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# Cycle ergometer training enhances plasma interleukin-10 in multiple sclerosis

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## ABSTRACT

The objective was to determine plasma levels of pro- (IL-12p70/IL-6) and anti-inflammatory (IL-10) cytokines before and after cycle ergometer training in healthy control (HC) and people with multiple sclerosis (pwMS), and to correlate plasma cytokines with physical/mental health. Study participants cycled for 30 minutes at 65-75% age-predicted maximal heart rate, twice a week for 8 weeks during supervised sessions. We determined that plasma IL-10 expression was lower in pwMS, compared to HCs, and that exercise augmented IL-10 in pwMS to baseline levels in HCs. Furthermore, plasma isolated from pwMS displayed enhanced expression of the pro-inflammatory cytokines IL-12p70/IL-6. Plasma cytokine signatures correlated with physical/mental health. Overall, this study highlights the potential of a short-term exercise programme to regulate circulating cytokine profiles with relevance to pwMS.

## Introduction

Neuroinflammation drives the pathogenesis of multiple sclerosis (MS), involving blood brain barrier dysfunction, activation of microglia/astrocytes and alterations in cytokines/chemokines in serum and cerebrospinal fluid (CSF) [1]. Indeed, dysregulation of pro-/anti-inflammatory cytokines, secreted by T-helper (Th)-1 and Th-2 cells, may dictate disease progression in patient groups [2].

Interleukin (IL)-10, a Th2 cytokine with anti-inflammatory propensity, is downregulated in the serum of people with MS (pwMS) during relapse [3], an important finding given that IL-10 can ameliorate Th1 cytokine synthesis. Furthermore, the expression of pro-inflammatory cytokines IL-12 [4] and IL-6 [5] are enhanced in the circulation of pwMS, indicating that pro- and anti-inflammatory cytokine signatures may indicate MS progression.

Indeed, the expression of serum/CSF cytokines correlate with expanded disability status scale (EDSS) [6] and depression [2] in pwMS.

MS therapies can be partially effective and with side effects. As a result there is a need to explore/quantify the benefits of non-drug interventions. Exercise activates an array of immunological/hormonal responses, and can ameliorate neuroinflammation by targeting inflammatory cytokines [1]. Our recent publication in this study cohort indicates that exercise improves both physical and mental health in pwMS, while reducing depressive symptomatology and cognitive dysfunction in MS [7]. Given such reports, we next determined whether short-term cycle ergometer training promoted an anti-inflammatory plasma cytokine signature that positively correlated with physical/mental health.

## Materials and methods

Research was carried out in accordance with the Declaration of Helsinki, except for database registration. Written informed consent was obtained from each participant and the study received ethical approval from the Clinical Research Ethics committee of the Cork Teaching Hospitals (ECM3(xxxxx14/04/15&4(o)01/07/14). As previously detailed in this MS cohort, exercise improved quality of life (QOL), symptoms of depression and cognitive function [7] using the Cambridge Neuropsychological Test Automated Battery (CANTAB®; Cambridge Cognition, LTD) software. The QOL of participants was assessed prior to week 1 of exercise, and at the end of 8 weeks of training using the MS Quality of Life-54 (MSQOL-54) questionnaire generating a physical and mental health composite score. The MSQOL-54 is a self-report questionnaire. All subjects also completed the self-rated 16-item Quick Inventory of Depressive Symptomatology (QIDS-SR<sub>16</sub>) questionnaire prior to week 1 of training, and at the end of 8 weeks of training. The total QIDS-SR<sub>16</sub> score (0–27) was blindly generated a quantitative result corresponding to depression severity (0–5 = None; 6–10 = Mild; 11–15 = Moderate; 16–20 = Severe; 21–27 = Very Severe). Study participants attempted and self-administered all the questions. PwMS with cardiovascular, respiratory, metabolic or autoimmune disease other than MS were excluded. Healthy control (HC) volunteers with no history of autoimmune, cardiovascular, respiratory or neurodegenerative disease were included. Exercise consisted of a supervised 30-minute session of cycle ergometry, repeated twice a week for 8-weeks [7]. Each study participant exercised at 65–75% age-predicted maximal heart rate. Participants continued their standard medical care during the period of the study and were asked to avoid participation in any additional exercise programmes during the prescribed 8-week exercise programme.

Plasma samples were separated following centrifugation of blood samples by density separation over Lymphoprep™ (Axis-Shield, Norway). Samples were stored at –80°C for analysis via V-PLEX pro-inflammatory assays (MesoScale Discovery, USA). Intra-individual variability exists in the levels of cytokines/chemokines, and the values presented herein are consistent with data we have previously published in human plasma [8].

Data were analysed using two-way analysis of variance (ANOVA) where indicated, followed by a Bonferroni *post test*. Where correlations are performed the Pearson's *r* value is reported. Data are expressed as mean±standard errors of the mean (SEM).

## Results

A description of the study cohort is outlined in our previous publication [7]. In summary, 19 subjects were assessed consisting of HCs ( $n = 10$ ; mean age = 36.0 years) and pwMS ( $n = 9$ ; mean age = 35.33 years), with average disease duration of  $5.9 \pm 1.2$  years. Data herein indicates that MS was associated with a significant reduction in plasma IL-10 at baseline prior to exercise, when compared to HCs ( $2372 \pm 126$  pg/L HC vs.  $1693 \pm 137$  pg/L MS,  $p < 0.05$ ; Table. 1). Importantly, exercise increased IL-10 expression in pwMS ( $1693 \pm 137$  pg/L pre-exercise

vs.  $2318 \pm 221$  pg/L post-exercise,  $p < 0.05$ ; Table. 1). Two-way ANOVA analysis revealed a significant interaction of time and disease status ( $p < 0.01$ ).

MS was also associated with higher expression of IL-12p70 and IL-6, compared to HCs; exercise reduced both plasma cytokines in pwMS (Table. 1). Indeed, elevated plasma profiles of IL-12p70 ( $39.79 \pm 4.42$  pg/L HC vs.  $59.59 \pm 6.99$  pg/L MS) and IL-6 ( $1041 \pm 75$  pg/L HC vs.  $1640 \pm 206$  pg/L MS) were determined between HCs and pwMS at baseline (Table. 1). Exercise reduced IL-12p70 ( $59.59 \pm 6.99$  pg/L pre-exercise vs.  $51.71 \pm 10.06$  pg/L post-exercise) and IL-6 ( $1640 \pm 206$  pg/L pre-exercise vs.  $1599 \pm 140$  pg/L post-exercise) in pwMS (Table. 1). Two-way ANOVA analysis indicated a significant influence of disease status on IL-6 and IL-12p70 ( $p < 0.05$ ), and a significant interaction of time and disease status on IL-12p70 ( $p < 0.05$ ).

An analysis of the ratio of anti- and pro-inflammatory cytokines indicated that IL-10:IL-12p70 ( $p < 0.01$ ; Fig. 1A) and IL-10:IL-6 ( $p < 0.001$ ; Fig. 1C) were significantly reduced in pwMS pre-exercise, and that exercise ameliorated this decline. Furthermore, linear regression analysis determined a significant positive correlation between plasma IL-10:IL-12p70 ( $p < 0.01$ ; Fig. 1B), IL-10:IL-6 ( $p < 0.05$ ; Fig. 1D) and physical/mental health as determined using the MSQOL-54 questionnaire. These findings indicate that MS is associated with a reduced anti-inflammatory cytokine signature in plasma that can be reversed by aerobic exercise.

## Discussion

In this study we sought to determine the anti-inflammatory role of a short-term (30 min/day, 2 days/week for 8-weeks) aerobic exercise training programme in pwMS. Data herein demonstrate that exercise enhanced plasma IL-10 in pwMS, and that plasma cytokine signatures correlated with physical/mental health scores. The study identifies an exercise programme that promotes anti-inflammatory changes in the circulation that correlates with the health of the individual.

Low, high and combined intensity cycle ergometry are safe protocols for pwMS and impact positively on MS symptomatology (reviewed [1]). Peripheral Th1/Th2 cytokines are dysregulated in MS, and importantly studies highlight that exercise has anti-inflammatory potential in MS, ameliorating pro-inflammatory cytokine production in plasma/immune cells [1]. Indeed, exercise enhances IL-10 (a Th2 anti-inflammatory cytokine produced by macrophages/T cells) mRNA in leukocytes from pwMS [9], and our findings support this, highlighting IL-10 as a key anti-inflammatory plasma factor that is positively targeted by ergometry training in MS. The control group consisted of a healthy control cohort, and the study did not include a non-exercising MS cohort, with assessment of plasma cytokines at baseline (week 0) and after 8 weeks. Therefore we cannot entirely rule out that some of the effects determined may be contributed by other factors. The present hypothesis should be further explored within a controlled study.

Our study also probed the impact of exercise on plasma pro-inflammatory cytokines, IL-12p70 and IL-6. IL-12p70 (active heterodimer of IL-12) is produced by monocytes/macrophages and dendritic cells, and IL-12 expression is enhanced in the circulation of pwMS [4], which is confirmed here. Although exercise had no significant effect on IL-12p70 in MS, a reduction was determined in MS following exercise, although a limitation of this study is that plasma cytokine profiles were not assessed in non-exercising MS individuals at baseline and after 8 weeks. Given that IL-10 has the proclivity to inhibit IL-12 in immune cells [10], it is reasonable to suggest that the decrease in plasma IL-10 determined in pwMS (compared to HCs) contributes to an augmented pro-inflammatory cytokine signature in pwMS, which can be “re-set” with exercise. Our findings demonstrating reduced IL-10:IL-

IL-12p70 in pwMS, compared to HCs at baseline, and that exercise augments this signature in pwMS, supports this.

IL-6, a pro-inflammatory cytokine produced by monocytes/macrophages and muscle cells, is enhanced in the circulation of pwMS [5], which is in line with data presented herein. Furthermore, plasma IL-6 levels increased in HCs following exercise, which is consistent with findings elsewhere [11]. It is known that exercise increases muscle-derived IL-6 [11], hence it is attractive to speculate that any anti-inflammatory effects of exercise on immune cell-derived IL-6 in pwMS is negated by muscle-derived IL-6. Future studies assessing the impact of exercise on immune cell subsets will address this.

A body of research continues to elucidate the therapeutic role of exercise in MS, with much data demonstrating that exercise skews cytokine profiles to an anti-inflammatory signature. The significant finding here is that a short 8-week training programme drives plasma IL-10 production in pwMS, and this induction promotes an anti-inflammatory cytokine signature that correlates with physical/mental health.

### Figure legends

**Fig 1.** An 8-week aerobic exercise programme enhances a plasma anti-inflammatory cytokine signature in pwMS, that correlates with physical and mental health. Plasma cytokine and QOL indices were assessed prior to week 1 of training, and at the end of 8 weeks of training, using V-PLEX human pro-inflammatory assays and the MSQOL-54 questionnaire, respectively. The ratio of plasma (a) IL-10:IL-12p70 and (c) IL-10:IL-6 was reduced in pwMS, compared to HCs. Exercise reversed the MS-associated decline in the ratio of plasma IL-10:IL-12p70 and IL-10:IL-6. (b) A significant positive correlation was determined between plasma IL-10:IL-12p70 and both physical ( $r=0.434$ ) and mental health ( $r=0.316$ ) in HCs and pwMS. (d) A significant positive correlation was determined between plasma IL-10:IL-6 and both physical ( $r=0.160$ ) and mental health ( $r=0.172$ ) in HCs and pwMS. Data are presented as the mean $\pm$ SEM and are representative of data from HCs ( $n=10$ ) and pwMS ( $n=9$ ). \*\* $p<0.01$ , \*\*\* $p<0.001$  compared to HCs pre-exercise training. HC: healthy control; MS: Multiple sclerosis.

### Disclosure statement

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Table 1.** Effect of exercise on the production of plasma cytokines from HC and MS individuals.

	HC		MS		E	D	ExD
	Pre-exercise (mean ± SEM)	Post-exercise (mean ± SEM)	Pre-exercise (mean ± SEM)	Post-exercise (mean ± SEM)			
IL-10 (pg/L)	2372 ± 126	1993 ± 159	1693 ± 137*	2318 ± 221 <sup>+</sup>			X
IL-12p70 (pg/L)	39.79 ± 4.42	39.46 ± 8.56	59.59 ± 6.99	51.71 ± 10.06		X	X
IL-6 (pg/L)	1041 ± 75	1276 ± 261	1640 ± 206	1599 ± 140		X	

\*  $p < 0.05$  compared to healthy individuals pre-exercise training.

<sup>+</sup>  $p < 0.05$  compared to MS individuals pre-exercise training.

E: significant effect of exercise; D: significant effect of disease; ExD: significant interaction between exercise and disease.

Statistical analysis: two-way ANOVA.

HC: healthy control; MS: multiple sclerosis



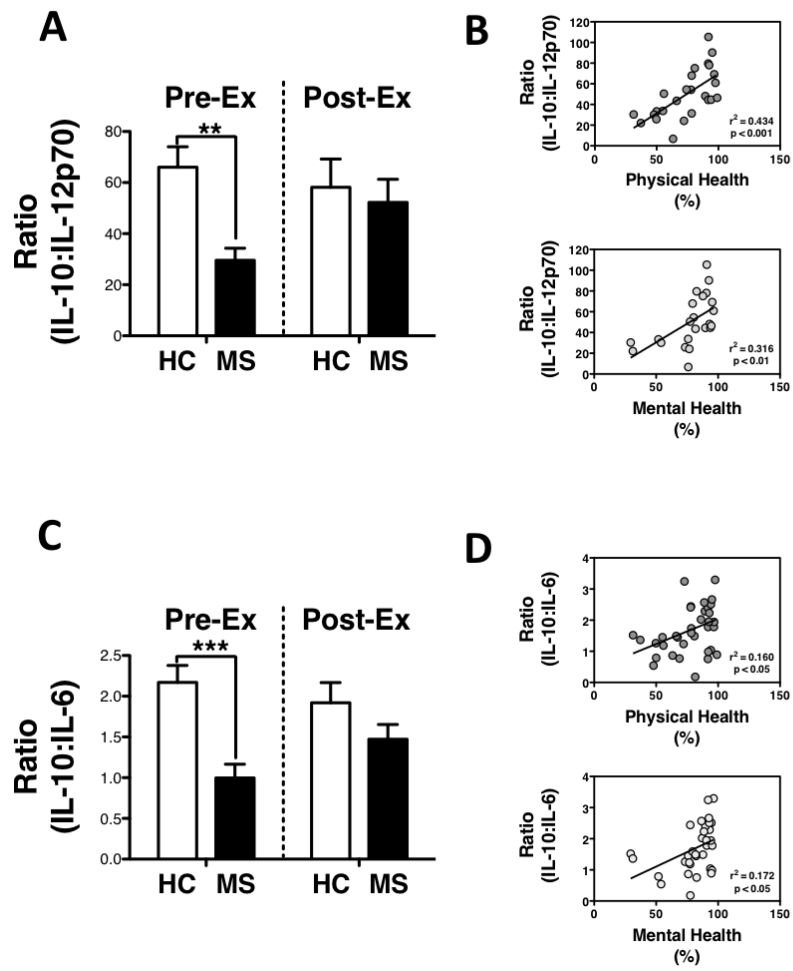


Figure. 1. Barry et al.,