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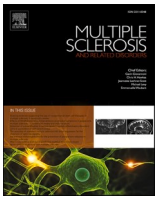

The serum kynurenine pathway metabolic profile is associated with overweight and obesity in multiple sclerosis

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ABSTRACT

Background: Overweight and obesity increase multiple sclerosis (MS) susceptibility, disease severity, and disability progression. Kynurenine pathway (KP) dysregulation is present in overweight and obesity, and in MS. Since the effect of overweight and obesity on KP dysregulation in persons with MS (pwMS) remains to be established, this study primarily aims to explore the effect of overweight and obesity on the serum KP metabolic profile in pwMS.

Methods: This cross-sectional study represents a secondary analysis of a randomized clinical trial at Valens rehabilitation clinic, Switzerland. Registration was performed on 22 April 2020 at clinicaltrials.gov (NCT04356248, https://clinicaltrials.gov/ct2/show/NCT04356248). The first participant was enrolled on 13 July 2020. Based on body mass index (BMI), 106 MS inpatients (Expanded Disability Status Scale (EDSS) score $\leq$ 6.5) were dichotomised to a lean group (LG, BMI $<$ 25 kg/m^2), and an overweight/obese group (OG, BMI $\geq$ 25 kg/m^2). Targeted metabolomics (LC-MS/MS) was performed to determine serum concentrations of tryptophan (TRP), KP downstream metabolites, and neopterin (Neopt). Correlations between BMI, kynurenine-to-TRP ratio (KTR), and serum concentrations of TRP, KP downstream metabolites, and Neopt were calculated. ANCOVA was used to determine differences in KTR, and serum concentrations of TRP, KP downstream metabolites and Neopt between OG and LG, and across MS phenotypes.

Results: Higher BMI correlated with higher KTR (r = 0.425, p <0.001) and serum concentrations of most KP downstream metabolites, but not with EDSS score. Higher KTR (r = 0.470, p < .001) and serum concentrations of most KP downstream metabolites correlated with a higher serum concentration of Neopt. The OG (n = 44, 59% female, 51.68 (9.98) years, EDSS: 4.71 (1.37)) revealed higher KTR (0.026 (0.007) vs. 0.022 (0.006), p=.001) and serum concentrations of most KP downstream metabolites than the LG (n = 62, 71% female, 48.37 (9.63) years, EDSS: 4.60 (1.29)). KP metabolic profiles did not differ between MS phenotypes.

Conclusion: Overweight and obesity are associated with a systemic elevation of KP metabolic flux and an accumulation of most KP downstream metabolites in pwMS. Further research is needed to clarify if KP involvement serves as a mechanism linking overweight and obesity with symptom expression, disease severity, and disability progression in pwMS.

1. Introduction

Multiple sclerosis (MS) is an autoimmune mediated chronic neuroinflammatory and neurodegenerative disease of the central nervous system (CNS) that represents the most common cause of neurological disability in early adulthood (Ward and Goldman, 2022). MS is marked by a globally rising incidence over the past decades, contributing to a prevalence of 2.8 million persons with MS (pwMS) worldwide (Koch-Henriksen and Magyari, 2021; Multiple Sclerosis International Federation, 2020). As this rise in incidence rate took place within a relatively short period of time, changes in genetic risk factors are considered unlikely. Instead, increased exposure to environmental and life-style

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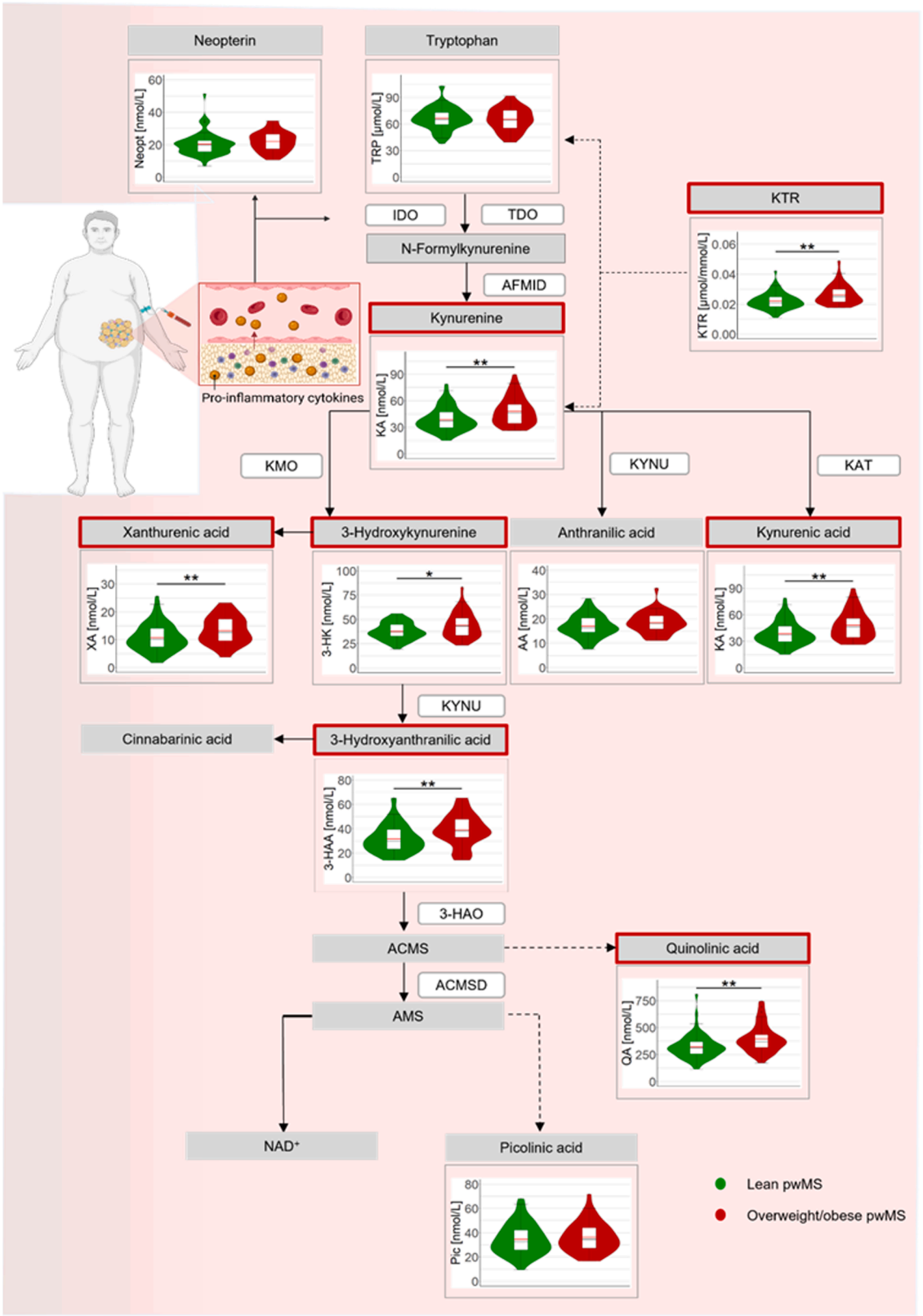

Fig. 1. Overweight/obese persons with multiple sclerosis reveal higher KTR and serum concentrations of most KP downstream metabolites. Abbreviations: KTR= kynurenine-to-tryptophan ratio (µmol/L by mmol/L); IDO= indolamine-2,3 dioxygenase; TDO= tryptophan dioxygenase; KMO= kynurenine 3-monooxygenase; KAT= kynurenine aminotransferases; KYNU= kynureninase; 3-HAO= 3-hydroxyanthranilic acid oxygenase; ACMS= 2-amino-3-carboxymuconate-6-semialdehyde; AMS= alpha-aminomuconate semialdehyde; ACSMD= 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase; NAD^+= nicotinamide adenine dinucleotide. Lean persons with multiple sclerosis (pwMS) (green; BMI < 25 kg/m^2); overweight/obese pwMS (red; BMI ≥ 25 kg/m^2). Serum concentrations are given as non-log-transformed values. Age- and EDSS-adjusted ANCOVA was performed on log10 transformed values. *between-group difference significant on a .05 level; **between-group difference significant on a .01 level. The figure was created with BioRender.com.

related risk factors is assumed to explain increased MS incidence (Marrodan et al., 2021). Among environmental and life-style related risk factors, such as Epstein-Barr virus infection, infectious mononucleosis, tobacco smoking, and vitamin D deficiency, overweight and obesity revealed to be key factors dramatically increasing MS susceptibility (Gianfrancesco and Barcellos, 2016). There is mounting evidence pointing towards an up to twofold likelihood of adult-onset MS in persons that have been overweight or obese in childhood, adolescence, and/or early adulthood (Marrodan et al., 2021; Olsson et al., 2017). Beyond increased MS susceptibility, overweight and obesity seem to worsen the course of MS, aggravating disease severity, and disability progression (Lutfullin et al., 2022).

Chronic systemic low-grade inflammation is a hallmark feature of overweight and obesity, is associated with elevated amounts of visceral adipose tissue (VAT), and is thought to be involved in MS pathophysiology. VAT dysfunction related to overweight and obesity is characterized by altered adipocyte function involving pro-inflammatory immune cells (e.g., macrophages), that release large amounts of pro-inflammatory cytokines, such as interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α). Originating from local VAT inflammation, pro-inflammatory cytokines spill over into the blood circulation, leading to the manifestation and maintenance of systemic inflammation, which is associated with CNS inflammation in overweight and obesity (O'Brien et al., 2017).

The kynurenine pathway (KP) is the main degradative pathway of the essential amino acid tryptophan (TRP), that via several KP downstream metabolites, ultimately leads to the formation of nicotinamide adenine dinucleotide (Fig. 1).

TRP degradation along the KP is mediated by three rate-limiting enzymes, that are tryptophan dioxygenase (TDO), indolamine-2,3 dioxygenase-1 (IDO-1), and indolamine-2,3 dioxygenase-2 (IDO-2), all of which catalyze the cleavage of TRP to the first KP downstream metabolite kynurenine (KYN). Under physiological conditions, hepatic KP activity mediated by TDO predominates (Badawy, 2017). However, high levels of pro-inflammatory cytokines encountered in inflammatory conditions, such as obesity and overweight and/or MS, increase extrahepatic TRP degradation by stimulating IDO-1, that is ubiquitously expressed in various tissues (e.g., VAT, endothelial cells, microglia) (Ji et al., 2021). Consequently, inflammation increases KP metabolic flux, as indicated by higher kynurenine-to-tryptophan ratio (KTR), and higher concentrations of KP downstream metabolites (Mellor and Munn, 2003; Murr et al., 2002). Systemic elevation of KP metabolic flux is likely to affect CNS concentrations of KP downstream metabolites, such as KYN and 3-hydroxykynurenine (3-HK) (Fukui et al., 1991). Elevated CNS concentrations of 3-HK, 3-hydroxyanthranilic acid (3-HAA), and quinolinic acid (QA) are associated with neuronal dysfunction and/or death via multiple mechanisms, including N-methyl-D-aspartate (NMDA) receptor excitotoxicity and oxidative stress, and have been associated with MS neurodegeneration and neuroinflammation (Lovelace et al., 2016; Nishizuka and Hayaishi, 1963). In contrast, kynurenic acid (KA) is considered a neuroprotectant, counteracting NMDA receptor excitotoxicity of quinolinic acid (QA) (Swartz et al., 1990). Independent of KP downstream metabolite concentrations in the CNS, systemic elevation of KP metabolic flux and serum concentrations of KP downstream metabolites, such as 3-HK and QA, seem to detrimentally affect disease severity, as indicated by correlations with higher Expanded Disability Status Scale (EDSS) score (Lim et al., 2017). While systemic KP dysregulation has been separately shown in overweight and obesity, and in MS, a potential interactive effect of overweight and obesity on KP metabolic flux and serum concentrations of KP downstream metabolites in pwMS remains to be established (Lim et al., 2017; Theofylaktopoulou et al., 2013).

Therefore, this cross-sectional study primarily aims to explore the effect of overweight and obesity on the serum KP metabolic profile in pwMS. We assume that higher body mass index (BMI) correlates with higher KTR and serum concentrations of KP downstream metabolites. Further, we hypothesize that overweight and obese pwMS reveal higher KTR and serum concentrations of KP downstream metabolites than lean pwMS, as classified by BMI. The secondary aim is to evaluate the correlations between KTR, serum concentration of TRP, and serum concentrations of KP downstream metabolites with the serum concentration of the immune activation marker neopterin (Neopt). Additionally, we aim to assess correlations between KTR, serum concentration of TRP, and serum concentrations of KP downstream metabolites with EDSS score. We expect that higher KTR, lower serum concentration of TRP, and higher serum concentrations of KP downstream metabolites correlate with a higher serum concentration of Neopt, and higher EDSS score. The third aim is to determine differences in the serum KP metabolic profile between MS phenotypes. Distinct differences in serum KP metabolic profiles that characterize relapsing-remitting (RRMS), secondary progressive (SPMS), and primary progressive MS phenotype (PPMS) are expected, as previously reported (Lim et al., 2017).

2. Materials and methods

2.1. Study registrations and patient consents

The study has been approved by the regional Swiss Ethics Committee (Ethikkommission Ostschweiz (EKOS)) on research involving human experimentation (EKOS20/050; project ID: BASEC2020–00797). For this secondary cross-sectional analysis, an additional EKOS approval was not required, as blood serum analyses were performed on already obtained biomaterial. Registration was performed at the *S*wiss National Clinical Trials Portal (SNCTP000003737) and at ClinicalTrials.gov (NCT04356248). All participants provided written informed consent. Study procedures were performed in accordance with the Declaration of Helsinki.

2.2. Data collection

PwMS that entered the Valens rehabilitation clinic, Switzerland, for inpatient rehabilitation between 13 July 2020 and 19 October 2021 have been consecutively screened for eligibility. Main inclusion criteria were adult age (> 18 years), MS diagnosis according to the 2017-revised McDonald criteria, EDSS score ≤ 6.5, and presence of substantial fatigue (Thompson et al., 2018; Kurtzke, 1983). PwMS with severe concomitant cardiovascular, metabolic, neurological, and/or psychiatric diseases, as well as those with severe orthopedic problems prohibiting exercise were excluded.

Demographic data (age in years, sex) and MS-related data (phenotype, EDSS score, time since diagnosis in months, current intake of disease-modifying drugs (DMD) with name of the active ingredient) were obtained from the medical records. Anthropometric data included height (in meters to the second decimal) and bodyweight (kilogram with one decimal). Height was self-reported. Bodyweight without footwear was determined after an overnight-fast by digital scales (Soehnle Style Sense Comfort 100, Soehnle, Nassau, Germany).

Fasting blood samples were obtained by vein puncture of the antecubital vein in rested supine position between 08:00 and 09:00 AM. Blood samples have been centrifuged at 3000 g for 10 min at 4 °C, and the supernatant was aliquoted. Serum samples were stored at −80 °C until analysis. Data collection was performed within three days after clinic admission. Further details on the procedures are provided elsewhere (Patt et al., 2021).

2.3. Targeted metabolomics

Targeted metabolomics was performed by blinded personnel at Bevital AS (Bergen, Norway). Using liquid chromatography-tandem mass spectrometry (LC-MS/MS), serum concentrations of TRP (μmol/L), KYN, (μmol/L), KA (nmol/L), anthranilic acid (AA, nmol/L), 3-HK (nmol/L), xanthurenic acid (XA, nmol/L), 3-HAA (nmol/L), QA

Table 1
Demographic, Anthropometric, and Multiple Sclerosis-related Characteristics of Participants.

	Overall sample $N = 106$	OG $n = 44$	LG $n = 62$	PPMS $n = 15$	RRMS $n = 53$	SPMS $n = 38$
Sex						
Male	36 (34.0%)	18 (40.9%)	18 (29.0)	7 (47.0%)	14 (26.4%)	15 (39.5%)
Female	70 (66.0%)	26 (59.1%)	44 (71.0)	8 (53.0%)	39 (73.6%)	23 (60.5%)
Age (years)	49.75 (9.87)	51.68 (9.98)	48.37 (9.63)	51.80 (7.99)	47.09 (10.79)	52.63 (8.25)
MS phenotype						
PPMS	15 (14.2%)	5 (11.4%)	10 (16.1%)	15 (100.0%)	0 (0.0%)	0 (0.0%)
RRMS	53 (50.0%)	24 (54.5%)	29 (46.8%)	0 (0.0%)	53 (100.0%)	0 (0.0%)
SPMS	38 (35.8%)	15 (34.1%)	23 (37.1%)	0 (0.0%)	0 (0.0%)	38 (100.0%)
EDSS score	4.64 (1.32)	4.71 (1.37)	4.60 (1.29)	4.97 (1.13)	4.29 (1.29)	5.00 (1.32)
Time since diagnosis (months)	159.38 (109.12)	167.39 (115.42)	153.69 (105.00)	76.27 (65.56)	143.74 99.13)	214.00 (110.16)
Current intake of DMD						
No DMD	38 (35.8%)	16 (36.4%)	22 (33.5%)	4 (26.7%)	13 (24.5%)	21 (55.3%)
DMD	68 (64.2%)	28 (63.6%)	40 (64.5%)	11 (73.3%)	40 (75.5%)	17 (44.7%)
Ocrelizumab	28 (26.4%)	9 (20.5%)	19 (30.6%)	9 (60%)	11 (20.8%)	8 (21.1%)
Natalizumab	10 (9.4%	8 (18.2%)	2 (3.2%)	0 (0.0%)	9 (17%)	1 (2.6%)
Rituximab	5 (4.7%)	1 (2.3%)	4 (6.5%)	1 (6.7%)	0 (0.0%)	4 (10.5%)
Fingolimod	10 (9.4%)	3 (6.8%)	7 (11.3%)	0 (0.0%)	9 (17%)	1 (2.6%)
Dimethylfumarat	7 (6.6%)	4 (9.1%)	3 (4.8%)	1 (6.7%)	5 (9.4%)	1 (2.6%)
Interferon-Beta	2 (1.9%)	1 (2.3%)	1 (1.6%)	0 (0.0%)	2 (3.8%)	0 (0.0%)
Alemtuzumab	1 (0.9%)	1 (2.3%)	1 (1.6%)	0 (0.0%)	1 (1.9%)	0 (0.0%)
Teriflunomid	1 (0.9%)	0 (0.0%)	1 (1.6%)	0 (0.0%)	1 (1.9%)	0 (0.0%)
Ofantumumab	2 (1.9%)	1 (2.3%)	1 (1.6%)	0 (0.0%)	2 (3.8%)	0 (0.0%)
Siponimod	2 (1.9%)	0 (0.0%)	2 (3.2%)	0 (0.0%)	0 (0.0%)	2 (5.3%)
Absolute VO_{2peak} ($mL \cdot min^{-1}$)	1349.58 (464.31)	1425.39 (443.75)	1295.77 (474.53)	1311.8 (405.98)	1385.4 (488.39)	1314.50 (458.81)
Relative VO_{2peak} ($mL \cdot min^{-1} \cdot kg^{-1}$)	19.03 (6.04)	16.51 (4.92)	20.82 (6.16)	18.95 (6.51)	19.242 (6.13)	18.76 (5.89)
Current smokers	28 (26.4%)	8 (18.2%)	20 (32.3%)	5 (33.3%)	11 (20.8%)	12 (31.6%)
BMI (kg/m^2)	24.91 (5.46)	30.01 (4.54)	21.28 (2.14)	25.32 (6.07)	25.43 (6.16)	24.01 (4.00)
Underweight ($BMI < 18.5\ kg/m^2$)	8 (7.5%) 17.50 (0.71)	0 (0.0%)	8 (12.9%); 17.50 (0.71)	2 (13.3%)	4 (7.5%)	2 (5.3%)
Normal weight ($BMI\ 18.5 – 24.9\ kg/m^2$)	54 (50.9%) 21.84 (1.65)	0 (0.0%)	54 (87.1%); 21.84 (1.65)	8 (53.3%)	25 (47.2%)	21 (55.3%)
Overweight ($BMI\ 25.0 – 29.9\ kg/m^2$)	26 (24.5%) 26.94 (1.27)	26 (59.1%) 26.94 (1.27	0 (0.0%)	1 (6.7%)	13 (24.5%)	12 (31.6%)
Obese ($BMI \geq 30.0\ kg/m^2$)	18 (17.0%) 34.46 (3.78)	18 (40.9%) 34.46 (3.78)	0 (0.0%)	4 (26.7%)	11 (20.8%)	3 (7.9%)

Abbreviations: MS= multiple sclerosis; RRMS= relapsing-remitting MS; SPMS= secondary progressive MS; PPMS= primary progressive MS; EDSS= Expanded Disability Status Scale; DMD= disease-modifying drug; BMI= body mass index; LG= 'lean' group (i.e., $BMI < 25\ kg/m^2$); OG= 'overweight'/'obese' group (i.e., $BMI \geq 25\ kg/m^2$). VO2peak= maximum oxygen uptake during cardiopulmonary exercise testing. Categorical data given as total number and percentage (%). Continuous data given as *M*(*SD*). For BMI-based grouping, sub-group size (total number and percentage (%)) and actual BMI *M*(*SD*) are provided separately for the lean (LG) and overweight/obese (OG) group, and with respect to BMI categories acc. to the World Health Organization (i.e., underweight, normal weight, overweight, obese).

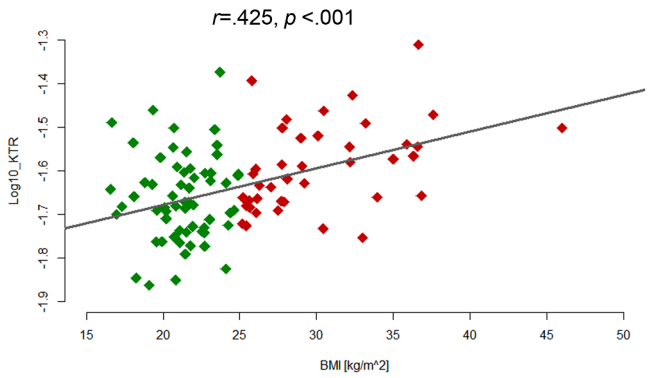

Fig. 2. Correlation between BMI and KTR (on a log scale). Abbreviations: KTR= kynurenine-to-tryptophan ratio (μmol/L by mmol/L); BMI= body mass index; LG (green)= 'lean' group (i.e., BMI < 25 kg/m^2); OG (red)= 'overweight'/'obese' group (i.e., BMI ≥ 25 kg/m^2). Two-tailed bivariate correlation analysis was performed on log10 transformed values on n = 101 using Pearson'r.

(nmol/L), picolinic acid (Pic, nmol/L), and Neopt (nmol/L) were determined as previously described (Midttun et al., 2009). The lower limit of detection (LOD) for the assay ranged from 0.01 to 8 nmol/L, and within- and between-day Coefficients of Variability (CVs) ranged from 3-8% and 4–10%, respectively.

2.4. Statistical analysis

Preliminary analyses. BMI was calculated as the index of the participant's weight divided by the square of his or her height (kg/m^2).

The World Health Organization defines BMI ≥ 25 kg/m^2 as cut-off value, above which abnormal or excessive fat accumulation is presumed (World Health Organization, 2022). Accordingly, the study sample was split into a lean group (LG, BMI < 25 kg/m^2), and an overweight/obese group (OG, BMI ≥ 25 kg/m^2). For a separate analysis, the study sample was split by MS phenotype (i.e., RRMS vs. SPMS vs. PPMS).

KTR was calculated as serum concentration of KYN (μmol/L) divided by serum concentration of TRP (mmol/L). For each missing value, its most likely nature was determined. In case that values have been 'missing at random' and/or that missing values totaled less than 5% of data, imputation was omitted. Subsequently, Z-scoring was performed to identify extreme statistical outliers, that have been defined as values three standard deviations (SD) above or below the arithmetic mean. Extreme outliers were excluded from consecutive statistical analysis.

Distribution of data was evaluated with the Shapiro-Wilk test. To account for statistically significant skewness, log10 transformations were performed on serum concentrations of TRP and all KP downstream metabolites. Positively skewed variables (i.e., TRP, KYN, KA, XA, QA, Neopt) were transformed with the formula log10(x). Negatively skewed variables (i.e., AA, 3-HK, 3-HAA) were transformed with the formula log10 (maximum value (x + 1)-x). According to the central-limit theorem, sample size was sufficient to perform parametric testing. Group differences between participants taking DMD, and participants that do not take DMD were computed using two-tailed independent Student's t-test.

Primary analyses. Demographic, anthropometric, and MS-related characteristics are presented as arithmetic mean and standard deviation *(M(SD))* for continuous variables, and as absolute numbers and frequencies (%) for categorial variables. Descriptive data are provided for the whole sample, as well as for all subgroups (i.e., OG, LG, PPMS, RRMS, SPMS).

For the whole sample, bivariate correlations between BMI and KTR, serum concentration of TRP, serum concentrations of KP downstream metabolites, serum concentration of Neopt, and EDSS score were calculated using Pearson's correlation coefficient. Bivariate correlations are reported by strength (r) and significance (p).

Age- and EDSS-adjusted ANCOVAs were performed to assess the group differences between OG and LG. Age-, EDSS-, and BMI-adjusted ANCOVAs were performed to assess group differences among participants with RRMS, SPMS, and PPMS phenotype.

For all results, $p \leq 0.05$ was considered as statistically significant. All statistical analyses were conducted with IBM SPSS Statistics 29 (Armonk, NY, USA). Scatterplots and violin plots were created with RStudio, Version 2022.07.2 (Build 576, PBC, Boston, MA).

2.5. Data availability

The data that support the findings of this study are publicly available in the repository B2share.eudat.eu at http://doi.org/10.23728/b2share.7c71f47410544bc8aebd368222e7b9b2, reference number [7c71f47410544bc8aebd368222e7b9b2].

Table 2
Correlations between BMI, EDSS, Neopt, KTR, TRP, and KP downstream metabolites.

	EDSS	Neopt	Trp	Kyn	KA	AA	3-HK	XA	3-HAA	QA	Pic	KTR
BMI	.057	.172	-.067	**.398*** **	**.411*** **	.089	**.376*** **	**.308** **	**0.369*** **	**0.432*** **	.001	**.425*** **
EDSS		-.004	-.046	-.015	-.052	.116	.007	-.037	-.187	.043	.001	-.010
Neopt			-.126	**.383*** **	**.266** **	**.279** **	**.398*** **	.117	**.211***	**.510*** **	.141	**.470*** **
TRP				.369***	.061	-.006	.124	.417***	.440***	-.018	.408***	-.421***
KYN					.548***	.445***	.707***	.549***	.618***	.710***	.420***	.688***
KA						.278**	.556***	.645***	.432***	.362***	.381***	.473***
AA							.284**	.170	.080	.276**	.321**	.415**
3-HK								.593***	.582***	.624***	.284**	.580***
XA									.662***	.318**	.580***	.184
3-HAA										.479***	.529***	.220*
QA											.153	.707***
Pic												.088

Abbreviations: BMI= body mass index; EDSS= Expanded Disability Status Scale; Neopt= neopterin; TRP= tryptophan: KYN= kynurenine; KA= kynurenic acid; AA= anthranilic acid; 3-HK= 3-hydroxykynurenine; XA= xanthurenic acid; 3-HAA= 3-hydroxyanthranilic acid; QA= quinolinic acid; Pic= picolinic acid; KTR= KYN-to-TRP ratio (μmol/L by mmol/L). Two-tailed bivariate correlation analysis was performed on log10 transformed values of KTR, TRP, and KP downstream metabolites using Pearson'r. *correlation is significant on a 0.05 level; **correlation is significant on a 0.01 level; *** correlation is significant on a <0.001 level, bold= significant correlations relevant to this study.

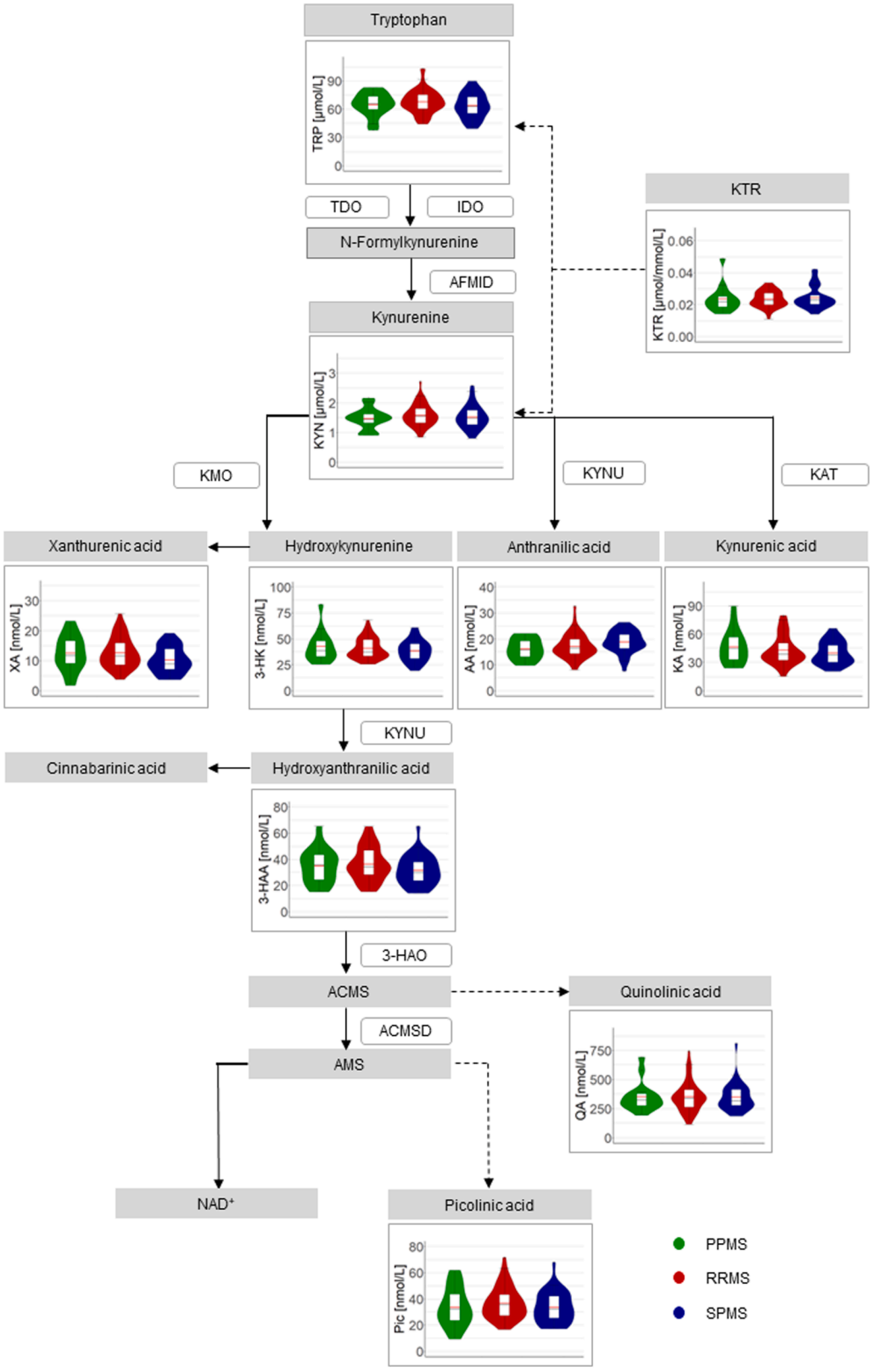

Fig. 3. KTR and serum concentrations of TRP and KP downstream metabolites do not differ among MS phenotypes. Abbreviations: MS= multiple sclerosis; RRMS (red)= relapsing-remitting MS; SPMS (blue)= secondary progressive MS; PPMS (green)= primary progressive MS; KTR= kynurenine-to-tryptophan ratio; IDO= indolamine-2,3 dioxygenase; TDO= tryptophan dioxygenase; KMO= kynurenine 3-monooxygenase; KAT= kynurenine aminotransferases; KYNU= kynureninase; 3-HAO= 3-hydroxyanthranilic acid oxygenase; ACMS= 2-amino-3-carboxymuconate-6-semialdehyde; AMS= alpha-aminomuconate semialdehyde; ACSMD= 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase; NAD^+= nicotinamide adenine dinucleotide.

3. Results

Complete datasets ($N = 106$) were available for the demographic, anthropometric, and MS-related characteristics of all participants. One blood sample was not analyzed due to an insufficient quantity. Serum concentrations of Neopt, AA, 3-HK, and 3-HAA were not determined in three samples due to severe hemolysis. In this case, the nature of missing values was regarded as 'missing at random'. All missing values totaled a maximum of 3.8%. The exclusion of extreme statistical outliers from further statistical analyses resulted in a maximum data loss of further

5.7%. Considering missing values and the exclusion of extreme statistical outliers, a minimum of 90.5% of data was available for each KP metabolite. The sample characteristics – whole sample with all subgroups – are presented in Table 1.

KTR and serum concentrations of TRP, KP downstream metabolites, and Neopt did not differ between participants under DMD treatment and those participants that did not take any DMD during the study participation (Table A.1).

3.1. Associations of overweight and obesity with KTR, and KP metabolites

Higher BMI correlated with higher KTR (Fig. 2) and serum concentrations of most KP downstream metabolites, including KYN, KA, 3-HK, XA, 3-HAA, and QA (Table 2, Table A.2).

Age- and EDSS-adjusted ANCOVA revealed higher KTR and serum concentration of KYN, KA, 3-HK, XA, 3-HAA, and QA in the OG compared to the LG. Serum concentrations of TRP, AA, and Pic did not differ between groups (Fig. 1, Table A.3).

3.2. Correlations between KTR, KP metabolites, and serum Neopt

Higher KTR and serum concentration of KYN, KA, AA, 3-HK, 3-HAA, and QA were correlated with higher serum concentrations of Neopt (Table 2, Table A.2).

3.3. Correlations between KTR, serum KP metabolites, and EDSS score

Neither KTR nor serum concentrations of TRP or KP downstream metabolites revealed correlations with EDSS score (Table 2, Table A.2).

3.4. Differences in KP metabolic profiles among MS phenotypes

BMI-, Age-, and EDSS-adjusted ANCOVA did not reveal any group difference in KTR and serum concentrations of TRP and KP downstream metabolites across MS phenotypes (Fig. 3, Table A.4).

4. Discussion

4.1. Principal findings

This study reveals that higher BMI correlates with higher KTR and serum concentrations of most KP downstream metabolites, indicating a BMI-dependent increase in the formation of KP downstream metabolites in pwMS. In accordance with these correlations, findings of this study indicate higher systemic KP metabolic flux, as indicated by KTR, and a peripheral accumulation of almost all KP downstream metabolites along the whole KP in overweight and obese, compared to lean pwMS. Higher KTR and serum concentrations of most KP downstream metabolites correlated with a higher serum concentration of Neopt, but not with EDSS score. Adjusted ANCOVAs, however, did not reveal any differences in KTR or serum concentrations of KP downstream metabolites in relation to MS phenotype.

4.2. BMI correlates with KTR and most KP downstream metabolites

Correlations of BMI with KTR and systemic concentrations of KP downstream metabolites have been extensively studied in overweight and obese individuals. The findings of this study agree with previous results on positive correlations between BMI and KTR in community-based and clinical samples of normal-weight, overweight, and obese individuals (Cussotto et al., 2020; Favennec et al., 2015). It has been shown that higher BMI correlates with higher systemic concentrations of KYN across the whole spectrum of BMI classes, and with higher concentrations of KYN, KA, and QA in obese individuals (Favennec et al., 2015; Carayol et al., 2017; Huang et al., 2022; Zhao et al., 2016).

4.3. KP metabolic profiles in overweight and obese compared to lean pwMS

Findings of this study show that overweight and obese pwMS have higher KTR and serum concentrations of KYN, KA, 3-HK, XA, 3-HAA, and QA compared to lean pwMS. These findings are in line with two previous studies providing evidence for higher KTR and serum concentrations of KYN in overweight and obese compared to normal-weight, and in obese compared to non-obese individuals (Mangge et al., 2014; Wolowczuk et al., 2012). Moreover, findings of this study agree with the results of the community-based Hordaland Health Study, demonstrating higher levels of nearly all investigated KP downstream metabolites (i.e., KYN, KA, 3-HK, XA, 3-HAA) except AA in overweight and obese compared to lean individuals (Theofylaktopoulou et al., 2013).

Overall, these findings strengthen the hypothesis that chronic systemic low-grade inflammation due to overweight and obesity represents a driving force of a general increase in systemic KP metabolic flux, with a consequent peripheral accumulation of KP downstream metabolites.

4.4. KTR and KP downstream metabolites in relation to immune activation

KTR has been proposed as an index of inflammation-driven KP upregulation due to obesity, arguing that the excessive local release of IFN-γ in VAT fosters IDO-1 activity, both, locally in VAT, as well as in various remote tissues (Oxenkrug et al., 2011). This hypothesis finds support from gene expression analyses, revealing that, compared to non-obese women, obese women presented with higher IDO-1 expression in VAT, as well as in subcutaneous adipose tissue and hepatic tissue, while expression levels of hepatic TDO and IDO-2 did not differ between groups.

Interestingly, this IDO-1 overexpression in each tissue was independently correlated with higher KTR and serum concentrations of KYN (Wolowczuk et al., 2012). Neopt serves as a sensitive marker that reliably reflects IFN-γ-mediated immune activation in autoimmunity, and in obesity (Murr et al., 2002; Oxenkrug et al., 2011; Murr et al., 2001; Schröcksnadel et al., 2006). Thus, serum concentration of Neopt was studied as an indicator of inflammation-driven upregulation of KP activity. Findings of this study reveal that not only higher KTR but also serum concentrations of most KP downstream metabolites correlate with higher serum concentration of Neopt. This observation is in line with results from a previous study that identified positive correlations between KTR and serum concentration of Neopt in a mixed clinical sample, composed of normal-weight and obese individuals. In addition, serum concentration of Neopt was significantly higher in the obese compared to the normal-weight individuals (Brandacher et al., 2006). As in our study, serum Neopt concentration did not differ between normal-weight, overweight, and obese individuals in the community-based Hordaland Health Study (Theofylaktopoulou et al., 2013).

4.5. KTR, KP downstream metabolites, EDSS score, and MS phenotypes

We aimed to assess the clinical relevance of higher KTR and serum concentrations of KP downstream metabolites by investigating their correlation with EDSS score. We found no correlation, which contrasts with results from two previous studies. While lower EDSS correlated with higher levels of 3-HK and XA in a recent investigation, another study revealed that higher EDSS correlated with higher KTR and serum concentrations of 3-HK and QA (Lim et al., 2017; Saraste et al., 2022).

Our third aim was to replicate findings of a prior study, suggesting that differences in serum KP metabolic profiles may discriminate between MS phenotypes, exemplified by higher serum concentrations of QA as indicative of a more progressive MS course (Lim et al., 2017). Our findings do not confirm the results of this study. However, it remains unclear, whether BMI was controlled for. We revealed that BMI is an important covariate, that significantly contributes to the unexplained

variance in ANCOVAs performed to assess between-group differences in KTR and serum concentrations of KYN, KA, 3-HK, XA, 3-HAA, QA, and Neopt across MS phenotypes.

4.6. Limitations

In line with both of the abovementioned studies, this study has a cross-sectional design, based on a single blood collection time point that prohibits any statement on causality (Lim et al., 2017; Saraste et al., 2022). No pro-inflammatory cytokines, as main drivers of KP metabolic flux, were assessed. BMI has been shown to correlate with direct measures of overweight and obesity (Sun et al., 2010).Additional performance of more advanced methods, such as dual energy x-ray absorptiometry and/or bioimpedance analysis, would have permitted to obtain more precise information on body composition, and adipose tissue distribution.

4.7. Clinical relevance for pwMS

Metabolomic profiling using targeted metabolomics uncovered that overweight and obesity are associated with a systemic elevation of KP metabolic flux and an accumulation of KP downstream metabolites in pwMS. Some of these KP downstream metabolites are pro-oxidant and excitotoxic agents with the potential to be neurotoxic at high CNS levels.

The clinical consequences of systemic KP metabolite accumulation on EDSS score have been explored within this and other studies, yielding equivocal results. Higher EDSS indicates higher disease severity, and a shorter time to reach disability milestones in pwMS due to obesity (Lutfullin et al., 2022). This highlights the relevance of lifestyle change, including dietary management and promotion of physical activity, to prevent and combat overweight and obesity in order to counteract their adverse impact on disease severity and disability progression in pwMS.

5. Conclusions

Overweight and obesity are associated with a systemic elevation of KP metabolic flux and an accumulation of most KP downstream metabolites in pwMS. Further research is needed to clarify if KP dysregulation in pwMS qualifies as a causal mechanism linking overweight and obesity with measures of disease severity, and to better understand the role of KP dysregulation in MS symptom expression, and disability progression.

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CRediT authorship contribution statement

Marie Kupjetz: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Nadine Patt:** Writing – review & editing, Visualization, Investigation. **Niklas Joisten:** Writing – review & editing, Formal analysis. **Per Magne Ueland:** Methodology, Writing – review & editing. **Adrian McCann:** Methodology, Writing – review & editing. **Roman Gonzenbach:** Conceptualization, Writing – review & editing. **Jens Bansi:** Conceptualization, Funding acquisition, Investigation, Writing – review & editing, Supervision, Project administration. **Philipp Zimmer:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing, Formal analysis, Project administration.

Declaration of Competing Interest

Per Magne Ueland is a paid employee at Bevital AS; Adrian McCann is a paid employee at Bevital AS. Bevital AS is owned by a not-for-profit foundation established to promote research into functional B-vitamin deficiency.

Marie Kupjetz: none, Nadine Patt: none, Niklas Joisten: none, Roman Gonzenbach: none, Jens Bansi: none, Philipp Zimmer: none.

Appendices

Tables A.1, A.2, A.3 and A.4

Table A.1
Detailed results of the two-tailed independent *t*-test comparing treatment status with disease-modifying drugs.

	N			Mean (SD)		Independent samples *t*-test				
	Total	None	DMD	No DMD	DMD	*t*	*df*	*p*	95% Confidence Interval	
									Lower	Upper
TRP (μmol/L)	103	38	65	67.899 (13.346)	64.851 (12.429)	1.098	101	.275	−0.016	.055
KYN (μmol/L)	102	37	65	1.609 (0.454)	1.508 (0.319)	.908	60.420	.368	−0.026	.068
KA (nmol/L)	102	37	65	42.093 (15.674)	42.672 (14.314)	−0.277	100	.782	−0.069	.052
AA (nmol/L)	99	36	63	18.996 (4.592)	16.830 (4.294)	2.180	97	**.032***	.005	.099
3-HK (nmol/L)	96	35	61	42.273 (12.717)	39.046 (9.634)	1.189	94	.237	−0.019	.077
XA (nmol/L)	103	38	65	11.658 (4.909)	11.867 (5.131)	−0.126	101	.900	−0.0908	.080
3-HAA (nmol/L)	99	36	63	34.683 (12.015)	34.449 (12.834)	.162	97	.871	−0.062	.073
QA (nmol/L)	102	37	65	364.405 (147.544)	344.261 (108.933)	.280	57.705	.780	−0.057	.076
Pic (nmol/L)	102	38	64	35.959 (12.324)	34.722 (12.565)	.566	100	.572	−0.046	.083
KTR	101	37	64	.024 (0.008)	.024 (0.005)	.047	58.744	.963	−0.048	.050
Neopt (nmol/L)	98	36	62	21.206 (6.125)	20.936 (7.102)	.389	96	.698	−0.046	.068

Abbreviations: DMD= disease-modifying drug; TRP= tryptophan; KYN= kynurenine; KA= kynurenic acid; AA= anthranilic acid; 3-HK= 3-hydroxykynurenine; XA= xanthurenic acid; 3-HAA= 3-hydroxyanthranilic acid; QA= quinolinic acid; Pic= picolinic acid; KTR= KYN-to-TRP ratio (μmol/L by mmol/L); Neopt= neopterin. Serum concentrations are given as non-log-transformed values. Independent samples Student's *t*-test was performed on log10 transformed values. *between-group difference is significant on a 0.05 level.

Table A.2
Detailed results of the bivariate two-tailed correlation analysis of BMI, KTR, TRP, KP downstream metabolites, Neopt, and EDSS score.

		BMI	EDSS	Neopt	Trp	Kyn	KA	AA	3-HK	XA	3-HAA	QA	Pic	KTR
BMI		1	.057	.172	−0.067	**.398*****	**.411*****	.089	**.376*****	**.308****	**.369*****	**.432*****	.001	**.425*****
	p		.559	.09	.503	<0.001	<0.001	.382	<0.001	.002	<0.001	<0.001	.992	<0.001
	N	106	106	98	103	102	102	99	96	103	99	102	102	101
EDSS		.057	1	−0.004	−0.046	−0.015	−0.052	.116	.007	−0.037	−0.187	.043	.001	−0.01
	p	.559		.969	.644	.882	.604	.252	.943	.714	.063	.667	.992	.918
	N	106	106	98	103	102	102	99	96	103	99	102	102	101
Neopt		.172	−0.004	1	−0.126	**.383*****	**.266****	**.279****	**.398*****	.117	**.211***	**.510*****	.141	**.470*****
	p	.09	.969		.219	<0.001	.008	.006	<0.001	.253	.038	<0.001	.17	<0.001
	N	98	98	98	97	98	97	96	95	97	97	98	96	97
TRP		−0.067	−0.046	−0.126	1	.369***	.061	−0.006	.124	.417***	.440***	−0.018	.408***	−0.421***
	p	.503	.644	.219		<0.001	.545	.952	.233	<0.001	<0.001	.855	<0.001	<0.001
	N	103	103	97	103	101	101	98	95	102	98	101	101	101
KYN		.398***	−0.015	.383***	.369***	1	.548***	.445***	.707***	.549***	.618***	.710***	.420***	.688***
	p	<0.001	.882	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N	102	102	98	101	102	101	97	96	101	98	102	100	101
KA		.411***	−0.052	.266**	.061	.548***	1	.278**	.556***	.645***	.432***	.362***	.381***	.473***
	p	<0.001	.604	.008	.545	<0.001		.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N	102	102	97	101	101	102	97	95	101	98	101	100	100
		BMI	EDSS	Neopt	Trp	Kyn	KA	AA	3-HK	XA	3-HAA	QA	Pic	KTR
AA		.089	.116	.279**	−0.006	.445***	.278**	1	.284**	.17	.08	.276**	.321**	.415**
	p	.382	.252	.006	.952	<0.001	.006		.005	.095	.436	.006	.001	.001
	N	99	99	96	98	97	97	99	96	98	97	97	97	96
3-HK		.376***	.007	.398***	.124	.707***	.556***	.284**	1	.593***	.582***	.624***	.284**	.580***
	p	<0.001	.943	<0.001	.233	<0.001	<0.001	.005		<0.001	<0.001	<0.001	.006	<0.001
	N	96	96	95	95	96	95	96	96	96	95	96	94	95
XA		.308**	−0.037	.117	.417***	.549***	.645***	.17	.593***	1	.662***	.318**	.580***	.184
	p	.002	.714	.253	<0.001	<0.001	<0.001	.095	<0.001		<0.001	.001	<0.001	.067
	N	103	103	97	102	101	101	98	96	103	98	101	101	100
3-HAA		.369***	−0.187	.211*	.440***	.618***	.432***	.08	.582***	.662***	1	.479***	.529***	.220*
	p	<0.001	.063	.038	<0.001	<0.001	<0.001	.436	<0.001	<0.001		<0.001	<0.001	.031
	N	99	99	97	98	98	98	97	95	98	99	98	97	97
QA		.432***	.043	.510***	−0.018	.710***	.362***	.276**	.624***	.318**	.479***	1	.153	.707***
	p	<0.001	.667	<0.001	.855	<0.001	<0.001	.006	<0.001	.001	<0.001		.13	<0.001
	N	102	102	98	101	102	101	97	96	101	98	102	100	101
Pic		.001	.001	.141	.408***	.420***	.381***	.321**	.284**	.580***	.529***	.153	1	.088
	p	.992	.992	.17	<0.001	<0.001	<0.001	.001	.006	<0.001	<0.001	.13		.388
	N	102	102	96	101	100	100	97	94	101	97	100	102	99
KTR		.425***	−0.010	.470***	−0.421***	.688***	.473***	.415***	.580***	.184	.220*	.707***	.088	1
	p	<0.001	.918	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	.067	.031	<0.001	.388	
	N	101	101	97	101	101	100	96	95	100	97	101	99	101

Abbreviations: BMI= body mass index; EDSS= Expanded Disability Status Scale; Neopt= neopterin; TRP= tryptophan: KYN= kynurenine; KA= kynurenic acid; AA= anthranilic acid; 3-HK= 3-hydroxykynurenine; XA= xanthurenic acid; 3-HAA= 3-hydroxyanthranilic acid; QA= quinolinic acid; Pic= picolinic acid; KTR= KYN-to-TRP ratio (μmol/L by mmol/L). Two-tailed bivariate correlation analysis was performed on log10 transformed values using Pearson'*r*. *correlation is significant on a 0.05 level; **correlation is significant on a 0.01 level; *** correlation is significant on a <0.001 level.

Table A.3
Detailed ANCOVA results comparing lean(LG) vs. overweight/obese(OG) participants.

	N			Mean (SD)		ANCOVA			
	Total	OG	LG	OG	LG	*F*	*df*	*p*	pη^2
TRP (μmol/L)	103	41	62	65.274 (12.945)	66.439 (12.780)	0.065	1	.799	.001
KYN (μmol/L)	102	41	61	1.693 (0.393)	1.444 (0.328)	10.728	1	**.001****	.099
KA (nmol/L)	102	40	62	48.171 (15.574)	38.779 (13.033)	10.403	1	**.002****	.096
AA (nmol/L)	99	40	59	18.490 (4.395)	17.027 (4.519)	2.377	1	.129	.024
3-HK (nmol/L)	96	39	57	43.840 (12.897)	37.747 (8.576)	7.007	1	**.010***	.071
XA (nmol/L)	103	42	61	13.298 (4.860)	10.752 (4.913)	7.840	1	**.006****	.073
3-HAA (nmol/L)	99	39	60	39.038 (12.924)	31.607 (11.361)	10.026	1	**.002****	.095
QA (nmol/L)	102	41	61	395.659 (128.650)	321.934 (112.355)	9.639	1	**.002****	.090
Pic (nmol/L)	102	42	60	36.185 (12.184)	34.483 (12.652)	1.409	1	.238	.014
KTR	101	40	61	.026 (0.007)	.022 (0.006)	11.216	1	**.001****	.104
Neopt (nmol/L)	98	39	59	22.028 (6.133)	20.379 (7.068)	2.331	1	.130	.024

Abbreviation: OG= overweight/obese group (i.e., BMI $\geq$ 25 kg/m^2); LG= lean group (i.e., BMI < 25 kg/m^2); TRP= tryptophan: KYN= kynurenine; KA= kynurenic acid; AA= anthranilic acid; 3-HK= 3-hydroxykynurenine; XA= xanthurenic acid; 3-HAA= 3-hydroxyanthranilic acid; QA= quinolinic acid; Pic= picolinic acid; KTR= KYN-to-TRP ratio (μmol/L by mmol/L); Neopt= neopterin. Serum concentrations are given as non-log-transformed values. Age- and EDSS-adjusted ANCOVA was performed on log10 transformed values. *between-group difference significant on a 0.05 level; **between-group difference significant on a 0.01 level.

Table A.4
Detailed ANCOVA results comparing primary progressive vs. relapsing-remitting vs, secondary progressive multiple sclerosis phenotype.

	N				Mean (SD)			ANCOVA			
	Total	PPMS	RRMS	SPMS	PPMS	RRMS	SPMS	*F*	*df*	*p*	$p\eta^2$
TRP (μmol/L)	103	15	51	37	64.827 (12.545)	68.008 (12.634)	63.639 (12.000)	1.101	2	.337	.022
KYN (μmol/L)	102	15	51	36	1.476 (0.361)	1.581 (0.366)	1.521 (0.396)	.535	2	.587	.011
KA (nmol/L)	102	15	50	37	46.653 (18.339)	43.014 (15.377	40.018 (11.985)	.476	2	.623	.010
AA (nmol/L)	99	14	51	34	16.074 (3.968)	17.229 (4.684)	18.839 (4.248)	2.248	2	.111	.046
3-HK (nmol/L)	96	14	49	33	42.664 (15.147)	40.788 (9.975)	38.347 (10.194)	.185	2	.831	.004
XA (nmol/L)	103	15	51	37	12.507 (5.971)	12.675 (5.052)	10.280 (4.314)	1.209	2	.303	.024
3-HAA (nmol/L)	99	15	50	34	34.853 (13.157)	36.456 (12.874)	31.568 (11.332)	.117	2	.890	.003
QA (nmol/L)	102	15	51	36	352.000 (129.997)	352.373 (129.444)	350.250 (116.893)	.297	2	.743	.006
Pic (nmol/L)	102	15	51	36	33.758 (14.993)	36.741 (12.183)	33.570 (11.694)	.771	2	.466	.016
KTR	101	15	50	36	.024 (0.008)	.023 (0.005)	.024 (0.007)	.670	2	.514	.014
Neopt (nmol/L)	98	15	50	33	22.667 (5.085)	19.971 (5.625)	21.906 (8.583)	2.366	2	.100	.049

Abbreviation: MS= multiple sclerosis; RRMS= relapsing-remitting MS; SPMS= secondary progressive MS; PPMS primary progressive MS;.TRP= tryptophan: KYN= kynurenine; KA= kynurenic acid; AA= anthranilic acid; 3-HK= 3-hydroxykynurenine; XA= xanthurenic acid; 3-HAA= 3-hydroxyanthranilic acid; QA= quinolinic acid; Pic= picolinic acid, KTR= KYN-to-TRP ratio (μmol/L by mmol/L); Neopt= neopterin. Serum concentrations are given as non-log-transformed values. Age-, EDSS- and BMI-adjusted ANCOVA was performed on log10-transformed values.

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