Baseline Inflammation but not Exercise Modality Impacts Exercise-induced Kynurenine Pathway Modulation in Persons With Multiple Sclerosis: Secondary Results From a Randomized Controlled Trial

International Journal of Tryptophan Research
Volume 17: 1–15

DOI: 10.1177/11786469241284423

Marie Kupjetz[1], Nadine Patt[2,3], Niklas Joisten[1,4], Per Magne Ueland[5], Adrian McCann[5], Roman Gonzenbach[2], Jens Bansi[2,6] and Philipp Zimmer[1]

[1]Division of Performance and Health (Sports Medicine), Institute for Sport and Sport Science, TU Dortmund University, Dortmund, Germany. [2]Department of Neurology, Valens Rehabilitation Centre, Clinics of Valens, Valens, Switzerland. [3]Graduate School for Health Sciences, University of Bern, Bern, Switzerland. [4]Division of Exercise and Movement Science, Institute for Sport Science, University of Göttingen, Göttingen, Germany. [5]Bevital AS, Bergen, Norway. [6]Department of Health, Physiotherapy, OST – Eastern Switzerland University of Applied Sciences, Sankt Gallen, Switzerland.

ABSTRACT

BACKGROUND: The kynurenine pathway (KP) is an important hub in neuroimmune crosstalk that is dysregulated in persons with multiple sclerosis (pwMS) and modulated by exercise in a modality-specific manner.

OBJECTIVES: To compare changes in the KP metabolite profile of pwMS (1) following combined treatments including either high-intensity interval training (HIIT) or moderate-intensity continuous training (MICT) during a 3-week multimodal rehabilitation, (2) to evaluate exercise response in relation to baseline systemic inflammation, and (3) to investigate associations of kynurenines with physical capacity and clinical outcomes.

METHODS: For this secondary analysis of a randomized controlled trial, serum concentrations of kynurenines at baseline and after 3 weeks were determined using targeted metabolomics (LC-MS/MS). Exercise-induced changes in the KP metabolite profile according to treatment and baseline systemic inflammation (neutrophil-to-lymphocyte ratio (NLR) <3.12 versus $\geqslant 3.12$) were investigated using covariance analyses.

RESULTS: Regardless of treatment, concentrations of tryptophan and most kynurenines decreased over time. Quinolinic acid concentration increased ($p < .001$). Participants with low and high NLR revealed differential exercise-induced changes in concentrations of kynurenines and NLR. The systemic inflammation markers neopterin ($p = .015$) and NLR ($p < .001$) decreased in the whole group and in participants with high NLR, respectively.

CONCLUSIONS: Combined treatments including HIIT or MICT do not differentially modulate the KP metabolite profile, with both reducing concentrations of most kynurenines. Baseline systemic inflammation may impact exercise-induced changes in the KP metabolite profile and anti-inflammatory effects of exercise in pwMS.

TRIAL REGISTRATION: clinicaltrials.gov (identifier: NCT04356248)

KEYWORDS: Multiple sclerosis, endurance training, tryptophan, kynurenine, inflammation, NLR

RECEIVED: May 20, 2024. **ACCEPTED:** August 29, 2024.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The source study was funded by the Swiss Multiple Sclerosis Society (SMSG-2020-1, 2020). The funding source had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: P.M.U. and A.M. are paid employees at Bevital AS. Bevital AS is owned by a not-for-profit foundation established to promote research into functional B-vitamin deficiency. M.K., N.P., N.J., R.G., J.B., and P.Z. declare that there is no conflict of interest.

CORRESPONDING AUTHOR: Philipp Zimmer, Division of Performance and Health (Sports Medicine), Institute for Sport and Sport Science, TU Dortmund University, Otto-Hahn-Str. 3, Dortmund 44227, Germany. Email: philipp.zimmer@tu-dortmund.de

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) resulting from aberrant adaptive and innate immunity. MS represents the leading cause of non-traumatic neurological disability in young adulthood and manifests as heterogeneous constellations of motor, sensory, cognitive, and neuropsychiatric symptoms that may progress over time.[1] Maintaining independent living in persons with MS (pwMS) requires rigorous multimodal treatment in which physical exercise plays an integral role due to its beneficial effects on various MS symptoms, physical capacity, and health-related quality of life (HRQoL).[2,3]

Physical exercise also exerts immunomodulatory effects.[4] Based on preclinical evidence, these immunomodulatory effects have been proposed to underpin the beneficial effects of physical exercise on neuroinflammation and neuroaxonal

damage in MS.[5] However, the exact molecular mechanisms remain poorly understood. Among others, the kynurenine pathway (KP) may contribute to the immunomodulatory effects of physical exercise in MS. The KP is the major route of tryptophan (Trp) metabolism which is present in various organ-specific and systemic peripheral cells, such as hepatocytes and immune cells, as well as CNS-resident cells, such as microglia and astrocytes.[6,7] Due to the immunoregulatory and neuroactive properties of some Trp downstream metabolites, referred to as *kynurenines*, the KP is recognized as an important hub in neuroimmune crosstalk.[7,8]

Chronic KP dysregulation is a common feature of numerous inflammation-related diseases and conditions, including MS.[8,9] Elevated levels of pro-inflammatory cytokines, such as interferon-gamma (IFN-γ), activate the enzyme indoleamine 2,3-dioxygenase 1 (IDO1). IDO1 catalyzes the first step of the KP, leading to an increased formation of kynurenine (Kyn). Further, inflammation directs Kyn degradation toward downstream kynurenines, such as 3-hydroxykynurenine (3-HK) or quinolinic acid (QA), which in abnormal concentrations exert neurotoxic effects at the CNS level, rather than neuroprotective kynurenines, such as kynurenic acid (KA).[7,10,11] KP dysregulation is observed at the CNS and systemic level which mutually interact as some kynurenines readily cross the blood-brain barrier.[8] Accordingly, higher QA concentration and QA/KA ratio have been observed in both cerebrospinal fluid and blood serum of pwMS compared to healthy individuals,[12] and higher concentration of circulating QA as well as QA/KA ratio and kynurenine-to-tryptophan ratio (KTR) have been reported to be associated with greater MS-related disability.[13] Against this backdrop, the KP has been investigated as a pharmacological target in MS[14] and has been addressed in research on non-pharmacological interventions, such as physical exercise.[15,16]

Exercise is a strong physiological stimulus capable of modulating the KP by affecting both CNS[17] and systemic concentrations of kynurenines, as has been shown in young healthy men.[17,18] In pwMS, single bouts of high-intensity interval training (HIIT), but not moderate-intensity continuous endurance training (MICT), have been shown to induce an *acute* increase in plasma KA concentration, accompanied by a transient reduction in the systemic QA/KA ratio.[19] This previous work highlighted intensity as a key factor in exercise-induced KP modulation and suggests that regular HIIT may also have a beneficial *chronic* effect on KP dysregulation in MS. Studies investigating chronic exercise-induced KP modulation in pwMS remain scarce and, of the limited research available, results are ambiguous.[19,20]

Therefore, the primary aim of this study was to evaluate the effects of repeated HIIT and MICT bouts, performed as part of combined treatments during 3 weeks of multimodal rehabilitation, in modulating the serum KP metabolite profile in pwMS. Based on previous evidence highlighting that HIIT but not MICT provokes acute changes in the KP, we hypothesized superiority of repeated HIIT compared to MICT bouts in modulating the serum KP metabolite profile. Secondarily, we aimed to assess differences in exercise-induced KP modulation when dichotomizing participants according to baseline systemic inflammatory status, using the neutrophil-to-lymphocyte ratio (NLR). We expected that effects of endurance exercise on the serum KP metabolite profile in pwMS differ dependent on the baseline inflammatory status of participants, given that systemic KP activity is stimulated by pro-inflammatory cytokines[7] and regular endurance exercise induces anti-inflammatory effects.[4] Thirdly, we aimed to explore associations of kynurenines and ratios of kynurenines with physical capacity and clinical outcomes at baseline and following the interventions. We hypothesized that kynurenines and ratios of kynurenines are associated with baseline physical capacity (ie, cardiorespiratory fitness, peak power output (PPO)) and clinical outcomes (ie, HRQoL, fatigue), and that changes in the serum KP metabolite profile over time are correlated with improvements in physical capacity and clinical outcomes.

Methods

Trial design

This study is a secondary analysis of a parallel-group randomized controlled superiority trial conducted within a 3-week multimodal inpatient setting at the Valens Rehabilitation Centre, Valens, Switzerland. The source study was powered to evaluate superiority of HIIT combined with inpatient energy management education (IEME) over MICT combined with progressive muscle relaxation (PMR) (usual care) on change in HRQoL from baseline until 6-month follow-up (N = 106). Results have been reported elsewhere.[21] Changes in serum concentrations of Trp, Kyn, QA, and KA as well as NLR from pre (T_0) to post (after 3 weeks, T_1) were prespecified as secondary outcomes.[22] Ethical approval was obtained from the regional Ethics Committee of Eastern Switzerland (EKOS20/050, ID: BASEC2020–00797, 09 April 2020). The trial was registered prospectively at ClinicalTrials.gov (NCT04356248, 22 April 2020). Study procedures were carried out in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent. For this secondary analysis, no additional ethical approval or consent was required. The manuscript was written in accordance with the CONSORT 2017 Statement for Randomized Trials of Nonpharmacologic Treatments.[23]

Participants

PwMS admitted for inpatient rehabilitation were consecutively screened and recruited on-site. The main inclusion criteria comprised age ⩾ 18 years, MS diagnosis according to the 2017-revised McDonald criteria, Expanded Disability Status Scale (EDSS) score ⩽ 6.5, and substantial fatigue (Fatigue Scale for Motor and Cognitive Functions total score

($FSMC_{tot}$) ⩾ 43).[24] The main exclusion criteria were concomitant disease (eg, other autoimmune, neuroinflammatory, and/or neurodegenerative diseases, cardiopulmonary diseases, acute infections), cognitive impairment, severe depressive symptoms, and recent stem cell treatment (⩽6 months). During the trial, participants did not receive corticosteroid treatment. Participants with the relapsing-remitting MS phenotype were in remission phase.

Concealed stratified randomization (1:1) to 1 of the 2 combined treatments (ie, HIIT combined with IEME or MICT combined with PMR) was conducted by a researcher not involved in the study using Randomization in Treatment Arms software (version 1.5.2, EVIDAT, Kiel, Germany). Strata included age, sex, MS phenotype, EDSS score, fatigue, PPO, and HRQoL assessed at baseline. Further details on sample size calculation and randomization (ie, sequence generation, allocation concealment, and implementation) are provided in the study protocol.[22]

Treatment regimens

Treatments started a maximum of 2 days after randomization. IEME is an educational group-based approach for fatigue management covering topics like break management or effective communication.[25] PMR includes muscle relaxation exercises combined with deep breathing. IEME and PMR were delivered twice weekly and are described in detail elsewhere.[22] HIIT and MICT were considered the potent physiological stimuli to induce modulation of the KP metabolite profile. Thus, groups are referred to as HIIT and MICT hereafter.

HIIT and MICT sessions were conducted on bicycle ergometers (Cybex 750C Bike, Cybex International, Medway, MA, USA) 3 times per week. Individual exercise intensity was calculated as percentage of peak heart rate (HR_{peak}) determined during baseline cardiopulmonary exercise testing (CPET).

HIIT sessions included five 1.5-minute intervals at high cadence (80-100 revolutions per minute), aimed at achieving 95% to 100% HR_{peak}. High-intensity intervals were separated by four 2-minute active rests at a reduced cadence (50-60 revolutions per minute) aimed at restoring HR to 60% $HR_{peak.}$ The total session duration of HIIT was 15.5 minutes excluding warm-up and cool-down periods. MICT required participants to cycle continuously at 60 to 70 revolutions per minute and 65% HR_{peak} for a duration of 24 minutes. HIIT and MICT sessions were enclosed by a 3-minute warm-up and cool-down period at 50% HR_{peak}. HR was monitored using wristwatches (Polar M200, Polar Electro Oy, Kempele, Finland) connected to chest belts (Polar T31, Polar Electro Oy, Kempele, Finland). Training sessions were conducted under the supervision of exercise scientists or a physiotherapist, individually (1:1) or in small groups of up to 3 participants (1:3). The supervising exercise scientists (n = 2) and the physiotherapist (n = 1) were master level clinicians, had received prior training, and were experienced with the supervision of HIIT and MICT in pwMS. Supervising personnel monitored HR throughout exercise sessions and adjusted the pedaling resistance (W) according to the target HR to ensure compliance with the prescribed exercise protocol. The participants were supported in getting on and off the ergometer as needed. Blinding participants or supervising personnel was not possible due to the study design. Attendance, protocol adherence, and potential adverse events were documented each session using case report forms. Relevant concomitant care included physiotherapy (30-60 minutes, 5 times per week), strength training (30-45 minutes, 3 times per week), and occupational therapy (30 minutes, 2-3 times per week). Personnel providing concomitant care was discouraged from including HIIT (and IEME) or MICT (and PMR) components in respective sessions. No changes were made to the trial design, recruitment, group allocation, or treatments after the start of the study.

Blood sampling and analytic procedures

Blood sampling was conducted at baseline (T_0) and after 3 weeks (T_1). Blood samples were collected at rest in sitting position between 08:00 and 09:00 AM after an overnight fast and at least 24 hours after the last HIIT or MICT session. Samples were drawn from the antecubital vein using ethylenediaminetetraacetic acid (EDTA)-containing tubes and serum tubes. After coagulation, serum samples were centrifuged at 3000*g* for 10 minutes at 4°C and the supernatant was stored at −80°C until analysis. Targeted metabolomics was performed using high-throughput liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) at Bevital AS, Bergen, Norway (https://bevital.no/key-data/#D), as described elsewhere.[26] The lower limit of detection (LOD) for the assay ranged from 0.01 to 8.00 $nmol*L^{-1}$ and within- and between-day coefficients of variation (CVs) ranged from 3% to 8% and 4% to 10%, respectively. EDTA samples were analyzed using an automated hematology analyzer (Sysmex XN-1000, Norderstedt, Germany) at Dr Risch laboratory, Buchs, Switzerland, to determine complete blood cell counts. The personnel conducting LC-MS/MS and hematology analysis was blinded to group allocation.

Tryptophan downstream metabolites: kynurenines and ratios of kynurenines

KP metabolite profiling included Trp ($\mu mol*L^{-1}$), Kyn ($\mu mol*L^{-1}$), KA ($nmol*L^{-1}$), anthranilic acid (AA, $nmol*L^{-1}$), 3-HK ($nmol*L^{-1}$), xanthurenic acid (XA, $nmol*L^{-1}$), 3-hydroxyanthranilic acid (3-HAA, $nmol*L^{-1}$), QA ($nmol*L^{-1}$), and picolinic acid (Pic, $nmol*L^{-1}$). Based on that, KTR, KA/Kyn ratio, QA/Kyn ratio, QA/KA ratio, and 3-HAA/AA ratio were calculated. KTR ($\mu mol*L^{-1}$ by $mmol*L^{-1}$) is a normalized measure of overall Trp degradation along the KP,[27] and, in combination with neopterin (Neopt), serves as a marker of *adaptive Th1-type immune system activation.*[28] KA/Kyn ratio and QA/Kyn ratio

(both nmol*L^{-1} by µmol*L^{-1}) reflect changes in Kyn catabolism toward an increased/decreased KA and QA formation. QA/KA ratio (nmol*L^{-1} by nmol*L^{-1}) is considered as a proxy marker of excitotoxic KP dysregulation.[13] 3-HAA/AA ratio (nmol*L^{-1} by nmol*L^{-1}) has been hypothesized to be a response marker to inflammatory stimulation and neurotoxic damage.[29]

Markers of inflammation: neopterin and neutrophil–to–lymphocyte ratio

Neopt concentration (nmol*L^{-1}) was determined within the LC-MS/MS assay. Neopt is released from macrophages upon IFN-γ stimulation by Th1-type lymphocytes.[30] Parallel assessment of Neopt helps to attribute KTR elevation to inflammatory KP stimulation.[28]

NLR was calculated as absolute neutrophil count (ANC) divided by absolute lymphocyte count (ALC), as determined via automated hematology analysis. NLR is a non-specific marker of *innate immune system* activation. NLR is chronically elevated across inflammation-related diseases and conditions, including MS.[31,32] In pwMS, NLR is associated with clinical outcomes, including disease activity,[33-36] disability,[34,35,37,38] and fatigue.[38]

Physical capacity and clinical outcomes

Measures of physical capacity included cardiorespiratory fitness (peak oxygen consumption divided by body weight in kilogram (relative $\dot{V}O_{2\ peak}$, mL*min^{-1}*kg^{-1})) and relative peak power output (PPO, maximum W divided by body weight in kilogram, W*kg^{-1}) determined via CPET. CPET was conducted as a ramp-type protocol on a bicycle ergometer (ergometrics er800 S, ergoline GmbH, Bitz, Germany) by a blinded investigator. The ramp-type protocol consisted of (a) a resting state measurement without pedaling while the participants were sitting on the bicycle ergometer (3 minutes); (b) subsequent pedaling at 20 W (3 minutes); (c) the testing phase with a progressive increase of 5 to 10 W/minute until subjective exhaustion (8-12 minutes); (d) followed by a cool-down of unloaded pedaling (3 minutes). HR was monitored continuously. Blood pressure and rate of perceived exertion (Borg CR-10 scale) were assessed every 2 minutes and within the last 10 seconds of the test. $\dot{V}O_2$ was monitored by direct and continuous measurements (breath by breath) using a Jaeger CPX CPET system (Jaeger, Würzburg, Germany). $\dot{V}O_{2\ peak}$ was defined as the highest 15 second averaged $\dot{V}O_2$ value when one or more of the following criteria were attained: respiratory equivalent ratio >1.10, HR_{peak} within 10 minutes^{-1} of the age-predicted maximum, and Borg CR-10 rating >8.5.[39]

HRQoL was queried using the Medical Outcomes Study 36-Item Short Form Health Survey (SF-36). SF-36 scores were aggregated into the Physical Component Summary (PCS) and the Mental Component Summary (MCS) score. Higher PCS and MCS scores indicate better HRQoL.[40] Fatigue was assessed using the $FSMC_{tot}$ score as well as the motor and cognitive subscale scores ($FSMC_{mot}$, $FSMC_{cog}$). Higher scores indicate greater fatigue.[30]

Statistical analysis

Data was screened for missing values. Replacement of missing values was waived as missing values totaled <5%. For all blood-derived variables (ie, Trp, kynurenines, Neopt, white blood cell count, ANC, ALC), reasons for missingness were determined. Non-imputed data was *Z*-scored to identify statistical outliers, defined as values higher or lower than the mean ± 3 SD. Statistical outliers were winsorized by replacement with the maximum or minimum value within 3 SD. Data distribution was assessed visually using histograms and *Q-Q* plots. Due to the positive skewness of most blood-derived variables, log10 transformation was uniformly applied to blood-derived variables, relative $\dot{V}O_{2\ peak}$, and relative PPO. Ratios were calculated based on log10-transformed values.

For the main analysis, repeated measures analyses of covariance (ANCOVAs) were computed with the treatment modality (HIIT versus MICT) and sex (female versus male) as between-subject factors and Trp, kynurenines, ratios of kynurenines, Neopt, and NLR as dependent variables. Covariates included age, body mass index (BMI), EDSS score, and the baseline value of the dependent variable. Significant time * modality * sex interactions were specified using Bonferroni-corrected post hoc tests. A similar ANCOVA model was computed for $\dot{V}O_{2\ peak}$ and PPO as dependent variables, serving as a manipulation check for the effectiveness of the endurance training protocols. As no significant time * group interactions were identified, treatment groups were pooled. Participants were then dichotomized according to baseline systemic inflammatory status, using an NLR cut-off value of 3.12 which has been previously described to predict MS-related disability and disease activity.[35] ANCOVAs were repeated with NLR subgroups (low NLR versus high NLR) and sex (female versus male) as between-subject factors. Due to NLR dichotomization, baseline adjustment for NLR was omitted. Significant time * NLR subgroup interactions were specified using Bonferroni-corrected post hoc tests.

Associations between Trp, kynurenines, ratios of kynurenines, Neopt, NLR, measures of physical capacity ($\dot{V}O_{2\ peak}$, PPO), and clinical outcomes (PCS, MCS, FSMC scores) at baseline were calculated using two-tailed Spearman's correlation analysis. Additionally, correlations of changes in blood-derived variables with changes in physical capacity and clinical outcomes were calculated for those metabolites/ratios that showed significant changes over time. Strength of correlation was interpreted as weak ($r_s = \pm 0.10$ to ± 0.29), moderate ($r_s = \pm 0.30$ to ± 0.49), or strong ($r_s = \pm 0.50$ to ± 1.0).[41] Results of ANCOVAs and correlation analyses were considered

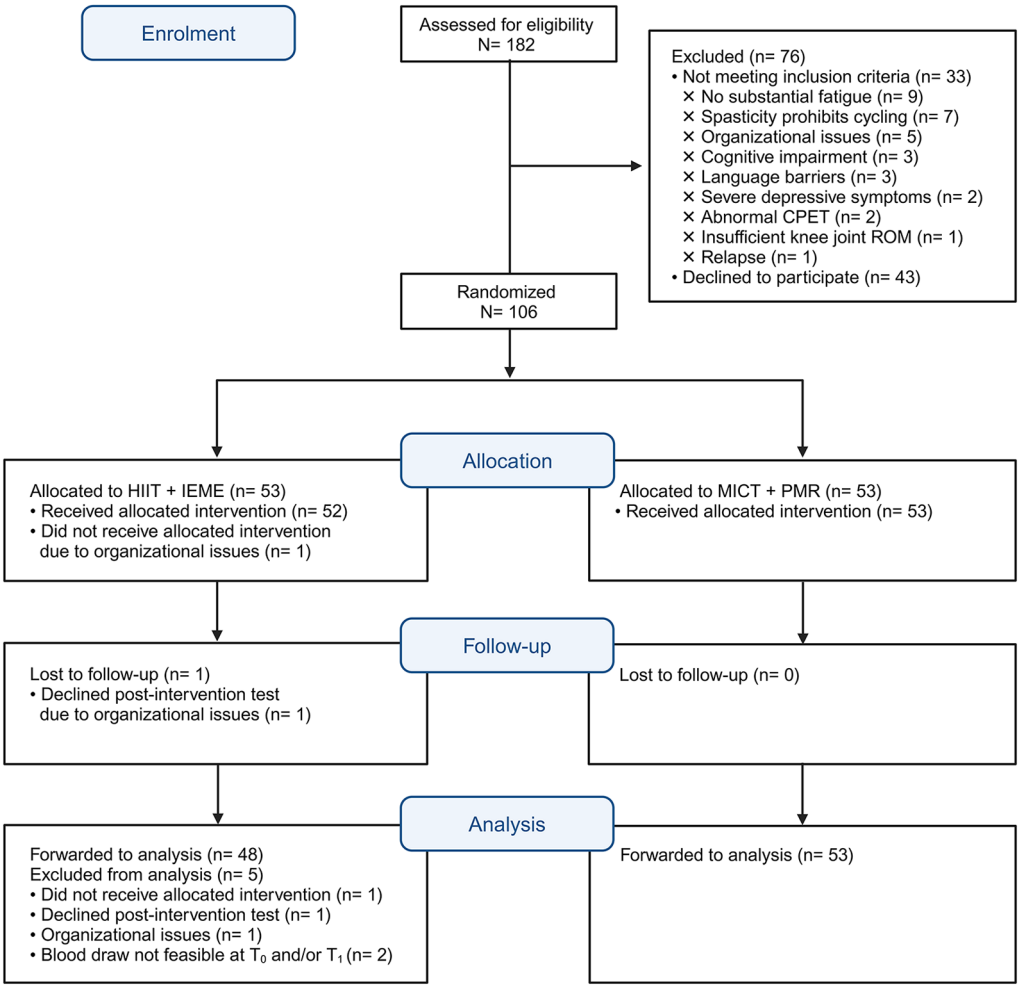

Figure 1. CONSORT flow diagram.
Source: This CONSORT flow diagram was created with biorender.com.
Abbreviations: CPET, cardiopulmonary exercise testing; HIIT, high-intensity interval training; IEME, inpatient energy management education; MICT, moderate-intensity continuous training; PMR, progressive muscle relaxation; ROM, range of motion; T_0, baseline assessment; T_1, follow-up assessment after 3 weeks of intervention.

significant if $p \leq .05$. All statistical analyses were computed using IBM SPSS Statistics 29 (Armonk, NY, USA). Charts were created using GraphPad Prism (version 10 for Windows, GraphPad Software, San Diego, CA, USA).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Participants were recruited between July 13, 2020 and October 19, 2021. The intervention phase was completed on November 5, 2021 (last blood sampling). Participants were randomized equally (HIIT: n = 53, MICT: n = 53) as intended. Participant flow for the period considered in this secondary analysis is illustrated in Figure 1. During the study period, no changes were made to the protocol design, intervention delivery, or outcome assessment. On average, participants completed 97.25% of the prescribed HIIT sessions and 97.85% of the prescribed MICT sessions. No adverse events occurred.

Participant characteristics are reported in Table 1. Blood samples were available for 105 participants at T_0, as in one participant, blood collection was not feasible. Among the 105 available samples at T_0 forwarded to analysis, hematology analysis (n = 1) and LC-MS/MS (n = 1) failed due to insufficient sample quantity. At T_1, blood samples were available for 101 participants. Reasons for missing blood samples at T_1 included administrative issues (n = 2), non-feasible blood collection (n = 2), and dropout (n = 1). Among the 101 available samples at T_1 forwarded to analysis, LC-MS/MS failed in n = 1 due to an insufficient sample quantity. Concentrations of AA, 3-HK, 3-HAA, and Neopt could not be determined in n = 3 at T_0, and in n = 2 at T_1 due to severe hemolysis. Missing values amounted to 4.48% and were considered "missing at random". Statistical outliers totaled 1.40% of individual blood-derived variables and 1.72% of subsequently calculated ratios. Baseline NLR was available for n = 104. Dichotomization according to the cut-off value of NLR = 3.12 classified 76 participants as having a *low NLR* and 28 participants as having a *high NLR* at baseline.

Table 1. Demographic, anthropometric, and MS-related characteristics of participants at baseline.

	OVERALL SAMPLE	HIIT	MICT	LOW NLR	HIGH NLR
	N = 106	n = 53	n = 53	n = 76	n = 28
Sex					
Male	35 (33.0%)	19 (35.8%)	16 (30.2%)	25 (32.9%)	9 (32.1%)
Female	71 (67.0%)	34 (64.2%)	37 (69.8%)	51 (67.1%)	19 (67.9%)
Age [y]	49.8 (9.9)	50.0 (10.9)	49.5 (8.8)	49.7 (9.7)	49.2 (10.5)
BMI [kg*m^{-2}]	24.91 (5.46)	24.33 (5.22)	25.48 (5.68)	25.04 (5.71)	24.40 (4.97)
MS phenotype					
RRMS	53 (50.0%)	27 (50.9%)	26 (49.1%)	35 (46.1%)	17 (60.7%)
SPMS	38 (35.8%)	20 (37.7%)	18 (34.0%)	30 (39.5%)	7 (25.0%)
PPMS	15 (14.2%)	6 (11.3%)	9 (17.0%)	11 (14.5%)	4 (14.3%)
EDSS score	4.64 (1.32)	4.61 (1.41)	4.67 (1.23)	4.65 (1.37)	4.54 (1.19)
TSD [months]	159.38 (109.12)	179.32 (112.51)	139.43 (102.82)	157.51 (113.34)	152.36 (91.87)
Current DMT					
No DMT	38 (35.8%)	16 (30.2%)	22 (41.5%)	32 (42.1%)	6 (21.4%)
DMT	68 (64.2%)	37 (69.8%)	31 (58.5%)	44 (57.9%)	22 (78.6%)
Ocrelizumab	28 (26.4%)	15 (28.3%)	13 (24.5%)	20 (26.3%)	7 (25.0%)
Natalizumab	10 (9.4%)	3 (5.7%)	7 (13.2%)	10 (13.2%)	0 (0.0%)
Fingolimod	10 (9.4%)	8 (15.1%)	2 (3.8%)	0 (0.0%)	9 (32.1%)
Dimethyl fumarate	7 (6.6%)	4 (7.5%)	3 (5.7%)	4 (5.3%)	3 (10.7%)
Rituximab	5 (4.7%)	4 (7.5%)	1 (1.9%)	4 (5.3%)	1 (3.6%)
Interferon beta	2 (1.9%)	0 (0.0%)	2 (3.8%)	2 (2.6%)	0 (0.0%)
Ofatumumab	2 (1.9%)	1 (1.9%)	1 (1.9%)	2 (2.6%)	0 (0.0%)
Siponimod	2 (1.9%)	1 (1.9%)	1 (1.9%)	1 (1.3%)	1 (3.6%)
Alemtuzumab	1 (0.9%)	1 (1.9%)	0 (0.0%)	0 (0.0%)	1 (3.6%)
Teriflunomide	1 (0.9%)	0 (0.0%)	1 (1.9%)	1 (1.3%)	0 (0.0%)
Baseline NLR[a]	2.97 (2.22)	3.54 (2.60)	2.41 (1.62)	1.93 (0.64)	5.79 (2.52)
Rel. $\dot{V}O_{2\ peak}$ [mL*min^{-1}*kg^{-1}]	19.03 (6.04)	19.67 (5.82)	18.39 (6.25)	19.10 (6.04)	19.09 (6.26)
Rel. PPO [W*kg^{-1}]	1.33 (0.57)	1.41 (0.55)	1.26 (0.58)	1.33 (0.58)	1.38 (0.56)
Current smoker	28 (26.4%)	12 (22.6%)	16 (30.2%)	22 (28.9%)	5 (17.9%)

Abbreviations: BMI, body mass index; DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; MS, multiple sclerosis; NLR, neutrophil-to-lymphocyte ratio (low NLR: NLR < 3.12; high NLR: NLR ⩾ 3.12); PPMS, primary progressive multiple sclerosis; rel. PPO, peak power output during cardiopulmonary exercise testing divided by kilogram body weight; rel. $\dot{V}O_{2\ peak}$, peak oxygen consumption during cardiopulmonary exercise testing divided by kilogram body weight; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; TSD, time since diagnosis.
Categorical data are given as total number and percentage (%). Continuous data are given as Mean (SD). Blood-derived measures have been winsorized.
[a]Baseline NLR was available for n = 104. Winsorized NLR values are presented.

Kynurenines are associated with neopterin but not neutrophil-to-lymphocyte ratio

Concentrations of most kynurenines, including Kyn, KA, AA, 3-HK, XA, 3-HAA, and QA, and KTR revealed weak to strong positive correlations with Neopt concentration. KA/Kyn ratio and QA/Kyn ratio showed moderate inverse correlations with Neopt concentration. No significant correlations were observed between Neopt and Trp concentration, Pic concentration, QA/

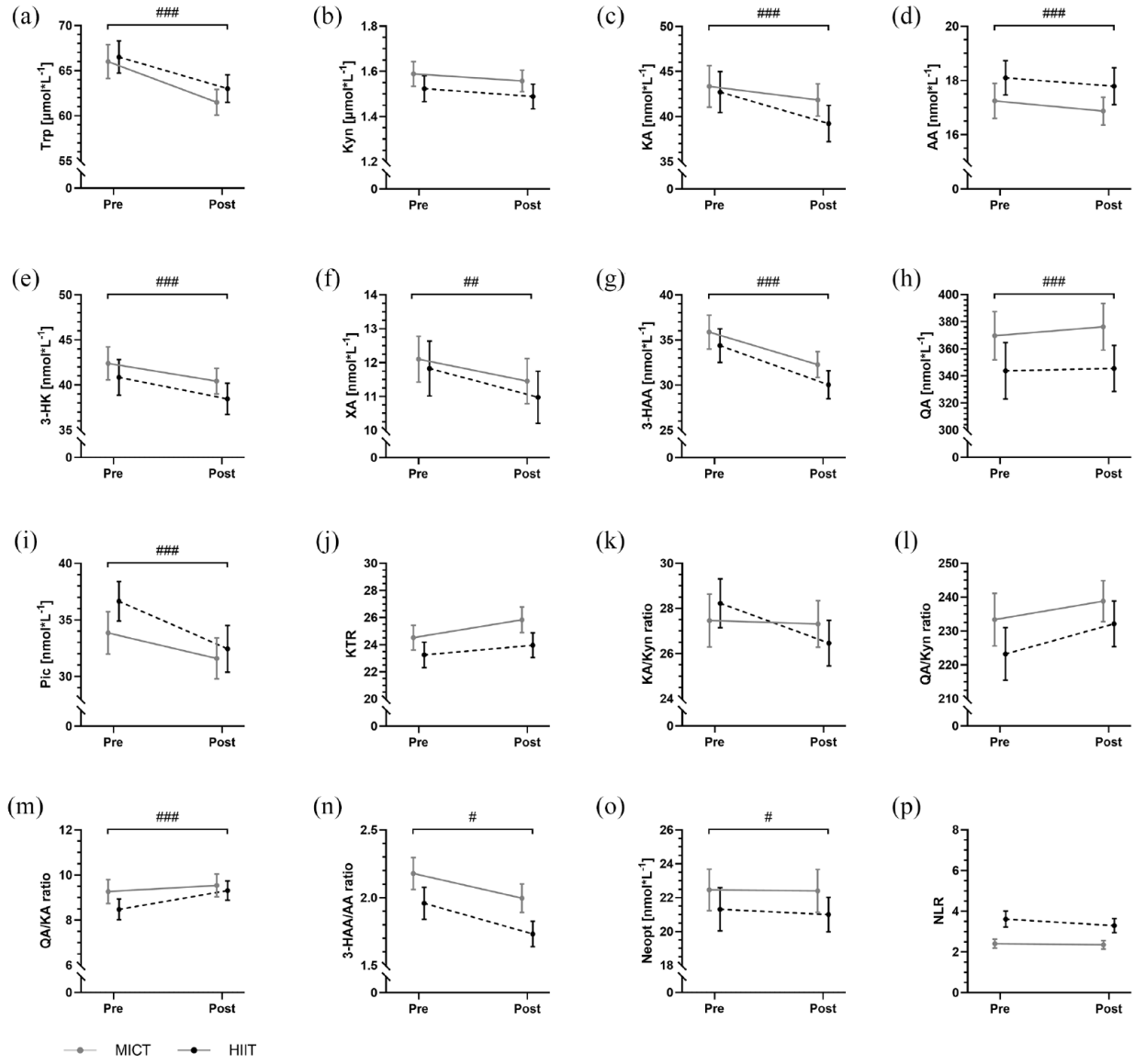

Figure 2. Exercise-induced changes in the kynurenine pathway metabolite profile and inflammation markers according to time (baseline, 3 weeks) and modality (HIIT, MICT).

Abbreviations: AA, anthranilic acid (d); HIIT, high-intensity interval training; KA, kynurenic acid (c); KA/Kyn ratio, kynurenic acid-to-kynurenine ratio [nmol*L^{-1} divided by µmol*L^{-1}] (k); KTR, kynurenine-to-tryptophan ratio [µmol*L^{-1} divided by mmol*L^{-1}] (j); Kyn, kynurenine (b); MICT, moderate-intensity continuous training; Neopt, neopterin (o); NLR, neutrophil-to-lymphocyte ratio (p); Pic, picolinic acid (i); QA, quinolinic acid (h); QA/KA ratio, quinolinic acid-to-kynurenic acid ratio [nmol*L^{-1} divided by nmol*L^{-1}] (m); QA/Kyn ratio, quinolinic acid-to-kynurenine ratio [nmol*L^{-1} divided by µmol*L^{-1}] (l); Trp, tryptophan (a); XA, xanthurenic acid (f); 3-HAA, 3-hydroxyanthranilic acid (g); 3-HK, 3-hydroxykynurenine (e); 3-HAA/AA ratio, 3-hydroxyanthranilic acid-to-anthranilic acid ratio [nmol*L^{-1} divided by nmol*L^{-1}] (n).

Line charts illustrate exercise-induced changes in kynurenines, ratios of kynurenines, and inflammation markers from pre (T_0; baseline) to post (T_1; after 3 weeks) per exercise modality (HIIT or MICT). Non-transformed winsorized data are presented as Mean (SEM). Hash signs indicate significant time effects at a 0.05 level (#), 0.01 level (##), or 0.001 level (###), as determined by ANCOVA performed on log10-transformed values (main analysis, Table 2).

KA ratio, or 3-HAA/AA ratio. Neither concentrations of kynurenines or ratios of kynurenines, nor Neopt correlated significantly with NLR (Supplemental Figure 1).

Endurance exercise modulates the serum kynurenine pathway metabolite profile

Both endurance training protocols proved effective in improving $\dot{V}O_{2\ peak}$ ($p < .001$, Supplemental Figures 2a and 4a) and PPO ($p = .025$, Supplemental Figures 2b and 4b) over time (Table 2). No significant time*group interactions were observed when comparing the effects of HIIT versus MICT on concentrations of Trp, kynurenines, ratios of kynurenines, Neopt concentration, and NLR. Significant reductions in concentrations of Trp ($p < .001$, Figure 2a), KA ($p < .001$, Figure 2c), AA ($p < .001$, Figure 2d), 3-HK ($p < .001$, Figure 2e), XA ($p = .005$, Figure 2f), 3-HAA ($p < .001$, Figure 2g), and Pic ($p < .001$, Figure 2i) as well as in 3-HAA/AA ratio ($p = .019$, Figure 2n), and Neopt concentration ($p = .015$, Figure 2o) were observed over time. QA concentration ($p < .001$, Figure 2h) and QA/KA ratio ($p < .001$, Figure 2m) increased significantly. No significant time effects were observed for Kyn concentration (Figure

Table 2. Exercise-induced changes in the kynurenine pathway metabolite profile and inflammation markers according to time (baseline, 3 weeks) and modality (HIIT, MICT).

	HIIT				MICT				ANCOVA					
	n = 53				n = 53									
	PRE (T_0)		POST (T_1)		PRE (T_0)		POST (T_1)		TIME*GROUP			TIME		
	n	*M* (SD)	n	*M* (SD)	n	*M* (SD)	n	*M* (SD)	*F*	*p*	$p\eta^2$	*F*	*p*	$p\eta^2$
Kynurenines														
Trp [μmol*L^{-1}]	51	66.50 (12.39)	48	63.01 (10.49)	53	65.99 (13.63)	52	61.49 (10.36)	1.637	.204	0.017	47.936	**<.001*** **	0.343
Kyn [μmol*L^{-1}]	51	1.52 (0.40)	48	1.49 (0.38)	53	1.59 (0.39)	52	1.56 (0.34)	0.035	.851	<0.001	2.615	.109	0.028
KA [nmol*L^{-1}]	51	42.72 (15.72)	48	39.23 (13.98)	53	43.34 (16.54)	52	41.84 (12.91)	0.543	.463	0.006	18.450	**<.001*** **	0.167
AA [nmol*L^{-1}]	49	18.09 (4.18)	44	17.79 (4.53)	52	17.25 (4.63)	51	16.87 (3.67)	2.561	.113	0.029	65.783	**<.001*** **	0.431
3-HK [nmol*L^{-1}]	49	40.85 (13.14)	44	38.46 (11.43)	52	42.39 (13.09)	51	40.43 (10.18)	0.164	.687	0.002	12.920	**<.001*** **	0.129
XA [nmol*L^{-1}]	51	11.83 (5.61)	48	10.98 (5.32)	53	12.10 (4.88)	52	11.45 (4.79)	0.026	.872	<0.001	8.251	**.005****	0.082
3-HAA [nmol*L^{-1}]	49	34.38 (12.40)	44	30.04 (10.28)	52	35.88 (13.47)	51	32.28 (10.17)	0.055	.815	0.001	23.160	**<.001*** **	0.210
QA [nmol*L^{-1}]	51	343.71 (144.53)	48	345.52 (117.80)	53	369.65 (128.86)	52	376.21 (124.44)	0.145	.705	0.002	36.785	**<.001*** **	0.286
Pic [nmol*L^{-1}]	51	36.64 (12.15)	48	32.44 (14.38)	53	33.84 (13.56)	52	31.59 (13.10)	0.093	.761	0.001	20.855	**<.001*** **	0.185
Ratios														
KTR	51	23.25 (6.51)	48	23.96 (6.34)	53	24.52 (6.59)	52	25.83 (6.82)	0.141	.708	0.002	2.769	.100	0.029
KA/Kyn ratio	51	28.23 (7.51)	48	26.47 (6.99)	53	27.46 (8.48)	52	27.31 (7.48)	0.099	.754	0.001	1.324	.253	0.014
QA/Kyn ratio	51	223.25 (53.90)	48	232.10 (46.65)	53	233.35 (56.17)	52	238.82 (43.66)	0.073	.787	0.001	1.239	.268	0.013
QA/KA ratio	51	8.48 (3.18)	48	9.31 (2.98)	53	9.27 (3.83)	52	9.54 (3.64)	0.599	.441	0.006	17.929	**<.001*** **	0.163
3-HAA/AA ratio	49	1.96 (0.78)	44	1.73 (0.62)	52	2.18 (0.85)	51	2.00 (0.76)	0.995	.321	0.011	5.683	**.019***	0.062
Inflammation markers														
Neopt [nmol*L^{-1}]	49	21.31 (8.50)	44	21.00 (6.78)	52	22.46 (8.78)	51	22.40 (9.04)	0.063	.803	0.001	6.153	**.015***	0.066
NLR	51	3.62 (2.69)	47	3.30 (2.36)	53	2.41 (1.62)	53	2.35 (1.52)	0.003	.955	<0.001	3.328	.071	0.036
WBC [10^3*μL^{-1}]	51	5.59 (1.55)	47	5.33 (1.80)	53	5.82 (1.89)	53	5.56 (1.77)	—	—	—	—	—	—
ANC [10^3*μL^{-1}]	51	3.48 (1.06)	47	3.16 (1.14)	53	3.28 (1.10)	53	3.15 (1.03)	—	—	—	—	—	—
ALC [10^3*μL^{-1}]	51	1.33 (0.71)	47	1.27 (0.69)	53	1.73 (0.89)	53	1.68 (0.88)	—	—	—	—	—	—
Physical capacity														
Rel. $\dot{V}O_{2\,peak}$ [mL*min^{-1}*kg^{-1}]	53	19.10 (5.33)	50	21.59 (5.81)	53	18.39 (6.25)	53	19.95 (6.36)	1.548	.216	0.016	21.899	**<.001*** **	0.187
Rel. PPO [W*kg^{-1}]	53	1.36 (0.53)	50	1.57 (0.59)	53	1.26 (0.58)	53	1.43 (0.63)	1.337	.250	0.014	5.222	**.025***	0.052

Abbreviations: AA, anthranilic acid; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; HIIT, high-intensity interval training; KA, kynurenic acid; KA/Kyn ratio, kynurenic acid-to-kynurenine ratio [nmol*L^{-1} divided by μmol*L^{-1}]; KTR, kynurenine-to-tryptophan ratio [μmol*L^{-1} divided by mmol*L^{-1}]; Kyn, kynurenine; MICT, moderate-intensity continuous training; Neopt, neopterin; NLR, neutrophil-to-lymphocyte ratio; Pic, picolinic acid; QA, quinolinic acid; QA/KA ratio, quinolinic acid-to-kynurenic acid ratio [nmol*L^{-1} divided by nmol*L^{-1}]; QA/Kyn ratio, quinolinic acid-to-kynurenine ratio [nmol*L^{-1} divided by μmol*L^{-1}]; rel. PPO, peak power output during cardiopulmonary exercise testing divided by kilogram body weight; rel. $\dot{V}O_{2\,peak}$, peak oxygen consumption during cardiopulmonary exercise testing divided by kilogram body weight; Trp, tryptophan; T_0, baseline; T_1, post 3 weeks of HIIT/MICT; WBC, absolute white blood cell count; XA, xanthurenic acid; 3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; 3-HAA/AA ratio, 3-hydroxyanthranilic acid-to-anthranilic acid ratio [nmol*L^{-1} divided by nmol*L^{-1}].

n indicates the number of available samples per variable at pre (T_0) and post (T_1), respectively. Mean (SD) is provided for participants with available T_0 and T_1 data. Asterisks and bold-type indicate significant time effects at a 0.05 level (*), 0.01 level (**), or 0.001 level (***), as determined by ANCOVA performed on log10-transformed values.

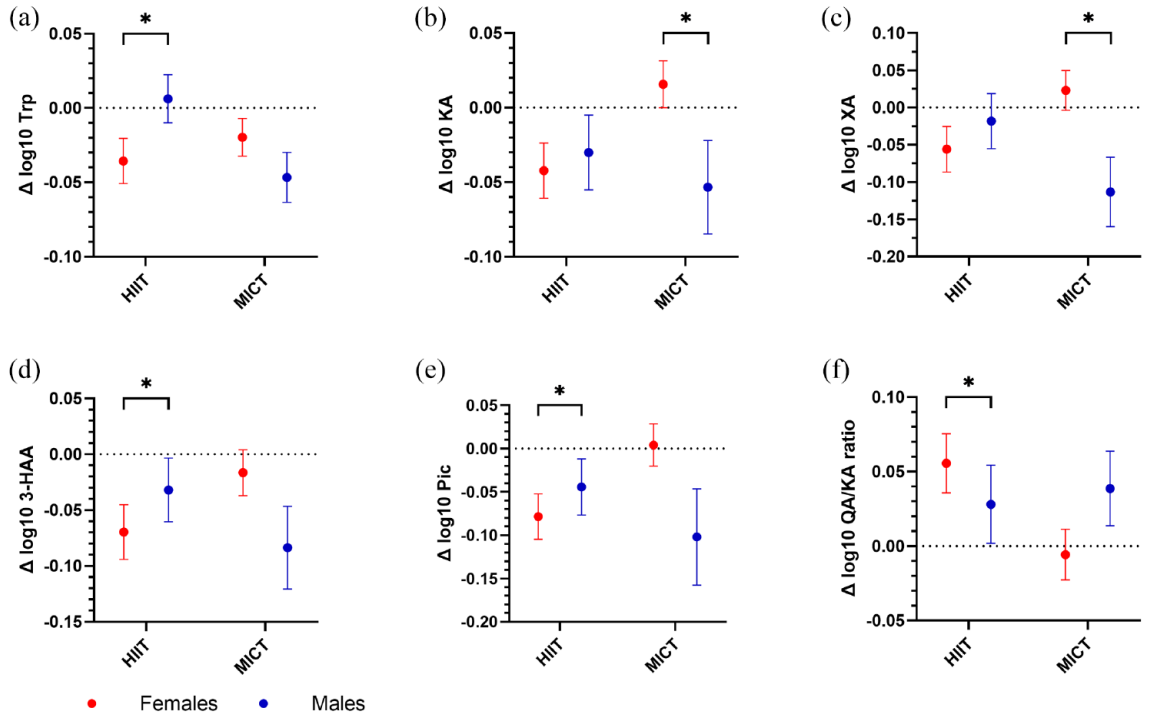

Figure 3. Significant sex differences in kynurenine pathway modulation according to time (baseline, 3 weeks) and modality (HIIT, MICT). Abbreviations: HIIT, high-intensity interval training; KA, kynurenic acid (b); MICT, moderate-intensity continuous training; Pic, picolinic acid (e); QA/KA ratio, quinolinic acid-to-kynurenic acid ratio [nmol*L^{-1} divided by nmol*L^{-1}] (f); Trp, tryptophan (a); XA, xanthurenic acid (c); 3-HAA, 3-hydroxyanthranilic acid (d). Interaction plots illustrate significant sex-specific changes in Trp, KA, XA, 3-HAA, and Pic concentration and QA/KA ratio from pre (T_0; baseline) to post (T_1; after 3 weeks) per exercise modality (HIIT or MICT). Log10-transformed delta (Δ) values of winsorized data are presented as Mean (SEM). Time*group*sex interactions were computed using ANCOVA on log10-transformed values (main analysis, Supplemental Table 1) and specified using Bonferroni-corrected post hoc tests. Asterisks indicate significant differences in changes of metabolites/ratios in female and male persons with multiple sclerosis per exercise modality (HIIT, MICT) at a 0.05 level (*), as determined by ANCOVA. Interaction plots for all variables are provided in Supplemental Figure 3.

2b), KTR (Figure 2j), KA/Kyn ratio (Figure 2k), QA/Kyn ratio (Figure 2l), or NLR (Figure 2p) (Table 2). Individual changes in the KP metabolite profile and inflammation markers in HIIT and MICT participants according to time are presented in Supplemental Figure 2c–r.

Significant time*group*sex interactions were observed for Trp ($p = .037$), KA ($p = .018$), XA ($p = .006$), 3-HAA ($p = .021$), and Pic ($p = .027$) concentrations and QA/KA ratio ($p = .032$) (Supplemental Table 1). Bonferroni-corrected post hoc tests revealed that reductions in Trp ($p_{adj} = .011$, Figure 3a), 3-HAA ($p_{adj} = .035$, Figure 3d), and Pic ($p_{adj} = .016$, Figure 3e) concentrations and the increase in QA/KA ratio ($p_{adj} = .046$, Figure 3f) were greater in female compared to male HIIT participants. Reductions in KA ($p_{adj} = .040$, Figure 3b) and XA concentrations ($p_{adj} = .027$, Figure 3c) were greater in male compared to female MICT participants (Supplemental Table 2). Interaction plots for non-significant results can be found in Supplemental Figure 3.

Participants with low and high neutrophil–to – lymphocyte ratio respond differently to endurance exercise

Significant time*group interactions were observed between low NLR and high NLR participants for Kyn concentration ($p = .033$, Figure 4b), KTR ($p = .025$, Figure 4j), and NLR ($p = .001$, Figure 4p) (Table 3). Kyn concentration ($p_{adj} = .016$), KTR ($p_{adj} = .018$), and NLR ($p_{adj} < .001$) significantly decreased in participants with high NLR but not in those with low NLR (Supplemental Table 3). Individual changes in the kynurenine metabolite profile and inflammation markers in low NLR and high NLR participants according to time are presented in Supplemental Figure 4.

Kynurenines are not or weakly associated with physical capacity and clinical outcomes

At baseline, QA concentration was inversely correlated with $\dot{V}O_{2\ peak}$ ($r_s = -.236$, $p = .016$) and PPO ($r_s = -.239$, $p = .015$). A positive correlation was observed between Pic concentration and $\dot{V}O_{2\ peak}$ ($r_s = .225$, $p = .022$). 3-HAA concentration correlated positively with $FSMC_{cog}$ subscale core ($r_s = .236$, $p = .017$). NLR correlated inversely with $FSMC_{mot}$ subscale score ($r_s = -.228$, $p = .020$). All significant correlations were weak. No significant correlations of Trp, kynurenines, ratios of kynurenines, or inflammation markers were observed for $FSMC_{tot}$, PCS, or MCS (Supplemental Table 4).

Change in AA concentration over time was positively correlated with change in $\dot{V}O_{2\ peak}$ ($r_s = .217$, $p = .034$) whereas change in Pic concentration was inversely correlated with change in PPO ($r_s = -.222$, $p = .026$). Change in QA concentration was positively correlated with change in both

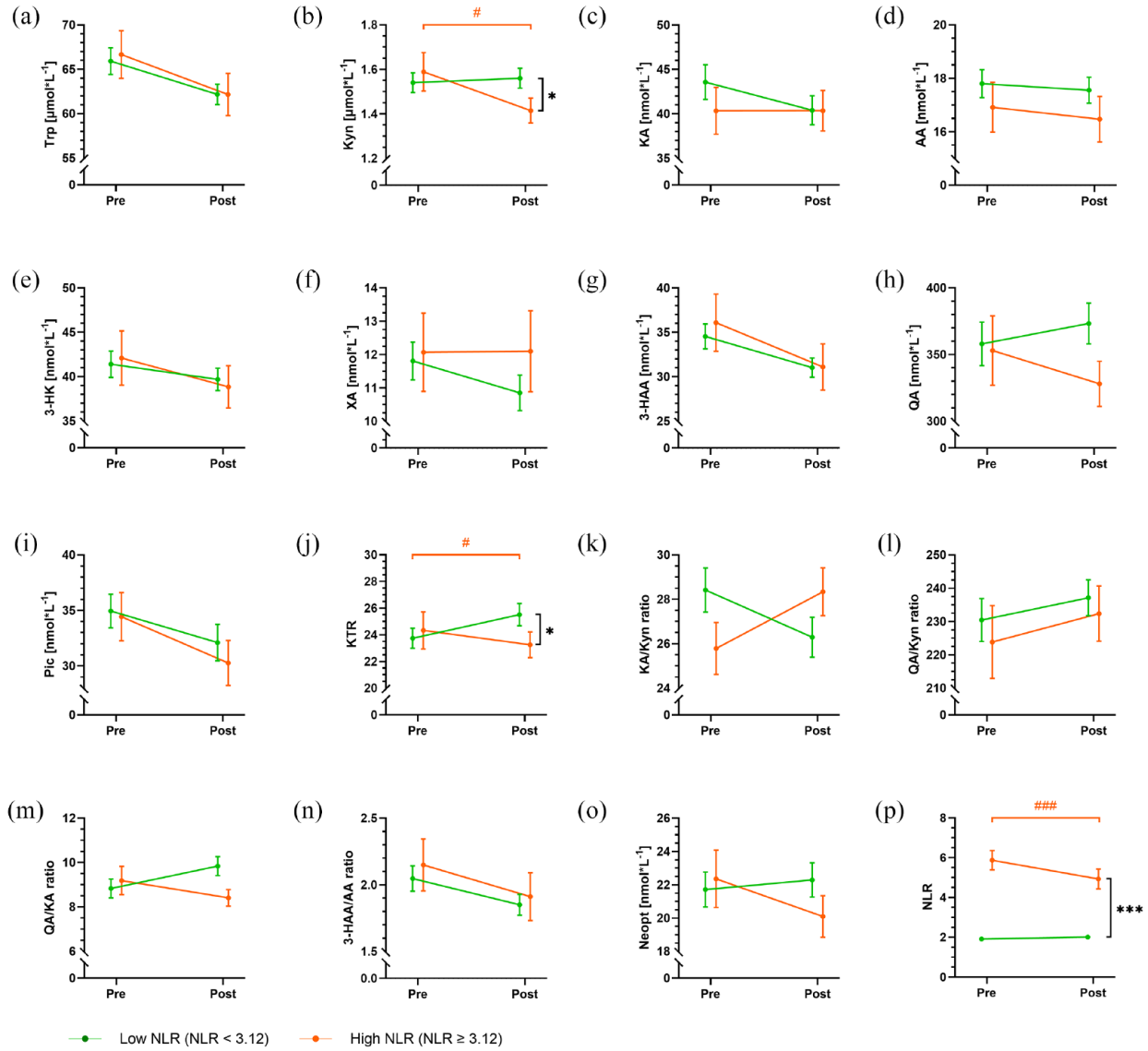

Figure 4. Exercise-induced changes in the kynurenine pathway metabolite profile and inflammation markers according to time (baseline, 3 weeks) and baseline systemic inflammation (low NLR, high NLR).
Abbreviations: AA, anthranilic acid (d); KA, kynurenic acid (c); KA/Kyn ratio, kynurenic acid-to-kynurenine ratio [nmol*L^{-1} divided by µmol*L^{-1}] (k); KTR, kynurenine-to-tryptophan ratio [µmol*L^{-1} divided by mmol*L^{-1}] (j); Kyn, kynurenine (b); Neopt, neopterin (o); NLR, neutrophil-to-lymphocyte ratio (p); Pic, picolinic acid (i); QA, quinolinic acid (h); QA/KA ratio, quinolinic acid-to-kynurenic acid ratio [nmol*L^{-1} divided by nmol*L^{-1}] (m); QA/Kyn ratio, quinolinic acid-to-kynurenine ratio [nmol*L^{-1} divided by µmol*L^{-1}] (l); Trp, tryptophan (a); XA, xanthurenic acid (f); 3-HAA, 3-hydroxyanthranilic acid (g); 3-HK, 3-hydroxykynurenine (e); 3-HAA/AA ratio, 3-hydroxyanthranilic acid-to-anthranilic acid ratio [nmol*L^{-1} divided by nmol*L^{-1}] (n).
Line charts illustrate exercise-induced changes in kynurenines, ratios of kynurenines, and inflammation markers from pre (T_0; baseline) to post (T_1; after 3 weeks of HIIT/MICT (combined)) per NLR subgroup (low NLR: NLR < 3.12; high NLR: NLR ≥ 3.12). Non-transformed winsorized data are presented as Mean (SEM). Asterisks indicate significant time*group interactions at a 0.05 level (*), 0.01 level (**), or 0.001 level (***). Hash signs indicate significant main effects of time at a 0.05 level (#) or 0.001 level (###), as determined by ANCOVA performed on log10-transformed values (exploratory analysis, Supplemental Table 3).

$FSMC_{tot}$ ($r_s = .203$, $p = .043$) and $FSMC_{mot}$ ($r_s = .240$, $p = .016$) (Supplemental Table 5).

Discussion

Principal findings

This secondary analysis of a parallel-group randomized controlled trial aimed to compare the effects of repeated HIIT and MICT bouts, both delivered as part of combined treatments during 3-weeks of inpatient rehabilitation, on the serum KP metabolite profile in pwMS. Contrary to our primary hypothesis, the effects of repeated HIIT and MICT bouts on the serum KP metabolite profile did not differ significantly. However, participants in both treatment groups revealed an exercise-induced modulation of the serum KP metabolite profile over time, as indicated by significant reductions in serum concentrations of most kynurenines (ie, Trp, KA, AA, 3-HK, XA, 3-HAA, Pic) and 3-HAA/AA ratio and significant increases in QA concentration and QA/KA ratio. We also observed a significant decrease in Neopt concentration over time. Within the HIIT and MICT groups, female and male persons with MS revealed some significant differences in exercise-induced KP modulation. For example, reductions in Trp, 3-HAA, and Pic and the

Table 3. Exercise-induced changes in the kynurenine pathway metabolite profile and inflammation markers according to time (baseline, 3 weeks) and baseline inflammation (low NLR, high NLR).

	LOW NLR				HIGH NLR				ANCOVA		
	n = 76				n = 28						
	PRE (T_0)		POST (T_1)		PRE (T_0)		POST (T_1)		TIME * GROUP		
	n	*M* (SD)	n	*M* (SD)	n	*M* (SD)	n	*M* (SD)	*F*	*p*	$p\eta^2$
Kynurenines											
Trp [μmol*L^{-1}]	75	65.93 (12.84)	70	62.18 (9.87)	28	66.65 (13.67)	26	62.16 (12.11)	0.034	.854	<0.001
Kyn [μmol*L^{-1}]	75	1.54 (0.38)	70	1.56 (0.38)	28	1.59 (0.44)	26	1.41 (0.29)	4.698	**.033***	0.049
KA [nmol*L^{-1}]	75	43.56 (16.59)	70	40.40 (13.99)	28	40.34 (13.43)	26	40.35 (11.63)	0.916	.341	0.010
AA [nmol*L^{-1}]	72	17.80 (4.36)	70	17.55 (4.08)	28	16.91 (4.56)	24	16.47 (4.18)	0.858	.357	0.010
3-HK [nmol*L^{-1}]	72	41.41 (12.50)	70	39.70 (10.60)	28	42.09 (14.99)	24	38.85 (11.64)	0.449	.505	0.005
XA [nmol*L^{-1}]	75	11.81 (4.87)	70	10.85 (4.56)	28	12.07 (5.99)	26	12.10 (6.19)	0.573	.451	0.006
3-HAA [nmol*L^{-1}]	72	34.54 (11.69)	70	31.02 (9.14)	28	36.08 (15.76)	24	31.10 (12.74)	0.271	.604	0.003
QA [nmol*L^{-1}]	75	357.96 (139.52)	70	373.33 (131.27)	28	353.00 (132.74)	26	328.08 (86.16)	2.697	.104	0.029
Pic [nmol*L^{-1}]	75	34.95 (12.95)	70	32.10 (14.11)	28	34.43 (11.10)	26	30.27 (10.35)	0.219	.641	0.002
Ratios											
KTR	75	23.74 (6.43)	70	25.51 (7.12)	28	24.33 (7.09)	26	23.25 (4.89)	5.214	**.025***	0.054
KA/Kyn ratio	75	28.42 (8.50)	70	26.29 (7.70)	28	25.79 (5.96)	26	28.34 (5.50)	0.368	.545	0.004
QA/Kyn ratio	75	230.46 (55.37)	70	237.13 (46.39)	28	223.82 (55.82)	26	232.36 (42.17)	0.352	.554	0.004
QA/KA ratio	75	8.83 (3.65)	70	9.84 (3.64)	28	9.19 (3.26)	26	8.41 (1.90)	3.885	.052	0.041
3-HAA/AA ratio	72	2.05 (0.80)	70	1.85 (0.66)	28	2.15 (0.92)	24	1.91 (0.84)	0.394	.532	0.005
Inflammation markers											
Neopt [nmol*L^{-1}]	72	21.71 (8.78)	70	22.28 (8.64)	28	22.35 (8.45)	24	20.10 (6.15)	2.291	.134	0.026
NLR	76	1.91 (0.65)	70	2.01 (0.86)	28	5.87 (2.53)	27	4.93 (2.61)	11.336	**.001*****	0.111
WBC [10^3*μL^{-1}]	76	5.84 (1.80)	70	5.65 (1.79)	28	5.37 (1.52)	27	4.92 (1.66)	—	—	—
ANC [10^3*μL^{-1}]	76	3.22 (1.05)	70	3.07 (1.01)	28	3.80 (1.06)	27	3.37 (1.24)	—	—	—
ALC [10^3*μL^{-1}]	76	1.84 (0.76)	70	1.74 (0.79)	28	0.74 (0.35)	27	0.81 (0.39)	—	—	—

(Continued)

Table 3. (Continued)

	LOW NLR			HIGH NLR			ANCOVA	
	n = 76			n = 28				
	PRE (T_0)	POST (T_1)		PRE (T_0)	POST (T_1)		TIME * GROUP	
Physical capacity								
Rel. $\dot{V}O_{2\,peak}$ [mL*min-1*kg^{-1}]	76	18.91 (5.98)	74	28	18.48 (5.49)	27	21.27 (5.70)	2.088	.152	0.022
Rel. PPO [W*kg^{-1}]	76	1.31 (0.58)	74	28	1.34 (0.53)	27	1.55 (0.58)	0.016	.900	<0.001

Abbreviations: AA, anthranilic acid; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; KA, kynurenic acid; KA/Kyn ratio, kynurenic acid-to-kynurenine ratio [nmol*L^{-1} divided by µmol*L^{-1}]; KTR, kynurenine-to-tryptophan ratio [µmol*L^{-1} divided by mmol*L^{-1}]; Kyn, kynurenine; Neopt, neopterin; NLR, neutrophil-to-lymphocyte ratio (low NLR: NLR < 3.12; high NLR: NLR ⩾ 3.12); Pic, picolinic acid; QA, quinolinic acid; QA/KA ratio, quinolinic acid-to-kynurenic acid ratio [nmol*L^{-1} divided by nmol*L^{-1}]; QA/Kyn ratio, quinolinic acid-to-kynurenine ratio [nmol*L^{-1} divided by µmol*L^{-1}]; rel. PPO, peak power output during cardiopulmonary exercise testing divided by kilogram body weight; rel. $\dot{V}O_{2\,peak}$, peak oxygen consumption during cardiopulmonary exercise testing divided by kilogram body weight; Trp, tryptophan; T_0, baseline; T_1, post 3 weeks of HIIT/MICT (combined); WBC, absolute white blood cell count; XA, xanthurenic acid; 3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; 3-HAA/AA ratio, 3-hydroxyanthranilic acid-to-anthranilic acid ratio [nmol*L^{-1} divided by nmol*L^{-1}]. *n* indicates the number of available samples per variable at pre (T_0) and post (T_1), respectively. Mean (SD) is provided for participants with available T_0 and T_1 data. Asterisks and bold-type indicate significant time * group interactions at a 0.05 level (*) or 0.001 level (***), as determined by ANCOVA performed on log10-transformed values. For metabolites/ratios with significant time * group interaction effects, Bonferroni-corrected post hoc tests were conducted to specify effects within NLR subgroups according to time (baseline, 3 weeks) (Supplemental Table 3).

increase in QA/KA ratio were greater in female compared to male HIIT participants. Additionally, we also aimed to evaluate differences in exercise-induced KP modulation as a function of systemic inflammation at baseline, dichotomizing participants according to NLR (cut-off value of 3.12). Exercise-induced changes in the KP metabolite profile of participants with high and low NLR differed significantly. Kyn concentration and KTR decreased only in participants with high NLR at baseline. These findings support our secondary hypothesis of differential serum KP metabolite profile modulation dependent on baseline systemic inflammation. In the high NLR subgroup of participants, we also observed a significant reduction in NLR over time. Concentrations of kynurenines and ratios of kynurenines were not or only weakly correlated with physical capacity (ie, $\dot{V}O_{2\,peak}$, PPO) and clinical outcomes (ie, HRQoL, fatigue) at baseline and following the treatments. These findings contradict our third hypothesis.

Endurance exercise-induced changes in the kynurenine pathway metabolite profile

Under the premise of differences in analytical methods and investigation of a limited number of metabolites, the primary results of this analysis are partially consistent with 2 previous studies of similar design.[19,20] In one of these studies, differential effects of HIIT and MICT on the serum KP metabolite profile were observed following single bouts of endurance exercise, pointing toward superior *acute* effects of HIIT over MICT in increasing the concentration of the neuroprotective and anti-inflammatory kynurenine KA.[19] Consistent with the absence of differential *chronic* effects on the KP in the present study, repeated HIIT and MICT bouts did not differentially modulate the concentrations of kynurenines in either study.[19,20] However, when considering KTR, a marker of cellular immune response,[42] the exercise-induced increase was shown to be greater following 3 weeks of HIIT compared to MICT.[19]

An increase in KTR results from a decrease in Trp concentration and/or an increase in Kyn formation. As observed previously,[20] we showed a significant decrease in Trp concentration over time but, in contrast, no concomitant increase in Kyn concentration. Instead, we observed a non-significant decrease in Kyn concentration and a significant reduction in the concentrations of KA and most other kynurenines, including AA, 3-HK, XA, 3-HAA, and Pic, which were not analyzed in earlier studies. The concentration of QA increased over time. Both the decrease in KA concentration and the increase in QA concentration caused a significant shift in QA/KA ratio. This finding may seem contradictory, given the associations of chronically increased QA/KA ratio as a result of KP dysregulation and its association with MS-related disability.[13] However, an exacerbation of KP dysregulation is considered unlikely, considering that the concentrations of most kynurenines decreased over time. Rather, an increase in QA concentration may indicate an increase

in KP flux in favor of NAD^+ production, given the primary role of the hepatic KP in the *de novo* synthesis of NAD^+, which is an indispensable co-substrate in cellular energy metabolism. QA is formed sequentially from 3-HK and 3-HAA and is degraded to NAD^+ via the rate-limiting enzyme quinolinic acid phosphoribosyltransferase (QPRT). In contrast, all other kynurenines are formed alternatively from Kyn (ie, KA, AA), 3-HK (ie, XA), and the QA precursor metabolite 2-amino-3-carboxymuconic acid-6-semialdehyde (ie, Pic) and do not or only to a limited extent contribute to NAD^+ synthesis.[6] Reductions in KA, AA, XA, and Pic concentrations in parallel to an increase in QA concentration may therefore represent an endogenous mechanism to meet the increased energy requirements due to the numerous physical stimuli participants were exposed to during the multimodal rehabilitation stay. To support the above hypotheses, the additional investigation of key KP enzymes, such as QPRT, and NAD^+ metabolite concentrations on a systemic and cellular level would have provided additional insight. Moreover, the identification of sex-specific responses to HIIT and MICT, including the greater increase in QA/KA ratio in female compared to male HIIT participants, underscores the necessity to design investigations that primarily aim to explore sex-specific differences in KP modulation in cohorts with an equal distribution of female and male participants.

The impact of baseline inflammatory status on exercise-induced kynurenine pathway modulation

Considering the well-described anti-inflammatory effects of regular endurance exercise,[4] the reductions in concentrations of several kynurenines could be considered as a preliminary indication that exercise may induce KP downregulation due to a reduced inflammatory KP activation. Consistent with this hypothesis, we show a significant reduction in 3-HAA/AA ratio, considered as a counterregulatory mechanism against inflammatory stimulation,[29] and Neopt concentration over time. Neopt serves as a measure of inflammatory stimulation by Th1-type lymphocytes[30] and was correlated with concentrations of most kynurenines and ratios of kynurenines at baseline. Contradicting this hypothesis, no significant reduction in NLR was observed when studying the cohort as a whole.

However, when pooling exercise groups and dichotomizing participants according to baseline NLR, we unveil a highly significant exercise-induced reduction in NLR among participants with high NLR at baseline. In participants with low baseline NLR, NLR remained constant. We also observed differences in exercise-induced KP modulation according to low and high NLR baseline status, despite NLR not being related to the KP metabolic profile at baseline. Thus, while changes in NLR and the KP metabolite profile appear to be independent of each other, endurance exercise likely induces metabolic and immunological effects that affect both the NLR and the KP metabolite profile.

Strengths and limitations

Strengths of this study include the comprehensive KP metabolite profiling in a well-characterized cohort of pwMS using reliable state-of-the-art methodology. Statistical analyses were controlled for BMI, age, and EDSS score, factors known to influence the KP metabolite profile.[13,43,44] Nevertheless, additional control for other potential confounding variables, such as diet and renal function, would have increased the validity of the results. HIIT and MICT were part of combined treatments, and, for ethical reasons, were delivered in addition to relevant concomitant care. We cannot rule out any additional physiological stimuli induced by treatments other than HIIT and MICT that caused *acute effects* on the serum KP metabolite profile within the 24-hour time window between the last exercise session and T_1 blood sampling. The changes in the KP metabolite profile over time in both the HIIT and MICT group are likely a result of combined effects, induced by the respective endurance training modality and several relevant concomitant care interventions, such as strength training, that were equally prescribed for both groups. The design of combined treatments (ie, HIIT combined with IEME versus MICT combined with PMR) was reasonable considering the primary aim of the source study, but may have introduced bias in between-group comparisons when investigating exercise-induced KP modulation. In this regard, also the absence of intensity * session duration matching between HIIT and MICT (ie, HR_{peak} * minutes) and the short intervention period of 3 weeks may have obscured any potential superiority of HIIT over MICT. Both the inclusion of a control/usual care group receiving only concomitant care and a comparison of two single interventions with matched workloads would have been preferable. Lastly, controlling for individual disease-modifying therapies would have been optimal. Differences between the NLR subgroups in response to HIIT and MICT may not only have depended on systemic inflammation per se but may also have involved interactions with disease-modifying therapy. For example, all fingolimod-treated participants (32.1%) were part of the high NLR group. Fingolimod induces pronounced monocytosis and lymphopenia, thereby increasing NLR.[38,45] Concurrently, all natalizumab-treated participants (13.2%) were part of the low NLR group. Natalizumab increases systemic counts of lymphocyte subpopulations which causes a reduction in NLR.[38,46] Although preferred, controlling for individual disease-modifying therapies was not feasible due to large heterogeneity among participants. While the present study is not without its limitations, the authors endeavored to have an ecologically valid approach to the research question, with a study located in a *real-world inpatient rehabilitation setting* incorporating interdisciplinary multimodal care that reflects best practice in MS management.[47] The results of this study are generalizable to pwMS with similar characteristics who are admitted to MS inpatient rehabilitation.

Conclusions

Results of this secondary analysis show that treatment combinations including repeated bouts of HIIT or MICT, embedded into a multimodal 3-week rehabilitation, modulate the serum KP metabolite profile in pwMS. Baseline systemic inflammation, as indicated by NLR, may have a significant impact on KP modulation and anti-inflammatory effects of exercise.

Findings from this analysis highlight the potential complexity of the KP, exercise-induced KP modulation, and interactions with pathophysiology and treatment response in inflammation-related diseases and conditions. Future studies investigating mechanistic effects of exercise in pwMS should complement KP metabolite profiling with comprehensive immunophenotyping and measures of neuroinflammation and neuroaxonal damage, taking into account the potential impact of baseline systemic inflammation and disease-modifying therapy.

Acknowledgements

We thank Ramona Sylvester for her support in supervising HIIT and MICT sessions. We thank Daniel Caminada and Francesca Ferrara for performing hematology analysis at Dr Risch laboratory, Buchs, Switzerland. We thank all participants involved in this study.

Supplemental Material

Supplemental material for this article is available online.

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