



## Peripheral and central kynurenine pathway abnormalities in major depression

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### ABSTRACT

Considerable data relate major depressive disorder (MDD) with aberrant immune system functioning. Pro-inflammatory cytokines facilitate metabolism of tryptophan along the kynurenine pathway (KP) putatively resulting in reduced neuroprotective and increased neurotoxic KP metabolites in MDD, in addition to modulating metabolic and immune function. This central nervous system hypothesis has, however, only been tested in the periphery. Here, we measured KP-metabolite levels in both plasma and cerebrospinal fluid (CSF) of depressed patients ( $n = 63/36$  respectively) and healthy controls ( $n = 48/33$ ). Further, we assessed the relation between KP abnormalities and brain-structure volumes, as well as body mass index (BMI), an index of metabolic disturbance associated with atypical depression. Plasma levels of picolinic acid (PIC), the kynurenic/quinolinic acid ratio (KYNA/QUIN), and PIC/QUIN were lower in MDD, but QUIN levels were increased. In the CSF, we found lower PIC in MDD. Confirming previous work, MDD patients had lower hippocampal, and amygdalar volumes. Hippocampal and amygdalar volumes were correlated positively with plasma KYNA/QUIN ratio in MDD patients. BMI was increased in the MDD group relative to the control group. Moreover, BMI was inversely correlated with plasma and CSF PIC and PIC/QUIN, and positively correlated with plasma QUIN levels in MDD. Our results partially confirm previous peripheral KP findings and extend them to the CSF in MDD. We present the novel finding that abnormalities in KP metabolites are related to metabolic disturbances in depression, but the relation between KP metabolites and depression-associated brain atrophy might not be as direct as previously hypothesized.

### 1. Introduction

Over the last four decades a considerable body of literature has amassed showing an association between major depressive disorder (MDD) and immune system dysregulation, particularly in immunometabolic/atypical depression (Lamers et al., 2020; Milaneschi et al., 2020). Increased circulating pro-inflammatory cytokines are among the most reliably observed findings in the biological psychiatry of MDD (Dowlati et al., 2010; Enache et al., 2019; Köhler et al., 2017). Moreover,

a causal link for inflammation in MDD is suggested by the antidepressant effects of anti-inflammatory medication (Bai et al., 2020) in addition to the normalization of abnormal inflammation following treatment with conventional antidepressants (Köhler et al., 2018). A promising connection between inflammation and conventional molecular pathways in MDD is provided by the finding that pro-inflammatory messengers can alter the metabolism of the serotonergic precursor tryptophan (Dantzer et al., 2011; Myint and Kim, 2003). Tryptophan is metabolized along two pathways, the serotonergic pathway, along

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which serotonin and melatonin are synthesized, and the kynurenine pathway, where tryptophan is catabolized to kynurenine and then to several neuroactive metabolites including kynurenic acid (KYNA) and quinolinic acid (QUIN; [Ruddick et al., 2006](#)).

Pro-inflammatory cytokines, such as interleukin (IL)-1 $\beta$ , IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$ , increase the degradation of tryptophan along the kynurenine pathway (KP) by activating the rate-limiting enzymes indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan dioxygenase 2 (TDO2; [Guillemin, 2012](#); [Schwarcz et al., 2012](#); [Schwieler et al., 2015](#); [Sellgren et al., 2016](#)). IDO1 and TDO2 catabolize tryptophan to *N*-formylkynurenine, which is then further metabolized into *L*-kynurenine (KYN). KYN can, in turn, either be converted to KYNA, an *N*-methyl-D-aspartate (NMDA) receptor antagonists, or to 3-hydroxy-kynurenine (3-HK), a generator of free radicals. A further bifurcation can see 3-HK converted either to picolinic acid (PIC) or QUIN, an NMDA receptor agonist with putatively excitotoxic properties at glutamate receptors ([Schwarcz and Stone, 2017](#)). QUIN can further be metabolized into nicotinamide (NAM), a precursor of the crucial co-factor nicotinamide adenine dinucleotide ([Schwarcz and Stone, 2017](#)), which possesses putatively neuroprotective properties ([Harrison et al., 2019](#)). A simplified overview of the KP can be found in Supplementary Fig. 1.

Abnormalities of KP metabolites have been observed in MDD. Plasma levels of KYNA, NAM, and PIC, all associated with neuroprotective effects ([Müller and Schwarz, 2008](#); [Stone, 2000](#); [Guillemin et al., 2007](#); [Beninger et al., 1994](#); [Grant et al., 2009](#); [Kalisch et al., 1994](#)), are reduced in patients with MDD ([Ogyu et al., 2018](#); [Colle et al., 2020](#); [Ryan et al., 2020](#)), while plasma levels of QUIN, associated with neurotoxic properties ([Beninger et al., 1994](#); [Grant et al., 2009](#); [Kalisch et al., 1994](#); [Lovelace et al., 2016, 2017](#)), are elevated in depression ([Doolin et al., 2018](#)). Moreover, neuroprotective to neurotoxic metabolite ratios (KYNA/QUIN, KYNA/3-HK, and PIC/QUIN) are reliably reduced in the periphery in depression ([Ogyu et al., 2018](#); [Ryan et al., 2020](#); [Meier et al., 2016](#); [Savitz et al., 2015](#)). While KP abnormalities have only been assessed in the periphery in MDD, these metabolites are assumed to act in the central nervous system (CNS) to produce depressogenic effects ([Dantzer et al., 2011](#); [Dantzer et al., 2008](#)). For example, a study by Savitz and colleagues ([Savitz et al., 2015](#)), assessed the relation between peripherally measured KYNA/QUIN ratio (a neuroprotective index) and hippocampal and amygdalar volumes in MDD patients and controls. While MDD patients and controls assessed in this study did not differ in peripheral KYNA/QUIN ratios, nor with respect to amygdalar or hippocampal volumes once adjusted for potential noise covariates, these structure volumes were, nonetheless, found to be positively related to this neuroprotective index in MDD patients only. This finding suggests a potential role for kynurenine metabolism in neurotoxic and neurotrophic processes in MDD ([Campbell et al., 2004](#); [Hamilton et al., 2008](#); [Videbech and Ravnkilde, 2004](#)). Given that abnormalities in KP metabolites are hypothesized to exert depressogenic effects in the brain and that within kynurenine's metabolic pathway only some metabolites (KYN, 3-Hydroxyanthranilic acid and 3-HK) can pass the blood–brain barrier ([Fukui et al., 1991](#)), we propose that a necessary step in the development of the kynurenine-depression literature is to compare depressed and healthy samples with regard to central levels of KP metabolites. A recent study has assessed KP metabolites in the cerebrospinal fluid (CSF) in a cohort of depressed patients and found a strong association between plasma and CSF levels of KYN and QUIN, but not other metabolites ([Haroon et al., 2020](#)). A previous study compared CSF levels of KYNA and QUIN in suicide attempters, some of whom met DSM criteria for depression, and healthy control (HC) participants and found increased central levels of QUIN in suicide attempting MDD patients compared to controls ([Erhardt et al., 2013](#)). Similarly, CSF KP metabolite levels have been assessed in a wide range of neurological disorders, such as Alzheimer's disease ([Jacobs et al., 2019](#)), Parkinson's disease ([Iwaoka et al., 2020](#)), amyotrophic lateral sclerosis ([Tan and Guillemin, 2019](#)), and in response to physical exercise in healthy participants

([Isung et al., 2021](#)), but data on MDD patients compared to healthy participants is missing.

Recent *meta*-analyses showed that effect sizes vary greatly between studies assessing kynurenine pathway metabolites in mood disorders ([Ogyu et al., 2018](#); [Arnone et al., 2018](#); [Marx et al., 2020](#)). This variability is reasonably explained in terms of the heterogeneous manifestation of MDD. It has been postulated recently that one subtype of depression is best characterized in terms of a constellation of abnormal inflammatory and metabolic factors ([Lamers et al., 2020](#); [Milaneschi et al., 2020](#)). In terms of the conventional nosology of MDD, this subtype has been linked most closely with atypical depression ([American Psychiatric Association, 2013](#)). Given that increased IDO1 activity and changes in kynurenine metabolite levels are associated with metabolic syndrome ([Oxenkrug et al., 2017](#); [Oxenkrug, 2010](#)), it is of interest in the context of MDD to examine the association between kynurenine metabolites and measures of metabolic syndrome, such as body mass index (BMI).

In the present study, we conducted a broad, multi-level assessment of abnormalities in the kynurenine pathway in the periphery as well as the CNS in a general, non-subtyped MDD sample. To do this we used ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) to assay blood plasma and CSF for several components of the KP in patients with MDD and in HC participants. Next, given that KP metabolites have been characterized in terms of their neurotoxic and neurotrophic effects, we examined the relation between levels of peripheral and central KP metabolites and volumes of the amygdala and hippocampus, which reliably show volumetric reduction in depression ([Campbell et al., 2004](#); [Hamilton et al., 2008](#); [Videbech and Ravnkilde, 2004](#)). Further, we assessed whether abnormal levels of KP metabolites in MDD were related to BMI, an index of metabolic disturbance. Finally, we estimated the relation between peripheral and central KP metabolites in depression and healthy control samples to attempt to replicate and extend — by assessing a broader array of KP metabolites — previous findings ([Haroon et al., 2020](#)). Based on previous research, we hypothesize that the primarily neuroprotective metabolites, KYNA, PIC, and NAM, the ratios of KYNA/QUIN, KYNA/3-HK and PIC/QUIN, as well as KYN, which has reliably been found to be reduced in MDD, will be decreased in depression, while the primarily neurotoxic metabolites 3-HK and QUIN will be increased. We further hypothesize a positive relation between neuroprotective KP metabolites as well as neuroprotective/neurotoxic ratios with brain volumes/BMI and a negative relation between neurotoxic KP metabolites and brain volumes/BMI.

## 2. Methods and materials

### 2.1. Participants

Participants were 63 treatment-seeking, formally diagnosed MDD patients and 48 age- and sex-matched HCs. Participant demographics and clinical information are presented in [Table 1](#). Treatment-seeking depressed participants were recruited via the adult psychiatric clinic at Linköping University Hospital, Sweden, through referral from general practitioners, and advertisements in local newspapers and social media. HC participants were recruited through advertisements in social media and posters in public spaces.

Trained interviewers assessed eligibility using the Mini-International Neuropsychiatric Interview (MINI) ([Sheehan et al., 1998](#)), a validated clinical tool for diagnosis of psychiatric disorders according to the DSM-5 ([American Psychiatric Association, 2013](#)) and ICD-10 ([World Health Organization WHO, 2004](#)) criteria. Prospective participants in the MDD group had to suffer from a current unipolar depression that was not accounted for by other medical conditions (e.g. hypothyroidism). Participants from the HC group had to be free of any current or previous severe psychopathologies as defined by DSM-5, determined by the MINI. Exclusion criteria specific for the MDD group were: a current DSM-5 diagnosis of substance use disorder, except nicotine; a psychotic

disorder, except depression with mood-congruent psychotic features; new antidepressant medication during the month before study participation (two months for fluoxetine); change of the dose of psychotropic medications over the last month (antidepressant and antipsychotic medication) or the last two months (mood stabilizers and anticonvulsants). It has been noted that, in response to antidepressant / mood-stabilizing drugs, mood remains in flux and side effects emerge and then either stabilize or remit over the course of about a month for antidepressants and two months for mood stabilizers. Excluding depressed persons in this phase of pharmacological response both reduces noise and removes one class of potential confounds associated with this state of medication-induced neuro-affective change due. General inclusion criteria were: age 18 – 65; working knowledge of Swedish; willingness and ability to provide informed consent. General exclusion criteria included: standard magnetic resonance imaging (MRI) contraindications (e.g. implanted ferrous metal, claustrophobia); pregnancy; medical conditions likely to influence cerebral blood flow or brain anatomy; standard clinical contraindications for a lumbar puncture (e.g. increased intracranial pressure); ongoing use of immune-modulators or anti-inflammatory drugs. Prospective participants were further excluded if they suffered from any medical condition that may affect immunological or metabolic function (e.g. diabetes type II, liver disease).

The work described in the present study was carried out in accordance with the code of ethics of the world medical association (declaration of Helsinki) for experiments including humans: “[wma.net/en/30publications/10policies/b3/](http://wma.net/en/30publications/10policies/b3/)”. The study was approved by the regional ethical review board, Linköping, Sweden, and all participants gave written consent to the use of their data for scientific purposes. Participants completed two sessions, both taking place in the mornings and within a maximum of seven days of one another. During the first session, participants underwent a multimodal MRI scanning session. During the second session, blood and CSF samples were drawn with the latter pending confirmation of absence of elevated intracranial pressure as determined by examination of structural MRI data acquired at the first session. Finally, participants were asked to fill in a set of self-report questionnaires online regarding potential factors of interest (e.g. demographics), including BMI.

## 2.2. MRI scan

Neuroimaging was carried out at the Center for Medical Imaging Science and Visualization at Linköping University Hospital, Sweden. A whole-head T1-weighted MPRAGE scan was conducted on a 3.0 Tesla Siemens MAGNETOM Prisma MR scanner with a 64-channel head coil (TR = 2300 ms, TE = 2.34 ms, FOV 250 × 187.2 mm, voxel size = 0.9 × 0.9 × 0.9 mm, flip angle = 8°). Participants were instructed to lie still during the 280-second scan and to close their eyes or look at soothing pictures on an in-scanner display.

## 2.3. MRI analyses

We obtained estimates of hippocampal, amygdalar, and cerebellar cortex volumes using automatic segmentation from FreeSurfer version 7 (<http://surfer.nmr.mgh.harvard.edu/>). GRIN2B encodes the protein of the GluN2B subunit of the NMDA receptor, through which the neurotoxic effects of QUIN are hypothesized to occur (Stone, 1993). High expression of GRIN2B is found in hippocampus and amygdala, while low GRIN2B expression occurs in the cerebellum ([gtexportal.org/home/gene/GRIN2B](http://gtexportal.org/home/gene/GRIN2B)). The cerebellum was chosen, therefore, as a control region. We reasoned that if there is a volumetric abnormality in MDD in

the hippocampus and amygdala, but not the cerebellum, then we can proceed with the assertion that QUIN excitotoxicity could play a role in volumetric reduction in hippocampus and amygdala. Segmentations were inspected visually to confirm their accuracy. Hippocampal and amygdalar volumes were obtained by summing volume estimates from left and right sides from subfield segmentations. Cerebellar cortex volumes were obtained by summing estimates from left and right cerebellar cortex from whole brain segmentations.

## 2.4. Blood and CSF sampling

Blood and CSF samples were obtained between 0800 and 1200 h. While blood was acquired from every participant, CSF was not, given that some participants did not consent to a lumbar puncture and some lumbar punctures were not successful. This resulted in fewer CSF samples in each group (MDD: n = 36, HC : n = 33). Venipuncture plasma samples were collected using BD Vacutainer EDTA tubes, immediately centrifuged for 10 min at 1500 g, 4 °C, apportioned into aliquots, and stored at –80 °C. A lumbar puncture was performed to obtain CSF as described in (Umhau et al., 2010). Briefly, following local anesthesia, a volume of up to 20 ml of CSF from the L3/L4 or L4/L5 interspace was collected in a silicone-coated tube and gently mixed to avoid gradient effects. Samples were then centrifuged at 2000 g, 8 °C, for 10 min to remove cells and other insoluble material, apportioned into aliquots and stored at –80 °C until further analyses.

## 2.5. Ultra performance liquid chromatography – Tandem mass spectrometry (UPLC-MS/MS)

KYN, KYNA, QUIN, PIC, 3-HK, NAM and nicotinic acid were quantified in CSF and plasma by UPLC-MS/MS system using a Xevo TQ-XS triple-quadrupole mass spectrometer (Waters, Manchester, UK) equipped with a Z-spray electrospray interface and a Waters Acquity UPLC I-Class FTN system (Waters, MA, USA). Full description of the CSF and plasma UPLC-MS/MS method, sample preparation and stability test of all metabolites can be found in (Schwieler et al., 2020; Trepci et al., 2020). In brief, the MS was operated in electrospray-positive multiple reaction monitoring (MRM) mode with a source temperature of 150 °C, capillary voltage of + 3.0 kV, desolvation temperature 650 °C, desolvation gas flow rate 1000 l/h and detector gain 1. Used column was Acquity HSS T3 2.1 × 150 mm, 1.8 μm (Waters, Product Number [PN]: 186,003,540) in a temperature of 50 °C. The two mobile phases were composed of A: 0.6% formic acid in water and B: 0.6% formic acid in methanol (UPLC grade). An isolator column (Waters, 2.1 × 50 mm column, PN: 186,004,476) was installed to retain contaminants from the mobile phase. The flow rate was set at 0.3 ml/min and the run time for each sample was 13.0 min. The *m/z* for the MRM transitions of each individual analyte were: KYN, 209 > 94; KYNA, 190 > 116; QUIN, 168 > 78; PIC, 124 > 78; NAM, 123 > 78; 3-HK, 225 > 110; Nicotinic acid, 124 > 80 and for the internal standards (IS): KYN-*d*<sub>4</sub>, 213 > 94; QUIN-*d*<sub>3</sub>, 171 > 81; KYNA-*d*<sub>5</sub>, 195 > 121; PIC-*d*<sub>4</sub>, 128 > 82; NAM-[<sup>13</sup>C<sub>6</sub>], 129 > 101; 3-HK-*d*<sub>3</sub>, 228 > 163 and Nicotinic acid-[<sup>13</sup>C<sub>6</sub>], 130 > 85. Nicotinic acid was detected in less than 50% of all samples and was included in the method to make sure we could distinguish it from the isomer PIC.

All metabolites measured in CSF and plasma samples were detected in higher concentrations than lowest level of quantification (LOQ, KYN, 0.25 nM; KYNA, 0.1 nM; QUIN, 5 nM; PIC, 3 nM; 3-HK, 1 nM; NAM, 10 nM). The variation (%CV) of quality controls within a run (intra-assay, n = 6, during 15 h) were less than 5% for all metabolites measured. The variation between two different experiments running over 2 days

**Table 1**  
Participant demographics.

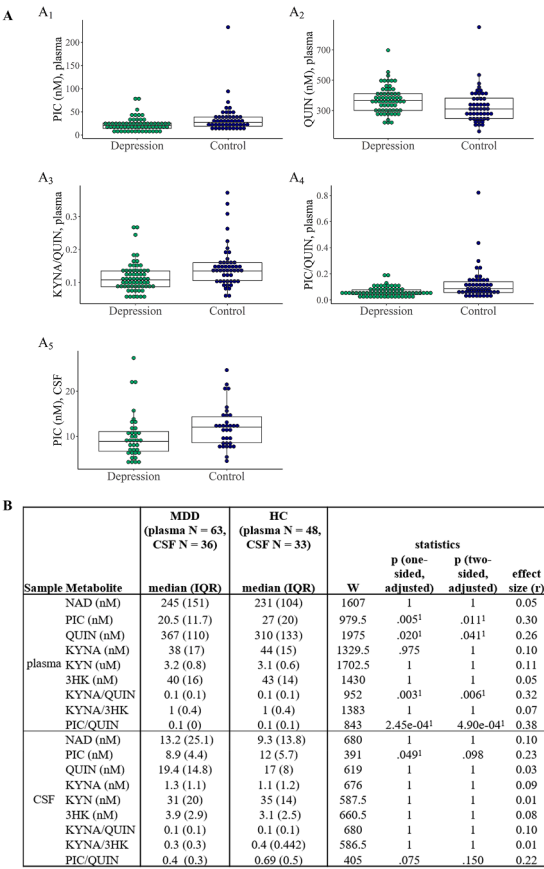
	MDD (n = 63)		Healthy Controls (n = 48)	p
	n (% of total)			
plasma	Gender (% women)	44 F (69.8)	34 F (70.8)	.910
	Antidepressant medication	28 (44.4)		
	Median (IQR)			
	Age	32 (24)	27.5 (17.2)	.160
	BMI	25.2 (8.4)	23.59 (3.3)	.038 <sup>1</sup>
	MADRS	28 (6.5)		
	MDD (n = 36)		Healthy Controls (n = 33)	p
	n (% of total)			
CSF	Gender (% women)	25 F (69.4)	22 F (66.7)	.805
	Antidepressant medication	16 (44.4)		
	Median (IQR)			
	Age	31 (18.3)	26 (17)	.271
	BMI	24.6 (6.7)	23.9 (2.5)	.211
	MADRS	28 (5)		

Note. P-values are based on Chi-square test (Gender) and Mann-Whitney-U test (Age, BMI). CSF was not available for all participants, resulting in smaller group sizes (36/33). BMI data of one MDD participant is missing and therefore based on 62/35 participants respectively. MDD: major depressive disorder, MADRS: Montgomery-Asberg Depression Rating Scale. <sup>1</sup>p < .05

(interassay n = 12) were less than 5% with an exception for NAM and PIC that had less than 10% variation.

2.6. Statistical analyses

Statistical analyses were conducted using RStudio (R Development Core Team, 2021) (including packages poolr (Cinar and PoolR, 2016), rcompanion (Mangiafico, 2016), and ggplot2(Wickham, 2016)). Data were visually inspected for measurement outliers and data distributions were assessed using Shapiro-Wilk tests. Given the non-normal distribution of the majority of KP metabolites, non-parametric statistical tests were conducted for all metabolites. Given strong a priori hypothesis regarding the directionality of the effects (Ogyu et al., 2018; Arnone et al., 2018; Marx et al., 2020), we conducted one-sided tests; given that the analyses conducted were not, however, direct replications of previously reported findings, we also present two-sided probabilities of our results. Using Mann-Whitney U tests, we compared KP metabolite levels and brain region volumes between MDD and HC groups. For the group comparison of KP metabolite levels we further, to account for multiple comparisons of potentially correlated dependent variables, calculated the number of effective tests,  $M_{eff}$  (Nyholt, 2004; Derringer, 2018), for plasma and CSF separately and adjusted statistical significance thresholds accordingly using Bonferroni correction. The effective number of tests,  $M_{eff}$ , was estimated to be 7 for both plasma and CSF KP metabolite levels, rendering a Bonferroni-corrected  $\alpha = 0.007$ . The  $M_{eff}$  method is explained further in the supplementary methods section. Group comparison p-values reported are accordingly adjusted to account for the family-wise error rate. Effect sizes were calculated using the non-



**Fig. 1.** A) Scatterplots and boxplots showing the distributions of kynurenine metabolite levels (nM) that differed significantly between patients with major depressive disorder (green) and healthy controls (blue). 1) plasma picolinic acid, 2) plasma quinolinic acid, 3) plasma kynurenic / quinolinic acid ratio, 4) plasma picolinic / quinolinic ratio, and 5) cerebrospinal fluid (CSF) picolinic acid; B) Median and interquartile range (IQR) of all kynurenine pathway metabolites levels in plasma and CSF of major depressive disorder (MDD) and healthy control (HC) participants. P-values are based on Mann-Whitney U tests. NAM: nicotinamide, PIC: picolinic acid, QUIN: quinolinic acid, KYNA: kynurenic acid, KYN: L-kynurenine, 3-HK: 3-hydroxy-kynurenine. CSF: cerebrospinal fluid (Lamers et al., 2020)p < .05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

parametric effect size estimate r, where  $r = Z/\sqrt{N}$  (Rosenthal et al., 1994).

To better understand the implications of KP metabolite abnormalities in MDD, we computed correlations of levels of KP metabolites that differed significantly between groups in either plasma or CSF against hippocampal, amygdalar, and cerebellar volumes as well as BMI using Spearman's rank correlation. Lastly, we assessed the relation between all peripheral and central KP metabolites in the two groups separately using Spearman's rank correlation.

Here, we assessed levels of KYN, KYNA, 3-HK, PIC, QUIN and NAM, as well as neuroprotective/neurotoxic ratios of KYNA/QUIN, KYNA/3-HK and PIC/QUIN in plasma and CSF.

2.6.1. Supplementary analyses

In the supplement, we present demographic and clinical characteristics of participants who did not consent to undergo a lumbar puncture, participants who had a successful lumbar puncture, and participants for whom the lumbar puncture was not successful. The latter two groups were compared statistically, with no significant differences observed (all  $p > 0.10$ ; Supplementary Table 1. In addition, given that about half of



Table 2

A) Group comparison of volumetric brain data and B) correlations between volumetric data and kynurenine metabolite levels for depressed and healthy samples.

A					
			MDD (n = 61)	HC (n = 48)	
Structure	p (one-sided)	p (two-sided)	Median (IQR) mm <sup>3</sup>	Median (IQR) mm <sup>3</sup>	
Hippocampus	.023 <sup>1</sup>	.046 <sup>1</sup>	7315.3 (917)	7489.9 (1010.5)	
Amygdala	.034 <sup>1</sup>	.069	3607.6 (418.6)	3771.7 (521.6)	
Cerebellar cortex	.104	.206	117810.6 (10012.3)	121911.8 (17314.6)	

B					
			MDD	HC	
			(n: plasma 61/ CSF 32)	(n: plasma 48/ CSF 33)	
Structure	metabolite	Spearman's ρ	p (one-sided)	p (two-sided)	Spearman's ρ
Hippocampus	plasma PIC	.06	.334	.669	.14
	plasma QUIN	.02	.574	.852	-.04
	plasma KYNA/QUIN	.28	.016 <sup>1</sup>	.031 <sup>1</sup>	.01
	plasma PIC/QUIN	.04	.375	.751	.12
	CSF PIC	.07	.341	.683	.16
Amygdala	plasma PIC	-.05	.361	.723	-.18
	plasma QUIN	-.03	.404	.809	-.16
	plasma KYNA/QUIN	.35	.003 <sup>1</sup>	.006 <sup>1</sup>	.03
	plasma PIC/QUIN	.06	.323	.647	.19
	CSF PIC	.11	.267	.534	-.02
Cerebellar cortex	plasma PIC	.08	.276	.821	-.25
	plasma QUIN	.14	.857	.287	-.12
	plasma KYNA/QUIN	.17	.095	.323	.05
	plasma PIC/QUIN	.13	.161	.750	-.01
	CSF PIC	-.03	.570	.860	-.33

Note. Correlations were computed only for kynurenine metabolites that differed significantly between MDD and HC samples. MDD: Major depressive disorder, HC: healthy control, QUIN: quinolinic acid, PIC: picolinic acid, KYNA: kynurenic acid, CSF: cerebrospinal fluid. <sup>1</sup>p < .05.

our MDD sample was taking antidepressant medication at the time of testing, we assessed associations between antidepressant use and KP metabolites (Supplementary Table 2). Further, for KP metabolites that differed significantly between groups, we assessed the relation to depression severity as measured using the Montgomery-Åsberg Depression Rating Scale (Montgomery and Åsberg, 1979) (Supplementary Fig. 2). In addition, we examined group differences in plasma markers of inflammation (IL-6 and TNF-α) and related those to KP metabolites (Supplementary Table 3). We further conducted parametric group comparisons, using age and gender as covariates (Supplementary Table 4). For KP metabolite ratios that correlated significantly with brain volumes, and for which individual KP-metabolite-by-brain-structure volume correlations were not presented, we tested if these correlations were driven by one of the KP metabolites by computing the correlation between individual KP metabolites and brain-structure volume (Supplementary Table 5). For our assessment of relations between levels of KP metabolites that differed between groups and brain-structure volumes, we ran an ancillary analysis controlling regional brain-structure volumes for total intracranial volume. We did this by correlating KP metabolites with ratios of hippocampal, amygdalar, and cerebellar volume relative to total intracranial volume (Supplementary Table 6). Lastly, we present analyses testing if BMI or depression severity (measured with the Montgomery-Åsberg Depression Rating Scale) mediate or moderate significant relations between KP metabolites and brain structure volumes (Supplementary Table 7).

3. Results

3.1. Group comparison of kynurenine pathway metabolites

Medians, interquartile ranges, and inferential statistics for all KP metabolites are presented in Fig. 1. In plasma, lower levels of PIC (one-sided p = .005, two-sided p = .011, r = 0.30), higher levels of QUIN (one-sided p = .020, two-sided p = .041, r = 0.26) and reduced levels of neuroprotective to neurotoxic metabolite ratios (KYNA/QUIN: one-sided p = .003, two-sided p = .006, r = 0.32; PIC/QUIN: one-sided p = 2.45e-04, two-sided p = 4.90e-04, r = 0.38) were found in MDD compared to HCs. In CSF, a significantly lower level of PIC (one-sided p = .049, two-sided p = .098, r = 0.23) was found in MDD compared to HCs. Importantly, none of these differences are attributable to psychotropic medication use in the MDD group (Supplementary Table 2).

3.2. MRI

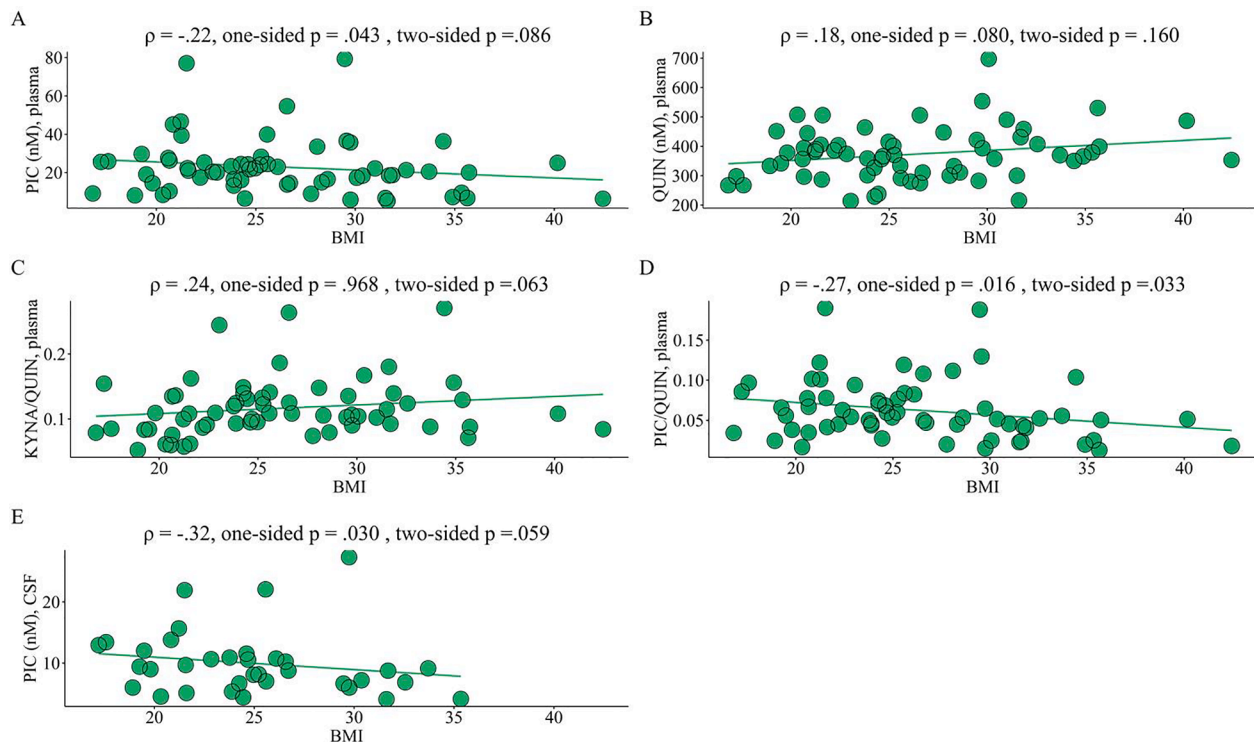
We lacked MRI data for two MDD patients; analyses were therefore conducted on data from 61 MDD patients with plasma samples, and 32 with CSF. All HC participants provided MRI data. In the MDD group we observed significantly smaller hippocampal volume (one-sided p = .023, two-sided p = .046), and smaller amygdalar volume (one-sided p = .034, two-sided p = .069). We also observed in the MDD group a significant positive relation between plasma KYNA/QUIN ratio and hippocampal volume (ρ = 0.28, one-sided p = .016, two-sided p = .031), as well as amygdalar volume (ρ = 0.35, one-sided p = .003, two-sided p = .006), indicating that as peripheral KYNA/QUIN ratios decrease in MDD, hippocampal and amygdalar volumes are reduced. In the HC group we did not observe any significant KP metabolite-by-brain-structure-volume correlations. Importantly, in spite of the proposed neuroprotective properties of PIC in addition to the CSF PIC reduction we observed in MDD, we did not observe significant correlations between CSF PIC and brain-structure volumes in MDD; all one-sided p > 0.27. See Table 2.

3.3. BMI

We lacked BMI for one MDD patient; we therefore assessed BMI-KP metabolite correlations using 62 plasma samples and 32 CSF samples for KP metabolites that significantly differed between MDD and HC groups. We observed higher BMI in the MDD relative to the HC group (one-sided p = 0.019, two-sided p = .038; see Table 1. BMI correlated significantly in MDD with plasma PIC (ρ = -0.22, one-sided p = .043, two-sided p = .086), plasma PIC/QUIN (ρ = -0.27, one-sided p = .016, two-sided p = .033), and CSF PIC (ρ = -0.32, one-sided p = .030, two-sided p = .059). We did not find significant correlations between BMI and plasma QUIN or KYNA/QUIN. See Fig. 2.

3.4. Relation between peripheral and central kynurenine pathway metabolites

In Fig. 3, we summarize the results and compare them to the findings of Haroon and colleagues (Haroon et al., 2020). Significant correlations between plasma and CSF KP metabolites in MDD patients were found for NAM (ρ = 0.44, one-sided p = .003, two-sided p = .007), PIC (ρ = 0.67, one-sided p = 3.88e-06, two-sided p = 7.76e-06), QUIN (ρ = 0.40, one-sided p = .008, two-sided p = .017), KYN (ρ = 0.46, one-sided p = .003, two-sided p = .005), and PIC/QUIN (ρ = 0.52, one-sided p = 6.37e-04, two-sided p = .001). In HCs, significant relations were found for PIC (ρ = 0.82, one-sided p = 2.14e-07, two-sided p = 5.75e-09), QUIN (ρ = 0.33, one-sided p = .032, two-sided p = .063), KYNA/3-HK (ρ = 0.30,



**Fig. 2.** Scatterplots showing distributions of BMI versus A) plasma picolinic acid, B) plasma quinolinic acid, C) plasma kynurenic /quinolinic acid ratio, D) plasma picolinic / quinolinic ratio, and E) cerebrospinal fluid picolinic acid. Spearman rho values with one-sided and two-sided significance levels shown. PIC: picolinic acid, QUIN: quinolinic acid, and KYNA: kynurenic acid.

one-sided  $p = .044$ , two-sided  $p = .089$ ), and PIC/QUIN ( $\rho = 0.79$ , one-sided  $p = 3.39e-07$ , two-sided  $p = 4.26e-08$ ).

#### 4. Discussion

In this investigation we compared diagnosed depressed patients and HC samples with respect to KP metabolites assessed at both peripheral and CNS levels. We further assessed the relations of abnormal KP metabolite levels in MDD to brain volumes and BMI, to better understand the implications of the primary findings in terms of neurotrophic and neurotoxic effects, as well as metabolic disturbances in MDD. We replicated some previous findings of elevated neurotoxic and reduced neurotrophic KP metabolites in the blood in depression. Indicating that at least some of these abnormalities also occur at the CNS level, we present the novel finding of reduced PIC concentrations in MDD. While decreased peripheral KYNA/QUIN ratios predicted decreased brain volumes in MDD, we observed no such relation between reductions in central levels of PIC and brain-structure volume reduction in depression. We also describe the additional novel finding that the extent of kynurenine metabolite abnormality both peripherally and centrally correlates in the directions predicted with the extent of general metabolic disturbance in depression as assessed with BMI. Finally, we observed significant correlations between some, but not all KP metabolite levels in the peripheral and central nervous system.

Our partial replication of findings of alterations in KP metabolite levels in the blood in depression strengthens the hypothesis that metabolites of the kynurenine pathway play a consistent role in the pathophysiology of MDD. While the design of our study does not afford

testing causal hypotheses, we point out here that among a large array of serotonergic and inflammation- and stress-related genes that could have been implicated in a recent genome-wide association study of MDD, only the KYNU gene—which codes for the enzyme kynureninase—was identified (Howard et al., 2019).

While recent meta-analyses of peripheral KP metabolite levels in MDD have shown robust reductions of KYN, KYNA, and KYNA/3-HK ratios (Ogyu et al., 2018; Arnone et al., 2018; Marx et al., 2020), we did not replicate these findings. Previous research has shown that antidepressant medication increases KYNA and KYNA/3-HK ratio levels in astroglial cells in a time-dependent manner (Kocki et al., 2012) and is further related to increases of central levels of KYN and KYNA in bipolar patients. In this study, approximately half of the MDD participants were stably medicated. While this is speculative, we found near-significant lower levels of plasma KYNA in antidepressant-free MDD patients (Supplementary Table 2). Our inclusion of MDD patients using antidepressant medications potentially rendered the MDD-HC comparison insensitive. Nonetheless, the treatment effect detected provides a partial replication of previous pre-clinical work (Kocki et al., 2012) and does so in a clinical sample. The effect detected in our currently depressed MDD sample also indicates that putative modulation of kynurenine pathway metabolites by antidepressant medication is insufficient to bring about remission of depressive episodes in some cases.

The reductions of PIC in the periphery that we observe here have been previously seen in MDD patients (Colle et al., 2020; Ryan et al., 2020); our CNS-level findings converge with those observed in patients showing suicidal behavior (Brundin et al., 2016). It has been shown in preclinical models that PIC attenuates the neurotoxic effects of QUIN without

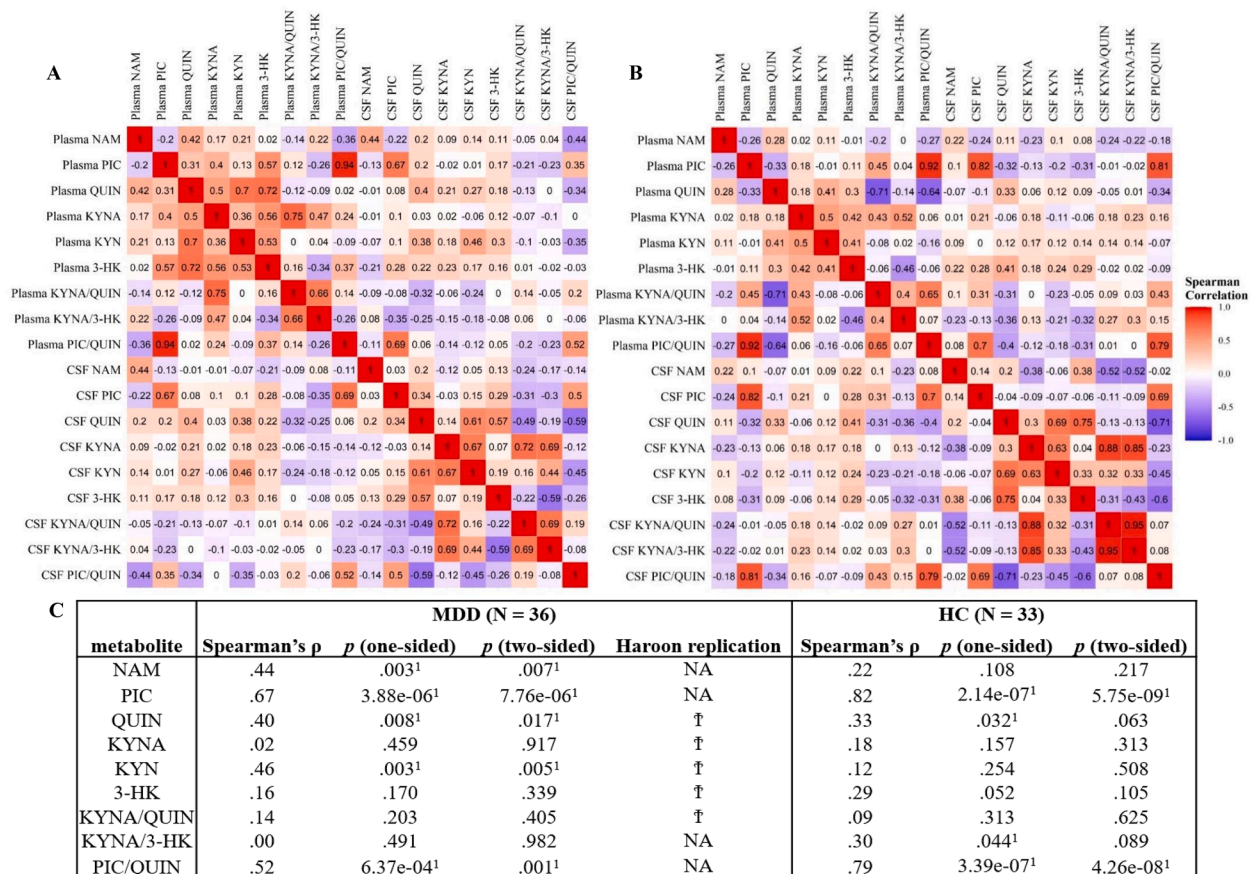
affecting its neuroexcitant properties (Beninger et al., 1994; Kalisch et al., 1994), which leaves the underlying mechanism of the neuroprotective effects of PIC unclear (Jhamandas et al., 2000; Vrooman et al., 1993). Given the protective effects of PIC in relation to QUIN and, more broadly, in the context of intracerebral infection (Blasi et al., 1993) we advocate for additional inquiry into the pathways underlying these effects.

Among the novel findings presented here, the most consequential is likely that both peripheral and central changes in KP metabolite levels in MDD were found to be associated with metabolic disturbance, as operationalized by elevated BMI in depression. This is important when considered in relation to the case for reconceptualizing vegetative, atypical depression as a metabolic-inflammatory subtype of MDD (Lamers et al., 2020; Milanese et al., 2020). This subtype has greater heritability and familial aggregation than the melancholic subtype and is characterized by elevated metabolic and inflammatory signaling, whereas melancholic depression is associated with a greater stress response (Lamers et al., 2013). While connections between stress-related, melancholic MDD and neural-level dysfunction have been identified and widely discussed (Sapolsky, 1996), the present findings could help bridge the gap between a putative metabolic-inflammatory subtype and abnormal brain function. While our cross-sectional investigation does not allow us to specify the directionality of the relation between metabolic and inflammatory factors in MDD, future experimental studies assessing the metabolic effects of inflammatory challenges and vice-versa can identify critical causal factors in what is currently considered an aggregate immuno-metabolic syndrome.

In the context of understanding brain-level dysfunction in MDD, the present results indicate that pathways connecting kynurenine

dysfunction to neural dysfunction in MDD are less direct than previously hypothesized. We found that only reduced peripheral KYNA/QUIN ratios predicted reductions in brain structure volume in MDD. This is unexpected, given that the neurotoxic effect of QUIN has been suggested to be mediated at the neural level. Since PIC blocks the neurotoxic effects of QUIN (Beninger et al., 1994; Grant et al., 2009; Kalisch et al., 1994), we expected to find reduced brain-structure volumes in relation to the reduced central PIC we observed in MDD patients. Assaying CSF samples provides an important and relatively practical means for understanding the relation between abnormal KP metabolite levels and neural abnormalities in MDD. However, we hasten to point out that, in their best light, CSF-derived markers are only general indicators of KP metabolite levels in the interstitial spaces of the brain and that, to the best of our knowledge, the relation between CSF and brain levels of the KP metabolites has not yet been thoroughly assessed. One study in gerbils found that QUIN levels were positively correlated between CSF and brain tissue (Heyes and Morrison, 1997); another study in rats, however, found that increases in brain levels of KYNA after inhibition of the metabolism of KYN to 3-HK were not reflected in CSF (Erhardt and Engberg, 2002). More comprehensive analyses of the relation between blood, CSF, and brain-tissue levels of all KP metabolites will be required before we can determine how well a proxy CSF levels of KP metabolites are for levels in their respective loci of action in the brain.

Finally, our results indicate that levels of some peripheral KP metabolites might serve as proxies for central KP metabolite levels in patients with MDD (NAM, PIC, QUIN, KYN, and PIC/QUIN) and HCs (PIC, QUIN, KYNA/3-HK, and PIC/QUIN). We replicated previously reported relations between plasma and CSF levels of all metabolites assessed by



**Fig. 3.** Correlations between all plasma and CSF kynurenine pathway metabolites in A) major depressive disorder and B) healthy controls. C) Peripheral to central correlations on a per metabolite basis and corresponding one- and two-sided p-values. NAD: nicotinamide, PIC: picolinic acid, QUIN: quinolinic acid, KYNA: kynurenic acid, KYN: L-kynurenine, 3-HK: 3-hydroxy-kynurenine. 1p < .05, † = replication of Haroon et al (Haroon et al., 2020).



Haroon et al. (Haroon et al., 2020) in MDD patients, and extended their findings by assessing additional metabolites in MDD in addition to the relation of peripheral and central metabolite levels in HCs. Differences in peripheral-central correlations between patients with MDD and HCs could be due to a more permeable blood–brain barrier in MDD. As mentioned previously, only some KP metabolites can pass the blood–brain barrier. Molecular changes in the blood–brain barrier, such as disrupted tight junctions, have been reported in MDD (Dudek et al., 2020; Greene et al., 2020), and could lead to heightened peripheral-central molecular exchange in this disorder. The data presented here will be useful for informing future research efforts with respect to whether assumptions about peripheral-central correspondences in the kynurenine pathway are warranted.

## 5. Limitations

Given that food intake can alter kynurenine pathway metabolite levels, a key limitation of the study is that samples were not acquired during fasting. Further, the size of the patient and control samples assessed prohibited conducting potentially informative subgroup analyses comparing, for example, associations between sex and KP metabolites as a function of diagnostic group. In addition, appropriate behavioural measures and a more rigorous assessment of depression subtypes would have allowed us to test in this investigation whether changes in KP metabolites are related to subtypes of MDD. Furthermore, our KP metabolite panel did not include all metabolites along the KP. Therefore, associations between MDD and potentially important KP metabolites such as xanthurenic and cinnabarinic acid remain uninvestigated. Lastly, the number of participants providing CSF in each group was small. A post-hoc power analysis assuming a moderate effect size using the largest absolute rho value of all correlation analyses (CSF PIC  $\times$  BMI;  $\rho = 0.32$ ) gave a power of 0.57 (for one-sided tests) and 0.45 (for two-sided tests). Therefore, strong conclusions on differences between correlations with peripherally versus centrally measured KP metabolite levels and BMI and brain-structure volume are not statistically warranted.

## 6. Conclusion

This study is the first to compare both peripheral and central kynurenine pathway markers in diagnosed depressed and never disordered samples. We confirmed previous findings from blood assays and show that at least some of these extend to the CSF level. Our observed associations between kynurenine pathway abnormalities and brain-structure volume require further investigation with larger samples.

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## Declaration of Competing Interest

Ms Paul, Dr Schwieler, Ms Boda, Ms Trepici, Mr Kämpe, Ms Asratian, Dr Holm, Mr Yngve, Dr Hamilton, and Dr Samuelsson declare no potential conflict of interest. Dr Erhardt discloses grant support from AstraZeneca and Jansen Pharmaceuticals as principal investigator and

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbi.2022.01.002>.

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