Journal of the Neurological Sciences 454 (2023) 120819

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

The role of the kynurenine pathway in cognitive functioning after stroke: A prospective clinical study

Lieke Bakker[a,b,c,d], Inez H.G.B. Ramakers[a,b,c,d], Simone J.P.M. Eussen[e,f,g], Kyonghwan Choe[b,c,d,1], Daniel L.A. van den Hove[b,c,d,h], Gunter Kenis[b,c,d], Bart P.F. Rutten[b,c,d], Robert J. van Oostenbrugge[c,f,i], Julie Staals[f,i], Arve Ulvik[j], Per M. Ueland[j], Frans R.J. Verhey[a,b,c,d], Sebastian Köhler[a,b,c,d,*]

[a] *Alzheimer Center Limburg, Maastricht University, 6229 ET Maastricht, the Netherlands*
[b] *Department of Psychiatry and Neuropsychology, Maastricht University, 6229 ER Maastricht, the Netherlands*
[c] *School for Mental Health and Neuroscience (MHeNs), Maastricht University, 6229 ER Maastricht, the Netherlands*
[d] *EURON European Graduate School of Neuroscience, 6229 ER Maastricht, the Netherlands*
[e] *Department of Epidemiology, Maastricht University, 6229 HA Maastricht, the Netherlands*
[f] *School for Cardiovascular Diseases (CARIM), 6229 ER Maastricht, the Netherlands*
[g] *Care and Public Health Research Institute (CAPHRI), 6229 ER Maastricht, the Netherlands*
[h] *Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, 97080 Wuerzburg, Germany*
[i] *Department of Neurology, Maastricht University Medical Center (MUMC+), 6229 HX Maastricht, the Netherlands*
[j] *BEVITAL AS, 5021 Bergen, Norway*

ARTICLE INFO

Keywords:
Kynurenine pathway
Kynurenines
Stroke
Post-stroke cognitive impairment
Cognition
Plasma

ABSTRACT

Background: The kynurenine pathway is the main metabolic pathway of tryptophan degradation and has been associated with stroke and impaired cognitive functioning, but studies on its role in post-stroke cognitive impairment (PSCI) are scarce. We aimed to investigate associations between metabolites of the kynurenine pathway at baseline and post-stroke cognitive functioning over time.

Methods: Baseline plasma kynurenines were quantified in 198 stroke patients aged 65.4 ± 10.8 years, 138 (69.7%) men, who were followed up over a period of three years after stroke. Baseline and longitudinal associations of kynurenines with PSCI and cognitive domain scores were investigated using linear mixed models, adjusted for several confounders.

Results: No evidence of associations between kynurenines and odds of PSCI were found. However, considering individual cognitive domains, higher plasma levels of anthranilic acid (AA) were associated with better episodic memory at baseline (β per SD 0.16 [0.05, 0.28]). Additionally, a linear-quadratic association was found for the kynurenic acid/ quinolinic acid ratio (KA/QA), a neuroprotective index, with episodic memory (Wald $\chi^2 = 8.27$, $p = .016$). Higher levels of KA were associated with better processing speed in women only ($p_{interaction} = .008$; β per SD 0.15 [95% CI 0.02, 0.27]). These associations did not change over time.

Conclusions: Higher levels of KA, AA and KA/QA were associated with better scores on some cognitive domains at baseline. These associations did not change over time. Given the exploratory nature and heterogeneity of findings, these results should be interpreted with caution, and verified in other prospective studies.

* Corresponding author at: Universiteitssingel 40, 6229 ER Maastricht, the Netherlands.
E-mail addresses: lieke.bakker@maastrichtuniversity.nl (L. Bakker), i.ramakers@maastrichtuniversity.nl (I.H.G.B. Ramakers), simone.eussen@maastrichtuniversity.nl (S. J.P.M. Eussen), k.choe@maastrichtuniversity.nl (K. Choe), d.vandenhove@maastrichtuniversity.nl (D.L.A. van den Hove), g.kenis@maastrichtuniversity.nl (G. Kenis), b.rutten@maastrichtuniversity.nl (B.P.F. Rutten), r.van.oostenbrugge@mumc.nl (R.J. van Oostenbrugge), j.staals@mumc.nl (J. Staals), arve.ulvik@bevital.no (A. Ulvik), per.ueland@uib.no (P.M. Ueland), f.verhey@maastrichtuniversity.nl (F.R.J. Verhey), s.koehler@maastrichtuniversity.nl (S. Köhler).
[1] Present address: College of Natural Sciences, Division of Life Science and Applied Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea.

https://doi.org/10.1016/j.jns.2023.120819
Received 25 November 2022; Received in revised form 26 September 2023; Accepted 28 September 2023
Available online 4 October 2023

1. Introduction

Stroke is the second largest cause of mortality and a major cause of disability worldwide. According to the Global Burden of Disease Study, stroke accounted for an estimated 13.7 million new cases and 5.5 million deaths in 2016 [1]. In high-income countries, mortality rates have decreased, likely due to improved general health care, stroke detection and secondary prevention strategies, increasing the number of stroke survivors and those with stroke-related disabilities [2,3]. Next to problems with speech, loss of function, and visual deficits, a common residual consequence of stroke includes cognitive impairment [4]. The biochemical pathways in post-stroke cognitive impairment (PSCI) are largely unknown. Due to its role in inflammation, (cerebro)vascular damage, and neurodegenerative mechanisms, the kynurenine pathway may be involved [5–11].

The kynurenine pathway (Fig. 1) is the main metabolic pathway of tryptophan (TRP) degradation. Formation of kynurenine (KYN) is catalyzed by indoleamine 2,3- dioxygenase (IDO), which is activated by pro-inflammatory cytokines, or by tryptophan 2,3-dioxygenase (TDO), activated by corticosteroids and elevated TRP [12–14]. Activated microglia and macrophages are producing a spectrum of kynurenines, including quinolinic acid (QA), which is an *N*-methyl-D-aspartate (NMDA) receptor agonist and a pro-oxidant [15]. The result is an excitotoxicity of glutamatergic neurons and the induction of endothelial apoptosis and dysfunction [9]. The latter can result in blood brain barrier (BBB) lesions [9] and, consequently, may cause vascular damage in the brain [8]. Although most kynurenines cannot readily cross the BBB under normal circumstances, they may cross the BBB where its microvascular endothelium is damaged [16,17].

There are few studies that suggest that IDO activation, reflected by the KYN/TRP (KTR) ratio, and the ratio between neuroprotective and neurotoxic metabolites, especially kynurenic acid (KA) and QA, might play a role in PSCI [18–20]. However, these studies are mainly cross-sectional and are restricted to a limited set of peripheral circulating kynurenines [18–20]. Therefore, it is still largely unclear whether the kynurenine pathway plays a role in cognitive symptoms after stroke. The present study aimed to investigate metabolites of the kynurenine pathway as potential early markers for cognitive impairment and as predictors of changes in cognitive functions over time in a clinical stroke population up to 3 years after stroke.

2. Materials and methods

2.1. Study population and design

Longitudinal data were derived from patients who participated in the Cognition and Affect after Stroke – a Prospective Evaluation of Risks (CASPER) study between 2013 and 2018. For the present study, we included those patients for whom baseline kynurenines in fasting plasma were quantified. The rationale and procedures of the study have previously been described in more detail elsewhere [21]. In short, the CASPER study is a prospective cohort study in patients after ischemic or hemorrhagic stroke who were admitted to the Stroke Unit of the Maastricht University Medical Center+ (MUMC+), the Stroke Unit of Zuyderland Medical Center, Sittard-Geleen, and Heerlen, or who visited the Transient Ischemic Attack clinic of MUMC+, the Netherlands. Inclusion criteria at baseline were ischemic or hemorrhagic stroke, age of 40 and older, a Mini-Mental-State Examination score of ≥15, and sufficient knowledge of the Dutch language. Exclusion criteria consisted of a subarachnoidal, traumatic or primary intraventricular hemorrhage, severe aphasia, evidence of pre-stroke dementia, and a preexisting psychiatric or neurological diagnosis known to affect cognition (such as schizophrenia, bipolar disorder, epilepsy, Parkinson's disease or substance abuse).

At baseline (10 to 12 weeks after stroke, T0), all patients underwent a comprehensive neuropsychological evaluation of cognitive and affective problems, a physical examination, a magnetic resonance imaging (MRI) of the brain, and collection of blood samples. The neuropsychological evaluation was repeated 6 (T1), 12 (T2) and 36 (T3) months later. All patients gave written informed consent prior to testing. The Medical Ethics Review Committee of MUMC+ provided ethical approval for the CASPER study. Research was conducted in accordance with the principles of the Declaration of Helsinki 59th WMA General Assembly, Seoul (October 2008) and the Dutch Medical Research Involving Human Subjects Act (WMO).

2.2. Blood collection, biochemical analysis

Processing and storage of plasma samples was done according to standard operating procedures at the Biobank Maastricht, the ISO (9001–2015) certificated Biobank of the MUMC+. Fasting blood samples were collected at baseline in 6 ml EDTA tubes, then centrifuged at 1000*g* for 15 min, followed by 10 min at 10000 g, and aliquoted into 0.5 ml vials, and stored at −80 °C in the Biobank until analysis. Liquid

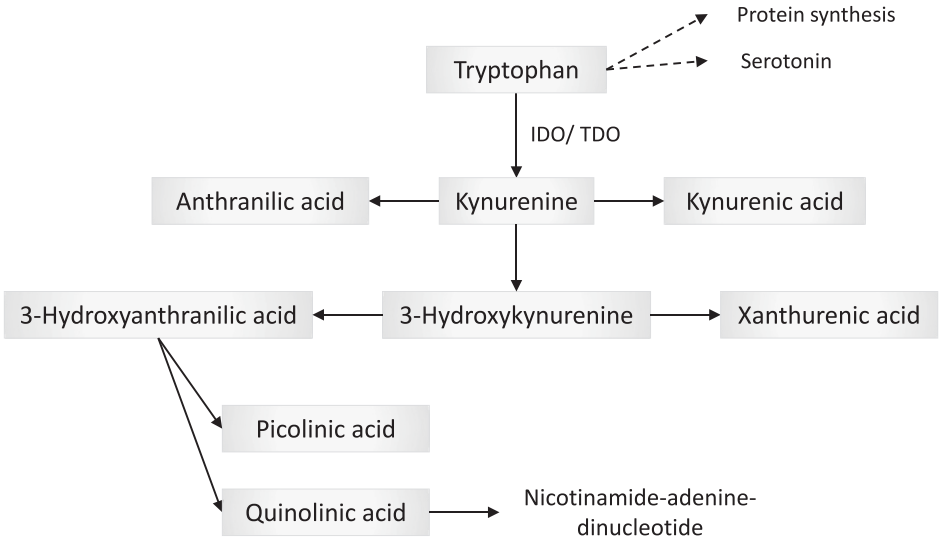

Fig. 1. The kynurenine pathway, a simplified representation.

chromatography-tandem mass spectrometry (LC-MS/MS; Bevital AS, Bergen, Norway) was used to determine plasma concentrations of TRP, KYN, 3-hydroxykynurenine (3-HK), KA, xanthurenic acid (XA), anthranilic acid (AA), 3-hydroxyanthranilic acid (3-HAA), picolinic acid (PIC) and QA [22,23]. For specific procedural details, see [23]. Limits of detection for the kynurenine metabolites were 0.4–7 nmol/l. Within-day and between-day coefficients of variation were 1.8–9.5% and 4.9–16.9%, respectively [23]. Additionally, ratios of KTR (KYN/TRP*1000) and KA/QA were calculated. The KTR is often used as an indication of IDO activity, whereas the KA/QA ratio represents a neuroprotective index through opposite effects of KA and QA on NMDA receptors.

Plasma B2 (riboflavin) and B6 (pyridoxal 5′-phosphate (PLP)) vitamers, neopterin and creatinine were determined in a single aliquot with kynurenine concentrations. Plasma creatinine was used to estimate glomerular filtration rate (eGFR), a measure of renal functioning, by the Chronic Kidney Disease Epidemiology collaboration equation (CKD-EPI; ml/min/1.73 m2) [24].

Lastly, C-reactive protein (CRP, μg/ml), serum amyloid A (SAA, μg/ml), intercellular adhesion molecule-1 (ICAM-1, ng/ml), IL-6 (pg/ml), IL-8 (pg/ml), and tumor necrosis factor alpha (TNF-α, pg/ml) were determined in EDTA plasma by multiplex assays from Meso Scale Discovery (Rockville, MD 20860 USA).

2.3. Neuropsychological assessment

Neuropsychological assessment consisted of a standardized test battery, including a wide variety of cognitive tests (Table 1). All raw scores were normalized into z-scores using age, gender and education adjusted regression-based norm scores. Where necessary, test scores were inverted so that higher scores indicated better cognitive functioning. Domain scores were calculated by averaging the z-scores of the tests of that particular domain (Table 1). PSCI was defined as a score of >1.5 standard deviations below the general population mean in one or more cognitive domains, based on available norm scores for age, gender, and level of education for the Dutch general population [25,26].

2.4. General characteristics and covariates

Structured interviews with the patient and informant obtained information on educational level (low, intermediate, high), alcohol use (none, low, high), smoking behavior (never, former, current), history of diabetes type 2, history of cardiovascular disease, presence of hypertension, and medication use. Height and weight were measured. Presence of a minor (MIND) or major depressive disorder (MDD) was assessed with the Mini International Neuropsychiatric Interview (MINI) [28]. The MINI is a semi-structured interview based on DSM-IV criteria.

Table 1
Neuropsychological assessment.

Domain	Instrument
Global cognition	Mini-Mental State Examination[27]
Episodic memory	15-Word Verbal Learning Test (immediate recall, delayed recall and recognition)[25]
Working memory	15-Word Verbal Learning Test (Trial 1)[25]
	Digit span (total; WAIS-III)
Information processing speed	Digit Symbol Substitution Test
	Trail Making Test (part A)[26]
Executive functioning	Trail Making Test (part B)[26]
	Verbal Fluency (animals and professions; GIT-2)
Depression	MINI[28]
	MADRS[29]
Functional ability	Barthel Index[30]

GIT-2 = Groninger Intelligence Test 2, MADRS = Montgomery-Åsberg Depression Rating Scale, MINI = Mini International Neuropsychiatric Interview, WAIS = Wechsler Adult Intelligence Scale.

The Montgomery-Åsberg Depression Rating Scale (MADRS) [29] was used to investigate the severity of depressive symptoms. The MADRS is a clinician-rated questionnaire that consists of 10-items and a maximum score of 60. Post-stroke depression (PSD) was defined as MADRS ≥7 or minor or major depressive disorder as assessed with the MINI as was done previously [31]. The Barthel Index [30] is a questionnaire with a maximum score of 20 points and was used to evaluate impairments in activities of daily living.

2.5. Data analysis

Statistical analyses were done using STATA version 17 for MacOS. Differences in baseline characteristics between patients with and without PSCI were investigated using ANOVA (Mann-Whitney *U* tests when assumptions were violated). For categorical variables, χ^2-squared tests were used. Next, linear mixed models, including a random intercept, were used to investigate associations between baseline levels of TRP, kynurenines, ratios, and neopterin with cognitive domain scores at baseline and over time. To investigate associations with PSCI, non-linear mixed models for binary outcomes with a logit-link function were used. To investigate potential non-linear associations, a quadratic term of the metabolites was included in the analyses. Goodness of fit was determined by likelihood ratio tests.

All analyses were adjusted for covariates. The order of these models was as follows: 1 = demographics (age, sex, educational level) and eGFR, 2 = model 1 + lifestyle factors (BMI, alcohol consumption and smoking behavior), 3 = model 2 + stroke type and disability (Barthel index). We expected that a current depression, hypertension, type 2 diabetes, cardiovascular disease, B vitamins and inflammatory markers might be potential mediators along the pathway of kynurenine concentrations and cognitive functioning, and that inclusion of these variables could result in overcorrection. Therefore, we controlled for these variables in a separate model (model 4). A composite score including inflammation markers (CRP, SAA, ICAM-1, IL-6, IL-8, and TNF- α) was calculated by transforming concentrations into z-scores and averaging. Lastly, this study investigated interactions with age and sex. For interactions with age, both age as a continuous variable and quartiles of age were investigated. Analyses were exploratory and did not control for multiple testing.

3. Results

3.1. Characteristics of study population

Of the 250 patients enrolled in the CASPER study, kynurenines were analyzed in 200 patients with available blood samples. Compared to those for whom no kynurenines were determined, patients included in the analyses were significantly younger, more often male, used alcohol more often, scored higher on the Barthel index, and performed better on all cognitive domains except for processing speed (Supplementary Table 2).

Two patients had extreme metabolite levels. For one of these patients these levels were likely due to severe kidney disease, whereas the other had very high levels of pro-inflammatory cytokines. Both patients were excluded from the analyses, leaving 198 patients (Fig. S1). Out of these 198 patients, 175 (88.4%) completed T1, 182 (91.9%) completed T2 and 95 (48.0%) completed T3. All three follow-up measurements were completed by 88 patients (44.4%). This last group had a significantly higher educational level (Supplementary Table 3) and higher levels of TRP and 3-HAA (Supplementary Table 4) compared to those who did not complete all follow-ups, but they did not differ on any other characteristic.

At baseline, 127 (64.1%) patients were classified as cognitively impaired (PSCI). This group had modestly lower scores on the Barthel index compared to those without cognitive impairment. No differences were found with respect to any of the other general characteristics, nor

with respect to concentrations of any of the kynurenines, PLP or riboflavin (Tables 2 and 3).

3.2. Associations between baseline metabolite levels and PSCI

There were no significant overall associations of any of the kynurenines with odds of PSCI. However, when interactions with age and sex were investigated for pooled associations over all time points, significant interaction effects were found for odds of PSCI between age and KYN ($p_{interaction}$ = .042) and between age and neopterin ($p_{interaction}$ = .023). For both KYN and neopterin, this interaction with age was significant for baseline PSCI ($p_{interaction}$ = .026 and .018, respectively) and did not moderate associations over time. Post hoc tests showed that, at baseline, higher levels of neopterin were associated with higher odds of PSCI in the youngest group only (OR per SD 20.27 [95% CI 2.38, 172.68]; Fig. 2). For KYN, a similar trend was found of an association with higher odds of PSCI in the youngest age group, but this association did not reach statistical significance (2.39 [0.76, 7.50]), neither did the association with lower odds of PSCI in the highest age group (0.28 [0.05, 1.54]).

Table 2
General characteristics and cognitive domain scores of patients with- and without post-stroke cognitive impairment.

	Total (n = 198)	No PSCI (n = 67)	PSCI (n = 127)	P value
Demographics				
Age, years	65.4 ± 10.8	66.0 ± 11.4	65.0 ± 10.6	0.547
Men, n (%)	138 (69.7)	47 (70.2)	87 (68.5)	0.814
Educational level, n (%)				0.539
Low	78 (39.6)	23 (34.3)	54 (42.5)	
Intermediate	67 (34.0)	25 (37.3)	41 (32.3)	
High	52 (26.4)	19 (28.4)	32 (25.2)	
Lifestyle factors				
Alcohol use, n (%)				0.658
None	62 (31.3)	19 (28.4)	42 (33.1)	
Low	115 (58.1)	42 (62.7)	71 (55.9)	
High	21 (10.6)	6 (9.0)	14 (11.0)	
Smoking, n (%)				0.872
Never	46 (23.2)	17 (25.4)	28 (22.0)	
Former (> 6 months)	116 (58.6)	38 (56.7)	75 (59.1)	
Current	36 (18.2)	12 (17.9)	24 (18.9)	
Cardiovascular risk factors				
BMI, kg/m^2	27.3 ± 4.5	27.0 ± 4.5	27.5 ± 4.6	0.495
eGFR, ml/min [1.73 m]$^{-2}$	79.4 ± 15.0	77.7 ± 15.5	80.3 ± 14.8	0.265
Stroke type, n (%)				0.928
Ischemic	186 (93.9)	63 (94.0)	119 (93.7)	
Hemorrhagic	12 (6.1)	4 (6.0)	8 (6.3)	
Stroke disability (Barthel index)	19.7 ± 1.0	19.9 ± 0.4	19.6 ± 1.2	**0.049**
Comorbidities, n (%)				
PSD	72 (36.4)	25 (37.3)	47 (37.0)	0.967
MDD	10 (5.1)	3 (4.5)	7 (5.6)	0.748
MIND	10 (5.1)	4 (6.0)	6 (4.7)	0.709
Hypertension	141 (71.2)	47 (70.2)	92 (72.4)	0.736
Diabetes mellitus, type 2	27 (13.6)	5 (7.5)	22 (17.3)	0.059
CVD	80 (40.4)	25 (37.3)	53 (41.7)	0.551
Medication use, n (%)				
Antihypertensive agent	135 (68.9)	44 (66.7)	89 (70.6)	0.571
Cholesterol-lowering agent	160 (81.6)	52 (78.8)	104 (82.5)	0.527
MMSE	28.2 ± 1.7	28.9 ± 1.1	27.9 ± 1.7	< **0.001**
Cognitive domain scores				
Episodic memory	0.13 ± 0.86	0.59 ± 0.61	−0.12 ± 0.87	< **0.001**
Working memory	0.09 ± 0.76	0.38 ± 0.72	−0.05 ± 0.75	< **0.001**
Processing speed	−0.01 ± 0.41	0.03 ± 0.43	−0.03 ± 0.41	0.327
Executive functioning	0.04 ± 0.62	0.37 ± 0.53	−0.14 ± 0.59	< **0.001**

Data are presented as means ± SD or n (%).
Difference between groups were investigated with ANOVA and Chi^2 tests. Data on cognitive status was available for 194 patients.
Abbreviations: BMI = Body Mass Index, eGFR = glomerular filtration rate, PSCI = post-stroke cognitive impairment, PSD = post-stroke depression, MDD = major depressive disorder, MIND = minor depression.

Table 3
Baseline concentrations of kynurenines, inflammation markers and B-vitamins of patients with- and without post-stroke cognitive impairment.

	Total (n = 198)	No PSCI (n = 67)	PSCI (n = 127)	P value
Metabolite				
TRP, μM	61.1 [12.1]	61.3 [11.0]	61.0 [12.6]	0.661
KYN, μM	1.86 [0.55]	1.82 [0.56]	1.86 [0.56]	0.907
3-HK, nM	51.5 [27.2]	51.7 [26.9]	50.8 [22.3]	0.491
KA, nM	58.0 [27.0]	58.1 [34.9]	58.6 [25.6]	0.854
XA, nM	13.1 [9.2]	13.0 [9.4]	13.2 [9.8]	0.843
AA, nM	15.5 [5.4]	15.8 [5.2]	15.3 [5.9]	0.732
3-HAA, nM	44.1 [21.8]	43.1 [23.8]	44.2 [21.5]	0.930
PIC, nM	34.1 [15.6]	34.1 [16.5]	34.1 [15.6]	0.885
QA, nM	477 [239]	482 [259]	477 [242]	0.898
KA/QA	0.12 [0.06]	0.12 [0.06]	0.12 [0.06]	0.535
Low-grade inflammation[a]	−0.06 [0.83]	−0.04 [0.97]	−0.05 [0.70]	0.985
Inflammation markers				
KTR	30.5 [8.7]	30.6 [12.6]	30.5 [8.1]	0.864
Neopterin, nM	17.5 [8.6]	15.7 [9.6]	18.2 [7.9]	0.129
CRP, μg/ml	1.91 [3.37]	1.74 [3.88]	1.93 [2.70]	0.625
SAA, μg/ml	1.89 [2.81]	1.80 [1.61]	1.97 [3.09]	0.286
ICAM-1, ng/ml	441.66 [153.25]	442.25 [167.96]	442.59 [148.84]	0.843
TNF-α, pg/ml	2.58 [0.99]	2.61 [1.27]	2.59 [0.93]	0.780
IL-6, pg/ml	0.88 [0.84]	0.91 [0.83]	0.85 [0.87]	0.855
IL-8, pg/ml	3.49 [1.98]	3.49 [1.83]	3.49 [2.14]	0.919
B-vitamins				
PLP, nM	37.3 [28.5]	37.4 [26.0]	37.1 [29.8]	0.245
Riboflavin, nM	9.02 [10.24]	9.89 [10.85]	8.79 [9.57]	0.297

Data are presented as median [IQR]. A Mann-Whitney U test was used to investigate differences in levels of metabolites, inflammation markers and B vitamers between patients with and without post-stroke cognitive impairment. For 3-HK, AA, 3-HAA, and neopterin data was available for 196 patients. Data on cognitive status was available for 194 patients.
[a] Composite score of CRP, SAA, ICAM-1, TNF-α, IL-6, and IL-8 (transferred into z-scores and averaged).

3.3. Associations between baseline metabolite levels and cognitive domain scores

An overview of all baseline associations between metabolites and cognitive domain scores for the fully adjusted model can be found in Supplementary Fig. 2. Averaged over all time points, higher levels of AA were associated with better episodic memory (β per SD 0.15 [95% CI 0.04, 0.26]) and working memory (0.11 [0.02, 0.20]) in main model 3. Additionally, a linear quadratic association was found of XA with working memory (Wald χ^2 = 7.91, p = .019), and of KA/QA with episodic memory (Wald χ^2 = 7.37, p = .025; Table 4).

To understand temporal relationships, subsequent analyses were done for these associations including the interaction term with time. For AA, higher levels were associated with better episodic memory at baseline (Fig. 3 and Table 4), and this association did not change over time ($p_{interaction}$ = .345). For AA and working memory, a similar trend was found, although the baseline association did not reach statistical significance (0.10 [−0.01, 0.20] and $p_{interaction}$ = .766, respectively).

For KA/QA and episodic memory, a linear quadratic association was found (Wald χ^2 = 8.27, p = .016) showing stronger positive associations at more extreme values, which also did not change over time ($p_{interaction}$ = .602). For XA and working memory, no associations were found at baseline, nor did they change over time ($p_{interaction}$ = .417).

Next, interactions with age and sex were investigated for pooled

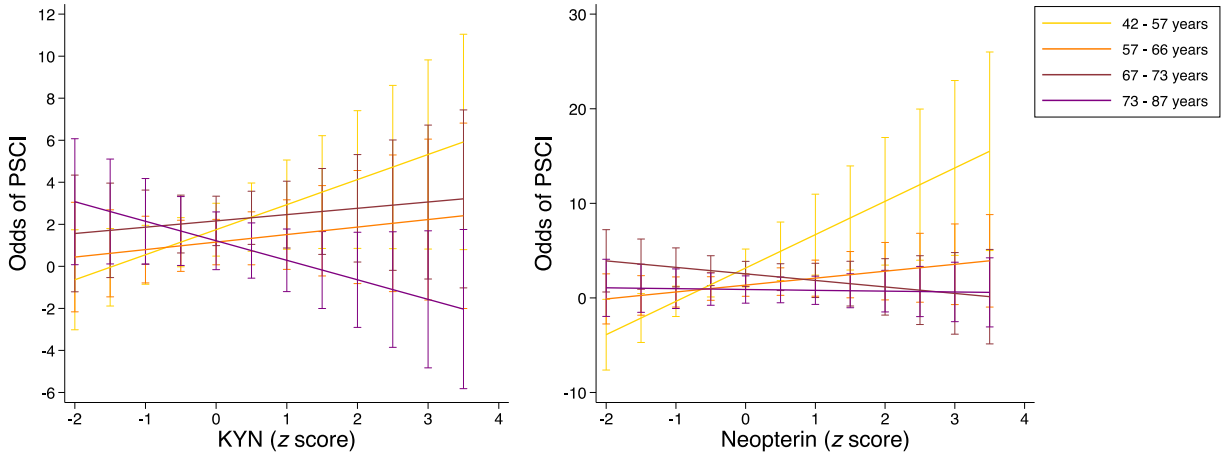

Fig. 2. Baseline associations of KYN and neopterin with odds of PSCI, separately for different age groups (quartiles).

associations over all time points. Significant interaction effects were found for processing speed between sex and KA ($p_{interaction}$ = .014). This interaction was only significant at baseline ($p_{interaction}$ = .008) and sex did not moderate change in cognition over time ($p_{interaction}$ = .158). Post hoc tests showed that, at baseline, higher levels of KA were associated with better processing speed in women (β per SD 0.15 [95% CI 0.02, 0.27]), but not in men ((−0.05 [−0.13, 0.04]; Fig. 4). No other significant interaction effects with age and sex were found.

3.4. Additional analyses

Most results were not altered by including the additional covariates PSD, hypertension, T2DM, history of CVD, or inflammatory markers in the model (Model 4; Tables 4 and S5-S6). Associations with individual test scores were similar to those found with the corresponding cognitive domain (Table S7).

4. Discussion

The present study explored associations between plasma kynurenines measured at baseline and cognitive functioning over time in 198 stroke patients up to 3 years after stroke. No associations between kynurenines and odds of PSCI were found. However, when investigating individual cognitive domains, higher levels of AA were associated with better episodic memory at baseline. Additionally, a non-linear association was found between KA/QA and episodic memory, with stronger positive associations at highest values of KA/QA. Lastly, higher levels of KA were associated with better processing speed in women, but not in men. These associations did not change over time. Given the exploratory nature and heterogeneity of findings, these results should be interpreted with caution and verified in other prospective studies.

4.1. KA/QA is associated with episodic memory after stroke

As mentioned previously, studies investigating the role of the kynurenine pathway in cognitive dysregulation after stroke are scarce and generally have relatively small sample sizes (n = 23–60) and a limited set of kynurenine metabolites (e.g. TRP, KYN, KA, QA), whereas downstream (e.g. 3-HAA and PIC) and side-branch (e.g. XA and AA) metabolites are often not investigated [18–20]. One recent study investigated associations between cognition after stroke and the ratio between KA and QA in a small sample of patients and reported that levels of QA/KA predicted cognitive impairment [20]. These findings mirror our observation, where high KA/QA levels correlate with better memory. Additionally, in this same study, levels of QA/KA were significantly increased and correlated with neuronal death and long-term memory in male diabetic mice that showed signs of cognitive impairment after permanent occlusion of the middle cerebral artery. In other in vivo studies with animal models of cerebral ischemia, experimental increases of KA levels provided neuroprotection as well [32–34]. However, higher KA levels were associated with higher mortality rates in patients within 3 weeks after stroke in another study [35], which might have been the result of other pathological processes increasing endogenous levels of KA. We also found a positive association between KA and processing speed at baseline, in women only. Studies suggest that blood levels of KA might be lower in women compared to men [36,37], which was also what we observed in our study. However, it is unclear why these sex-specific associations with cognition were found and research on this topic is scarce.

KA has both anti-glutamatergic and anti-inflammatory properties and is therefore considered neuroprotective. More specifically, KA is an *N*-methyl-D-aspartate (NMDA) receptor antagonist but also inhibits the α7-nicotinic-acetylcholine receptor [8,38]. QA, on the other hand, is considered neurotoxic and mediates oxidative stress [39], by the production of reactive oxygen species (ROS) and may play a role in neuronal apoptosis [8,40,41]. Additionally, in contrast to KA, QA is an NMDA receptor agonist and has the potential to induce glutamatergic toxicity [5,42]. Glutamate is the primary excitatory neurotransmitter in the brain and under normal circumstances has an important role in neuronal plasticity and cognitive functioning [43]. However, in cases where the release of glutamate is excessive, this can result in glutamate mediated neurotoxicity, one of the processes implicated in stroke [44]. As such, an imbalance between the synthesis of neurotoxic and neuroprotective kynurenines might play a role in post-stroke cognitive functioning.

4.2. AA is associated with better episodic memory after stroke

In the present study, higher levels of AA were associated with better episodic memory at baseline. A similar but non-significant trend was observed for working memory. Interestingly, these findings are not in line with recent large population-based cohort studies that suggest inverse or no associations between AA and cognitive functioning [45–47]. For instance, we recently found a non-linear association between AA and cognitive functioning in a large population study, with more extreme values being associated with higher odds of cognitive impairment and lower scores on executive functioning in individuals with prediabetes [45]. Additionally, in another study, baseline levels of AA predicted the development of all-type dementia and AD approximately 16 years later [46]. However, another study reported no associations between AA and tests evaluating memory and executive functioning [47].

CSF AA levels were not associated with AD pathological markers of

Table 4
Associations between baseline metabolite levels and cognitive test scores for model 1–4.

Metabolite	Pooled associations over time	Baseline associations
Episodic memory *(15-Word Verbal Learning Test)*		
AA		
Model 1[a]	**0.152 [0.042, 0.262]** **	**0.163 [0.044, 0.281]** **
Model 2[b]	**0.156 [0.045, 0.267]** **	**0.168 [0.048, 0.288]** **
Model 3[c]	**0.152 [0.042, 0.262]** **	**0.164 [0.045, 0.283]** **
Model 4[d]	**0.166 [0.055, 0.276]** **	**0.179 [0.060, 0.299]** **
KA/QA[+]		
Model 1[a]	linear: 0.004 (−0.116, 0.124) quadratic: **0.076 (0.014, 0.138)***	linear: −0.010 (−0.139, 0.119) quadratic: **0.089 (0.022, 0.157)***
Model 2[b]	linear: 0.003 (−0.118, 0.123) quadratic: **0.077 (0.014, 0.139)***	linear: −0.011 (−0.141, 0.119) quadratic: **0.092 (0.024, 0.160)****
Model 3[c]	linear: 0.003 (−0.117, 0.122) quadratic: **0.075 (0.014, 0.137)***	linear: −0.012 (−0.142, 0.117) quadratic: **0.091 (0.024, 0.159)****
Model 4[d]	linear: −0.029 [−0.156, 0.099] quadratic: **0.074 [0.010, 0.137]** *	linear: −0.052 [−0.189, 0.085] quadratic: **0.091 [0.022, 0.161]** *
Working memory *(Trial 1 of 15-Word Verbal Learning Test, Digit span)*		
AA		
Model 1[a]	**0.110 [0.015, 0.205]** *	0.096 [−0.009, 0.202]
Model 2[b]	**0.114 [0.018, 0.209]** *	0.101 [−0.004, 0.207]
Model 3[c]	**0.110 [0.015, 0.204]** *	0.098 [−0.006, 0.203]
Model 4[d]	**0.117 [0.021, 0.213]** *	**0.108 [0.002, 0.214]** *
XA[+]		
Model 1[a]	linear: **0.143 (0.017, 0.269)*** quadratic: **−0.076 (−0.134, −0.018)***	linear: 0.132 (−0.005, 0.270) quadratic: −0.057 (−0.120, 0.006)
Model 2[b]	linear: **0.155 (0.029, 0.280)*** quadratic: **−0.081 (−0.139, −0.024)****	linear: **0.147 (0.010, 0.285)*** quadratic: −0.063 (−0.126, 0.000)
Model 3[c]	linear: **0.145 (0.021, 0.269)*** quadratic: **−0.079, −0.137, −0.022)****	linear: 0.131 (−0.006, 0.268) quadratic: −0.059 (−0.122, 0.003)
Model 4[d]	linear: **0.133 [0.006, 0.261]** * quadratic: **−0.077 [−0.135, −0.019]** **	linear: 0.124 [−0.016, 0.263] quadratic: −0.058 [−0.121, 0.006]

Data are presented as β per SD [95% CI] for pooled associations over time, as well as baseline associations. For non-linear associations both the linear and quadratic term are shown.

[+] Non-linear association according to likelihood ratio test and visual inspection.

[a] Model 1: adjusted for demographics (age, sex, educational level) and eGFR.

[b] Model 2: 1 + lifestyle factors (BMI, alcohol consumption and smoking behavior).

[c] Model 3 (main model): 2 + stroke type and disability (Barthel index).

[d] *Model 4*: 3 + PSD, hypertension, type-2 diabetes, history of CVD, B vitamins (PLP, riboflavin) and low-grade inflammation (composite score of CRP, SAA, ICAM-1, IL-6, IL-8 and TNF-α).

* $p < .05$.

** $p < .01$.

$A\beta^{42}$, p-tau or t-tau in CSF in patients with AD [48], nor were these markers significantly different in clinical studies in CSF [48,49] or blood [48,50,51] samples of patients with AD compared to healthy controls. However, higher levels of AA have been observed in patients with a variety of different neurological and psychiatric disorders with an inflammatory component including stroke, chronic brain injury, Huntington's disease and depression [35,52,53] and, in patients with Huntington's disease, AA was associated with inflammatory status [54].

AA can inhibit 3-hydroxyanthranilic acid oxidase, which might serve as a potential neuroprotective mechanism by inhibiting 3-HAA being catabolized into QA [53,55] and could, hypothetically, be one reason for the positive associations found in the present study. According to another study, AA showed anti-oxidant effects and is an efficient iron chelator [56]. As such, enhanced expression of AA could potentially limit iron-mediated redox damage in neurological diseases such as stroke. Yet again, other studies suggest that the biological function of AA is inert [53,57]. The exact molecular mechanisms involved in associations between AA and cognitive functioning are therefore still largely unclear, especially after stroke, and should be verified in future studies.

4.3. Neopterin is associated with odds for PSCI in middle aged adults

Next to glutamate mediated excitotoxicity, inflammation plays an important role after stroke. Neopterin is believed to reflect the activation of macrophages and microglia, as well as pro-inflammatory cytokines [58]. In our study neopterin was associated with higher odds of PSCI in the youngest age group (42–57 years). We also found a non-significant trend for associations between higher levels of KYN and higher odds of PSCI in this same group. Through its role in inflammation, neopterin has been associated with activation of the kynurenine pathway [59]. As such, these results might suggest a role for inflammation and activation of the kynurenine pathway in post-stroke cognitive impairment in middle aged adults and are in line with previous studies that reported higher levels of KYN [18] and KTR [19] in patients with cognitive impairment after stroke compared to those without cognitive impairment.

Although associations of neopterin with PSCI have not been investigated before, a recent systematic review and meta-analysis reported higher levels of neopterin in patients with ischemic stroke in which they were associated with disease severity and mortality [60]. However, studies suggest that blood levels of KYN, KTR and neopterin all increase with aging [61–63]. Additionally, neopterin levels were associated with lower cognitive performance in a population-based study in community-dwelling older adults [47] and in patients with AD [64]. It is therefore surprising that the positive association with odds of PSCI in our study was only found in the youngest age group and needs verification in future studies.

4.4. No evidence of associations between kynurenines and odds for PSCI

We did not find evidence for associations of kynurenines and PSCI, which might indicate that the effects of kynurenines were only modest or do not exist. Other reasons for differences between our results and those from existing literature [18–20] might be the heterogeneity of previous studies, including differences in how cognitive impairment was defined. Additionally, our baseline measurements took place in the post-acute phase (10–12 weeks) after stroke to avoid confounding by factors in the acute stroke state, such as acute care, rehabilitation, sickness behavior and generic inflammatory responses. Most previous studies investigated associations of kynurenines with cognitive functioning in the acute phase after stroke [19,20]. Lastly, the group with PSCI in the present study consists of patients along the entire spectrum of mild cognitive impairment to dementia and might therefore be too heterogeneous to detect differences in kynurenine levels. Nevertheless, results from our study suggest that kynurenines measured in the post-acute phase after stroke are not associated with PSCI 3 to 36 months after stroke.

4.5. Strengths and limitations

Strengths of the present study consist of a relatively large sample size, in which a large number of metabolites of the kynurenine pathway were quantified. Another strength is the availability of rich clinical data, including extensive assessment of cognitive functioning across multiple

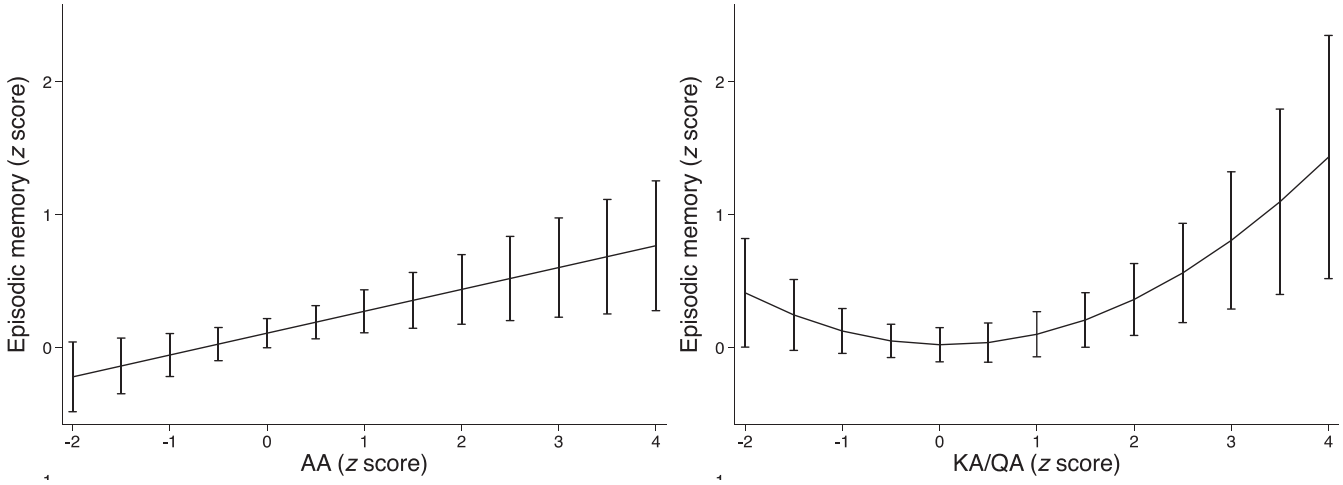

Fig. 3. Significant baseline associations of metabolites and ratios with domain scores.

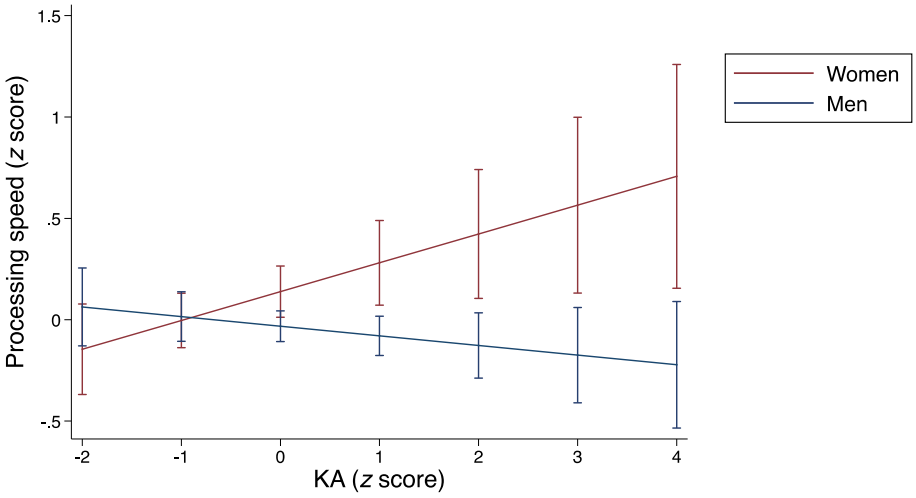

Fig. 4. Baseline associations of KA with processing speed, separately for men and women.

domains. Additionally, the longitudinal design of the CASPER study with a relatively long follow-up up to 3 years post-stroke made it possible to investigate associations between baseline kynurenines and cognitive dysregulation over time. At the same time, results should be interpreted with caution, since all analyses in this study were observational, exploratory and therefore uncorrected for multiple testing. It is also likely that the CASPER study includes patients at the less severe end of the stroke spectrum because of self-selection. For instance, ceiling effects were identified on a questionnaire that evaluates impairments in activities of daily living, indicating that most patients were at least functionally independent [30]. Patients in this study were also younger, had higher MMSE scores, and a lower percentage of diabetes and hypertension compared to a previous study [19]. As such, patients might have experienced milder stroke compared to a general stroke population. Additionally, the number of patients with cognitive impairment and the severity of their symptoms might be underestimated. Lastly, metabolites were only determined at baseline, making it impossible to link changes in cognitive dysregulation to changes in metabolite levels over time.

5. Conclusion

In the present study, higher levels of KA, AA and KA/QA were associated with better scores on cognitive domains at baseline. These associations did not change over time. Results need to be verified in other prospective studies, preferably by using larger sample sizes and by measuring all kynurenines at different points in time after stroke.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This project was funded by Maastricht University, Health Foundation Limburg, and the Adriana van Rinsum-Ponsen Stichting. This work was performed as a partial fulfilment towards the International Master in Affective Neuroscience of Maastricht University and the University of Florence. The authors would like to thank all patients who participated in the CASPER study and Nico Rozendaal for his work on the electronic database.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2023.120819.

References

[1] Collaborators, G.B.D.S, Global, regional, and national burden of stroke, 1990-2016: a systematic analysis for the global burden of disease study 2016, Lancet Neurol. 18 (5) (2019) 439–458.
[2] V.L. Feigin, et al., Global and regional burden of stroke during 1990-2010: findings from the global burden of disease study 2010, Lancet 383 (9913) (2014) 245–255.
[3] R.L. Sacco, C.H. Dong, Declining stroke incidence and improving survival in US communities evidence for success and future challenges, J. Am. Med. Assoc. 312 (3) (2014) 237–238.
[4] W.M. van der Flier, et al., Vascular cognitive impairment, Nat. Rev. Dis. Prim. 4 (2018) 18003.
[5] G.J. Guillemin, B.J. Brew, Implications of the kynurenine pathway and quinolinic acid in Alzheimer's disease, Redox Rep. 7 (4) (2002) 199–206.
[6] O.C. Kuster, et al., Novel blood-based biomarkers of cognition, stress, and physical or cognitive training in older adults at risk of dementia: preliminary evidence for a role of BDNF, Irisin, and the kynurenine pathway, J. Alzheimers Dis. 59 (3) (2017) 1097–1111.
[7] K. Ogyu, et al., Kynurenine pathway in depression: a systematic review and meta-analysis, Neurosci. Biobehav. Rev. 90 (2018) 16–25.
[8] L. Vecsei, et al., Kynurenines in the CNS: recent advances and new questions, Nat. Rev. Drug Discov. 12 (1) (2013) 64–82.
[9] Q. Wang, et al., Activation of NAD(P)H oxidase by tryptophan-derived 3-hydroxykynurenine accelerates endothelial apoptosis and dysfunction in vivo, Circ. Res. 114 (3) (2014) 480–492.
[10] C. Duran, A. San Martin, Do endothelial cells eat tryptophan to die? Circ. Res. 114 (3) (2014) 406–408.
[11] J. Savitz, The kynurenine pathway: a finger in every pie, Mol. Psychiatry 25 (1) (2020) 131–147.
[12] A. Macchiarulo, et al., Highlights at the gate of tryptophan catabolism: a review on the mechanisms of activation and regulation of indoleamine 2,3-dioxygenase (IDO), a novel target in cancer disease, Amino Acids 37 (2) (2009) 219–229.
[13] A.L. Mellor, et al., Indoleamine 2,3-dioxygenase, immunosuppression and pregnancy, J. Reprod. Immunol. 57 (1–2) (2002) 143–150.
[14] Y. Mandi, L. Vecsei, The kynurenine system and immunoregulation, J. Neural Transm. (Vienna) 119 (2) (2012) 197–209.
[15] R. Schwarcz, et al., Kynurenines in the mammalian brain: when physiology meets pathology, Nat. Rev. Neurosci. 13 (7) (2012) 465.
[16] N.H. Hunt, et al., Immunopathogenesis of cerebral malaria, Int. J. Parasitol. 36 (5) (2006) 569–582.
[17] R.J.H. Martens, et al., Microvascular endothelial dysfunction is associated with albuminuria: the Maastricht study, J. Hypertens. 36 (5) (2018) 1178–1187.
[18] M. Liu, et al., Potential of serum metabolites for diagnosing post-stroke cognitive impairment, Mol. BioSyst. 11 (12) (2015) 3287–3296.
[19] A.B. Gold, et al., The relationship between indoleamine 2,3-dioxygenase activity and post-stroke cognitive impairment, J. Neuroinflammation 8 (2011).
[20] A. Cogo, et al., Increased serum QUIN/KYNA is a reliable biomarker of post-stroke cognitive decline, Mol. Neurodegener. (2021) 16(1).
[21] E. Douven, et al., The cognition and affect after stroke - a prospective evaluation of risks (CASPER) study: rationale and design, BMC Neurol. 16 (2016) 65.
[22] S. Hustad, et al., Kinetic modeling of storage effects on biomarkers related to B vitamin status and one-carbon metabolism, Clin. Chem. 58 (2) (2012) 402–410.
[23] Ø. Midttun, S. Hustad, P. Ueland, Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 23 (9) (2009) 1371–1379.
[24] A.S. Levey, et al., A new equation to estimate glomerular filtration rate, Ann. Intern. Med. 150 (9) (2009) 604–612.
[25] W. Van der Elst, et al., Rey's verbal learning test: normative data for 1855 healthy participants aged 24-81 years and the influence of age, sex, education, and mode of presentation, J. Int. Neuropsychol. Soc. 11 (3) (2005) 290–302.
[26] W. Van der Elst, et al., The concept shifting test: adult normative data, Psychol. Assess. 18 (4) (2006) 424–432.
[27] M.F. Folstein, S.E. Folstein, P.R. McHugh, "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician, J. Psychiatr. Res. 12 (3) (1975) 189–198.
[28] I. Overbeek, K. Schruers, E. Griez, Mini International Neuropsychiatric Interview: Nederlandse Versie 5.0. 0. DSM-IV [Dutch Version], Universiteit Maastricht, Maastricht, the Netherlands, 1999.
[29] S.A. Montgomery, M. Åsberg, A new depression scale designed to be sensitive to change, Br. J. Psychiatry 134 (4) (1979) 382–389.
[30] F.I. Mahoney, D.W. Barthel, Functional evaluation: the Barthel index, Md. State Med. J. 14 (1965) 61–65.
[31] E. Douven, et al., Co-occurrence of depressive symptoms and executive dysfunction after stroke: associations with brain pathology and prognosis, J. Neurol. Neurosurg. Psychiatry 89 (8) (2018) 859–865.
[32] L. Gellert, et al., Neuroprotection with a new kynurenic acid analog in the four-vessel occlusion model of ischemia, Eur. J. Pharmacol. 667 (1–3) (2011) 182–187.
[33] H. Robotka, et al., Neuroprotection achieved in the ischaemic rat cortex with L-kynurenine sulphate, Life Sci. 82 (17–18) (2008) 915–919.
[34] K. Sas, et al., Kynurenine diminishes the ischemia-induced histological and electrophysiological deficits in the rat hippocampus, Neurobiol. Dis. 32 (2) (2008) 302–308.
[35] L.G. Darlington, et al., Altered kynurenine metabolism correlates with infarct volume in stroke, Eur. J. Neurosci. 26 (8) (2007) 2211–2221.
[36] A.A. Badawy, D.M. Dougherty, Assessment of the human kynurenine pathway: comparisons and clinical implications of ethnic and gender differences in plasma tryptophan, kynurenine metabolites, and enzyme expressions at baseline and after acute tryptophan loading and depletion, Int. J. Tryptophan. Res. 9 (2016) 31–49.
[37] T.B. Meier, et al., Kynurenic acid is reduced in females and oral contraceptive users: implications for depression, Brain Behav. Immun. 67 (2018) 59–64.
[38] E. Vamos, et al., The role of kynurenines in disorders of the central nervous system: possibilities for neuroprotection, J. Neurol. Sci. 283 (1–2) (2009) 21–27.
[39] C.L. Allen, U. Bayraktutan, Oxidative stress and its role in the pathogenesis of ischaemic stroke, Int. J. Stroke 4 (6) (2009) 461–470.
[40] L. Szalardy, et al., Mitochondrial disturbances, tryptophan metabolites and neurodegeneration: medicinal chemistry aspects, Curr. Med. Chem. 19 (13) (2012) 1899–1920.
[41] W.M.H. Behan, et al., Oxidative stress as a mechanism for quinolinic acid-induced hippocampal damage: protection by melatonin and deprenyl, Br. J. Pharmacol. 128 (8) (1999) 1754–1760.
[42] G.J. Guillemin, Quinolinic acid, the inescapable neurotoxin, FEBS J. 279 (8) (2012) 1356–1365.
[43] G. Riedel, B. Platt, J. Micheau, Glutamate receptor function in learning and memory, Behav. Brain Res. 140 (1–2) (2003) 1–47.
[44] R. Bullock, et al., Massive persistent release of excitatory amino acids following human occlusive stroke, Stroke 26 (11) (1995) 2187–2189.
[45] L. Bakker, et al., Associations between plasma kynurenines and cognitive function in individuals with normal glucose metabolism, prediabetes and type 2 diabetes: the Maastricht study, Diabetologia 64 (11) (2021) 2445–2457.
[46] V. Chouraki, et al., Association of amine biomarkers with incident dementia and Alzheimer's disease in the Framingham study, Alzheimer's Dementia 13 (12) (2017) 1327–1336.
[47] S.H. Solvang, et al., The kynurenine pathway and cognitive performance in community-dwelling older adults. The Hordaland health study, Brain Behav. Immun. 75 (2019) 155–162.
[48] K.R. Jacobs, et al., Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid-beta and tau, Neurobiol. Aging 80 (2019) 11–20.
[49] V. van der Velpen, et al., Systemic and central nervous system metabolic alterations in Alzheimer's disease, Alzheimers Res. Ther. 11 (1) (2019) 93.
[50] L.M. Giil, et al., Kynurenine pathway metabolites in Alzheimer's disease, J. Alzheimers Dis. 60 (2) (2017) 495–504.
[51] E. Gulaj, et al., Kynurenine and its metabolites in Alzheimer's disease patients, Adv. Med. Sci. 55 (2) (2010) 204–211.
[52] B. Kepplinger, et al., Alterations of tryptophan metabolites in cerebrospinal fluid and serum after stroke, J. Neurol. Sci. 357 (2015), e385.
[53] L. Darlington, et al., On the biological importance of the 3-hydroxyanthranilic acid: Anthranilic acid ratio, Int. J. Tryptophan. Res. 3 (2010) 51–59.
[54] C.M. Forrest, et al., Blood levels of kynurenines, interleukin-23 and soluble human leucocyte antigen-G at different stages of Huntington's disease, J. Neurochem. 112 (1) (2010) 112–122.
[55] G.J. Guillemin, et al., Characterization of the kynurenine pathway in human neurons, J. Neurosci. 27 (47) (2007) 12884–12892.
[56] V. Chobot, et al., Iron chelation and redox chemistry of anthranilic acid and 3-hydroxyanthranilic acid: a comparison of two structurally related kynurenine pathway metabolites to obtain improved insights into their potential role in neurological disease development, J. Organomet. Chem. 782 (2015) 103–110.
[57] J.R. Ocampo, et al., Kynurenines with neuroactive and redox properties: relevance to aging and brain diseases, Oxidative Med. Cell. Longev. 2014 (2014).
[58] K. Schroecksnadel, et al., Neopterin to monitor clinical pathologies involving interferon-gamma production, Pteridines 15 (3) (2004) 75–90.
[59] J. de Bie, et al., Central kynurenine pathway shift with age in women, J. Neurochem. 136 (5) (2016) 995–1003.
[60] L.L. Meng, L. Cao, Serum neopterin levels and their role in the prognosis of patients with ischemic stroke: a systematic review and meta-analysis, J. Clin. Neurosci. 92 (2021) 55–60.
[61] D. Theofylaktopoulou, et al., A community-based study on determinants of circulating markers of cellular immune activation and kynurenines: the Hordaland health study, Clin. Exp. Immunol. 173 (1) (2013) 121–130.
[62] L. Capuron, et al., Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms, Biol. Psychiatry 70 (2) (2011) 175–182.
[63] V. Valdiglesias, et al., Immune biomarkers in older adults: role of physical activity, J. Toxicol. Environ. Health-Part A Curr. Issues 80 (13–15) (2017) 605–620.
[64] P. Wissmann, et al., Immune activation in patients with Alzheimer's disease is associated with high serum phenylalanine concentrations, J. Neurol. Sci. 329 (1–2) (2013) 29–33.