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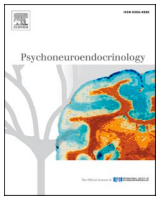

Kynurenine metabolites and ratios differ between Chronic Fatigue Syndrome, Fibromyalgia, and healthy controls

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ABSTRACT

Background: There is growing evidence that the kynurenine pathway is involved in the pathology of diseases related to the central nervous system (CNS), because of the neuroprotective or neurotoxic properties of certain metabolites, yet the role of each metabolite is not clear. The pathology of Chronic Fatigue Syndrome (CFS) and Fibromyalgia (FM) is currently under investigation, and the overlapping symptoms such as depression suggest that the CNS may be involved. These symptoms may be driven by enhanced neurotoxicity and/or diminished neuroprotection. However, the kynurenine metabolite status has not been well studied in these two possible related disorders of CFS and FM.

The objective of this study was to investigate the metabolites and ratios of the kynurenine pathway in CFS and FM compared to healthy controls and examine the possible correlations with symptoms of anxiety and depression.

Method: In this study, females aged 18–60 were included: 49 CFS patients; 57 FM patients; and 54 healthy controls. Blood plasma was analysed for the following metabolites involved in the kynurenine pathway: Tryptophan, kynurenine, kynurenic acid (KA), 3-hydroxykykynurenine (HK), anthranilic acid, xanthurenic acid (XA), 3-hydroxyanthranilic acid, quinolinic acid (QA) and picolinic acid. The concentrations of these metabolites, as well as the ratios of different metabolites indicating enzymatic activity, were compared between the groups. Findings were controlled for age, body mass index (BMI), and symptoms of anxiety and depression.

Results: QA differed between CFS and FM patients ($\beta = .144$, $p = .036$) and was related to higher levels of BMI ($\beta = .017$, $p = .002$). The neuroprotective ratio given by KA/QA was lower for CFS patients compared to healthy controls ($\beta = -.211$, $p = .016$). The neuroprotective ratio given by KA/HK was lower for FM patients compared to healthy controls, and this lower neuroprotective ratio was associated with increased symptoms of pain. The kynurenine aminotransferase II (KAT II) enzymatic activity given by XA/HK was lower for FM patients compared to healthy controls ($\beta = -.236$, $p = .013$). In addition, BMI was negatively associated with enhanced KAT II enzymatic activity ($\beta = -.015$, $p = .039$). Symptoms of anxiety and depression were not associated with the metabolites or ratios studied.

Conclusion: Our study indicates associations between kynurenine metabolism and CFS and FM as well as characteristic symptoms like fatigue and pain. Forthcoming studies indicating a causative effect may place kynurenine metabolites as a target for treatment as well as prevention of these conditions in the future.

1. Introduction

Kynurenines are suggested to play a central role in psychiatric diseases such as depression (Branchi et al., 2020). Symptoms of depression are frequently accompanying Chronic Fatigue Syndrome (CFS) and Fibromyalgia (FM) (Groven et al., 2019). Thus, exploring kynurenines also in CFS and FM is of interest. CFS and FM are two related disorders with unknown pathology (Clauw and Chrousos, 1997; Rasouli et al., 2019). Both disorders are common, with prevalence ranging from 0.5% to 2.5% for CFS (Estévez-López et al., 2020) and up to 5% for FM (Heidari et al., 2017). The personal burden of individuals struggling to maintain daily tasks and activities added to the high cost on society, through its strain

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on the work force (Global Burden of Disease Study (GBD) 2013, 2015), increase the significance of these conditions. Although their aetiology is unclear, there are several indications of disturbed immunological responses in both CFS and FM (Anderson et al., 2014; Coskun Benlidayi, 2019; Groven et al., 2019, 2020).

Kynurenines are the metabolites from the kynurenine pathway, following the breakdown of tryptophan (Try), through a cascade involving several enzymes. Immune activity is known to affect the kynurenine metabolic pathway and has been suggested to play a role in the pathophysiological mechanisms of both CFS and FM (Blankfield, 2012; Anderson et al., 2014, 2018). We have previously reported increased C-reactive protein (CRP) in CFS and FM patients (Groven et al., 2019). The upregulation of enzymatic activity in the kynurenine pathway follows increased inflammation (Dantzer et al., 2008). Specific pro-inflammatory cytokines activate the enzyme indoleamine 2–3-dioxygenase (IDO), enhancing Try conversion into Kyn and its metabolites at the cost of serotonin production. Serotonin is involved in many central mechanisms, ranging from sleep regulation to digestion, and is a key neurotransmitter in depression. Some metabolites of the kynurenine pathway are considered either neurotoxic (quinolinic acid [QA]) or neuroprotective (kynurenic acid [KA]), as they act as agonists (neurotoxic) or antagonists (neuroprotective) in glutamate nerve transmission (Colin-Gonzalez et al., 2013, Schwarcz and Stone, 2017). A shunt towards of Try breakdown into the kynurenine pathway and increased neurotoxic metabolites may affect fatigue, pain (Rojewska et al., 2018), and depression (Ogyu et al., 2018, Branchi et al., 2020) and explain mechanisms behind syndromes involving these symptoms, all of which are commonly found in CFS and FM.

In depression the neuroprotective ratios (KA/QA and KA/HK) are decreased compared to healthy controls (Ogyu et al., 2018). This is likely caused by variations in the activity of the enzymes involved, which may tip the balance into more neurotoxic products and effect the neuropsychiatric outcomes.

The importance of the neurotoxic metabolites of kynurenine in psychiatry are conceivable but we know very little about their effects neither directly nor indirectly on CFS and FM. The upregulation of enzymatic activity in the kynurenine pathway and potentially decreased availability of serotonin, coupled with the frequent comorbidity of depression in CFS and FM patients may suggest a shared background and requires a deeper investigation. The kynurenine pathway has been poorly studied in these patient groups, and studies comparing both CFS and FM are lacking. To investigate this, we conducted a study comparing kynurenine metabolites between patients with CFS and FM with healthy controls.

The present study on kynurenine and its metabolites in CFS and FM is part of a larger study on defined subgroups of patients with CFS and FM (Groven et al., 2019, 2020).

In the present paper we explore levels of kynurenines in the three groups CFS, FM and healthy controls. The hypothesis of this study was that patients with CFS and patients with FM have altered levels and ratios of tryptophan and its metabolites in the kynurenine pathway compared to healthy controls. A relation to symptoms of depression and anxiety was suggested. Confounders such as age, BMI anxiety and depression were explored.

[Fig. 1 Illustrates the kynurenine pathway with the metabolites used in this study.].

2. Method

2.1. Sample population

2.1.1. Patient groups

As previously reported (Groven et al., 2019), female patients aged 18–60 years admitted to the Multidisciplinary Pain Centre at St. Olav's University Hospital, Norway, found to qualify for the diagnoses CFS and FM were eligible for the study. Patients with challenging clinical pictures regarding problems such as CFS and FM are referred to this centre by general practitioners in Mid-Norway.

Each participant went through a comprehensive clinical examination and was thoroughly evaluated by an expert team of medical doctors, physiotherapists, and psychologists for inclusion- and exclusion criteria. FM patients ($n = 58$) were diagnosed by using the 1990 ACR criteria (Wolfe et al., 1990). CFS patients ($n = 49$) were diagnosed according to the CDC/Fukuda criteria (Fukuda, Straus et al., 1994). Exclusion criteria were in accordance with diagnostic criteria including known inflammatory diseases.

2.1.2. Healthy controls

A group of 53 healthy females aged 18–60 years was consecutively recruited by advertising through websites among the staff of the Norwegian University of Science and Technology (NTNU) and St. Olav's University Hospital. Their health was assessed by taking a structured medical history and by questionnaires evaluating the symptoms of CFS and FM (see 2.4 Questionnaires and 2.5 Interview).

2.2. Procedure

The CFS patients were informed about the study by a letter sent by the hospital prior to or shortly after their clinical examination or during their evaluation at the centre. The FM patients were given an information letter by the staff during the examination and evaluation of their FM diagnosis. Both patient groups were then contacted by phone by a member of staff and invited to participate in the study, and an appointment was scheduled for those who accepted to join.

2.3. Study design and ethics

The assessment lasted 30–40 min and included an interview, questionnaires, and blood sampling as formerly defined by Groven et al. (2019) in the period from March 2015 to December 2016. The study was

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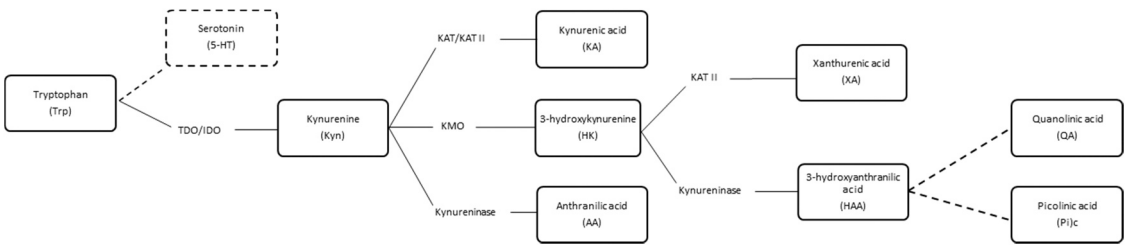

Fig. 1. The Kynurenine pathway (Groven, 2021), Activity of enzymes involved could be described as the ratio of the converted metabolite over the previous metabolite, Enzymes involved are: TDO = Tryptophan 2,3-dioxygenase. IDO = Indoleamine 2,3-dioxygenase. KAT = Kynurenine aminotransferase. KMO = Kynurenine 3-monooxygenase.

approved by the Regional Committee for Medical and Health Research Ethics (REK no. 2014/711). Written informed consent was obtained from all participants.

2.4. Questionnaires

The Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983; Bjelland et al., 2002) was used for symptoms of anxiety and depression. This scale divided into HADS-D (depression) and HADS-A (anxiety) sub-scores of 0–14, with higher scores indicating more severe symptoms.

The Chalder Fatigue Scale (Chalder et al., 1993; Loge et al., 1998) is used to evaluate (the severity of) fatigue in CFS patients. The total sum of each of the 11 items, scored on a 0–3 Likert scale, total sum ranging from 0 to 33, is applied; higher scores imply more severe fatigue.

A Numeric Rating Scale (NRS) was used to evaluate the subjective feeling of experienced pain on average in the last week. NRS is taken from the Brief Pain Inventory (Cleeland, 1991; Klepstad et al., 2002) which is a Likert scale ranging from 0 ("no pain") to 10 ("maximal possible pain").

The Fibromyalgia Survey Diagnostic Criteria (FSDC) is a self-report questionnaire that is used for diagnostics and classification in clinical and epidemiological studies (Wolfe et al., 2016; Fors et al., 2020). The FSDC consists of two sub-scales: Widespread Pain Index (WPI), scores 0–19; and Symptom Severity Scale (SSS), scores 0–12. WPI and SSS are summarised into a third score, i.e. the Fibromyalgia Severity (FS) score, ranging from 0 *(no symptoms)* to 31 (*most severe symptoms*) and indicate the severity of symptoms.

2.5. Interview

For each participant, age, height, and weight were recorded mainly by self-report, and a structured clinical interview was performed. History regarding infections, immune disorders, illness in general (somatic as well as mental), medication, menstrual cycle, menarche, use of contraceptives, status of menopause, duration of illness (if applicable), smoking or other nicotine use, and level of physical activity during the previous two weeks were recorded. The latter was scored on a scale from 1 (bedridden) to 4 (conducting regular exercise more than two times per week).

2.6. Blood sampling and analyses

There were no restrictions regarding fasting, medication or caffeine intake given prior to blood sampling. Plasma and serum samples for all study participants were collected and sent to the St. Olav's University Hospital clinical laboratories for further analysis. The samples were screened for deviating white blood cells, hsCRP, and serology against mycoplasma pneumonia, borrelia burgdorferi, cytomegalo-, Epstein-Barr -, hepatitis B - and hepatitis C virus, and total plasma IgE. Any sign of infection led to exclusion from the study.

Blood samples for tryptophan (Try) and its metabolites kynurenine (Kyn), kynurenic acid (KA), 3-hydroxykynurenine (HK), anthranilic acid (AA), xanthurenic acid (XA), 3-hydroxyanthranilic acid (HAA), quinolinic acid (QA) and picolinic acid (Pic) (Box 1) were collected in EDTA plasma tubes, immediately put on ice, centrifuged (1500 g, 15 min, 4 °C) and aliquoted into cryovials and frozen at − 80 °C until further analyses. The frozen samples were shipped to Bevital AS, Bergen, Norway, and analysed according to the company's protocols (bevital.no) by the means of liquid chromatography/tandem mass spectrometry (LC-MS/MS). The ratios between the metabolites could expresses the different breakdown-indexes in the kynurenine pathway, or the ratios between neuroprotective and neurotoxic metabolites. The metabolite ratios calculated were: Kyn/Try, KA/Kyn, XA/HK, HK/Kyn, AA/Kyn and HAA/HK; which involved the following neuroprotective indexes: KA/QA and KA/HK (Box 1).

2.7. Statistical analysis

The statistical analyses were performed using the Statistical Software package IBM Statistics (SPSS) for Windows, version 22. The criteria for using parametric statistics were met when all variables were transformed into natural log (ln), i.e. lnTry, lnKyn, lnKA, lnHK, lnAA, lnXA, lnHAA, lnQA, and lnPic, and these ln-values were used throughout this

Box 1
Abbreviations of kynurenine metabolites and ratios.

Metabolites:

Try	Tryptophan.
Kyn	Kynurenine.
KA	Kynurenic acid.
HK	3-hydroxykynurenine.
AA	Anthranilic acid.
XA	Xanthurenic acid.
HAA	3-hydroxyanthranilic acid.
QA	Quinolinic acid.
Pic	Picolinic acid.

Metabolite ratios (corresponding enzyme / ratio)[1]:

Kyn/Try	(TDO/IDO).
KA/Kyn	(KAT / KAT II).
XA/HK	(KAT II).
HK/Kyn	(KMO).
AA/Kyn	(Kynureninase).
HAA/HK	(Kynureninase).
KA/QA	(NPR1).
KA/HK	(NPR2).

[1]TDO = Tryptophan 2,3-dioxygenase. IDO = Indoleamine 2,3-dioxygenase. KAT = Kynurenine aminotransferase. KMO = Kynurenine 3-monooxygenase. NPR = Neuroprotective ratio.

study. The metabolite ratios were the ratios between one log transformed metabolite over another log transformed metabolite: [lnKyn]/[lnTry], [lnKA]/[lnKyn], [lnXA]/[lnHK], [lnHK]/[lnKyn], [lnAA]/[lnKyn] [lnHAA]/[lnHK], [lnKA]/[lnQA], and [lnHK]/[lnKA].

A linear regression model (model 1) with the covariates age, BMI, HADS-A and HADS-D was applied, serving as the basis model in this study (Box 2).

When the group showed significant effects on the kynurenine pathway metabolites or the ratios, an additional model (model 2) was applied. Model 2 included the co-factors of model 1 (age BMI, HADS-A and HADS-D scores), and adding the co-factors: fatigue scores, pain scores, FS scores, use of nicotine/smoking, and allergy (natural log-transformed [ln] total plasma concentration of IgE) (Box 2).

Student's t-test was used for post-hoc pair-wise comparison between the CFS, FM and control groups. Significance levels were set to $p < .05$.

3. Results

3.1. Population

A total of 160 participants were included in this study, consisting of 49 CFS patients, 58 FM patients, and 54 healthy controls. The demographic of the sample population is summarised in Table 1. The CFS patients in this study were significantly younger than the FM and control group ($p < .001$ and $p = .010$, respectively). The FM group had significantly higher BMI than the CFS and control group ($p = .007$ and $p = .049$, respectively). Both patient groups had significantly higher HADS-A scores, HADS-D scores, fatigue scores and FS scores compared to controls. There were less nicotine users (29%) than non-users (71%) in the total population, but more nicotine users in the two patient groups CFS ($n = 16$) and FM ($n = 22$) than in the control group ($n = 8$).

3.2. Tryptophan and the kynurenine pathway

3.2.1. Metabolites

3.2.1.1. Quinolinic acid (QA). Group differences were found for quinolinic acid (QA), where the CFS group had significantly higher levels compared to the FM group ($\Delta R^2 = .029$, $\beta = .114$, SE = 0.054, $t(153) = 2.11$, $p = .036$, overall adjusted $R^2 = .078$). Neither CFS nor the FM differed from controls (Table 2). BMI had an effect on QA ($\Delta R^2 = .094$, $\beta = .017$, SE = 0.004, $t(153) = 3.90$, $p < .001$). In the second model, BMI still had an effect on QA ($\Delta R^2 = .068$, $\beta = .014$, SE = 0.004, $t(149) = 3.18$, $p = .002$, overall adjusted $R^2 = .096$). Nicotine use also had an effect on the differences in QA levels ($\Delta R^2 = .029$, $\beta = -.091$, SE = 0.046, $t(149) = -2.05$, $p = .043$). (Supplementary Table 1.).

3.2.1.2. Anthranilic acid (AA). Group differences were found for anthranilic acid (AA), where the CFS group had lower levels compared to controls ($\Delta R^2 = .049$, $\beta = -.145$, SE = 0.073, $t(152) = -1.98$, $p = .049$, overall adjusted $R^2 = .026$). This effect disappeared in model 2 (Supplementary Table 2), where fatigue and nicotine had effect on AA ($\Delta R^2 = .036$, $\beta = -.014$, SE = 0.006, $t(148) = -2.26$, $p = .026$; and $\Delta R^2 = .070$, $\beta = -.185$, SE = 0.058, $t(148) = -3.20$, $p = .002$; adjusted $R^2 = .159$). The CFS patients could not be distinguished from the FM group, nor could the FM patients be distinguished from the control group (Table 2).

None of the other metabolites in the kynurenine pathway (Try, Kyn,

Box 2
Overview of the two statistical regression models used in this study.

Model 1:

Dependent variable: Metabolite / Metabolite ratio.

Fixed factor: Group variable (CFS, FM, healthy controls).

Co-factors:

1) Age
2) BMI
3) HADS-A
4) HADS-D

Model 2:

Dependent variable: Metabolite / Metabolite ratio.

Fixed factor: Group variable (CFS, FM, healthy controls).

Co-factors:

1) Age
2) BMI
3) HADS-A
4) HADS-D
5) fatigue scores
6) pain scores
7) FS scores (fibromyalgianess)
8) Nicotine
9) IgE (allergy)

[1),2)]Variables included because of differences between the diagnostic groups in this study (and their assumed relationship with inflammation). [3),4)]Variables included based on the hypothesis of this study. [5),6),7)]Variables included based on primary symptoms in CFS and FM. [8),9)]Variables included based on the possibility of their relationship with inflammation.

Table 1
Descriptives of age, Body Mass Index (BMI), depression and anxiety scores in CFS, FM and controls.

	CFS				FM				Control				
	(*n*=49)				(*n*=58)				(*n*=53)				
Parameter	Missing (*n*)	*M (SD)*	*Mdn*	*Range (min– max)*	Missing (*n*)	*M (SD)*	*Mdn*	*Range (min– max)*	Missing (*n*)	*M (SD)*	*Mdn*	*Range (min– max)*	*p*
Age	0	33.8 (11.3)	35.0	18–60	0	42.0 (9.1)	42.5	22–60	0	39.4 (10.4)	39.0	23–59	< .001[a]
BMI	2	24.0 (3.6)	23	18.1–34.6	1	26.7 (5.6)	26	16.3–40.4	0	24.7 (4.0)	24	16.3–41.7	.017[a]
HADS depression	0	6.0 (4.2)	5.0	0–17	1	6.4 (3.9)	6.0	0–16	1	1.3 (1.8)	1.0	0–8	< .001[a]
HADS anxiety	0	5.9 (4.6)	5.0	0–19	1	8.4 (4.1)	8.0	0–17	1	3.2 (2.6)	3.0	0–10	< .001[a]
Fatigue score	0	36.5 (5.3)	37	23–44	1	33.5 (5.3)	34	18–44	1	21.3 (3.2)	21	14–33	< .001[a]
FS score	0	15.2 (5.5)	13	7–29	1	20.1 (5.2)	20	3–30	1	3.1 (2.5)	3	0–11	< .001[a]
lnIgE[b]	0	3.19 (1.58)	3.22	0–7.84	2	3.31 (1.41)	3.37	0.69–7.39	1	3.20 (1.40)	2.94	0.69–7.26	.895[c]

Note: BMI = body mass index. HADS = Hospital Anxiety and Depression Scale (0–21, respectively). FS = (0–31) Fibromyalgia Severity. Fatigue = Chalder Fatigue Score (0–33).

[a] Kruskall-Wallis test.

[b] ln-transformed concentration of total plasma IgE.

[c] One-way ANOVA.

KA, HK, XA, HAA, and Pic) showed any difference between CFS, FM and controls in model 1 (controlling for age, BMI, and HADS scores).

3.2.2. Ratios

3.2.2.1. XA/HK (KAT II enzymatic activity). Group differences were found for the ratio between xanthurenic acid and 3-hydroxyanthranilic acid [lnXA]/[lnHK], where the FM group showed lower value than both the control group ($\Delta R^2 = .041$, $\beta = -.236$, SE = 0.094, $t(152) = -2.508$, $p = .013$; overall model adjusted $R^2 = .113$), and CFS patients ($p = .032$). The CFS group could not be distinguished from the control group (Table 2). Age, HADS-A and HADS-D did not affect the ratio. However, BMI had an effect, although no longer significant, on [lnXA]/[lnHK] ratio ($\Delta R^2 = .029$, $\beta = -.015$, SE = 0.007, $t(152) = -2.08$, $p = .039$). In model 2, the group effect disappeared. BMI still had an effect, although no longer significant, on [lnXA]/[lnHK] ($\beta = -.013$, SE = 0.008, $t(148) = -1.74$, $p = .084$; overall model adjusted $R^2 = .151$). Also in the second model, pain scores and nicotine could explain a significant proportion of the [lnXA]/[lnHK] ratio ($\Delta R^2 = .055$, $\beta = -.059$, SE = 0.021, $t(148) = -2.81$, $p = .006$; and $\Delta R^2 = .034$, $\beta = -.170$, SE = 0.078, $t(148) = -2.19$, $p = .030$). (Supplementary Table 3).

3.2.2.2. KA/QA (neuroprotective ratio 1). Group differences were found for the neuroprotective ratio between kynurenic acid and quinolinic acid [lnKA]/[lnQA], where the CFS group had lower levels compared to the control group ($\Delta R^2 = .039$, $\beta = -.211$, SE = 0.086, $t(153) = -2.44$, $p = .016$; overall model adjusted $R^2 = .045$). The CFS group could not be distinguished from the FM group, nor could FM patients be distinguished from the control group (Supplementary Table 3). Neither age, BMI, HADS-A nor HADS-D had any effects on [lnKA]/[lnQA] in this model. The group effect disappeared in model 2 ($\beta = -.058$, SE = 0.134, $t(149) = -0.43$, $p = .665$; overall model adjusted $R^2 = .033$). (Supplementary Table 4.).

3.2.2.3. KA/HK (neuroprotective ratio 2). Group differences were found for the ratio between kynurenic acid and 3-hydroxyanthranilic acid [lnKA]/[lnHK], where the FM group showed lower value than the control group ($\Delta R^2 = .027$, $\beta = -.046$, SE = 0.023, $t(152) = -2.00$, $p = .048$; overall model adjusted $R^2 = .041$). This group effect disappeared in model 2. CFS could not be distinguished from controls ($\Delta R^2 = .008$, $\beta = .039$, SE = 0.036, $t(148) = 1.083$, $p = .281$; overall model adjusted $R^2 = .122$). The CFS group could not be distinguished from the control group nor the FM group (Table 2). In model 2 only pain scores could explain a significant proportion of the [lnKA]/[lnHK] ratio ($\Delta R^2 = .066$, $\beta = -0.015$, SE = 0.005, $t(148) = -3.10$, $p = .002$).
(Supplementary Table 5.).

The results for all metabolites and ratios (Box 1) are found in Supplementary Tables 1–17.

4. Discussion

In this study we found reduced neuroprotective [lnKA]/[lnQA] ratio in CFS patients compared to healthy controls, and lower ratios of both [lnXA]/[lnHK] and neuroprotective [lnKA]/[lnHK] in FM patients compared to healthy controls. These differences persisted when controlled for age, BMI, and symptoms of anxiety depression. There were no differences between the CFS and FM groups. Anxiety and depression scores did not have any effect on any of the metabolites or ratios of the tryptophan-kynurenine pathway. Age, BMI, fatigue, pain scores and nicotine use affected several of the findings. Furthermore, we observed higher quinolinic acid (QA) concentrations in CFS patients.

4.1. Metabolite concentrations

4.1.1. Quinolinic acid (QA)

The CFS group had significantly higher levels of QA compared to the FM group when controlling for age, BMI, HADS-A, and HADS-D (model 1). In addition, when controlling for more co-factors (model 2), higher BMI indicated higher levels of QA ($\beta = .014$, SE = 0.004).

BMI in the CFS group was lower than the other groups, and yet this did not mask the effect of higher QA levels in the CFS group. The FM group however, had lower QA levels, yet significantly higher BMI than the CFS group. This could result in opposing effects on the model (model 1), and may be the reason why the FM group effect on QA disappeared when additionally controlling for fatigue, pain, FS scores, nicotine use and ln-IgE in model 2 (Supplementary Table 1). Likewise, more nicotine use in FM patients compared to the other groups combined with a slight, negative effect of nicotine on QA may neutralize the same group effects. Correcting for multiple co-factors didn't increase the overall power of the model, and this could indicate that the CFS patients may indeed have higher concentrations of QA compared to controls.

Table 2
Comparisons of the (ln-transformed) kynurenine pathway metabolites and ratios with group effects.

	Control				CFS					FM				
	Mean	SE	95% CI		Mean	SE	95% CI	β	p^a	Mean	SE	95% CI	β	p^a	p^b
lnAA[c]	2.686	0.050	2.588	2.785	2.541	0.050	2.443	2.639	−.145	**.049**	2.593	0.046	2.502	2.685	−.093	.207	.455
lnQA[d]	5.796	0.038	5.721	5.872	5.888	0.038	5.812	5.964	.092	.106	5.775	0.036	5.703	5.846	−.022	.106	**.036**
[lnKA]/[lnQA][c]	−1.985	0.059	−2.097	−1.864	−2.191	0.059	−2.308	−2.075	−.211	**.016**	−2.087	0.055	−2.196	−1.978	−.106	.223	.207
[lnXA]/[lnHK][e]	−0.898	0.064	−1.025	−0.772	−0.941	0.064	−1.067	−0.815	−.043	.647	−1.135	0.060	−1.252	−1.017	−.236	**.013**	**.035**
[lnKA]/[lnHK][f]	0.015	0.014	−0.012	0.042	−0.021	0.015	−0.051	0.008	−.036	.074	−0.032	0.013	−0.059	−0.006	−.047	**.015**	.600

Note: Covariates appearing in the model are evaluated at the following values: Age at test = 38.8; Body Mass Index ≈ 25; HADS-A = 5.9; HADS-D = 4,5. SE = standard error. CI = confidence interval.

[a] The control group is set as the reference group. β and *t* are the estimated from the control group and p^a are the significance values for CFS and FM compared to the control group.

[b] Significance levels for pair-wise comparison between CFS and FM.

[c] CFS lower than the control group.

[d] CFS higher than the FM group.

[e] FM lower than the CFS and control groups.

[f] FM lower than the control group.

4.1.2. Anthranilic acid (AA)

Anthranilic acid (AA) was significantly lower in CFS patients compared to controls when corrected for age, BMI, anxiety, and depression. This group difference disappeared when extending this model to include more co-factors (model 2). The extended model showed that fatigue and nicotine exhibited significant effects on AA (Supplementary Table 2). Since both patient groups (CFS and FM) had higher scores on fatigue and there were more nicotine users compared to controls, this indicates that fatigue and nicotine use are more likely than the diagnostic group variable to explain changes in this metabolite.

If less AA is derived from Kyn, this could mean that more Kyn is converted into KA or HK and eventually into the neurotoxic metabolite QA (which we claim were elevated in the CFS group discussed above). Still, these findings need further exploration, preferably in other study populations with relevant symptoms. It would be useful to specify "fatigue" to see if elements of the kynurenine pathway are related to mere physical and/or mental fatigue.

4.1.3. Metabolite ratios

4.1.3.1. Xanthurenic acid (XA) and 3-hydroxykynurenine (HK) – expression of KAT II activity). HK is converted into XA by the enzyme kynurenine aminotransferase II (KAT II), and the [lnXA]/[lnHK] ratio could be indicative of the activity of this enzyme. The FM group showed lower ratio value for [lnXA]/[lnHK] than controls and CFS patients. The CFS group could not be distinguished from the control group. However, Model 2 (Supplementary Table 3) was better at explaining the changes in the [lnXA]/[lnHK] ratio, and in this model the differences between the FM patients, CFS patients and the control group disappeared, suggesting that BMI and nicotine use, rather than the FM group, is more likely to have an effect on this ratio.

The potential clinical relevance of this is not known. To our knowledge there are no other studies exploring the role of neither higher weight nor pain associations with lowered KAT II activity. Both higher BMI and pain scores are solid findings in our FM patients, and they had significantly higher use of nicotine. The initial finding of reduced KAT II activity in FM patients could be indicative of symptoms that follow this disorder. Chronic pain conditions are common and affect approximately 25% of the population (Landmark, Romundstad et al. 2012). The involvement of kynurenines in pain sensation and chronification is plausible due to the involvement of glutamate in the processing of pain (Jovanovic and Candido et al., 2020). Deviating findings of Try–Kyn metabolites such as elevated QA and XA have been reported in a large sample ($n = 17{,}834$) of chronic pain patients (Gunn et al., 2020), and serum samples of 119 chronic migraine patients showed increased levels of Try, AA and XA and decreased Kyn, KA, HK, HAA and QA (Curto et al., 2015). Pain intensity was associated with the Kyn/Try ratio and Try plasma levels in 17 patients with temporomandibular myalgia (Barjandi et al., 2019). Pain scores could explain a significant proportion of the [lnXA]/[lnHK] ratio in our study, and it is therefore likely that the increased severity of pain is related to lower [lnXA]/[lnHK] ratio. Interestingly, in the absence of any group differences, we also discovered that pain scores were positively associated with kynurenine Mono-Oxygenase activity [lnHK]/[lnKyn] (Supplementary Table 6), and negatively associated with kynureninase activity [lnHAA]/[lnHK] (Supplementary Table 7).

4.1.3.2. Neuroprotective ratio 1 – expressed by kynurenic acid (KA) and quinolinic acid (QA). The [lnKA]/[lnQA] ratio is regarded "neuroprotective" because of the anticipated neuroprotective properties of KA and the neurotoxic properties of QA (Schwarcz and Stone, 2017). Our findings suggest that the "neuroprotective ratio" in CFS is lower than in healthy controls. An imbalance between the neurotoxic and neuroprotective metabolites is described in several neurodegenerative disorders and depression (Maddison and Giorgini, 2015; Savitz, 2017), which

implies that similar mechanisms could be found in CFS patients, but further studies are warranted before any conclusions can be drawn.

4.1.3.3. Neuroprotective ratio 2 – expressed by kynurenic acid (KA) and 3-hydroxykynurenine (HK). Similar to the KA/QA ratio, the KA/HK ratio could also be regarded as "neuroprotective" because of the above-mentioned neuroprotective properties of KA and the anticipated neurotoxic properties of HK (Colin-Gonzalez et al., 2013). In this study group differences were found for [lnKA]/[lnHK], where the FM group showed lower ratio value than the control group when controlling for age, BMI, HADS-A and HADS-D (model 1). The FM group could not be distinguished from the CFS group. Model 2 was better overall at explaining the [lnKA]/[lnHK] ratio, and the differences between the FM group and controls disappeared (Supplementary Table 5). In this model, only pain scores could explain a significant proportion of the [lnKA]/[lnHK] ratio, with higher pain scores indicating lower [lnKA]/[lnHK] ratio. To our knowledge there are no other studies reporting these ratios in neither FM nor CFS.

4.1.4. Neuroprotection and neurotoxicity

The neuroprotective properties of KA lies with its ability to block *N*-methyl-D-aspartate (NMDA) glutamatergic receptors (Schwarcz and Stone, 2017). Excessive glutamate signalling through NMDA receptors leads to neuronal loss (Colin-Gonzalez et al. 2013; Schwarcz and Stone, 2017). It is suggested that QA has neurotoxic effects by binding to NMDA receptors, and by promoting oxidative stress (Santamaría et al., 2001). The neurotoxic properties of HK depend on its physiological concentrations and results should be interpreted with caution since it can act as both agonist and antagonist of NMDA receptors (Colin-Gonzalez et al., 2013). Glutamate signalling through NMDA receptors can increase pain sensation by producing hypersensitivity of spinal neurons (Bannister et al., 2017) and it is thus possible that enhanced HK or QA could lead to increased pain sensation independent of reduced KA. Interestingly, pain NRS scores were negatively associated to KA in our study (Supplementary Table 8). It is tempting to speculate that this indicates a negative neuroprotective state for patients with CFS of FM. It may offer an explanation to how QA influences the overall symptom picture of these patients, although the mechanisms behind these observations need further investigation.

Try is an essential amino acid that is converted into Kyn by indoleamine 2–3-dioxygenase (IDO) in macrophages and glial cells (Schwarcz et al., 2012), and thus the [lnKyn]/[lnTry] ratio is indicative of IDO-activity. IDO activity is increased by the pro-inflammatory cytokines IFN-γ and TNF-α. Although we did not find increased IFN-γ and TNF-α levels in the patients, we did find that CFS and FM patients had other increased inflammatory markers, such as CRP (Groven, 2011; Groven et al., 2019). Since hsCRP status can be a proxy of the CFS and FM status controlling for hsCRP in this study may conceal any associations between the patient groups and the kynurenine pathway. When we added CRP in the model, it did not alter the results.

Inflammatory agents such as IL-6 are also produced in fatty tissue and this explains the relationship between higher BMI and inflammation. Altered tryptophan breakdown index (Kyn/Try) has been reported in obese subjects with systemic inflammation (Cussotto et al., 2020). We also found that BMI was positively associated with [lnKyn]/[lnTry] ratio (Supplementary Table 9). The link between BMI and inflammation on enzyme activity and neurotoxic metabolites of the kynurenine pathway, and how this relates to CFS and FM is a potential useful approach for further studies.

As Try is also converted into serotonin, altered levels of Try and altered activity in the enzymes of the kynurenine pathway could affect the production of serotonin. Studies have indicated lower serotonin levels in FM patients (Alnigenis and Barland, 2001) and increased serum concentrations of serotonin in sub-groups of CFS patients (Badawy et al., 2005). Abnormal Try and Kyn metabolites and neuroprotective ratios / higher neurotoxic ratios are found in patients suffering from depression (Savitz, 2017). Depressive symptoms are often seen in CFS and FM, as also shown in this study (higher HADS-D score). Tricyclic antidepressants (TCAs) and selective serotonin and noradrenaline reuptake inhibitors (SSRI/SNRIs) (both increasing levels of serotonin in synapses) have been reported to have an effect on pain and depression (but not fatigue) in FM (Hauser et al., 2009; Macfarlane et al., 2017; Welsch et al., 2018). In this study we did not find any differences in the Try concentrations or [lnKyn]/[lnTry] ratio between CFS, FM and controls, nor did the use of anti-depressants alter any of ours results (data not shown). Serotonin was not measured.

4.2. Limitations and strengths

The present study is a cross sectional study and conclusions on causality cannot be drawn. The time of day for blood samples collection varied between 9 AM and 18 PM. There were no dietary restrictions prior to the collection of blood samples, and information on nutrition or supplements was not recorded. Furthermore, only females ages 18–60 were included, also limiting generalisability. For example, children and adolescents, elderly and people with other known somatic disorders may have symptoms of CFS and FM with completely different biological background. Another weakness is that the control group consisted of university and hospital staff, which may not be representative of the general population.

Strength of the study: Other factors that are linked to inflammation or a shift in immunological responses such as infection, age, pregnancy, and BMI have all been taken into account. Participants with active infections or pregnancy were excluded. Both patient populations were recruited from a specialist care clinic. This is a strength as diagnoses were thoroughly evaluated and confounding comorbidities were excluded. There were no inequalities in the socioeconomic status in our study population.

To our knowledge this is the first report comparing the kynurenine metabolites and their ratios in CFS and FM and the findings need to be further explored.

5. Conclusion

CFS patients may have lower neuroprotection due to higher levels of QA and lower neuroprotective ratio (KA/QA) than healthy controls. Fatigue and pain – central factors in CFS and FM – seem to be particularly related to AA, QA, and KAT II activity. Body weight reduction and smoking cessation may be beneficial in chronic fatigue and pain conditions. Kynurenine metabolites and ratios can be promising indicators and targets of diagnosis and treatment of both FM and CSF. However, caution should be taken because of the complexity of the symptoms in these patients, such as fatigue and pain, and their underlying mechanisms, independent of diagnostic groups.

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Conflict of interest

All the authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105287.

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