of peak oxygen uptake (VO<sub>2Peak</sub>), a gold-standard measure of cardiorespiratory fitness (Balady et al., 2010). Tests were conducted at the Center for Studies in Psychobiology and Exercise, scheduled within 2–7 days after the psychometric/blood sampling visit. Patients were asked to abstain from coffee 24 h before the test and to restrain from food 2 h before the test. The workload increment was set to provoke volitional exhaustion within the

precozied 8–12 min (Balady et al., 2010). Participants received strong verbal encouragement as they started to exhibit visual sign of fatigue. Respiratory gas exchange measurements were obtained breath-by-breath through an open-circuit computerized spirometry system (Quark CPET, COSMED, Rome, Italy) and 30-s averages were calculated for analysis. The  $VO_{2Peak}$  identification met at least 2 of the following criteria: 1) to reach the age-predicted heart rate; 2) respiratory exchange ratio  $> 1.1$  and 3) rate of perceived effort  $\geq 18$  (Balady et al., 2010). The gas analyser and spirometer were calibrated before each test using known gas concentrations and a 3-l syringe. An integrated 12-lead digital electrocardiogram and software (ERGO PC 13, MICROMED, Brasília, DF, Brazil) was used for heart rate monitoring.

### 3. Sample size and statistical analysis

This sample was of convenience, and we did not perform *a priori* sample size calculations. Shapiro-Wilk's statistic was performed for testing data distribution. Differences between migraine and control groups were calculated by Student's *t*-test.

In order to explore the correlations between mood/anxiety scores, cardiorespiratory fitness, and cytokines, we performed 6 stepwise multiple linear regression models, one for each cytokine selected as the dependent variable. The independent variables were age, BMI, participant diagnosis (control or migraine), mood/anxiety scores, and  $VO_{2Peak}$ . The variable regarding participants' diagnosis was transformed into dummy variables to enter the regression model. Statistical tests were computed by the SPSS software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY), and graphs were generated by GraphPad Prism® software (GraphPad Software Inc., Version 5.0, San Diego, CA, USA). The *p* value  $< 0.05$  was considered statistically significant.

### 4. Results

Fig. 1 summarizes the participant's flow in the study. Thirty-seven participants (age =  $34 \pm 10$ , BMI =  $26.5 \pm 4.9$ ) concluded the study and were analysed. Table 1 summarizes anthropometric, cardiorespiratory fitness, psychometric, and clinical data. Migraine group showed higher scores for anxiety-related scores POMS Tension-Anxiety and GAD-7 than control groups (Table 1).

For cytokines analyses, IL-10 was undetectable in 1 (5.8%) participant from the control group and in 13 (65%) participants from the migraine group. IL-1 $\beta$ , IL-6 and IL-8 were also undetectable in 5 (25%), 8 (40%), and 3 (15%) migraine patients, respectively.

Compared to control group, migraine group showed increased IL-12p70 [ $3.1 \pm 1.4$  pg/mL vs  $1.9 \pm 0.2$  pg/mL, respectively; mean difference (95%CI) =  $1.2(0.5, 2.0)$ ,  $p = 0.001$ ] and TNF- $\alpha$  [ $1.7 \pm 0.5$  pg/mL vs  $1.2 \pm 0.2$  pg/mL, respectively; mean difference (95%CI) =  $0.43 (0.11, 0.74)$   $p = 0.009$ ], and lower IL-10 [ $0.7 \pm 0.5$  pg/mL vs  $1.8 \pm 0.1$  pg/mL, respectively; mean difference (95%CI) =  $-1.0(-1.3, -0.7)$ ,  $p < 0.001$ ], IL-6 [ $0.6 \pm 0.6$  pg/mL vs  $1.8 \pm 0.1$  pg/mL, respectively; mean difference (95%CI) =  $-1.19(-1.5, -0.87)$ ,  $p < 0.001$ ], and IL-8 [ $0.7 \pm 0.5$  pg/mL vs  $1.6 \pm 0.3$  pg/mL, respectively, mean difference (95%CI) =  $-0.93(-1.2, -0.59)$ ,  $p < 0.001$ ] (Fig. 2). There was no difference between migraine and control groups for IL-1 $\beta$ .

Correlations between cytokines in the whole cohort are shown in the Table 2.

In the multiple linear regression models, migraine was associated with increased IL-12p70 and TNF- $\alpha$ , and decrease IL-6, IL-8, and IL-10 (Table 3). IL-12p70 was also associated with higher anxiety symptoms scores, while TNF- $\alpha$  was associated with lower cardiorespiratory fitness (Table 3).

### 5. Discussion

In agreement with other studies (Bockowski et al., 2010; Uzar et al.,

2011), we found lower circulating IL-10 concentrations in migraine patients during the interictal period, undetectable in most patients. Also, akin with the reciprocal immunomodulatory actions of Th1/Th2 cytokines (Elenkov et al., 2000), the Th1 cytokines IL-12p70 and TNF- $\alpha$  were increased in migraine patients and were strongly inversely correlated with IL-10, a cytokine present in the Th2 response. This study shows for the first time that this unbalanced TNF- $\alpha$  and IL-12p70/IL-10 profile is associated with anxiety scores, which constitute well-known symptomatology/comorbidity of migraines (Peres et al., 2017, 2002), and lower cardiorespiratory fitness, which has been associated with migraines (Hagen et al., 2016).

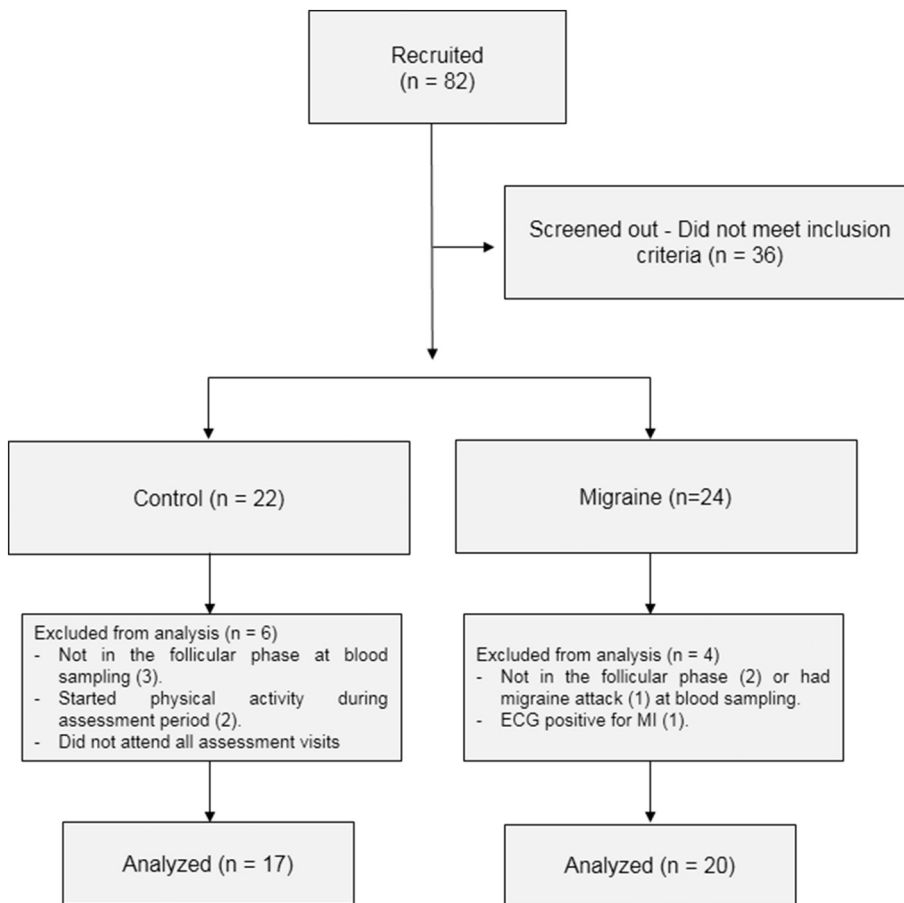
Contrary to others studies measuring interictal cytokines concentrations in migraine patients (Duarte et al., 2014; Perini et al., 2005; Sarchielli et al., 2006), there were no differences between migraine and control groups for IL-1 $\beta$ , while IL-6 and IL-8 were lower rather than increased compared to control group. We ascribe these discrepancies to ethnic heterogeneity of the samples in these studies, clinical history of allergies and atopic disease, psychiatric disorders, migraine subtype (e.g., migraine with or without aura), differences in technical methods, biological matrix (serum vs plasma), and possibly the timing of blood sampling with regard the proximity of migraine attacks.

In migraine patients, a Th2-dominant immune response with elevated IL-10 production was once thought to be involved in the aetiology of attacks, since plasma IL-10 concentration was increased during attacks and posteriorly reduced after administration of the migraine abortive medication sumatriptan (Munno et al., 2001). However, given its potent inhibitory action on pro-inflammatory cytokines and antinociceptive effects (Bao et al., 2014; Tai et al., 2009; Vale et al., 2003), increased plasma IL-10 levels during attacks and its progressive reduction with the temporal distancing from the attack has been ascribed as a compensatory mechanism operative in the resolution of migraine attacks by limiting neurogenic inflammation, rather than responsible for the precipitation of attacks (Fidan et al., 2006; Perini et al., 2005). In agreement with this view, translational studies have shown that IL-10 is upregulated 24 h after administration of sumatriptan (Vause and Durham, 2012), while it partly mediates the anti-nociceptive effects of gabapentin (Bao et al., 2014) and amitriptyline (Tai et al., 2009), all of the constituting prophylactic anti-migraine medication (Silberstein et al., 2012). Likewise, IL-10 upregulation has been associated with the neuroprotective and anti-inflammatory effects of melatonin by immunoregulatory actions on central and peripheral T cell subpopulation in a translational model of multiple sclerosis (autoimmune encephalomyelitis) (Álvarez-Sánchez et al., 2015). Interestingly, melatonin supplementation has been shown to promote anti-migraine effects, with clinical efficacy comparable to amitriptyline (Gonçalves et al., 2016; Peres et al., 2004). These studies suggest that enhancing IL-10 might potentially be the focus of therapeutic approaches for migraine prevention. It is unknown, however, whether there would be systemic or peripheral side effects of a Th2/IL-10-mediated response with such approaches.

Regarding its role in the regulation of behavioural processes, IL-10 attenuates lipopolysaccharide-induced sickness behavior in rodents (Eyre et al., 2013), it is downregulated in patients with depression (Eyre et al., 2013; Patel, 2013), while IL-10 receptors polymorphism has been linked to severe fatigue in women (but not in men) with lung cancer (Reyes-Gibby et al., 2013). These data corroborate with the idea considering this cytokine a player in pain perception and mood regulation, and lower concentration of IL-10 could be involved in mood/anxiety symptomatology of migraine.

Regarding elevated IL-12p70 levels, our study does not provide straightforward evidence whether IL-12p70 may play a direct role in migraine pathophysiology and mood/anxiety-associated symptomatology, or it could affect these processes by suppressing the anti-nociceptive and anxiolytic actions of IL-10. For example, in a study with a larger cohort with patients with general anxiety disorder, circulating cytokines concentrations were not different between healthy

Fig. 1. Participants' flow in the study.



individuals and patients (Hou et al., 2017b). However, patients showed higher ratio of TNF- $\alpha$ /IL-10, TNF- $\alpha$ /IL-4, INF- $\gamma$ /IL-10, and INF- $\gamma$ /IL-4 (Hou et al., 2017b). IL-12p70 was not assessed in this study.

Our analyses suggest a potentially significant role for IL-12p70 in anxiety-related behavior, and partly explain the common overlap between migraine and anxiety (Lucchetti et al., 2013; Mercante et al., 2011; Peres et al., 2017). For example, evidence from translational studies concurs with our data and show a participation of IL-12p70 on nociception and angiogenic behavior. IL-12p70 promotes mechanical hyperalgesia mediated by endothelin receptors (type-B) in rats, an

effect blocked by dexamethasone or morphine, but not blocked by antiserum against TNF- $\alpha$  or IL-18 (Verri et al., 2005). However, to date this is the only study to investigate the participation of IL-12p70 in pain. Likewise, the influence of IL-12p70 on mood and anxiety disorders is poorly studied. One evidence stems from rats of the Lewis strain, which have been validated as an animal model of post-traumatic syndrome disorder, as these animals exhibit higher fear and others angiogenic behavior on a myriad of behavioural paradigms (Goswami et al., 2012). Agreeably, Lewis rats also exhibit dominant Th1 cytokine phenotype, with increased IL-12p70/IL-10 ratio, mostly due to

**Table 1**  
Participants' anthropometric, clinical, cardiorespiratory, and psychometric data.

	Control (n = 17)	Migraine (n = 20)	Mean difference (95% CI)	p*
Age (yrs)	33.7 $\pm$ 9.0	33.8 $\pm$ 10.5	0.9 (-6.5, 6.7)	0.86
Weight (kg)	70.0 $\pm$ 9.8	68.8 $\pm$ 15.6	-1.2 (-10.1, 7.6)	0.77
Height (m)	1.62 $\pm$ 0.07	1.61 $\pm$ 0.05	-0.1 (-0.5, 0.3)	0.56
BMI (kg·m <sup>2</sup> )	26.7 $\pm$ 4.7	26.4 $\pm$ 5.3	-0.3 (-3.7, 3.0)	0.82
Time living w/disease (yrs)	-	16.8 $\pm$ 11.8	-	-
Days w/headaches (/month)	-	8.0 $\pm$ 0.3.2	-	-
Attacks frequency (/month)	-	5.4 $\pm$ 2.3	-	-
Disability (0–3)	-	1.77 $\pm$ 0.3	-	-
Medication (/month)	-	5.20 $\pm$ 3.8	-	-
VO <sub>2Peak</sub> (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	32.6 $\pm$ 7.9	31.2 $\pm$ 6.4	-1.4 (-6.2, 3.3)	0.54
POMS tension-anxiety	9.2 $\pm$ 4.4	13.1 $\pm$ 6.4	3.8 (0.08, 7.6)	0.046
POMS depression	6.0 $\pm$ 10.2	10.1 $\pm$ 8.9	4.1 (-2.2, 10.5)	0.19
POMS anger-hostility	6.4 $\pm$ 8.2	8.5 $\pm$ 7.5	2.1 (-3.1, 7.4)	0.41
POMS vigor	16.8 $\pm$ 4.6	14.9 $\pm$ 5.8	-1.9 (-5.4, 1.6)	0.28
POMS fatigue	6.5 $\pm$ 5.4	9.5 $\pm$ 5.0	3.0 (-0.47, 6.5)	0.056
POMS confusion	5.7 $\pm$ 3.9	7.2 $\pm$ 4.3	1.4 (-1.3, 4.2)	0.3
GAD-7	4.0 $\pm$ 2.7	7.4 $\pm$ 5.0	3.3 (0.62, 6.1)	0.018

VO<sub>2Peak</sub>: Peak oxygen uptake; POMS: Profile of Mood State Questionnaire; GAD-7: 7-items Generalized Anxiety Disorder Questionnaire.

\* Student's t-test.

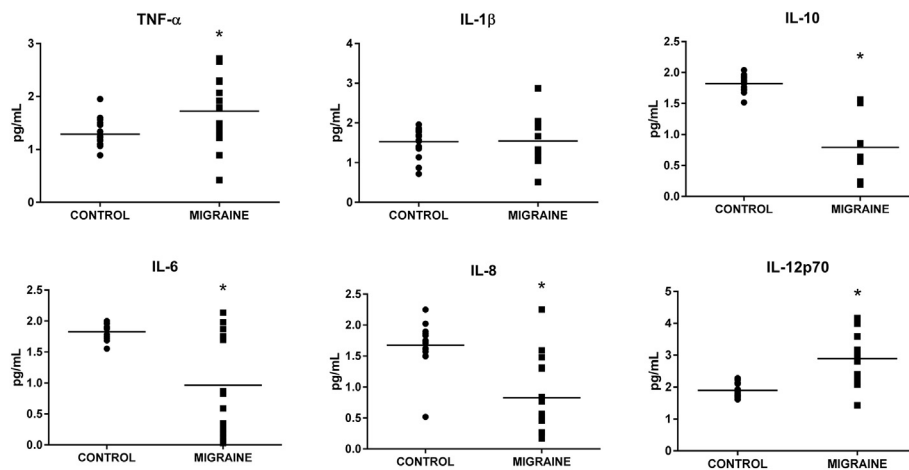


Fig. 2. Plasma cytokines concentrations. \**p* < 0.01; Student's *t*-test.

**Table 2**  
Product-moment bivariate Pearson's correlations between cytokines (whole cohort).

	IL-12p70	IL-10	TNF-α	IL-1β	IL-6	IL-8
IL-12p70	–	– 0.82**	0.44**	0.06	– 0.46**	– 0.51**
IL-10		–	– 0.79**	– 0.07	0.87**	0.72**
TNF-α			–	0.37*	– 0.24	– 0.15
IL-1β				–	0.20	0.15
IL-6					–	0.90**
IL-8						–

\* *p* < 0.05.  
\*\* *p* < 0.01.

**Table 3**  
Multiple linear regression models testing participants' diagnosis (control or migraine), GAD-7 and mood scores, age, BMI, and VO<sub>2Peak</sub> as predictors of plasma cytokines concentration.

	Adjusted R <sup>2</sup>	B	SE	β	t	<i>p</i>
IL-12p70						
GAD-7	0.38	0.146	0.038	0.51	3.8	0.001
Migraine	0.45	0.78	0.33	0.31	2.3	0.023
TNF-α						
VO <sub>2Peak</sub>	0.15	– 0.027	0.01	– 0.37	– 2.6	0.012
Migraine	0.27	0.39	0.14	0.38	2.7	0.01
IL-10						
Migraine	0.69	– 1.02	0.14	– 0.84	– 7.1	< 0.001
IL-6						
Migraine	0.65	– 1.1	0.15	– 0.81	– 7.6	< 0.001
IL-8						
Migraine	0.46	– 0.93	0.16	– 0.69	– 5.5	< 0.001

GAD-7: 7-items generalized anxiety disorder questionnaire; VO<sub>2Peak</sub>: Peak oxygen uptake.

monocytes IL-12p70 overproduction, since they show normal monocyte IL-10 production (Elenkov et al., 2008). Yet, no study has mechanistically tested the relationship between behavioural outcomes and these cytokines in this rodent strain.

Migraine has been associated with several inflammatory and allergic/atopic diseases characterized by either Th1- or Th2-dominant response, with increased IL-12p70 and IL-10 production, respectively (Cámara-lemarroy et al., 2016; Davey et al., 2002; Loewendorf et al., 2016; Martin et al., 2016, 2014). For example, asthma is often comorbid with migraine (Davey et al., 2002; Loewendorf et al., 2016), in which a Th2-mediated response with increased IL-10 and deficient IL-12 receptor signaling have a pivotal pathophysiological role (Elenkov et al., 2000; Xu et al., 2013). On the other hand, several Th1-mediated inflammatory gastrointestinal disorders causing IL-12p70

overproduction in the epithelial cells of the gastric mucosa are also associated with migraine (Cámara-lemarroy et al., 2016; Pellicano et al., 2007). It is worth noting that IL-12 has been considered a relevant player in the emerging complex mechanisms of neuroinflammation derived from the gut-brain axis, with implication in the pathogenesis of mood disorders (Powell et al., 2017). As such, it is intriguing that conditions exhibiting either Th1 or Th2 response are associated with migraine. We believe that compensatory mechanisms working continuously through the reciprocal regulation of IL-12p70 and IL-10 may be operative in migraine patients, resulting in a Th1-dominant response with higher IL-12p70 production, and the neuro-behavioral effects associated with this cytokines (i.e., pro-nociceptive, angiogenic effects).

Additionally, this peculiar cytokine pattern involving the IL-12p70/IL-10 balance may add further evidence for an involvement of mast cells in the aetiology of migraines (Levy et al., 2007; Loewendorf et al., 2016; Theoharides et al., 2005; Zhang et al., 2007). Mast cells produce a myriad of vasoactive and nociceptive molecules and are implicated in the activation and sensitization of meningeal and dural nociceptors, the neuroanatomical substrate of head pain (Levy et al., 2007; Zhang et al., 2007). Interestingly, mast cells release both IL-12p70 (Nakano et al., 2007) and IL-10 (Moller et al., 1998), while both endothelin-1-mediated IL-12p70 production and IL-10-mediated Th2-dominant immune responses can degranulate mast cells (Coulombe et al., 2002; Elenkov and Chrousos, 2002). Moreover, IL-10 genes expression after mast cells degranulation peaks around 24 h (Moller et al., 1998), suggesting that late IL-10 production may work as a compensatory mechanism to terminate pro-inflammatory (pro-nociceptive) processes. Thus, it is tempting to speculate that perhaps IL-12p70 and IL-10 derived from mast cells might partly explain the paroxysmal and self-limiting characteristic of migraines, in the sense that these immune cells can release cytokines with the potential of either inducing or suppressing a migraine attack. However, there is still no study available to confirm such pattern in humans mast cells, or even in a tissue-specific manner (e.g., in meningeal or dural mast cells).

The association between TNF-α and VO<sub>2Peak</sub> in our regression models imply that this skewed Th1 cytokine profile may underlie behavioural and physiological deterioration, and could explain poor overall function commonly observed in migraine patients (Bond et al., 2015; Peres et al., 2002; Stronks et al., 2004). We did not find differences between groups in cardiorespiratory fitness, possibly due to our small sample size and the restrictive inclusion criterion of low physical activity levels. Nevertheless, TNF-α was also negatively correlated with cardiorespiratory fitness, which may play a role in lower physical activity levels (Le et al., 2011; Varkey et al., 2008) and cardiorespiratory fitness (Hagen et al., 2016) observed in migraine patients in population-based studies. These findings could be related to the inhibitory

influence of TNF- $\alpha$  on physical activity behavior (e.g., through the anhedonic, avoidance component of the sickness-like behavior) (Eyre et al., 2013) and the negative effect of TNF- $\alpha$  on skeletal muscles energy metabolism by impairing insulin signaling (Nieto-Vazquez et al., 2008) and mitochondrial function (Sente et al., 2016). Accordingly, the plasma concentrations of TNF- $\alpha$  soluble receptors I and II were inversely correlated with poor cardiorespiratory fitness ( $VO_{2Peak} < 18 \text{ mL kg min}^{-1}$ ) in patients with heart failure (Itoh et al., 2005). Still, a recent study investigating the effect of baseline cytokines and changes in cardiorespiratory fitness after aerobic exercise training in patients with heart failure, showed a blunted improvement response among individuals with above-median baseline plasma TNF- $\alpha$  levels compared to individuals with below-median levels (Fernandes-Silva et al., 2017).

In fact, perhaps this might represent a mechanism underlying abnormal mitochondrial function in migraine patients (Sparaco et al., 2006), and the clinical benefit of aerobic exercise in managing migraines through improvement in cardiorespiratory fitness (Varkey et al., 2011, 2009).

This study has several limitations, and our data should be interpreted with caution. First, because of our small sample and the number of variables used in the statistical analyses, type I error was more likely to occur. Large samples are mandatory to reproduce the findings in this study. Second, patients were not assessed by a psychologist or psychiatrist to receive proper anxiety or depression diagnoses applying DSM criteria. Rather, the data referred only to continuous values from the psychometric scores. However, a previous study showed that the number of symptoms related to the GAD-7 items progressively increased the risk for having migraines, irrespective of anxiety diagnosis, but reaching the highest odds ratios with the concurrent clinical diagnosis of anxiety (Lucchetti et al., 2013). Therefore, the findings here may represent immunological biomarkers of the close relationship between anxiety and migraines (Lucchetti et al., 2013; Mercante et al., 2011; Peres et al., 2017). Still, the psychometric interviews were not performed blindly to participant's assignment. This could result in disproportional care and attention during interviews, thus biasing the participants' response to the questionnaires. Also, this is a per-protocol study, in which bias is a major concern. Lastly, due to unbalanced drop-out/exclusion among groups, there was an undermatching in our sample. All these factors limit the generalizability of our results.

## 6. Conclusions

Our study, albeit presented as a preliminary evidence, suggests the participation of a skewed TNF- $\alpha$ , and IL-12p70/IL-10 cytokine pattern underlying the link between migraine and its major psychological and physical symptomatology.

## Authorship

ABO contributed to study's design, supervised the cardiopulmonary exercise tests and conducted psychometric interviews, wrote the manuscript, and analysed the data. ALLB was responsible for blood samples analyses, data interpretation, and manuscript editing. MFPP and RTR screened participants and contributed to study's design, data analyses/interpretation and manuscript editing. ST and MTM contributed to study's design and manuscript editing.

## Conflict of interest

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

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