Tryptophan and kynurenine pathway metabolites in neuropsychiatric disorders

Tore Ivar Malmei Aarsland

Thesis for the degree of Philosophiae Doctor (PhD)
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Tore Ivar Malmei Aarsland

December 21th 2023

Scientific environment

The work presented in this thesis was carried out at the K.G. Jebsen Centre for Neuropsychiatric Disorders/Department of Biomedicine at the University of Bergen, Norway, in collaboration with colleagues at the Division of Psychiatry, Haukeland University Hospital, Bergen, Norway, and Bevital A.S., Bergen, Norway. It started in 2013, as a project in The Medical Student Research Program at the Faculty of Medicine, University of Bergen, and continued as a PhD project from 2019.

Main supervisor

Professor Jan Haavik, MD, PhD, Department of Biomedicine, University of Bergen, Norway, and Division of Psychiatry, Haukeland University Hospital, Bergen, Norway.

Co-supervisors

Associate professor Ute Kessler, MD, PhD, Department of Clinical Medicine, University of Bergen, Norway, and Division of Psychiatry, Haukeland University Hospital, Bergen Norway.

Professor Per Magne Ueland, MD, PhD, Department of Clinical Science, University of Bergen, Norway.

UNIVERSITY OF BERGEN
Faculty of Medicine

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Abbreviations

AA	anthranilic acid
ADHD	attention-deficit hyperactivity disorder
ACMS	2-amino-3-carboxymuconate-6-semialdehyde
ACMSD	amino-β-carboxymuconate-semialdehyde-decarboxylase
AhR	aryl hydrocarbon receptor
ASRS	Adult ADHD self-report scale
BBB	blood-brain-barrier
BDNF	brain-derived neurotrophic factor
CRP	C-reactive protein
ECT	electroconvulsive therapy
FAD	flavin adenine dinucleotide
GPR35	G protein-coupled receptor 35
GWAS	genome-wide association study
HAA	3-hydroxyanthranilic acid
HAAO	3-hydroxyanthranilate 3,4-dioxygenase
HDRS (HAM-D)	Hamilton depression rating scale
HK	3-hydroxykynurenine
HPA	hypothalamic-pituitary-adrenal
ICC	intraclass correlation coefficient
IDO	indoleamine 2,3-dioxygenase
IFN-γ	interferon gamma
IL	interleukin
KAT	kynurenine aminotransferase
Kyn	kynurenine

KYNU	kynureninase
KTR	kynurenine-tryptophan ratio
MADRS	Montgomery-Åsberg depression rating scale
MDD	major depressive disorder
LC/MS/MS	liquid chromatography/tandem mass spectrometry
mGlu	metabotropic glutamate receptor
NAD	nicotinamide adenine dinucleotide
NEFA	non-esterified fatty acids
NMDAr	N-methyl-D-aspartate receptor
NF-κB	nuclear factor-κB
Pic	picolinic acid
PLP	pyridoxal 5'-phosphate
QA	quinolinic acid
ROS	reactive oxygen species
TDO	tryptophan 2,3-dioxygenase
TGF-β	transforming growth factor beta
TNF-α	tumour necrosis factor alpha
TPH	tryptophan hydroxylase
Trp	tryptophan
WURS	Wender Utah rating scale
XA	xanthurenic acid

Abstract

Background: The kynurenine pathway constitutes the major route for metabolism of the essential amino acid tryptophan (Trp), also a precursor of serotonin. Kynurenine pathway metabolites, collectively termed kynurenines, have many biological properties, including regulation of glutamatergic signalling and immune activity, production of cellular energy, as well as the production and scavenging of reactive oxygen species. The pathway activity is upregulated by pro-inflammatory processes, which has also been reported in psychiatric disorders. Thus, with its relation to both neurotransmitters and inflammation, the kynurenine pathway could potentially be involved in the pathophysiology of psychiatric disorders. Meta-analyses have showed altered levels of Trp and kynurenines in patients with major depressive disorder, bipolar disorder, and schizophrenia compared to controls. However, the nature of the relationship between kynurenines and psychiatric disorders is unclear, and there is limited knowledge about how inflammation, comorbidity, medication, and lifestyle factors affect this association.

Aims: We aimed to investigate the kynurenine pathway in patients with attention-deficit hyperactivity disorder (ADHD) and depression, and to explore methodological issues in such studies. The specific aims were to 1) examine the status of Trp, kynurenines, and B vitamins in adults with ADHD and depression compared to healthy controls, to 2) investigate changes in levels of Trp, kynurenines and neopterin in patients with depression after electroconvulsive therapy (ECT), and to 3) review factors that affect analyses of Trp and kynurenines in clinical studies and propose strategies for future studies on Trp metabolism in psychiatric disorders.

Materials and methods: We investigated levels of Trp and kynurenines, B vitamins, and cotinine in 133 adults with ADHD and 131 adult controls from the project "*ADHD in adults in Norway"* in Bergen, Norway. The same markers, and the inflammatory marker neopterin, were compared between 27 adults with severe, treatment-resistant depression referred to ECT and 14 healthy controls from the project "*ECT and neuroradiology"* in Bergen, Norway. Changes in biomarker levels after ECT were analysed using paired Wilcoxon signed-rank tests in all patients and in subgroups based

on treatment response and remission. The same method was used to analyse changes in these biomarkers after ECT in 48 depressed patients from the "*Mood Disorders in Elderly treated with Electroconvulsive Therapy"* (MODECT) study in Amsterdam, the Netherlands. Here potential roles of inflammation and somatic comorbidity were explored in two subgroups based on changes in neopterin, and in two other subgroups based on the presence of somatic disorders. Lastly, we performed a systematic literature search for studies on changes in Trp and/or kynurenines after ECT and used vote counting based on the direction of effect to establish if there was any evidence for significant changes in single metabolites or ratios after ECT. We also extracted data on known determinants of Trp and kynurenines, as well as factors related to patient characteristics, intervention, and study design, to shed light on factors that could affect analyses of change.

Results: Adults with ADHD had significantly lower serum concentrations of KA, XA, HAA, riboflavin and vitamin B6, and higher cotinine compared to controls. There was also a significant inverse correlation between levels of Trp and kynurenine and ADHD symptom scores in the whole sample. Compared to healthy controls, depressed patients in study II had lower levels KA, XA and Pic, as well as lower KA/Kyn, KA/QA, XA/HK and Pic/QA. After ECT, the levels of HAA, Pic, Pic/QA and neopterin increased significantly. Depressed patients in study III showed a reduction in KA/HK after ECT. However, summarising the results of 19 studies included in the systematic review, there was no evidence for change in free Trp, total Trp, Kyn, KA or KTR after ECT. There was large variation in study designs and clinical characteristics of participants, and no consistent handling of determinants of Trp and kynurenine pathway metabolite levels.

Conclusions: Our results showed altered baseline levels of Trp and kynurenines in patients with ADHD and severe depression. Changes after ECT were inconsistent but indicated a role for pro inflammatory processes. Future studies on kynurenine metabolism in psychiatric disorders should use larger and better-defined patient samples. Such studies may also shed light on the role of life-style factors and inflammation in these conditions.

Abstrakt

Bakgrunn: Kynureninar er ei gruppe metabolittar av den kosthaldsavhengige aminosyra tryptofan (Trp) som er involverte i ei rekkje biologiske mekanismar, mellom anna inflammasjon og reguleringa av nevrotransmitteren glutamat. Det er funne forandringar i kynureninnivå hjå pasientar med ulike psykiatriske lidingar samanlikna med kontrollar, men det er usikkert kva som ligg bakom denne samanhengen.

Mål: Me ynskte å 1) undersøkja kynureninstoffskiftet hjå vaksne med attention-deficit hyperactivity disorder (ADHD) og depresjon, 2) å studera endringar i nivået av kynureninar etter elektrokonvulsiv behandling (ECT), 3) samt å kartleggja faktorar som kan påverka analysar av Trp og kynureninar i kliniske studiar.

Materialar og metodar: 1) Me samanlikna serumnivået av Trp, kynureninar og relaterte stoff hjå 133 vaksne med ADHD mot 131 vaksne kontrollar, og hjå 27 vaksne med alvorleg depresjon mot 14 vaksne kontrollar. 2) Endringar i serumnivået av desse biomarkørane vart vurdert i to grupper på 21 og 48 pasientar behandla med ECT. 3) Dei samla resultata frå studiar på endring etter ECT og tilhøyrande metodologiske utfordringar vart diskuterte i ein systematisk litteraturgjennomgang.

Resultat: 1) Me fann relativt låge serumnivå av fleire kynureninar både hjå vaksne med ADHD og vaksne med depresjon. 2) Etter ECT steig nivået av fleire metabolittar i den eine gruppa, men ikkje i den andre. I begge gruppene var det teikn til at endringane i kynureninverdiane hang saman med endringar i inflammasjon. 3) I oppsummeringa vår av 19 studiar fann me ingen prov for endringar i nivået av Trp eller tre andre kynureninmarkørar etter ECT. Det var stor variasjon i studiedesign, deltakarar og handsaminga av faktorar som påverkar serumverdiane av kynureninar.

Konklusjonar: Me fann forandringar i serumveridar av kynureninar og relaterte stoff hjå vaksne med ADHD og depresjon. Endringane etter ECT var inkonsistente, men tyda på at inflammasjon kan spela ei rolle. Framtidige studiar på kynureninstoffskiftet ved psykiatriske lidingar bør nytta større og betre definerte pasientgrupper. Det trengst òg auka kunnskap om kva rolle livsstilsfaktorar og inflammasjon speler for samanhengen mellom kynureninstoffskiftet og psykiatriske lidingar.

List of publications

Study I: Aarsland, T. I., Landaas, E. T., Hegvik, T. A., Ulvik, A., Halmoy, A., Ueland, P. M., & Haavik, J. (2015). Serum concentrations of kynurenines in adult patients with attention-deficit hyperactivity disorder (ADHD): a case-control study. *Behav Brain Funct, 11*(1), 36. doi:10.1186/s12993-015-0080-x

Study II: Aarsland, T. I., Leskauskaite, I., Midttun, O., Ulvik, A., Ueland, P. M., Oltedal, L., Erchinger, V. J., Oedegaard, K. J., Haavik, J., Kessler, U. (2019). The effect of electroconvulsive therapy (ECT) on serum tryptophan metabolites. *Brain Stimul, 12*(5), 1135-1142. doi:10.1016/j.brs.2019.05.018

Study III: Aarsland, T.I.M., Haavik, J., Ulvik, A., Ueland, P.M., Dols, A., Kessler U. (2023) The effect of electroconvulsive therapy (ECT) on serum kynurenine pathway metabolites in late-life depression. *Journal of Affective Disorders Reports* 12 (2023) 100578. doi: 10.1016/j.jadr.2023.100578

Study IV: Aarsland, T.I.M., Instanes, J.T., Posserud, M.R., Ulvik, A., Kessler, U., Haavik, J. (2022) Changes in tryptophan-kynurenine metabolism in patients with depression undergoing ECT – a systematic review. *Pharmaceuticals* 2022, 15, 1439. doi: 10.3390/ph15111439

Related publications

Elisabeth Toverud Landaas, Tore Ivar Malmei Aarsland, Arve Ulvik, Anne Halmøy, Per Magne Ueland and Jan Haavik (2016) Vitamin levels in adults with ADHD. *BJPsych Open*. 2(6). 377-384. doi: 10.1192/bjpo.bp.116.00.3491

All studies are available through open access.

1. Introduction

The role of the kynurenine pathway in psychiatric disorders has been investigated in a wide range of studies since the 1960s. Originally, the interest in this branch of tryptophan metabolism was tightly linked to research on serotonin, an important neurotransmitter that has long been central to investigations of the pathophysiology of depression [1]. Different versions of the "tryptophan depletion theory" postulated that the human body have mechanisms to consume this essential amino acid in case of infection, to aid the immune system by starving pathogens, or to supress T-cells during pregnancy to avoid fetal rejection [2]. Such activation of the kynurenine pathway seizes tryptophan and could impair serotonin production. This could be a possible mechanism for low serotonin levels in subjects with depression, while also providing a possible explanation for the apparent association between infectious diseases and psychiatric disorders [3,4]. However, meta-analyses indicate that experimental tryptophan depletion does not lower mood in healthy individuals [1].

Later, the focus has largely been shifted towards the properties of kynurenines themselves [5]. The kynurenine pathway yields metabolites that play a role in several biological systems that may be related to the pathophysiology of psychiatric disorders, including serotonin synthesis [1], glutamatergic signalling [6], immune system regulation [7], cellular energy production [8], regulation of the hypothalamic-pituitary-adrenal (HPA) axis [9], and generation and scavenging of reactive oxygen species (ROS) [10]. Specifically, many investigations have focused on the balance between neurotoxic and neuroprotective kynurenines which seems to be disturbed in a range of psychiatric disorders [11,12]. The last decade, multiple meta-analyses have concluded that patients with major depressive disorder, bipolar disorder and schizophrenia have decreased levels of Trp and Kyn, and signs of an altered balance between neuroactive kynurenines [13-20]. This is possibly related to the increasingly well documented role of inflammation in psychiatric disorders [7,21,22], that is also connected to the kynurenine metabolism [23]. Moreover, a key pathway enzyme, kynureninase (KYNU), was found to be significantly associated with depression symptoms in a recent genome-wide association study (GWAS) [24]. In parallel, the kynurenine pathway has been investigated

in relation to a wide range of somatic conditions, including neurodegenerative diseases [25,26], metabolic diseases [27,28], cardiovascular disease [29], kidney disease [30], and cancer [31,32].

In summary, the kynurenine pathway could be an important link between several important biological systems and the brain [6,33-35]. Importantly, the pathway also presents a range of possible targets for treatment, several of which are already under testing, that could be of considerable clinical importance [36-39]. While kynurenine levels in patients with depression are much studied, there is still limited knowledge about their relationship to clinical symptoms and possible changes after depression treatment. Moreover, kynurenines in relation to ADHD has also been studied [40], but not to the same degree as other psychiatric disorders.

Research on kynurenines in relation to neuropsychiatric disorders raises several fundamental questions about the roles and properties of these metabolites in the human body. Some central questions that underly this research include the following:

1. Are there altered kynurenine levels in psychiatric disorders?
2. Are the levels of these metabolites changed after treatment?
3. Is there a causal relationship between changes in kynurenines and symptom relief after treatment?

The various hypotheses regarding tryptophan metabolism in psychiatric disorders have already guided years of studies that seek to answer these fundamental questions, how to design proper studies, and to interpret the results. Meanwhile, several papers have been published discussing various methodological challenges, such as the considerable heterogeneity in study design [41], clinical characteristics and psychiatric diagnoses [42], and difficulties with establishing causal relationships [4,43].

1.1 The kynurenine pathway of tryptophan metabolism

The following sections will cover some central aspects of the kynurenine pathway of tryptophan metabolism (Figure 1), including the major components of the pathway, their origins and fate, their relevant biochemical properties, important determinants and covariates, as well as some notes on tissue differences and normal variation.

1.1.1 Tryptophan, kynurenine pathway metabolites, and related molecules

Tryptophan (Trp)

Trp is one of twenty proteinogenic amino acids in humans and one of nine essential amino acids, i.e. not synthesised in the body but mainly supplied through the diet. After entering the body, Trp is utilised in protein synthesis or as precursor for several biologically important molecules. Through the activity of tryptophan hydroxylase (TPH) Trp is converted to serotonin, which can be further metabolised to melatonin. Trp can also be decarboxylated into tryptamine [44]. Trp is additionally an important substrate for microbial metabolism in the gut, where it is a precursor for indoles, some of which are important signalling molecules [45]. The largest portion of Trp is metabolised through the kynurenine pathway [46], which yields NAD+/niacin and Acetyl-CoA, with the two enzymes tryptophan 2,3-dioxyganase (TDO) and indoleamine 2,3-dioxygenase (IDO) regulating the initial step to N-formylkynurenine. In the blood, most of the Trp is bound to albumin, with a free fraction of about ten percent under physiological conditions [47]. Trp can be displaced from albumin by competition from non-esterified fatty acids (NEFA) and various drugs, potentially making more Trp available for uptake and metabolism [48]. Trp is dependent on active transport through the large neutral amino acid transporter (LAT1, SLC7A5) across cell membranes, and here it competes with other large neutral amino acids. The ratio of Trp to these competing amino acids is thought to affect the availability of Trp in tissues including the brain [48].

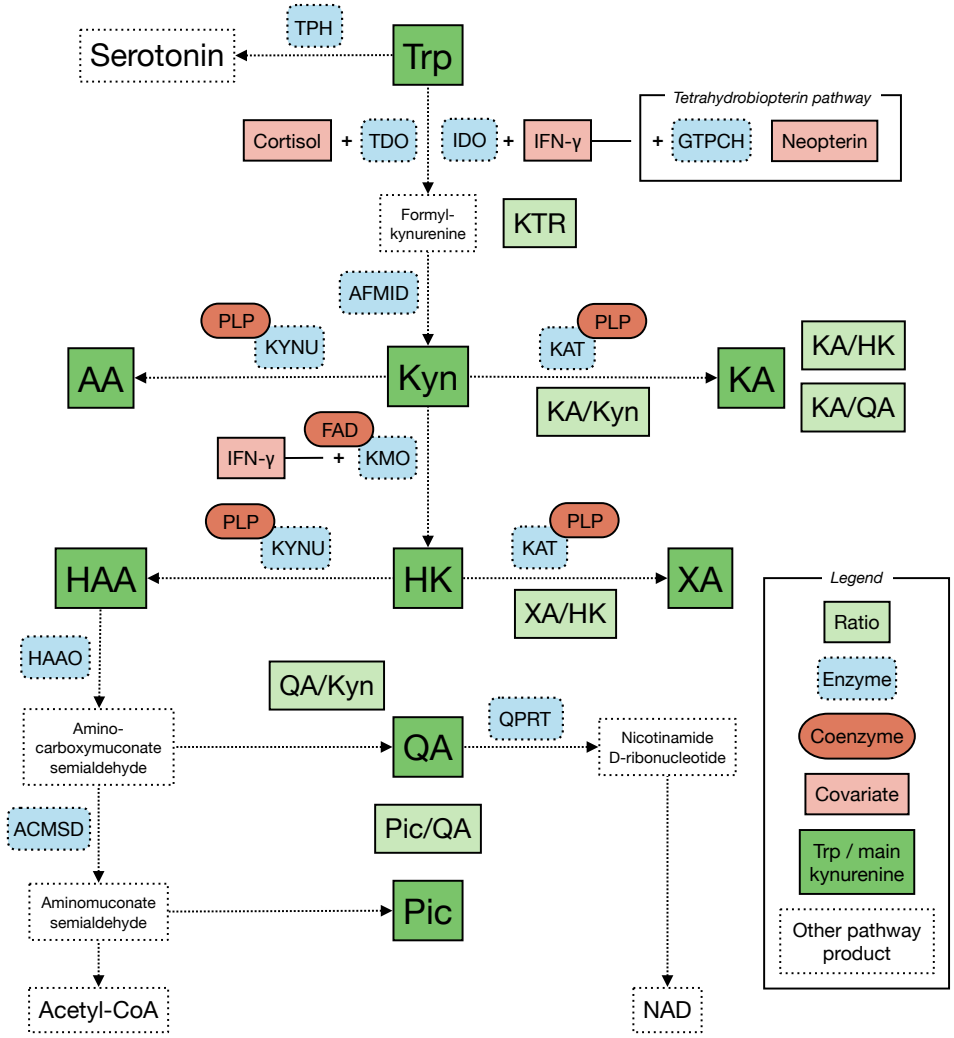


Figure 1. The kynurenine pathway of Trp metabolism. Adapted from study IV. Abbreviations: Acetyl-CoA, acetyl coenzyme A; ACMSD, aminocarboxymuconate semialdehyde decarboxylase; AA, anthranilic acid; AFMID, arylformamidase; FAD, flavin adenine dinucleotide; CAA, competing amino acid; GTPCH, GTP cyclohydrolase; HAA, 3-hydroxyanthranilic acid; HAAO, 3-hydroxyanthranilate 3,4-dioxygenase; HK, 3-hydroxykynurenine; IDO, indoleamine 2,3-dioxygenase; KA, kynurenic acid; KAT, kynurenine aminotransferase; KMO, kynurenine monooxygenase; KTR, kynurenine-tryptophan ratio; Kyn, kynurenine; KYNU, kynureninase; LAT1, L-type amino acid transporter; NEFA, non-esterified fatty acid; NAD, nicotinamide adenine dinucleotide; Pic, picolinic acid; PLP, pyridoxal 5′-phosphate;QA, quinolinic acid; QPRT, quinolinate phosphoribosyltransferase; TDO, tryptophan 2,3-dioxygenase; IFN-γ, interferon gamma; Trp, tryptophan; TPH, tryptophan hydroxylase; XA, xanthurenic acid.

Kynurenine (Kyn)

Derived from N-formylkynurenine by formamidase, Kyn is the first stable and the most abundant metabolite of the kynurenine pathway [49]. Notably, it is a ligand of the transcription factor aryl hydrocarbon receptor (AhR) and can act as an immunosuppressor [50]. Kyn is further metabolised to kynurenic acid (KA), to anthranilic acid (AA), or to 3-hydroxykynurenine (HK) which is the next metabolite in the main branch of the pathway towards NAD, through which most Kyn is utilised [46]. Excess Kyn is effectively handled by the kidneys and excreted in the urine [49].

Kynurenic acid (KA)

One of the first kynurenines to be characterised, KA is still one of the most studied kynurenine metabolites due to its role as an endogenous antagonist of glutamate receptors and its possible neuroprotective properties [51]. It is produced from Kyn in a step catalysed by a family of transaminases named kynurenine aminotransferase (KAT) I-IV [52]. KA is documented to bind to three classes of receptors involved in glutamate signalling, i.e. NMDA receptors, kainate receptors, and AMPA receptors [53], as well as the α7 nicotinic acetylcholine receptor (α7nAChR) [54]. Experiments in rats have shown that even a small increase in KA is accompanied by reduced extracellular levels of glutamate and dopamine in the brain [55,56]. Likewise, inhibition of KATII is followed by increased levels of glutamate, dopamine, and acetylcholine [6]. KA has also been shown to bind to G protein-coupled receptor 35 (GPR35) [57,58] involved in cell adhesion and mobility [49], and AhR through which it promotes immunosuppression and immune tolerance [51]. KA is also an antioxidant and ROS scavenger [59]. KA levels have been reported to be increased in the central nervous system of patients with bipolar disorder and schizophrenia [60-62], but also relatively low in blood from patients with schizophrenia, psychotic bipolar disorder and major depressive disorder [35].

3-Hydroxykynurenine (HK)

HK is derived from kynurenine by kynurenine monooxygenase (KMO) and is one of two main neurotoxic metabolites [36,63]. It generates ROS [64-66] and has been reported to disrupt TCA cycle metabolism [67], induce mitochondrial dysfunction [66] and cause cell death in high concentrations [65]. Experimental increase of HK in human neurons has

shown considerable increase in oxidative stress [66]. HK is metabolised further along the main branch to 3-hydroxyanthranilic acid (HAA), to xanthurenic acid (XA), or excreted through the kidneys.

Xanthurenic acid (XA)

XA is the product of transamination of HK by KATs [63]. It can attenuate glutamate signalling between neurons by inhibiting vesicular glutamate transporters (VGLUT) [68]. Like KA, XA is a ligand of AhR and can contribute to immunosuppression [23]. XA is also thought to be an endogenous agonist of the metabotropic glutamate receptor 2 (mGluR2) [69], a potential target for pharmacological therapy in both depression and schizophrenia. Like several other kynurenines, it is also a ROS scavenger [70]. Furthermore, it can interact with insulin and has been implicated in the pathophysiology of diabetes [71]. XA is mainly excreted in the urine.

Anthranilic acid (AA)

AA is converted from Kyn by kynureninase (KYNU). It forms complexes with iron and functions as an antioxidant [72]. A range of gut microbes are able to produce anthranilic acid and could be important contributors to serum levels of this metabolite [45]. Increased blood levels of AA have been found in patients with schizophrenia as well as in patients with various auto-immune disorders [73].

3-hydroxyanthranilic acid (HAA)

HAA is generated from HK in a step catalysed by KYNU, or through spontaneous hydroxylation from AA [8]. HAA can generate ROS and has been described as a neurotoxic metabolite [74], but it has also antioxidant properties [75]. Furthermore, it is a ligand of the inflammatory transcription factor nuclear factor-κB (NF-κB) [76] and supresses T cell activity and expansion [77]. It has also been shown to cause apoptosis in Th1 cells [78]. Relatively low blood levels of HAA have been reported in several neurological conditions [79], something that could be related to the high redox reactivity of the metabolite [72]. HAA is metabolised further to 2-amino-3-carboxymuconate-6-semialdehyde (ACMS) by 3-hydroxyanthranilic acid-oxygenase (HAAO), but can also form cinnabarinic acid through spontaneous condensation [69].

Quinolinic acid (QA)

QA is formed spontaneously from ACMS. It is an agonist of the NMDA receptor and can increase the synaptic concentration of glutamate by stimulating glutamate release but also by inhibiting its breakdown by astroglial glutamine synthase [80]. By forming a complex with iron, it can also produce ROS that can cause damage to cell membranes by lipid peroxidation [80]. QA likely plays a role in inflammatory response and pathogen clearance [81], but is also characterised as a neurotoxin on the basis of its biological properties [80]. It is cytotoxic even at low levels in neurons and astrocytes [82]. Experiments with neuronal cell cultures from mice, rats and humans have demonstrated rapid toxic effects from micromolar concentrations of QA, but also damage to cells that have prolonged exposure to sub-micromolar concentrations [76]. Increased levels of QA are associated with a range of pathological conditions [83]. Elevated QA levels have been reported in CSF of suicide attempters [84]. QA levels also correlate with amyloid and tau levels in post-mortem hippocampal tissue from patients with Alzheimer's disease [26], and reduced cognitive function has been associated with high levels of QA in patients with dementia [85]. QA is converted to nicotinamide-D-ribonucleotide by the enzyme quinolinate phosphoribosyltransferase (QPRT).

Picolinic acid (Pic)

Pic is formed from aminomuconate semialdehyde which is a metabolite of ACMS, the precursor of QA. Unlike QA, its formation is dependent on the enzyme amino-β-carboxymuconate-semialdehyde-decarboxylase (ACMSD). Pic is an iron and zinc chelator and is involved in the intestinal absorption of zinc [47]. It acts as a modulator of immune activity and has important antiviral and antimicrobial properties [86]. Like KA, Pic is a potential neuroprotectant that has been shown to block the neurotoxic effects of QA, possibly by attenuating calcium dependent glutamate release [87]. Reduced blood Pic and Pic/QA has been found in suicide attempters [88], and Pic levels have also been found to correlate negatively with tau in patients with Alzheimer's [26].

Vitamin B3 and nicotinamide adenine dinucleotide (NAD+)

Nicotinic acid (niacin), nicotinamide and nicotinamide riboside are the three vitamers of vitamin B3 and precursors of NAD+. They are supplied through the diet but are also

products of biological reactions using NAD+. With its ability to transfer electrons between molecules, NAD+ plays an essential role in many processes in human biology. It is central to cellular energy production, DNA repair, and the function of a range of enzymes [81]. Deficiency of niacin leads to pellagra, a disease characterized by dermatitis, diarrhoea and delirium [89]. Under physiological conditions, NAD+ is mainly formed from vitamin B3. However, it is also a product of the main branch of the kynurenine pathway, which serves as an important source for NAD+ in cases of niacin deficiency, or increased demand in case of immune challenge [81,90]. Indeed, genetic inactivation and pharmacological inhibition of IDO have been shown to hamper NAD+ production in macrophages [90].

Pyridoxal 5'-phosphate (PLP)

The B6 vitamer pyridoxal-5'-phosphate (PLP) is an essential coenzyme of hundreds of different enzymes, including KYNU and KAT that are catalysing several steps in the metabolism of Trp. Plasma measures of PLP are inversely correlated with a range of inflammatory markers, and have been observed to be low in several somatic conditions including diabetes, cardiovascular disease and cancer [91].

Flavin adenine dinucleotide (FAD)

FAD is one of three interconvertible circulating forms of vitamin B2, the others being riboflavin and flavin mononucleotide (FMN). FAD is the coenzyme of many enzymes, including the kynurenine pathway enzyme KMO.

Neopterin

Neopterin is a pteridine that is produced by macrophages and dendritic cells upon stimulation by interferon gamma (IFN-γ), a Th1-type cytokine involved in cellular immune activation [92]. It is relatively stable in plasma and a useful marker of IFN-γ production [92]. IFN-γ is also the principal inducer of IDO, and blood levels of neopterin often correlate well with the kynurenine-tryptophan ratio (KTR) [93-97]. Neopterin is therefore often included in studies of kynurenines to shed light on the contribution of inflammation on the pathway's activity.

1.1.2 Enzymes

The metabolism through the kynurenine pathway is dependent on a series of enzymes. Genetic variants affecting these enzymes have been associated with various diseases [98], although the strength of associations and causal effects still remain to be validated for some of these reports. Furthermore, several of these enzymes have been investigated as potential pharmacological targets. Examples include inhibitors of IDO, KMO, KYNU and HAAO, all intended to reduce the level of the potentially neurotoxic metabolites HK, HAA and QA [33].

Tryptophan 2,3-dioxygenase (TDO)

TDO is the main enzyme responsible for metabolising Trp to N-formylkynurenine and is mainly expressed in the liver. It is activated by Trp itself and by heme. It is induced by glucocorticoids such as cortisol, and is inhibited by nicotinamide adenine dinucleotide phosphate (NADP+) and its reduced form NADPH in a negative feedback loop [46].

Indoleamine 2,3-dioxygenase (IDO)

IDO is the main enzyme responsible for tryptophan metabolism outside of the liver. Like TDO, IDO is dependent on heme as cofactor [46]. It is induced mainly by the pro-inflammatory cytokine interferon gamma IFN-γ [99], and this induction is potentiated by tumour necrosis factor alpha (TNF-α) [23], another prominent pro-inflammatory cytokine. IDO is inhibited by anti-inflammatory cytokines like interleukin-4 (IL-4) and transforming growth factor beta (TGF-β), by nitric oxide [76] and Trp itself [23]. It can also be inhibited pharmacologically by l-methyltryptophan [76]. The regulation of IDO is a key aspect of the relationship between the kynurenine pathway and the immune system, and IDO is abundant in white blood cells, especially macrophages.

Kynurenine aminotransferase (KAT)

KA and XA are synthesised with the help of four PLP-dependent kynurenine aminotransferases (KATs) [52]. A range of transaminase and KAT inhibitors have been identified and are studied for potential therapeutic use, especially in relation to schizophrenia [6,36]. For instance, inhibition of KATII has been shown to have pro-

cognitive effects in rats and to counter act working memory deficits in monkeys treated with ketamine [6], and to alleviate sleep disturbance in rats [100].

Kynurenine 3-monooxygenase (KMO)

KMO is dependent on FAD as a coenzyme and catalyses the formation of HK. Significant increase in HK has been demonstrated in experimental overexpression of KMO in human neurons [66]. Being a key enzyme in the synthesis of HK and QA further downstream, KMO is a possible therapeutic target in disorders related to these neurotoxic metabolites. Inhibition of KMO has been associated with improvement in murine models of Alzheimer's disease and Huntington's disease [101].

Kynureninase (KYNU)

KYNU is involved in two steps of the kynurenine pathway: the conversion of Kyn into AA, and HK into HAA. Like KAT, it is dependent on PLP as a coenzyme. Dysfunction of KYNU leads to accumulation of XA, exemplified by mutations in the *KYNU* gene accompanied by increased urinary excretion of XA [102]. Experimental knockout of KYNU has also been used to increase levels of HK in IDO-active cancer cells, in order to induce apoptosis [67]. KYNU expression in macrophages can be increased by IFN-γ and TNF-α [103]. KYNU was significantly associated with depression symptoms in a recent genome-wide association study [24].

3-hydroxyanthranilic acid 3,4-dioxygenase (3-HAO / HAAO)

By the action of HAAO, HAA is oxidised into amino-carboxymuconate-semialdehyde. The enzyme utilises iron as cofactor.

Amino-β-carboxymuconate-semialdehyde-decarboxylase (ACMSD)

From amino-carboxymuconate-semialdehyde, the kynurenine pathway splits into the QA branch leading to the synthesis of NAD+, and to Pic or acetyl CoA. The latter is dependent on ACMSD. A mutation in the *ACMSD* gene was found to be associated with increased CSF levels of QA and was more prevalent in suicide attempters compared to population-based controls [88].

Quinolinate phosphoribosyltransferase (QPRT)

From QA, QPRT catalyses the formation of nicotic acid mononucleotide [81]. Like IDO, KMO and KYNU, QPRT is upregulated by IFN-γ [104].

1.1.3 Ratios

Kynurenine-tryptophan ratio (KTR)

KTR is the most widely used ratio and describes the activity of the kynurenine pathway by comparing the level of the product Kyn to the precursor Trp. It is particularly useful as an indicator of inflammation, as IDO induction by pro-inflammatory cytokines cause a substantial reduction in Trp and increase in Kyn in blood [103]. Due to this, KTR is often considered together with markers of inflammation such as neopterin, C-reactive protein (CRP) or cytokines. KTR has been shown to correlated with age, BMI, alcohol consumption, and HDL [105,106]. Reduced cognitive function has been associated with high levels KTR in older community-dwelling adults [107].

Ratios of KA to main branch metabolites (KA/Kyn, KA/HK, KA/QA)

The duality in biochemical properties of kynurenines continues to be of central interest in the context of neuropsychiatric disorders. The neuroprotective property of KA is thought to attenuate the neurotoxic effects of HK and QA, and it is of interest to monitor the balance between these two effects. Ratios of KA over the neurotoxic metabolites HK and QA, or over the common precursor Kyn, are used for this purpose, as well as estimating the activity of KATs.

Markers for B6 availability (HK/XA, HK-ratio)

PLP is an important coenzyme for pathway enzymes KAT and KYNU, and several ratios have been used to inform about the availability of B6. HK-ratio, which puts HK over the sum of the PLP dependent metabolites KA, AA, XA and HAA, has been demonstrated as the best marker for PLP status [108].

Ratio of HAA to AA (HAA/AA)

Several neurological conditions and autoimmune disorders have been associated with reduced HAA/AA [79].

Ratio of picolinic acid to quinolinic acid (Pic/QA)

Pic represents an alternative direction of the kynurenine pathway after HAA, away from the neurotoxic QA. Pic/QA is a measure of this, and of the activity of ACMSD, the enzyme responsible for producing Pic.

1.1.4 Determinants and covariates

Diet

As an essential amino acid, Trp needs to be supplied through the diet. It is the least abundant of all amino acids found in proteins [109]. Dietary sources rich in Trp include turkey, chicken, milk, cheese, bread, oats, banana, and chocolate [110], whereas maize has an especially low Trp content [89]. Kyn can be found in yogurt, white cheese and kefir, while honey, broccoli, potato, cocoa, beer, and wine contain relatively high levels of KA [111,112]. Vitamin B3 (the precursor of NAD+) and the pathway coenzymes FAD and PLP are also supplied through the diet. Blood levels of Trp and kynurenines can be directly affected by dietary intake of Trp, but there is limited knowledge about the effect of specific diets or standardised meals on levels of kynurenines. Severe Trp and B3 deficiency, pellagra, has historically been associated with maize based diet, but is also a possible result of alcoholism and long-term diarrhoea [89]. Niacin-free diets are also shown to decrease blood Trp, and stimulate the NAD+ producing branch of the kynurenine pathway in rats [2]. Regarding the effect of common diets in humans, an intervention study using a two week caloric restriction weight loss diet resulted in a significant reduction in both Trp and Kyn [113]. A crossover study using ten healthy subjects found increased Trp after a four days' fast-food diet, and a reduction after four days with a Mediterranean diet [114]. A high fat diet also increased plasma KTR in mice [115], while a ketogenic diet increased brain KA in rats [116].

The availability of blood Trp for metabolism, and the rate of Trp turnover, is influenced by the intake of other amino acids. For instance, administration of leucine can be used for an experimental increase of Trp metabolism in humans [46], and Trp-free diets and amino acid mixtures can cause Trp-depletion [117]. Incubation of rat brain slices with a mixture of free amino acids reduced KA formation [118]. Furthermore, NEFA, which can displace Trp from albumin and increase the Trp availability for breakdown, increase in concentration during fasting [47]. Supplements of NEFA have been found to be associated with lower HK and KTR [109], suggesting a slower rate of Trp breakdown. Similarly, carbohydrate intake can reduce Trp availability by stimulating the release of insulin which causes lipolysis [119]. More knowledge is nevertheless needed concerning the effects of fasting and standardised meals on circulating levels of Trp and kynurenines in humans.

Age

Higher age is associated with lower serum concentrations of Trp (reviewed in [48]), as well as higher levels of Kyn, AA, KA, HK, and KTR [120]. This may be related to the increased inflammation, reduced kidney function, and somatic comorbidity accompanying higher age.

Kidney function

The main mechanism for disposal of kynurenines is through renal excretion [121]. Reduced kidney function is associated with accumulation of most kynurenines [120-123]. Moreover, kynurenine metabolism is active in renal tissue cells. Prolonged high levels of kynurenines, for instance in the context of chronic inflammation, can cause damage to these tissues, reduce the kidney function and consequently also the excretion of kynurenines [30]. Except for IDO which is stimulated, the activity of kynurenine pathway enzymes seems to be unaltered in patients with kidney disease [30].

Inflammation

In accordance with the tight regulatory connection between kynurenine pathway metabolism and the immune system, inflammation is an essential determinant of blood levels of kynurenines. Through induction of several pathway enzymes, especially IDO

and KMO, pro-inflammatory signals can increase the metabolism of Trp through the pathway down to QA and the synthesis of NAD+ [81,124]. This is probably an important mechanism for the maintenance of cellular metabolism under inflammatory conditions [81,90,125]. QA especially, is substantially increased in various immune cells in response to immune stimuli [81,90,125]. Inflammation is also related to reduced PLP levels [103,126]. Strong covariation between immune markers and kynurenines has been found both in CSF and plasma in clinical studies [127]. Thus, measures of immune markers can yield important information when investigating kynurenines in clinical samples.

B vitamin status

Plasma levels of PLP were positively correlated with Trp, AA, KA, HAA and XA, and negatively correlated with HK and KTR in a large community sample [126]. Plasma HK has also been shown to be lower in users of B6 supplements compared to non-users [128]. Correspondingly, plasma XA/HK, HK-ratio, and urinary excretion of XA are functional markers of PLP status [91,108]. In cases of PLP deficiency, the urinary excretion of Kyn, HK and XA increases [103]. Similarly, increased urinary excretion of KA and Kyn, and decreased excretion of HK, HAA and XA has been reported in relation to vitamin B2 deficiency [46]. In the population study of Theofylaktopoulou and colleagues, riboflavin, a marker of B2 status, was positively associated with HAA and XA, but not correlated with HK [126]. The level of HK thus seems to be influenced more by the rate of PLP-dependent consumption than FAD-dependent formation [126].

Body mass index

Blood levels of Trp, Kyn, KA, HK, HAA, XA and KTR have been shown to be positively correlated with BMI [120,129,130]. The relationship between BMI and kynurenine metabolism could be different between children and adults, and abdominal fat content has been suggested to be more closely related to KTR in overweight adults [130].

Stress

Acute stress in mice has been associated with increased IDO1 expression in various tissues [131], reduced levels of Trp and increased levels of Kyn both in plasma and in the brain [131,132]. In a study of stress associated with academic examination, there was significant increase in cortisol, correlating with increased perceived stress, but there was no significant increase in Trp or kynurenines [133].

Tobacco

Cotinine is a stable metabolite of nicotine that is commonly used as a marker of tobacco use in clinical studies. Cotinine has been found to be inversely correlated with the plasma concentrations of Trp, Kyn, KA, AA, XA and HAA [120] and with KTR [134]. It is also associated with lower blood levels of PLP and riboflavin [135].

Exercise

A significant reduction in blood levels of Trp and Kyn [127,136] and increase in blood KA [137,138] has been found in healthy adults after various forms of physical training. Activation of skeletal muscle has also been shown to consume peripheral Trp and Kyn in a study on mice and could lead to a reduction in the production of neurotoxic metabolites in the central nervous system [139]. However, there is limited knowledge on how different training forms, durations and intensities differ regarding the effect on Trp metabolism [136,140].

Sex hormones

Several sex hormones have been shown to inhibit the activity of several enzymes in the kynurenine pathway [44,141], and levels of KA has been reported to be comparatively low in oral contraceptive users [142].

1.1.5 Liver, blood, and the central nervous system

Most of the Trp metabolism through the kynurenine pathway takes place in the liver. Hepatocytes, the main cell type of the liver, contain all the necessary enzymes and coenzymes to produce NAD or acetyl CoA [81]. Immune cells, muscle cells, glial cells and many other cell types in various tissues also metabolise Trp using IDO [46,81]. Trp and

kynurenines are actively transported across cell membranes and the blood-brain-barrier (BBB), mainly by LAT1. However, a recent study also described a role for the organic cation transporter 2 in Trp availability in relation to SSRI treatment [143].

The concentrations of Trp and kynurenines in the blood is affected by intake and rate of metabolism. Under physiological conditions, TDO is the main regulator of pathway activity, but the effect of IDO increases under pro-inflammatory conditions. In clinical studies, KTR is often used as a proxy for IDO activity, though there is still uncertainty regarding how IDO activation affects blood levels of kynurenine pathway levels in humans [144,145]. The levels of single metabolites are determined both by the rate of synthesis and rate of consumption, and the relative blood levels of kynurenine pathway metabolites depend on the activity of the various pathway enzymes. For instance, increased levels of Kyn and KTR that result from IDO induction can be masked by simultaneous increase in KMO activity and higher consumption of Kyn. Excess of Trp and kynurenines are reflected in their urinary concentrations, for instance as a result of accumulation due to enzyme insufficiency, like in B6 deficiency [91] or in xanthurenic aciduria which is caused by a mutation in the *KYNU* gene [102].

The concentration of TDO and IDO in the brain is lower than in peripheral tissues. The main source for kynurenine metabolism in the brain is Kyn, which crosses the BBB by the LAT1 [35] and is taken up into glial cells [6]. Up to 50% of infused Trp can be transported across the BBB in a single pass through the brain capillary bed [47], but this fraction is affected by the level of albumin binding and the concentration of competing amino acids [119]. HK and XA also seem to cross the BBB [36,69], while KA and QA are thought not to cross in any considerable amount, with their levels in the brain therefore mostly being the result of local metabolism in astrocytes and microglia [6]. As several amino acids compete for LAT1 transport, these amino acids can affect the active transport of Trp and Kyn and possibly reduce KA production in the brain [118]. The production of QA in the brain is usually low, with low nanomolar concentrations [35,81]. In case of inflammation and IDO-induction, however, they show rapid and massive increase in the production of QA that functions both as a tool in pathogen clearance and as a precursor of NAD which is in high demand in pro-inflammatory states [81]. Brain

efflux is the most important mechanism for disposal of KA and QA from the brain [6], and under physiological conditions the concentration of QA in the brain is held below blood levels by active transport out across the BBB [146]. Analyses of cerebrospinal fluid (CSF) or brain tissue can yield information of the concentration of Trp and kynurenines in the central nervous system. However, plasma levels of Kyn, HK, AA and Pic have been shown to correlate well with CSF levels [60,147,148].

1.1.6 Normal ranges, within-person reproducibility, and analytical variation

There are no established physiological ranges or pathological thresholds for Trp or kynurenines, and there is a wide normal range in blood concentrations (Table 1). In a recent meta-analysis of 120 studies the grand means of Trp were 60.52 ± 15.38 μmol/L in serum and 51.45 ± 10.47 μmol/L in plasma, while the grand means of Kyn were 1.96 ± 0.51 μmol/L in serum and 1.82 ± 0.54 μmol/L in plasma [149]. The community sample of Theofylaktopoulou and colleagues shows how the lowest levels of kynurenines are generally associated with high kidney function and young age, while the highest levels are related to low kidney function, high age, and high BMI [120]. The within-person reproducibility has been evaluated by comparing blood levels from the same individuals at multiple timepoints, and is considered good, with intraclass correlation coefficients (ICCs) ranging from 0.5 to 0.7 [150]. There is important analytical variation, with potentially large differences between different laboratories, different analytical methods and between batches of blood samples, that should be considered when comparing concentrations between studies [149].

Table 1. Plasma levels of Trp, kynurenines, PLP, riboflavin and neopterin from four different studies [120,150-152]

	Theofylaktopoulou et al. (2013) Community study (HUSK), non-fasting samples from two age groups: 45-46 years (n = 3723) and 70-72 years (n = 3229)		Midttun et al. (2009) 94 non-fasting, presumed healthy individuals	Midttun et al. (2014) 40 postmenopausal women (NHS)	Ulvik et al. (2013) 2628 non-fasting adults with coronary artery disease (WENBIT)
Metabolite	5% - 95%	lowest 5% - highest 5% (clinical characteristics)	Median (5% - 95%)	Geometric mean (5% - 95%)	Median (5% - 95%)
Trp (µmol/l)	44.4 - 95.5	(heavy smokers) 41.6 - 98.2 (men, young)	63.1 (42.8 - 88.7)	67.1 (64.6 - 69.7)	68.0 (47.3–92.5)
Kyn (µmol/l)	0.94 - 2.63	(highest eGFR, young) 0.88 - 2.94 (lowest eGFR, high age)	1.62 (0.97 - 2.86)	1.62 (1.59 - 1.65)	1.7 (1.1–2.6)
AA (nmol/l)	7.7 - 28.8	(heavy smoker, young) 6.9 - 31.8 (lowest eGFR, high age)	12.7 (6.7 - 33.4)	13.8 (13.4 - 14.2)	13.8 (7.7–26.8)
KA (nmol/l)	24.1 - 92.7	(highest eGFR, young) 21.6 - 121.2 (lowest eGFR, high age)	35.4 (20.4-93.2)	48.1 (46.7 - 49.6)	48.5 (26.0–92.6)
HK (nmol/l)	17.4 - 65.0	(highest eGFR, young) 16.7 - 78.1 (lowest eGFR, high age)	24.8 (12.5 - 65.8)	29.0 (28.1 - 29.9)	29.1 (15.3–58.8)
HAA (nmol/l	17.8 - 64.5	(heavy smoker, young) 15.4 - 70.9 (BMI>30, high age)	23.3 (9.4 - 55.4)	33.9 (32.8 - 35.0)	34.6 (16.0–67.1)
XA (nmol/l)	6.1 - 33.8	(heavy smoker, high age) 5.0 - 36.5 (BMI>30, high age)	11.4 (4.1 - 32.8)	13.9 (13.4 - 14.4)	14.2 (6.1–30.5)
KTR	13.4 - 43.9	(highest eGFR, young) 12.6 - 52.4 (lowest eGFR, high age)		26.8 (26.1 - 27.5)	23.8 (15.8–39.4)
Neopt. (nmol/l)	4.7 - 15.6	(highest eGFR, young) 4.3 - 18.6 (lowest eGFR, high age)	8.4 (5.4 - 25.4)	8.16 (7.97 - 8.37)	7.8 (5.2–14.5)
Ribo. (nmol/l)			11.7 (5.2 - 66.8)	12.5 (11.8 - 13.2)	11.1 (4.5–44.3)
PLP (nmol/l)			37.2 (14.2 - 181.6)	40.6 (39.1 - 42.3)	39.9 (18.6–101)

Abbreviations: AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; HUSK, Hordaland Health Study; KA, kynurenic acid; KTR, kynurnine-tryptophan ratio; Kyn, kynurenine; Neopt., neopterin; NHS, Nurses' Health Study; PLP, pyridoxal 5'phosphate; Ribo., riboflavin; Trp, tryptophan; WENBIT, Western Norway B-Vitamin Intervention Trial; XA, xanthurenic acid

1.2 Attention-deficit hyperactivity disorder, depression and electroconvulsive therapy

1.2.1 Attention-deficit hyperactivity disorder (ADHD)

ADHD is a neuropsychiatric disorder characterised by hyperactivity, impulsivity and/or inattention to such a degree that it leads to impairments in daily functioning. The prevalence of ADHD is estimated to be around 5.9% in children and 2.8% in adults [153]. ADHD has a childhood-onset, but often persists into adulthood [154] with implications for social, academic, and occupational success, and with a significantly increased risk of substance abuse, accidents and death compared to the general population [155-157]. The diagnosis of ADHD is based on symptom assessment and clinical interviews. There are various self-report questionnaires that can be of use when diagnosing ADHD in adults, including the Adult ADHD Self-Report Scale (ASRS) for assessment of current symptoms, and the Wender Utah Rating Scale (WURS) for retrospective assessment of symptoms in childhood.

As determined using twin studies, ADHD has a relatively high heritability, around 74% [158]. It also has a large genetic overlap with other psychiatric disorders, including autism spectrum disorders, anxiety, and depression [159], with which it also often co-occurs [160]. In addition, ADHD has been shown to be associated with a range of somatic disorders, including obesity, diabetes, migraine, asthma, epilepsy, cardiovascular disease, and psoriasis [161-163]. Known environmental risk factors include prematurity, maternal smoking, toxins, unhealthy eating, and adverse life experiences, but these factors have been difficult to separate from the genetic risk [164]. ADHD is generally considered a multifactorial disorder, with no single explanatory factor [165]. According to a traditional hypothesis of ADHD pathophysiology, some ADHD symptoms may be related to a dysregulation of monoaminergic neurotransmission (dopamine, norepinephrine, and serotonin), the principal target of pharmacological treatment. Stimulants, like methylphenidate and amphetamine, are the first line pharmacological option for children and adults alike.

There are no validated biomarkers for ADHD [164,166]. Currently, the most promising biomarkers for ADHD are derived from psychological tests, neuroimaging, and EEG

[153,166]. However, there are multiple candidate biochemical biomarkers related to monoamine synthesis, including homovanillic acid, 3-methoxy-4-hydroxyphenylglycol, 5-hydroxyindoleacetic acid and Trp metabolites [166]. Other noteworthy candidates are inflammatory markers [167], markers of glial function (for instance S100b [168]), markers of neuroplasticity (brain-derived neurotrophic factor (BDNF) [169]), and micronutrients, like vitamin D, lead, magnesium [164]. Importantly a range of genetic variants are associated with ADHD and have been central in the search for biomarkers. Additionally, the genetic profile itself, in the form of a polygenic risk score of ADHD, has some usefulness in predicting ADHD symptoms in the population and people with a high score are more likely to be diagnosed with ADHD [153].

1.2.2 Depression

Depression is a common psychiatric disorder and a major cause of disability and reduced life quality world-wide [170]. Depression is characterised by depressed mood or diminished interest or pleasure in all or most activities most of the day every day, together with other symptoms such as problems with concentration, hopelessness, feelings of worthlessness or inappropriate guilt [171]. To receive a diagnose, symptoms must cause significant distress or impairment in important areas of functioning. There are several clinician-administered questionnaires for assessing the severity of depression symptoms that are often used in clinical studies, including Montgomery-Åsberg Depression Rating Scale (MADRS) and Hamilton Depression Rating Scale (HDRS/HAM-D). Depressive episodes can be isolated or recurrent and can be part of a major depression disorder or a bipolar disorder [171].

Major depressive disorder (MDD) is a diagnosis from the Diagnostic and Statistical Manual of Mental Disorders (DSM) [172], that is commonly used in clinical studies world-wide. MDD has a life-time prevalence of about 16.6% [173]. The clinical presentation of MDD is very heterogenous, both in severity and symptomatology, and there are multiple clinical subgroups [171]. There are many risk factors for depression, including

genetic risk [174], psychological trauma [175], somatic disease [21], immunotherapy [176], and diet [177]. The twin based genetic risk of MDD seems to be lower than for ADHD [174].

Like ADHD, MDD is a multifactorial disorder, possibly with multiple different aetiologies [178]. Theories of pathophysiology include monoamine deficiency [1,179], dysfunctional glutamate signalling [180], inflammation and immune dysfunction [4,7,181], reduced neurogenesis and neuroplasticity [182], HPA-axis dysregulation [183] and metabolic dysfunction [184], all of which indicate a role for kynurenine metabolism.

There are no validated biomarkers of depression, but various promising candidates, including TNF-α, IL-6, IL-1 beta and CRP [185], BDNF [185,186], cortisol [187], homovanillic acid [188] and n-3 polyunsaturated fatty acids [185]. Furthermore, mRNA levels of IL-1 and MIF [189] and polymorphisms in various genes like the multidrug-resistance gene (MDR1) [185] have been associated with the outcome of pharmacological treatment.

Comorbid psychiatric and somatic conditions are common, with notable examples being obesity, metabolic syndrome, diabetes, hypertension, renal disease, coronary disease, Alzheimer's disease, and cancer [190].

Bipolar disorder (BD) is characterised by fluctuations in mood between episodes of depression and elevated mood in the form of hypomania or mania. It has a life-time prevalence of about 2.1% [191] and a high heritability [192]. Depression in BD is similar to major depression, but is typically associated with an earlier age of onset, episodes are often more abrupt and shorter in duration and are more often accompanied by psychosis, psychomotor retardation and catatonia [193].

Antidepressants are the first-line biological treatment of MDD, but the response rates in the first round of treatment are modest and many patients need sequential treatment. Bipolar depressive episodes are often treated with mood stabilisers such as lithium, valproate, and carbamazepine, as well as atypical antipsychotics. Antidepressants may also be used, in combination with mood stabilisers, to prevent switch to mania. Lack of clinical response after a minimum of two trials with different antidepressants in adequate doses is commonly considered as treatment resistance.

1.2.3 Electroconvulsive therapy (ECT)

ECT is a treatment option for severe, treatment-resistant depression, both in MDD and BD, with a high response rate and comparatively rapid clinical effect. ECT is performed by passing an electric current through the brain and thereby triggering a seizure. The treatment is carried out in general anaesthesia. It is typically administered two to three times per week until remission or lack of further clinical response. Some pre-treatment characteristics are associated with higher response to ECT, including higher age, the presence of psychotic symptoms, higher CRP, higher TNF-α, higher homovanillic acid, and lower S-100B [194-196].

The details of the therapeutic effects of ECT are still unknown. Possible mechanisms include altered neurotransmission [197], changes in endocrine signalling [198], immune modulation [199-201], and neurotrophic effects [202]. Several studies have shown increased levels of BDNF after ECT [203,204], a protein involved in neuroplasticity [202]. ECT also seems to cause a broad volumetric expansion in grey matter [205], reportedly as early as two hours after the first ECT session [206]. Moreover, single ECT sessions have been associated with an acute inflammatory response [200,201]. Modulation of kynurenine pathway metabolism has also been investigated as a possible mechanism in ECT, but results have been inconclusive [41,200]

2. Aims

The overarching aim of this work was to examine the tryptophan and kynurenine pathway in patients with various neuropsychiatric disorders, both compared to controls without such disorders and in the context of therapy. Additionally, we sought to identify factors that can affect tryptophan and kynurenine pathway metabolite concentrations and the analysis of these concentrations in clinical studies.

2.1 Specific aims

1. Examine the serum levels of tryptophan, kynurenines, and B vitamins in adults with ADHD and depression compared to healthy controls. **Paper I, II**
2. Investigate changes in serum concentrations of tryptophan, kynurenines and neopterin in patients with depression after ECT. **Papers II, III and IV**
3. Review factors that affect analyses of tryptophan and kynurenines in clinical studies and propose strategies for future studies on tryptophan metabolism in patients with psychiatric disorders. **Paper IV**

3. Material and methods

Table 2. Material and methods.

Studies	Materials	Samples	Design
I	*ADHD in adults in Norway*	133 adults with ADHD and 131 adults without ADHD	Case/control
II	*ECT and neuroradiology*	27 patients with depression and 14 healthy controls	Case/control and repeated measures
III	*MODECT*	48 patients with late-life depression	Repeated measures
IV	Embase, MEDLINE, PsycInfo and PubMed	17 published studies including 386 patients and 27 controls	Systematic review

3.1 Materials and methods

Study I: ADHD Norway, case-control

ADHD in adults in Norway: from clinical characterization to molecular mechanisms is an ongoing clinical project initiated at the University of Bergen (UoB) in Bergen, Norway, in 2004.

Study I included data from 133 adults with ADHD and 131 adults without ADHD participating in this project. The blood samples were mainly collected at Haukeland University Hospital, but some were collected by primary care physicians in Bergen and various other locations in Norway and transported to the laboratory by mail. Fasting was not required. Two self-report questionnaires, ASRS and WURS, were used to collect information on current ADHD symptoms and symptoms in childhood for all participants. Student's t-test was used to compare clinical continuous variables, while Chi squared was used to compare sex, alcohol, drug abuse, comorbid disorders. The main analysis was performed using logistic regression with ADHD status as outcome per tertile of each metabolite in two models, one adjusted for sex, and another adjusted for both sex and tryptophan. Additionally, Spearman's correlation analyses were

performed to investigate the association between ADHD symptom severity and levels of biochemical variables.

Study II: ECT Bergen, case-control, repeated measures

ECT and neuroradiology is a prospective and observational study conducted at Haukeland University Hospital in Bergen from 2015 that aims to clarify the pathophysiology of MDD and the mechanisms of action for ECT [207]. The study includes patients with depression referred to ECT, as well as two control groups consisting of patients undergoing electrical cardioversion for atrial fibrillation and healthy volunteers. The patients receive three ECT sessions per week until remission, or until no further improvement, with individual stimulus dose and a maximum of 20 sessions. Data from neuropsychological tests, clinical assessments, blood samples and MRI are collected for all participants. Blood samples are collected after at least 8 hours fasting. The renal function marker creatinine is measured at baseline.

In study II, we included blood samples and assessed symptom severity from 27 patients before and after a full series of ECT, as well as baseline data from 14 age- and sex-matched healthy volunteers. Response was defined as a reduction of 50% or more in MADRS, and remission as a post treatment MADRS lower than 10. Log-transformed serum concentrations at baseline were compared between patients and controls using linear regression with and without adjustment for smoking. Changes in serum concentrations after ECT were analysed using paired Wilcoxon signed-rank tests in 21 patients and in subgroups based on ECT response and remission.

Study III: ECT Amsterdam, repeated measures

Mood Disorders in Elderly treated with Electroconvulsive Therapy (MODECT) is a naturalistic, longitudinal study on older patients with depression treated with ECT [208]. Patients with depression aged 55 years and older that were referred to ECT were recruited from GGZ inGeest in Amsterdam, the Netherlands, and University Psychiatric Center, KU Leuven, Belgium. The main aims of the project were to compare demographics and clinical characteristics between inclusion sites, to explore clinical and structural brain characteristics and to identify predictors of ECT response.

Each patient was scored with MADRS before and during therapy, and was treated with ECT until remission, or until there was no further improvement the last two weeks of treatment.

Study III included 48 patients from the Amsterdam branch of MODECT, with two venous samples for each patient, one at baseline and one after the completed ECT series. Using paired Wilcoxon signed-rank tests, changes in tryptophan, kynurenines and neopterin were analysed in all 48 patients, and in subgroups based on remission, neopterin change after ECT, and somatic comorbidity.

Study IV: Systematic review

The systematic review was conducted following the PRISMA guideline. Using free text and index terms for tryptophan, kynurenine pathway metabolites and ECT, we carried out a systematic literature search in the four databases Embase, MEDLINE, PsychInfo, and PubMed. The criteria for inclusion were treatment with ECT and pre- and post-treatment measures of either Trp, kynurenines or both. The systematic review identified seventeen studies eligible for inclusion, with publication year spanning from 1973 to 2022. From the included studies, data was extracted on biomarker concentrations as well as on a wide range of factors known to be associated with tryptophan metabolism or depression treatment. Vote counting based on the direction of effect was used to establish if there was evidence for significant changes in single metabolites or ratios after ECT.

3.2 Biochemical analyses

Serum samples from study I-III were all analysed in the laboratory of Bevital A.S. in Bergen, Norway. Serum levels of Trp, Kyn, KA, HK, XA, AA, HAA, QA and Pic, as well as nicotine marker cotinine, inflammatory marker neopterin, vitamin B2 marker riboflavin, and B6 vitamers PLP and pyridoxal (PL) were measured using a high throughput liquid chromatography/tandem mass spectrometry (LC/MS/MS) [151].

3.3 Ethics

Studies that included human subjects (I, II and III) were all carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent to participate. Study I was approved by the Regional Committee for Medical Research Ethics in West Norway (REK Vest, 2013/543). Study II was approved by REK in South East Norway (2013/1032). Study III was approved by REK North (2018/721) and by the Medical Ethical Committee of the Amsterdam UMC, location VUmc.

4. Main results

Study I

We found significantly lower serum concentrations of KA, XA, HAA, riboflavin and vitamin B6 (PL + PLP), and higher cotinine in adult patients with ADHD compared to healthy controls. In logistic regression with tertiles of biochemical variables, low levels of Trp, KA, XA, HAA and riboflavin and high cotinine levels were associated with increased risk of ADHD. Differences in HAA, riboflavin and cotinine remained significant when adjusting for sex and serum Trp.

There was a significant inverse correlation between age and Trp in the material, and the median age was significantly higher in the ADHD group (28.0 vs 22.5 years). When comparing two ADHD age groups, lower levels of Trp and XA, and higher riboflavin and KTR were associated with higher age (34-40 years, n = 36). Comparing patients and controls with similar age (97 patients aged 19-33 vs 131 controls aged 18-33), lower levels of Trp, HAA and riboflavin, and higher cotinine were all associated with ADHD.

We also found a significant inverse correlation between Trp and kynurenine and current and past ADHD symptom scores (ASRS and WURS) when including all participants and controlling for cotinine and age. Correlation analyses within the ADHD group showed that levels of Trp and vitamin B6 were inversely correlated with total ASRS and the inattentive subscale. No significant correlations were found in controls alone. There were no significant differences in biochemical variables between patients with or without self-reported anxiety/depression or bipolar disorder, or between medicated and drug-naïve patients.

Study II

Three out of 30 patients were excluded, one due to missing baseline blood samples, two due to high serum creatinine (>120 μmol/l). There were 36% smokers among controls and 52% among patients. The median MADRS score was 1 for controls and 34 for patients. Compared to healthy controls, patients with depression had lower levels

of KA, XA, Pic, KA/Kyn, KA/QA, XA/HK and Pic/QA. When adjusting for cotinine levels, KTR was significantly higher in patients.

After ECT, MADRS was reduced from 34.0 to 15.0. Out of 21 patients with repeated measures, twelve patients responded to treatment. There was no significant difference in number of treatments between responders and non-responders, but non-remitters had significantly more sessions than remitters. There was significant increase in HAA, Pic, Pic/QA and neopterin after ECT. Responders had significant increase in HK and Pic, and there was significant increase in neopterin in both responders and non-responders as well as in non-remitters.

Study III

After a full ECT series, MADRS decreased from a median of 32.5 to 6.5, with 30 remitters and 18 non-remitters. There were no significant changes in single metabolites, but a reduction in KA/HK after treatment. In sub-analyses based on remission status, remitters had increased post treatment levels of HK, XA and HAA, while non-remitters had reduced levels of QA after ECT. In patients that had increased neopterin after ECT (n = 25), HK and KTR were also significantly increased, while PLP and KA/HK were reduced. In patients with reduced post-treatment neopterin (n = 23), AA, QA and KTR were also significantly reduced. Eleven patients with no registered somatic comorbidity had significant increased TRP, HK and HAA after ECT, while patients with one or more somatic diagnoses (n = 37), had no significant changes in serum biomarkers.

Study IV

The systematic review included 19 reports, as well as previously unpublished data from the control group of study II, for a total of 386 patients with depression treated with ECT and 27 controls. Using vote counting based on the direction of effect, there was no overall evidence of an effect of ECT on free Trp, total Trp, Kyn, KA or KTR, the five biomarkers that were available in an adequate number of studies to enable analysis. The most conspicuous patterns of change were trends of increased levels of HK, AA, HAA, XA, and Pic in the three studies investigating a large panel of kynurenines.

In addition, we reviewed a range of factors related to Trp metabolism, including inflammation, age, kidney function, BMI, sex, and B vitamin status, as well as details regarding the study populations, study designs, sample handling and treatment delivery. Our review showed that there were large variations both in study populations, study design and in the declaration and handling of such variables relevant for interpretation of changes and study comparison. Furthermore, large differences in baseline concentrations and a lack of clear changes after ECT suggested a large unexplained variability both between studies, between individuals and within individuals.

5. Discussion

5.1 Do patients with depression or ADHD have altered kynurenine metabolism?

Abnormal levels of Trp and kynurenines compared to healthy controls have been demonstrated in several meta-analyses of patients with major depression disorder [13,15-17,19] and bipolar disorder [16-20] as well as schizophrenia [14,17]. A recurring finding is that patients with psychiatric disorders have lower Trp and Kyn compared to healthy controls [17].

The central hypothesis states that imbalance between neurotoxic (mainly HK and QA) and neuroprotective metabolites (mainly KA) disturbs glutamatergic signalling and facilitates a neurotoxic environment [11]. Meta-analyses indicate that this might be the fact, at least for depressed patients who have comparatively low levels of KA and its ratios KA/Kyn, KA/HK and KA/QA [17]. These differences between patients and controls are suggestive of impaired neuroprotection in depressed subjects [17].

Depression

Our own results of study II, published in 2019, coincide with these meta-analyses, with low serum levels of KA, XA and Pic, and low KA/Kyn, KA/QA, XA/HK and Pic/QA in patients with uni- or bipolar depression compared to healthy controls. When adjusting for smoking, patients also had relatively high levels of KTR, indicative of an inflammation related stimulation of the pathway.

Results from neuroimaging studies also lend support to the findings of abnormal kynurenine metabolism in depression (reviewed in [209]), with observed correlations between hippocampal volume and plasma KA/QA [210], and between striatal volume and plasma KTR [211]. KA/HK has also been found to be a moderating factor in the relationship between MDD and cortical thinning [212]. Furthermore, MR spectroscopy of adolescents with MDD has shown associations between plasma Kyn and HAA, and striatal levels of choline, a marker of cell membrane metabolism that is often increased in depression [213]. A higher serum KA/QA ratio has also been found to be associated

with increased white matter integrity in several brain areas [214]. These reports add to the evidence of a role of kynurenines in depression.

Correlations have also been shown between kynurenines and symptom load in depressed patients, including an inverse relationship between KA and MADRS [215], AA and HAM-D [216], and KA/QA and anhedonia [217]. Depression symptoms have also been positively associated with KTR in patients with hepatitis C treated with IFN-α [124]. Similar results have also been found independent of the depression diagnosis, with a significant correlation between KTR and depression symptoms in a population based study [105]. KA/QA has also been identified as a predictor of ketamine response [218], and low KA has been reported as a predictor of response to treatment with escitalopram [219].

However, results of these studies are inconsistent, and there are multiple studies that have not found significant associations between kynurenine levels, MR markers and depression scores [209,220].

ADHD

In contrast to the literature on depression, kynurenine levels in patients with ADHD have only been described in a handful of studies (reviewed in [40,221]), and have not been subject to meta-analyses.

In study I, we found that adult patients with ADHD had lower serum levels of Trp, KA, XA and HAA compared to healthy controls. Furthermore, total symptom scores were inversely correlated with Trp and Kyn levels. In contrast, studies on children with ADHD have found higher blood levels of Trp [168,222] and Kyn [222-224], and higher urine levels of Trp [225] in patients compared to children without ADHD. Evangelisti and colleagues also found higher KTR in patients, but lower levels of KA, AA and XA [222], while Saglam and colleagues found lower levels of HK, HK/Kyn and KA/Kyn [224]. Another study found no differences in Trp, KA, XA, AA or QA between children with ADHD and their control group consisting of healthy siblings [226].

Some animal studies also suggest a role of kynurenines in ADHD. Patched domain containing protein 1 (*PTCHD1*) mutation is a known genetic risk factor for autism.

PTCHD1 knockout mice display ADHD symptoms, and have been shown to have increased levels of Kyn, AA, KA, HK, KTR and KA/Kyn compared to wild type [227]. The level of Kyn significantly decreased after treatment with atomoxetine, together with a reduction in symptom levels [227]. Plasma KA has also been positively associated with ADHD-like symptoms in dogs in a non-targeted study [228].

Peripheral vs central levels

The results discussed above are mainly from studies on peripheral levels of kynurenines, often regarded as a proxy for levels in the central nervous system. Analyses of CSF are likely better suited for evaluating levels of kynurenines in the context of psychiatric disorders. But although several studies have done this, there seems to be more obstacles when designing and carrying out a study based on CSF compared to peripheral measures. In the meantime, methods such as neuroimaging/MR spectrometry [210,213] and correlation analyses between central and peripheral measures [60,147,148,229], contribute to bridge the gap in knowledge between of peripheral and central kynurenine metabolism.

Challenges with the case-control design

Although meta-analyses show some significant differences between patients and controls, many of the studies in this area are small. They are vulnerable to the effect of outliers and are susceptible to bias. In study IV, we discussed various challenges with outlining the kynurenine metabolism in patients with depression, most of which are transferrable to studies of kynurenines in other neuropsychiatric disorders. Establishing good references for comparison is one such challenge. One of the main problems of single, small scale clinical studies/pilot studies, aiming to shed light on kynurenine metabolism in a patient group, is the reliance on small control groups that do not provide a reliable baseline for comparison.

In study IV, we found considerable variations in levels of Trp and kynurenines both between studies, and between patients and controls within studies. The between-study variation is most likely linked to differences in pre-analytical and analytical factors and underline the general difficulty of establishing good references for evaluating patient

levels and the need for control groups. However, these control groups need to be carefully selected to minimise potential bias arising from sample collection, sample handling and clinical characteristics such as life-style factors, somatic or psychiatric illness, and medication.

Poor matching is a well-known and common issue, and differences between controls and patients in variables that are not a feature of the disorder could introduce bias to the analyses. Two common examples are age and sex, and in many studies, patients and controls are age- and sex-matched to minimise this kind of bias. In study I however, the ADHD group had a wider age range, and a significantly higher mean age. Age is a known determinant of Trp and kynurenines, and sub-analyses based on age groups showed that it was indeed an important factor when comparing metabolite levels between patients and controls. The handling of other clinical features such as socio-economic status, education or life-style factors are more complicated, as they are often tightly related with the disorders and not suitable for matching. Choosing controls that reflect the general population can often intensify the importance of these factors, and so a conflict emerges between choosing a control group that reflects the general population or a control group that minimises the effect of these factors. The frequency of smokers in study I provide a valuable example. Smoking was very common in the ADHD group, but quite rare in control group, something that could affect comparison of kynurenine levels. If, on the other hand, there had been an equal number of smokers among the controls, they would not be representative of the general population and there would be a high risk of overcontrolling.

Similarly, undiagnosed psychiatric disorders in the control group can compromise its value as a reference point. In study I, 6.2% of the healthy controls had an ASRS score above the standard cut-off for ADHD, and 3.8% reported severe anxiety or depression. While these are not evaluated clinically and cannot be considered as diagnoses, this may obscure biomarker differences between the groups, as indicated for instance in a meta-analysis of inflammatory markers and depression [230]. Similarly, the presence of somatic disorders in patient or control groups can also affect the differences in levels of kynurenines between the groups [230].

In conclusion, meta-analyses indicate that patients with neuropsychiatric disorders have altered tryptophan-kynurenine metabolism, with low Trp and Kyn being a recurrent finding. Furthermore, especially in patients with depression, KA seems to be low relative to other kynurenine metabolites. Kynurenine metabolism in ADHD is still little explored, but the low levels of Trp and several kynurenines in our study are in line with the trend across other psychiatric diagnoses. However, there are several challenges when comparing concentrations between groups, like the handling of life-style factors, and comorbidity. In addition, Trp availability and levels of kynurenines in the central nervous system need to be further investigated.

5.2 Do levels of kynureniens change after depression treatment?

Given that patients with depression have abnormal blood levels of Trp and kynurenines, and that abnormal kynurenine levels seem to be an aspect of the pathophysiology of depression, it can be hypothesised that these metabolites are restored to normal concentrations after effective treatment [231].

In studies II and III, we investigated change in Trp, kynurenines and related markers after ECT. In study II, we found increased levels of Pic, HAA and neopterin after a series of ECT for 21 patients with uni- or bipolar depression. In study III, there was a reduction in KA/HK after a series of ECT for 48 patients with late life depression. These results does not seem to be in line with the hypothesis of a normalisation of the balance between neuroactive metabolites, which would mean an increase in KA relative to toxic metabolites like HK and QA.

Several similar studies have been carried out, but the results have been inconsistent. Blood levels of Trp and Kyn have been reported both to increase [232] and decrease [233] after ECT, and have remained unchanged in other studies [234,235]. Regarding the balance between neuroactive kynurenines, some studies have reported improved neuroprotection, with increased levels of KA and KA/HK [233], and increased level of

KA/QA after ECT [235]. Other studies have found opposite trends, with significant increase in HK [232] and reduction in KA/HK [234], which is more similar to the results of study II and III.

We summarised the results from studies on change in Trp and kynurenines after ECT, including studies II and III, in a systematic review presented in study IV (Table 3). Using vote counting based on the direction of effect we found no evidence for an effect of ECT on the levels of Trp, Kyn, KA or KTR. These analyses did not consider the studies' quality or sample size. Still, the lack of significant changes is consistent with a recent meta-analysis that found moderate evidence for no effect of ECT on plasma Trp [41].

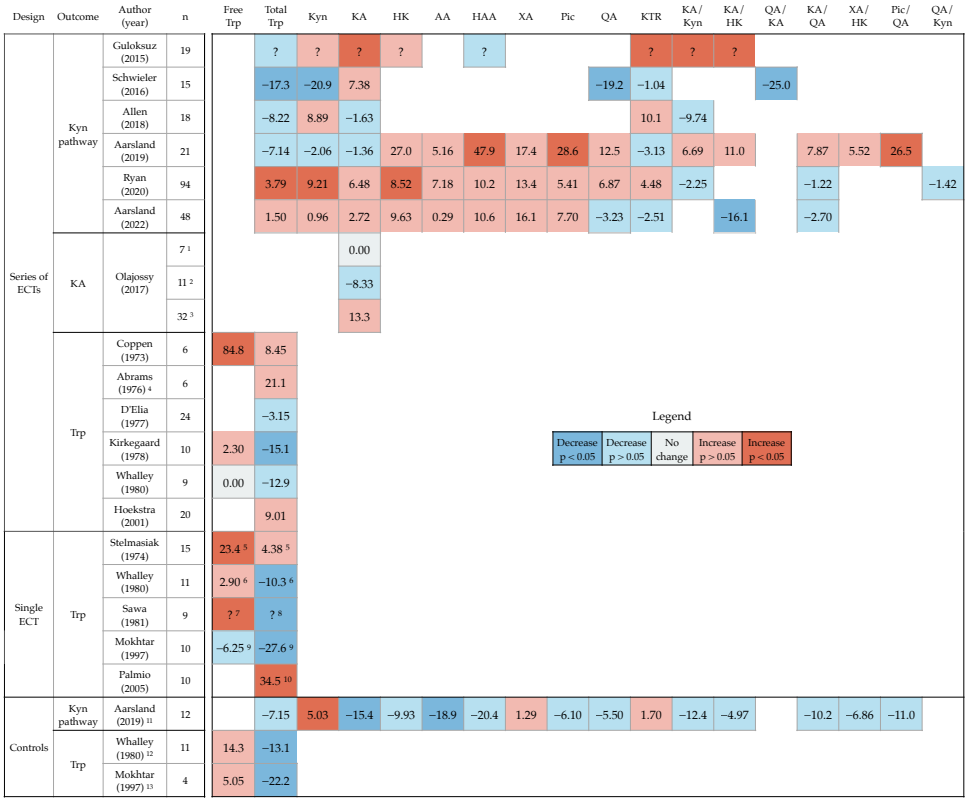
Relation to the clinical effect

It is possible that changes in kynurenines are more prominent in individuals who respond to treatment or who reach remission. We therefore performed sub-analyses in groups of responders (study II) and remitters (study III). In study II, those who responded to ECT had increased levels of HK, Pic and neopterin after treatment. Non-responders also had an increase in neopterin, but no significant changes in kynurenines. In study III, remitters also had increased HK, as well as XA and HAA after treatment, while non-remitters had reduced QA. Similar to the results from the main analyses discussed above, post-treatment increase in levels of HK is contrary to what is expected from the hypothesis that the neuroprotective balance should be normalised after treatment. Among the other reports reviewed in study IV, one found increased Trp and neopterin in 15 ECT responders that was absent in non-responders (n = 5) [236], while two other ECT studies found no significant changes in responders [234] or remitters [237].

If there are acute effects of ECT on inflammation, stress, or Trp metabolism, it is possible that also non-responders and non-remitters could display changes in metabolite concentrations due to the exposure to ECT and its effect on the body. In study II, non-remitters had significantly more ECT sessions than remitters, and had a significant increase in neopterin after ECT. Increase in IL-6 after ECT has also been found to correlated positively with the stimulus dose [238]. A higher degree of exposure

to ECT in non-responders could cause an association between inflammation, non-response and treatment that could potentially obscure kynurenine changes.

Table 3. Summary of results from studies on changes in Trp and kynurenines after ECT.



Adapted from study IV. The table shows percentage change calculated from the included studies (see study IV for references). Question marks indicate missing data on concentrations. Comments on participant diagnosis, sample type, and sample timing: 1) schizoaffective disorder, 2) depression in bipolar disorder, 3) recurrent depressive disorder, 4) CSF samples; samples collected 5) 15 min after ECT, 6) 10 min after first ECT, 7) 1 min after ECT, 8) 5 min after ECT, 9) 60 min after ECT, 10) 2 h after ECT, 11) with eight weeks in between, 12) after recovery from anaesthesia, and 13) 15 min after start of surgery. Abbreviations: AA, anthranilic acid; ECT, electroconvulsive therapy; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine-tryptophan-ratio; Kyn, kynurenine; Pic, picolinic acid; QA, quinolinic acid; Trp, tryptophan; XA, xanthurenic acid.

Kynurenine changes after other depression treatments

Changes in Trp and kynurenines have also been investigated in the context of other depression treatments, including transcranial magnetic stimulation (TMS), ketamine and oral antidepressants. Like ECT, TMS is a non-invasive brain stimulation technique available for treating patients with treatment-resistant depression. Increased Trp was found in plasma of 13 patients after repeated TMS (rTMS) in one study [239], and increased Kyn in CSF of 5 patients in another [240], but there were no significant changes in kynurenines after TMS in five other studies (reviewed in [41]).

Ketamine is a NMDA-receptor antagonist, like KA, and is a pharmacological treatment option for severe depression. The effect of ketamine treatment on kynurenine metabolism has also been studied (reviewed in [241]). One study reported increased Kyn, KA and KA/Kyn, and reduced QA/Kyn after treatment [242], while another found increased KA and KA/Kyn in responders [243]. A third study found a trend of decrease in Kyn after first infusion in responders [234].

Regarding oral antidepressant treatments, increased plasma Trp has been documented after 18 weeks with fluoxetine treatment, but also in a control group receiving psychiatric counselling [244]. Another study reported reduced serum levels of HK, QA and HK/kyn, QA/Trp, KA/QA and QA/HK in 30 patients after 12 weeks of escitalopram monotherapy [245]. Increased plasma Kyn has also been found after 6 weeks treatment with lithium or mirtazapine, but not in patients treated with amitriptyline [246]. In metabolomics study, Zhu and colleagues found reduced Kyn/melatonin and HK/melatonin after treatment compared to baseline in patients that responded to sertraline [247]. Another metabolomics study, where 290 patients received either citalopram or escitalopram, showed no changes in plasma Trp, Kyn or HK after 4 or 8 weeks of treatment, although 5HIAA/5HT and indole-3-acetic acid were significantly increased [248].

Some of the studies mentioned above report a relative increase in KA, something that could be taken as a sign of increased neuroprotection after treatment. However, like for the studies of ECT, the results are highly inconsistent.

Challenges with repeated measures

Like with case-control studies, there are multiple challenges when working with repeated measures. In study IV, we extracted information about study design and sample handling from the reviewed studies, and discussed several factors that makes it difficult to make a proper assessment of changes in Trp and kynurenines after treatment.

The time between treatment and blood sampling could be an essential factor when assessing biomarker levels. For studies of ECT, it is a challenge to distinguishing the acute effect of ECT itself from the potential biochemical changes associated with clinical improvement. While acute changes after ECT are best investigated with samples collected in the following hours or days, changes that are related to the accumulated effect of a treatment series may be better demonstrated in samples drawn after the acute phase has passed. In study II and III, we only included samples at baseline and after the full treatment series. Ideally, studies should utilise both methods, with blood samples both right after ECT and after the whole treatment series.

In their review and meta-analysis of studies on non-invasive brain stimulation and Trp metabolism, Giron and colleagues found strong evidence for heterogeneity, and concluded that the absence of effect likely stemmed from the considerable differences in ECT protocols and biomarker sampling time points [41]. In our studies, we did not find significant correlations between number of ECTs and kynurenine levels, but as previously discussed, non-remitters had more ECT sessions than remitters as well as increased levels of neopterin. Variations in sampling times between patients are probably very likely to cause heterogenous results and obscure biomarker changes. In study III, 11 patients had their baseline blood sample drawn after the first ECT. However, excluding these patients from the main analyses did not affect the results.

The normal variation of kynurenines constitutes another challenge when trying to identify the treatment effect. In the material of study II, we had repeated measures for 12 healthy controls, that could provide a reference point when analysing change in patient samples after treatment. However, analyses revealed that the control group had

significant changes in Kyn, KA and AA at eight weeks follow up, even though these participants did not receive any treatment or other intervention. Although the within-person reproducibility of these metabolites is considered good, there seems to be enough variation during the span of a clinical study that it is difficult to separate the effect of treatment from normal fluctuations. Larger samples are possibly needed to provide reference for changes in kynurenine pathway metabolites in clinical studies.

In studies II and III, there were considerable differences between patients not only in baseline levels of blood biomarkers but also in clinical characteristics such as age, depression severity, medication, and somatic comorbidity. It is not clear what effect this kind of heterogeneity has on the potential for change in kynurenine levels, or to what degree it affects the potential of identifying metabolite changes related to treatment. One example is kidney function (measured as serum creatinine in studies II and III), which varies considerably between individuals and constitutes an important determinant for serum levels of kynurenines [120]. It is possible that differences in kidney function, as well as other somatic comorbidity, could affect the potential for change in kynurenines. In study III, there were strong correlations between baseline creatinine and levels of kynurenines both before and after treatment. However, we found no association between baseline creatinine and change in kynurenines. There were also considerable differences in metabolite levels between patients with and without somatic comorbidity, and patients without somatic diagnoses had significant increase in levels of Trp, HK and HAA that were not seen in patients with somatic disease.

Lastly, like with the question of abnormal baseline levels of kynurenines, investigations of change after treatment are usually based on peripheral measures, which leaves much uncertainty about changes in the central nervous system. Even without significant changes in blood, there could be relevant changes in kynurenine metabolism in the brain. Only a few studies have analysed changes in Trp and kynurenines in CSF after ECT, but without consistent results [41].

In conclusion, there is so far no consistent evidence of changes in kynurenines after ECT. This is in line with studies on other antidepressant treatments, which have not yet

documented convincing results regarding kynurenine pathway changes. There are multiple methodological challenges related to this research, and low samples sizes as well as heterogeneity in participants, treatment delivery, and blood sampling are possible explanations for the lack of significant results.

5.3 Interpretations and overarching methodological considerations

As discussed in the sections above, patients with depression and ADHD seem to have altered kynurenine metabolism, but there are no consistent results from studies on changes after treatment. The next questions are what mechanisms might be involved, and whether or not Trp metabolism and the kynurenine pathway are causally linked with the disorders in question.

5.3.1 Potential mechanisms

There are multiple possible explanations for the discrepancy in Trp and kynurenine levels between patients with psychiatric disorders and healthy controls, but differences between participants in factors that have a large impact on kynurenine metabolism stick out as a major candidate. In our review in study IV, we discussed several factors that can affect levels of Trp and kynurenines, and it was clear that there is no consistent consideration of these factors in clinical studies.

Nutritional status and other life-style factors

Dietary habits can affect Trp metabolism [109], and the potential usefulness of dietary interventions has been studied in relation to both ADHD [249] and depression [109]. There is a vast literature that indicates a poorer dietary quality in patients with psychiatric disorders. In short, intake of fish, fruits and vegetables has been inversely associated with risk of both ADHD [249] and depression [250,251]. Furthermore, summaries of observational studies utilising various dietary indices have shown that healthy eating,

or adherence to certain specific diets, is associated with lower risk of depression [252,253]. Dietary improvement has been shown to reduce symptoms of depression in randomised control trials of nonclinical depression [254]. Bipolar disorder is also associated with higher intake of energy and sodium, proxies for poor diet quality [255]. Similarly, unhealthy diet has been associated with ADHD traits [256] and diagnosis [257], and high consumption of sugar and unhealthy food also seems to interact with genetic risk factors for ADHD [258]. Youths with ADHD also had relatively low levels of omega-3 polyunsaturated fatty acids in a meta-analyses [259].

Regarding Trp specifically, one study reported an inverse association between Trp intake and prevalence of depression symptoms in Japanese students and their mothers [260]. A low intake of Trp has also been found in elderly with mild to moderate depression compared to healthy young adults and elderly without depression [261]. A small cross-sectional study on diet and ADHD in children, found that higher intake of aspartate and glycine, and lower intake of glutamate, was associated with increased risk of ADHD, but found no significant effect for Trp intake [262].

In the studies of this thesis, we had no direct information on dietary habits for patients or controls. In study I, adult ADHD patients had comparatively low levels of Trp, KA, XA, HAA and riboflavin. In a related study on the same sample, ADHD was also associated with relatively low levels of vitamins B6 and B9 [263]. Low levels of riboflavin and PLP could contribute to low serum levels of HAA and XA, two metabolites that are dependent on the co-enzymatic activity of these vitamins [126]. In their study on children with ADHD, Dolina and colleagues also interpreted the altered Trp metabolism as a possible deficit in vitamin B6 and postulated that pyridoxine supplements could correct the abnormal Trp metabolism [225]. Low levels of B2, B3 and PLP have also been reported in patients with depression, with 30% of patients having low levels of PLP in one study [264]. However, we found no significant differences in levels of PLP or riboflavin between patients with depression and healthy controls in study II.

Additional life-style factors could also be of importance. Smoking was significantly more common among patients with ADHD than controls in study I. The relatively low levels of Trp, KA, XA, HAA, PLP and riboflavin observed in these patients are in line with previously reported inverse relationships between smoking and blood levels of Trp, kynurenines and B vitamins [120,135]. In study II, there was no significant difference in the number of smokers, with 36% among controls and 52% among patients. However, when adding cotinine as a variable in baseline comparisons, KTR and neopterin were significantly higher in patients. KTR has previously been reported to be comparatively low in smokers [134], and smoking could potentially mask differences in inflammation and kynurenine metabolism between patients and controls in study II.

Physical exercise is associated with lower mental health burden [265], and with lower risk of developing depression [266]. However, a review of intervention studies on exercise, mental health and Trp metabolism, found no consistent changes in Trp or kynurenines [140]. We did not have information on physical exercise in our materials. Similarly, high alcohol consumption, which is associated with malnutrition and increased morbidity, was significantly more frequent among patients compared to controls in study I and could potentially play a role for Trp and kynurenine levels.

In sum, poor nutritional status is a possible explanation for low levels of Trp, kynurenines and B vitamins in adults with ADHD observed in study I. Similarly, it could be part of the explanation for the low levels of Trp and Kyn reported in meta-analyses of patients with depression. On the other hand, high levels of Kyn, KTR, or metabolites on the main branch such as HK and QA, are suggestive of a higher Trp availability or increased Trp turnover for instance due to inflammation related induction of IDO.

Inflammation

Inflammation is a central aspect of both psychiatric disorders and kynurenine metabolism (reviewed in [7,23,35,209,267,268]). Meta-analyses have found lower levels of TNF-α and higher levels of IL-6 in subjects with ADHD [269,270] and higher levels of TNF-α,

IL-3, IL-6, IL-12, IL-18, soluble IL-2 receptor (sIL-2R) as well as neopterin in patients with depression compared to healthy controls [106,230,271-277]. Higher levels of inflammatory markers at baseline have been associated with poor response to treatment with anti-depressants [189,278], but also better response to ECT [279,280]. Furthermore, cytokine therapy has been shown to increase the risk of depression [176], and depression treatment is associated with decrease in inflammatory markers in several meta-analyses [278,281-284].

Reduced levels of Trp and increased Kyn, KTR and QA have been observed in the context of cytokine treatments, and correlate with an increase in depression symptoms [176,285,286]. Similarly, administration of endotoxin in healthy subjects has been followed by depression symptoms, and an increase in blood levels of IL-6, TNF-α, KTR, Kyn and KA, as well as reduced levels of Trp, with a significant positive correlation between depressive mood and levels of Kyn and QA [287]. Furthermore, blocking of TNF-α has been shown to prevent IDO expression and damage to cortical neurons in mice exposed to chronic stress [288], and LPS-induced depression-like behaviour in mice has been blocked by inhibiting IDO [289]. However, despite the well-established relationship, it is still unclear if inflammation is a feature of depression in general [275], or if it is linked to specific subgroups of patients with depression [290,291].

In study I, we had no measures of immune markers other than the kynurenine metabolites. KTR was not significantly different between patients and controls and was not correlated with ADHD symptom scores. Thus, there were no signs of chronic inflammation. Comparatively low levels of KA, XA and Pic in the patient group of study II could potentially be related to inflammation and an activation of the main pathway branch towards QA. The patients also had higher KTR and netopterin than healthy controls, but only when adjusting for cotinine.

The results from meta-analyses suggest that there is an acute inflammatory response immediately after an ECT session, but that the levels of inflammatory markers are normalised after a full treatment series [200,201]. MRI studies have also shown correlations between volumetric increase in hippocampus and peripheral reduction in IL-6 and TNF-α after ECT [292]. In studies II and III, measures of neopterin were used to monitor

immune activation or suppression following ECT, and to shed light on the possible role for kynurenine pathway changes after treatment. After a full series of ECT, neopterin was significantly increased in study II, suggesting an immune response. The observed increase in Pic and HAA in these patients after treatment could be related to increased immune activity. In study III, we did not find the same significant change in neopterin in the whole patient group after ECT. Instead, there was a variety of changes in both directions. When dividing the patients into two groups based on direction of neopterin change, however, there were significant changes in several kynurenines corresponding with the neopterin change. Thus, although the general immune response of study II was not replicated, inflammation was nevertheless a central factor for changes in kynurenine metabolites. The change in neopterin was closely linked to changes in kynurenines, and consistent with the known interaction between tryptophan metabolism and inflammation. As such, both studies pointed to inflammation as a central component in analyses of kynurenines in the context of ECT and underline the need for careful study design and improved methods for analyses.

Somatic comorbidity

Inflammation and abnormal kynurenine metabolism are prominent aspects of a wide range of somatic diseases including neurodegenerative disorders [10,25,293], cardiovascular disease [29], kidney disease [30], endocrine disorders [294,295] and various forms of cancer [32]. Importantly, obesity has been identified in several studies as a moderating factor in the relationship between depression and inflammation [105,106,273,296]. Obesity is also an important comorbidity of ADHD [297]. Moreover, serum levels of neopterin have also been shown to be positively correlated to BMI [298].

Differences in somatic health could be a factor when comparing levels of Trp and kynurenine between patients and controls. Study II included baseline measures of creatinine, and several patients had levels indicating moderate or severe kidney failure. Two patients were excluded from the analyses for this reason, but the role of somatic comorbidity was not further examined. Study III also included baseline creatinine measures, which correlated significantly with levels of multiple kynurenines both before and after treatment. There was, however, no correlation between baseline

creatinine and change in kynurenines. In study III, we also had information on a range of somatic diagnoses. When we compared patients with and without somatic comorbidity separately, there were significantly higher baseline levels of Trp, Kyn, KA, HK, HAA, QA and Pic in those with one or more somatic diagnoses, but no difference in neopterin. After ECT, there was a significant increase in Trp, HK and HAA in patients without somatic diagnoses, but no significant changes in patients without these conditions. Study I did not include detailed information on somatic comorbidity, and none of the studies I-III included data on BMI.

Stress

Stress has been associated with an increased risk for depression, possibly through glucocorticoid resistance and increased immune activity [9,187,299]. As such, stress could be a common factor for anxiety, depression, trauma, suicidal ideation, inflammation, and kynurenine alterations. However, a meta-analysis did not find evidence for abnormal cortisol levels in ADHD compared to healthy controls [300]. None of our three clinical studies included measures of cortisol or other markers of stress, and the potential role of stress could therefore not be evaluated.

Enzyme function

Dysfunctional enzymes could potentially cause abnormal levels of kynurenines in psychiatric disorders. Single nucleotide polymorphisms in enzymes including TPH2, KMO and KAT have been associated with depression [301]. In a study of postpartum depressive symptoms, women with a specific KMO polymorphism also had comparatively high serum HK and HK/Kyn [302]. A genetic variant that decreases KMO activity has also been associated with elevated KA levels in CSF of BD patients with psychosis [303]. Furthermore, a polymorphism in the ACMSD gene has been associated with higher levels of QA in CSF of suicide attempters [88]. Dysfunction of ACMSD is a potential explanation for low Pic and Pic/QA in depressed patients like in study II and similar studies [232,304]. Similarly, KAT and KYNU deficits have also been suggested as potential causes for low levels of KA and AA in ADHD [222].

Medication

Lastly, medication could affect levels of Trp and kynurenines in patients with psychiatric disorders. A range of drugs have been shown to affect the levels of Trp in blood [119]. Amphetamine and salicylate could increase the free fraction of TRP, while insulin has been reported to reduce free Trp [119]. TDO activity has been shown to be inhibited by several antidepressants [305], and reduced Trp breakdown in the liver and increased brain synthesis of serotonin has for instance been reported in rats treated with the SSRI paroxetine [306]. Rats treated with the anticonvulsant drug carbamazepine had increased brain synthesis of KA [307]. Studies of glial cultures from rats incubated in four antidepressants (citalopram, fluoxetine, amitriptyline and imipramine) found increased expression of KAT I and II, and reduced production of HK and increased production of KA [231]. Besides antidepressants, other common drugs have also been suggested to affect Trp and kynurenine levels in clinical studies, including anti-inflammatory drugs [308], oral contraceptives [142] and anti-rheumatics [309].

The use of medications can thus conceivably influence the baseline levels of Trp and kynurenines of both patients and controls. In the ECT studies reviewed in study IV, the use of antidepressants, muscle relaxants and anaesthetics were commonly discussed as a limitation. The overall role of medication on baseline kynurenines and changes after treatment is difficult to assess due to the large variation in drug use between individuals and their washout times. Additionally, it can be ethically problematic to discontinue therapeutic or useful drugs in biomarker studies.

5.3.2 Sample handling, biochemical analyses, and statistical analyses

Pre-analytical factors

Various pre-analytical factors can affect the analyses of kynurenines. Firstly, variations in time of blood sampling could influence blood concentrations, as repeated sampling of CSF from healthy subjects across 24 hours has shown substantial diurnal variation in Trp levels [310]. In studies II and III, all blood samples were collected in the morning,

while there was no specific timing for blood collection in study I. Secondly, fasting could have a large impact on metabolite levels. In studies II and III, samples were collected after at least 8 hours fasting or fasting overnight. In study I, on the other hand, fasting was not required, potentially strengthening the role of variations in diet between participants. Thirdly, the concentrations of metabolites are dependent on sampling handling. The blood level of AA increases when stored in room temperature, while HK, HAA and PLP levels decrease [311]. One meta-analysis showed that significant differences in kynurenines levels were observed in studies on plasma but not in similar studies using serum [17]. This is likely related to a lower stability of kynurenines in serum compared to plasma when samples are not handled optimally. In study II, all blood samples were collected at Haukeland University Hospital, and serum was separated and stored at -80°C within a few hours. Most samples of study I were handled in the same way, but some were collected in other parts of the country and sent by mail, thus being exposed to ambient temperatures.

Statistical analyses

In studies I-III, the kynurenine pathway metabolites were mostly considered separately, although they are interdependent. The focus on single metabolites is a limitation for investigations of both baseline levels and changes over time. We therefore explored the relationship between the different kynurenines through extensive use of correlation analyses, both of raw metabolite levels and variables of change in levels after ECT in studies II and III. In study I, we also investigated the impact of precursor variations between participants, by including Trp as an adjusting variable in logistic regression. However, for a better understanding of the status of the pathway and changes after treatment, the levels of kynurenines should have been evaluated using methods that consider all metabolites together as part of a pathway.

The handling of factors affecting the concentrations of Trp, kynurenines and B vitamins is also a challenge. As discussed above, there are known correlations between kynurenines and many clinical and biochemical factors, including inflammation, B-vitamins, smoking, alcohol, stress, medication, BMI, and various somatic diseases. In one study comparing kynurenines between depressed and healthy controls, significant

differences disappeared when adjusting for age, sex, education, smoking status, alcohol consumption and chronic diseases [291]. However, several of these factors, especially diet, stress, and inflammation, are possible intermediaries between psychiatric disorders and kynurenine pathway metabolism. Adjusting for these factors will likely cause an underestimate. Analyses in subgroups based on these factors is an alternative approach, but usually means reducing the statistical power.

5.3.3 Challenges in establishing biomarkers for psychiatric disorders

This thesis represents a small contribution to a large, collective process of identifying biomarkers for depression and ADHD. The methodological issues discussed above constitute a major challenge for understanding the relationship between kynurenines and psychiatric conditions. There are also more fundamental challenges to be considered when evaluating these studies in the larger context of biomarkers in psychiatry.

Current knowledge indicates a multifactorial pathophysiology in psychiatric disorder, with numerous small effects and a high degree of complexity. Simplicity often is an important quality of a scientific hypothesis (Occam's razor). However, a lack of correspondence with a complex reality favours simplistic studies, that may be inadequate to explain the relationships between biomarkers and complex disorders. Interpretation of the results is likely to be guided by the hypothesis and the related expectations, with the risk of setting aside alternative models. For kynurenines, their neuroactive properties must be considered in the context of their diverse and multifaceted biological role as well as the tight regulation of the kynurenine pathway. Hypotheses regarding the neuroactive effects in isolation, can lead to exaggerated, overly simplistic, or even misplaced conclusions.

The utility of the hypothesis must also be considered, and the hypothesis of kynurenines in depression is difficult to test for several reasons: Firstly, psychiatric disorders are dimensional constructs, with unclear boundaries versus normality [312].

Small study samples collected at different sites are certain to be diverse, non-representative of other samples, and with an unclear relationship to the core concept being tested. The literature on kynurenines and psychiatric disorders covers a wide variety of approaches, including animal models, self-reported mood states in the general population, specific depression symptoms, or severe clinical cases and outcomes such as accidents, drug abuse and suicide. It is difficult to integrate the results of such diverging approaches, and to evaluate the collective findings in relation to a core concept and hypothesis. Secondly, as reviewed extensively in the introduction, the biological role of kynurenines is similarly multifaceted, and there is limited knowledge about the physiological state of the pathway, normal ranges and variation, the metabolites' pathological concentrations, and the role of life-style factors and comorbidity on metabolite levels. Thirdly, and tightly related to the above points, there is no proper reference point for defining evidence for or against the hypothesis.

The results of the studies in this thesis must be interpreted in the light of the fact that, despite numerous investigations, there are still no established biochemical biomarkers for ADHD [164,166], depression [185] or for the effect of ECT [196,313]. A possible reason for this is that the ontological framework for psychiatric disorders has originated in relation to clinical practice rather than being structured to support studies of the underlying biological mechanisms. Psychiatric diagnoses are, for instance, based on clinical evaluation and are generally disconnected from genetic and biochemical data. Several approaches have been suggested to remedy this incongruity. The Research Domain Criteria of the National Institute of Mental Health suggest that studies of psychiatric disorders should be based on domains of neurobehavioral function rather than traditional diagnostic categories [314]. Alternative approaches such as nomothetic psychiatry [315] and precision psychiatry [316] suggest that psychiatric illnesses should be considered as systemic disorders rather than brain disorders. Supporters of such approaches advocate for the incorporation of biological variables into the diagnostic paradigm, to get more precise definitions and better tools for identifying specific endophenotypes. Another view is that we still lack a proper understanding of the clinical diagnoses, and that large longitudinal studies with structured data collection is needed to identify better defined clinical phenotypes [317]. Still others argue that it is

unlikely that clinical aspects and biological mechanisms can be reconciled, and that we need different ontologies for research and clinical practice[318].

Kynurenine abnormalities are seemingly related to psychiatric disorders in general [35], but have also been studied in a wide range of clinical subgroups, including patients with suicidal ideation [88,215,319,320] and cognitive dysfunction [321]. Childhood trauma [175,290] and chronic stress are examples of additional clinical characteristics that could be related to inflammation and kynurenine metabolism across diagnoses. These latter examples, however, underline the possibility that the relationship between kynurenine metabolism and psychiatric disorders is mediated by a third factor, were diet, inflammation and stress are major candidates. Moreover, it is also possible that kynurenine abnormalities are a consequence of the clinical disorder, rather than the opposite. Knowledge of the causal relationship in mental disorders is currently scarce [43].

The studies in this thesis focus on subjects with established diagnoses of ADHD, major depressive disorder, and bipolar disorder. Studying groups of such clinically diagnosed individuals provides a relatively well-defined and specific scope, that is likely to contain at least some part of the relationship in question. However, the diagnoses still make room for plenty of heterogeneity in symptom presentation, severity, and comorbidity. For instance, patients with ADHD in study 1 had considerable self-reported psychiatric comorbidity, though in this case we found no differences in levels of kynurenines between patients with or without depression/anxiety or bipolar disorder, or between medicated and medication-naïve patients. There is considerable variation in clinical characteristics both between individuals in a single study, and between various studies. Consequently, the results from these studies are not readily transferable to other samples, to the general population or to the general concept of depression or ADHD.

Alternatively, studying the relation of biomarkers with continuous symptom scores either in patient groups or in the general population can provide information independent of clinical cut-offs and the patient-control paradigm. We used this

approach in study 1 to compliment the main analyses. However, consistent and meaningful correlations between biomarkers and clinical symptoms are difficult to identify, even when considering symptom rating scales.

As explained above, there are good reasons to believe that alterations in kynurenine metabolism are related to the pathophysiology of psychiatric disorders, including depression and ADHD [35]. However, the nature of this relationship is not well established, and it is unclear if there is a direct causal relationship in any direction. Given the complex nature of depression and the diverse roles of the kynurenine pathway in human biology, it seems unlikely that an unbalanced kynurenine pathway is a prerequisite for depression, or that remission is dependent on normalisation of kynurenines. In any case, the relationship between depression and kynurenine metabolism is most likely dependent on other important and systemic variables like nutrition, stress, and inflammation.

5.4 Conclusion and future perspectives

Multiple meta-analyses have shown that patients with unipolar and bipolar depression have different levels of Trp and kynurenines than healthy controls. Similar trends have been found in ADHD. The two first studies of this thesis also show abnormal kynurenine levels in patients with ADHD and depression, possibly connected to differences in age, inflammation, smoking, and diet.

In studies II and III, we found associations between kynurenines and inflammation, but no clear relationship between changes in kynurenines and clinical treatment effect. We found no convincing changes in kynurenines after ECT that could support the hypothesis of reduced neuroprotection in depression. There are several possible reasons for this, including low sample size, inappropriate sampling times, and the use of peripheral measures. Alternatively, changes in kynurenines may be limited to specific clinical subgroups. It may be that altered kynurenines constitute a persisting vulnerability to depressive episodes, and that patients continue to have abnormal levels

of kynurenines even though they are successfully treated. However, there is also the possibility that the changes in the levels of various kynurenines are not essential for or related to depression symptom relief.

Kynurenines have been shown to affect several cellular functions in the CNS. Their activity is closely linked to that of the immune system, which has also been shown to be altered in psychiatric disorders. Thus, the kynurenine pathway could be an important factor in the pathophysiology of psychiatric disorders. Measures of kynurenines could aid diagnostics, possibly as part of a wider biomarker profile for psychiatric disorders and could potentially be of help in treatment decisions. Furthermore, the pathway contains several established protein targets for therapeutic interventions.

Still, given the complex nature of depression and the multifaceted role of kynurenines, it may be overly simplistic to expect a strong correlation between psychiatric symptoms and kynurenine levels, for example based on the hypothesis of glutamatergic regulation. Moreover, many potential intermediary variables are well known, and some well characterised, but often not adequately considered in clinical studies. Future work needs to continue developing the hypotheses regarding the role of kynurenines in psychiatric disorders, and test these hypotheses in larger samples, with appropriate study designs and careful interpretations in the light of known determinants. More knowledge is also needed regarding the normal ranges of Trp and kynurenines, their relationship with inflammatory processes, the effects of diet, malnutrition and fasting, and the role of somatic comorbidity.

6. References

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7. Errata

Page 38, “including a model adjusted for age, sex and cotinine” corrected to “in two models, one adjusted for sex, and another adjusted for both sex and tryptophan”.

Page 39, “paired Wilcoxon signed-rank test in all patients” corrected to “paired Wilcoxon signed-rank test in 21 patients”.

8. Studies I – IV

Study I

I

Aarsland *et al. Behav Brain Funct (2015) 11:36*
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BEHAVIORAL AND BRAIN FUNCTIONS

RESEARCH

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Serum concentrations of kynurenines in adult patients with attention-deficit hyperactivity disorder (ADHD): a case–control study

Tore Ivar Malmei Aarsland[1,2*], Elisabeth Toverud Landaas[1,2], Tor-Arne Hegvik[1,2], Arve Ulvik[3], Anne Halmøy[1,2,4], Per Magne Ueland[5] and Jan Haavik[1,2,4]

Abstract

Background: The essential amino acid tryptophan is catabolised mainly through the kynurenine pathway. Altered circulating levels of kynurenines have been reported in chronic inflammatory conditions and in several neuropsychiatric disorders, including depression and schizophrenia. Candidate gene studies suggest that genes related to the kynurenine catabolism may be associated with attention-deficit hyperactivity disorder (ADHD). Additionally, ADHD patients often report comorbid depression or anxiety. In this study we investigated serum levels of kynurenines in Norwegian adult ADHD patients and adult controls.

Methods: We compared serum levels of tryptophan and the seven tryptophan metabolites kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid, 3-hydroxyanthranilic acid and quinolinic acid in 133 adult patients with ADHD and 131 adult controls (18–40 years). Riboflavin (vitamin B2), total vitamin B6 and the nicotine metabolite cotinine were also measured. Serum samples were analysed using mass spectrometry. Patients and controls reported comorbid disorders and past (childhood) and current ADHD symptoms using the Wender Utah Rating Scale (WURS) and the Adult ADHD Self-report Scale (ASRS). Logistic regression was used to calculate odds ratios for having an ADHD diagnosis for different serum levels of each metabolite. In addition, we used Spearman's correlation analysis to investigate the correlation between serum levels of tryptophan and kynurenines and ADHD symptom scores.

Results: Lower serum concentrations of tryptophan [odds ratio 0.61 (95 % confidence interval 0.45–0.83)], kynurenic acid [0.73 (0.53–0.99)], xanthurenic acid [0.65 (0.48–0.89)] and 3-hydroxyanthranilic acid [0.63 (0.46–0.85)], and higher levels of cotinine [7.17 (4.37–12.58)], were significantly associated with ADHD. After adjusting for tryptophan levels, only 3-hydroxyanthranilic acid and cotinine remained significant. Lower levels of tryptophan and kynurenine were also found to be correlated with higher total ASRS score and higher total WURS score, when adjusting for smoking and age.

Conclusions: Our results suggest that there may be differences in serum levels of tryptophan and kynurenines between adult ADHD patients and adult controls. Although our findings do not suggest a chronic immune activation in ADHD, the underlying mechanisms and possible clinical implications of the differences should be further explored.

Keywords: Attention-deficit hyperactivity disorder, Kynurenine, Tryptophan, Vitamin B, Cotinine, Inflammation, Biomarker

*Correspondence: aarsland.toreivar@gmail.com
[1] Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway
Full list of author information is available at the end of the article

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Background

Attention-deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterised by inattention, hyperactivity and impulsivity and has a pooled prevalence rate of about 2.5 % in the adult population [1]. The symptoms are often severe and may cause serious difficulties in the daily life of affected individuals [2]. The disorder often coexists with other neuropsychiatric disorders like depression and bipolar disorder, with which it also shares symptoms [3, 4]. The aetiology of ADHD is complex and is most likely explained by the combined impact of many environmental and genetic factors [5, 6]. In a recent study on ADHD and its relation to low birth weight, it was suggested that genetic variants in the kynurenine pathway might contribute to ADHD symptom severity [7].

The kynurenine pathway (Fig. 1) constitutes the major route for catabolism of the essential amino acid tryptophan [8]. More than 90 % of tryptophan is catabolised to kynurenine, mainly in the liver by the tryptophan specific enzyme tryptophan 2,3-dioxygenase (TDO), but also in lungs, kidneys, spleen, placenta and blood by the enzyme indole 2,3-dioxygenase (IDO) [9]. In the brain, the catabolism of tryptophan to kynurenine is driven by TDO and IDO located in astrocytes and microglia [9]. The activity of TDO depends on the concentration of tryptophan and is stimulated by high levels of cortisol, while IDO activity is enhanced mainly by pro-inflammatory cytokines, such as interferon-γ, and reduced by the anti-inflammatory cytokine interleukin 4 [9]. Kynurenine is further converted into kynurenic acid (KA), by kynurenine aminotransferase (KAT I, II, III), or by kynurenine-3-monooxygenase (KMO) into 3-hydroxykynurenine (HK), which is metabolised to 3-hydroxyantranilic acid (HAA) and eventually quinolinic acid (QA) [9]. These steps through the kynurenine pathway are dependent on the coenzymes pyridoxal 5′-phophat (PLP), the active form of vitamin B6 [8, 10], and flavin adenine dinucleotide (FAD), the active form of riboflavin (vitamin B2) [10]. Thus, diet may influence tryptophan metabolism either directly or via vitamin levels. Moreover, serum levels of tryptophan and vitamin B2 and B6 may be affected by smoking [11, 12].

KA and QA have neuroactive properties and exert multiple effects in the central nervous system. KA is an antagonist of the glutamatergic NMDA receptor and the cholinergic nicotinic α7-receptor and as such an endogenous protector against excitotoxicity [9]. High cerebrospinal fluid (CSF) levels of KA have been associated with cognitive dysfunction both in animals [13] and

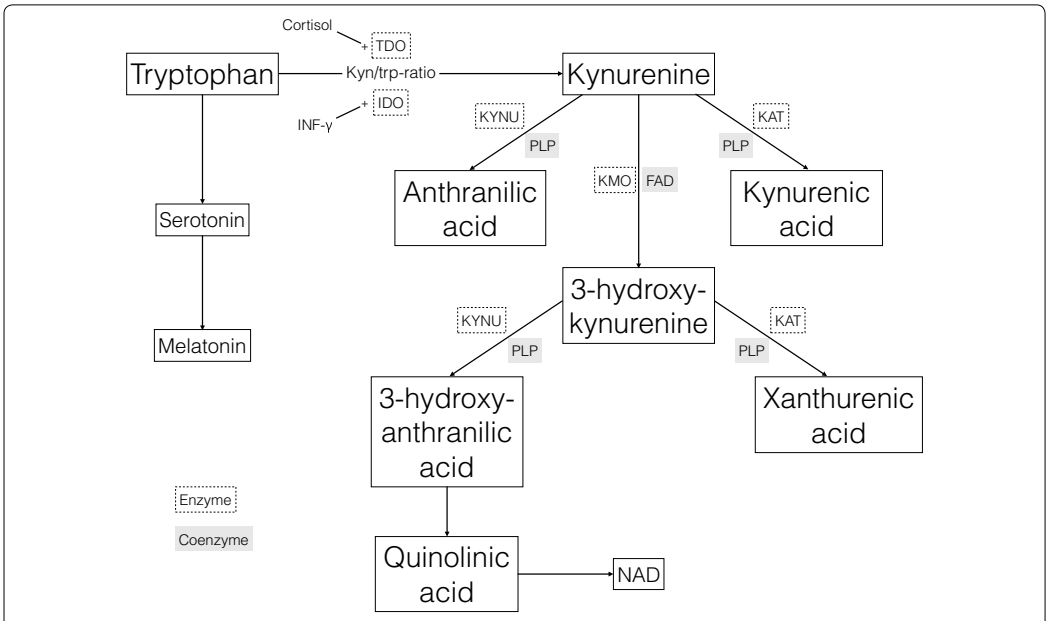

Fig. 1 The kynurenine pathway of tryptophan catabolism. *INF-γ* interferon gamma, *TDO* tryptophan 2,3-dioxygenase, *IDO* indole 2,3-dioxygenase, *KYNU* kynureninase, *KAT* kynurenine aminotransferase, *KMO* Kynurenine mono-oxygenase, *PLP* pyridoxal 5′-phosphate (vitamin B6), *FAD* flavin adenine dinucleotide (vitamin B2), *NAD* nicotinamide adenine dinucleotide

in humans, including patients with schizophrenia [14, 15]. Reduced plasma levels of KA have been reported in depression [16] and in patients with bipolar disorder [17]. QA is an agonist of the NMDA receptor and may cause excitotoxicity [18]. High levels of QA have been found in several neurodegenerative diseases such as Huntington's disease and Alzheimer's disease [18].

ADHD often coexists with other neuropsychiatric disorders that have been associated with altered levels of metabolites of the kynurenine pathway, such as major depression and bipolar disorder [3, 4]. Furthermore, known environmental risk factors for ADHD, such as preeclampsia, postnatal infection and malnutrition, may involve abnormal tryptophan catabolism [19, 20]. Neuropsychological deficits, for example in executive functioning, are often found in ADHD [21], and are thought to be related to a hypofunctional dopamine system [22]. Tryptophan metabolites can modulate several neurotransmitter systems, including dopaminergic transmission [9]. Moreover, in addition to kynurenine, tryptophan is also the precursor of serotonin (5-hydroxy-tryptamine, 5-HT), a neurotransmitter that has been suggested as an important agent in several neuropsychiatric disorders including ADHD [23].

In this study we sought to compare serum levels of kynurenines between adult ADHD patients (N = 133) and adult controls (N = 131) and also across continuous scales of self-reported past and present ADHD symptom scores (N = 264).

Methods

Participants

ADHD patients and controls were recruited as part of the Norwegian study "ADHD in adults in Norway: from clinical characterization to molecular mechanisms", initiated at the University of Bergen in 2004. Most patients were recruited by responding to invitation letters sent to people listed in a Norwegian national registry of adult ADHD patients. Some patients were recruited directly from psychiatrists or outpatient clinics, as described earlier [24]. All patients had been previously diagnosed with ADHD using either DSM-IV [25] or ICD-10 [26]. The ICD-10 criteria were adapted to the DSM-IV criteria by allowing the inattentive subtype as sufficient for the ADHD diagnosis, and by accepting the coexistence of other neuropsychiatric disorders as long as they appeared after the criteria of ADHD were fulfilled. For the present study, only participants from whom we had access to blood samples, who were between 18 and 40 years, were of Norwegian ancestry, and who volunteered to participate after receiving oral and written information about the project were included (N = 264). The patient group consisted of 133 adults (71 females, 62 males) and the control group consisted of 131 students (75 females, 56 males) recruited locally at the University of Bergen.

Measures

The majority of blood samples were collected locally at the Haukeland University Hospital campus in Bergen. Some samples were also collected by primary care physicians in Bergen and at other locations in Norway. Fasting was not required before blood sampling. Blood samples were collected in serum tubes with gel separator. Samples collected outside the hospital were transported by mail to the laboratory. During transport, samples were kept at ambient temperature. Immediately upon arrival at the laboratory serum was separated and stored at −80 °C. All samples were visually inspected and samples with signs of haemolysis or degradation were excluded (N = 2). To avoid batch effects, control and patient samples were analysed together. Every second sample was from either controls or patients. Tryptophan and the seven kynurenines, kynurenine, kynurenic acid (KA), anthranilic acid (AA), xanthurenic acid (XA), 3-hydroxykynurenine (HK), 3-hydroxyanthranilic acid (HAA) and quinolinic acid (QA), as well as riboflavin (vitamin B2), B6 vitamers pyridoxal (PL) and pyridoxal-5′-phosphate (PLP), and the nicotine metabolite cotinine [27], were measured using stable isotope dilution liquid chromatography-tandem mass spectrometry, as described [28]. All biochemical analyses were performed by Bevital AS (http://www.bevital.no), Bergen.

All participants completed questionnaires with information on mental health, education, occupational status, and lifetime psychiatric co-morbidity. The latter was assessed by questions like "Have you ever experienced significant anxiety and/or depression?" and "Have you ever had problems with alcohol?" [3]. For assessment of current ADHD symptoms, all participants filled in the 18-item Adult ADHD Self-report Scale (ASRS) consisting of 9 questions specific to symptoms of inattentiveness and 9 questions on symptoms of hyperactivity and impulsivity, all with a 0–4 point Likert scale of symptom severity (0 = never/seldom, 4 = very often) [29]. In addition the 25-item version of the Wender Utah Rating Scale (WURS) was used for retrospective assessment of ADHD symptoms in childhood, each question with a Likert scale of 0–4 indicating the severity of the symptom (0 = not at all/very slightly, 4 = very much) [30].

Statistical analyses

To explore the material, age and ADHD symptom scores in the patient and control group were compared using Student's t test, while χ^2 test were used for comparing the categorical variables sex, alcohol and drug abuse, comorbid disorders and subgroups of ADHD. Total vitamin

B6 level was calculated as the sum of PL and PLP [31]. Kynurenine/tryptophan ratio (KTR), a marker of immune activation, was calculated as the serum concentration of kynurenine divided by the concentration of tryptophan, multiplied with 1000. About 80 % of the biochemical variables were non-normally distributed, and levels in patients and controls were therefore investigated using Mann–Whitney U tests. Spearman's correlations for biochemical variables, age and sex were used to further explore the data.

For use in logistic regression all biochemical variables were split in tertiles, except for cotinine which was divided in three categories corresponding to the serum level of cotinine in non-smokers (<80 nmol/l), moderate smokers (80–1000 nmol/l) and heavy smokers (>1000 nmol/l) [32]. These three-way categorisations were chosen to assess possible effects of low, medium and high levels of the biochemical variables/smoking. Blood cotinine can also be derived from nicotine in smokeless tobacco/snuff tobacco. Although we could not discriminate between different sources of nicotine, we considered the contribution from smokeless tobacco to be small in our study. At the time of sample collection, regular smoking was much more common than smokeless tobacco consumption. Some users also combine several sources of tobacco/nicotine (https://helsedirektoratet.no/english/tobacco-control). Logistic regression with patient status (ADHD yes/no) as outcome was performed for each biochemical variable in two models, one with sex as covariate and another adjusting for both sex and tryptophan. Odds ratio (OR) with 95 % confidence interval (CI) for ADHD was calculated per tertile of each metabolite (per category in the case of cotinine).

There was a greater age span in the ADHD group than in the control group, and it is known that tryptophan levels may decrease with age. Logistic regressions were therefore performed with two age subgroups of ADHD patients as outcome for each biochemical variable with adjustment for sex. The first age group, 19–33 years (N = 97), corresponded to the age span of the control group, while the second group contained the patients with no age-matched controls (34–40 years, N = 36). Associations between biochemical variables and patients' comorbid disorders, i.e. bipolar disorder and anxiety/depression, were also investigated using logistic regressions adjusted for sex. A last logistic regression analysis was performed to explore the effect of current treatment with central stimulants.

Partial correlation analyses (Spearman's) were used to explore associations between ADHD symptom severity and levels of biochemical variables, while adjusting for smoking (cotinine level) and age. The analyses were performed in all participants and for patients and controls separately.

Student's t test, χ^2 test, Mann–Whitney U test and regular Spearman's correlation analysis were performed in Statistical Package for Social Sciences version 22 (IBM Corp., Armonk, New York) for Apple OSX. All regression models as well as partial Spearman's correlation were performed using R (The R Project for Statistical Computing) for Apple OSX. All p values were two-sided, and statistical significance was defined at the 5 % level.

Ethics

All patients gave written informed consent and the protocol was approved by the Regional Committee for Medical Research Ethics, REK Vest (IRB #3 (FWA00009490, IRB00001872)).

Results

Clinical data

There were 264 participants included in this study, 133 adult ADHD patients and 131 adult controls. A comparison of clinical data is shown in Table 1. Women were slightly overrepresented in both groups (53 and 57 %). The median age of the patient group was significantly higher than that of the control group (28.0 versus 22.5 years). In the patient group, 65.1 % reported a lifetime history of significant anxiety or depression, while this was reported by only 3.8 % of the controls. Among the ADHD patients, 9.9 % also reported having a comorbid bipolar disorder, compared to none in the control group. There was a significant difference in the number of smokers, with 66.2 % of the patients having a serum cotinine level of >80 nmol/l, compared to only 12.2 % of the controls. As expected, median childhood ADHD symptom scores (WURS) and median present ADHD symptom scores (ASRS) were much higher in the patient group (60 and 47 versus 12 and 22 in the control group). According to standard cut-offs for ASRS, 6.2 % of the controls screened positive for ADHD.

Among patients for whom we had information on medication status, 113 patients (out of 123, 92 %) had a history of central stimulant treatment. When the blood samples were collected, 91 patients (out of 114, 80 %) still received such treatment. Twenty-five patients were currently using anti-depressants.

Biochemical data

Correlations

All kynurenine metabolites were positively correlated to kynurenine levels [Spearman's rho (r): 0.24–0.58] (Table 2). Tryptophan levels showed a moderate correlation with KA, XA and HAA, (r: 0.34–0.41), and a weak correlation with HK and QA (r: 0.14, 0.18). KA, XA, HAA and QA showed positive correlation with both vitamin

Table 1 Clinical and biochemical data

	ADHD	N	Control	N	p value
		133		131	
Female, N (%)	71 (53.4)	133	75 (57.3)	131	0.52[a]
Age (range 18–40), median (SD)	28.0 (6.5)	133	22.5 (2.8)	131	<0.001***[b]
Alcohol problems, N (%)	24 (19.2)	125	1 (0.8)	131	<0.001***[a]
Problems with illicit drugs, N (%)	34 (27.0)	126	0 (0.0)	131	<0.001***[a]
Serious anxiety and/or depression, N (%)	82 (65.1)	126	5 (3.8)	126	<0.001***[a]
Bipolar disorder, N (%)	12 (9.9)	121	0 (0.0)	131	<0.001***[a]
Moderate smokers[d], N (%)	51 (38.3)	133	12 (9.2)	131	<0.001***[a]
Heavy smokers[d], N (%)	27 (27.8)	133	4 (3.1)	131	<0.001***[a]
Total WURS (range 0–100), median (SD)	60.0 (16.9)	118	12.0 (10.4)	129	<0.001***[b]
Total ASRS (range 0–72), median (SD)	47.0 (11.6)	123	22.0 (7.7)	128	<0.001***[b]
Combined type[e], N (%)	65 (52.8)	123	3 (2.3)	128	<0.001***[a]
Hyperactive type[e], N (%)	3 (2.4)	123	1 (0.8)	128	0.32[a]
Inattentive type[e], N (%)	31 (25.2)	123	4 (3.1)	128	<0.001***[a]
Tryptophan[f, i]	77.3 (68.1–92.3)	133	83.6 (76.6–94.0)	130	0.004**[c]
Kynurenine[f, i]	1.51 (1.29–1.78)	133	1.57 (1.40–1.76)	130	0.15[c]
Kynurenine/tryptophan ratio (KTR)[g, i]	19.0 (16.5–21.6)	133	18.4 (16.7–20.8)	130	0.46[c]
3-hydroxykynurenine (HK)[h, i]	31.8 (22.2–39.4)	131	30.6 (26.1–28.1)	130	0.43[c]
Kynurenic acid (KA)[h, i]	38.9 (30.9–52.2)	131	46.7 (34.0–57.5)	130	0.03*[c]
Xanthurenic acid (XA)[h, i]	14.2 (11.0–22.4)	131	18.9 (13.7–24.7)	130	0.004**[c]
Anthranilic acid (AA)[h, i]	19.4 (13.2–24.3)	131	17.6 (14.9–22.8)	130	0.52[c]
3-hydroxyanthranilic acid (HAA)[h, i]	29.8 (20.0–40.1)	131	35.4 (29.2–46.5)	130	<0.001***[c]
Quinolinic acid (QA)[h, i]	303 (258–387)	131	306 (274–364)	130	0.76[c]
Riboflavin (Vit. B2)[h, i]	18.1 (12.7–25.9)	131	19.9 (16.1–28.3)	130	0.02*[c]
Total vitamin B6[h, i]	58.5 (41.8–83.9)	131	67.1 (49.0–88.4)	130	0.05*[c]
Cotinine[h, i]	678 (2.48–1088)	131	1.2 (0.55–6.06)	130	<0.001***[c]

Significance <0.001: '***', <0.01: '**', <0.05: '*'

[a] χ^2 test

[b] Student's T test

[c] Mann–Whitney U test

[d] Calculated based on serum cotinine levels: 80–1000 nmol/l: moderate smoker, >1000 nmol/l: heavy smoker

[e] Calculated based on ASRS scores: A score of 21 or higher on the nine questions on inattentiveness is indicative of inattentive type, while a score of 21 or higher on the hyperactivity/impulsive questions is indicative for hyperactive/impulsive type. Combined type requires a score of 21 or higher on both subscales

[f] µmol/L

[g] µmol/µmol

[h] nmol/L

[i] Median (25–75 percentile)

B2 (r: 0.15–0.27) and total vitamin B6 (r: 0.18–0.24). All correlations were statistically significant ($p < 0.05$).

There was a significant inverse correlation between age and tryptophan in this material as a whole (r: −0.24) and in the patient group (r: −0.30) in particular. A positive, although non-significant, correlation between age and tryptophan was also found in the control group (r: 0.10). There was a significant but weak inverse correlation between cotinine and tryptophan (r: −0.13), XA (r: −0.16), HAA (r: −0.20), riboflavin (r: −0.13) and total vitamin B6 levels (r: −0.16) when including all participants.

Metabolite levels according to ADHD status

Mann–Whitney U test showed significantly lower serum concentrations in the ADHD group for tryptophan, KA, XA, HAA, vitamin B2 (riboflavin) and total vitamin B6 (PL + PLP), as well as a significantly higher level of cotinine, compared with the control group (Table 1). Distribution of raw biochemical variables in tertiles for patients and controls are shown in Fig. 2. OR with 95 % confidence interval (CI) for ADHD was calculated per tertile/category of each metabolite (Fig. 3; Table 3), using two models: one adjusted for sex, and one adjusted for sex

Table 2 Unadjusted Spearman's correlations

	Trp	Kyn	KTR	HK	KA	XA	AA	HAA	QA	Vit. B2	Vit. B6	Cot	ADHD status
All (N = 264)													
Age	−0.24*	−0.05	0.13*	−0.13*	−0.13*	−0.26*	0.09	−0.27*	−0.10	−0.04	−0.23*	0.26*	0.47*
Sex	0.19*	0.18*	0.01	−0.11	0.21*	0.01	0.08	−0.04	−0.03	−0.14*	0.11	0.15*	0.04
Trp		0.49*	−0.46*	0.14*	0.34*	0.41*	0.10	0.34*	0.18*	0.17*	0.32*	−0.13*	−0.18*
Kyn			0.48*	0.39*	0.48*	0.24*	*0.36**	0.32*	0.58*	0.16*	0.19*	−0.04	−0.09
Vit. B2			−0.06	0.02	0.15*	0.23*	0.11	0.15*	0.27*		0.32*	−0.13*	−0.14*
Vit. B6			−0.12	−0.12	0.22*	0.20*	0.03	0.24*	0.19*			−0.16*	−0.12*
Cotinine			0.05	−0.06	−0.08	−0.16*	0.02	−0.20*	−0.03				0.52*
ADHD (N = 133)													
Age	−0.30*	−0.08	0.22*	−0.26*	−0.11	−0.31*	0.05	−0.27*	−0.08	0.22*	−0.16	−0.04	
Sex	0.15	0.14	0.03	−0.04	0.08	0.03	−0.02	0.09	−0.00	−0.18*	0.09	0.13	
Trp		0.55*	−0.41*	0.21*	0.34*	0.48*	0.01	0.35*	0.32*	0.18*	0.40*	−0.07	
Kyn			0.46*	0.45*	0.50*	0.29*	0.32*	0.39*	0.64*	0.17	0.21*	0.07	
Vit. B2			−0.03	−0.01	0.15	0.29*	0.10	0.15	0.30*		0.32*	−0.13	
Vit. B6			−0.17	−0.01	0.28*	0.26*	−0.03	0.28*	0.24*			−0.27*	
Cotinine			0.08	0.03	−0.11	−0.19*	−0.03	−0.08	−0.01				
Control (N = 131)													
Age	0.10	0.10	−0.01	0.05	0.01	−0.03	0.10	−0.06	−0.10	−0.19*	−0.24*	0.14	
Sex	0.28*	0.25*	−0.03	−0.19*	0.36*	−0.01	0.20*	−0.11	−0.08	−0.06	0.16	0.20*	
Trp		0.41*	−0.48*	0.04	0.31*	0.27*	0.24*	0.19*	−0.02	0.13	0.21*	0.06	
Kyn			0.55*	0.31*	0.46*	0.17	0.42*	0.16	0.50*	0.10	0.13	0.05	
Vit. B2			−0.10	0.03	0.09	0.12	0.13	0.03	0.21*		0.33*	0.06	
Vit. B6			−0.06	−0.26*	0.14	0.11	0.12	0.14	0.11			0.14	
Cotinine			−0.02	−0.11	0.17	0.03	0.09	−0.10	−0.03				

Significance: <0.05 '*'

Trp tryptophan, *Kyn* kynurenine, *KTR* kynurenine/tryptophan ratio, *HK* 3-hydroxykynurenine, *KA* kynurenic acid, *XA* xanthurenic acid, *AA* anthranilic acid, *HAA* 3-hydroxyanthranilic acid, *QA* quinolinic acid, *Cot* cotinine

and tryptophan. Adjusting for sex, lower levels of tryptophan, KA, XA, HAA and vitamin B2 were associated with increased risk of having ADHD. In addition, higher levels of cotinine were strongly associated with ADHD. In the second model, adjusting for sex and tryptophan, only HAA, riboflavin and cotinine remained significant predictors of ADHD/control status, while KA and XA were no longer significant.

Metabolite levels in relation to age, comorbidity and medication

Logistic regression using two age groups of ADHD patients, i.e. 19–33 years and 34–40 years, as outcome were performed to investigate the effect of age. Lower levels of tryptophan [OR: 0.58 (95 % CI 0.35–0.93, p value: 0.03)] and XA [0.56 (0.22–0.91, 0.02)] as well as higher levels of riboflavin [2.13 (1.32–3.54, 0.003)] and higher KTR [1.65 (1.02–2.71, 0.04)] were significantly associated with the age 34–40 group (N = 36). When the age 19–33 ADHD subgroup (N = 97) was compared to the control group (18–33, N = 131), lower levels of tryptophan [0.71 (0.51–0.99, 0.05)], HAA [0.66 (0.47–0.91, 0.01)] and riboflavin [0.53 (0.37–0.75, <0.001)], and higher levels of cotinine [7.84 (4.63–14.22, <0.001)] were significantly associated with increased risk of having ADHD. No significant differences in serum concentrations were found between ADHD patients with or without self-reported anxiety/depression (all p values >0.13) or bipolar disorder (all p values >0.09). Comparisons of levels of tryptophan metabolites between medicated and drug-naïve patients showed a trend towards lower levels of KA and HAA in non-medicated patients in preliminary analyses. However, logistic regression analyses did not yield any significant differences. The number of patients currently using antidepressants was too small to allow for comparisons.

Metabolite levels according to symptom scores

Correlation analyses adjusted for cotinine and age showed significant inverse correlations between the serum concentration of tryptophan and kynurenine and the current and past ADHD symptom scores ASRS and WURS, when including all participants (N = 264) (Table 4). Analyses on symptom scores in the ADHD group separately (N = 133) confirmed that lower levels

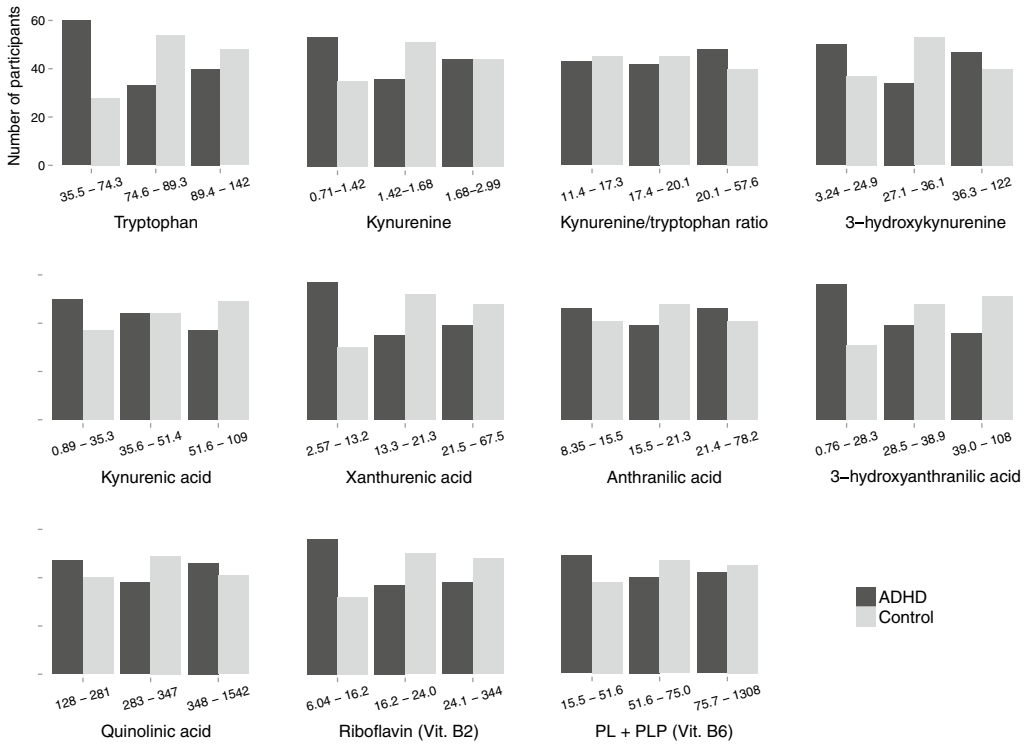

Fig. 2 Distribution in tertiles of biochemical variables. Each pair of columns contains a third of the participants. All participants are included (N = 264). ADHD patients are strongly represented in the lower concentrations of several metabolites, including tryptophan, XA, HAA and riboflavin. Controls are in majority in the middle concentrations of all metabolites except for KA

of tryptophan and total vitamin B6 were correlated with higher total ASRS score and higher score on the ASRS inattentive subscale (Table 4). Lower levels of tryptophan were also correlated with higher WURS score. In the control group (N = 131), there were no significant correlations between tryptophan and ADHD symptom scores (Table 4).

Discussion

In this study of 133 adult patients with ADHD and 131 adult controls, we found significantly lower concentrations of tryptophan, KA, XA and HAA in the patient group (Table 3). These results are strengthened by the observed inverse correlations between levels of tryptophan and kynurenine and total scores on both ASRS and WURS adjusted for smoking and age (Table 4). Together, our findings suggest a connection between severity of ADHD symptoms and serum levels of tryptophan and tryptophan metabolites. Furthermore, significantly lower levels of riboflavin and higher levels of cotinine were found among the ADHD patients compared to the controls (Table 3). The kynurenine/tryptophan ratio (KTR) was not significantly different between the two groups in any of the analyses, and did not show any correlation to ADHD symptom scores (Table 4). Thus, there is no strong indication of chronic immune activation in the ADHD patients.

Our results are different from an earlier, exploratory study on kynurenines in children with ADHD which reported higher serum levels of tryptophan and lower levels of HK in children with ADHD (N = 35) compared to controls (N = 28) [33–35]. We do not have any clear explanation for these different findings for tryptophan levels. If the inverse relation between age and tryptophan levels in the patient versus control groups in our sample is a true finding, we would expect to find different results in tryptophan metabolite studies of children versus adults with ADHD. However, published studies

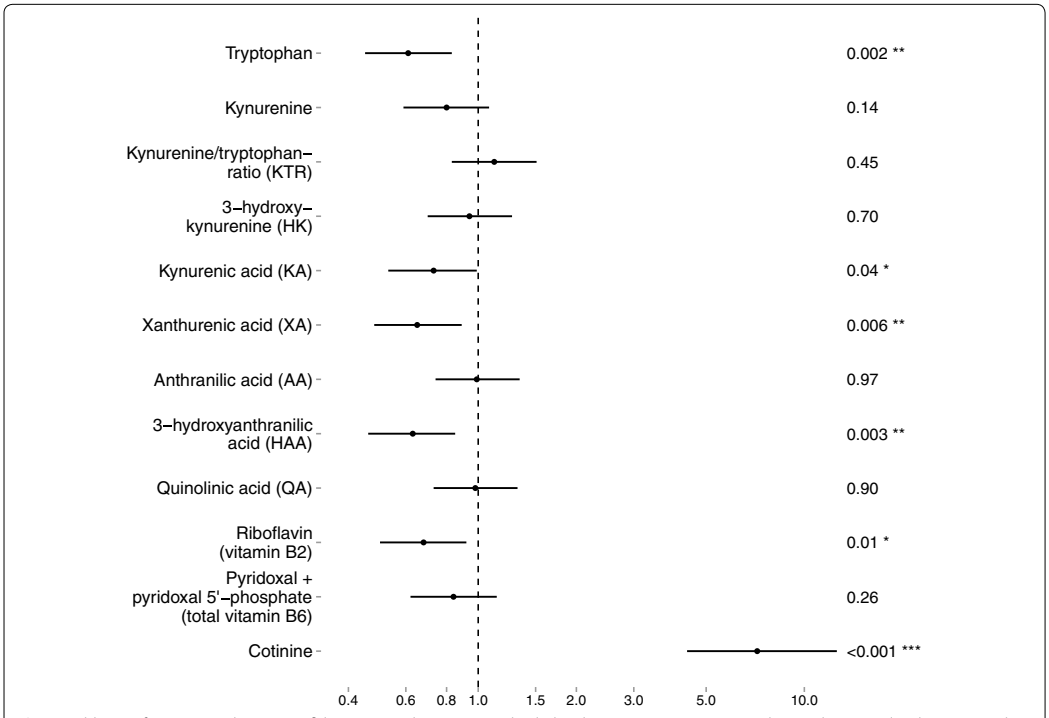

Fig. 3 Odds ratio for ADHD with 95 % confidence intervals. Cotinine is divided in three categories corresponding to the serum level in non-smokers, smokers and heavy smokers. High levels of tryptophan, KA, XA, HAA and riboflavin were associated with lower odds ratio for ADHD. High levels of cotinine were associated with higher odds ratio for ADHD. Significance <0.001: '***', <0.01: '**', <0.05: '*'

Table 3 Logistic regression

	Adjusted for sex			Adjusted for sex and tryptophan		
	B	p value	Odds ratio (95 % confidence interval)	B	p value	Odds ratio (95 % confidence interval)
Tryptophan	−0.50	0.002**	0.61 (0.45–0.83)			
Kynurenine	−0.23	0.14	0.80 (0.59–1.08)	−0.04	0.80	0.96 (0.69–1.34)
Kyn/Trp-ratio (KTR)	0.12	0.45	1.12 (0.83–1.51)	−0.11	0.53	0.90 (0.64–1.25)
3-hydroxykynurenine (HK)	−0.01	0.70	0.94 (0.70–1.27)	0.01	0.94	1.01 (0.74–1.38)
Kynurenic acid (KA)	−0.32	0.04*	0.73 (0.53–0.99)	−0.21	0.21	0.81 (0.59–1.12)
Xanthurenic acid (XA)	−0.43	0.006**	0.65 (0.48–0.89)	−0.30	0.07	0.74 (0.54–1.03)
Anthranilic acid (AA)	−0.01	0.97	0.99 (0.74–1.34)	0.04	0.79	1.04 (0.77–1.42)
3-hydroxyanthranilic acid (HAA)	−0.47	0.003**	0.63 (0.46–0.85)	−0.38	0.02*	0.69 (0.50–0.94)
Quinolinic acid (QA)	−0.02	0.90	0.98 (0.73–1.32)	0.04	0.82	1.04 (0.76–1.41)
Riboflavin (vitamin B2)	−0.39	0.01*	0.68 (0.50–0.92)	−0.34	0.03*	0.71 (0.52–0.97)
Total vitamin B6	−0.17	0.26	0.84 (0.62–1.14)	−0.09	0.55	0.91 (0.67–1.24)
Cotinine	1.97	<0.001***	7.17 (4.37–12.58)	1.94	<0.001***	6.96 (4.22–12.25)

Odds ratio of ADHD per tertile of variable. Cotinine is divided into three categories corresponding to the serum level in non-smokers, smokers and heavy smokers. All participants included: N = 264. Significance <0.001: '***', <0.01: '**', <0.05: '*'

Table 4 Spearman's correlations for ADHD symptom scores and serum variables, adjusted for cotinine and age

		Trp	Kyn	KTR	HK	KA	XA	AA	HAA	QA	Vit. B2	Vit. B6
All												
N = 248	ASRS total score	−0.15*	−0.14*	0.04	0.04	−0.08	0.01	0.04	−0.05	−0.04	−0.13*	−0.06
N = 250	ASRS inattentive	−0.15*	−0.13*	0.06	0.02	−0.08	−0.01	0.06	−0.03	−0.01	−0.06	−0.03
N = 251	ASRS hyperactive/impulsive	−0.14*	−0.16*	0.02	0.03	−0.09	0.01	0.02	−0.07	−0.07	−0.17*	−0.05
N = 243	WURS total score	−0.18*	−0.15*	0.03	−0.01	−0.14*	−0.08	0.05	−0.08	−0.05	−0.09	−0.01
ADHD												
N = 121	ASRS total score	−0.20*	−0.16	0.07	0.11	−0.05	0.01	0.05	−0.01	−0.14	−0.17	−0.22*
N = 122	ASRS inattentive	−0.20*	−0.16	0.08	0.11	−0.03	0.06	0.02	0.07	−0.08	−0.09	−0.19*
N = 123	ASRS hyperactive/impulsive	−0.17	−0.14	0.03	0.09	−0.03	−0.02	0.06	−0.05	−0.12	−0.17	−0.14
N = 115	WURS total score	−0.27*	−0.13	0.13	−0.08	−0.18	−0.16	0.15	−0.09	−0.16	0.05	−0.04
Control												
N = 127	ASRS total score	−0.05	−0.11	0.03	−0.11	−0.12	0.01	−0.05	−0.07	−0.10	−0.14	−0.04
N = 128	ASRS inattentive	0.01	−0.02	0.03	−0.13	−0.10	−0.04	0.02	−0.01	−0.01	−0.07	0.03
N = 128	ASRS hyperactive/impulsive	−0.10	−0.19*	−0.01	−0.09	−0.13	0.05	−0.12	−0.10	−0.17	−0.19*	−0.08
N = 128	WURS total score	−0.14	−0.14	0.03	−0.07	−0.11	−0.07	−0.07	−0.06	−0.08	−0.11	−0.01

The 18-item ASRS (Adult ADHD Self-report Scale) is used to assess current ADHD symptom burden, with nine questions specific to hyperactivity/impulsivity and nine questions specific to inattentiveness. WURS (Wender Utah Rating Scale) consists of 25 items for retrospective assessment of childhood ADHD symptom burden. Significance: <0.05 '*'

Trp tryptophan, *Kyn* kynurenine, *KTR* kynurenine/tryptophan ratio, *HK* 3-hydroxykynurenine, *KA* kynurenic acid, *XA* xanthurenic acid, *AA* anthranilic acid, *HAA* 3-hydroxyanthranilic acid, *QA* quinolinic acid, *Cot* cotinine

are still too small and few in number to allow for definite conclusions.

High levels of tryptophan have also been shown in urine from ADHD children along with low HAA/HK ratios which made the authors suggest that this was due to a low activity of B6-dependent enzymes [36]. We observed low levels of B6 in our patient group, though not significantly different from controls. Like the low levels of tryptophan observed in our patients, low B6 concentrations could be a sign of poor nutritional status.

Levels of tryptophan and kynurenines

Tryptophan levels, age and IDO/TDO activity

Differences in tryptophan concentrations between ADHD patients and controls could be a result of an abnormal IDO/TDO activity with increased catabolism through the kynurenine pathway. Increased catabolism of tryptophan to kynurenine has been found in patients with depression [16, 37], schizophrenia [38, 39], women with postpartum depression [40] and women with preeclampsia [19]. Tryptophan has also been shown to decrease with age in response to increased immune activity [12]. Increased IDO and TDO activity may increase KTR and the level of kynurenines. However, we found no significant difference in KTR between patients and controls (Table 3). Instead we observed positive correlations between concentration of tryptophan and kynurenine in both the patient and control groups, indicating a normal conversion of tryptophan to kynurenine (Table 2). Furthermore, the levels of kynurenines in the ADHD group were not elevated, but were either similar to those of the control group (Kyn, HK, AA and QA) or lower than in controls (KA, XA and HAA) (Tables 1, 3).

Age and tryptophan were inversely associated in the patient group, but not in the control group, which had a smaller age span (18–33 years). Because of the strong association between age and patient/control-status, we chose to compare two age subgroups of patients, 19–33 years and 34–40 years, in order to control for the effect of age. Analyses showed that lower levels of tryptophan [OR: 0.58 (95 % CI 0.35–0.93, p value 0.03)] and XA [0.56 (0.22–0.91, 0.02)], and higher KTR [1.65 (1.02–2.71, 0.04)], were associated with the age 34–40 subgroup. However, using ADHD-status as outcome when including only controls and patients in the age 19–33 subgroup, there were still significantly lower levels of tryptophan [OR: 0.71 (95 % CI 0.51–0.99, p: 0.05)] and HAA [0.66 (0.47–0.91, 0.01)] in patients. Furthermore, in the partial correlation analysis, tryptophan and kynurenine were both inversely correlated to ADHD symptom scores, even when adjusting for the effect of smoking and age (Table 4). Thus, although there seems to be an association between higher age, lower tryptophan and increased KTR, low level of tryptophan in the ADHD group cannot be fully explained by higher IDO or TDO activity. It seems likely that the difference in tryptophan levels could be partially due to a relative tryptophan deficiency in the ADHD group compared to the control group.

Vitamin B2 and B6 levels

We found significantly lower levels of riboflavin (vitamin B2) in the ADHD group, in addition to lower HAA and XA. Our findings confirm a previous study reporting a positive association of HAA and XA levels to concentrations of PLP and riboflavin [10]. Similarly, plasma levels of HK have been observed to be inversely associated with plasma PLP [41]. We found an inverse correlation between ASRS score and total vitamin B6 level in the ADHD group, adjusted for smoking and age (Table 4). It could be that low levels of total vitamin B6 among patients cause accumulation of HK and decreased levels of the other kynurenine metabolites [36, 42, 43]. This is supported by the fact that HAA was still significantly associated with ADHD status when adjusting for tryptophan levels in logistic regression analyses (Table 3). Analyses with KA and XA as predictors also suggest a difference, though not significant (Table 3).

Smoking

The high number of smokers, 38.3 % moderate smokers and 27.8 % heavy smokers, is an important and well-known characteristic of the ADHD group. Tryptophan and kynurenines (except HK) have been found to be inversely associated with smoking [12], and associations have been observed between blood levels of the nicotine metabolite cotinine and KTR [44]. Serum levels of riboflavin and PLP are also known to be decreased in smokers [11]. With 66 % smokers, it is likely that the levels of kynurenines in the ADHD group are affected by smoking either directly or via decreased levels of B-vitamins. Still, we found no strong correlation between cotinine and tryptophan, KTR, kynurenines or vitamins in our material when using Spearman's correlation analyses (Table 2). Furthermore, there were significant inverse correlations between ADHD symptom scores and tryptophan and kynurenine even when adjusting for smoking and age (Table 4).

Strengths and limitations

Our study included a relatively large number of participants (N = 264) and many measured metabolites, including riboflavin, total vitamin B6 and cotinine, which are important as they influence tryptophan catabolism. There are still no generally accepted objective measures or biomarkers available for diagnosing ADHD. ASRS and WURS are widely used tools for evaluation of present and past ADHD symptoms. The inclusion of ADHD symptom scores, although self-reported, allowed us to analyse the relationship between ADHD and biochemical markers as continuous variables.

The limitations of our study include base-line differences between the patient and the control groups regarding age, comorbid conditions and the proportion of smokers, the possible effect of central stimulants and uncertainty regarding the quality of some blood samples. In our analyses we have explored the possible effect of these factors.

We had no information on dietary habits or the relationship between food intake and time of blood collection. We are therefore unable to conclude regarding the effect of nutrition upon serum levels of vitamin B2, vitamin B6 and tryptophan.

Comorbid conditions are common in ADHD patients, and some of these conditions, notably depression and bipolar disorder, have been linked to alterations in tryptophan catabolism. While regression analysis did not show any differences between ADHD patients with or without anxiety/depression or between patients with or without bipolar disorder, we cannot rule out the possibility that comorbid conditions also could affect tryptophan catabolism.

Little is known about the effect of central stimulants on tryptophan catabolism. The small number of drug-naïve patients in our study did not allow any real comparison between medicated and non-medicated patients. The trend of lower levels of tryptophan metabolites in non-medicated patients in our study is however in line with a previous exploratory study in children with ADHD, showing a potential normalising effect of methylphenidate on tryptophan levels [36]. If this represents a true effect, our results could be an underestimation of the differences in tryptophan levels between ADHD patients and controls.

Some blood samples were shipped by mail, and we do not have access to detailed information about their transit time before they were stored at −80 °C. Storing serum samples in room temperature may increase the levels of AA and reduce HK and HAA within a few days [31]. Thus, we cannot rule out the possibility that the concentrations of these metabolites were also affected by pre-analytic effects. In contrast, the concentration of tryptophan has been shown to be stable [31].

Lastly, the results of statistical analyses of the different compounds were not adjusted for multiple testing. The main reason for this choice was that since the biochemical variables are non-independent (Fig. 1), correction for all regression analyses would be too conservative. Instead, if calculating Bonferroni correction by dividing the critical significance level by the number of group comparisons performed by logistic regression, i.e. 0.05/7, the threshold of significant would be 0.007. In the main logistic regression analysis using patient/control-status as outcome (Table 3) tryptophan ($p = 0.002$), XA ($p = 0.006$) and HAA ($p = 0.003$) would remain significant when applying this correction. Likewise, controlling

for the false-discovery rate (FDR-correction) for the 12 analyses in the main logistic regression model (Table 3) would also yield significant differences for tryptophan ($p = 0.01$), XA ($p = 0.02$), HAA ($p = 0.01$) and riboflavin ($p = 0.03$).

Summary and conclusion

We found lower levels of tryptophan, KA, XA and HAA in adult patients with ADHD compared to adult controls. The levels of kynurenines are dependent mainly on the concentration of tryptophan, the rate of conversion by the enzymes TDO and IDO, and the level of circulating riboflavin and PLP. High TDO and IDO activity does not seem to explain these results, since we observed a normal kynurenine/tryptophan ratio (KTR) and generally low levels of both tryptophan and kynurenines in the ADHD group. The patient group also had low levels of riboflavin (vitamin B2) and total vitamin B6 (PL + PLP), something that could affect the balance of kynurenines in favour of HK. The oxidative effect of smoking on circulating B vitamins may be the cause of low levels of riboflavin and total vitamin B6, and possibly also the low level of tryptophan. Still, the level of the nicotine metabolite cotinine was only weakly correlated with tryptophan, the kynurenines and the vitamins. Also, low levels of tryptophan were correlated with high ADHD symptom scores, even when adjusting for smoking and age. This was observed not only when all participants were included but also in the patient group alone. Thus, low levels of tryptophan, KA, XA and HAA seem to be best explained by a deficiency in tryptophan and in vitamin B2 and B6. We cannot, however, exclude that differences in age, smoking habits and comorbid disorders could contribute to the observed differences in levels of tryptophan and kynurenines. Further independent and carefully controlled studies are needed to clarify the relationship of tryptophan catabolism and ADHD.

Difference in nutritional status is a possible explanation of both low levels of tryptophan and B vitamins. As has been observed in other studies [2, 45], it may be that patients with high symptom scores have a more disordered lifestyle and possibly also a poorer nutritional status. There is now increasing interest in nutrition and possible effects on ADHD and related symptoms, and it has been shown that dietary interventions are able to reduce symptom burden in children with ADHD [46, 47]. Signs of low activity in PLP dependent enzymes in ADHD patients suggest that pyridoxine treatment may have an effect [36]. It is possible that also tryptophan supplements could be beneficial for some patients with ADHD, but more studies on larger populations are needed to further investigate the relation between ADHD and tryptophan catabolism.

Abbreviations

AA: anthranilic acid; ADHD: attention-deficit hyperactivity disorder; ASRS: Adult ADHD Self-Report Scale; CI: confidence interval; HAA: 3-hydroxyanthranilic acid; HK: 3-hydroxykynurenine; IDO: indole 2,3-dioxygenase; KAT: kynurenine aminotransferase; KMO: kynurenine 3-monooxygenase; KTR: kynurenine/tryptophan ratio; Kyn: kynurenine; KA: kynurenic acid; NMDAr: *N*-methyl-D-aspartate receptor; OR: odds ratio; QA: quinolinic acid; TDO: tryptophan 2,3-dioxygenase; Trp: tryptophan; WURS: Wender Utah Rating Scale; XA: xanthurenic acid.

Authors' contributions

TIMA, ETL, PMU and JH designed the study. TIMA performed the statistical evaluation of the data and drafted the manuscript. All authors contributed to data analyses and writing of the paper. All authors read and approved the final manuscript.

Author details

[1] Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway. [2] K.G. Jebsen Centre for Research on Neuropsychiatric Disorders, University of Bergen, Bergen, Norway. [3] Bevital A/S, Bergen, Norway. [4] Division of Psychiatry, Haukeland University Hospital, Bergen, Norway. [5] Section for Pharmacology, Department of Clinical Science, University of Bergen, Bergen, Norway.

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Competing interests

The authors declare that they have no competing interests with respect to the authorship and/or publication of this article.

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Study II

II

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The effect of electroconvulsive therapy (ECT) on serum tryptophan metabolites

Tore Ivar Aarsland [a, b], Ieva Leskauskaite [c], Øivind Midttun [d], Arve Ulvik [d], Per Magne Ueland [d, e], Leif Oltedal [f, g], Vera Jane Erchinger [f], Ketil Joachim Oedegaard [b, c, f], Jan Haavik [a, b, c], Ute Kessler [c, f, *]

[a] Department of Biomedicine, University of Bergen, Bergen, Norway
[b] K.G. Jebsen Centre for Neuropsychiatric Disorders, University of Bergen, Bergen, Norway
[c] Division of Psychiatry, Haukeland University Hospital, Bergen, Norway
[d] Bevital AS, Bergen, Norway
[e] Laboratory Medicine and Pathology, Haukeland University Hospital, Bergen, Norway
[f] Department of Clinical Medicine, University of Bergen, Bergen, Norway
[g] Mohn Medical Imaging and Visualization Centre, Department of Radiology, Haukeland University Hospital, Bergen, Norway

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ABSTRACT

Background: Prior studies suggest that activation of the tryptophan catabolism via the kynurenine pathway by proinflammatory cytokines may be involved in the pathophysiology of depression. Electroconvulsive therapy (ECT) is an effective treatment for major depression (MD) with immunomodulation as one of the proposed modes of action.
Objective: The aim of this study was to investigate serum concentrations of tryptophan and kynurenine pathway metabolites in MD patients and healthy controls, and to explore the effect of ECT on components of the kynurenine pathway.
Methods: The study included 27 moderately to severely depressed patients referred to ECT. Blood samples were collected prior to treatment and after the completed ECT-series. Baseline samples were also collected from 14 healthy, age- and sex-matched controls. Serum concentrations of tryptophan, kynurenine, 3-hydroxykynurenine (HK), kynurenic acid (KA), xanthurenic acid (XA), anthranilic acid (AA), 3-hydroxyanthranilic acid (HAA), quinolinic acid (QA), picolinic acid (Pic), pyridoxal 5′-phosphat (PLP), riboflavin, neopterin and cotinine were measured.
Results: Patients with MD had lower levels of neuroprotective kynurenine-pathway metabolites (KA, XA and Pic) and lower metabolite ratios (KA/Kyn and KA/QA) reflecting reduced neuroprotection compared to controls. The concentration of the inflammatory marker neopterin was increased after ECT, along with Pic and the redox active and immunosuppressive metabolite HAA.
Conclusion: In this pilot study, we found increased concentrations of inflammatory marker neopterin and putative neuroprotective kynurenine metabolites HAA and Pic in MD patients after ECT. Further research in larger cohorts is required to conclude whether ECT exerts its therapeutic effects via changes in the kynurenine pathway.
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Introduction

Major depression (MD) is a severe and potentially life-threatening psychiatric illness that accounts for a large part of the overall global burden of disease [1]. The neurobiology of depression is complex and not fully understood [2]. However, it has been shown that MD often is associated with increased levels of pro-inflammatory cytokines, suggestive of a mild to moderate immune and inflammation activation [3,4].

The kynurenine pathway of tryptophan metabolism [5] (Fig. 1) has been proposed as a link between inflammatory processes and depressive symptoms [6,7]. The essential amino acid tryptophan is mainly (90%) metabolised to kynurenine (Kyn) and a small

* Corresponding author. Division of Psychiatry, Haukeland University Hospital, Haukelandsbakken 11, Pb 1, 5021 Bergen, Norway.
E-mail address: ute.kessler@helse-bergen.no (U. Kessler).

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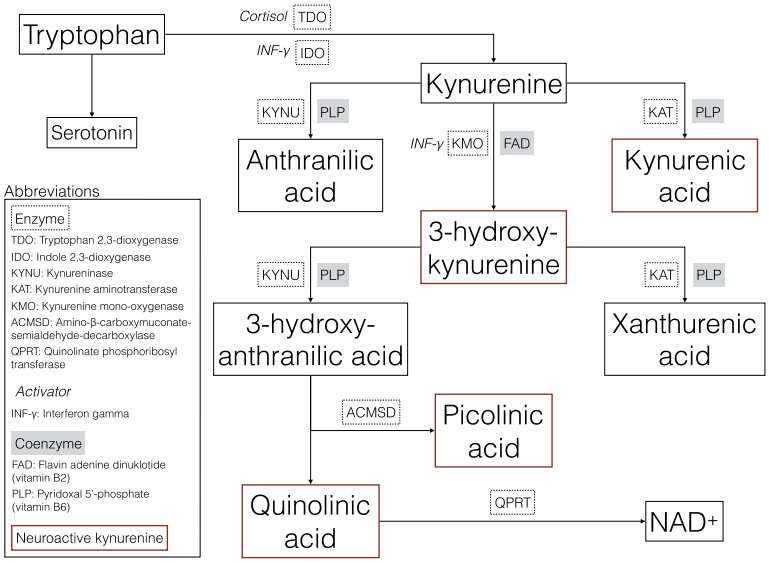

Fig. 1. The kynurenine pathway of tryptophan metabolism.

portion serves as precursor of serotonin. Conversion of tryptophan to kynurenine is regulated by tryptophan 2,3-dioxygenase (TDO) and indole 2,3-dioxygenase (IDO). The activity of IDO is stimulated by proinflammatory cytokines, especially interferon gamma (INF-γ), but also tumor necrosis factor alpha (TNF-α) and interleukin-6, whereas TDO is activated mainly by cortisol [8]. Through activation of IDO, inflammation leads to enhanced catabolism of tryptophan via the kynurenine pathway. The kynurenine to tryptophan ratio (KTR) functions as a proxy measure of INF-γ mediated activation of cellular immunity and this ratio has been shown to correlate positively with the concentration of other immune markers, like neopterin an established marker of cellular immune activation [9]. However, while conversion of tryptophan towards kynurenine is induced by both IFN-γ, through up-regulation of IDO, and by TDO, formation of neopterin is induced by IFN-γ only. Thus, circulating concentrations of neopterin are considered more specific to immune activation than is KTR. Kyn is metabolised further by the enzyme kynurenine aminotransferase (KAT) to kynurenic acid (KA), an *N*-methyl-D-aspartate receptor (NMDAr) antagonist and neuroprotective agent [8], or by kynurenine 3-monooxygenase (KMO) to 3-hydroxykynurenine (HK). HK is then metabolised through 3-hydroxyanthranilic acid (HAA) to either picolinic acid (Pic) or quinolinic acid (QA). Both HK and the NMDAr agonist QA are thought to exert neurotoxic effects [8]. Like IDO, KMO is activated by proinflammatory cytokines, directing metabolism through the neurotoxic branch of the kynurenine pathway and thus disrupting the balance between neuroprotective KA and the neurotoxic metabolites HK and QA [8,10]. Several steps in the kynurenine pathway are dependent on the coenzymes pyridoxal 5′-phosphate (PLP), the active form of vitamin B6, and flavine adenine dinucleotide (FAD), the active form of riboflavin (vitamin B2) [11] (Fig. 1). The serum level of these vitamins is affected by smoking [12]. Cotinine, a metabolite of nicotine, is a commonly used serum marker of recent nicotine exposure [13].

The status of the kynurenine pathway can be described by a set of ratios starting with KTR as a marker of the first and rate-limiting step catalysed by INF-γ-responsive enzyme, IDO. The direction of the Kyn breakdown and the flux through the downstream enzymes, KAT and KMO, are reflected by KA/Kyn and HK/Kyn, while KA/HK and KA/QA reflect the balance between the two main branches of the pathway [14]. Several studies have shown that MD patients have significantly lower plasma concentration of KA and lower KA/KYN and KA/QA than healthy controls, indicating altered balance in favour of neurotoxic metabolites [6,14–17]. The ratio XA/HK is a useful marker for vitamin B6 [18], an important coenzyme in several steps in the kynurenine pathway. Finally, the enzyme aminocarboxymuconate semialdehyde decarboxylase (ACMSD) limits QA formation by competitive production of the putative neuroprotective metabolite Pic. It has been suggested that QA might induce suicidal symptoms by affecting glutamate neurotransmission [19]. Furthermore, a study assessing the CFS and plasma Pic to QA ratio in suicide attempters supported the hypothesis that a reduced ACMSD activity underlies excess of neurotoxic QA production observed in patients exhibiting suicidal behavior [20]. The ratio of Pic and QA (Pic/QA) can be used as an estimate of ACMSD activity.

Electroconvulsive therapy (ECT) is considered the most effective treatment option for severe or treatment resistant MD [21]. It has been suggested that ECT may act by modulating immunological mechanisms [22–24]. Studies on how ECT impacts the immune system have indicated that a single session of ECT might induce an acute activation of immune response [25–27], while repetitive ECT treatment can down-regulate proinflammatory markers [27–29]. Through this immunomodulating effect, ECT might also affect the tryptophan metabolism [24]. Studies suggest that ECT in MD patients might shift the tryptophan metabolism towards metabolites with neuroprotective properties, with increase in KA and KA/HK [22] and decrease in QA after treatment with ECT [16]. However, other studies found no significant changes in KA [30] or in KYN, KA and KA/KYN [17].

The aim of this study was to investigate serum concentrations of tryptophan and a large panel of kynurenine pathway metabolites in MD patients referred to ECT in comparison with healthy controls and to explore the effect of ECT on the kynurenine pathway over a whole course of ECT.

Material and methods

Study design

In this prospective, observational study we collected blood samples and assessed the severity of depressive symptoms in major depression patients before and after a series of ECT. Additionally, the study included a group of age- and sex-matched healthy controls that contributed with the same baseline data. The study protocol has previously been reported in detail [31].

Ethical considerations

The study was approved by the Regional Committee for Medical Research Ethics in South East Norway (2013/1032). All participants provided informed written consent to participate in the study.

Participants

Between September 2013 and November 2016, 30 patients and 14 age- and sex-matched healthy controls from Hordaland, Norway, were included into the study. Patients (age > 18) were referred to and accepted for ECT because of a moderate to severe uni- or bipolar depressive episode with or without psychotic symptoms. The diagnosis was established by the treating clinician based on a clinical interview and information from medical records on symptoms, course of illness, family history, and past treatment. The following criteria were used for exclusion of patients: ECT within the last 12 months and moderate kidney failure (serum creatinine > 120 μmol/L). Data on clinical characteristics were recorded along with medication use both before and after treatment. Healthy controls were recruited by advertisement distributed in Bergen, in Hordaland, Norway. Only those that had no current somatic disease, no use of medication except hormonal birth control agents, and no history of psychiatric disorder were included. The healthy controls underwent the same baseline investigations as the ECT patient group, but did not receive ECT or anaesthesia.

ECT treatment

All patients received the standard ECT treatment as it is provided at the ECT-department at the Haukeland University Hospital in Bergen, Norway, administered with right unilateral electrode placement and a Thymatron System IV device (Somatics Inc., Venice, FL, USA), providing brief- or ultra-brief-pulse (0.25–0.5 ms), square wave, constant current (900 mA). Anaesthesia was obtained with the short acting anaesthetic thiopental. Muscle relaxation was obtained with succinylcholine (1 mg/kg). Three sessions per week were given until remission or until no further improvement of symptoms was expected, with a maximum of 20 sessions. The initial stimulus dose was determined based on age, and subsequent adjustments were made after each treatment based on electroencephalographic parameters such as seizure duration, δ-waves and postictal suppression, as well as reorientation time and clinical effect.

Assessments

Symptom intensity was measured with Montgomery and Åsberg Depression Rating Scale (MADRS) [32] by the treating clinician before and after completed ECT-series. Response was defined as a reduction of more than 50% in MADRS score over the treatment series, and remission as a MADRS score lower than 10 after ECT.

Blood samples

Venous blood samples were collected after at least 8 h of fasting at two time points for each patient: prior to treatment and one to two weeks after the completed ECT-series (median = 10 days, interquartile range = 6 days). For controls, samples were collected at baseline. The samples were centrifuged and the serum separated and stored at −80 °C until analysis. Serum concentrations of tryptophan and eight metabolites kynurenine (Kyn), 3-hydroxykynurenine (HK), kynurenic acid (KA), xanthurenic acid (XA), anthranilic acid (AA), 3-hydroxyanthranilic acid (HAA), quinolinic acid (QA) and picolinic acid (Pic), as well as riboflavin (vitamin B2) and pyridoxal 5′-phosphat (PLP, vitamin B6), inflammatory marker neopterin and the nicotine metabolite cotinine were measured by Bevital (www.bevital.no) using liquid chromatography-tandem mass spectrometry [33]. QA and Pic, as well as isotope labelled internal standards $^{2}H_{3}$-QA and $^{2}H_{4}$-Pic, were added to the published assay [34] by including the ion pairs 168.0/78.9, 124.2/78.0, 171.0/81.0, and 128.2/82.0, respectively. Within-day and between-day CVs were 4–7% for QA and Pic, precision data for the other biomarkers analysed by this assay can be found in previous publication [34]. The renal function marker creatinine was also measured at baseline for evaluation of renal function [34].

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM Corp., Armonk, New York) and RStudio version 1.1.383 [35] with core package *stats* and additional packages *Tidyverse* and *ggsignif*. Baseline clinical data for controls and patients were compared using chi-square test for categorical variables and Mann-Whitney *U* test for continuous variables. Baseline biochemical data were compared using linear regression for log-transformed variables both unadjusted and adjusted for smoking using log-transformed levels of cotinine. Changes in patients' serum concentrations from before to after treatment were analysed using Wilcoxon paired test. The same analyses were also performed for patients divided in subgroups based on ECT response and remission.

Results

Demographics and clinical characteristics

Out of the 30 patients recruited, three were excluded - one due to missing baseline blood sample and two due to high serum creatinine values (>120 μmol/L). The 27 remaining patients (15 female and 12 male) had a median age of 46.0 years while the 14 controls (8 female and 6 male ($p = 1.00$)) had a median age of 42.5 ($p = 0.57$). There were 5 (36%) smokers in the control group and 14 (52%) among the patients ($p = 0.51$). There was a significant difference in depression symptom load as measured with MADRS, with a median score of 1.0 for the controls and 34.0 for the patients ($p < 0.001$). Details on clinical characteristics and medication for patients are given in Table 1.

ECT treatment variables and symptom severity before and after treatment

Anaesthesia was given with a median of 3.88 (IQR = 1.88) mg thiopental per kg body weight. ECT was delivered with a median

Table 1
Clinical characteristics and medication.

	Total	*n* (%)[1]/ Median (IQR)[2]		Min.	Max.
Unipolar depression[1]	24	19	(79.2)		
Bipolar depression[1]	24	5	(20.8)		
Age at inclusion[2]	27	46	(21.0)	22	65
Age at debut of depressive symptoms[2]	26	20	(11.8)	10	60
Years since debut[2]	26	19.5	(25.3)	1	42
Number of depressive episodes[2]	20	3	(3.25)	1	50
Length in weeks of current depressive episode[2]	24	39	(44.2)	3	156
Psychotic symptoms in current depressive episode[1]	26	4	(15.4)		
Previous ECT treatment[1]	26	2	(7.69)		
No medication[1]	27	0	(0.00)		
Only litium[1]	27	1	(3.70)		
Only quetiapin[1]	27	2	(7.40)		
Two or more medications[1]	27	24	(88.8)		

Only patients were included (n = 27). Medication refers to the use of antidepressants, mood stabilisers and/or antipsychotics.Abbreviations: IQR, interquartile range.

charge of 237.8 mC (IQR = 134) and the median seizure length was recorded as 50.7 s (IQR = 16). The median MADRS score decreased from 34 pre-treatment to 15 post-treatment. Twelve patients responded to treatment (57.1%), whereas remission occurred in eight patients (38.1%). While the number of treatments did not differ between the 12 responders and 9 non-responders (10.3 and 12.1, respectively), there was a significant difference in the number of treatments between the 8 remitters and the 13 non-remitters (8.3 and 12.8, respectively, $p = 0.008$).

Tryptophan metabolites in patients and controls

The comparison of serum concentrations of tryptophan and metabolites for patients and the age and gender matched healthy controls are given in Table 2. In the unadjusted analyses, patients had significantly lower concentrations of KA, XA and Pic, as well as lower KA/Kyn, KA/QA, XA/HK and Pic/QA, while there were no statistical differences in measures of Trp, Kyn, HK, AA, HAA and QA or KTR between the groups. Adjusted for cotinine, KTR was higher while XA, KA/Kyn, KA/QA, XA/HK and Pic/QA were lower in the patient group compared to controls.

Changes in tryptophan metabolites in MDD patients after ECT

Post-treatment blood samples were available for 21 patients, of whom 12 responded to ECT while 9 did not. Wilcoxon analyses showed significant increase of HAA ($p = 0.028$), Pic ($p = 0.013$), Pic/QA ($p = 0{,}018$) and neopterin ($p < 0.001$) (Fig. 2, Supplementary Table 1). With patients divided in subgroups based on treatment response, there was significant increase in HK and Pic among

Table 2
Baseline concentrations and ratios or tryptophan metabolite and related metabolites in MDD patients compared to healthy controls.

	Baseline values				Linear regression			
	Control		Patient		Unadjusted		Adjusted for	
	(*n* = 14)		(*n* = 27)				cotinine	
	Median (IQR)		Median (IQR)		Estimate	*p*-value	Estimate	*p*-value
Trp, μmol/L	77.2	(9.45)	75.2	(10.3)	−0.07	0.18	−0.09	0.11
Kyn, μmol/L	1.39	(0.47)	1.53	(0.42)	0.01	0.87	0.04	0.54
KA, nmol/L	45.3	(12.8)	37.4	(18.1)	−0.21	**0.04**	−0.16	0.11
HK, nmol/L	37.8	(7.77)	37.3	(13.3)	−0.07	0.46	−0.02	0.79
XA, nmol/L	15.9	(4.83)	10.2	(6.80)	−0.47	**0.00**	−0.44	**0.00**
AA, nmol/L	19.2	(9.15)	15.6	(6.75)	−0.11	0.26	−0.10	0.32
HAA, nmol/L	38.0	(13.6)	29.2	(12.6)	−0.20	0.10	−0.17	0.17
QA, nmol/L	318	(114)	329	(155)	0.09	0.38	0.14	0.15
Pic, nmol/L	32.9	(13.7)	25.2	(14.0)	−0.29	**0.01**	−0.28	0.02
KTR, ratio[a]	18.1	(4.07)	20.1	(5.62)	0.08	0.18	0.13	**0.02**
KA/Kyn, ratio[a]	31.9	(2.87)	26.1	(8.01)	−0.22	**0.00**	−0.20	**0.00**
KA/HK, ratio[b]	12.6	(1.97)	10.0	(3.20)	−0.14	0.16	−0.13	0.20
KA/QA, ratio[c]	15.2	(4.59)	10.9	(3.62)	−0.30	**0.00**	−0.30	**0.00**
XA/HK, ratio[c]	45.6	(10.8)	28.5	(14.4)	−0.40	**0.01**	−0.42	**0.00**
Pic/QA, ratio[a]	106	(37.3)	73.5	(31.3)	−0.38	**0.00**	−0.42	**0.00**
PLP, nmol/L	63.5	(14.4)	49.1	(34.9)	−0.19	0.21	−0.17	0.27
Riboflavin, nmol/L	11.9	(4.13)	14.6	(5.80)	0.07	0.54	0.13	0.23
Creatinine, μmol/L	71.8	(10.7)	73.8	(18.7)	0.04	0.42	0.06	0.25
Neopterin, nmol/L	14.3	(7.10)	17.7	(9.30)	0.23	0.07	0.29	0.02
Cotinine, nmol/L	0.49	(250)	298	(1120)	1.51	0.16		

Estimates and *p*-values from linear regression for log-transformed variables with and without adjustment for log-transformed cotinine. *p*-values below significance threshold 0.05 are marked in bold. Abbreviations: Trp, tryptophan; Kyn, kynurenine; HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; PLP, pyridoxal 5′-phosphat; IQR, interquartile range. Ratios are multiplied by.

[a] 1000.
[b] 10 or.
[c] 100.

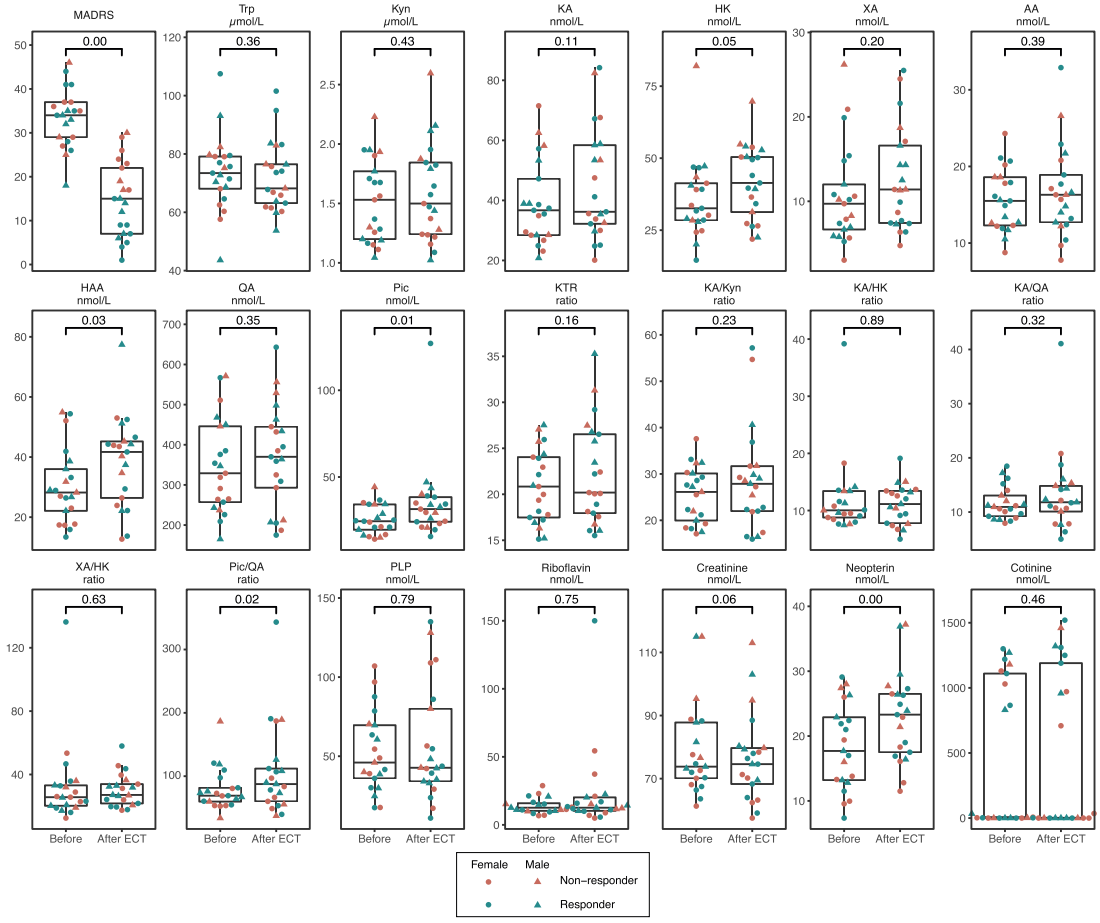

Fig. 2. MADRS and biomarker levels before and after ECT treatment. The horizontal box lines show the first (Q1), second (Q2) and third quartile (Q3). The whiskers cover all values between Q1 - 1.5 * IQR and Q3 + 1.5 * IQR. The *p*-value from Wilcoxon test of values before and after ECT is displayed for each variable. Y-axis scale is indicated below each variable's name. Abbreviations: MADRS, Montgomery and Åsberg Depression Rating Scale; Trp, tryptophan; Kyn, kynurenine; KA, kynurenic acid; HK, 3-hydroxykynurenine; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; PLP, pyridoxal 5′-phosphat; Ribo, riboflavin; Creat, creatinine; Neopt; neopterin; Cot, cotinine.

responders (Table 3). There was also a significant increase in neopterin concentration both in responders and non-responders. Other metabolites concentrations and ratios remained unchanged. Analyses in remitters ($n = 8$) showed the same direction of effect as in patients with treatment response though no changes were significant. In the non-remitters ($n = 13$) there were significantly increased levels of neopterin (Supplementary Table 2).

Discussion

This study aimed to investigate serum concentrations of kynurenine metabolites in MD patients referred to ECT in comparison with healthy controls and to assess the effect of ECT on the kynurenine pathway. There were three main findings:

i) Compared to healthy controls, patients had low levels of kynurenine metabolites KA, XA and Pic and ratios KA/Kyn, KA/QA, XA/HK and Pic/QA, indicative of an imbalance in favour of neurotoxic substances.
ii) Comparing post-treatment to baseline concentrations, there was no reduction of KTR as a proxy measure for inflammation, nor in the concentration of inflammation marker neopterin. On the contrary, the concentration of neopterin was significantly increased after ECT.
iii) After treatment there was an increase in patient concentrations of HAA and Pic, metabolites with putative neuroprotective properties, and in the Pic to QA ratio.

Altered kynurenine pathway metabolism has been proposed as a link between mild chronic inflammation and depressive symptoms [5–7]. Inflammation can affect the kynurenine pathway both by activation of IDO, reflected by an increased KTR, and by activation of KMO, increasing metabolism through the HK branch of the pathway and causing an imbalance between neuroprotective and neurotoxic metabolites. In our study, like in a recent meta-analysis

Table 3
Changes in tryptophan metabolite concentrations and ratios after ECT treatment for responders and non-responders.

	Responders ($n = 12$)					Non-responders ($n = 9$)				
	Before ECT		After ECT		p-value	Before ECT		After ECT		p-value
	Median (IQR)		Median (IQR)			Median (IQR)		Median (IQR)		
MADRS, score	34.0	(5.50)	8.00	(6.75)	**0.00**	35.0	(8.00)	23.0	(7.00)	**0.01**
Trp, μmol/L	73.1	(7.67)	73.9	(19.7)	1.00	75.2	(16.6)	66.9	(13.8)	0.13
Kyn, μmol/L	1.52	(0.53)	1.72	(0.41)	0.08	1.53	(0.64)	1.28	(0.26)	0.65
KA, nmol/L	38.0	(7.25)	38.7	(26.9)	0.23	29.5	(29.8)	35.6	(20.9)	0.50
HK, nmol/L	32.2	(13.7)	42.7	(12.2)	**0.03**	33.6	(12.9)	36.5	(26.5)	0.57
XA, nmol/L	8.79	(6.24)	11.2	(7.46)	0.20	9.71	(2.73)	11.4	(8.42)	0.65
AA, nmol/L	15.2	(4.05)	15.7	(6.53)	0.17	17.8	(6.30)	16.3	(4.90)	0.73
HAA, nmol/L	29.0	(11.3)	43.0	(20.2)	0.06	27.0	(14.1)	40.3	(17.5)	0.36
QA, nmol/L	351	(163)	367	(158)	0.14	319	(181)	385	(152)	1.00
Pic, nmol/L	25.0	(9.10)	34.2	(14.6)	**0.03**	21.3	(18.6)	29.4	(8.30)	0.20
KTR, ratio[a]	19.6	(6.89)	22.8	(9.11)	0.09	20.8	(4.68)	20.1	(4.29)	1.00
KA/Kyn, ratio[a]	27.4	(8.64)	25.3	(11.0)	0.47	25.5	(11.0)	28.5	(6.28)	0.36
KA/HK, ratio[b]	10.7	(4.66)	10.9	(4.49)	0.85	9.51	(1.99)	12.6	(5.77)	0.65
KA/QA, ratio[c]	10.9	(6.39)	12.1	(4.51)	0.52	10.9	(1.95)	10.7	(4.73)	0.50
XA/HK, ratio[c]	24.3	(13.9)	26.8	(11.3)	0.91	26.1	(9.13)	31.2	(12.5)	0.57
Pic/QA, ratio[a]	7.48	(3.43)	9.84	(3.84)	0.06	5.99	(1.94)	6.65	(4.13)	0.25
PLP, nmol/L	40.1	(35.0)	40.9	(16.0)	0.47	48.9	(30.3)	56.5	(66.8)	0.73
Riboflavin, nmol/L	13.7	(5.25)	13.0	(6.90)	0.96	11.4	(5.32)	12.2	(12.1)	0.73
Creatinine, μmol/L	73.5	(13.6)	75.5	(10.4)	0.47	76.6	(18.7)	71.3	(16.5)	**0.03**
Neopterin, nmol/L	19.4	(9.43)	24.4	(8.00)	**0.03**	16.0	(12.7)	21.4	(10.4)	**0.01**
Cotinine, nmol/L	432	(1138)	480	(1265)	0.12	2.34	(1030)	0.00	(709)	0.55

Wilcoxon paired test. p-values below the significance treshold 0.05 are marked in bold. Only patients without missing data were included ($n = 21$). Abbreviations: MADRS, Montgomery and Åsberg Depression Rating Scale; Trp, tryptophan; Kyn, kynurenine; HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; PLP, pyridoxal 5′-phosphat; IQR, interquartile range.Ratios are multiplied by.

[a] 1000.

[b] 10 or.

[c] 100.

[36], there was no significant difference in KTR between healthy controls and patients with MD in the unadjusted analyses. However, adjusted for cotinine, KTR was higher in patients, indicating increased INF-γ mediated activation of cellular immunity. Furthermore, there were lower levels of KA, XA and Pic, and lower KA/Kyn and KA/QA in patients compared to controls. This is in line with other studies on blood and CSF samples from depressed or suicidal patients showing an imbalance in the kynurenine pathway in favour of neurotoxic metabolites [6,14–17,30,37,38]. Comparing patients with healthy controls, these studies have shown higher levels of neurotoxic kynurenines [37,38], lower levels of neuroprotective kynurenines [6,17,30,38] and altered kynurenine ratios with lower KA/Kyn [6,17] and KA/QA [14–16]. However, one study found normal levels of kynurenines in depressed patients compared to healthy controls [39].

ECT has been found to elevate KTR in a study with 23 patients with MD [40]. Like two other studies assessing changes of KTR during ECT [16,17], we found no such change in KTR after treatment. However, after treatment we found significant increase in the patient concentrations of the inflammation marker neopterin, indicating an inflammatory response. Inflammation as response to ECT has been demonstrated in several studies [24]. Increased levels of proinflammatory cytokines have been observed as a short-term effect of single ECT sessions [25–27]. In our study, the post-treatment blood sample was drawn several days (median = 10 days, IQR = 6 days) after the last session in a series of ECT. Full series of ECT treatments like this have mostly been associated with a decrease in inflammation markers [27–29]. However, in a study by Hoekstra et al. a significant increase in neopterin serum concentration was detected in 20 severely depressed patients after ECT series [41]. Similarly, after a series of ECT, Freire and colleagues found increased levels of the proinflammatory cytokines TNF-α and INF-γ, both potent activators of kynurenine pathway enzymes IDO and KMO, although IL-6 concentration was reduced [42].

Previous studies on changes in the balance between neuroprotective and neurotoxic kynurenines after ECT have yielded inconsistent results: Schwieler and colleagues [16] reported a reduction in QA as well as in QA/KA in blood samples from 19 patients after ECT treatment. In addition to increased KTR, Guloksuz and colleagues found increased levels of KA, KA/Kyn and KA/HK after ECT [40]. In contrast, Olajossy and colleagues [30] found low levels of KA in pre-treatment blood samples of 50 patients across three diagnostic groups, but no significant increase in KA after treatment. Similarly, Allen and colleagues [17] found low plasma concentrations of KA and low KA/Kyn in patients before treatment, but no increase in KA after treatment, independent of response status. In the current study, only two kynurenine metabolites, HAA and Pic, were significantly increased after treatment. These metabolites both belong to the KMO branch of the kynurenine pathway starting with the KMO mediated conversion of Kyn to HK. It is interesting to note that both Pic and HAA are proposed as neuroprotective substances and that Pic is though of as an escape route preventing high levels of the neurotoxic QA (Fig. 1) [20,43,44].

In sum, it is possible that an ECT associated inflammation response has caused increased metabolism through KMO and the neurotoxic branch of the kynurenine pathway. KMO is stimulated by the same pro-inflammatory cytokines that cause activation of neopterin producing macrophages [9,10], and higher KMO activity could explain the observed increase in HAA and Pic.

To our knowledge this is the first study assessing a large panel of metabolites of the kynurenine pathway of tryptophan degradation and metabolite ratios reflecting enzymes involved in patients with MD before and after ECT treatment. The kynurenine pathway represents a potential mechanistic link between low-grade inflammation in depression and neuroplasticity. However, the small sample size, the lack of a control group of depressed patients not receiving ECT, and the complex contribution of the various kynurenine metabolites to the pathogenesis of depression, make it

difficult to distinguish the antidepressant mechanisms of action of ECT from other, nonspecific effects. Furthermore, there are important variables, such as systemic inflammation, nutrition, BMI and time of blood sampling, which could affect the tryptophan metabolism that we were unable to adjust for in this study. We excluded patients with renal failure, which may increase plasma concentration of metabolites with high renal clearance. Patients with somatic disorders other than renal failure were not excluded, and medications for somatic and psychiatric disorder may possibly affect concentrations of some metabolites. However, for each individual, medication was essentially stable during the study period, as only minor changes were done in drug therapy, mainly reduction of benzodiazepines and other substances raising seizure threshold. Compared to population-based studies [45], the response and remission rate in the current study are relatively low. This is probably due to a selection bias, as the included patients were younger and had a longer duration of the current episode, both factors known to be associated with lower response rates. The small sample size is a limitation of the study, as is the heterogeneous study population consisting of both bipolar and unipolar depression patients. The statistical power and the ability to detect "true" associations may be further reduced by normal variation in metabolite concentration over time [46], and such attenuations are likely because metabolite concentration was measured only at a single time point before and after ECT. However, the ability to detect biomarker status from a single measurement has been evaluated in terms of intraclass correlation constants (ICCs) for most kynurenine investigated, and ICCs varies in the range from 0.5 to 0.7 [47], which is considered as moderate to strong within-subject reproducibility [48].

In summary, the current study explored the impact of ECT on a large panel of kynurenine metabolites possibly involved in the pathogenesis of depression. The results from the current study are preliminary and should be followed up by studies in larger cohorts, also including a control group of depressed patients not receiving ECT. Future studies should also seek to measure a broader panel of inflammation markers and should ideally include measurements from cerebrospinal fluid (CSF). Furthermore, metabolites should be measured before start of treatment and after a predefined number of treatments, as well as at multiple time points after the final treatment.

Conclusion

Patients with major depression referred to ECT showed lower levels of neuroprotective kynurenine-pathway metabolites (KA, XA and Pic) as well as lowered neuroprotection ratios (KA/Kyn and KA/QA) compared to age- and sex-matched healthy controls. The results from this pilot study indicate that concentration of the inflammation marker neopterin was increased after ECT along with increased levels of Pic and HAA, two kynurenine metabolites with putative neuroprotective properties. Further research in larger cohorts is required to conclude whether ECT exerts its therapeutic effects via changes in the kynurenine pathway.

Authors' contributions

This study was designed and executed by UK, JH, TIMA, IL, VJE, LO and KØ. ØM, AU and PMU performed biochemical analyses. TIMA performed the statistical analyses and drafted the manuscript together with IL. All authors read and approved the final manuscript.

Declarations of interest

None.

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Abbreviations

AA	anthranilic acid
ACMSD	aminocarboxymuconate semialdehyde decarboxylase
ECT	electroconvulsive therapy; HAA, 3-hydroxyanthranilic acid
HK	3-hydroxykynurenine
IDO	indole 2,3-dioxygenase
KA	kynurenic acid
KAT	kynurenine aminotransferase
KMO	kynurenine 3-monooxygenase
Kyn	kynurenine
MADRS	Montgomery and Åsberg Depression Rating Scale
MD	major depression
NMDAr	*N*-methyl-D-aspartate receptor
PLP	pyridoxal 5′-phosphat; Pic, picolinic acid
QA	quinolinic acid
TDO	tryptophan 2,3-dioxygenase
Trp	tryptophan
XA	xanthurenic acid

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2019.05.018.

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Study II

Supplementary material

Supplementary table 1

Changes in tryptophan metabolite concentrations and ratios after ECT treatment

	Pre-ECT (n = 21)	Post-ECT (n = 21)	
	Median (IQR)	Median (IQR)	p-value
MADRS, score	34.0 (8.00)	15.0 (15.0)	**0.00**
Trp, µmol/L	73.5 (11.0)	68.2 (13.3)	0.36
Kyn, µmol/L	1.53 (0.57)	1.50 (0.60)	0.43
KA, nmol/L	36.7 (18.8)	36.2 (26.2)	0.11
HK, nmol/L	32.6 (12.9)	41.4 (19.1)	0.05
XA, nmol/L	9.71 (5.34)	11.4 (9.17)	0.20
AA, nmol/L	15.5 (6.30)	16.3 (6.20)	0.39
HAA, nmol/L	28.2 (13.9)	41.7 (18.8)	**0.03**
QA, nmol/L	329 (189)	370 (152)	0.35
Pic, nmol/L	24.5 (14.6)	31.5 (14.2)	**0.01**
KTR, ratio[a]	20.8 (6.56)	20.2 (8.58)	0.16
KA/Kyn, ratio[a]	26.1 (10.2)	27.9 (9.70)	0.23
KA/HK, ratio[b]	10.0 (4.63)	11.1 (5.66)	0.89
KA/QA, ratio[c]	11.0 (3.83)	11.8 (4.73)	0.32
XA/HK, ratio[c]	25.7 (12.9)	27.1 (12.2)	0.63
Pic/QA, ratio[a]	69.2 (21.8)	87.6 (51.7)	**0.02**
PLP, nmol/L	46.0 (33.6)	42.7 (45.9)	0.79
Riboflavin, nmol/L	12.6 (5.60)	12.6 (9.90)	0.75
Creatinine, µmol/L	73.8 (17.7)	74.6 (11.4)	0.06
Neopterin, nmol/L	17.7 (9.70)	23.3 (9.00)	**0.00**
Cotinine, nmol/L	7.20 (1110)	1.92 (1190)	0.46

Wilcoxon paired test. Only patients without missing data were included (n = 21). p-values below the significance treshold 0,05 are marked in bold. Abbreviations: Trp, tryptophan; Kyn, kynurenine; HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; PLP, pyridoxal 5'-phosphat; IQR, interquartile range. Ratios are multiplied by a: 1000, b: 10 or c: 100.

Supplementary table 2

Changes in tryptophan metabolite concentrations and ratios after ECT treatment for remitters and non-remitters

	Remitters (n = 8)			Non-remitters (n = 13)		
	Before ECT	After ECT		Before ECT	After ECT	
	Median (IQR)	Median (IQR)	p-value	Median (IQR)	Median (IQR)	p-value
MADRS, score	34.5 (9.00)	6.50 (2.75)	**0.01**	34.0 (8.00)	19.0 (9.00)	**0.00**
Trp, µmol/L	73.1 (6.43)	73.9 (19.8)	0.95	75.2 (16.6)	66.9 (14.5)	0.31
Kyn, µmol/L	1.52 (0.46)	1.83 (0.39)	0.05	1.53 (0.70)	1.37 (0.34)	0.50
KA, nmol/L	37.7 (15.9)	38.7 (26.3)	0.46	34.9 (10.5)	35.6 (23.4)	0.24
HK, nmol/L	32.2 (12.7)	42.7 (12.1)	0.11	33.6 (12.9)	39.3 (23.1)	0.22
XA, nmol/L	10.5 (5.72)	11.0 (8.71)	0.38	8.28 (3.94)	11.4 (7.92)	0.38
AA, nmol/L	15.2 (5.58)	17.2 (5.50)	0.15	15.6 (6.30)	15.7 (4.70)	1.00
HAA, nmol/L	34.6 (15.7)	45.5 (14.7)	0.15	27.0 (6.90)	37.4 (17.5)	0.13
QA, nmol/L	316 (167)	364 (189)	0.38	329 (181)	385 (141)	0.65
Pic, nmol/L	25.0 (13.3)	36.4 (8.68)	0.05	24.0 (12.0)	29.4 (9.50)	0.11
KTR, ratio[a]	17.8 (6.89)	24.0 (9.72)	0.05	20.9 (4.68)	20.1 (5.32)	0.95
KA/Kyn, ratio[a]	28.1 (9.08)	22.6 (9.51)	0.84	25.5 (10.4)	29.0 (6.28)	0.22
KA/HK, ratio[b]	10.7 (3.37)	10.3 (3.63)	0.84	9.60 (4.63)	12.6 (5.77)	0.74
KA/QA, ratio[c]	10.5 (6.41)	11.8 (5.05)	0.55	10.9 (1.95)	12.2 (4.73)	0.50
XA/HK, ratio[c]	24.3 (18.3)	25.3 (10.1)	0.95	26.1 (12.6)	31.8 (9.97)	0.45
Pic/QA, ratio[a]	7.76 (3.52)	9.73 (5.46)	0.11	6.89 (2.25)	8.36 (5.27)	0.13
PLP, nmol/L	37.3 (20.8)	41.5 (27.5)	1.00	54.5 (29.6)	42.7 (47.0)	0.68
Riboflavin, nmol/L	12.9 (5.52)	12.3 (8.13)	0.80	12.6 (4.90)	12.6 (8.60)	0.91
Creatinine, µmol/L	71.2 (6.35)	74.6 (12.2)	0.84	77.6 (15.7)	78.0 (10.2)	**0.00**
Neopterin, nmol/L	17.4 (8.45)	21.2 (9.20)	0.11	19.4 (12.7)	23.9 (8.20)	**0.00**
Cotinine, nmol/L	988 (609)	1220 (594)	0.07	2.28 (33.4)	0.00 (35.1)	0.34

Wilcoxon paired test. p-values below the significance treshold 0,05 are marked in bold. Only patients without missing data were included (n = 21). Abbreviations: MADRS, Montgomery and Åsberg Depression Rating Scale; Trp, tryptophan; Kyn, kynurenine; HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; PLP, pyridoxal 5'-phosphat; IQR, interquartile range. Ratios are multiplied by a: 1000, b: 10 or c: 100.

Study III

III

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Research Paper

The effect of electroconvulsive therapy (ECT) on serum kynurenine pathway metabolites in late-life depression

Tore Ivar Malmei Aarsland [a,*], Jan Haavik [a,f], Arve Ulvik [b], Per Magne Ueland [c], Annemiek Dols [d,e], Ute Kessler [f,g]

[a] Department of Biomedicine, University of Bergen, Bergen, Norway
[b] Bevital AS, Bergen, Norway
[c] Department of Clinical Science, University of Bergen, Bergen, Norway
[d] Department of Psychiatry, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht, The Netherlands
[e] Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Psychiatry, Amsterdam Neuroscience, Mood, Anxiety, Psychosis, Sleep & Stress, Amsterdam, The Netherlands
[f] Division of Psychiatry, Haukeland University Hospital, Bergen, Norway
[g] Department of Clinical Medicine, University of Bergen, Bergen, Norway

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ABSTRACT

Background: Depression is reportedly associated with alterations in kynurenine pathway metabolites (kynurenines). Several kynurenines are involved in glutamate signaling, and some have potentially neurotoxic effects while others are considered neuroprotective. The pathway is upregulated under inflammatory conditions, which is associated with depression. Modulation of kynurenine metabolism has been investigated as a potential mechanism in electroconvulsive therapy (ECT), an effective treatment for major depressive disorder, particularly in late-life depression. However, results have been inconclusive. Here we aimed to investigate changes in tryptophan and kynurenines in older patients treated with ECT.

Methods: We analyzed levels of tryptophan, eight kynurenine pathway metabolites and the inflammation marker neopterin in serum samples collected at baseline and after a full ECT series for 48 patients with late-life depression from the Dutch MODECT study.

Results: There were no significant changes in the concentration of single metabolites after ECT, but a significant reduction in the ratio of kynurenic acid to 3-hydroxykynurenine (KA/HK). Analyses of change in kynurenines after ECT in remitters and non-remitters revealed no clear patterns or link to the therapeutic effect of ECT. There was considerable covariation between neopterin and several kynurenines.

Limitations: Variations in diet and serum collection timing may have impacted the results.

Conclusions: This study did not show consistent changes in the kynurenine pathway activation or balance between neuroactive metabolites after ECT. Still, changes in kynurenines were strongly related to changes in neopterin concentrations. This demonstrates the importance of considering inflammation when investigating the effect of ECT on the kynurenine pathway.

1. Introduction

The kynurenine pathway of tryptophan (Trp) metabolism (Fig. 1) has been implicated in the pathophysiology of depression (O'Farrell and Harkin, 2017; Savitz, 2020). This pathway includes several neuroactive metabolites, most notably the N-methyl-D-aspartate receptor (NMDAr) antagonist kynurenic acid (KA), considered neuroprotective (Foster et al., 1984), and the NMDAr agonist quinolinic acid (QA) and the free radical generator 3-hydroxykynurenine (HK). The latter two metabolites are considered neurotoxic (Guillemin, 2012; Okuda et al., 1998). The

Abbreviations: AA, anthranilic acid; ECT, electroconvulsive therapy; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; IFN-γ, interferon gamma; KA, kynurenic acid; KTR, kynurenine-tryptophan-ratio; Kyn, kynurenine; MD, major depression; NMDAr, N-methyl-D-aspartate receptor; Pic, picolinic acid; PLP, pyridoxal 5-phosphate; QA, quinolinic acid; Trp, tryptophan; XA, xanthurenic acid.

* Corresponding author.
E-mail address: tore.aarsland@uib.no (T.I.M. Aarsland).

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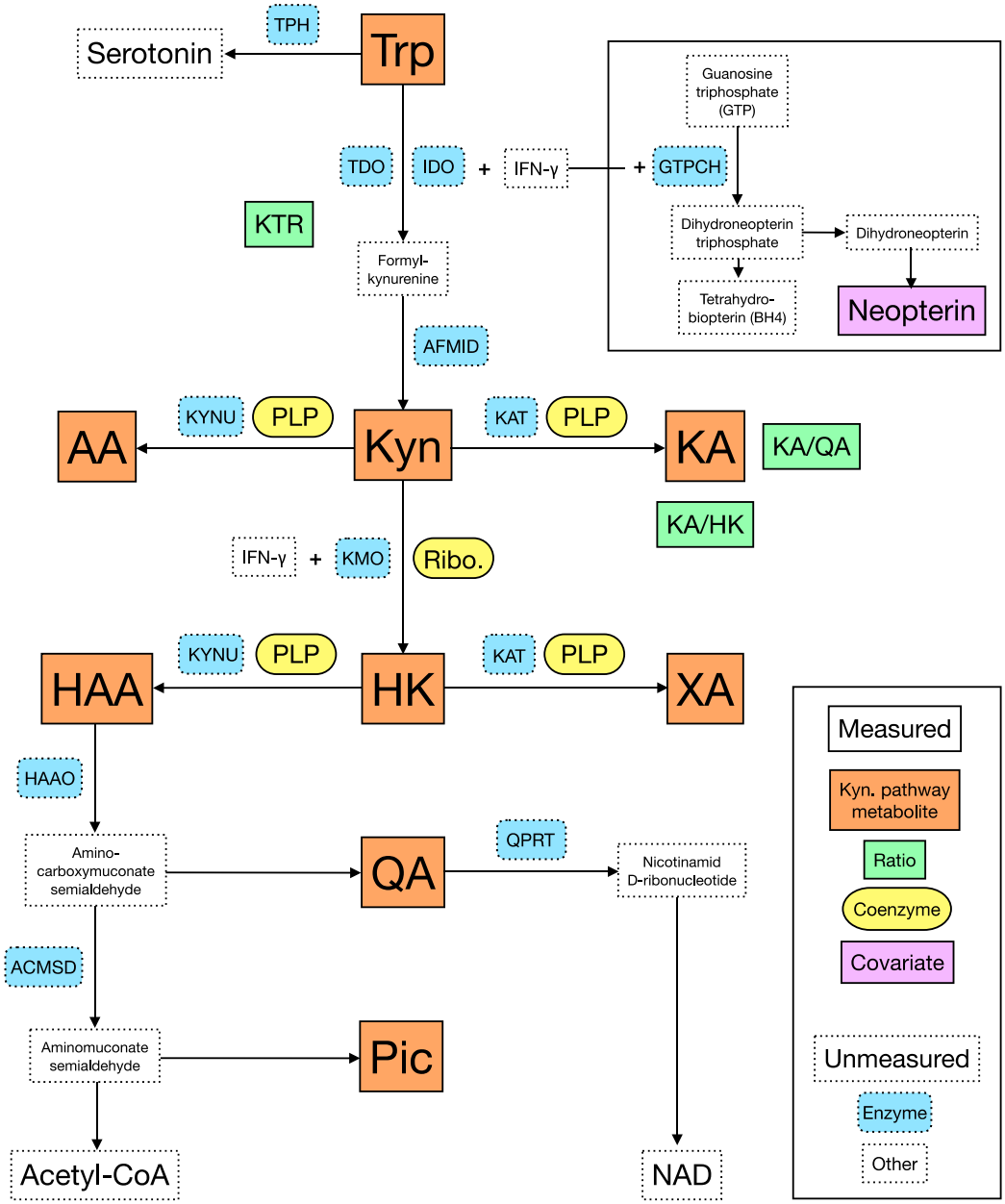

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Fig. 1. The kynurenine pathway of tryptophan metabolism. Kynurenine (Kyn) formation from tryptophan (Trp) is dependent on one of two enzymes, tryptophan 2,3-dioxygenase (TDO) in the liver, and indole 2,3-dioxygenase (IDO) in other tissues. Kyn is converted to kynurenic acid (KA) by kynurenine aminotransferase (KAT), to anthranilic acid (AA) by kynureninase (KYNU), or to 3-hydroxykynurenine (HK) by kynurenine 3-monooxygenase (KMO). KAT and KYNU are dependent on the coenzyme pyridoxal 5′-phosphate (PLP), an active form of vitamin B6, while KMO is dependent on flavin adenine dinucleotide (FAD), derived from riboflavin (vitamin B2). HK can be metabolized further to xanthurenic acid (XA) by KAT, or down the main branch to 3-hydryxyanthranilic acid (HAA) by KYNU. From HAA, the pathway leads to the production of nicotinamide adenine dinucleotide (NAD) via quinolinic acid (QA) or to aminomuconate semialdehyde which yields picolinic acid (Pic) or acetyl-coenzyme A (Acetyl-CoA). KA is an antagonist of the N-methyl-D-aspartate receptor (NMDAr) and is considered to be neuroprotective.[3] In contrast, QA is known to exert neurotoxic effects by stimulation of the NMDAr and through generation of free radicals.[4] While TDO activity is regulated mainly by Trp availability and by glucocorticoids, IDO and KMO can be induced by pro-inflammatory cytokines, especially interferon gamma (INF-γ).[19] The inflammatory marker neopterin is produced by macrophages upon stimulation with INF-γ and is correlated with increased metabolism through the kynurenine pathway measured as increased kynurenine to tryptophan ratio (KTR).[41] The ratios of KA to two metabolites of the main pathway branch, HK (KA/HK) and QA (KA/QA), are measures of the relative KA availability and represents the balance between neuroprotective and neurotoxic effects. Abbreviations: ACMSD, aminocarboxymuconate semialdehyde decarboxylase; AFMID, arylformamidase; GTPCH, GTP cyclohydrolase 1; HAAO, 3-hydroxyanthranilate 3,4-dioxygenase; TPH, tryptophan hydroxylase; QPRT, quinolinate phosphoribosyltransferase.

ratios between pathway metabolites, such as that of KA to QA (KA/QA), may reflect the balance between different neuroactive effects. It has been hypothesized that imbalance between neuroactive metabolites of the kynurenine pathway may contribute to neuronal toxicity and loss of neuronal tissue in depression (Maes et al., 2011; Muller and Schwarz, 2007; Myint and Kim, 2003; Savitz, 2020; Wichers et al., 2005). A recent meta-analysis of 59 studies supports this hypothesis by confirming that patients with major depression have lower levels of Trp, kynurenine (Kyn) and KA as well as lower ratios KA/Trp, KA/HK and KA/QA compared to healthy controls (Marx et al., 2020). Likewise, late-life depression has been associated with lower levels of Trp, Kyn and KA, and an increased Kyn to Trp ratio (KTR) compared to healthy older controls (Wu et al., 2018).

Electroconvulsive therapy (ECT) is a treatment option for severe or treatment-resistant depression with a relatively rapid effect and high response rate, especially in older patients (van Diermen et al., 2018). Hypothetically, successful treatment with ECT could be accompanied by normalized levels of KA, HK, QA, and other kynurenine pathway metabolites and ratios, that have been shown to be affected in depression. Several studies have investigated this possibility and show varying results (reviewed in Aarsland et al., 2022, Giron et al., 2022). In accordance with this hypothesis, Schwieler and colleagues reported a reduction in plasma Trp, Kyn and QA as well as reduced QA/KA after ECT, interpreted as a normalization of the imbalance between neuroprotective KA and neurotoxic QA (Schwieler et al., 2016). Similarly, increased KA was found by Guloksuz and collegues, along with increased KTR, KA/Kyn and KA/HK, taken as a sign of a strengthened neuroprotective effect (Guloksuz et al., 2015). In contrast, KA/HK decreased after ECT in another study (Yilmaz et al. (2022), and Ryan and colleagues found increased concentration of Trp and Kyn after an ECT series, with no significant increase in KA or reduction in QA (Ryan et al., 2020). A fifth study, from Allen and colleagues, found no significant changes in tryptophan kynurenine metabolites or ratios after ECT (Allen et al., 2018). In a previous study, we found a post treatment increase in 3-hydroxyanthranilic acid (HAA) and picolinic acid (Pic), in addition to an increased concentration of the inflammatory marker neopterin in adults with major depression after ECT series (Aarsland et al., 2019). These results could indicate a general stimulation of the kynurenine pathway in conjunction with an inflammatory response to ECT.

Inflammation plays an important role in the regulation of the kynurenine pathway (Hunt et al., 2020; Muller and Schwarz, 2007). As illustrated in Fig. 1, enzymes in the first and second steps of the main kynurenine pathway branch, responsible for the conversion of Trp to Kyn and Kyn to HK, are both induced by pro inflammatory cytokines, especially interferon gamma (IFN-γ) (Mandi and Vecsei, 2012). IFN-γ also stimulates macrophages to produce neopterin, an established inflammatory marker that often correlates strongly with KTR (Fuchs et al., 1991; Maes et al., 1994). Chronic low-grade inflammation is associated with depression, as shown in several meta-analyses (Kohler et al., 2017; Smith et al., 2018). Furthermore, a recent analysis of data from the UK Biobank showed that patients with depression had significantly higher levels of CRP than those without depression, independent of genetic, health and psychosocial factors (Pitharouli et al., 2021). Modulation of immune system activity is a potential effect of ECT (Yrondi et al., 2018). Summaries of studies on ECT and inflammation markers have suggested that single sessions increase inflammation, while full treatments series may reduce inflammation (Yrondi et al., 2018). Thus, inflammation constitutes a shared factor in the relationship between kynurenine metabolism, depression and ECT.

The natural aging process as well as many somatic pathological conditions are also associated both with changes in the kynurenine pathway (Badawy, 2017b; Savitz, 2020; Theofylaktopoulou et al., 2013) and with increased inflammatory activity (Franceschi et al., 2000; Pawelec, 2018). Higher age is associated with lower levels of Trp, and higher levels of most kynurenine pathway metabolites as well as higher KTR (Theofylaktopoulou et al., 2013). Importantly, the efficacy of ECT also increases with age, with especially high response rates in older patients (Kessler et al., 2018; van Diermen et al., 2018). Some studies suggest that kynurenine pathway modulation could be especially relevant in depressed patients with an inflammatory profile (Milaneschi et al., 2021) who possibly also benefit more from ECT (Carlier et al., 2019, 2021). Inflammation, age, and somatic disorders are therefore all relevant for the status of the kynurenine pathway in patients with late-life depression, and for changes in kynurenine pathway metabolite concentrations in conjunction with an ECT series.

To our knowledge, no previous studies have been published on kynurenine pathway metabolite changes after ECT in older patients with depression, a population where ECT is especially effective. In this study therefore, we aimed to investigate changes after ECT in kynurenine pathway metabolites in patients with late-life depression, and to investigate the role of inflammation and somatic disorders in this context.

2. Material and methods

This study was carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent to participate in the study. The MODECT study was approved by the Medical Ethical Committee of the Amsterdam UMC, location VUmc. The current study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics North (REK Nord 2018/721).

2.1. Patients

The participants in this study ($n = 48$) were recruited as part of Mood Disorders in Elderly treated with Electroconvulsive Therapy (MODECT) study (Dols et al., 2017). They were patients aged 55 years or older, diagnosed with unipolar depression and referred to ECT at GGZ inGeest, Amsterdam, in the period between January 1, 2011, and December 31, 2013. All patients were diagnosed by psychiatrists and the diagnosis confirmed with the Mini International Neuropsychiatric Interview (MINI). Patients with schizoaffective disorder, bipolar disorder or major neurologic illness were excluded. Previous treatment resistance was

defined as at least two failed trials of antidepressants in adequate doses. Montgomery and Åsberg Depression Scale (MADRS) was used to assess severity of depression before treatment, weekly during treatment, and after the completed ECT series. A post treatment MADRS score below 10 was considered as remission. Comorbid somatic disorders and medication use were assessed using a semi-structured interview, and baseline serum concentration of kidney function marker creatinine was measured for all patients. Psychotropic medication was either discontinued at least one week before ECT or kept unchanged from six weeks before ECT until the end of the ECT series.

2.2. ECT

Patients received brief-pulse (0.5–1.0 ms) right unilateral ECT twice a week, administered with the Thymatron System IV (Somatics, LLC, Lake Bluff, IL, USA) (maximum energy 200%, 1008 mC). Treatment response was evaluated weekly, and the treatment was concluded when the patient reached a MADRS score of less than 10 at two consecutive ratings. The treatment was switched to bilateral ECT if the clinical condition worsened or if there was no clinical improvement after six unilateral sessions. If there was no further improvement during the last two weeks of treatment after a minimum of six unilateral and six bilateral sessions, treatment was discontinued.

2.3. Blood sampling and analyses

Two venous blood samples were collected for each patient, one at baseline and one after the completed ECT series (median = 5 days, IQR = 5.25). For 37 of the patients, the baseline blood sample was collected before the treatment series (median = 5 days, IQR = 4), but for 11 patients it was collected after the first ECT (median = −5 days, IQR = 4). Blood was drawn between 7.30 and 9.30 A.M. after an overnight fast. Serum concentrations were measured for tryptophan (Trp) and the eight kynurenine pathway metabolites kynurenine (Kyn), kynurenic acid (KA), 3-hydroxykynurenine (HK), xanthurenic acid (XA), anthranilic acid (AA), 3-hydroxyanthranilic acid (HAA), quinolinic acid (QA) and picolinic acid (Pic). In addition, levels of riboflavin (vitamin B2), pyridoxal 5′-phosphate (PLP, vitamin B6), the nicotine metabolite cotinine and the inflammatory marker neopterin were measured. All serum analyses were performed by Bevital (www.bevital.no) in Bergen, Norway, using liquid chromatography/tandem mass spectrometry (Midttun et al., 2009).

2.4. Statistical analyses

Statistical analyses were done using RStudio version 1.2.1335 RStudio (Team, 2018) with packages *ggplot2, reshape2, tidyverse* and *ggsignif*. Three ratios of metabolite concentrations were calculated: KTR (1000*[Kyn]/[Trp]); KA/HK (10*[KA]/[HK]) and KA/QA (100*[KA]/[QA]). The main analyses of changes in serum concentrations from before to after treatment were performed using paired Wilcoxon signed-rank test (wilcox.test, paired=TRUE) for all patients with complete biochemical data. Percentage change for metabolite and ratio levels were calculated as 100*([post] – [pre])/[pre] for each individual to use as the primary measure of change. To control for the effect of variation in the collection time of the baseline blood samples, we also performed this analysis without the 11 patients for whom the baseline blood sample was collected after the first ECT. Additional subgroup analyses were performed with patients split in dichotomous groups by three variables: remission status, neopterin change after ECT, and diagnosis of somatic disease. Remission groups consisted of remitters and non-remitters. Neopterin change groups were created with patients with increased neopterin after ECT in one group, and those with reduced neopterin after ECT in the other. Somatic disease groups were defined as patients with no diagnosis of somatic disease in one group, and those with one or more such diagnoses in the other. Linear regression with natural logarithm transformed biochemical variables as outcome were used to compare baseline concentrations between patients with and without diagnoses of somatic disease. The linear regressions were adjusted for sex, age, smoking status, and serum creatinine concentration, all four possible confounders selected a priori. Like in the main analyses, Paired Wilcoxon signed rank test was used to investigate change after ECT in each of the six groups. Unadjusted Spearman correlation analyses was performed to investigate the relationship between kynurenine pathway metabolites, neopterin and baseline serum creatinine. Here, change in kynurenine pathway metabolites and neopterin was calculated as [post] – [pre]. The relationship between change in neopterin and change in QA was also investigated using a linear regression model (RStudio, ggplot2, geom_smooth with the lm() function) for all patients and in remission and somatic disease groups. Because of extreme neopterin values, one participant was excluded from all correlation analyses. Due to the tight relationship between the investigated kynurenine pathway metabolites, correction for multiple testing was not applied.

3. Results

3.1. Clinical data

MADRS decreased from a median of 32.5 (IQR = 13.5) to 6.5 (IQR = 11.75) after the full treatment series (Fig. 2). Age, sex, time of blood sample collection, number of ECT sessions, depression characteristics, data on medication, and somatic disorders for all patients as well as remitters and non-remitters are shown in Table 1. Corresponding data on subgroups of patients based on neopterin change and somatic comorbidity are available in Supplementary Table 1.

3.2. Changes in tryptophan and kynurenine pathway metabolites in all patients after ECT

Serum levels for kynurenine pathway metabolites and ratios, riboflavin, PLP and neopterin at baseline and after ECT together with results from paired Wilcoxon signed-rank tests on all patients (n = 48) are shown in Fig. 2 and listed in Supplementary Table 2. We observed a significant reduction in KA/HK (median = −16.1%, IQR = 29.2, p = 0.02) after the ECT series, whereas the levels of other kynurenine pathway metabolites or ratios were unchanged in the patient group as a whole. We also compared metabolite levels after excluding the 11 patients who had baseline blood samples collected after the first ECT. The overall changes in the metabolites and ratios did not diverge from analyses of the full set (data not shown). Similar trends were present, with the same reduction in KA/HK being closest to the significance threshold (p = 0.07).

3.3. Subgroups based on remission status

Thirty patients were classified as remitters and 18 as non-remitters. Performing paired Wilcoxon signed-rank tests for remitters and non-remitters separately, there were increased post treatment levels of HK, XA and HAA in remitters, and reduced QA concentrations after ECT in non-remitters, see Table 2 and Fig. 2.

3.4. Subgroups based on neopterin changes after ECT

After ECT, 25 patients had increased neopterin concentrations. For the other 23 patients neopterin concentrations were reduced. In paired Wilcoxon signed-rank tests, patients who had increased neopterin after ECT also had significantly increased HK (median = 20.6%, p = 0.03) and KTR (median = 8.06%, p = 0.04) and reduced levels of PLP (median = −23.1%, p = 0.02) and KA/HK (median = −17.2%, p = 0.01) after ECT (Fig. 3, Supplementary Table 3). Patients with reduced neopterin after ECT had significantly reduced levels of AA (median = −6.64%, p =

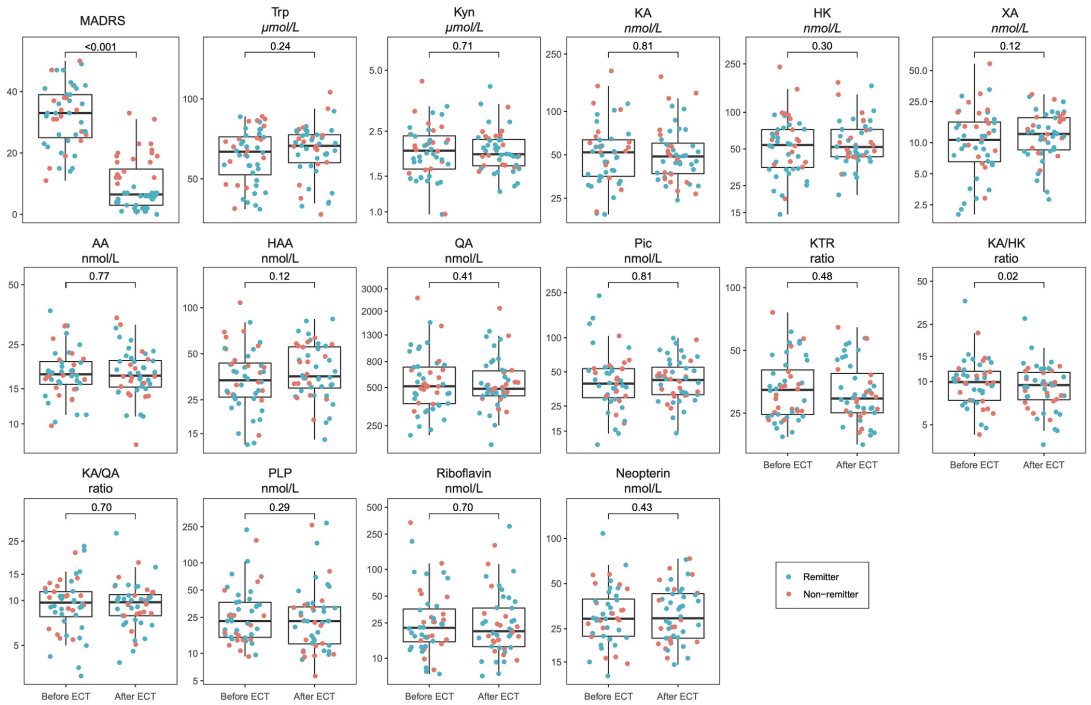

Fig. 2. MADRS scores and levels of kynurenine pathway metabolites, ratios, and coenzymes and neopterin at baseline and after ECT treatment for all patients (n = 48). P values from paired Wilcoxon signed-rank analyses of change after ECT are shown for each variable. Except for MADRS, the y-axes display serum measures logarithmically, providing a better visual resolution for lower values of metabolites with a large concentration spread, like QA, PLP and riboflavin. The middle horizontal line of the box indicates the median of the data (Q2), while the bottom and top horizontal lines indicate first and third quartile (Q1 and Q3). The bottom and top whiskers are calculated as Q1 – (1.5 x IQR) and Q3 + (1.5 x IQR). Abbreviations: MADRS, Montgomery and Åsberg Depression Rating Scale; Trp, tryptophan; Kyn, kynurenine; KA, kynurenic acid; HK, 3-hydroxykynurenine; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; KTR, kynurenine-tryptophan-ratio; PLP, pyridoxal 5′phosphate.

0.03), QA (median = −15.0%, p = 0.002) and KTR (median = −12.4%, p = 0.005) and increased concentration of Trp (median = 6.78%, p = 0.048) after ECT (Fig. 3, Supplementary Table 3).

In unadjusted Spearman correlation analyses, change in neopterin after ECT was significantly correlated with change in HK (rho 0.33, p = 0.02), AA (rho = 0.40, p = 0.01), QA (rho = 0.47, p = 0.0009), and KTR (rho = 0.37, p = 0.01). Changes in neopterin was also significantly and positively correlated with change in change in KTR (rho = 0.56, p = 0.02) in non-remitters. Linear regression analyses in 47 patients, showed positive relationship between change in neopterin and change in QA after ECT in the whole patient group, in remitters, and in patients with and without somatic comorbidity (Fig. 4).

3.5. Subgroups based on diagnoses of somatic disease

Among the patients, 35.4% had a history of heart or vessel disease, 33.3% a history of hypertension, 22.9% a cancer diagnosis and 6.2% diabetes (Table 1). Eleven patients had none of these somatic conditions, while 37 had one or more. Patients with one or more diagnoses of somatic disease had significantly higher Trp (1.19 times, p = 0.03), Kyn (1.41 times, p = 0.0001), KA (1.38 times, p = 0.02), HK (1.66 times, p = 0.001), AA (1.24 times, p = 0.04), HAA (1.56 times, p = 0.01), QA (1.39 times, p = 0.01) and Pic (1.56 times, p = 0.04) at baseline than those without somatic disease (Supplementary Table 4). Patients without diagnosis of somatic disease had significant increase in Trp (median = 10.2%, p = 0.002), HK (median = 15.6%, p = 0.02) and HAA (median = 31.6%, p = 0.01) concentrations after ECT (Supplementary Table 4). There were no other significant changes in any metabolites or ratios after ECT in either somatic disease group.

In unadjusted Spearman correlation analyses for all patients, baseline serum creatinine was significantly correlated to baseline Kyn (rho = 0.43, p = 0.003), KA (rho = 0.46, p = 0.001), HK (rho = 0.43, p = 0.003), QA (rho 0.54, $p < 0.001$) and KTR (rho = 0.54, $p < 0.001$), and post treatment concentrations of Kyn (rho = 0.54, $p < 0.001$), KA (rho = 0.50, $p < 0.001$), HK (rho = 0.56, $p < 0.001$), QA (rho = 0.59, $p < 0.001$) and KTR (rho = 0.59, $p < 0.001$), but not to change in these metabolites (data not shown).

4. Discussion

In this study of 48 patients with late-life depression treated with ECT we analyzed changes in kynurenine pathway metabolites and ratios. We found no significant changes in any of the metabolites after ECT, only reduced level of KA/HK. The most notable changes in kynurenine pathway metabolites were in two subgroups of patients with opposite neopterin change after ECT, illustrating the importance of including inflammation markers in investigation of changes in kynurenine pathway metabolism.

Hypothetically, successful treatment with ECT could be accompanied by normalized levels of KA, HK, QA and other kynurenine pathway metabolites and ratios that have been shown to be affected in depression (Marx et al., 2020; Myint and Kim, 2003). Several studies have measured

Table 1
Clinical characteristics for all patients and for remitters and non-remitters.

	All patients ($n = 48$)			Remitters ($n = 30$)			Non-remitters ($n = 18$)		
	median	(IQR)	range	median	(IQR)	range	median	(IQR)	range
Age (years)	73	(12.3)	55 - 92	74.5	(14)	57 - 92	69	(11.8)	55 - 82
Baseline MADRS [a]	32.5	(13.5)	11 - 50	33	(16)	14 - 49	31.5	(11.5)	11 - 50
Number of ECT sessions [b]	10	(8)	4 - 29	9	(3.75)	4 - 28	14	(9.75)	4 - 29
Days from from baseline blood sample to first ECT [c]	2	(5)	−12 - 30	2	(4.75)	−12 - 11	3	(7)	−5 - 30
Days from last ECT to blood sample [d]	5	(5.25)	−9 - 16	4	(5)	−9 - 16	5.5	(3.25)	1 - 13
Number of previous episodes	2	(4)	0 - 16	2	(3)	0 - 16	3	(4)	0 - 15
Age at onset of depression (years)	57	(24)	18 - 87	57.5	(24)	18 - 87	50.5	(21)	23 - 77
Length of index episode (weeks)	6	(12.5)	1 - 144	4	(11)	1 - 144	12	(18)	2 - 84
Baseline serum creatinine (μmol/L)	78.5	(23.8)	52 - 197	76.5	(21)	61 - 135	79.5	(32)	52 - 197
	n	(%)		n	(%)		n	(%)	
Female	30	(62.5)		17	(56.7)		13	(72.2)	
Non-smoker	38	(79.2)		23	(76.7)		15	(83.3)	
Never alcohol [e]	31	(72.1)		18	(69.2)		13	(76.5)	
Late onset of depression [f]	26	(54.2)		18	(60.0)		8	(44.4)	
MDD with psychotic features	25	(52.1)		17	(56.7)		8	(44.4)	
Previous treatment resistance [h]	25	(52.1)		15	(50.0)		10	(55.6)	
No medication	27	(56.2)		0	(0.00)		10	(55.6)	
Antidepressants	15	(31.2)		9	(30.0)		6	(33.3)	
Only antidepressants	13	(27.1)		7	(23.3)		6	(33.3)	
Antipsychotics	6	(12.5)		4	(13.3)		2	(11.1)	
Only antipsychotics	6	(12.5)		4	(13.3)		2	(11.1)	
Heart and vessel	17	(35.4)		11	(36.7)		6	(33.3)	
Hypertension	16	(33.3)		12	(40.0)		4	(22.2)	
Diabetes	3	(6.2)		2	(6.70)		1	(5.60)	
Stroke	4	(8.3)		4	(13.3)		0	(0.00)	
Cancer	11	(22.9)		5	(16.7)		6	(33.3)	
Migrain	0	(0.0)		0	(0.0)		0	(0.0)	
Parkinson's disease	0	(0.0)		0	(0.0)		0	(0.0)	
Neurological disorder	0	(0.0)		0	(0.0)		0	(0.0)	

[a] For one patient, MADRS could not be collected at baseline, and was replaced by MADRS at two weeks into the treatment series.
[b] Number of ECT sessions in the current treatment series.
[c] For eleven patients, the baseline blood sample was collected after the first ECT, indicated by a negative number of days for this variable.
[d] For one patient, the post ECT blood sample was collected before the last ECT session (nine days). This patient received a total of 14 ECT sessions over seven weeks.
[e] Data on alcohol consumption was missing for five patients.
[f] First depressive episode at 55 years or older.
[h] Previous treatment resistance was defined as at least two failed trials of antidepressants in adquate doses. Abbreviations: IQR, inter quartile range; MADRS, Montgomery and Åsberg Depression Scale; MDD, major depression disorder.

Table 2
Baseline serum metabolite concentrations and effect of ECT on metabolites according to remission status.

	Remitters ($n = 30$)							Non-remitters ($n = 18$)						
	Before ECT		After ECT		% change			Before ECT		After ECT		% change		
Variable	Median	(IQR)	Median	(IQR)	Median	(IQR)	p	Median	(IQR)	Median	(IQR)	Median	(IQR)	p
Trp, μmol/L	61.1	(21.0)	65.8	(16.3)	5.32	(25.9)	0.20	67.5	(18.6)	67.7	(15.4)	−0.04	(35.2)	0.74
Kyn, μmol/L	1.87	(0.76)	1.96	(0.57)	2.16	(16.0)	0.50	2.13	(0.67)	1.89	(0.68)	−3.98	(19.2)	0.20
KA, nmol/L	46.2	(24.0)	46.7	(20.8)	13.7	(39.2)	0.39	56.9	(24.6)	50.4	(31.5)	−4.34	(39.2)	0.15
HK, nmol/L	48.6	(31.7)	49.8	(30.0)	15.9	(51.7)	**0.02**	59.8	(52.1)	53.3	(24.3)	−3.31	(33.2)	0.20
XA, nmol/L	9.16	(7.61)	11.6	(8.37)	22.8	(92.1)	**0.04**	14.4	(11.0)	14.1	(6.65)	−0.45	(56.3)	1.00
AA, nmol/L	18.2	(4.08)	18.1	(5.30)	1.28	(25.0)	0.81	17.4	(5.57)	16.8	(5.33)	−5.41	(18.9)	0.38
HAA, nmol/L	30.0	(16.1)	34.9	(23.5)	21.9	(57.1)	**0.01**	41.2	(26.3)	40.4	(24.2)	−10.4	(69.8)	0.61
QA, nmol/L	451	(404)	482	(298)	0.09	(34.4)	0.57	522	(194)	499	(99.0)	−9.26	(15.1)	**0.03**
Pic, nmol/L	37.8	(22.5)	43.6	(31.2)	19.9	(68.6)	0.29	43.7	(22.2)	38.6	(14.3)	−19.0	(49.3)	0.33
KTR, ratio	32.4	(20.4)	30.0	(18.9)	1.93	(25.6)	0.84	31.6	(11.0)	28.7	(8.99)	−4.15	(20.0)	0.39
KA/HK, ratio	10.2	(3.55)	9.55	(4.03)	−14.7	(33.4)	0.06	9.79	(4.25)	9.03	(3.88)	−16.5	(27.2)	0.23
KA/QA, ratio	9.13	(3.25)	9.74	(3.30)	−0.80	(40.8)	0.90	10.7	(3.73)	9.74	(3.15)	−4.68	(27.8)	0.67
PLP, nmol/L	23.5	(20.8)	22.8	(20.4)	−19.0	(51.8)	0.40	18.8	(17.5)	21.2	(21.2)	−14.3	(44.1)	0.50
Riboflavin, nmol/L	20.6	(19.2)	21.0	(28.5)	1.54	(67.7)	0.54	25.5	(28.7)	18.8	(13.0)	−9.48	(52.3)	0.16
Neopterin, nmol/L	29.1	(16.7)	33.9	(18.9)	3.72	(34.4)	0.42	30.4	(18.2)	25.7	(24.6)	0.51	(47.4)	0.77

Notes: Median and interquartile range (IQR) before and after ECT, percentage change and p-value from paired Wilcoxon signed-rank test are shown for remitters and non-remitters. p-values below the significance threshold 0.05 are marked in bold.
Abbreviations: Trp, tryptophan; Kyn, kynurenine; KA, kynurenic acid; HK, 3-hydroxykynurenine; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; KTR, kynurenine-tryptophan-ratio; PLP, pyridoxal 5′phosphate.

kynurenine pathway metabolites in the context of ECT (Allen et al., 2018; Guloksuz et al., 2015; Ryan et al., 2020; Schwieler et al., 2016; Yilmaz et al., 2022; Aarsland et al., 2019). but the results have been inconsistent. Changes in the two first metabolites of the pathway, Trp-and Kyn, and their ratio KTR, provide information about the activation of the pathway. Trp-and Kyn were both increased after ECT in one study (Ryan et al., 2020). Increase in Kyn and KTR levels was also associated with reduced depression scores after ECT, and the authors concluded that ECT seemed to be activating the kynurenine pathway, at least in patients with unipolar depression (Ryan et al., 2020). In

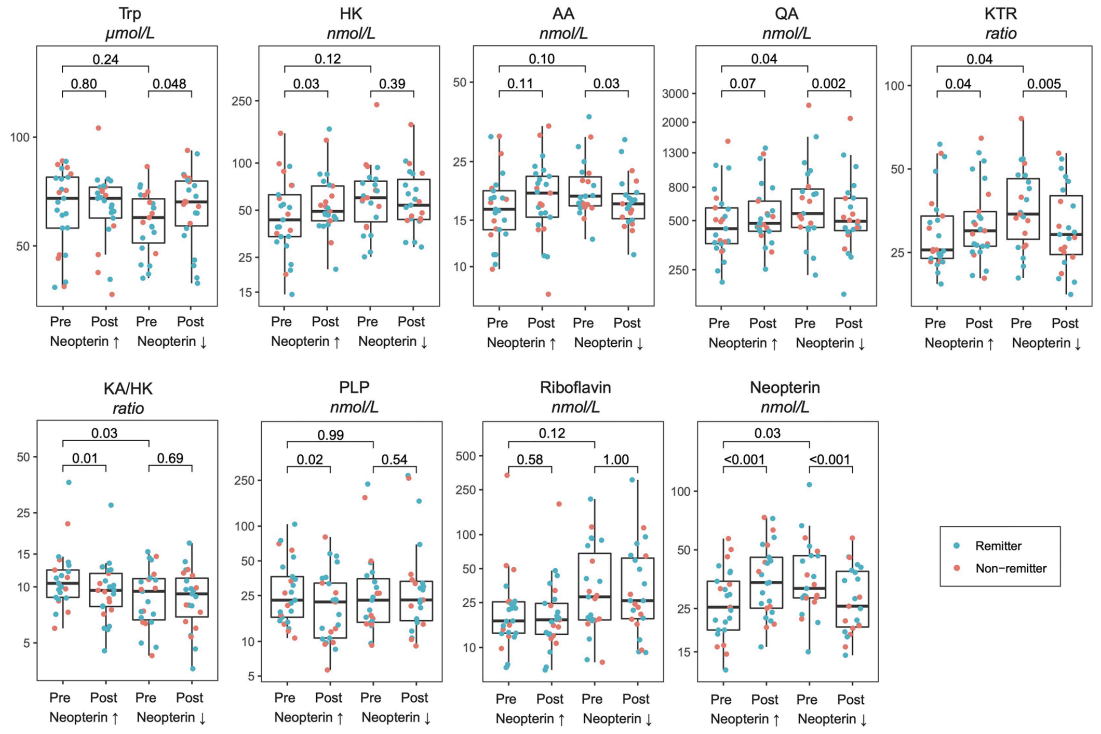

Fig. 3. Changes in selected biochemical variables after ECT in two groups of patients based on their change in neopterin concentration after treatment. Three p values are shown for each variable; two from paired Wilcoxon signed-rank tests of change after ECT in each group, and one from comparison of baseline values between the two groups by linear regression adjusted for sex, smoking status, and baseline serum creatinine. The y-axis serum measures are displayed on a logarithmic scale, providing a better visual resolution for lower values of metabolites with a large concentration spread, like QA, PLP and riboflavin. Abbreviations: Trp, tryptophan; HK, 3-hydroxykynurenine; AA, anthranilic acid; QA, quinolinic acid; KTR, kynurenine-tryptophan-ratio; PLP, pyridoxal 5′phosphate.

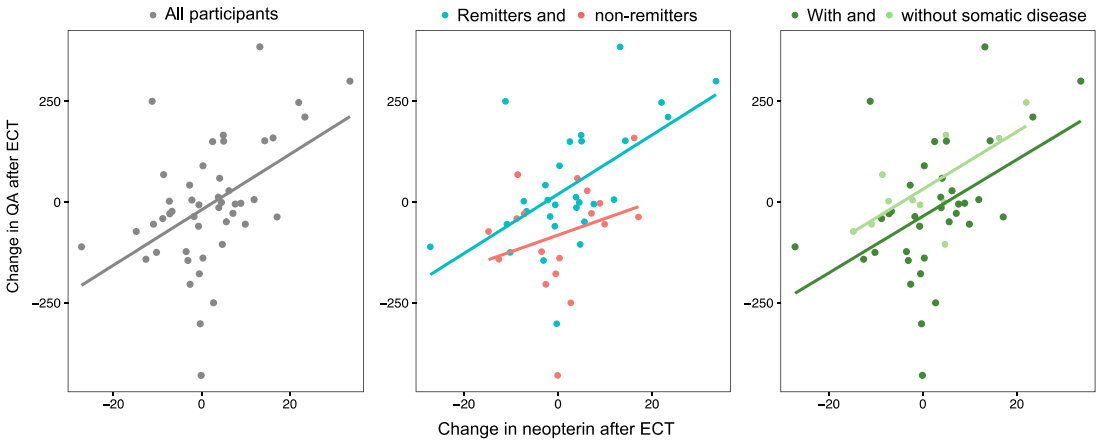

Fig. 4. The relationship between change (nmol/L) in neopterin and change (nmol/L) in QA after ECT in all patients ($n = 47$), in remitters ($n = 29$) and non-remitters ($n = 18$), and in patients with ($n = 36$) and without somatic comorbidity ($n = 11$). One patient was excluded from the analyses due to extreme values of neopterin. The dots show change for each patient. The lines show the relationship between the two change variables using a linear model for all patients ($\beta = 6.89$, $p < 0.001$), remitters ($\beta = 7.35$, $p = 0.001$), non-remitters ($\beta = 4.12$, $p = 0.26$), patients with no somatic diseases ($\beta = 7.03$, $p = 0.005$) and patients with one or more somatic diseases (7.13, $p = 0.01$).

contrast, Trp and Kyn were both reduced after ECT in another study (Schwieler et al., 2016), while in a third study KTR was increased after ECT without significant changes in Trp or Kyn (Guloksuz et al., 2015). In two other studies there were no changes in Trp, Kyn or KTR after ECT (Allen et al., 2018; Aarsland et al., 2019). Similarly, in the current study, Trp, Kyn and KTR all remained stable after ECT in the main analyses. There was therefore no apparent change in the activation of the pathway after treatment. This coincides with the conclusion of a recent meta-analyses that found moderate evidence for no effects of ECT on levels of TRP in eight studies (Giron et al., 2022), though the analysis only included plasma levels, and not serum TRP.

Regarding the relative concentrations of metabolites, two studies have found changes in markers indicating reduced neurotoxicity markers after ECT, with increased KA/QA in one (Schwieler et al., 2016), and increased KA and KA/HK in the other (Guloksuz et al., 2015). One other study did not find any such changes in ratios suggestive of reduced neurotoxicity (Allen et al., 2018). Furthermore, a fourth study found decreased KA/HK after ECT (Yilmaz et al., 2022), and another found that HK was significantly increased after ECT (Ryan et al., 2020), although not after correction for multiple testing. HK is a potentially neurotoxic metabolite (Okuda et al., 1998) on the main pathway branch that is produced from Kyn by kynurenine monooxygenase (KMO), a riboflavin dependent enzyme that can be induced by IFN-γ (Mandi and Vecsei, 2012) (Fig. 1). In the current study, like in the study of Yilmaz and collegues, we found an isolated reduction in KA/HK after ECT. Thus, we could not confirm a hypothesis of reduced neurotoxic markers after ECT when looking at the whole patient group.

We suspected, however, that changes in metabolite and ratio levels could be more prominent when analyzing remitters and non-remitters separately. After the ECT series, patients who remitted had significantly increased concentrations of HK, XA and HAA. XA and HAA are both formed directly from HK. The level of XA is dependent on kynurenine-aminotransferase (KAT) and its essential coenzyme PLP. Similar to KA, XA can inhibit glutamate transmission by blocking vesicular glutamate transportation and thereby reducing extracellular glutamate levels (Sathyasaikumar et al., 2017). HAA is formed by kynureninase (KYNU), which is also PLP dependent, and it is a metabolite on the main pathway branch and precursor of QA and Pic. The neuroactive effect of HAA seems to be context dependent, but it has been suggested to be neuroprotective under inflammatory conditions (Badawy, 2017a; Krause et al., 2011). In a previous study (Aarsland et al., 2019), we also found an increase in HAA after ECT, together with increase in the concentration of Pic which is also suggested to have neuroprotective effects (Brundin et al., 2016). These changes were accompanied by increased neopterin, a sign of increased inflammation after ECT. In the current study, there were no changes in the concentrations of riboflavin or PLP, and no increase in KTR or neopterin to suggest inflammation related increase in KMO activity and increased pathway flux in either remission group. The neurotoxic QA, downstream of HK and HAA, was stable in remitters after ECT, as were levels of KA. Non-remitters, on the other hand, had an isolated, significant reduction in the concentration of QA after ECT.

Like in the analyses of change in all patients, the findings from remitters do not immediately fit with the hypothesis of normalized kynurenine pathway metabolites after treatment. Rather, they suggest increased levels of metabolites in the main pathway branch leading up to QA. Still, there was no significant change in neopterin levels after ECT in the main analyses, nor in remitters or in non-remitters. There was, however, substantial variance between individuals in neopterin change following ECT, from a reduction of 69.7% to an increase of 33.6%. By creating two subgroups based on the direction of change in neopterin after ECT, we found several significant changes in kynurenine pathway metabolites and ratios in both groups.

KTR was significantly increased in patients with increased neopterin after ECT, and significantly reduced in those with reduced neopterin, in accordance with previous findings (Frick et al., 2004). Furthermore, patients with increased concentration of neopterin after ECT had significantly increased HK, and significantly reduced levels of PLP and KA/HK. As discussed above, PLP is an essential coenzyme for the conversion of HK to HAA, and, in general, a lack of this vitamer is associated with accumulation of HK (Theofylaktopoulou et al., 2014). Moreover, PLP is known to be reduced under inflammatory conditions (Ulvik et al., 2014). Thus, the pattern of increased neopterin and reduced PLP after ECT fits well with the increase in HK and reduction in KA/HK in this group (Ulvik et al., 2013). In the other group of neopterin change, reduction in neopterin was accompanied with increased Trp, and reduced AA and QA. This pattern is consistent with reduced activation through the pathway as was also reflected by the reduced level of KTR in this group. Additionally, in Spearman correlation analyses for 47 patients change in neopterin concentrations after ECT was strongly and positively correlated with changes in Kyn, HK, AA and QA.

All these changes are consistent with the role of neopterin as a marker of INF-γ activity which in turn induces activation of kynurenine pathway metabolism. Thus, it seems that inflammation is an important factor when assessing ECT-related changes in the kynurenine pathway in this material, even though neopterin was not significantly altered when looking at all participants together or grouped by remission status. A systematic review of inflammation in ECT suggests that there is an acute inflammatory response after ECT, but that a full treatment course is accompanied by reduced levels of inflammatory markers (Yrondi et al., 2018). In the current material, there seems to be large interindividual variation in neopterin changes after ECT, though the reason for this is unclear.

Somatic diseases are potential factors that could be associated both with inflammation and kynurenine changes. Somatic diseases could affect the levels kynurenine pathway metabolites, not only through increased inflammatory activity but also through altered nutritional status and organ function (Schrocksnadel et al., 2006; Strasser et al., 2017). In the current study diagnoses of heart or vessel disease, hypertension, cancer and diabetes, were registered, all of which are associated with inflammatory processes that may impact tryptophan metabolism (Cervenka et al., 2017; Strasser et al., 2017). Thirty-seven patients had one or more of these diagnoses, and had significantly higher baseline levels of Trp, Kyn, KA, HK, AA, HAA, QA and Pic compared to those without registered diagnosis of somatic disease. There was no baseline difference in neopterin concentrations between the two groups. After ECT, patients with no diagnosis of somatic disease had increased concentrations of Trp, HK and HAA, similar to the remitters discussed above. Patients with one or more such diagnoses had no significant changes in metabolites or ratios.

Kidney function is one factor that could be affecting the kynurenine pathway. In the current study there was substantial positive correlation between baseline serum creatinine and pre- and post-treatment concentrations of Kyn, HK, KA, QA and KTR. This is to be expected since kynurenine pathway metabolites are eliminated through the kidneys, thus potentially accumulating in the case of reduced kidney function (Pawlak et al., 2003). Importantly, there were no significant correlations of baseline creatinine to changes in these metabolites. This suggests that kidney function, although related to the absolute levels of kynurenine pathway metabolites, does not affect their potential for change in this material.

4.1. Strengths and limitations

We recently published a systematic review on changes in tryptophan and kynurneines after ECT, in which the current study is included (Aarsland et al., 2022). There, we discuss a wide range of factors that may affect the results, including patient characteristics, ECT delivery method, medication, and study design. A strength of the current study is that we included a large panel of metabolites and ratios, as well as coenzymes riboflavin and PLP, neopterin, cotinine and creatinine. We investigated these metabolites in a relatively homogenous group of

patients with age 55 years or higher. Furthermore, all psychotropic medication, some of which can affect tryptophan metabolism (Badawy, 2010), was either discontinued or kept stable during the whole treatment. On the other hand, we did not have information on nutritional status, physical activity, or body mass index, and could therefore not evaluate the possible effect of these factors. However, since this is a longitudinal study, the role of these factors should be of less importance than in cross sectional studies given that these traits are stable throughout the study. Although all patients were considered to have late life depression, about half of them had their first depressive episode before the age of 55 years (early onset). Differences in etiology, comorbidity and treatment efficacy between early and late onset depression could impact kynurenine metabolism. There was considerable variation in timing of blood sample collection at baseline, with 11 samples collected after the first ECT. However, from what we could see in sensitivity analyses without these 11 patients, this did not seem to have any significant effect on the direction or size of change in metabolites and ratios after ECT. The number of ECT sessions also varied between individuals, something that could bias the analyses of both kynurenines and inflammation markers. Ideally, the study should have included additional blood samples at a fixed number of ECT sessions (Aarsland et al., 2022). Neopterin was the only included measure of inflammation outside the kynurenine pathway, and a larger panel of inflammatory markers should preferably have been included. The sample size of the study was relatively low, especially when looking at subgroups of somatic disease. Furthermore, there was no available control group of healthy older adults, that could provide baseline control and inform on biological variation over time, nor a group of older patients with depression, treated with other treatment options than ECT to compare serum concentrations to before and after treatment. Such comparators could be very helpful when interpreting the kynurenine pathway metabolites in older patients with depression.

5. Conclusion

In this study of changes in kynurenine pathway metabolites after ECT, we did not find changes that suggest normalization of metabolite levels or ratios. On the contrary, reduced KA/HK in all patients and increased levels of HK and other metabolites in the main pathway branch in remitters, point to a reduction of the relative concentration of KA compared to neurotoxic HK and QA. The overall impression, however, is that large interindividual differences due to multiple uncontrolled sources of variance makes it difficult to isolate changes related to ECT and depression symptom relief. Inflammation is one such source of variability that could be related both to depression and the effect of ECT. When looking at patients with increased and reduced neopterin after ECT separately, we found more coherent changes in kynurenine pathway metabolites. Although effect modification by inflammation was expected, the substantial variation in neopterin change after ECT and close relationship to changes in kynurenine pathway metabolites illustrated the importance of including measures of inflammation and the need for further studies on the effect of ECT on tryptophan kynurenine metabolism.

Authors' contributions

This study was designed and executed by AD, UK, TIMA, JH, AU and PMU. AD contributed to data collection. AU and PMU were responsible for blood sample analyses. TIMA performed the formal analyses and writing - original draft. All authors read and approved the final manuscript.

Data availability

Individual deidentified participant data will be available at reasonable request.

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Declaration of Competing Interest

Jan Haavik has received lecture honoraria as part of continuing medical education programs sponsored by Shire, Takeda, Medice and Biocodex. All other authors declare that they have no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jadr.2023.100578.

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Study III

Supplementary material

Supplementary table 1

	Increased neopterin after ECT (n = 25)				Reduced neopterin after ECT (n = 23)				No somatic disease (n = 11)				One or more somatic diseases (n = 37)			
	n	x̃ (IQR)	min	max	n	x̃ (IQR)	min	max	n	x̃ (IQR)	min	max	n	x̃ (IQR)	min	max
Age (years)	25	75 (14)	55	87	23	72 (11)	60	92	11	66 (7)	59	77	37	75 (13)	55	92
Baseline MADRS [a]	25	36 (10)	15	50	22	26.5 (15.5)	11	49	10	31.5 (14.8)	15	50	37	33 (13)	11	49
Number of ECT sessions [b]	25	10 (6)	4	29	23	10 (9)	4	28	11	10 (3.5)	4	22	37	10 (8)	4	29
Days from from baseline blood sample to first ECT [c]	25	2 (5)	−12	30	23	2 (5)	−10	11	11	5 (8)	−6	11	37	2 (5)	−12	30
Days from last ECT to blood sample [d]	25	5 (5)	−9	15	23	5 (4.5)	1	16	11	5 (4)	0	8	37	5 (5)	−9	16
Number of previous episodes	24	2.5 (4.25)	0	16	12	1 (3.5)	0	7	10	0 (2)	0	9	37	3 (4)	0	16
Age at onset of depression (years)	25	57 (23.0)	18	85	23	57 (22.5)	23	87	11	58 (20.5)	33	77	37	57 (25.0)	18	87
Length of index episode (weeks)	25	6 (10)	1	84	21	6 (14)	1	144	10	6 (12)	1	24	36	6 (11.5)	1	144
Baseline serum creatinine (µmol/L)	25	73 (19)	52	195	23	82 (24)	55	197	11	74 (21)	52	107	37	79 (24)	55	197
	n	(%)			n	(%)			n	(%)			n	(%)		
Female	17	(68)			13	(56.5)			6	(54.5)			24	(64.9)		
Non-smoker	19	(76)			19	(82.6)			10	(90.9)			28	(75.7)		
Never alcohol [e]	17	(73.9)			14	(70)			5	(55.6)			26	(76.5)		
Remitter	16	(64)			14	(60.9)			8	(72.7)			22	(59.5)		
Late onset of depression [f]	13	(52)			13	(56.5)			5	(45.5)			21	(56.8)		
MDD with psychotic features	13	(52)			12	(52.2)			6	(54.5)			19	(51.4)		
Previous treatment resistance [h]	13	(52)			12	(52.2)			4	(36.4)			21	(56.8)		
No medication	14	(56)			13	(56.5)			7	(63.6)			20	(54.1)		
Antidepressants	11	(44)			4	(17.4)			2	(18.2)			13	(35.1)		
Only antidepressants	9	(36)			4	(17.4)			2	(18.2)			11	(29.7)		
Antipsychotics	0	(0)			6	(26.1)			2	(18.2)			4	(10.8)		
Only antipsychotics	0	(0)			6	(26.1)			2	(18.2)			4	(10.8)		
Heart and vessel	8	(32)			9	(39.1)			0	(0)			17	(45.9)		
Hypertension	11	(44)			5	(21.7)			0	(0)			16	(43.2)		
Diabetes	2	(8)			1	(4.3)			0	(0)			3	(8.1)		
Stroke	2	(8)			2	(8.7)			0	(0)			4	(10.8)		
Cancer	6	(24)			5	(21.7)			0	(0)			11	(29.7)		
Migrain	0	(0)			0	(0)			0	(0)			0	(0)		
Parkinson's disease	0	(0)			0	(0)			0	(0)			0	(0)		
Neurological disorder	0	(0)			0	(0)			0	(0)			0	(0)		

[a] For one patient, MADRS could not be collected at baseline, and is replaced by MADRS at two weeks into the treatment series. [b] Number of ECT sessions in the current treatment series. [c] For eleven patients, the baseline blood sample was collected after the first ECT, indicated by a negative number of days for this variable. [d] For one patient, the post ECT blood sample was collected before the last ECT session (nine days). This patient received a total of 14 ECT sessions over seven weeks. [e] Data on alcohol consumption was missing for five patients. [f] First depressive episode at 55 years or older. [h] Previous treatment resistance was defined as at least two failed trials of antidepressants in adquate doses. Abbreviations: IQR, inter quartile range; MADRS, Montgomery and Åsberg Depression Scale; MDD, major depression disorder; x̃, median.

Supplementary table 2

	Before ECT		After ECT		Percentage change			Paired Wilcox
Variable	Median	(IQR)	Median	(IQR)	Min.	Median	Max.	p
Trp, µmol/L	63.3	(20.2)	66.6	(15.9)	−27,9	1.50	89.3	0.24
Kyn, µmol/L	2	(0.75)	1.92	(0.59)	-40.1	0.96	86.4	0.71
KA, nmol/L	52	(28.2)	48.7	(23.3)	-48.0	2.72	79.4	0.81
HK, nmol/L	53.9	(37.0)	51.9	(29.3)	-46.8	9.63	129	0.30
XA, nmol/L	10.7	(9.33)	12.2	(8.95)	-75.7	16.1	413	0.12
AA, nmol/L	17.8	(4.75)	17.5	(5.50)	-47.1	0.29	90.6	0.77
HAA, nmol/L	33.5	(17.5)	35.6	(25.7)	-70.4	10.6	163	0.12
QA, nmol/L	512	(352)	489	(247)	-54.6	-3.23	118	0.41
Pic, nmol/L	39.4	(23.8)	42.4	(23.5)	-74.2	7.70	117	0.81
KTR, ratio	32.3	(15.7)	29.4	(13.7)	-54.1	-2.51	41.6	0.48
KA/HK, ratio	9.91	(4.39)	9.44	(4.12)	-48.4	-16.1	83.6	**0.02**
KA/QA, ratio	9.68	(3.67)	9.74	(3.04)	-54.9	-2.70	179	0.70
PLP, nmol/L	22.8	(21.6)	22.8	(19.9)	-59.9	-17.5	402	0.29
Riboflavin, nmol/L	22.0	(20.6)	20.2	(23.3)	-76.9	-1.12	240	0.70
Neopterin, nmol/L	29.2	(17.2)	29.4	(21.2)	-64.5	1.30	86.6	0.43

Median and interquartile range (IQR) for all patients (n = 48) at baseline and after ECT. p values are from paired Wilcoxon signed rank test of change after ECT. Change is shown for each variable as smallest, median, and largest percentage change. Abbreviations: Trp, tryptophan; Kyn, kynurenine; KA, kynurenic acid; HK, 3-hydroxykynurenine; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-Hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; KTR, kynurenine-tryptophan-ratio; PLP, pyridoxal 5'phosphate.

Supplementary table 3

	Participants with increased neopterin after ECT (n = 25)				Participants with reduced neopterin after ECT (n = 23)			
	Before ECT	After ECT	% change		Before ECT	After ECT	% change	
Variable	Median (IQR)	Median (IQR)	Median (IQR)	p	Median (IQR)	Median (IQR)	Median (IQR)	p
Trp, µmol/L	67.7 (21.5)	67.7 (13.0)	-4.81 (28.4)	0.80	59.9 (16.6)	66.2 (18.8)	6.78 (26.6)	**0.048**
Kyn, µmol/L	1.95 (0.57)	1.91 (0.56)	2.71 (11.8)	0.14	2.07 (0.81)	2.05 (0.68)	-8.79 (18.1)	0.09
KA, nmol/L	52.8 (28.9)	49.1 (24.2)	11.7 (29.0)	0.41	49.6 (23.6)	48.3 (21.1)	-1.75 (43.0)	0.40
HK, nmol/L	43.4 (28.9)	49.2 (28.6)	20.6 (46.2)	**0.03**	60.1 (34.5)	53.8 (35.3)	-5.66 (33.4)	0.39
XA, nmol/L	10.6 (8.02)	12.1 (8.72)	6.16 (52.8)	0.52	10.7 (8.52)	12.2 (8.61)	19.0 (107)	0,12
AA, nmol/L	16.5 (5.60)	19 (6.60)	5.56 (31.6)	0.11	18.5 (4.90)	17.3 (3.70)	-6.64 (17.8)	**0.03**
HAA, nmol/L	35.7 (15.6)	34.6 (18.6)	7.87 (68.6)	0.34	31.2 (24.8)	36.7 (27.1)	16.4 (57.7)	0.22
QA, nmol/L	446 (237)	481 (228)	3.66 (37.4)	0.10	552 (327)	495 (252)	-15.0 (16.8)	**0.002**
Pic, nmol/L	42.6 (23.8)	41.7 (18.9)	7.16 (59.9)	0.87	37.9 (22.7)	43.1 (31.7)	17.2 (86.7)	0.86
KTR, ratio	25.5 (10.0)	29.9 (8.77)	8.06 (20.9)	**0.04**	34.4 (18.2)	29.0 (15.5)	-12.4 (21.9)	**0.005**
KA/HK, ratio	10.5 (3.54)	9.57 (3.97)	-17.2 (16.8)	**0.01**	9.46 (4.42)	9.17 (4.23)	-11.0 (38.4)	0.69
KA/QA, ratio	10.5 (3.26)	10.2 (2.54)	-6.07 (27.6)	0.25	9.03 (5.05)	8.89 (2.52)	1.36 (51.0)	0.54
PLP, nmol/L	22.8 (20.3)	22.0 (21.4)	-23.1 (34.0)	**0.02**	22.8 (20.4)	22.9 (18.1)	1.39 (51.7)	0.32
Riboflavin, nmol/L	17.2 (12.0)	17.6 (11.5)	-4.96 (54.2)	0.58	28.1 (51.6)	26.0 (44.1)	-0.56 (61.0)	1.00
Neopterin, nmol/L	25.4 (15.1)	34.0 (20.7)	25.8 (23.7)	**0.00**	31.7 (18.3)	25.7 (18.7)	-19.5 (22.5)	**0.00**

Median and interquartile range (IQR) before and after ECT, percentage change and p-value from paired Wilcoxon signed rank test are shown for patients with increased neopterin and patients with reduced neopterin after ECT. Abbreviations: Trp, tryptophan; Kyn, kynurenine; KA, kynurenic acid; HK, 3-hydroxykynurenine; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-Hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; KTR, kynurenine-tryptophan-ratio; PLP, pyridoxal 5'phosphate.

Supplementary table 4

	No somatic disease (n = 11)				One or more somatic diseases (n = 37)				Baseline comparison	
	Before ECT	After ECT	% change		Before ECT	After ECT	% change		Linear regression	
Variable	Median (IQR)	Median (IQR)	Median (IQR)	p	Median (IQR)	Median (IQR)	Median (IQR)	p	exp(est)	p
Trp, µmol/L	62.8 (15.8)	68.6 (15.4)	10.2 (14.1)	**0.002**	63.7 (24.0)	66.4 (16.1)	-2.02 (31.3)	0.96	1.19	**0.03**
Kyn, µmol/L	1.46 (0.28)	1.64 (0.40)	12.7 (25.9)	0.11	2.15 (0.69)	2.07 (0.46)	-0.40 (13.9)	0.23	1.41	**0.0001**
KA, nmol/L	39.1 (18.4)	37.1 (18.0)	4.42 (36.9)	0.92	53.0 (29.0)	49.4 (30.2)	0.38 (44.0)	0.77	1.38	**0.02**
HK, nmol/L	34.0 (16.0)	42.6 (8.80)	15.6 (26.1)	**0.02**	60.1 (39.6)	56.5 (38.3)	1.85 (47.8)	0.85	1.66	**0.001**
XA, nmol/L	7.54 (9.01)	12.7 (5.54)	25.7 (81.0)	0.06	11.6 (8.05)	12.1 (9.10)	6.16 (64.9)	0.46	1.56	0.10
AA, nmol/L	16.0 (2.45)	16.8 (4.20)	5.30 (15.3)	0.10	18.3 (4.40)	17.9 (5.70)	-2.29 (28.1)	0.33	1.24	**0.04**
HAA, nmol/L	26.8 (6.50)	34.6 (16.1)	31.6 (35.4)	**0.01**	37.8 (17.4)	36.0 (25.6)	3.87 (59.7)	0.59	1.56	**0.01**
QA, nmol/L	370 (146)	453 (187)	0.44 (36.8)	0.52	552 (308)	502 (299)	-5.11 (25.9)	0.22	1.39	**0.01**
Pic, nmol/L	37.9 (21.2)	43.3 (38.0)	50.7 (82.7)	0.06	42.6 (24.0)	41.7 (18.9)	3.28 (60.0)	0.44	1.56	**0.04**
KTR, ratio	25.2 (5.90)	26.4 (8.98)	-4.21 (21.8)	0.77	34.1 (21.8)	30.7 (19.0)	-1.55 (21.8)	0.47	1.19	0.06
KA/HK, ratio	11.0 (2.64)	9.68 (4.05)	-15.9 (12.0)	0.05	9.67 (4.56)	9.42 (3.63)	-16.4 (37.0)	0.12	0.83	0.21
KA/QA, ratio	10.3 (1.76)	8.77 (3.32)	-7.20 (25.7)	0.24	9.53 (4.95)	10.0 (3.12)	0.08 (39.1)	0.79	0.99	0.97
PLP, nmol/L	26.7 (34.2)	30.0 (10.9)	-19.4 (49.4)	0.58	18.3 (19.0)	20.3 (21.4)	-16.4 (45.5)	0.42	0.73	0.22
Riboflavin, nmol/L	17.4 (7.60)	17.8 (11.4)	15.8 (27.9)	0.10	25.4 (25.0)	21.4 (21.3)	-5.23 (66.7)	0.24	1.35	0.35
Neopterin, nmol/L	29.1 (10.8)	23.9 (12.6)	-4.00 (46.5)	1.00	29.5 (20.5)	34.8 (20.8)	1.41 (38.9)	0.35	0.94	0.66

Median and interquartile range (IQR) before and after ECT, percentage change and p-value from paired Wilcoxon signed rank test are shown for patients with and without diagnoses of somatic disease. The last two columns contain back transformed estimates (exp(estimtate)) and p values from comparison of baseline values of the two groups using linear regression adjusted for sex, age, smoking status and baseline serum creatinine. The estimate of the linear regression describes the somatic disease group's concentration as percentage of the no somatic disease group. Abbreviations: Trp, tryptophan; Kyn, kynurenine; KA, kynurenic acid; HK, 3-hydroxykynurenine; XA, xanthurenic acid; AA, anthranilic acid; HAA, 3-Hydroxyanthranilic acid; QA, quinolinic acid; Pic, picolinic acid; KTR, kynurenine-tryptophan-ratio; PLP, pyridoxal 5'phosphate.

Study IV

IV

pharmaceuticals

MDPI

Review

Changes in Tryptophan-Kynurenine Metabolism in Patients with Depression Undergoing ECT—A Systematic Review

Tore Ivar Malmei Aarsland [1,*], Johanne Telnes Instanes [1], Maj-Britt Rocio Posserud [2,3], Arve Ulvik [4], Ute Kessler [2,3] and Jan Haavik [1]

[1] Department of Biomedicine, University of Bergen, 5020 Bergen, Norway
[2] Department of Clinical Medicine, University of Bergen, 5020 Bergen, Norway
[3] Division of Psychiatry, Haukeland University Hospital, 5021 Bergen, Norway
[4] Bevital A/S, Laboratoriebygget, 5020 Bergen, Norway
* Correspondence: tore.aarsland@uib.no

Abstract: The kynurenine pathway of tryptophan (Trp) metabolism generates multiple biologically active metabolites (kynurenines) that have been implicated in neuropsychiatric disorders. It has been suggested that modulation of kynurenine metabolism could be involved in the therapeutic effect of electroconvulsive therapy (ECT). We performed a systematic review with aims of summarizing changes in Trp and/or kynurenines after ECT and assessing methodological issues. The inclusion criterium was measures of Trp and/or kynurenines before and after ECT. Animal studies and studies using Trp administration or Trp depletion were excluded. Embase, MEDLINE, PsycInfo and PubMed were searched, most recently in July 2022. Outcomes were levels of Trp, kynurenines and ratios before and after ECT. Data on factors affecting Trp metabolism and ECT were collected for interpretation and discussion of the reported changes. We included 17 studies with repeated measures for a total of 386 patients and 27 controls. Synthesis using vote counting based on the direction of effect found no evidence of effect of ECT on any outcome variable. There were considerable variations in design, patient characteristics and reported items. We suggest that future studies should include larger samples, assess important covariates and determine between- and within-subject variability. PROSPERO (CRD42020187003).

Keywords: electroconvulsive therapy; depression; tryptophan; kynurenine; quinolinic acid; inflammation; kidney function; stress; age; comorbidity

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1. Introduction

An increasing number of cross-sectional studies, intervention studies and meta-analyses suggest that the kynurenine pathway of tryptophan (Trp) metabolism (Figure 1) is involved in the pathophysiology of depression and other psychiatric disorders (reviewed in [1–6]). The kynurenine pathway includes multiple metabolites (collectively known as kynurenines), several of which have important properties related to cellular energy (reviewed in [7,8]), glutamatergic signaling (reviewed in [9]), regulation of immune system activity (reviewed in [10]) and production and scavenging of reactive oxygen species (reviewed in [11]). Correspondingly, the kynurenine pathway has been implicated in a wide range of somatic and psychiatric conditions (reviewed in [2,12–14]).

Two main hypotheses link the Trp-kynurenine metabolism to depression (reviewed in [15]). The original hypothesis focuses on brain serotonin production, which can be limited by low levels of Trp, its essential precursor [16]. The kynurenine pathway is the main metabolic pathway for Trp [17] and is even more active under inflammatory conditions [8], which has been associated with depression [18–21]. Pathway activation is often assessed by measuring the relative concentrations of Trp and kynurenine (Kyn), the first stable metabolite of the pathway, i.e., the kynurenine-tryptophan ratio (KTR),

together with levels of inflammatory markers. Depression has been hypothesized to be related to suboptimal serotonin signaling [15], and low availability of Trp for serotonin synthesis could potentially contribute to depression. Deficiency of Trp, for instance through activation of the kynurenine pathway, has therefore been investigated as a potential cause for reduced serotonin production in depression [22].

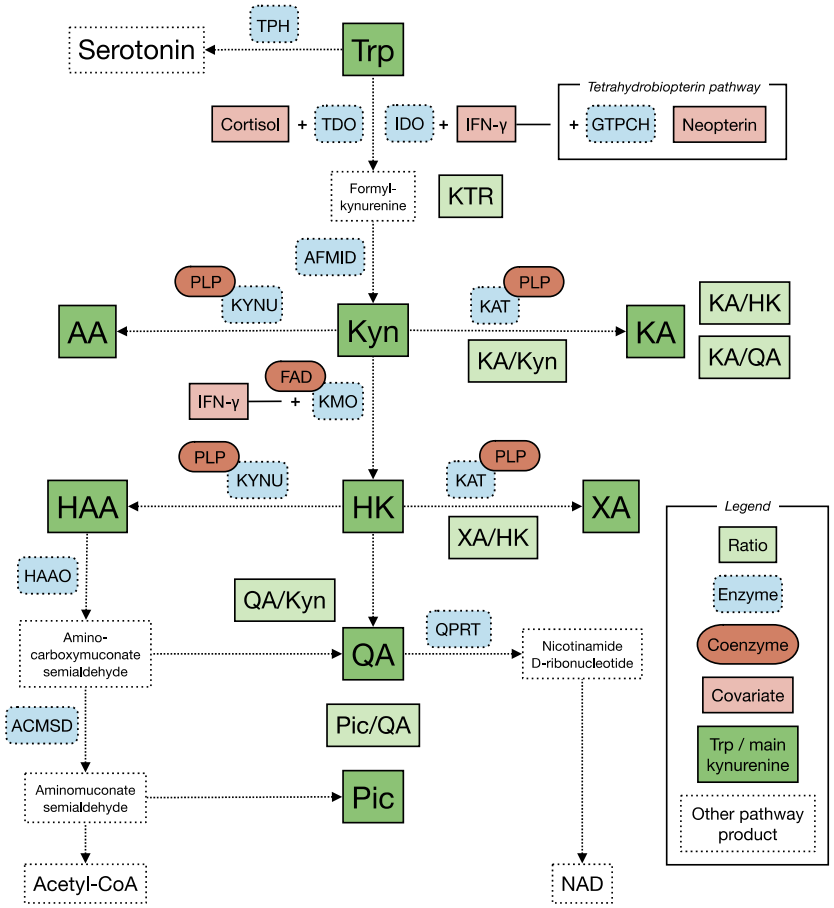

Figure 1. The kynurenine pathway of Trp metabolism. Abbreviations: Acetyl-CoA, acetyl coenzyme A; ACMSD, aminocarboxymuconate semialdehyde decarboxylase; AA, anthranilic acid; AFMID, arylformamidase; FAD, flavin adenine dinucleotide; CAA, competing amino acid; GTPCH, GTP cyclohydrolase; HAA, 3-hydroxyanthranilic acid; HAAO, 3-hydroxyanthranilate 3,4-dioxygenase; HK, 3-hydroxykynurenine; IDO, indoleamine 2,3-dioxygenase; KA, kynurenic acid; KAT, kynurenine aminotransferase; KMO, kynurenine monooxygenase; KTR, kynurenine-tryptophan ratio; Kyn, kynurenine; KYNU, kynureninase; LAT1, L-type amino acid transporter; NEFA, non-esterified fatty acid; NAD, nicotinamide adenine dinucleotide; Pic, picolinic acid; PLP, pyridoxal 5′-phosphate;QA, quinolinic acid; QPRT, quinolinate phosphoribosyltransferase; TDO, tryptophan 2,3-dioxygenase; IFN-γ, interferon gamma; Trp, tryptophan; TPH, tryptophan hydroxylase; XA, xanthurenic acid.

An alternative hypothesis suggests that alterations in concentrations of kynurenines themselves could play a role in depression [22–25]. Here, the most focus has been placed on the pathway's neurotoxic potential and on three key pathway metabolites: kynurenic

acid (KA), 3-hydroxykynurenine (HK) and quinolinic acid (QA). KA is a pathway branch product and an antagonist of the N-methyl-D-aspartate receptor (NMDAr) [26] that can inhibit presynaptic glutamate release and disrupt excitatory synaptic function [27]. QA exerts the opposite effect as an NMDAr agonist [26]. It is increased under inflammatory conditions and may cause neurotoxicity through multiple mechanisms [28]. Similarly, HK has been shown to have a neurotoxic potential, mainly as a free radical generator [29]. Thus, QA and HK are generally considered neurotoxic, while KA is considered neuroprotective [23]. The balance between the neuroprotective and neurotoxic effects of kynurenine pathway metabolites has been considered in many studies of depression, by analyzing levels of single metabolites and ratios such as KA to QA (KA/QA) and KA/HK. Several meta-analyses have shown that patients with major depressive disorder and bipolar depression have reduced neuroprotection compared to non-depressed individuals, with comparatively lower levels of KA and lower KA/HK and KA/QA in the blood [1,4,30,31]. Therapeutic interventions have been postulated to normalize these metabolite levels and restore a balance between their neuroactive effects [32].

Electroconvulsive therapy (ECT) is an effective treatment option for severe or treatment resistant depressive episodes, with a relatively rapid onset of effect compared to pharmacological therapy. The exact therapeutic mechanisms of action are still unknown, and several studies have been performed to investigate the possible role of Trp and the kynurenine pathway, including measures related to Trp availability and kynurenine pathway activation, and the balance between neuroactive metabolites. While some studies have reported an improved neuroprotective balance after ECT [33,34], other studies have not been able to replicate these findings [35–37]. The reasons for this inconsistency are unclear. There are multiple methodological challenges when analyzing changes in Trp metabolism in relation to ECT. These include potential intermediary or confounding effects from a wide range of factors such as inflammation, diet, medication and somatic disease [14,38,39].

In this systematic review, we wanted to summarize available results from studies on ECT and Trp-kynurenine metabolism and to use this as context for a discussion of methodology and the way forward. The aims of this review were to:

(1) summarize the findings of studies on changes in concentrations of Trp and kynurenines after ECT;
(2) review important factors that could potentially affect the analyses of these metabolites in relation to therapeutic outcome;
(3) consider the clinical role of measures of Trp and kynurenines;
(4) propose how future studies should be designed to meet methodological issues and clarify the role of Trp metabolism in ECT.

2. Methods

2.1. Literature Search and Study Selection

2.1.1. Protocol

This systematic review was conducted following the guidelines presented in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [40] and in Synthesis Without Meta-Analysis (SWiM) [41]. A review protocol was submitted to the international prospective register of systematic reviews (PROSPERO) (https://www.crd.york.ac.uk/prospero/) accessed on 5 May 2020, and published on 5 July 2020 (registration number CRD42020187003).

2.1.2. Eligibility Criteria

The inclusion criterium for this review was measurements of Trp and/or kynurenines before and after ECT or description of change in these markers after ECT. There were no restrictions on participant characteristics, report type or language integrated in the search, except for in PubMed where animal studies were excluded. Thus, the search syntax was structured with two parts: (1) terms related to Trp and the kynurenine pathway, and (2) terms related to ECT. In the subsequent screening, the following reports were excluded:

animal studies, letters, conference abstracts, case reports, commentaries or reviews and reports of studies using Trp administration or Trp depletion, unless the study also included a patient group that received ECT alone or with placebo.

2.1.3. Information Sources

The systematic literature search was developed by TIMA, UK and JTI. It was conducted by TIMA on 3 June 2020, in four databases: (1) Embase 1974 to 2 June 2020, (2) Ovid MEDLINE and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily 1946 to 2 June 2020, (3) APA PsycInfo 1806 to May Week 4 2020 and (4) PubMed (up to 3 June 2020). The search used free text and index terms for Trp-kynurenine metabolism and ECT in titles and abstracts. See Supplementary document for full search syntax. An additional systematic literature search was conducted by TIMA on 15 June 2022, in (1) Embase 1974 to 19 July 2022, (2) Ovid MEDLINE and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily 1946 to 19 July 2022, (3) APA PsycInfo 1806 to July Week 2 2022 and (4) PubMed. Here, the searches were limited to years 2020–2022 for the first three databases and 4 June 2020–20 July 2022 for PubMed. In addition, reference lists in included studies were scanned for relevant studies potentially not found through the systematic literature search.

2.1.4. Study Selection

All records collected from the original systematic search were screened by UK and TIMA independently, based on title and abstract. Records collected in the additional search were screened by TIMA. Studies that fulfilled one or more exclusion criteria were excluded. For remaining studies, the full text was retrieved. The full text was also retrieved for records where title and abstract did not provide enough information to determine eligibility. Full-text reports that met the inclusion criterium, and none of the exclusion criteria, were considered eligible and included in the review. Disagreement regarding study eligibility was resolved through discussion (UK, TIMA).

2.2. Data Collection

The data collection was performed by TIMA. Each study's aims, inclusion and exclusion criteria, outcome variables, analyses methods, main results, discussion points and conclusions were recorded for the purpose of study presentation.

For the first review aim (changes in concentrations of Trp and kynurenines after ECT), the following data were collected as primary outcomes: measures of Trp, Kyn, KA, anthranilic acid (AA), HK, xanthurenic acid (XA), 3-hydroxyanthranilic acid (HAA), picolinic acid (Pic) and QA, and of metabolite ratios KTR, KA/Kyn, KA/HK, KA/QA, XA/HK, PA/QA and QA/Kyn. These could be concentrations at any timepoint before, during or after ECT, or measures of change from before ECT to after ECT.

To address the third aim (assessment of the clinical potential of Trp and kynurenine measures), concentrations of these markers were also collected as primary outcomes for control groups at baseline and follow up when available. Associated summary statistics and *p*-values from analyses of change were also collected. For three studies [33,34,36], concentrations of the primary outcome variables were not present in the report. For two of these studies [34,36], these data were provided by the authors after written request. For the third study, the authors were contacted but could not provide the concentrations [33].

To meet the second aim of this study (effect modifiers and confounders), a wide range of pre-selected data items that could be relevant for baseline levels and analyzes of changes in Trp and kynurenines after ECT were extracted from each of the included studies. Such factors include known determinants of blood levels of Trp and kynurenines, predictors of treatment outcome and potential confounders affecting both aspects of the treatment and the outcome measure. This aspect of the review substituted the "study risk of bias assessment" described in the PRISMA 2020 checklist, with the purpose of providing points for discussion rather than assigning weights to each study in the context of a

meta-analysis. Thus, from each included study we extracted data on factors related to Trp-kynurenine metabolism (inflammation, age, kidney function, BMI, sex, nutrition, fasting, B vitamin status, stress, alcohol consumption, smoking, non-esterified fatty acids (NEFAs), large neutral amino acids/competing amino acids (CAAs), glucose, medication and liver function), patient characteristics (depression characteristics, medication and comorbidity), and intervention and study design (clinical measures, ECT details, anesthesia, sample timepoints, and evaluation of treatment response or remission).

2.3. Synthesis and Presentation

2.3.1. Grouping

The included studies were categorized first by treatment design (series or single ECT), secondly by primary outcome (kynurenines or Trp) and thirdly by year of publication. This grouping was used for all tables and figures.

2.3.2. Effect Measures

When available, unadjusted mean or median concentrations before and after ECT were used to calculate percentage change ($100 \times ([post]-[pre])/[pre]$) for each primary outcome variable for each study. This was also performed for concentration changes in control groups with repeated measures. To enable direct comparison, all concentrations were presented as μmol/L and nmol/L.

2.3.3. Synthesis

Vote counting based on the direction of effect is a method that can be used to check if there is any evidence of an effect [42]. This was performed using the Exact Binomial Test in RStudio (binom.test) [43]. For primary outcomes reported in five or more studies (the minimal number of outcomes necessary for finding a p-value below 0.05), the number of studies reporting increased concentration was divided by the total number of studies reporting this outcome variable. The test null hypothesis was that two possibilities of increase and decrease were equally likely.

2.3.4. Data Presentation Methods

All levels of Trp and kynurenine pathway metabolites and ratios before and after ECT were collected in a table. The calculated percentage change in these markers after ECT for patients and controls from all included studies were gathered in a table and color coded by direction and the reported statistical significance. Baseline concentrations of the collected primary outcome variables were collected in a figure for comparison of studies in relation to the third review aim.

3. Results

3.1. Databases, Search Structure and Study Selection

The first systematic search identified 657 records (Figure 2). Of these, 182 were duplicates, and a total of 474 records were screened. After exclusion of 439 records based on the information available in title and abstract, full-text reports were sought for 35 records. Four of these could not be retrieved [44–47]. Fifteen more were excluded: four articles were letters, commentaries or reviews [48–51]; eight publications were reports of original studies, but did not have post treatment measures, did not use ECT, used treatment that involved Trp administration or Trp depletion, or did not measure Trp or kynurenines [52–59]; three articles were reports of eligible studies (same as D'Elia 1977b and Olajossy 2017) but did not provide additional information relevant to this review [60–62].

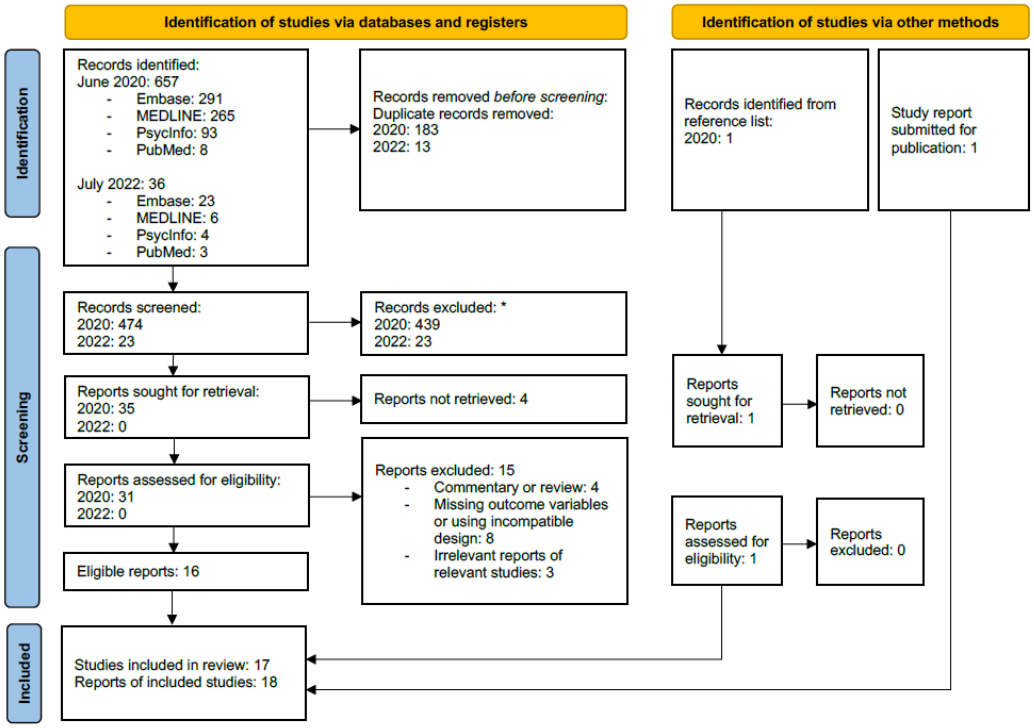

Figure 2. PRISMA 2020 flow diagram for new systematic reviews, adapted from Page et al. (2020) [40] under the terms of the Creative Commons Attribution License. * No automation tools were used.

The second systematic search from July 2022 provided 36 records (Figure 2). Thirteen duplicates were removed, including two records that were present in the original search [37,56]. The remaining 23 records were screened and excluded based on title and abstract. Thus, no studies or reports were included from this search.

Outside of the systematic searches, one record was found from scanning reference lists from other included reports and was assessed for eligibility [63]. Additionally, a study report in submission was also eligible for inclusion [64].

3.2. Included Studies

Nineteen reports of seventeen studies were included [33–37,63–76]. Previously unpublished follow-up measures for 12 controls in Aarsland 2019 were also included. Together, these studies contained repeated measures or analyses of change in Trp or kynurenines for 386 patients with depression treated with ECT and 27 controls. The studies are presented in detail in Figure 3, and Supplementary Table S1 shows all collected biomarker concentrations (primary outcome variables).

3.3. Baseline Concentrations and Changes in Levels of Tryptophan, Kynurenines and Ratios after ECT

Baseline concentrations of Trp and kynurenines for patients and controls are presented in Figure 4.

Percentage change after ECT of Trp, kynurenines and kynurenine pathway ratios are shown in Figure 5. Free Trp, total Trp, Kyn, KA and KTR were available in an adequate

number of studies to utilize vote counting based on effect direction (exact binomial test, binom.test).

(A)

Author (year)	Aim	Study design	Participants	Inclusion and exclusion criteria	ECT procedure and sampling time	Biochemical and clinical outcomes	Analysis method	Main relevant results	Discussion and conclusions
Guloksuz (2015) [33]	To investigate wether ECT influences the kynurenine pathway, whether such changes are related to depression scores, and whether baseline kynurenine pathway metabolite levels predict response to ECT	Serum samples before, during and after a series of ECT	23 patients with unipolar or bipolar depression	- Inclusion: no significant clinical improvement after at least two trials with antidepressants from different pharmacologic classes - Exclusion: age <18 or >65, illiteracy, major medical or psychiatric conditions	- Two ECT session per week until remission or no further improvement - Multiple samples: Before first ECT, then before every second ECT, and every second week for six weeks after last ECT	- Serum: Trp, Kyn, KA, HK, HAA, 3-HIAA, KTR, KA/Kyn, KA/HK, 3-HIAA/Kyn - Clinical: HDRS, BDI	Multilevel linear regression for analysis of change in metabolites. Adjusted for a priori confounders age and sex (n = 19). Significance threshold 0.05	- Increased KA, KTR, KA/Kyn and KA/HK over time during the study period - Baseline concentrations of Trp and kynurenines did not predict time to ECT response	- ECT influences the kynurenine pathway with a shift towards neuroprotection correlating with antidepressant effect of ECT - Small sample size - Antidepressants, anesthetics and muscle relaxants might confound the results
Schwieler (2016) [34]	To analyze cytokines and kynurenines in treatment-resistant patients with major depressive disorder and healthy controls	Plasma samples before and after a series of three ECT sessions	- 19 patients with major depressive disorder - 14 healthy male controls - 22 healthy population-based controls	Exclusion: Age < 18, involuntarily committed, subjected to ECT within 3 month prior to the study or not able to understand the verbal and written information	- Three ECT sessions in the morning - Two samples: Between 8:00 and 11:00 am 1) before first ECT and 2) after three ECT sessions.	- Plasma: KA, tryptophan, kynurenine, QA, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, TNF-α, IFN-γ, GM-CSF - Clinical: MADRS	Wilcoxon matched-paired signed-rank test for comparison of concentrations before and after treatment (n = 15). Significance threshold 0.05	- Reduced Trp, Kyn, QA and QA/KA after three ECT sessions - No change in KA after ECT. - No changes in cytokine levels after ECT	- ECT reduced plasma QA levels and QA/KA - No correlation between change in QA and response as measured with MADRS - Changes in serum might not correspond with changes in CSF - Pharmacological treatment could influence concentrations of cytokines or kynurenines
Allen (2018) [36]	To examine the neurobiology of major depression via cortisol awakening response, plasma cytokine levels and kynurenine pathway metabolites, both in comparison with healthy controls, and if they were altered by ketamin infusion or ECT	Whole blood samples collected before and after a series of ketamine infusion or a series of ECT	- 17 patients with major depressive disorder treated with ketamine - 20 patients with major depressive disorder treated with ECT - 20 healthy controls	- Inclusion: DSM-IV major depressive disorder, at least two adequate trials of antidepressant medications - Exclusion: significant physical illness or more than 10% above ideal body weigh.	- Two ECT sessions per week - Two samples: 1) 8-11 a.m. before first ECT and 2) 4-7 days after last ECT	- Serum: IL-6, IL-8, IL-10, IFN-γ, Trp, Kyn,, KTR, KA, KA/Kyn - Saliva: cortisol - Clinical: HDRS	T-test for comparison of transformed concentrations before and after treatment (n = 15)	No significant changes in tryptophan or kynurenines after ECT	- Patients were not treatment naive, and antidepressant medication may have already normalized some of the underlying biology - The antidepressant effect of the interventions might be related to other mechanisms besides kynurenine metabolism
Aarsland (2019) [35]	To investigate the effect of ECT on serum concentrations of tryptophan and kynurenine pathway metabolites	Serum measures for healthy controls and patients at baseline and for patients after a series of ECT	- 27 patients with moderate to severe unipolar or bipolar depression - 14 healthy controls matched on age and sex	Exclusion: ECT last 12 months, serum creatinine > 120 μmol/L	- Three ECT sessions per week until remission or no further improvement. Max 20 sessions. - Two samples: 1) before and 2) after treatment series	- Serum: Trp, Kyn, KA, HK, XA, AA, HAA, QA, Pic, KTR, KA/Kyn,, KA/HK, KA/QA, XA/HK, Pic / QA, PLP, riboflavin, creatinine, neopterin, cotinine - Clinical: MADRS	Unadjusted paired Wilcoxon signed-rank test. Significance threshold 0.05	Increased HAA, Pic and neopterin after ECT	Possible ECT associated inflammation response with stimulation of kynurenine metabolism

Abbreviations: AA, anthranilic acid; BDI, Beck Depression Inventory; CSF, cerebrospinal fluid; ECT, electroconvulsive therapy; GM-CSF, granulocyte-macrophage colony-stimulating factor; HAA, 3-hydroxyanthranilic acid; HDRS, Hamilton Depression Rating Scale; HK, 3-hydroxykynurenine; IFN, interferon; IL, interleukin; KA, kynurenic acid; KTR, kynurenine-tryptophan-ratio; Kyn, kynurenine; MADRS, Montgomery and Åsberg Depression Rating Scale; Pic, picolinic acid; PLP, pyridoxal 5′-phosphate; QA, quinolinic acid; TNF-α, tumor necrosis factor alpha; Trp, tryptophan; XA, xanthurenic acid.

(B)

Author (year)	Aim	Study design	Participants	Inclusion and exclusion criteria	ECT procedure and sampling time	Biochemical and clinical outcomes	Analysis method	Main relevant results	Discussion and conclusions
Ryan (2020a) [37] and (2020b) [76]	To investigate plasma concentrations of Trp and kynurenine pathway metabolites in medicated patients with depression compared to age- and sex-matched healthy controls and after ECT, taking account of co-variables including ECT modality and heterogenous psychopathology	Plasma samples before and after a series of ECT, including 3- and 6-month follow-up	- 94 patients with unipolar, bipolar and psychotic depression - 57 age- and sex-matched healthy controls	- Inclusion: Age > 18 years, referral to ECT for a major depressive episode (DSM-IV), pre-treatment HAM-D24 ≥ 21 - Exclusion: immune disorder, major neurological illness, substance misuse in the previous 6 months, medically unfit for general anesthesia, dementia or other axis I diagnosis, ECT in the previous 6 months	Two ECT session per week. - Five samples: Between 07:30 and 09:30 am 1) before the first ECT, 2) 1-3 days after the complete treatment series, 3) at 3 month follow-up; 4) at 6 month follow-up, 5) and at relapse	- Plasma: Trp, Kyn, QA, KA, HK, AA, XA, HAA, Pic, KTR, KA/Kyn, QA/Kyn, KA/QA, Pic/QA, CRP, TNF-α, thiamine, thiamine monophosphate, riboflavin, flavin mononucleotide, nicotinamide, N1-methylnicotinamide, PLP, pyridoxic acid, pyridoxal - Blood RNA sample: TNF-α mRNA - Clinical: HAM-D24	Unspecified linear model analysis of change in log10-transformed metabolite levels after ECT. Wilcoxon Signed Rank test for change in ratios after ECT. Adjusted for electrode placement, depression type, presence of psychotis and baseline HAM-D24. Significance threshold set at 0.006 after Bonferroni correction	- No significant change in Trp, kynurenines or ratios after ECT in adjusted or unadjusted analyses - Increase in metabolites associated with improved mood and response to ECT in subgroups - Remission associated with increased HK and HAA after ECT - Multiple metabolites increased in a subset of patients at follow-up compared to pre-ECT	- Although there were no significant changes in metabolites or ratios after ECT in the patient group as a whole, the combined results indicate that ECT mobilizes the kynurenine pathway - Results from analyses of TNF-α are consistent with a ECT-driven TNF-α response that could contribute to mobilization of the kynurenine metabolism
Aarsland (2022) [64]	To investigate the effect of ECT on serum concentrations of tryptophan and kynurenine pathway metabolites and evaluate the role of remission status, inflammation and somatic disease	Serum measures before and after a series of ECT	48 patients with severe unipolar depression	- Inclusion: Age > 55 years. - Exclusion: schizoaffective disorder, bipolar disorder, major neurologic illness.	- Two ECT sessions per week until remission or no further improvement. - Two samples: 1) before and 2) after treatment series	- Serum: Trp, Kyn, KA, HK, XA, AA, HAA, QA, Pic, KTR, KA/HK, KA/QA, PLP, riboflavin, neopterin, cotinine - Clinical: MADRS	Unadjusted paired Wilcoxon signed-rank test. Significance threshold 0.05	- Reduced KA/HK after ECT - Increased HK and KTR in patients with increased neopterin after ECT, and reduced QA and KTR in patients with reduced neopterin after ECT.	- No convincing signs of normalization of kynurenines after ECT - Pronounced covariation between neopterin and kynurenines
Olajossy (2017) [70]	To compare serum concentrations of KA in patients with recurrent depressive disorder, depression in bipolar disorder and schizoaffective disorder before ECT and after the first, sixth, and twelfth ECT	Serum samples before ECT and after the first, sixth, and twelfth ECT	- 32 patients with recurrent depressive disorder - 11 patients with depression in bipolar disorder - 7 patients with schizoaffective disorder - 48 age-matched healthy controls	No information	- Two ECT sessions per week for a total of 12 sessions. - Four samples: 1) Before the first ECT and thirty minutes after the 2) first, 3) sixth, and 4) twelveth ECT	- Serum: KA - Clinical: MADRS, GAF	Friedman's ANOVA and Kendall's coefficient of concordance of investigation of change in KA after first, sixth, and twelfth ECT	The serum level of KA after ECT was not significantly different from the baseline level for any of the patient groups	KA in patients were lower than in healthy controls at baseline, but did not change significantly during ECT

Abbreviations: ANOVA, analysis of variance; AA, anthranilic acid; BDI, Beck Depression Inventory; CSF, cerebrospinal fluid; ECT, electroconvulsive therapy; GAF, Global Assessment of Functioning; HAA, 3-hydroxyanthranilic acid; HAM-D24, Hamilton Depression Rating Scale; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine-tryptophan-ratio; Kyn, kynurenine; MADRS, Montgomery and Åsberg Depression Rating Scale; Pic, picolinic acid; PLP, pyridoxal 5′-phosphate; QA, quinolinic acid; TNF-α, tumor necrosis factor alpha; Trp, tryptophan; XA, xanthurenic acid.

Figure 3. *Cont.*

(C)

Author (year)	Aim	Study design	Participants	Inclusion and exclusion criteria	ECT procedure and sampling time points	Biochemical and clinical outcomes	Analysis method	Main relevant results	Discussion and conclusion
Coppen (1973) [63]	To investigate changes in total and free plasma-tryptophan in female patients before and after recovery from depression	Plasma samples before and after ECT (clinical recovery)	- 22 patients recovered from depression, 6 of which were treated with ECT - 24 treatment naive depressed patients - 26 healthy controls	No information	Two samples: At a standardized time 1) before and 2) after ECT	Plasma: Total Trp, Free Trp, Free Trp as % of total Trp	Unspecified comparison of levels before and after ECT	Increase in free Trp, and in free Trp as percent of total Trp after ECT	- Patients were medication free, had a full hospital diet and were tested at a standardized time after overnight fasting - Plasma-free-fatty-acids were not measured but could play a role for levels of free Trp
Abrams (1976) [65]	To investigate the effect of ECT on 5-HIAA, HVA and tryptophan in CSF	CSF measures before and after a series of ECT	6 patients with endogenous depression	Inclusion: drug-free for at least one week	Two samples: 1) before first ECT and 2) 4 or 24 hours after last ECT	- CSF: 5-HIAA, HVA, Trp - Clinical: DRS	Unspecified comparison of levels before and after ECT	No significant change in CSF Trp levels after ECT	- Low sample size - ECT did not influence CSF Trp levels
D'Elia (1977a) [66] and (1977b) [78]	To compare the effect of ECT + tryptophan with that of ECT + placebo	Serum samples before, during and after a series of ECT + Trp or ECT + placebo	24 patients with unipolar or bipolar depression	- Inclusion: pervasive depression as central symptom, endogenous etiology; severity that require ECT - Exclusion: age > 65 years, somatic disease that could be related to the depressive period, pregnancy, ECT last three months	Multiple samples: 1-2 days before first ECT, then right before each ECT. Always between 9 and 10 am.	- Serum: Total Trp - Clinical: CODS	Student's t-test for comparison of levels before and during ECT	No significant change in total Trp during ECT + placebo	Total Trp levels before and during ECT does not seem to be of pathogenic or therapeutic importance or of value in predicting patients as responders to Trp treatment combined with ECT or to ECT alone
Kirkegaard (1978) [68]	To evaluate the effect of intravenous administration of Trp in addition to ECT	Plasma and CSF samples before and after a series of ECT + Trp or ECT + placebo	20 patients with endogenous depression, unipolar and bipolar	Inclusion: baseline HDRS > 18 points	- Two ECT sessions per week until remission - Two samples: Between 8.00 and 8.30 am 1) before the first ECT, and 2) the day before the last HRS	- Plasma: total Trp, free Trp, free Trp as % of total, albumin, total protein - CSF: total Trp - Clinical: HDRS	Paired Wilcoxon tests for comparison of concentrations before and after treatment (n = 10). Significance threshold 0.05	Reduced total Trp and increased free Trp as % of total in plasma after ECT + placebo	The finding of 15% decrease in Trp in the placebo group was unexpected, and could not be explained by change in serum protein concentrations
Whalley (1980) [74]	To examine the hypothesis that the anti-depressant action of ECT is associated with changes in total or free plasma tryptophan levels, and to distinguish the acute effects from the chronic effects of ECT	Plasma samples before and after a single ECT and a series of ECT	- 12 unipolar depressed patients - 11 patients undergoing anesthesia and diagnostic cystoscopy	Inclusion: willingness to take part, no ECT in the previous year, and depressive illness with no symptoms suggestive of schizophrenia. At least	Seven samples: Just before anaesthesia and 10 minutes after 1/2) first ECT, 3/4) second ECT and 5/6) last ECT as well as 7) twelve months after the last ECT	- Plasma: free Trp, total Trp - HDRS	Paired Student t-test for comparison of concentrations at baseline with those after first (n = 11), second (n = 11) and last ECT (n = 9) as well as concentrations at 12 weeks after last ECT (n = 7). Significance threshold 0.05	- Reduced total plasma Trp in depressed patients after a single ECT - Reduced total plasma Trp in controls on recovery from anaesthesia	- Large within-patient variability in free plasma Trp, possibly due to adrenocortical activation and increase in circulation free fatty acids - Thiopentone or anesthesia could be the cause of reduced total Trp in both patients and controls - The results did not support the hypothesis that the anti-depressant action of ECT was associated with changes in total or free plasma Trp levels
Hoekstra (2001) [67]	To investigate status of biotetin, neopterin, tryptophan and competing amino acids in severly depressed compared to healthy controls and changes of these markers after ECT	Plasma samples before and after a series of ECT	- 20 patients with major depression (DSM-IV) free of antidepressant medication - 29 healthy controls	- Exclusion: Pregnancy, serious diseases known to influence biopterin metabolism	- Twice a week until remission or no further improvement - Two samples: 1) in the morning before first ECT, and 2) in the morning after the last ECT	- Plasma: biopterin, neopterin, phenylalanine, tyrosine, tryptophan, isoleucine, leucine, valine, Trp ratio - Clinical: HRS-D	Student's t-test for comparison of concentrations before and after treatment. Significance threshold 0.05	- No significant change in total Trp after ECT - Increased Trp after ECT in responders	- Antidepressant medication, although discontinued 7 days before ECT, and anaesthetics could influence the plasma measures - Results suggest that ECT influences the availability of Trp

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; CODS, Cronholm-Ottosson Depression Scale; CSF, cerebrospinal fluid; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition; ECT, electroconvulsive therapy; DRS, Depression Rating Scale; HDRS / HRS / HRS-D / HAM-D24, Hamilton Depression Rating Scale; HVA, homovanillic acid; Trp, tryptophan.

(D)

Author (year)	Aim	Study design	Participants	Inclusion and exclusion criteria	ECT procedure and sampling time points	Biochemical and clinical outcomes	Analysis method	Main relevant results	Discussion and conclusion
Stelmasiak (1974) [73]	To study whether plasma fatty acid and free tryptophan increase upon ECT of human subjects	Plasma samples before and after a single ECT session	18 patients with endogenous depression	No information	- Five samples: 1) Before first ECT and 2) 1, 3) 15, 4) 30 and 5) 60 minutes after ECT	- Plasma: NEFAs, free Trp, total Trp	Student paired t-test for comparison of concentrations at each time point versus baseline concentration (n = 18). Significance threshold 0.05	- Increased plasma free Trp at 1 and 15 minutes after a single ECT session - Increased plasma fatty acids at 1 minute after a single ECT session	- ECT rapidly leads to transient increases of both unesterified fatty acid and free Trp in plasma without concurrent changes of total Trp - Subanalyses suggested that the changes were not caused by anaesthetic or muscle relaxant
Sawa (1981) [72]	To investigate plasma concentrations of plasma cyclic-AMP, tryptophan, non-esterified fatty acids and tyrosine in patients with endogenous depression before and after a single ECT	Plasma samples before and after the first ECT session of a full treatment series	Nine patients with endogenous depression (ICD-9)	No information	- A single ECT at 10:00 am - Seven samples: 1) at 9:30 just before anaestesia, 2) at 9:55; 3) at 10:01 just after ECT, 4) at 10:05, 5) at 10:10, 6) at 10:30 and 7) at 11:00	- Plasma: cyclic-AMP, free Trp, total Trp, tyrosine, NEFAs - Clinical: Self-rating depression scale, Hamilton's depression scale, Bojanovsky's depression scale	t-test for comparison of concentrations at each time point versus the baseline concentration. Significance threshold 0.05	- Reduced total Trp compared to baseline at five and ten minutes after ECT - Increased free Trp compared to baseline one minute after ECT	- Changes in plasma markers could be related to intravenous anesthesia by amobarbital - Free Trp was increased after ECT despite a significant reduction in NEFA - The mechanism and metabolic meaning of reduced total Trp after ECT is not clear
Mokhtar (1997) [69]	To investigate the effect of a single ECT on the availability of Trp to the brain in comparison with a control group of control subjects receiving similar premedication	Serum samples before and after a single ECT session	- 10 patients with endogenous depression, unipolar and bipolar. - 4 ear, nose and throat minor surgical subjects	Inclusion: baseline HDRS > 17 points (to warrant referral for ECT)	- A single ECT session between 9 and 10 am. - Five samples: 1) Just before ECT and 2) 15; 3) 30; 4) 45; 5) 60 minutes after ECT	Serum: total Trp, free Trp, competing amino acids (valine, leucine, isoleucine, phenylalanine, tyrosine), NEFAs glucose, cortisol, albumin	One-way analysis of variance (ANOVA) for comparison of concentrations before and after ECT. Significance threshold 0.05	- Reduced total Trp compared to baseline at 45 and 60 minutes after ECT - Reduced total Trp at 15, 30 and 60 min in controls after surgery	- Although total Trp was increased, the ratio of Trp over CAA, marker Trp availability to the brain, was not increased - ECT does not affect parameters known to influence the rate of brain serotonin synthesis
Palmio (2005) [71]	To measure the acute effects of a single administration of ECT on the plasma levels of amino acids in depressed patients	Plasma samples before and after a one ECT session during a treatment series (first ECT for three patients, third to seventh ECT for seven patients)	10 with major depressive disorder (DSM-IV)	No information	- A single ECT session at noon. - Five samples: 1) Before ECT and at 2) 2, 3) 6, 4) 24, og 5) 48 hours after ECT	- Plasma: alanine, amino-n-butyrate, arginine, asparagine, aspartate, citrulline, GABA, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, serine, taurine, threonine, tryptophan, tyrosine, valine - Clinical: MADRS	Paired samples t-test for change between the various timepoints and baseline. Significance threshold 0.05	Increased plasma total Trp compared to baseline at two, six, and twenty-four hours after single ECT sessions	- Samples were collected after different ECT sessions for each patient in the treatment series - Single ECT sessions were associated with acute changes in tryptophan and several other amino acids

Abbreviations: ANOVA, analysis of variance; CAA, competing amino acid; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition; ECT, electroconvulsive therapy; GABA, gamma-aminobutyric acid; HDRS / HRS / HRS-D / HAM-D24, Hamilton Depression Rating Scale; ICD-9, International Classification of Diseases Ninth Revision; NEFA, non-esterified fatty acid; Trp, tryptophan.

Figure 3. Summary of included studies: Studies of tryptophan and kynurenines after a series of ECT. (**A**,**B**) Summary of included studies: Studies of tryptophan and kynurenines after a series of ECT. (**C**) Summary of included studies : Studies of tryptophan after a series of ECT. (**D**) Summary of in-cluded studies : Studies of tryptophan after a single ECT.

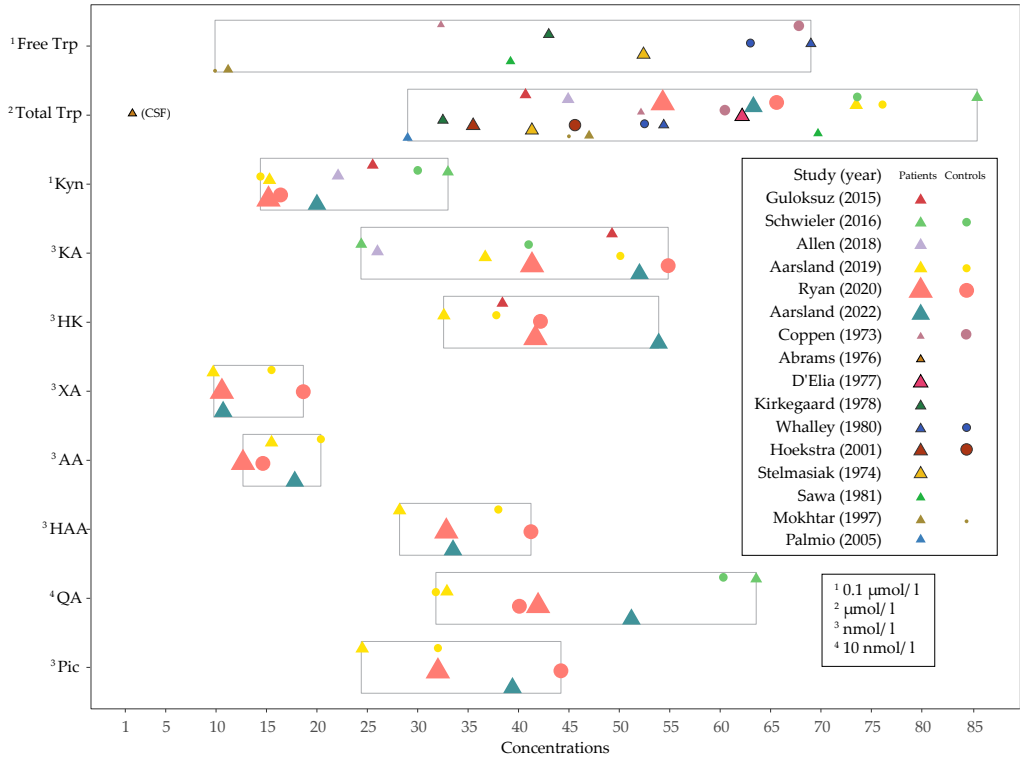

Figure 4. Baseline concentrations of Trp and kynurenines in patients and controls for each study. The size of each symbol represents the number of measured samples. The vertical position of the symbols within each biomarkers box corresponds with the order of the studies in the legend. See Supplementary Table S1 for detailed information on all collected outcome concentrations. References: Guloksuz (2015) [33], Schwieler (2016) [34], Allen (2018) [36], Aarsland (2019) [35], Ryan (2020) [37], Aarsland (2022) [64], Coppen (1973) [63], Abrams (1976) [65], D'Elia (1977) [66], Kirkegaard (1978) [68], Whalley (1980) [74], Hoekstra (2001) [67], Stelmasiak (1974) [73], Sawa (1981) [72], Mokhtar (1997) [69], Palmio (2005) [71].

Free Trp was measured before and after ECT in seven studies. Setting statistical significance aside, five out of seven studies reported increased concentrations of free Trp after ECT. Vote counting based on these effect directions did not reject the null hypothesis that increase and decrease in free Trp after ECT were equally likely (71.4% cases of increase (95% confidence interval (95%CI): 29.1% to 96.3%), $p = 0.45$). For total Trp, seven studies found increased concentration while ten studies found decreased concentration after ECT, and as with free Trp, the null hypothesis was not rejected (35.3% cases of increase (95%CI: 18.4% to 67.1%), $p = 0.41$). Seven studies investigated concentrations of one or more kynurenines before and after a series of ECT. Kyn was measured in six of these studies, four of which found increased concentrations after ECT (66.7% cases of increase (95%CI: 22.3 to 95.7), $p = 0.69$). KA was the only metabolite measured in all seven studies, one of which investigated change in three patient groups separately. It was increased in five out of nine analyses (55.6% cases of increase (95%CI: 21.2% to 86.3%), $p = 1$). Finally, KTR increased after ECT in three studies and decreased in three others (50% cases of increase (95%CI: 11.8% to 88.2%), $p = 1.00$). Thus, there was no overall evidence of an effect of ECT on levels of Trp, Kyn, KA or KTR.

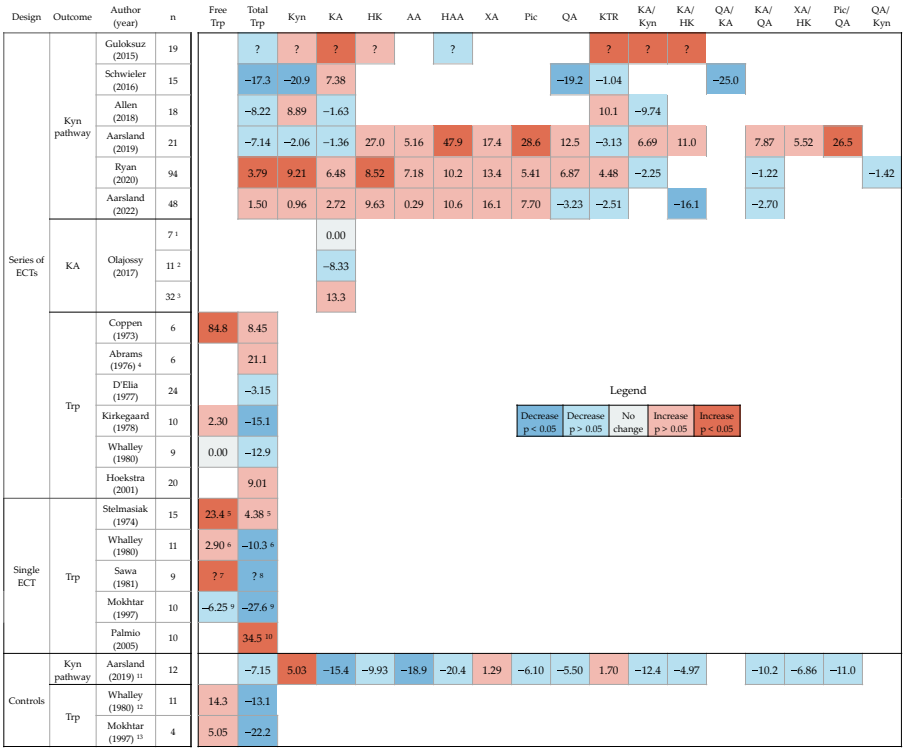

Figure 5. Percentage change in tryptophan, kynurenines and ratios after ECT. Percentage change was calculated based on concentrations before and after ECT collected from the included studies, except for Ryan (2020) [37] and Aarsland (2022) [64], where changes in percentage were collected from the reports. See Supplementary Table S1 for a detailed overview of the collected outcomes from each of the reviewed studies. The question marks indicate that the size of change was unknown due to missing data on concentrations. Comments on participant diagnosis, sample type, and sample timing: 1 schizoaffective disorder, 2 depression in bipolar disorder, 3 recurrent depressive disorder; 4 cerebrospinal fluid samples; samples collected 5 15 min after ECT, 6 10 min after first ECT, 7 1 min after ECT, 8 5 min after ECT, 9 60 min after ECT, 10 2 h after ECT, 11 with eight weeks between, 12 after recovery from anesthesia, and 13 15 min after start of surgery. Abbreviations: AA, anthranilic acid; ECT, electroconvulsive therapy; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine-tryptophan-ratio; Kyn, kynurenine; Pic, picolinic acid; QA, quinolinic acid; Trp, tryptophan; XA, xanthurenic acid. References: Guloksuz (2015) [33], Schwieler (2016) [34], Allen (2018) [36], Aarsland (2019) [35], Ryan (2020) [37], Aarsland (2022) [64], Olajossy (2017) [70], Coppen (1973) [63], Abrams (1976) [65], D'Elia (1977) [66], Kirkegaard (1978) [68], Whalley (1980) [74], Hoekstra (2001) [67], Stelmasiak (1974) [73], Sawa (1981) [72], Mokhtar (1997) [69], Palmio (2005) [71].

The three studies that included the largest panel of kynurenines all found trends of increase in HK, AA, HAA, XA and Pic. A fourth study also found increased HK and reduced HAA after ECT. However, neither of these kynurenines, nor QA or any of the pathway ratios, were reported in a sufficient number of studies to perform a vote counting.

Three studies had repeated measures from controls (Figure 5). In Whalley et al., eleven patients undergoing cystoscopy served as controls and had significant reduction in total Trp after anesthesia [74]. In Mokhtar et al., four anesthesia controls had significant reduction in total Trp 15 min after start of surgery [69]. In Aarsland et al., healthy controls (n = 12),

with no intervention, had significant reduction in Kyn, KA and AA in follow-up samples collected eight weeks after baseline [35].

3.4. Factors That Can Affect Analyses of Tryptophan and Kynurenines

Figure 6 (simplified version, see Section 4.2.2) and Supplementary Table S2 (detailed version) show a summary of factors known to be associated with either Trp-kynurenine metabolism, ECT response, or both and that were extracted from the included studies. The tables were limited to include factors reported in at least one of the reviewed studies. Declaration and investigations of these factors as possible mediators or confounders differed widely, with most studies only considering a few.

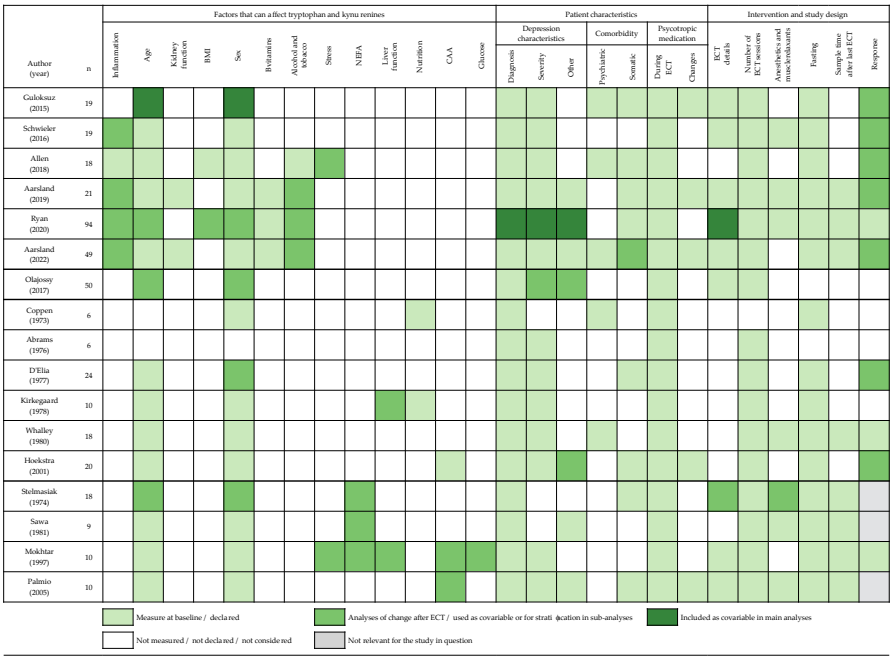

Figure 6. Simplified overview of the review studies' declaration and handling of factors that can affect analyses of tryptophan and kynurenines in the context of ECT. The table is limited to factors that have been included in at least one of the reviewed studies. See Supplementary Table S2 for a detailed version with more information about the reported data for each study. Abbreviations: BMI, body mass index; CAA, competing amino acid; ECT, electroconvulsive therapy; NEFA, non-esterified fatty acid. References: Guloksuz (2015) [33], Schwieler (2016) [34], Allen (2018) [36], Aarsland (2019) [35], Ryan (2020) [37], Aarsland (2022) [64], Olajossy (2017) [70], Coppen (1973) [63], Abrams (1976) [65], D'Elia (1977) [66], Kirkegaard (1978) [68], Whalley (1980) [74], Hoekstra (2001) [67], Stelmasiak (1974) [73], Sawa (1981) [72], Mokhtar (1997) [69], Palmio (2005) [71].

4. Discussion

4.1. Effect of ECT on Tryptophan, Kynurenines and Ratios

In this systematic review, the primary aim was to summarize changes in Trp and kynurenines and their ratios after ECT for patients with depression. We identified 17 studies that were eligible for inclusion. Sixteen of these reported measures of total Trp, seven reported measures of KA, four reported measures of QA, and three studies reported measures of a large panel of kynurenines and ratios. Vote counting based on direction of effect found no evidence for an effect of ECT on the levels of free Trp, total Trp, Kyn, KA or

KTR. Three studies with a large panel of kynurenines all found trends of increase in HK, AA, HAA, XA and Pic, but these were too few for synthesis.

As described in the introduction, Trp availability, which is tightly associated with kynurenine pathway activation, and the balance between neuroactive kynurenines are two main aspects of Trp-kynurenine metabolism in relation to depression, and both were addressed by the reviewed studies. Pathway activation and balance have been suggested to be related, as inflammation induced activation of the pathway potentially causes a larger increase in HK and QA relative to the side-branch metabolite KA [2,77,78].

Considering Trp availability, our synthesis did not provide evidence of an effect of ECT on free Trp or total Trp. Measures of free Trp have been important for investigations of the role of Trp availability for cerebral serotonin production in depression. Increase in free Trp suggests that more Trp is available for metabolism [39], potentially facilitating more serotonin production that could contribute to depression symptom relief. Dependent on the cause of the increase in free Trp, various concomitant changes could be expected in total Trp. If due to strong Trp displacement from albumin, increased utilization of Trp could be reflected as a reduction in total Trp concentration [39]. Conversely, if the increase in free Trp was due to inhibition of Trp metabolizing enzymes, total Trp would also be expected to increase [39]. In the current review, three out of five studies with numerically increased free Trp after ECT also found numerically reduced total Trp after ECT. However, only one of three studies with significant increase in free Trp after ECT found significantly reduced total Trp. None of these studies on free Trp included kynurenines, so kynurenine pathway activation as a possible explanation for decreased Trp was not elucidated there. Instead, these studies investigated the possible role of other factors that could affect the balance of free and total Trp, including free fatty acids [69,72,73], competing amino acids [69,71], albumin concentration [68] and anesthesia [72–74], though without any conclusive results.

Kynurenine pathway activation was assessed in the six studies that included both Trp and kynurenines. As with free and total Trp, the results were inconsistent. The only study that found a significant decrease in total Trp after ECT also found a significant decrease in Kyn and QA, with a stable KTR [34], suggestive of an overall reduction in levels of Trp and kynurenines after ECT. In contrast, the single study that found significant increases in Trp after ECT also found significant increase in Kyn and HK, with a stable KTR [37], indicating a general increase in the availability of Trp. KTR was significantly altered after ECT in only one study (Guloksuz 2015). Here, it was increased, indicating increased pathway activity, with corresponding but non-significant increase in Kyn and decrease in Trp [33]. The most conspicuous pattern of change, however, was the trend of increased levels of HK, AA, HAA, XA and Pic in the three studies investigating a large panel of kynurenines, including the study with the most participants (94 patients with depression and 57 controls) [37]. Here, Trp was increased in two studies [37,64] and reduced in the third [35], and it is unclear if the apparent pathway activation was a consequence of increased Trp availability or, on the contrary, a reason for Trp decrease. As will be discussed further below, this aspect of analyses of change in kynurenine pathway activation could be elucidated through measures of factors affecting pathway enzymes, most importantly inflammatory markers, but potentially also glucocorticoids and vitamin B6 status. Ryan and colleagues found a significant decrease in TNF-α after ECT, suggesting reduced inflammation after ECT [37]. This is often associated with reduced kynurenine pathway activity and, therefore, seemingly inconsistent with the general trend of increase in pathway metabolites in this study. In the study of Schwieler and colleagues, several cytokines were measured, but none of them were changed significantly after ECT [34]. In two other studies, changes in the inflammatory marker neopterin coincided with changes in kynurenines, suggesting a role of altered cellular Th1-immune activation after ECT [35,64].

Markers related to the balance between neuroactive effects of kynurenines were available in seven studies. With a stable concentration of KA, and a significant reduction in QA and QA/KA, Schwieler 2016 pointed to a possible increased neuroprotection after ECT. Similarly, Guloksuz found increased KA, KA/Kyn and KA/QA after ECT, together

with increased KTR. In contrast, two other studies found signs of lowered neuroprotection, with increased HK (and QA in adjusted analyses) in one [37] and reduced KA/HK in the other [64]. The three remaining studies found no significant changes related to pathway balance. From the synthesis, there was no evidence of an effect of ECT on KA. HK and QA, two main neuroactive metabolites suspected to cause neurotoxicity in relation to depression, were not available in a sufficient number of studies to perform a synthesis. Like with Trp, Kyn and KTR, KA and QA changed in both directions and in various degrees, as did various ratios used for estimating the balance between neuroactive effects: KA/Kyn, KA/HK and QA/KA (KA/QA).

The overall lack of consistent results coincides with findings in related biomarker literature, both in studies investigating the mechanisms of ECT (for a general overview of biomarkers for ECT, see [79,80]) and in studies analyzing the effect of other anti-depressant interventions on Trp-kynurenine metabolism (reviewed in [5]). The effect of ECT has been investigated on many other biochemical systems, some of which are tightly linked to kynurenine metabolism. Most importantly, change in the concentration of inflammatory markers after ECT has been the topic of many recent studies (reviewed in [81]). There, the overall results pointed to a short-term increase in inflammation markers IL-1 and IL-6 after ECT and a reduction in TNF-α and IL-6 levels in the long term. Similarly, there were findings of short-term increase in plasma cortisol after ECT, indicating an acute stress response, but also a long-term decrease in cortisol after a full treatment series. The review authors noted, however, that the studies were too few to be conclusive. Due to the activating role of cortisol, and the mutual regulation between kynurenines and inflammation systems, these fields are highly important for the investigations of kynurenine metabolism in relation to ECT. More and larger studies are therefore needed that investigate the relationship between ECT, inflammation and stress responses, including temporal aspects.

The effect of other anti-depressant treatments, including ketamine and selective serotonin reuptake inhibitors (SSRIs), on kynurenine metabolism has also been investigated. Like KA, ketamine is an antagonist of NMDAr and involved in regulation of immune activity [82]. The effects of ketamine on tryptophan metabolites have been investigated in a handful of studies (reviewed in [82]). One study showed increased levels of Kyn, KA and KA/Kyn and reduced levels of IDO and QA/Kyn after a series of ketamine infusions [83]. Another study, that was also included in the current review, compared the effects of ECT and ketamine [36] and found no effect of ketamine on kynurenines when looking at the whole ketamine treatment group. They found a trend, however, towards a decrease in Kyn at 2 h after the first infusion in ketamine responders. Similarly, a third study found increased KA and KA/Kyn in ketamine responders (Zhou 2018). The effects of ketamine on inflammatory markers have also been investigated, with some studies demonstrating decrease in peripheral levels of IL-1β, IL-6 and TNF-α [82]. A few studies provided data on kynurenines in relation to treatment with SSRIs (reviewed in [5]). Halaris and colleagues found reduced HK, QA and KA/QA in 15 patients with depression after 12 weeks of escitalopram treatment [84]. In a metabolomics study, Zhu and colleagues found reduced Kyn/melatonin and HK/melatonin in sertraline responders [85]. Finally, Mackay and colleagues found increased Trp at 6 and 12 weeks of fluoxetine therapy, but no change in kynurenines [86]. The same was found for a group of patients receiving counselling [86]. Like for ketamine, there were indications that SSRI treatment is associated with reduction in levels of inflammatory cytokines, specifically IL-1β and IL-6 [87].

Overall, there is still little solid evidence both of effect of ECT on other biochemical markers and of other anti-depressant treatments on kynurenine pathway metabolism. This general lack of convincing findings, both in relation to the mechanisms of ECT and the role of Trp metabolism in treatment of psychiatric disorders, is important as a context for interpreting the results of the current review. Most importantly, the lack of solid reproduced findings shows that the field is still in an exploratory phase, and that larger studies are probably needed to detect changes in kynurenines in relation to ECT. Moreover, the underlying mechanisms are complex and better understanding of the physiology,

including normal variation, key determinants and other influential factors, are needed to unravel the role of Trp and the kynurenine pathway in this context.

4.2. Effect Modifiers and Mediators

It is apparent that clinical and methodological differences play a role when comparing studies and looking for overarching patterns. Due to large differences in patient characteristics and methods, comparing studies and summarizing findings is challenging. Given the supposed relationship between Trp and kynurenine pathway abnormalities and depression symptom severity, results of studies can vary, not only due to variables affecting Trp metabolism or measures of Trp metabolites, but potentially also due to differences in treatment response. As reported above, we collected information on some central factors (summarized in Figure 6/Supplementary Table S2) that can affect baseline concentrations, metabolite changes after ECT as well as the patients' response to ECT. In the following, we discuss their relevance for Trp-kynurenine metabolism, and the implications for cross sectional comparisons and analyses of changes after ECT.

4.2.1. The Kynurenine Pathway of Tryptophan Metabolism (Figure 1)

Trp is an essential amino acid, i.e., not synthesized in the human body, and is supplied from diet and protein degradation. In blood, about 90 percent of Trp is bound to albumin, and the remaining unbound fraction, free Trp, is available for metabolization [88]. Displacement of Trp from albumin increases the free fraction of Trp in blood, potentially increasing Trp availability for serotonin synthesis in the brain [89]. Trp levels in the central nervous system, however, are dependent on transport across the blood brain barrier (BBB) by L-type amino acid transporter (LAT1) [88]. Total Trp in serum is, therefore, dependent on nutritional supply, the concentration of albumin, the rate of binding to and release from albumin, transport into other tissues and its subsequent metabolization.

The first step of the kynurenine pathway is the conversion of Trp to formyl-kynurenine by one of two enzymes, tryptophan 2,3-dioxygenase (TDO) and indolamine 2,3-dioxygenase 1 (IDO1). TDO is activated by Trp itself and induced by glucocorticoids such as cortisol [89]. IDO1, on the other hand, is induced by pro-inflammatory cytokines, especially interferon gamma (IFN-γ) [90]. Formyl-kynurenine is rapidly converted to Kyn, which can be metabolized to HK by kynurenine monooxygenase (KMO), to KA by kynurenine aminotransferases (KATs) or to AA by kynureninase (KYNU). KYNU is also necessary for the further conversion of HK to 3-hydroxyanthralinic acid (HAA), which can be metabolized to QA or to picolinic acid (Pic). HK can also be converted to xanthurenic acid (XA) by KAT. Two B vitamins are central cofactors in the pathway. Pyridoxal 5′-phosphate (PLP), an active form of vitamin B 6, is cofactor of KAT and KYNU, and therefore necessary for the enzymatic steps leading to KA, AA, XA and HAA. Flavine adenine dinucleotide (FAD), the active form of vitamin B2, is cofactor of KMO and necessary for the conversion of Kyn to HK [91]. The activity of the pathway enzymes affects both upstream and downstream metabolites, as high activity consumes precursors, while low activity can cause precursor accumulation.

While most of Trp metabolism is handled by TDO in the liver, the activity of IDO1 in other tissues can increase dramatically under pro-inflammatory conditions [8]. Like IDO1, KMO is induced by IFN-γ [8]. Inflammation related induction of the pathway is often reflected in a higher KTR and can result in increased levels of HK and QA [2]. Kynurenine pathway metabolites are eliminated from the body mainly by renal excretion [92]. The concentration of kynurenines in the central nervous system is dependent on local metabolism and transport across the blood brain barrier (BBB). Like Trp, Kyn is transported across the BBB by LAT1. The concentration of IDO and TDO is substantially lower in the brain than in other tissues, and most of the local metabolism seems to be based on Kyn [24]. The ability of other kynurenines to cross the BBB is debated, but the primary view is that QA, and especially KA, cross poorly and that their concentration therefore is dependent on local metabolism in glial cells [24]. Like in other tissues, local inflammation in the brain can cause

dramatic increase in IDO activity accompanied by increased production of kynurenine metabolites, both by glial cells and by infiltrating macrophages [8].

There are many factors that determine the metabolism of Trp through the kynurenine pathway and the fate of kynurenine metabolites and, hence, may affect the findings. Figure 6 (Supplementary Table S2 for detailed version) lists some of the most important factors that can affect Trp and the kynurenine pathway metabolism that have been measured, declared, or discussed in at least one of the reviewed studies. In the following, we will present and discuss these factors.

4.2.2. Factors That Can Affect Levels of Tryptophan and Kynurenines

Inflammation

Reduced levels of Trp and increased levels of kynurenines have been demonstrated in a range of clinical conditions involving immune activation, including infection, autoimmune disorders, cancer, neurodegenerative diseases and more (reviewed in [93,94]). Neopterin is an inflammatory marker that, like the kynurenines, is increased in concentration upon IFN-γ stimulation, and it often correlates with KTR under inflammatory conditions [95–98]. Measures of neopterin are therefore useful to clarify whether observed changes in KTR, or kynurenines in general, are related to inflammation.

The influence of inflammation on kynurenine pathway activity is important in the context of ECT, as depression has consistently been shown to be associated with chronic low-grade inflammation (review and meta-analysis: [18], recent original paper on CRP: [20]). Increased concentrations of kynurenines in depressed patients as compared with controls may be confined to patients with elevated levels of inflammatory markers, such as CRP and TNF-α [99]. Moreover, the risk of depression is increased in patients undergoing cytokine treatment, and this association has been linked to activation of the kynurenine pathway [100]. Remission from depressive episodes has been shown to be accompanied by reduced levels of inflammation [101]. Investigations in patients with depression and in animal models of depression also suggest that antidepressant medications have anti-inflammatory effects [102,103]. Furthermore, as discussed above, a meta-analysis suggests that ECT may affect inflammation, with short-term increase in inflammatory markers after a single ECT, and a long-term decrease after a full treatment series [81]. Given the strong relationship between depression, inflammation and kynurenine metabolism, it must be suspected that changes in levels of kynurenines after ECT depends on altered inflammatory status. Inflammation markers should therefore be assessed when investigating change in kynurenines.

Among the original reports included in this review, five reported levels of inflammatory markers, including CRP, TNF-α, IL-6, IL-8, IL-10, IFN-γ and neopterin [34–37,64]. Four studies [33,36,37,67] explicitly stated the presence of infection, immune disorders or inflammatory diseases as an exclusion criterion, presumably to reduce some of the noise potentially introduced by this factor on analyses of kynurenines.

Age

In general, higher age is associated with lower levels of Trp (reviewed in [89]). In the largest study to date, investigating two distinct community-based age groups (age 45–46 years (n = 3723) and age 70–72 years (n = 3329)), higher age was also associated with higher levels of Kyn, AA, KA, HK and neopterin, as well as higher KTR [38]. Increased inflammation with higher age could be part of the explanation for this association [104]. CSF levels of neopterin and kynurenine pathway metabolites have been found to be positively correlated with each other and with age in 49 healthy women [105].

The mean or median age of patients included in the reviewed studies ranged from 40 to 73 years, indicating that the age differences could have contributed to the variability across studies.

Kidney Function

Several studies have shown high levels of kynurenines in individuals with reduced kidney function compared to individuals with normal kidney function [38,106–109]. In

Theofylaktopoulou and colleagues' work, levels above 95% of normal distribution for Kyn, AA, KA, HK, KTR and neopterin were all associated with kidney dysfunction. Positive correlations of KA and QA with creatinine have also been shown [107], consistent with the importance of renal excretion for elimination of these metabolites. However, kidney dysfunction may also be associated with increased immune activation with increased neopterin [110], CRP levels [107] and increased IDO activity [111]. Altered concentrations of kynurenines in the context of kidney dysfunction is therefore probably due to a combination of reduced excretion, increased activities of TDO [106,112], increased inflammation [107] and possibly other mechanisms. It has further been established that kidney disease is a risk factor for depression [113], and kidney disease incidence was associated with depression symptom scores in a recent prospective cohort study [114].

Of the included papers in this review, only two included measures of kidney function [35,64].

Body Mass Index (BMI)

There is an intricate relationship between metabolic regulation and the kynurenine pathway (reviewed in [115,116]). Trp, Kyn, KA, HK, HAA, XA and KTR have all been found to be higher in obese compared to normal-weight individuals [38]. Positive associations between BMI and KTR [117,118] and between BMI and Kyn [118] have also been documented. Furthermore, neopterin was also associated with BMI in 426 clinically defined healthy individuals [119], and IDO gene expression was found to be enhanced in adipose tissue of people with obesity [120]. A recent study found higher BMI in patients with MDD compared with healthy controls, though without association to QA or QA/KA, and the authors suggested that altered kynurenine metabolism in depression could be related to metabolic disturbances [121].

Two of the reviewed studies included data on BMI [36,37].

Sex

In the community-based study of Theofylaktopoulou et al., levels of Trp, Kyn, KA, HAA and XA were higher in men than in women [38]. In another cohort study of 2436 healthy young adults, Trp, Kyn, KA, AA and HAA were also higher in men [122]. There are also indications of differential responses of interventions on kynurenine metabolism in women and men, with women exhibiting greater changes in concentrations after Trp administration or IFN-treatment [25].

In general, the reviewed studies included more females than men, resulting in a stronger representation of females overall (242 vs. 146).

B Vitamins, Tobacco and Alcohol

Low levels of PLP are associated with high concentrations of HK, and low concentrations of KA, AA, HAA and XA [123]. PLP concentration has been found to be reduced in many inflammatory conditions (reviewed in [124]), in smokers [125] and in subjects with high alcohol consumption [122]. Conversely, vitamin B6 supplements have been associated with lower HK concentrations [122]. High levels of nicotinamide (vitamin B3) can also inhibit TDO in a negative feedback mechanism [89]. There is an inverse association between smoking and concentration of several kynurenines [38] as well as KTR [126]. This could be related both to an anti-inflammatory effect of smoking and a reduction in circulating B-vitamin levels due to oxidative stress [125].

B-vitamin concentrations were reported in three studies [35,64,76], smoking in four studies [35–37,64] and alcohol consumption in one study [64].

Other Factors

Glucocorticoids, especially cortisol, are important inducers of TDO, and stress has long been recognized as a potential link between depression and kynurenine metabolism [15]. Both short-term increase and long-term decrease in cortisol have been described after ECT [81], potentially influencing peripheral metabolism through TDO activation. A number of other factors are also suggested to affect the availability of Trp for serotonin production, either through regulating the free fraction of Trp in blood, the transport across BBB or cell membranes or metabolization by TDO (reviewed in [89]). Trp can be displaced from

albumin by non-esterified fatty acids (NEFAs), which could be increased in response to ECT as part of an acute stress response [89]. Some studies have also suggested that impaired liver function with reduced albumin production can cause increased circulating free Trp levels [89]. Dietary supply is essential for Trp levels, and intake of Trp has, for instance, been reported to be reduced in elderly with mild-to-moderate depression compared to healthy elderly controls [127]. Protein intake can, however, alter the ratio between Trp and other large neutral amino acids that compete for LAT1 transport, so called competing amino acids (CAAs). For example, administration of leucine, a CAA with high affinity for LAT1, has been shown to prevent depression-like behavior upon lipopolysaccharide stimulation in mice by blocking Kyn transport across the BBB [128]. Glucose has also been suggested to affect Trp levels through an inhibitory effect on TDO [89]. A range of common medications are furthermore suspected to affect levels of Trp and kynurenines, including anti-inflammatory drugs [129], oral contraceptives [130], salicylate [131], antirheumatic drugs [132] and more [39]. Still, knowledge about the clinical significance in humans of Trp displacement, TDO inhibition, various medications and the competitive action of CAAs for LAT1 transport is limited.

4.2.3. Patient Characteristics

Patient characteristics are related both to expected treatment response and to the impact of the factors discussed above. Patients with depression constitute a highly heterogenous group and vary greatly between studies.

One main division is between major depressive disorder and depression in bipolar disorder, two closely related conditions that nevertheless have important differences, related to clinical characteristics, treatment methods, outcome and probably also etiology [133]. Furthermore, although alterations in kynurenine pathway metabolites have been found in patients with depression in general, many studies have suggested that pathway abnormalities may be more pronounced or relevant for various clinical subgroups. This includes depressed with high baseline concentrations of inflammatory markers [99,134], suicidal ideation [135–138] (reviewed in [138]) and psychotic features [139]. Similarly, comorbidity is also of importance, as kynurenine metabolism is often altered in somatic diseases (review in [94]), such as cancer, kidney disease, inflammatory disease, neurologic disease, diabetes [140], and psychiatric conditions such as schizophrenia [141]. Other relevant clinical characteristics, including depression severity [142], duration of depression [23], melancholy and anhedonia [143,144], cognitive function [145,146], and somatization [147], have all been related to inflammation and/or unbalance in kynurenine metabolism. Moreover, depression characteristics, age and inflammation are important predictors of treatment effect [148]. To address these aspects, stratification based on clinical data or correlation between kynurenines and clinical or biochemical scores were commonly applied in subanalyses in the reviewed studies, though with limited statistical power.

Finally, medication is another important aspect of the study population. Whether patients are medication naïve or on anti-depressant medication could affect the response to ECT [149] and possibly kynurenine metabolism itself, for instance, through TDO inhibition [150]. This topic was central to the methodology and discussion of several of the reviewed studies. In general, most patients received treatment with antidepressants, antipsychotics or mood stabilizers during the study period, with limited statistical strength to draw any conclusions about the role of medication.

4.2.4. Intervention and Study Design

As with patient characteristics, variation in study design and treatment delivery could contribute to differences in results. The method of ECT delivery varies between studies and could affect response [151] and potentially contribute to the variation in observed changes in Trp and its metabolites. The number of ECT sessions is a crucial factor since it is the main difference between two types of study design, single versus treatment series, and since the length of a series typically varies from patient to patient. It could be a factor acting directly

onto the biomarker levels, as well as a proxy for treatment effect. Patients with a delayed treatment response of ECT are likely to receive more sessions. This could potentially lead to larger effects on inflammation and kynurenine metabolism in non-responders.

The medication used in context of ECT, anesthesia and muscle relaxants, could conceivably affect the Trp and kynurenine levels, and this was a topic discussed in several of the reviewed reports. Whalley and colleagues discussed these medications as the potential cause of change in Trp in both patients and controls [74]. Stelmasiak and Curzon concluded, based on the results of sub-analyses, that anesthetics did not play a significant role for changes in free or total Trp [73]. Less commonly discussed was the potential role of fasting, which could be substantial if blood samples are collected with different fasting length, such as in the single ECT design. Given the dietary dependency of Trp and the effect of cortisol on TDO, both Trp and kynurenines could conceivably show diurnal and seasonal variation. Collecting blood in the morning or in the evening, or at different seasons, could potentially yield different concentrations. There could also be an effect of meals received at the clinic or improved nutritional status as a consequence of reduced symptom burden or other treatment (e.g., hospitalization). Moreover, time between last ECT and post-treatment blood sampling is possibly of high importance when analyzing kynurenines after ECT. In the current review, the post-treatment sample time varied substantially both within and between studies that declared this information. Systematic sampling time differences could be an explanation both for baseline differences between patients and controls, and for changes over time, both in patients receiving therapy and for healthy controls. This is relevant, not only for investigations into the short-term effect that ECT by itself exerts on Trp metabolism, but also for analyses of changes related to reduced symptom severity. For instance, follow-up samples could reveal important biomarker alterations, such as the significant changes in a sub-group of patients at 3 months follow-up after ECT in the work of Ryan and colleagues [37].

Treatment response is another important measure in this type of study. Given the hypothesis of association between symptom severity and levels of kynurenines, changes after ECT are expected to be more pronounced in responders or remitters than in non-responders or non-remitters. Stratification by treatment effect, or analyses of correlations between changes in biochemical and clinical measures, could reveal differences in kynurenine changes after ECT that would otherwise be concealed. These strategies were utilized in several of the reviewed studies [34–36,64,67].

4.2.5. Summary of the Role of Factors That Can Influence Analyses of Tryptophan and Kynurenines in the Context of ECT

This review indicates that many factors that are important for analyses of Trp-kynurenine metabolism are often not standardized, measured or reported in studies that investigate changes in these metabolites after ECT. The impact of these factors may vary according to study design. They can have an especially large impact when comparing groups of participants, such as baseline concentrations in patients and healthy controls. Controls are often selected to match patients on age and sex, but inflammation, kidney function, nutritional status, BMI, somatic disease and use of medication can potentially be important effect modifiers. For example, the observed differences in KA between 1100 depressed patients and 642 healthy controls were no longer significant after adjustment for age, sex, education, smoking status, alcohol consumption and chronic diseases [99].

In studies that utilize repeated measures, determinants of Trp and kynurenines should have limited effect on the analyses of change if they are stable throughout the treatment series. However, some important factors, such as inflammation, stress, B vitamin levels and medication, can change during the study period and could affect the outcome measures. Moreover, such changes could be an effect of ECT and, therefore, not readily adjusted for in statistical analyses without introducing bias. Additionally, it is still not clear to what degree the baseline concentrations of Trp or kynurenines affect the potential for change or treatment response. Many of the variables discussed above are potential determinants of

baseline concentrations. As part of the focus on Trp availability as essential for cerebral serotonin production, two of the review studies investigated whether administration of the essential amino acid could be beneficial to the treatment effect of ECT [66,68]. While the use of Trp administration in depression treatment is controversial [116], the underlying question of the importance of baseline concentrations for remission remains unanswered. Some of the reviewed studies investigated the relationship between baseline levels of Trp/kynurenines and variables such as treatment response and pre- and post-treatment inflammatory marker levels and symptom scores [33,34,37,66]. Such analyses are important to shed light on the clinical role of baseline levels, for instance, as predictors of clinical response, but should also take into account the determinants of kynurenines to avoid confounded results.

The heterogeneity of depression and differences between studies in terms of diagnoses and clinical characteristics of included patients are well-known challenges in the search for biomarkers. Diversity in patient characteristics could contribute to differences between studies in baseline measures and changes after treatment, due to both variable weight of the factors discussed above and differences in treatment outcome. Consequently, the generalizability of each study's findings could be limited. Especially for the purpose of evaluating the comparability of studies in the context reviews and meta-analyses, it is important that information is available for the most relevant and influential factors.

4.3. Challenges Regarding the Clinical Use of Tryptophan and Kynurenine Measures

Some of the variables discussed above may reduce the precision of change estimates or even cause misleading results. However, even with sufficient handling of such factors, there are additional challenges regarding the interpretation and application of Trp and kynurenine measures in a clinical setting. These are, especially: (1) insufficient knowledge on normal ranges and variability, (2) uncertainty regarding the value of blood measures as opposed to CSF measures in the context of neuropsychiatric disorders, and (3) difficulties relating to study design and statistical analyses, including a lack of methods to interpret changes in the pathway as an interactive network instead of single markers.

4.3.1. Normal Ranges and Variability

Community studies shows that the normal range of Trp and kynurenine pathway metabolite concentrations are quite wide, with Trp ranging from 41.6 to 98.2 μmol/L, Kyn from 0.94 to 2.86 μmol/L and KA from 20.4–93.2 nmol/L [38,152]. Extreme values are related specially to kidney function, BMI and smoking [38]. Looking at the baseline levels of the studies included in the current review (Figure 3), there was also large variation in baseline concentrations of Trp and kynurenines, both for patients and controls.

Clinically harmful ranges of kynurenines are not well established, and it is not known what a given concentration of kynurenines means for an individual's health. Abnormal levels are usually defined in each individual study, based on comparisons between patients and groups of healthy individuals. However, given the wide normal range and the large spread of concentration means in the reviewed studies, it seems unreliable, at least for small studies, to use control groups as a reference point for determining whether biomarker concentrations in patients are abnormal and in what direction they may change. Instead, population studies and meta-analyses should be used to provide points of reference.

Similarly, there are important challenges related to interpretation of change. In the current review, three included studies found significant changes in controls groups: in two studies samples were collected before and after anesthesia [69,74], and in the third, at baseline and at eight-week follow-up without any intervention [35]. These control groups were small (n = 4, 11 and 12 respectively), but the results suggest that the study design has important weaknesses. While the first two hint to a role of anesthesia and fasting in this type of study design, the changes in the third study were unexpected and the reasons unclear.

Investigations of metabolite levels depend on these concentrations being relatively stable over time, so that any observed changes can be attributed to the intervention rather than normal individual variation. Levels of Trp and kynurenines have been investigated with plasma measures in two cohorts without intervention, one with two samples 1–2 years apart (n = 40), and another with two samples 3.5 years apart (n = 402–545) [152]. Here, intraclass correlation coefficients (ICCs) were used to evaluate how much of the total sample variance was attributable to within-person variance as opposed to between-person variance. A high ICC indicates that the concentration of metabolite is quite stable if measured at two or more time points from the same individual, and the total sample variance is mainly due to concentration differences between individuals. High ICCs are preferable in intervention studies, as large within-person variance makes it difficult to distinguish the intervention effect from the normal individual variations. In the samples taken 3.5 years apart, Kyn, HK, KA, XA, AA and HAA all changed significantly. With ICCs corresponding to a reproducibility of fair-to-good (0.4–0.75), this study indicates that a substantial portion of the total variation was due to within-person variance. To our knowledge, there are no published studies on changes over a shorter period of time, mimicking clinical therapy trials. However, shorter time between samples and larger number of sample sizes both contribute to higher ICC and more reliable estimation of the intervention effect.

Alternatively, control groups that follow the same study structure as the depressed patients, only without the intervention, could provide a reference for normal variation over the timespan of the study [33,69]. This could also be useful for correction for the effect of factors relating to treatment, such as fasting and anesthesia. Such control groups could, for instance, be patients referred to procedures involving anesthesia.

4.3.2. Peripheral and Central Concentrations

Among the studies reviewed here, only two collected CSF samples (Abrams (1976), Kirkegaard (1978)). The problem with using peripheral blood versus cerebrospinal fluid measures and the question of the relevance of blood samples are commonly discussed in studies of biomarkers in relation to neuropsychiatric disorders, including kynurenine metabolism. There is a general lack of studies investigating kynurenines in CSF. More and larger CSF studies could be important to study the biology of psychiatric disorders. However, recent studies point to a high correlation between serum and CSF levels, in healthy individuals, but also in depression [121,153,154] and other conditions such as Alzheimer's disease [155].

4.3.3. Research Questions, Study Design and Methods of Analysis

There were two main categories of studies included in this review: studies that investigated changes after a single ECT and those that investigated changes after a series of ECT. These two designs present two quite different approaches to the topic of Trp metabolism in relation to ECT. The former considers the effect of ECT independent of the anti-depressant effect, while the latter addresses the changes in depression symptoms that follows from the treatment.

For investigating the first mechanism, one or a few ECT sessions with blood measures before and after each session might suffice to investigate changes in concentrations related to the effect of ECT. Here, it is important that patients are matched, and that each patient receive the same numbers of ECT sessions, preferably with the equivalent settings, so that the study exposure is as similar as possible between subjects. Although the results are intrinsically linked to the diagnostic criteria for referral to ECT, the patients' clinical response is not the focus. This design is especially vulnerable, however, to intervention related effects such as anesthesia and fasting. To shed light on the second mechanism, ECT serves as a convenient setting that can bring about dramatic changes in depression symptom levels. Here, the clinical response is an essential variable and should preferably be closely monitored. However, observed covariation, or change in response groups, is not easily separated from the direct effect of ECT considered using the first approach. Therefore, the

two designs should preferably be combined and the two mechanisms addressed together in the same study, with samples collected both before and after single sessions and complete treatment series (such as in [74]).

Analytical methods have evolved during the period covered in this review. While the earlier studies mainly utilized fluorescence-based detection, recent studies have mainly used liquid chromatography combined with mass spectrometry (Supplementary Table S3). Differences in analytical procedures may have contributed to differences in results. However, such effects are likely of minor relevance compared to variations in sample collection and handling and in patient and control populations.

Finally, all the included studies in this review considered Trp or kynurenine pathway changes through single metabolites or ratios of two metabolites. Given the large number of metabolites in the pathway and their mutual dependence, a systems biology approach may be warranted as a complement to investigations of each metabolite in isolation.

4.4. Suggestions for Future Studies

The relationship between depression, ECT and the kynurenine metabolism is complex, and as put forward in this review, the current study groups are too small to reliably detect the effect of ECT on Trp and kynurenines. Future studies should investigate changes in Trp and kynurenines while reporting, considering and adjusting for factors that could affect Trp metabolism, ECT outcome or both. Studies should seek to include larger patient groups with standardized intervention, fasting and timepoints for sampling and matched control groups that follow the same procedures and timeline. In such analyses, kynurenines should be considered interdependent, and attempts should be made to analyze changes at the pathway level, not only in single metabolites or ratios. Besides the studies on ECT, studies are also needed that investigate between- and within-subject variability in kynurenines under normal physiological conditions, as well as the effect of fasting. Future studies should also analyze changes in light of response or remission status and consider if there are clinical subgroups in which alterations in Trp metabolism could be a more decisive aspect than in others, e.g., older patients. Additionally, a focus on dimensional scores as opposed to diagnoses could reduce variation between study populations and aid the search for generalizable results regarding the effects of ECT. To ensure comparability between studies, inclusion and exclusion criteria should be clearly described, and studies should strive for open datasets for transparency and to enable meta-analyses.

4.5. Strengths and Limitations

This review was based on a systematic literature search in four databases using a wide selection of relevant terms, with a Supplementary search in June 2022. No exclusion criteria were used in the search process. In addition, references of included studies were scanned for studies not found in the systematic search. Still, since the search only assessed title and abstract, studies may have been overlooked. In the synthesis of this review, changes in biomarkers were presented using percentage change. Alternative ways of analyzing changes could have yielded more or other types of information. Effect sizes could have been calculated based on mean and SD, and the difference between timepoints could have been evaluated in relation to the standard error to estimate clinical significance of the reported changes. Furthermore, more attention could have been given to analyses in subgroups of patients, for example, response or remission subgroups, or to correlation analyses of change in biomarkers in relation to change in symptom scores. Finally, the vote counting synthesis method does not consider size or significance of change, nor the quality of the included studies [42]. However, conclusions drawn from this material, even based on more advanced synthesis methods, would have high risk of bias given the large heterogeneity between studies, small study groups and the exploratory nature of this field.

5. Conclusions

In this systematic review, there was no overall evidence of change in Trp, kynurenines or ratios after ECT. This could reflect that the kynurenine pathway is not altered by this intervention. Alternatively, it may be due to limitations of the cited studies, such as the relatively low number of participants in each study, and the challenge of isolating the effect of ECT from the influence of other factors such as inflammation, stress and medication. Additionally, patients with depression are a heterogenous group, and differences in pathophysiology, clinical characteristics, treatment response and baseline concentrations of biomarkers could be of great importance. Finally, there is limited knowledge about the between- and within-individual variability in kynurenine metabolism and about the effect of fasting and the significance of differences in blood sample timing. However, despite the challenges involved, it is important to continue investigating the role of kynurenine metabolism in depression treatment, as this pathway could be crucial for understanding the pathophysiology of mood disorders and contains several potentially important targets for therapeutic interventions.

Supplementary Materials: The Table following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ph15111439/: Table S1: Reported levels of tryptophan, kynurenines, ratios and related biomarkers before and after a series of ECT and corresponding analyses of change; Table S2: Detailed overview of the reviewed studies' declaration and handling of factors that can affect analyses of tryptophan and kynurenines in the context of ECT; Table S3: Data on storage and analytical procedures in the reviewed studies. Document S1: Syntax for systematic literature search, july 2022.

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Abbreviations

AA	anthranilic acid
BMI	body mass index
CAA	competing amino acid
ECT	electroconvulsive therapy
FAD	flavin adenine dinucleotide
HAA	3-hydroxyanthranilic acid
HK	3-hydroxykynurenine
ICC	intraclass correlation coefficient
IDO	indoleamine 2,3-dioxygenase
IFN	interferon; KA, kynurenic acid
KYNU	kynureninase
Kyn	kynurenine

KAT	kynurenine aminotransferase
KMO	kynurenine monooxygenase
KTR	kynurenine-tryptophan-ratio
NEFA	non-esterified fatty acid
NMDAr	N-methyl-D-aspartate receptor
Pic	picolinic acid
PLP	pyridoxal 5′-phosphate
QA	quinolinic acid
SSRI	selective serotonin reuptake inhibitor
TDO	tryptophan 2,3-dioxygenase
TNF	tumor necrosis factor
Trp	tryptophan
XA	xanthurenic acid

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Study IV

Supplementary material

Supplementary Document: Syntax for systematic literature search, july 2022

The syntax for the original literature search for june 2020 was identical to the one presented below. The number of records identified by each index term or free text in the syntax is indicated at the end of each line. Unlike in the original search, the results showen here were filtered on publication date.

Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily <1946 to July 19, 2022>

1 (Kynurenine pathway or tryptophan kynurenine pathway or tryptophan kynurenine metabolism or tryptophan catabolism or tryptophan catabolite pathway or TRYCAT).mp. 2682
2 tryptophan.mp. 65112
3 kynurenine.mp. 6883
4 (kynurenine tryptophan ratio or KTR).mp. 817
5 (kynurenic acid or kynurenate or KYNA).mp. 4130
6 anthranilic acid.mp. 1776
7 (hydroxykynurenine or 3-hydroxykynurenine).mp. 831
8 xanthurenic acid.mp. 674
9 (hydroxyanthranilic acid or 3-hydroxyanthranilic acid).mp. 710
10 (quinolinic acid or QUIN).mp. 4048
11 picolinic acid.mp. 1106
12 tryptophan 2,3-dioxygenase.mp. 649
13 indoleamine 2,3-dioxygenase.mp. 4297
14 (kynureninase or kynurenine hydrolase or KYNU).mp. 407
15 kynurenine aminotransferase.mp. 311
16 kynurenine 3-monooxygenase.mp. 343
17 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 77159
18 exp tryptophan/ 35459
19 exp kynurenine/ 3876
20 exp kynurenic acid/ 2501
21 exp ortho-aminobenzoates/ 8070
22 exp xanthurenates/ 3030
23 exp 3-hydroxyanthranilic acid/ 340
24 exp quinolinic acid/ 1790
25 exp picolinic acids/ 3590
26 exp tryptophan oxygenase/ 1993
27 exp indoleamine-pyrrole 2,3,-dioxygenase/ 3261
28 exp kynurenine 3-monooxygenase/ 252
29 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 54079
30 17 or 29 89654
31 (Electroconvulsive or Electroshock or Electroconvulsive Therapy or Electroconvulsive Therapies or Electroshock Therapy or Electroshock Therapies or Electric Convulsive Therapy or Electric Convulsive Therapies or Electric Shock Therapy or Electric shock Therapies or Electroconvulsive Shock Therapy or Electroconvulsive Shock Therapies or Electroconvulsive shock or ECT).mp. 33799
32 exp Electroconvulsive Therapy/ 13965
33 31 or 32 33799
34 30 and 33 267
35 limit 34 to yr="2020 -Current" 6

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1 (Kynurenine pathway or tryptophan kynurenine pathway or tryptophan kynurenine metabolism or tryptophan catabolism or tryptophan catabolite pathway or TRYCAT).mp. 3581
2 tryptophan.mp. 79946
3 kynurenine.mp. 9464
4 (kynurenine tryptophan ratio or KTR).mp. 1919
5 (kynurenic acid or kynurenate or KYNA).mp. 5093
6 anthranilic acid.mp. 4175
7 (hydroxykynurenine or 3-hydroxykynurenine).mp. 1152
8 xanthurenic acid.mp. 899
9 (hydroxyanthranilic acid or 3-hydroxyanthranilic acid).mp. 907
10 (quinolinic acid or QUIN).mp. 5581
11 picolinic acid.mp. 2210
12 tryptophan 2,3-dioxygenase.mp. 1813
13 indoleamine 2,3-dioxygenase.mp. 9057
14 (kynureninase or kynurenine hydrolase or KYNU).mp. 605
15 kynurenine aminotransferase.mp. 584
16 kynurenine 3-monooxygenase.mp. 553
17 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 100720
18 exp tryptophan/ 49648
19 exp kynurenine/ 7397
20 exp kynurenic acid/ 4162
21 exp anthranilic acid/ 1770
22 exp 3-hydroxykynurenine/ 905
23 exp xanthurenic acid/ 759
24 exp 3-hydroxyanthranilic acid/ 623
25 exp quinolinic acid/ 3530
26 exp picolinic acid/ 1189
27 exp tryptophan 2,3 dioxygenase/ 1647
28 exp indoleamine 2,3 dioxygenase/ 7720
29 exp kynureninase/ 451
30 exp Kynurenine 3 monooxygenase/ 520
31 exp Kynurenine aminotransferase/ 456
32 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 65535
33 17 or 32 100720
34 (Electroconvulsive or Electroshock or Electroconvulsive Therapy or Electroconvulsive Therapies or Electroshock Therapy or Electroshock Therapies or Electric Convulsive Therapy or Electric Convulsive Therapies or Electric Shock Therapy or Electric Shock Therapies or Electroconvulsive Shock Therapy or Electroconvulsive Shock Therapies or Electroconvulsive Shock or ECT).mp. 30370
35 exp Electroconvulsive Therapy/ 20071
36 34 or 35 30370
37 33 and 36 310
38 limit 37 to yr="2020 -Current" 23

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1 (Kynurenine pathway or tryptophan kynurenine pathway or tryptophan kynurenine metabolism or tryptophan catabolism or tryptophan catabolite pathway or TRYCAT).mp. 491
2 tryptophan.mp. 4461
3 kynurenine.mp. 840
4 (kynurenine tryptophan ratio or KTR).mp. 47
5 (kynurenic acid or kynurenate or KYNA).mp. 747

6 anthranilic acid.mp. 35
7 (hydroxykynurenine or 3-hydroxykynurenine).mp. 98
8 xanthurenic acid.mp. 42
9 (hydroxyanthranilic acid or 3-hydroxyanthranilic acid).mp. 35
10 (quinolinic acid or QUIN).mp. 515
11 picolinic acid.mp. 28
12 tryptophan 2,3-dioxygenase.mp. 49
13 indoleamine 2,3-dioxygenase.mp. 252
14 (kynureninase or kynurenine hydrolase or KYNU).mp. 12
15 kynurenine aminotransferase.mp. 35
16 kynurenine 3-monooxygenase.mp. 54
17 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 5459
18 exp tryptophan/ 2384
19 17 or 18 5615
20 (Electroconvulsive or Electroshock or Electroconvulsive Therapy or Electroconvulsive Therapies or Electroshock Therapy or Electroshock Therapies or Electric Convulsive Therapy or Electric Convulsive Therapies or Electric Shock Therapy or Electric shock Therapies or Electroconvulsive Shock Therapy or Electroconvulsive Shock Therapies or Electroconvulsive shock or ECT).mp. 18654
21 exp Electroconvulsive Shock Therapy/ 7077
22 20 or 21 18654
23 19 and 22 101
24 limit 23 to yr="2020 -Current" 4

Pubmed from 2020/6/3 - 2022/7/20

((kynurenine pathway[Title/Abstract] OR tryptophan kynurenine pathway[Title/Abstract] OR tryptophan kynurenine metabolism[Title/Abstract] OR tryptophan catabolism[Title/Abstract] OR tryptophan catabolite pathway[Title/Abstract] OR TRYCAT[Title/Abstract] OR tryptophan[Title/Abstract] OR kynurenine[Title/Abstract] OR kynurenine tryptophan ratio[Title/Abstract] OR KTR[Title/Abstract] OR kynurenic acid[Title/Abstract] OR kynurenate[Title/Abstract] OR KYNA[Title/Abstract] OR anthranilic acid[Title/Abstract] OR hydroxyanthranilic acid[Title/Abstract] OR 3-hydroxyanthranilic acid[Title/Abstract] OR quinolinic acid[Title/Abstract] OR QUIN[Title/Abstract] OR picolinic acid[Title/Abstract] OR tryptophan 2,3,-dioxygenase[Title/Abstract] OR indoleamine 2,3-dioxygenase[Title/Abstract] OR kynureninase[Title/Abstract] OR kynurenine hydrolase[Title/Abstract] OR KYNU[Title/Abstract] OR kynurenine aminotransferase[Title/Abstract] OR kynurenine 3-monooxygenase) AND (electroconvulsive[Title/Abstract] OR electroshock[Title/Abstract] OR electroconvulsive therapy[Title/Abstract] OR electroconvulsive therapies[Title/Abstract] OR electroshock therapy[Title/Abstract] OR electroshock therapies[Title/Abstract] OR electric convulsive therapy[Title/Abstract] OR electric convulsive therapies[Title/Abstract] OR electric shock therapy[Title/Abstract] OR electric shock therapies[Title/Abstract] OR electroconvulsive shock therapy[Title/Abstract] OR electroconvulsive shock therapies[Title/Abstract] OR electroconvulsive shock[Title/Abstract] OR ECT[Title/Abstract)) 87

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Supplementary Table 1: Reported levels of tryptophan, kynurenines, ratios and related biomarkers before and after a series of ECT and corresponding analyses of change

		Author (year)	**Guloksuz** (2015)	**Schwieler** [1] (2016)		**Allen** [1] (2018)	**Aarsland** [2] (2019)		**Ryan** (2020)		**Aarsland** (2022)	**Olajossy** (2018)			
		Participants	Patients (n=19)	Patients (n=15)	Controls (n=14)	Patients (n=18)	Patients (n=21)	Controls (n=12)	Patients (n=94)	Controls (n=57)	Patients (n=48)	SAD (n=7)	DBD (n=11)	RDD (n= 32)	Controls (n=48)
		Unit	µg/ml (total Trp), ng/ml, ratio	µmol/L, nmol/L, ratio	µmol/l, nmol/l, ratio	ng/ml, ratio	µmol/L, nmol/L, ratio	µmol/l, nmol/l, ratio	µmol/L, nmol/L, ratio	µmol/l, nmol/l, ratio	µmol/L, nmol/L, ratio	Not reported			Not reported
		Distribution	Mean (SD)	Mean (SD)	Mean (SD)	Mean	Median (IQR)	Median (IQR)	Mean (SD)	Mean (SD)	Median (IQR)	Median			Median
		Test / estimate	Multilevel linear regression / B	Wilcoxon matched-paired signed-rank test / ?		t-test / t	Wilcoxon paired test / ?		Linear model (for log(metabolite)), Wilcoxon signed rank (for ratios) / F, Z		Paired Wilcoxon signed-rank test / Median percentage change	Friedman's ANOVA and Kendalls coefficient of concordance / χ^2			
Biomarkers															
Trp	Total Trp	**Pre**	8.34 (1.23)	85.5 (24.3)	73.6 (12.4)	9173 (2825)	73.5 (11.0)	76.1 (10.9)	54.3 (10.99)	65.6 (12.47)	63.3 (20.2)				
		Post	?	70.7 (18.2)		8419 (2400)	68.2 (13.3)	70.7 (8.19)	56.36 (10.60)		66.6 (15.9)				
		Estimate / p-value	-0.003 / 0.448	? / <0.05		t=? / n.s.	? / 0.36	-7.15 / 0.15	F=4.02 / 0.048		1.50 / 0.24				
Kynurenines	Kyn	**Pre**	535.44	3.30 (1.15)	3.00 (0.73)	461 (144)	1.53 (0.57)	1.44 (0.49)	1.52 (0.47)	1.64 (0.41)	2.00 (0.75)				
		Post	?	2.61 (0.89)		502 (268)	1.50 (0.60)	1.51 (0.57)	1.66 (0.47)		1.92 (0.59)				
		Estimate / p-value	0.92 / 0.061	? / <0.01		t=? / n.s.	? / 0.43	5.03 / 0.02	F=7.362 / 0.008		0.96 / 0.71				
	KA	**Pre**	9.35	24.4 (8.07)	41.9 (12.4)	4.92 (2.44)	36.7 (18.8)	50.1 (12.4)	41.34 (19.42)	54.85 (19.42)	52.0 (28.2)	0.16	0.12	0.15	0.20
		Post		26.2 (9.69)		4.84 (3.53)	36.2 (26.2)	42.4 (14.3)	44.02 (20.61)		48.7 (23.3)	0.16	0.11	0.17	
		Estimate / p-value	0.04 / 0.001	? / n.s.		t=? / n.s.	? / 0.11	-15.4 / 0.003	F=2.18 / 0.143		2.72 / 0.81	0.33 / 0.88	0.66 / 0.95	1.41 / 0.70	
	HK	**Pre**	8.57				32.6 (12.9)	37.8 (7.25)	41.68 (15.64)	42.18 (12.03)	53.9 (37.0)				
		Post	?				41.4 (19.1)	34.0 (10.3)	45.23 (18.0)		51.9 (29.3)				
		Estimate / p-value	0.02 / 0.161				? / 0.05	-9.93 / 0.30	Z=2773.5 / 0.041		9.63 / 0.30				
	AA	**Pre**					15,5 (6,30)	20.4 (8.78)	12.67 (3.98)	14.64 (4.07)	17.8 (4.75)				
		Post					16,3 (6,20)	16.5 (3.15)	13.58 (4.82)		17.5 (5.50)				
		Estimate / p-value					? / 0.39	-18.9 / 0.03	F=3.89 / 0.052		0.29 / 0.77				
	HAA	**Pre**	54.57				28,2 (13,9)	38.0 (13.3)	32.84 (14.64)	41.23 (20.83)	33.5 (17.5)				
		Post	?				41,7 (18,8)	30.2 (15.7)	36.20 (17.76)		35.6 (25.7)				
		Estimate / p-value	-0.08 / 0.294				? / 0.03	-20.4 / 0.13	F=3.73 / 0.056		10.6 / 0.12				
	XA	**Pre**					9,71 (5,34)	15.5 (5.65)	10.58 (6.63)	18.65 (9.72)	10.7 (9.33)				
		Post					11,4 (9,17)	15.7 (10.8)	12.0 (7.01)		12.2 (8.95)				
		Estimate / p-value					? / 0.20	1.29 / 0.47	Z=2683 / 0.089		16.1 / 0.12				
	Pic	**Pre**					24,5 (14,6)	32.0 (13.0)	32.0 (20.72)	44.21 (26.96)	39.4 (23.8)				
		Post					31,5 (14,2)	30.0 (15.5)	33.73 (25.21)		42.4 (23.5)				
		Estimate / p-value					? / 0.01	-6.10 / 0.31	F=0.748 / 0.389		7.70 / 0.81				
	QA	**Pre**		635.7 (239.2)	603.2 (215.0)		329 (189)	318 (127)	419.4 (216.3)	400.8 (143.7)	512 (352)				
		Post		513.8 (256.5)			370 (152)	301 (98.8)	448.2 (210.2)		489 (247)				
		Estimate / p-value		? / <0.001			? / 0.35	-5.5 / 0.48	F=3.44 / 0.067		-3.23 / 0.41				
Ratios	KTR	**Pre**	63.75	0.0384 (0.008342)	0.04 (0.007845)	0.0527284 (0.0177521)	20.8 (6.56)	18.8 (5.14)			32.3 (15.7)				
		Post	?	0.038 (0.009411)		0.05807656 (0.01647596)	20.2 (8.58)	19.1 (5.32)			29.4 (13.7)				
		Estimate / p-value	0.14 / 0.001	? / n.s.		t=? / n.s.	? / 0.16	1.70 / 0.52	? / 0.065		-2.51 / 0.48				
	KA/Kyn	**Pre**	17.18			0.009784233 (0.004211415)	26.1 (10.2)	31.9 (2.36)							
		Post	?			0.008830895 (0.003578453)	27.9 (9.70)	28.0 (8.56)							
		Estimate / p-value	0.07 / <0.001			t=? / n.s.	? / 0.23	-12.4 / 0.13	? / 0.647						
	KA/HK	**Pre**	1.23				10.0 (4.63)	12.7 (2.16)			9.91 (4.39)				
		Post	?				11.1 (5.66)	12.1 (5.39)			9.44 (4.12)				
		Estimate / p-value	0.01 / 0.008				? / 0.89	-4.97 / 0.38			-16.1 / 0.02				
	QA/KA	**Pre**		28.4 (13.7)	14.8 (4,93)										
		Post		21.3 (7.83)											
		Estimate / p-value		? / <0.05											
	KA/QA	**Pre**					11.0 (3.83)	15.2 (4.45)			9.68 (3.67)				
		Post					11.8 (4.73)	13.6 (6.47)			9.74 (3.04)				
		Estimate / p-value					? / 0.32	-10.2 / 0.05			-2.70 / 0.70				

Ratios	XA/HK	**Pre**	25.7 (12.9)	44.3 (13.8)
		Post	27.1 (12.2)	41.3 (24.0)
		Estimate / p-value	? / 0.63	-6.86 / 0.79
	Pic/QA	**Pre**	69.2 (21.8)	104.7 (39.7)
		Post	87.6 (51.7)	93.2 (36.4)
		Estimate / p-value	? / 0.02	-11.0 / 0.68
	QA/Kyn	**Pre**		
		Post		
		Estimate / p-value		p = 0.702

Other biomarkers	Other 1	**Biomarker name**	MADRS	Creatinin
		Pre	34.0 (8.00)	73.5 (10.9)
		Post	15.0 (15.0)	72.1 (11.1)
		Estimate / p-value	? / 0.00	-1.90 / 0.003

Other biomarkers	Other 2	**Biomarker name**	Neopterin	Neopterin	CRP	CRP	Neopterin
		Pre	17.7 (9.70)	14.8 (6.03)	0.63 (0.92)	0.26 (0.42)	29.2 (17.2)
		Post	23.3 (9.00)	16.6 (9.50)	0.62 (0.74)		29.4 (21.2)
		Estimate / p-value	? / 0.00	12.2 / 0.33	Z=1805 / p=0.27		1.30 / 0.43
	Other 3	**Biomarker name**	Riboflavin	Riboflacin	TNF-α	TNF-α	Riboflavin
		Pre	12.6 (5.60)	13.0 (4.45)	3.34 (1.00)	2.68 (0.69)	22.0 (20.6)
		Post	12.6 (9.90)	14.5 (4.73)	3.47 (1.14)		20.2 (23.3)
		Estimate / p-value	? / 0.75	11.2 / 0.53	F3.84 / 0.05		-1.12 / 0.70
	Other 4	**Biomarker name**	PLP	PLP	TNF-α mRNA	TNF-α mRNA	PLP
		Pre	46.0 (33.6)	63.5 (9.48)	0.97 (0.30)	1.12 (0.32)	22.8 (21.6)
		Post	42.7 (45.9)	65.0 (18.4)	1.04 (0.27)		22.8 (19.9)
		Estimate / p-value	? / 0.79	2.28 / 0.85	F=4.37 / p=0.04		-17.5 / 0.29

Thick outline indicate significant change. [1] Biomarker levels were provided by the authors after written request. [2] Second time-point measures and percentage change for controls were not reported in the original published report. Abbreviations: AA, anthranilic acid; ANOVA, analysis of variance; CRP, C-reactive protein; DBD, depression in bipolar disorder; ECT, electroconvulsive therapy; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; IQR, interquartile range; KA, kynurenic acid; Kyn, kynurenine; KTR, kynurenine-tryptophan-ratio; MADRS, Montgomery and Åsberg Depression Rating Scale; mRNA, messenger ribonucleid acid; Pic, picolinic acid; QA, quinolinic acid; RDD, recurrent depressive disorder; SAD, schizoaffective disorder; SD, standard deviation; TNF-α, tumor necrosis factor alpha; Trp, tryptophan; XA, xanthurenic acid.

Supplementary Table 1 (continued): Reported levels of tryptophan and related biomarkers before and after a series of ECT and corresponding analyses of change

		Author (year)	**Coppen** (1973)		**Abrams** (1976)	**D'Elia** (1977)	**Kirkegaard** (1978)	**Whalley** (1980)	**Hoekstra** (2001)	
		Participants	Patients (n=6)	Controls (n = 26)	Patients (n=6)	Patients (n=24)	Patients (n=10)	Patients (n=9)	Patients (n=20)	Controls (n=29)
		Unit	µg/ml, %	µg/ml, %	ng/ml	mg%	nmol/ml	nmol/ml	µmol/l	µmol/l
		Distribution	Mean (SE)	Mean (SE)	Mean (SE)	Median (SD)	? (?)	Mean (SD)	Mean (SD)	Mean (SD)
		Test / estimate	? / ?	? / ?	? / ?	Student's t-test / ?	Paired Wilcoxon test / ?	Paired Student t-test / ?	Student's t-test / ?	
Biomarkers										
Trp	Free Trp	**Pre**	0.66 (0.10)	1.39 (0.07)			4.3 (1.0)	6.9 (2.0)		
		Post	1.22 (0.10)				4.4 (0.8)	7.2 (1.2)		
		Estimate / p-value	? / <0.01				? / n.s.	? / n.s.		
	Total Trp	**Pre**	10.65 (0.67)	12.4 (0.3)	347.0 (90.5)	1.27 (0.30)	32.5 (6.1)	54.4 (8.9)	35.5 (9.0)	45.6 (6.1)
		Post	11.55 (0.73)		420.2 (22.9)	1.23 (0.48)	27.6 (4.9)	47.4 (6.5)	38.7 (7.7)	no measure
		Estimate / p-value	? / n.s.		F=2.8 / p=n.s.	not tested	? / <0.01	? / <0.01	? / n.s.	
Other biomarkers	Other 1	**Biomarker name**	Free Trp as % of total Trp		DRS	Total CODS	Free Trp as % of total Trp		HRS-D	
		Pre	6.32 (1.12)	11.2 (0.5)	20.8 (6.5)	13,8	13.4 (2.4)		31,00	
		Post	10.58 (0.72)		3.0 (0.6)	2,3	15.8 (2.2)		11,00	
		Estimate / p-value	? / <0.005		F = 63.3 / 0.001	not tested	? / <0.05			
	Other 2	**Biomarker name**				Total NRS	Albumin, ? / ?, g/l		Neopterin, nmol/l	Neopterin, nmol/l
		Pre				22	39.7 (2.5)		21.7 (8.2)	19.6 (6.5)
		Post				6,5	40.8 (2.1)		23.6 (8.2)	no measure
		Estimate / p-value				not tested	? / n.s.		? / 0.03	
Comments					Total Trp was measured in CSF	No statistic test reported for comparison of pre and post ECT concentrations.			Patients' pre-treatment Trp levels were significantly lower than that of controls (p<0.0005).	

Thick outline indicate significant change. Abbreviations: CODS, Cronholm-Ottosson Depression Scale; CSF, cerebrospinal fluid; DRS, Depression Rating Scale; ECT, electroconvulsive therapy; HRS-D, Hamilton Depression Rating Scale; NRS, Nurses' rating scale; n.s., non-significant; SD, standard deviation; SE, standard error; Trp, tryptophan.

Supplementary Table 1 (continued): Reported levels of tryptophan and related biomarkers before and after a single ECT and corresponding analyses of change

		Author (year)	**Stelmasiak** (1974)	**Whalley** (1980)		**Sawa** (1981)	**Mokhtar** (1997)		**Palmio** (2005)
		Participants	Patients (n=18, 15 at 15min)	Patients (n=11)	Controls (n=11) undergoing anesthesia and cystoscopy	Patients (n=9)	Patients (n=10)	Controls (n=4) undergoing anesthesia and minor surgery	Patients (n=10)
		Unit	µg/ml	nmol/ml	nmol/ml	nmol/ml	µg/ml (free Trp), µmol/ml (total Trp), ratio	µg/ml (free Trp), µmol/ml (total Trp), ratio	µmol/l
		Distribution	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SEM)	Mean (SEM)	Mean (SD)
		Test / estimate	Paired Student's t-test / ?	Paired Student's t-test / ?	Paired Student's t-test / ?	t-test / ?	ANOVA / ?	ANOVA / ?	Paired sample t-test / ?
Biomarkers	Time points								
Free Trp	Baseline	**value**	1.07 (0.41)	6.9 (2.0)	6.3 (1.4)	3.92 (1.23) (range: 1.81 - 5.89)	1.12 (0.11)	0.99 (0.06)	
	First post measure	**time**	1 min	10 min after convulsion	10 min after recovery	1 min	15 min		
		value	1.42 (0.50)	7.1 (2.0)	7.2 (1.8)	increased	1.00 (0.12)	1.04 (0.05)	
		Estimate / p-value	? / <0.01	? / n.s.	? / n.s.	? / <0.01	? / n.s.	? / n.s.	
	Second post measure	**time**	15 min			5 min	30 min		
		value	1.32 (0.58)			normalised	1.06 (0.12)	1.06 (0.08)	
		Estimate / p-value	? / 0.02			? / n.s.	? / n.s.	? / n.s.	
	Third post measure	**time**	30 min			10 min	45 min		
		value	1.26 (0.35)			decreased	1.03 (0.11)	1.03 (0.11)	
		Estimate / p-value	? / n.s.			? / n.s.	? / n.s.	? / n.s.	
	Fourth post measure	**time**	60 min			30 min	60 min		
		value	1.05 (0.43)			decreased	1.05 (0.08)	0.99 (0.05)	
		Estimate / p-value	? / n.s.			? / n.s.	? / n.s.	? / n.s.	
	Fifth post measure	**time**				60:00.000			
		value				decreased			
		Estimate / p-value				? / <0.05			
	pooled	**Estimate / p-value**					F = ? / n.s.	F = ? / n.s.	
Total Trp	baseline	**value**	8.44 (1.87)	54.4 (8.9)	52.5 (9.4)	69.7 (22.5) range: 45.2 - 124.5	47 (3)	45 (3)	29.5 (6.7)
	First post measure	**time**	1 min	10 min after convulsion	10 min after recovery	1 min	15m 0s 0ms		2h
		value	8.03 (2.39)	48.8 (6.6)	45.6 (7.3)	decreased	40 (2)	35 (1)	39.0 (8.6)
		Estimate / p-value	? / n.s.	? / <0.05	? / <0.01	? / n.s	? / n.s.	? / <0.05 (unspecified)	? / <0.05
	Second post measure	**time**	15 min			5 min	30m 0s 0ms		6h
		value	8.81 (2.99)			decreased	40 (3)	34 (3)	42.4 (8.9)
		Estimate / p-value	? / n.s.			? / <0.01	? / n.s.	? / <0.05 (unspecified)	? / <0.005
	Third post measure	**time**	30 min			10 min	45m		24h
		value	8.67 (2.88)			decreased	38 (3)	36 (3)	39.3 (12.2)
		Estimate / p-value	? / n.s.			? / <0.01	? / <0.05	? / n.s.	? / <0.05

Total Trp	Fourth post measure	**time**	60 min		30 min	60 min	48h
		Value	7.43 (2.19)		decreased	34 (3)	30.1 (6.9)
		Estimate / p-value	? / n.s.		? / n.s.	? / <0.005	? / n.s.
	Fifth post measure	**time**			60 min		
		value			normalised		
		Estimate / p-value			? / n.s.		
	pooled	**Estimate / p-value**				F = 2.81 / <0.37	F = 3.83 / <0.24
Biomarkers							
Other biomarkers	Other 1	**Biomarker name**				NEFA, mmol/L	
		Pre				0.35 (0.06)	0.34 (0.05)
		Post				0.48 (0.07)	0.49 (0.08)
		Estimate / p-value					
	Other 2	**Biomarker name**				TRP/CAA	
		Pre				0.088 (0.006)	0.099 (0.004)
		Post				0.076 (0.008)	0.081 (0.002)
		Estimate / p-value					
Comments				Before and after first ECT	Before and after anesthesia	"Others" measured at 60 min post.	"Others" measured at 60 min post.

Thick outline indicate significant change. Abbreviations: CAA, competing amino acid; ECT, electroconvulsive therapy; NEFA, non-esterified fatty acid; n.s., non-significant; SD, standard deviation; SE, standard error; SEM, standard error of the mean; Trp, tryptophan.

Supplementary table 2 Detailed overview of the reviewed studies' declaration and handling of factors that can affect analyses of tryptophan and kynurenines in the context of ECT

Author (year)	n *	Factors that can affect levels of tryptophan and kynurenines													
		Inflammation	Age	Kidney function	BMI	Sex	Nutrition	Fasting	Vitamin status	Stress	Alcohol and tobacco	NEFA	CAA	Medication	Glucose	Liver function
Guloksuz (2015)	19		52.6 (14.4) [1]. Included as covariable in all analyses.			13♀ / 6♂. Included as covariable in all analyses.		Yes, overnight						Exclusion criterion (immuno-modulating medications)		
Schwieler (2016)	19 (15)	IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, TNF-α, IFN-γ, GM-CSF. Analyses of change.	41.0 (25.0-54.0) [2]			8♀ /11♂		Yes, since the evening before blod sample collection								
Allen (2018)	18	IL-6, IL-8, IL-10, IFN-γ	57.4 (14.7) [1]		Body weight. Exclusion criterion (>10% of ideal body weight).	12♀ / 6♂		Before ECT: yes. After ECT: yes		Saliva cortisol (cortisol awakening response). Analysis of change.	Smokers. (n = 16)					
Aarsland (2019)	21	Neopterin. Analysis of change.	47.0 (20.0) [2]	Creatinine. Exclusion criteria (>120µmol/l).		13♀ / 8♂		Before ECT: yes. After ECT: yes	PLP, riboflavin		Smokers (n = 14, 52%). Cotinine included as covariable in group comparison.					
Ryan (2020)	94	CRP, TNF-α, TNF α mRNA. Analyses of change.	55.48 (14.72) [1]. Included as covariable in group comparison.		26.76 (5.01) [1]. Included as covariable in group comparison.	58♀ / 36♂. Included as covariable in group comparison.		Yes, not further specified	PLP, riboflavin (Ryan 2020b)		Smokers (n = 40, 42.55%). Included as covariable in group comparison.					
Aarsland (2022)	48	Neopterin. Used for stratification in sub-analyses.	73.0 (12.3) [2]	Baseline creatinine		30♀ / 18♂		Before ECT: yes. After ECT: yes	PLP, riboflavin		Non-smokers (n = 38, 78.9%). Included as covariable in group comparison. Never alcohol (n = 31, 72.1%).					
Olajossy (2017)	50		49.4 (12.7) [1] 44.7 (13.8) [1], 33.3 (8.6) [1]. Used in sub-analyses.			19♀ / 13♂. 7♀ / 4♂. 4♀ / 3♂. Used in sub-analyses.										
Coppen (1973)	6					6♀	Full hospital diet for at least a week prior to ECT	Yes, since the evening before each test								
Abrams (1976)	6															
D'Elia (1977)	24		48.0 (12.5) [1] (range 22-64). Exclusion criterion (>65 years).			15♀ / 9♂. Used for stratification.		Yes, since 12 p.m. the day before testing								
Kirkegaard (1978)	10		63 (37-77) [3]			7♀ / 3♂	Ordinary hospital diet	Before ECT: yes. After ECT: yes								Albumin. Analysis of change.
Whalley (1980)	12 (11)		49 (13.9) [1]			9♀ / 3♂		Yes, overnight for all samples								
Hoekstra (2001)	20		52 (13.1) [1]			13♀ / 7♂		Before ECT: yes. After ECT: not specified					Phenylalanine, tyrosine, isoleucine, leucine, valine, Trp-ratio			
Stelmasiak (1974)	18 (15)		48 (20-70) [3]. Used in sub-analyses.			11♀ / 7♂. Used in sub-analyses.		Yes, since six hours before ECT				Fatty acids. Analyses of change.				
Sawa (1981)	9		40.7 (20-52) [3]			5♀ / 4♂		Yes, for 17 hours before ECT				NEFA. Analyses of change.				
Mokhtar (1997)	10		48.5 (4.3) [4] (range 24-66)			5♀ / 5♂		Yes, since the evening before ECT		Serum cortisol. Analysis of change.		NEFA. Analyses of change.	Phenylalanine, tyrosine, isoleucine, leucine, valine, Trp-ratio. Analyses of change.		Glucose. Analysis of change.	Albumin. Analysis of change.
Palmio (2005)	10		55.6 (28-70) [3]			7♀ / 3♂		Yes, since the evening before ECT					Phenylalanine, tyrosine, isoleucine, leucine, valine. Analyses of change.			

Measure at baseline / declared

Analyses of change after ECT / used as covariable or for stratification in sub-analyses

Included as covariable in main analyses

Not measured / not declared / not considered

The table is limited to factors that have been included in at least one of the reviewed studies. * The number in parentheses indicates the number of patients with repeated measures. [1] mean (standard deviation); [2] median (interquartile range); [3] mean (range); [4] mean (standard error of the mean). Abbreviations: BMI, body mass index; CAA, competing amino acid; CRP, C-reactive protein; ECT, electroconvulsive therapy; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; mRNA, messenger ribonucleic acid; NEFA, non-esterified fatty acid; PLP, pyridoxal 5'-phosphate; TNF, tumor necrosis factor; Trp, tryptophan.

Supplementary table 2 (continued) Detailed overview of the reviewed studies' declaration and handling of factors that can affect analyses of tryptophan and kynurenines in the context of ECT

Author (year)	n *	Patient characteristics								Intervention and study design					
		Depression characteristics			Psychotropic medication			Comorbidity		Clinical measures	ECT details	Anesthetics and muscle relaxants	Number of ECT sessions	Sample time after last ECT	Symptom score after ECT, response, remission
		Diagnosis	Severity	Other	During ECT	Discontinued before ECT	Changes during ECT	Psychiatric	Somatic						
Güloksuz (2015)	19	Unipolar (12) or bipolar (7) depression	HDRS [11,12]23.4 (6.4) [1] [13]BDI [12]32.8 (11.1) [1]		Yes (n = 19, 100%), antidepressants, mood stabilizers, antipsychotics	Benzodiazepine	No	Exclusion criterion (cancer, cerebrovascular disorders, organic psychiatric syndroms, drug abuse, mental retardation, dementia, neurodegenerative disorders, inflammatory condition)		HDRS. Used in sub-analyses	Seizure duration: 52 s (10-200 s) [9] [14]Charge: 462 mC [15](150-90 mC) [3]		Mean = 6.1 [16](range: 3-11)		HDRS after ECT utilized to assess baseline Trp metabolite and ratio levels as predictors for time to ECT response
Schwieler (2016)	19 (15)	Major depressive disorder	MADRS [17]37.0 (28.8-44.3) [2]		Lithium (n = 1), antipsychotics (n = 3), benzodiazepines (n = 8), and hypnotics (n = 12)					MADRS	Right unilateral, ultrabrief or brief pulse. Dose adjusted by age, sex and outcome.	Atropine, thiopental and succinylcholine	3		MADRS: 20.5 (14.0-26.5) [2] Analyses of correlation between change in QA and change in MADRS after ECT.
Allen (2018)	18	Major depressive episode	HDRS [18]20.2 (1.5) [4]		Yes (n = 15), SSRI, SNRI, TCA, mirtazapine			Anxiety (n = 10), history of drug dependence [18](n = 1)	Exclusion criterion (infection, endocrine, immune or metabolic disorder such as autoimmune disorders, inflammatory bowel disease or acquired immunodeficiency syndrom)	HDRS			Median = 8 [18](range: 5-12) (from Allen et al. 2015)		9 responders (50%). Analyses of correlation between change in kynurenines and change in HDRS after ECT.
Aarsland (2019)	21	Moderate to severe unipolar or bipolar depression	MADRS [19,20]34.0 (8.0) [2]	Age at debut of depressive symptoms, years since debut, number of episodes, length of current episode, previous ECT, psychotic symptoms (n = 4, 15.4%).	Yes (n = 27, 100%), antidepressants, mood stabilizers, antipsychotics		No		Exclusion criterion (kidney failure)	MADRS	Right unilateral, brief or ultrabrief pulse. Constant current (900 mA).	Thiopental and succinylcholine	Responders: median = 12.3, Non-responders median = 12.2	10 days (6 days) [2] after last ECT	MADRS: 15.0 (15.0) [2] [18]12 responders (57.1%) and 9 non-responders. Used for stratification in sub-analyses.
Ryan (2020)	94	Unipolar (n = 73) or bipolar (n = 21) depression.	HAM-D24 [21]31.0 (6.57) [1]	Psychotic features [18](n = 21)	SSRI (n = 22), SNRI (n = 48), TCA (n = 25), MAOI (n = 10), mirtazapine (n = 32), bupropion [18](n = 1), lithium (n = 34), sodium valproate (n = 6), antipsychotics [18](n = 65), benzodiazepines (n = 49), other hypnotics (n = 56), pregabaline (n = 5), other (n = 16)				Exclusion criterion (immune disorder, major neurological illness, medically unfit for general anesthesia)	MADRS	Unilateral (n = 48) or bitemporal (n = 46)	Methohexital and succinylcholine	7.96 (2.47) [1]	1 - 3 days after last ECT	HAM-D24: 10.44 (8.20) [1] [18]50 remitters (53.19%)
Aarslund (2022)	49	Severe unipolar depression	MADRS [18]33.0 (14.0) [2]	Number of previous episodes, length of index episode, age at onset, psychotic features (n = 25, 52.1%)	Yes (n = 21) [18]antidepressants (n = 15), antipsychotics (n = 6) [18]		No	Exclusion criterion (schizoaffective disorder, bipolar disorder)	Exclusion criterion (major neurological illness). Presence of other somatic comorbidity was used for stratification in sub-analyses	MADRS	Right unilateral, brief puls. Switch to bilateral on specified conditions.		10 (8) [21] [18](range = 4-29)	5 days (5.25 days) [2] after last ECT	30 remitters and 18 non-remitters. Used for stratification in sub-analyses.
Olajossy (2017)	50	Major depression in recurrent depressive disorder, bipolar disorder and schizoaffective disorder	MADRS, GAF. Used in sub-analyses	Duration of symptoms, number of phases, duration of the current phase. Used in sub-analyses.	Antidepressants (majority of patients)	Barbiturat, benzodiazepin, litium and other mood-stabilizing drugs that significantly affected seizure threshold				MADRS, GAF. Used in sub-analyses	Bilateral, fronto-temporal		12		
Coppen (1973)	6	depression			Medication free	None		No history of manic illness		Clinical recovery					
Abrams (1976)	6	endogenous depression	DRS [10]20.8 (6.5) [1]		Medication free since at least one week before start of treatment					DRS before and after			6.7 (4-8) [3]		
D'Elia (1977)	24	unipolar (11), bipolar (2) or not yet classified (11) depression	CODS [22]median = 13.8		Antidepressants [23,24](n = 12, 50%)				Exclusion criterion (somatic disease which could have a relation to the depressive period)	CODS and NRS before ECT and 4 days after last ECT			6.1 (3-12) [2]		Correlation analyses of baseline Trp and post-treatment CODS and NRS
Kirkegaard (1978)	10	Endogenous depression, unipolar (n = 3) or not classified [18](n = 7)	HDRS [18]mean = 26.0		Medication free since one week before start of treatment, except diazepam and kloraldol					HDRS			10.5 (2.6) [1]		
Whalley (1980)	12 (11)	Unipolar depression	HDRS [18]26.2 (7.6) [1]		Amitriptyline (n = 7), imipramine (n = 1), [18]naïve (n = 4)			Symptoms of schizophrenia was exclusion criterion		HRS		Thiopentone and suxamethonium	Range = 4-9	10 minutes after convulsion	HRS: 11.4 (4.2) [1] after the full ECT series
Hoekstra (2001)	20	Major depression	HDRS [25,26]mean = 31.0	Psychotic features (n = 10). Used for stratification in sub-analyses.	Medication free since at least seven days before start of treatment		Changes for some patients between last ECT and last blood sample		Exclusion criterion (serious disease known to influence biopterin metabolism, like infectious disease, autoimmune disorders, neoplastic disorders, Parkinson disease or dementia)	HDRS			Mean = 11		HDRS: mean = 11.0. 15 responders (75%). Used for stratification in analyses of change after ECT.
Stelmasiak (1974)	18 (15)	Endogenous depression			Imipramine, amitriptyline, barbiturate				Free of serious organic disease		Unilateral. Dose: 15-45 joules. Seizure duration used in sub-analyses.	Barbiturate and succinylcholine. Plasma concentration of barbiturate used in sub-analyses.	One single ECT	1, 15, 30 and 60 minutes after ECT	
Sawa (1981)	9	Endogenous depression, unipolar (n = 8) or bipolar (n = 1)	Self-rating depression scale, Hamilton's depression scale, Bojanovksy's depression scale	Number of phase, time since onset of current phase and beginning of medication	Amitriptyline [27,28](n = 8) [28]clomipramine [18](n = 1)					Self-rating depression scale, Hamilton's depression scale, Bojanovksy's depression scale		Amobarbital	Samples taken before and after the first ECT of a full treatment series	1, 5, 10, 30 and 60 minutes after ECT	
Mokhtar (1997)	10	Endogenous depression, unipolar (n = 7) or bipolar (n = 3)	HDRS > 17		Antidepressants [29,30](n = 10, 100%)					HDRS	Bilateral. [31,32]Duration: 4 seconds [33]7.93 J		One single ECT	15, 30, 45 and 60 minutes after ECT	Good seizure quality in 9/10 patients.
Palmio (2005)	10	Major depressive disorder	MADRS [18,34]29 (5.9) [1]	Psychotic feautres (n = 4)	Neuroleptics (n = 8), antidepressants [18](n = 7), benzodiazepines [18](n = 6)		Unchanged before and during ECT		One case of ischemic heart disease, otherwise none	MADRS	Brief-pulse, bilateral [18,34]Charge: mean = 272.2 mC [18,34]Seizure duration: mean = 45.7	Propofol (n = 5) [18]or methohexital [18](n = 5), and succinylcholine	Samples taken at different stage in the treatment series for each patient	2, 6, 24 and 48 hours after ECT	MADRS: 8.9 (8.9) [1] after the full ECT series.

Measure at baseline / declared

Analyses of change after ECT / used as covariable or for stratification in sub-analyses

Included as covariable in main analyses

Not measured / not declared / not considered

Not relevant for the study in question

The table is limited to factors that have been included in at least one of the reviewed studies. * The number in parentheses indicates the number of patients with repeated measures. [1] mean (standar deviation); [2] median (interquartile range); [3] mean (range); [4] mean (standard error of the mean). Abbreviations: CODS, Cronholm-Ottosson Depression Scale; ECT, electroconvulsive therapy; GAF, Global Assessment of Functioning; HAM-D24 / HDRS / HRS, Hamilton Depression Rating Scale; MADRS, Montgomery and Åsberg Depression Rating Scale; MAOI, monoamine oxidase inhibitor; NEFA, non-esterified fatty acid; NRS, Nurses' rating scale; SNRI, serotonin-norepinephrine reuptake inhibitor, SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant;

Supplementary Table 3 Data on storage and analytical procedures in the reviewed studies

Author (year)	Storage	Analytical procedure	Articles cited
Guloksuz (2015)	"Serum was seperated and stored at -80◦C"	"High performance liquid chromatography (HPLC) was used []". Metabolites were detected "spectrophotometrically", "fluorimetrically" or "by UV detection".	Hervé et al. (1996) *Determination of tryptophan and its kynurenine pathway metabolites in human serum by high-performance liquid chromatography with simultaneous ultraviolet and fluorimetric detection.* J. Chromatogr. B 675, 157–161. Oades et al. (2010a) *Attention-deficit hyperactivity disorder (ADHD) and glial integrity: S100B, cytokines and kynurenine metabolism-effects of medication.* Behav. Brain Funct. 6, 29. Oades et al. (2010b) *Attention-deficit hyperactivity disorder (ADHD) and glial integrity: an exploration of associations of cytokines and kynurenine metabolites with symptoms and attention.* Behav. Brain Funct. 6, 32.
Schwieler (2016)	"Blood samples were centrifuged [], and plasma was collected and stored at −70 °C until analysis"	"Analysis of KYNA was measured by high-performance liquid chromatography (HPLC) with fluorescence detection." "[For tryptophan, kynurenine and QUIN the] detection was performed using a [] mass spectrometer []."	Olsson et al. (2010) *Elevated levels of kynurenic acid in the cerebrospinal fluid of patients with bipolar disorder.* J Psychiatry Neurosci. 35 (3): 195–9.
Allen (2018)	"Samples were centrifuged immediately and frozen at −80 °C."	"[] analysis on the HPLC system (UV and FLD detection)."	Clarke et al. (2009) *Tryptophan degradation in irritable bowel syndrome: evidence of indoleamine 2,3-dioxygenase activation in a male cohort.* BMC Gastroenterol. 9 (1), 6.
Aarsland (2019)	"[] serum separated and stored at -80 °C until analysis."	"[] liquid chromatography-tandem mass spectrometry."	Midttun et al. (2009) *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): 1371–1379.
Ryan (2020)	"Plasma was stored in aliquots at −80 °C until analysis."	"Liquid chromatography-tandem mass spectrometry"	Midttun et al. (2009) *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): 1371–1379. Midttun et al. (2013) *High-throughput, low-volume, multi-analyte quantification of plasma metabolites related to one-carbon metabolism using HPLC-MS/MS.* Anal. Bioanal. Chem. 405, 2009–2017.
Aarsland (2022)	Not described	"[] liquid chromatography / tandem mass spectrometry."	Midttun et al. (2009) *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): 1371–1379.
Olajossy (2017)	"[] the supernatant was collected and frozen at −72°C."	"The content of KYNA in serum was assessed [] using a Varian Pro Star 210 liquid chromatograph []."	Turski et al (1988) *Identification and quantification of kynurenic acid in human brain tissue.* Brain Res. 454(1–2): 164–169.
Coppen (1973)	"[] samples of the ultra filtrate and plasma were deep-frozen."	"Free and total acid-soluble tryptophan were [] estimated by a modification of the method of Denckla and Dewey." (Spectrofluorometry)	Denckla and Dewey (1967) *The determination of tryptophan in plasma, liver and urine.* J. lab. clin. Med. 69, 160-169.
Abrams (1976)	"The first 8-10 ml of CSF was placed without preservative in a freezer at -20 C within 5 min of removal and was stored until analysis."	"A third aliquot was assayed for TRYP contend using an adaptation of a tissue assay for this aromativ amino acid (Tagliamonte et al., 1971)." (Spectrofluorometry)	Taglimonte et al. (1971) *Effect of psychotropic drugs and tryptophan in the rat brain.* J. Pharmacol. Exptl. Therap. 177: 475.
D'Elia (1977)	Not described	"Analyses of total L-TP concentrations were performed according to Denckla & Dewey (1967) []." (Spectrofluorometry)	Denckla and Dewey (1967) *The determination of tryptophan in plasma, liver and urine.* J. lab. clin. Med. 69, 160-169.

Kirkegaard (1978)	"The EDTA-2Na stabilized plasma was stored at -20ºC."	"[] the ultrafiltration and the determination of total and free tryptophan was carried out as described elsewhere (Møller et al. (1976))." (Spectrofluorometry)	Møller et al. (1976): Plasma amino acids as an index for subgroups in manic depressive psychosis: correlation to effect of tryptophan. Psychopharmacology 49, 205-213. In which "The total acid-soluble and the free TRY were determined according to Denckla and Dewey (1967)"
Whalley (1980)	"Plasma and ultrafiltrate were stored at -20ºC until assayed for total and free tryptophan, respectively."	"Plasma and ultrafiltrate were stored at -20ºC until assayed for total and free tryptophan, respectively." (Spectrofluorometry)	Hess and Udenfreid (1959) *A fluorometric procedure for the estimatin of tryptamine in tissues.* Journal of Pharmacology and Experimental Therapeutics 127, 175-177
Hoekstra (2001)	"Immediately after venipuncture, plasma was prepared [] and stored at -80ºC"	"The amino acids [] were measured by means of high-performance liquid chromatography." (HPLC + fluorescence detection)	Fekkes et al. (1995) *Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated derivatization with o-phthaldialdehyde.* Journal of Chromatography B 669, 177-186.
Stelmasiak (1974)	"The blood was collected into heparinized tubes, centrifuged immediately and the plasma kept frozen at -20°C until it was used for determinations within 1 week."	"Tryptophan was determined using the method of Denckla and Dewey (1967) in whole plasma and also in an ultrafiltrate []." (spectrofluorometry)	Denckla and Dewey (1967) *The determination of tryptophan in plasma, liver and urine.* J. lab. clin. Med. 69, 160-169.
Sawa (1981)	"Immediately after blood collection, blood was centrifuged at 1000 g for 15 min at ice cold, and the plasma was kept frozen at -20°C till measurement."	"The contents of free (non-albumin bound) tryptophan and total tryptophan were determined spectrofluorometrically."	Denckla and Dewey (1967) *The determination of tryptophan in plasma, liver and urine.* J. lab. clin. Med. 69, 160-169.
Mokhtar (1997)	"Serum was prepared within 60-120 min from venesection and was analyzed either freshly or within 2 days of storage at -20°C."	"Free (ultra-filterable) and total (free plus albumin-bound) Trp concentrations were determined fluorimetrically as described previously (Badawy and Evans 1976)."	Badawy AA-B, Evans M (1976): *Animal liver tryptophan pyrrolases - Absence of apoenzyme and of hormonal induction mechanism from species sensitive to tryptophan toxicity.* Biochem J 158:79-88. which uses a modified version of Denckla and Dewey (1967).
Palmio (2005)	"The samples were [] stored at -70°C before the analyses."	"[] high-performance liquid chromatography."	

The information presented in this table is selected excerpts from the reviewed studies' method sections, and not the complete method descriptions.

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