The kynurenine pathway in cognition, dementia, and aging

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Thesis for the degree of Philosophiae Doctor (PhD)
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To my parents,

SCIENTIFIC ENVIRONMENT

This research project was performed at the Department of Internal Medicine at Haraldsplass Deaconess Hospital (HDS), Bergen, Norway. There have been weekly meetings at HDS with my main supervisor, Lasse Melvær Giil, focused on planning studies, statistical analysis, and scientific writing. I have also had frequent meetings with my co-supervisors and these discussions have been a source of inspiration. My co-supervisor, professor emeritus Jan Erik Nordrehaug, has made this project possible through his expertise and vast experience. My co-supervisor, professor emeritus Grethe S. Tell, has contributed much-needed expertise concerning The Hordaland Health Study (HUSK) and epidemiological research. In addition, I have gained knowledge, inspiration, and support from discussions with my fellow researchers and colleagues at HDS. Measurements of kynurenines in serum, plasma, and cerebrospinal fluid and sample handling were facilitated by Bevital AS. Professor emeritus Per Magne Ueland and his laboratory have provided substantial biochemical expertise to conduct these studies, relying on their extensive experience of measuring and studying the metabolites of the kynurenine pathway. This work would not have been possible without both national and international collaboration from the COGNORM, the Dementia Study of Western Norway (DemVest), HUSK, the Melbourne Collaborative Cohort Study (MCCS), and the Western Norway B Vitamin Intervention Trial (WENBIT).

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PREFACE

The kynurenine pathway catabolizes the essential amino acid tryptophan and has been studied since the early part of the 20th century. The neurobiology of the kynurenine pathway was not extensively studied for years as scientists viewed brain tryptophan metabolism mostly through the lens of serotonin. In recent years, research efforts have focused on the ability of kynurenines to influence neurotransmitter systems and modulate the immune system. Essential organs and tissues, including the liver, immune cells, brain, muscle, and the gastrointestinal tract, express the kynurenine pathway's enzymes. Indeed, the kynurenine pathway, induced by inflammatory cytokines, is now implicated in metabolic, cardiovascular, gastrointestinal, psychiatric, and neurological disorders. Despite significant experimental evidence linking the kynurenines to cognition, dementia, and aging, there is a lack of rigorous clinical studies investigating the kynurenines in these areas of research.

ACKNOWLEDGMENTS

First, I wish to thank all the participants, researchers, and staff in the COGNORM, the Dementia Study of Western Norway (DemVest), the Hordaland Health Study (HUSK), the Melbourne Collaborative Cohort Study (MCCS), and the Western Norway B Vitamin Intervention Trial (WENBIT). I gratefully acknowledge the support and generosity of the Norwegian Health Association, Dementia Research Program, without which the present study could not have been completed. Their continuous efforts to help patients with dementia and their caregivers are commendable.

Post-doctor Lasse Melvær Giil has been my main supervisor. Thank you, Lasse, for always supporting and inspiring me to improve and work hard. You have taught me how to work with discipline and methodically day-by-day with a long-term goal in sight. You are always available for discussions and questions. For that, I am very grateful. Your creative mind, combined with statistical insight and expertise, has been invaluable to interpreting our research data.

I am grateful for having had professor emeritus Jan Erik Nordrehaug and professor emeritus Grethe S. Tell as co-supervisors. Thank you, Jan Erik, for all the advice, support and words of encouragement you have given me. You have made this project possible through your excellent input throughout all stages of the process, and I feel privileged for getting the opportunity to learn from you. Thank you, Grethe, for always looking out for me, giving me great advice, and for sharing your expertise and experience with me. I feel honored to get the opportunity to learn from you. I appreciate that you included me in your research group and invited me to participate in meetings, scientific seminars, and social gatherings. Such opportunities have given me insight into the scientific process beyond my doctoral studies and the chance to meet other scientists and expand my network.

I am especially grateful for all the help I have received from professor emeritus Per Magne Ueland. Thank you, Per Magne, for taking the time to teach me how to best present research data in figures and tables and for your significant contributions to this project, particularly regarding the interpretation of biochemical data. Without you, this work would not have been possible. I would also like to thank the Bevital researchers Adrian McCann, Øivind Midttun, and Arve Ulvik, for their work on measurements of kynurenines, C-reactive protein, and neopterin, and their valuable insight, comments, and contributions to these studies. It has been a real pleasure collaborating with you all.

I am grateful to professor Ottar Nygård for providing scientific expertise on cardiovascular disease, biochemistry, and WENBIT, of which he is the principal investigator. Thank you, Ottar, for a great collaboration and valuable feedback on **study I and III**.

Thank you, professor Dag Aarsland, for providing your scientific expertise and experience on clinical dementia research and DemVest, and for your valuable feedback on **study II**. I would also like to thank my colleague, Ragnhild Skogseth, who has been instrumental with data collection for the DemVest study here in Bergen. Thank you, Ragnhild, for always being supportive, encouraging, and available for discussions and questions.

I would like to thank the principal investigator of the COGNORM, Dr. Leiv Otto Watne. Thank you, Leiv Otto, for sharing your scientific insight and facilitating access to cerebrospinal fluid (CSF) data, your words of encouragement and enthusiasm, and for your valuable feedback on **study III**. I would also like to thank, Ane-Victoria Idland and Nathalie B. Halaas, for your impressive work in collecting serum and CSF samples from patients included in COGNORM.

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I feel privileged to work at Haraldsplass Deaconess Hospital, which focuses on geriatric and palliative medicine research. I want to thank my colleagues for supporting me and taking an interest in my work. A special thanks to Christian Alsing, Ida Kristine Sangnes, Irit Titlestad, Marit Stordal Bakken, Katinka Nordheim Alme, Anders Lund, and Guri Fossdal for your friendship, discussions, and for always being supportive.

During my time as a doctoral student, Neuro-SysMed, a center of excellence for clinical research investigating neurological diseases in which Haraldsplass Deaconess Hospital is a partner, was established in Bergen. Its mission is to discover and test new and effective therapies for dementia, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis. I am proud that my research environment is participating in this center.

Finally, I would like to thank Maria Eilertsen, for her patience, understanding, and support during my years as a doctoral student. I am very grateful to my family, and especially my parents, Olaug Hafstad and Vidar Solvang, who have always helped and encouraged me to pursue academia and to believe in myself. They have taught me the value of education, hard work, and always being kind to others. I would also like to thank my late grandparents Asbjørg (1919-2013) and Oddvin Hafstad (1911-2008), for their unconditional support throughout my youth.

ABBREVIATIONS

3-HAO	–	3-hydroxyanthranilic acid 3,4-dioxygenase
α7nAChR	–	α-7 nicotinic acetylcholine receptor
Aβ	–	Amyloid-beta
AA	–	Anthranilic acid
AD	–	Alzheimer's disease
ACMS	–	α-amino-α-carboxymuconic-ω-semialdehyde
ACMSD	–	α-amino-ß-carboxymuconate-ε-semialdehyde decarboxylase
AhR	–	Aryl hydrocarbon receptor
BBB	–	Blood-brain barrier
CDR	–	Clinical Dementia Rating
COWAT	–	Controlled Oral Word Association Test
CRP	–	C-reactive protein
CSF	–	Cerebrospinal fluid
DLB	–	Dementia with Lewy bodies
DST	–	Digit Symbol Test
FE	–	Fixed effects
GFR	–	Estimated glomerular filtration rate
HAA	–	3-hydroxyanthranilic acid
HADS	–	The Hospital Anxiety and Depression Scale

HK	–	3-hydroxykynurenine
IDO	–	Indoleamine 2, 3 dioxygenase
IFN-γ	–	Interferon gamma
IQR	–	Interquartile range
KA	–	Kynurenic acid
KATs	–	Kynurenine aminotransferases
KKR	–	Kynurenic acid to kynurenine ratio
KMO	–	Kynurenine 3-monooxygenase
KOLT	–	Kendrick Object Learning Test
KTR	–	Kynurenine to tryptophan ratio
Kyn	–	Kynurenine
KYNU	–	Kynureninase
LBD	–	Lewy body dementia (group refers to both patients with dementia with Lewy bodies and Parkinson's disease dementia).
MMSE	–	Mini-Mental State Examination
NAD^+	–	Nicotinamide adenine dinucleotide
NMDAR	–	*N*-methyl-*D*-aspartate receptor
NPI	–	Neuropsychiatric Inventory
p	–	p-value
PIC	–	Picolinic acid
PLP	–	Pyridoxal 5'-phosphate

Q	–	Q-value
QA	–	Quinolinic acid
QPRT	–	Quinolinate phosphoribosyl transferase
R_s	–	Spearman's Rho
SD	–	Standard deviation
TDO	–	Tryptophan 2, 3 dioxygenase
Trp	–	Tryptophan
XA	–	Xanthurenic acid

ABSTRACT

Background: Experimental studies implicate the kynurenine pathway in cognitive function, dementia, aging, and longevity. Comparatively, clinical studies are few and most lack comprehensive targeted metabolomic profiling of the kynurenine pathway.

Aims: To investigate associations between circulating kynurenines and cognitive function in older adults (**Study I**) and between kynurenines, cognitive and neuropsychiatric prognosis in mild dementia (**Study II**). Lastly, to assess the relationship between aging and concentrations of metabolites of the kynurenine pathway in blood and cerebrospinal fluid (CSF) using longitudinal cohorts (**Study III**).

Methods: Tryptophan (Trp) and nine kynurenines were measured in serum, plasma and cerebrospinal fluid. Associations between the kynurenines and cognitive performance were estimated using Zellner's regression in community-dwelling older adults (**Study I**, n = 2174), and between the kynurenines, cognitive performance and neuropsychiatric symptoms in patients with mild dementia using a multilevel model (**Study II**, n = 155). In **Study III**, associations between age and the kynurenines were investigated in multilevel models in two longitudinal studies (n = 970 and n = 604), and non-parametrically in a small cohort with CSF samples (n = 109). Associations between the kynurenines and frailty were assessed using regression, mortality using Cox regression, and minor age differences using a multinomial logit model. The results of **studies I** and **II** were adjusted for multiple comparisons.

Main findings: Higher kynurenine to tryptophan ratio (KTR) and neopterin concentrations were linearly associated with lower cognitive test performance, whereas kynurenine (Kyn) had a non-linear, quadratic association with cognitive test performance (**Study I**). The quadratic association between cognitive test performance and Kyn was also present in mild dementia, where higher kynurenic acid to kynurenine ratio (KKR) was further associated with more neuropsychiatric symptoms over time (**Study II**). In **Study III**, the strongest associations between age and the kynurenines were with Kyn, quinolinic acid (QA), and KTR which were positively associated with age and increased the most over time. Trp was inversely associated

with age and decreased over time. Kyn, 3-hydroxykynurenine, kynurenic acid, 3-hydroxyanthranilic acid, QA and KTR were associated with frailty. Higher Trp concentrations were associated with lower all-cause mortality, whereas higher QA and KTR concentrations were associated with higher all-cause mortality in two cohorts of community-dwelling adults. Kyn and QA increased in the CSF over a period of four years and correlated the most with age. Compared to serum concentrations, age was more strongly correlated to CSF concentrations of Kyn and particularly QA.

Conclusions: We found a non-linear relationship between Kyn and cognitive performance in both community-dwelling older adults and patients with mild dementia, where higher and lower Kyn concentrations were associated with poorer cognitive performance. Further, it appears that activation of the kynurenine pathway, reflected by increased KTR, is associated with poorer cognitive performance, aging, frailty, and mortality. However, of the downstream kynurenines, QA showed the strongest association with aging, frailty, and mortality and was more strongly correlated with age in the CSF relative to serum over time. Accordingly, the aging brain could be exposed to a disproportionate increase in the excitotoxic QA. Higher KKR, which may reflect increased kynurenine aminotransferase activity, was associated with more neuropsychiatric symptoms over time.

LIST OF STUDIES

I. Solvang SH, Nordrehaug JE, Tell GS, Nygård O, McCann A, Ueland PM, Midttun Ø, Meyer K, Vedeler CA, Aarsland D, Refsum H, Smith AD, Giil LM. **The kynurenine pathway and cognitive performance in community-dwelling older adults. The Hordaland Health Study.** Brain Behav Immun. 2019 Jan;75:155-162.

II. Solvang SH, Nordrehaug JE, Aarsland D, Lange J, Ueland PM, McCann A, Midttun Ø, Tell GS, Giil LM. **Kynurenines, Neuropsychiatric Symptoms, and Cognitive Prognosis in Patients with Mild Dementia.** Int J Tryptophan Res. 2019 Sep 29;12:1178646919877883.

III. Solvang SH, Hodge A, Watne LO, Cabral-Marques O, Nordrehaug JE, Giles GG, Milne RL, Dugué PA, Nygård O, Ueland PM, McCann A, Idland AV, Midttun Ø, Ulvik A, Tell GS, Giil LM. **Kynurenine pathway metabolites in the blood and cerebrospinal fluid are associated with human aging.** Unpublished manuscript, submitted to Molecular Psychiatry in February 2021.

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INTRODUCTION

Tryptophan (Trp) is mainly metabolized by the kynurenine pathway with minor quantities used to generate serotonin and melatonin. The metabolites of the kynurenine pathway are collectively referred to as the kynurenines. They are involved in antioxidant activity, inflammation, immune regulation, and neurotransmission. Significantly, the kynurenine pathway provides the substrate for nicotinamide adenine dinucleotide (NAD^+, or vitamin B_3) synthesis, a central cofactor of cellular metabolism. [1] Experimental studies have implicated the kynurenine pathway in cognitive function, neurodegeneration, aging, and longevity. [2-6] In contrast, clinical studies are relatively few and have often relied on measuring a few select metabolites of the kynurenine pathway. This thesis aims to fill some of the knowledge gaps outlined below by using comprehensive targeted metabolomic profiling of the kynurenine pathway using data from several cohort studies.

AN OVERVIEW OF KYNURENINE METABOLITES

Tryptophan Metabolism

Trp is an essential amino acid obtained through meat, dairy products, and fruit. [7] Once ingested, Trp is absorbed in the gut, passes into the portal circulation, and reaches the tissues by way of the liver. [1] However, gut microbiota can metabolize Trp to tryptamine, serotonin, kynurenines, and indoles prior to absorption. [7] Trp and the kynurenines can, in turn, influence the enteric nervous system and intestinal motility. [8] Under physiological conditions, the liver degrades around 90% of Trp. Cells take up circulating Trp for synthesis and turnover of proteins. Intracellular proteolysis and protein catabolism partly regenerate Trp for subsequent protein synthesis. [7]

Expression of Kynurenine Pathway Enzymes

The enzymes that catalyze the chemical reactions of the kynurenine pathway are differentially expressed in organs, tissues, and cells. [9] In humans, the enzymes are fully expressed in hepatocytes, antigen-presenting cells, fibroblasts, and vascular

endothelial cells. [9] In the brain, there is a differential expression of enzymes in astrocytes compared to microglia. [10] The kynurenine pathway has two rate-limiting enzymes: indoleamine 2, 3 dioxygenase (IDO) and tryptophan 2, 3 dioxygenase (TDO). Both catalyze the conversion of Trp to kynurenine (Kyn). TDO is mainly expressed in the liver. [11] Compared to TDO, IDO has wider tissue distribution, including cells of the immune system, most significantly antigen-presenting cells such as macrophages and dendritic cells. [12] Further, IDO is expressed in the lung, intestine, placenta, kidney, liver, and brain. [9] Skeletal muscle also metabolizes Trp through the kynurenine pathway. [1] The contribution of extrahepatic Trp metabolism along the kynurenine pathway is relatively minor (5-10%) under physiological conditions. However, this becomes more significant following activation of the immune system. [13]

THE BIOCHEMISTRY OF THE KYNURENINE PATHWAY

Rate-Limiting Enzymes

IDO and TDO are members of the heme-dependent family of enzymes. Specifically, the heme dioxygenase enzymes which uniquely use heme for catalysis. [14] The enzymes are functionally very similar but structurally different and are thus considered autologous enzymes. IDO and TDO have likely evolved independently, as gene duplication has not been identified. [15] Several IDO homologs have been identified in different species, but IDO1 and IDO2 are the main homologs expressed in humans. [9] These homologs are encoded on genes adjacent to each other, suggesting that the homologs arose from gene duplication. [16]

Metabolism of Tryptophan by the Kynurenine Pathway

The kynurenine pathway degrades Trp by several enzymatic reactions to quinolinic acid (QA). [11] Briefly, the metabolic pathway that gives rise to QA starts with the formation of Kyn from Trp by way of the intermediate metabolite N-formylkynurenine. Next, Kyn gives rise to 3-hydroxykynurenine (HK), which is converted to hydroxyanthranilic acid (HAA). α-amino-α-carboxymuconic-ω-

semialdehyde (ACMS) is generated from HAA by the oxidoreductase enzyme 3-hydroxyanthranilic acid 3,4-dioxygenase (3-HAO), and subsequently converted to either picolinic acid (PIC), or QA. For conceptual purposes, the kynurenine pathway can be divided into the main pathway and branches from the main pathway, where Kyn and HK give rise to kynurenic acid (KA), anthranilic acid (AA), and xanthurenic acid (XA). Figure 1 on the next page shows a summary of the kynurenine pathway.

In the main pathway, the first step of the kynurenine pathway is the oxidation of Trp to N-formylkynurenine by either IDO or TDO. [17] Kynurenine formamidase, a hydroxylase, catalyzes the hydrolysis of N-formylkynurenine to Kyn, [18] a pivotal metabolite of the kynurenine pathway. Kynurenine 3-monooxygenase (KMO), an oxidoreductase, catalyzes the conversion of Kyn to HK using oxygen and nicotinamide adenine dinucleotide phosphate (NADPH). [19] Kynureninase (KYNU), belonging to the family of aminoreductases, catalyzes the conversion of HK to HAA. [20]
3-HAO catalyzes the conversion of HAA and O_2 to ACMS. In the main pathway, ACMS is spontaneously converted to QA. [10] However, α-amino-ß-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD), a zinc-dependent amidohydrolase, [21] preferentially catalyzes the conversion of ACMS to PIC. Saturation of ACMSD shifts the conversion of ACMS towards QA. [22] Quinolinate phosphoribosyl transferase (QPRT) is a glycosyltransferase enzyme that catalyzes the formation of nicotinic acid mononucleotide from QA and 5-phosphoribosyl-1-pyrophosphate, fueling NAD^+ synthesis. [10]

There are primarily three important branches; First, Kyn can be metabolized to KA, by four kynurenine aminotransferases (KATs). [23] Second, KYNU can convert Kyn to AA. [10] Third, KATs can convert HK to XA.

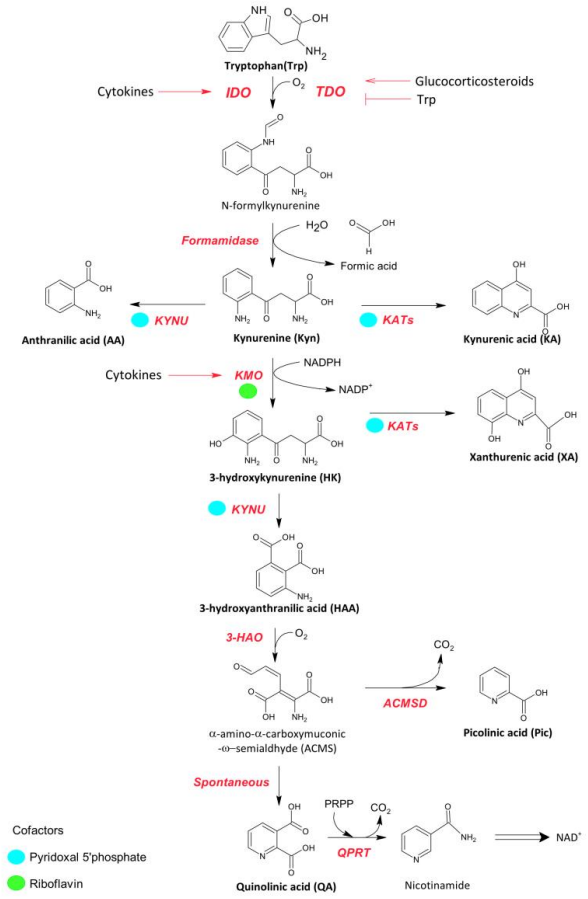

Figure 1. The Kynurenine Pathway. Trp is oxidized to N-formylkynurenine by the rate-limiting enzymes indoleamine 2, 3 dioxygenase (IDO) or tryptophan 2, 3 dioxygenase (TDO). Formamidase catalyzes the formation of Kyn from N-formylkynurenine. Kynurenine 3-monooxygenase (KMO) metabolizes Kyn to HK, which is converted to HAA by kynureninase (KYNU). ACMS is formed from HAA, catalyzed by 3-hydroxyanthranilic acid 3,4-dioxygenase (3-HAO). ACMS converts spontaneously to QA, which is further metabolized by the action of quinolinate phosphoribosyl transferase (QPRT) and several intermediary steps to nicotinamide adenine dinucleotide (NAD^+). AA is produced from Kyn by KYNU. Kynurenine aminotransferases (KATs) generate KA from Kyn and XA from HK. α-amino-ß-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) converts ACMS to the intermediary metabolite 2-aminomuconic-6-semialdehyde (not shown), which is spontaneously converted to picolinic acid (Pic). Adapted from Schwarcz et al[10].

Regulators of Enzyme Activity in the Kynurenine Pathway

Tryptophan Availability

Under physiological conditions, the activity of the kynurenine pathway is mostly determined by plasma free Trp. Generally, 90-95% of Trp in the bloodstream is bound to albumin, with 5-10% in an unbound state. While Trp induces TDO, Kyn acts as an allosteric inhibitor, resulting in a negative feedback loop. [9]

Glucocorticosteroids

Glucocorticosteroids increase in response to physiological stressors [24] and induce TDO expression by acting on glucocorticoid-responsive elements of the TDO gene. [25]

Pro-Inflammatory Cytokines

Cytokines can be broadly classified as pro-inflammatory or anti-inflammatory. IDO is mainly activated by the pro-inflammatory cytokine interferon-γ (IFN- γ). [26] However, other pro-inflammatory cytokines such as interferon-α, [27] tumor-necrosis factor-α, interleukin-1β, and interleukin-2 can activate IDO to a lesser degree. Anti-inflammatory cytokines (interleukin-4, interleukin-10, and transforming growth factor-β) inhibit IDO induction by IFN-γ. [28] Thus, the balance between pro- and anti-inflammatory cytokines is of importance to IDO-activity. [27]

Enzymatic Cofactors of the Kynurenine Pathway

Pyridoxal 5'-phosphate

The active form of vitamin B_6, pyridoxal 5'-phosphate (PLP), is an enzymatic cofactor for transferring biological amines in multiple metabolic pathways. [29] Including KATs and KYNU, the two aminotransferase enzymes of the kynurenine pathway. [30]

Riboflavin

Riboflavin (vitamin B_2) is a water-soluble vitamin present in milk, meat, fish, fruit, and vegetables, and its biologically active forms are involved in redox reactions. The activity of the oxidoreductase enzyme KMO can be decreased in riboflavin deficiency, as suggested by a ten-fold decrease in HK and a two-fold increase in KA in riboflavin-deficient baboons. [31]

IMMUNOMODULATION BY KYNURENINE METABOLITES

Experimental evidence suggests that the kynurenine pathway is not only induced by cytokines but has immunomodulatory and immunosuppressive actions. [32,33] IDO contributes to immune regulation by three main mechanisms. First, by acting as a signaling molecule influencing nutrient-sensing systems. Second, by depleting Trp, which activates amino-acid-sensing signal transduction pathways. Third, by producing Kyn, which acts as a natural ligand for the aryl hydrocarbon receptor (AhR), a transcription factor that inhibits immune responses. [2] By way of downstream signaling pathways, this suppresses $CD8^+$ and $CD4^+$ T-cells and stimulates regulatory T-cells (Tregs), promoting resolution of inflammation. [9] IDO deficient mice do not develop spontaneous autoimmune diseases. However, IDO inhibition reduces acquired tolerance to new antigens. [34] For example, pharmacological inhibition of IDO results in the rejection of allogenic fetuses in mice. [35]

KYNURENINE METABOLISM IN THE BRAIN

The enzymes of the kynurenine pathway are expressed in the brain, with some variation between brain regions. [10] In the brain, IDO- and TDO-expression levels are relatively low. [36] Approximately 60% of brain kynurenine metabolism stems from circulating Kyn [37] which readily crosses the blood-brain barrier (BBB) and enters glial cells. [38]

The Blood-Brain Barrier

The BBB restricts the influx of most compounds from the blood to the brain, generating an optimal internal milieu for neurotransmission. [39] Trp, Kyn, and HK cross the BBB by way of the large amino acid transporter. Conversely, KA and QA do not cross the BBB due to their polarity and lack of transporters. [40] Experimentally, extracellular KA and QA can be detected after intracerebral injections of Kyn and HAA, respectively. [41,42] KA and QA are cleared from the brain interstitial fluid by cellular uptake. [9,43]

Cellular Compartmentalization

The enzymes of the kynurenine pathway are differentially expressed in cells of the brain. Astrocytes express KAT enzymes, but not KMO, and account for the biosynthesis of KA. [44] In contrast, microglia have a much lower expression of KATs, [45] but express KMO and generate HK, further converted to downstream kynurenines such as QA. Please see Figure 2 on the next page for a summary. After synthesis, both KA and QA are released into the extracellular space to affect their neuronal targets. [10] HK and PIC are synthesized in neurons. [46] In addition, oligodendrocytes express KYNU, KMO, 3-HAO, and QPRT, but not IDO or KATs. [47]

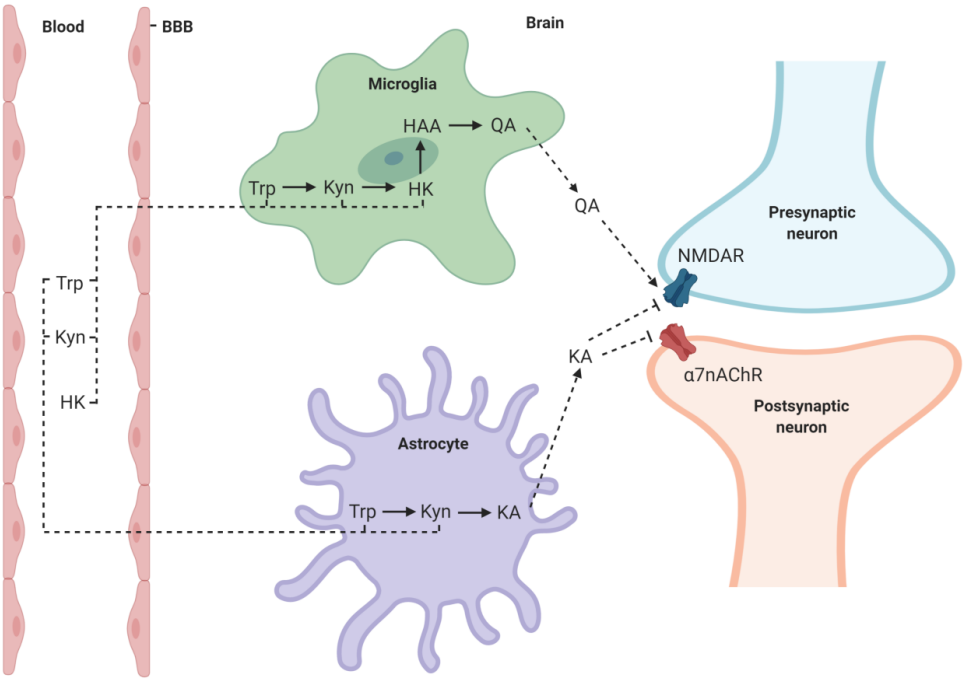

Figure 2. Kynurenines and the blood-brain barrier. Tryptophan (Trp), kynurenine (Kyn), and 3-hydroxykynurenine (HK) cross the blood-brain barrier (BBB), where kynurenic acid (KA) is mainly synthesized in astrocytes and quinolinic acid (QA) in microglial cells. Kyn is considered the primary precursor of kynurenines in the brain. After synthesis, QA is released into the extracellular space to affect the *N*-methyl-*D*-aspartate receptor (NMDAR) as an agonist, whilst KA acts as an antagonist on the NMDAR, and at the α-7 nicotinic acetylcholine receptor (α7nAChR). Adapted from Schwarcz et al[10].

Neuroactivity

Some kynurenines display neuroactive properties. KA is an antagonist of the the *N*-methyl-*D*-aspartate receptor (NMDAR), whilst QA is an NMDAR agonist. [48] Additional receptors where antagonist activity has been reported for KA include α-7 nicotinic acetylcholine receptor (α7nAChR), the kainate receptor, and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor. However, KA has the highest affinity for the glycine co-agonist site of the NMDAR. [9] Increased concentrations of QA may disrupt glutamatergic transmission in NMDA-expressing neurons, induce apoptosis in astrocytes, and amplify neuroinflammation. [49]

Experimental studies in rats suggest that KA is neuroprotective in brain ischemia and seizures by reducing excitotoxicity. [11] In contrast, intraventricular injection of QA produces convulsions in mice. [50] Further, QA injection into the prefrontal cortex of mice leads to cognitive and behavioral impairment and reduces hippocampal neuroplasticity. [51]

THE KYNURENINES IN GERIATRIC MEDICINE

Clinical and experimental studies, mostly focused on Trp, Kyn, and KA, have identified associations between the kynurenine pathway and cognitive function, neurodegenerative disorders, and aging. These are major aspects of geriatric medicine. Notably, psychosis and mood disorders are prevalent in elderly patients, often secondary to neurological disease, and are in the context of brain disease referred to as neuropsychiatric symptoms. A number of high-quality studies on younger individuals have identified altered kynurenine pathway metabolite levels in blood, cerebrospinal fluid (CSF) and post-mortem brain samples from patients with schizophrenia and bipolar disorder with psychotic symptoms. [52] This highlights the need to investigate whether the kynurenines are associated with the development of neuropsychiatric symptoms.

Cognitive Function

Results from experimental studies investigating acute inflammation suggest that the kynurenine pathway may be a mediator of inflammation-related cognitive impairment. In rats, IDO-inhibition prevented sepsis-induced cognitive impairment after cecal ligation and perforation. [3] In line with this, IDO knockout prevented cognitive impairment in mice following lipopolysaccharide injection. [5]

Two cross-sectional studies have investigated the potential association between cognitive test performance and concentrations of metabolites of the kynurenine pathway in blood in patients without neurocognitive disorders (see Table 1 on the next page). However, both studies included small clinical populations with either severe cardiovascular disease [53] or renal failure, [54] conditions which could affect circulating

levels of the kynurenines. Due to the neuroactivity of the kynurenines, experimental links to cognitive function, and immunomodulatory actions, there is a need to investigate the relationship between kynurenine metabolites and cognition in cohorts more representative of the general population. Notably, all metabolites are regulated around physiological concentrations, and thus non-linear associations could be present. However, the studies to date have focused on linear relationships.

Table 1. Cross-sectional Studies on Kynurenines and Cognitive Function in Persons without Brain Disease

Author (year)	Sample size	Population	Fluid	Measures	Main findings
Forrest et al (2011) [53]	56	Cardiac bypass surgery/ thoracic surgery	Serum	KA, Kyn, KTR, neopterin	Higher concentrations of KA, Kyn, KTR, and neopterin were associated with lower cognitive performance.
Karu et al (2016) [54]	27	Stage IV renal failure	Serum	KA, Kyn, QA, XA, neopterin	Higher concentrations of KA were associated with lower cognitive function.

Abbreviations: KA, kynurenic acid; Kyn, kynurenine; KTR, kynurenine to tryptophan ratio; QA, quinolinic acid; XA, xanthurenic acid.

Psychiatric Disease and Behavioral Impairment

The kynurenine pathway has been linked to major psychiatric disorders in patients without organic brain disease. [12] Post-mortem and CSF studies of schizophrenia show elevated concentrations of KA[55] with similar findings in bipolar disorder. [52] Higher Kyn concentrations have also been linked to schizophrenia. [52] Higher KTR and lower KA plasma concentrations are also associated with depression. [56] In addition, patients who attempted suicide display higher QA and lower KA in plasma and CSF. [57] Mice with reduced NMDAR expression display severe behavioral abnormalities reminiscent of schizophrenia and autism. [58] In contrast, chronic excitotoxicity is linked to neurodegenerative disorders. [59] Despite these clinical and experimental findings,

investigations into a potential role of the kynurenines in relation to the pathophysiology of neuropsychiatric symptoms observed in patients with organic brain disease are yet to be undertaken.

Neurodegenerative Disease Leading to Dementia

Several cross-sectional studies have investigated possible differences in circulating Trp and other kynurenines in patients with dementia compared to controls, focusing on Alzheimer's disease (AD), the most common cause of dementia. These studies are summarized in Table 2 on the next page and have generally found lower Trp concentrations in AD patients compared to controls. However, the findings regarding concentrations of downstream kynurenines have been inconsistent. Plasma HAA, XA, and QA were lower in histopathologically confirmed AD. [60] However, Gulaj et al[61] found lower plasma KA and higher QA in patients with AD. KA concentrations in CSF were not significantly altered in patients with dementia with Lewy bodies (DLB) compared to controls. [62]

The kynurenine pathway is linked to both underlying inflammation, immunomodulation, and potential activity at the NMDAR, all of which are hypothesized to play a role in cognitive deterioration in dementia. [63] Several experimental studies have investigated the possible relationship between the kynurenine pathway and dementia. For example, IDO inhibition in AD knockin mice was related to less neurodegeneration and improved cognitive performance. [4] Previous clinical studies have identified that higher concentrations of QA are associated with lower cognitive function in elderly patients with AD. [60,61] Despite these clinical and experimental studies, there have been no investigations into whether kynurenine metabolites can predict longitudinal cognitive outcomes in patients with dementia.

Neuropsychiatric symptoms are highly prevalent in dementia and have an adverse impact on patients' quality of life and cognitive prognosis. [64] Notably, patients often have a range of neuropsychiatric symptoms such as psychotic symptoms, aggression, disinhibition, depression, anxiety, and aberrant motor behavior. [65] However, the pathophysiology that leads some patients with dementia to develop neuropsychiatric

symptoms whereas others do not is unclear. Studies on patients with bipolar disorder and schizophrenia have found higher KA concentrations, suggesting that the kynurenine pathway may be related to psychiatric disease with psychotic symptoms. [52] Thus, investigations into the potential role of the kynurenine pathway in both cognitive prognosis and neuropsychiatric symptoms in patients with dementia are warranted.

Table 2. Cross-sectional Studies on Kynurenines Comparing Patients with Dementia to Controls

Author (year)	Sample size	Population	Fluid/Tissue	Measures	Main findings
Heyes et al (1992) [66]	39 AD, 30 Ctrls	AD patients vs. Ctrls	Cerebrospinal fluid	Trp, Kyn, KA, QA	KA concentrations were lower in AD patients compared to Ctrls.
Baran et al (1999) [67]	11 AD, 13 Ctrls	AD patients vs. Ctrls	Brain tissue	Kyn, KA, HK, PLP	KA was lower in the caudate and putamen of AD patients compared to Ctrls.
Widner et al (2000) [68]	21 AD, 20 Ctrls	AD patients vs. Ctrls	Serum	Trp, Kyn, KTR	Trp was lower, and Kyn and KTR were higher in AD patients compared to Ctrls.
Hartai et al (2007) [69]	28 AD, 13 Ctrls	AD patients vs. Ctrls	Plasma/Red blood cells	Kyn, KA	KA concentrations were lower in AD patients compared to Ctrls.
Gulaj et al (2010) [61]	34 AD, 18 Ctrls	AD patients vs. Ctrls	Plasma	Trp, Kyn, HK, KA, AA, QA	Trp and KA were lower, and QA higher in AD patients compared to Ctrls.
Wennström et al (2014) [62]	19 AD, 18 DLB, 20 Ctrls	AD, DLB vs. Ctrls	Cerebrospinal fluid	KA	KA concentrations were not altered in either AD or DLB patients compared to Ctrls.
Giil et al (2017) [60]	65 AD, 65 Ctrls	AD patients vs. Ctrls	Plasma	All[a], PLP, neopterin	Trp, HAA, XA, and QA concentrations were lower in AD patients compared to Ctrls.
Jacobs et al (2019) [70]	20 AD, 18 Ctrls	AD patients vs. Ctrls	Plasma / Cerebrospinal fluid	All[a], neopterin, p-tau, t-tau	Plasma Kyn and PIC inversely correlated with CSF p-tau and t-tau. Higher HK/Kyn ratio correlated with CSF p-tau and t-tau.

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; Ctrls, controls; DLB, dementia with Lewy bodies; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; Kyn, kynurenine; KTR, kynurenine to tryptophan ratio; PIC, picolinic acid; PLP, pyridoxal 5'-phosphate; QA, quinolinic acid; Trp, tryptophan; XA, xanthurenic acid.

[a] HAA, HK, KA, Kyn, PIC, QA, Trp, XA.

Aging and Immunosenescence

The chronic low-grade inflammation of aging, called "inflammaging" is likely to activate the kynurenine pathway with increasing age, [2] as aging is associated with increased interferon gamma (IFN-γ) and other pro-inflammatory cytokines. [71] Indeed, multiple cross-sectional studies suggest that this occurs. [72-80] C-reactive protein (CRP) is a commonly used biomarker to study inflammaging. [81] The kynurenines are immunoactive and could participate in the broader process of immunosenescence, characterized by a dysregulated immune system with vulnerability to infections, decreased self-tolerance, and reduced cancer surveillance. [9] The kynurenine pathway is also associated with adverse age-related outcomes, such as myocardial infarction, [82] cancer, [83] frailty, [84] type II diabetes, [85] and obesity. [86] Due to this, and their capacity for immune regulation, the kynurenines have been proposed as biomarkers of immunosenescence. [2]

As proposed by Ingram et al[87], a biomarker of aging should show 1) significant cross-sectional correlation with age and 2) significant longitudinal change with age consistent with the cross-sectional correlation. It should also display significant alterations with small age changes/increments.

Table 3 on the next page summarizes previous studies that have investigated metabolism and the kynurenines in aging. All studies have used a cross-sectional design. The studies demonstrate cross-sectional correlation between chronological age and kynurenine pathway metabolites in serum/plasma and CSF, suggesting a relationship with human aging. However, this is not completely clarified as the absence of longitudinal studies makes it difficult to rule out a cohort effect. [88] Moreover, most studies in Table 3 have focused on only a few select metabolites. Other studies have focused on select groups such as women or clinical populations, further limiting their external validity. [89,90] Taken together, longitudinal investigations into altered concentrations of kynurenine metabolites in human aging are warranted. Ideally, such investigations should include comprehensive measurements of kynurenine pathway metabolites in multiple cohorts that include community-dwelling

persons. Further, due to the neuroactive properties of some kynurenine metabolites, CSF sampling would be of value.

Table 3. Cross-sectional studies on Tryptophan and Kynurenines in Human Aging

Author (year)	Sample size	Population	Fluid	Metabolites	Main findings
Frick et al (2004) [91]	43	Healthy persons 34-93 years old	Serum	Trp, Kyn, neopterin	KTR and neopterin concentrations were positively correlated with older age.
Kepplinger et al (2005) [80]	27	Acute headache patients	CSF Serum	KA	CSF-KA concentrations were positively correlated with age.
Coggan et al (2009) [79]	241	Suspected meningitis patients	CSF	PIC	PIC was positively correlated with age.
Capuron et al (2011) [78]	284	Non-institutionalized persons >65 years old	Serum	Trp, Kyn, neopterin	Trp was inversely associated with age, whilst Kyn, KTR, and neopterin were positively associated with age.
Yu et al (2012) [77]	2886	Persons without metabolic diseases	Serum	Trp	Trp was inversely associated with age.
Collino et al (2013) [76]	396	Centenarians, off-spring of centenarians	Serum	Trp	Trp was inversely associated with age.
Theofylaktopoulou et al (2013) [75]	7052	Community-dwelling persons born during 1925-27 and 1950-51	Plasma	Trp, Kyn, HK, KA, AA, HAA, XA, CRP, and neopterin	Kyn, KA, AA, HK, KTR, and neopterin were higher in the older- versus middle-aged participants.
De Bie et al (2015) [74]	49	Healthy women 20-90 years old	CSF	Trp, Kyn, HK, HAA, KA, PIC, QA, neopterin	Trp and HK were inversely correlated with age. KTR, PIC, and QA were positively correlated with age.
Rist et al (2017) [73]	301	Healthy adults 18-80 years old	Plasma	Trp	Trp concentrations were inversely associated with age.
Ramos-Chávez et al (2018) [72]	77	Women over 50 years old with normal cognitive function	Serum	Trp, Kyn, HK, KA.	Trp was inversely associated with age, whilst the KA/Trp and HK/Trp ratios were positively associated with age.

Abbreviations: AA, anthranilic acid; CSF, cerebrospinal fluid; CRP, C-reactive protein; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine to tryptophan ratio; Kyn, kynurenine; PIC, picolinic acid; QA, quinolinic acid; Trp, tryptophan; XA, xanthurenic acid.

THE RATIONALE FOR THE THESIS

There is substantial experimental evidence linking the kynurenine pathway to aging, cognitive function, and dementia. However, there are gaps in the current knowledge. First, there have been no large community-based studies on relations between kynurenines and cognitive function. Second, there have been no longitudinal studies investigating whether the kynurenines predict cognitive and neuropsychiatric prognosis in dementia. Third and finally, due to the hitherto cross-sectional nature of the aging studies on kynurenines among selected populations with limited metabolite measurements, it is not clear whether and how aging may impact the kynurenine pathway. [72,73,75-78,91-93]

AIMS OF THE STUDIES

1. To investigate cross-sectional associations between the kynurenines and cognitive function in community-dwelling older adults.
2. To assess longitudinal associations between kynurenines, and cognitive prognosis in patients with mild dementia.
3. To determine whether these associations (aims 1 and 2) were linear or non-linear.
4. To assess longitudinal associations between kynurenines, and neuropsychiatric symptoms in patients with mild dementia.
5. To assess the relationship between the kynurenine pathway and human aging using several cohorts, including longitudinal studies with repeated metabolite measurements in the blood.
6. To assess whether the kynurenine pathway is altered in the cerebrospinal fluid with aging and whether this is different from serum.
7. To assess associations between kynurenines, frailty, and all-cause mortality.
8. To compare kynurenine metabolites to CRP as aging biomarkers.

METHODS

PARTICIPANTS IN THE STUDY AND CASE DEFINITIONS

Study I

The Hordaland Health Study (HUSK) is a community-based health survey conducted during 1992/93 and 1997/99 (https://husk-en.w.uib.no/). The principal aim of HUSK was to investigate lifestyle epidemiology and chronic diseases such as cancer, cardiovascular disease, osteoporosis, anxiety, depression, obesity, and diabetes. HUSK is an interdisciplinary collaborative study, which includes subprojects targeting psychosocial health, occupational medicine, musculoskeletal diseases, and cognitive function. Significantly, most participants donated blood samples to a biobank, including plasma and whole blood in 1997/99. The study focused on persons born during 1925-27 and 1950-52, of which we have included solely the oldest group where a large subgroup underwent cognitive testing. [94]

In 1997/99, 4338 community-dwelling older adults born in 1925-27, residing in Hordaland County, who had participated in 1992/93, were invited by mail to participate in a follow-up study. In all, 3328 older adults (76.7%) agreed to participate. The invitational letter included a self-administered questionnaire on, among other questions, education, smoking habits, use of medications, physical exercise, alcohol consumption, and history of angina pectoris, myocardial infarction, stroke, phlebitis, thrombosis, diabetes, and hypertension. Additionally, the participants answered the Hospital Anxiety and Depression Scale (HADS), designed to assess symptoms of anxiety and depression. Further, a comprehensive self-administered food-frequency questionnaire to assess habitual food intake was included. Study staff collected blood samples at the clinical examination, and recorded height, weight, waist and hip circumferences, upper-arm circumference, and blood pressure. [95]

For the cognitive sub-study, 2841 individuals born in 1925-27 were invited to participate. The selection of these participants was based on their residence as the time-consuming nature of the cognitive test battery would be challenging for participants undertaking longer journeys to the study center. The included participants resided in the city of Bergen and three immediate surrounding municipalities. Participants were invited by letter and 2197 participants (77.3%) agreed to participate in the cognitive sub-study. Trained nurses at the study center administered a standardized cognitive test battery. Altogether, 2174 participants who underwent cognitive testing also had available blood samples and are included in our study.

Study II

The Dementia Study of Western Norway (DemVest) is a longitudinal cohort study of patients with mild dementia from multiple centers in Western Norway. [96] The principal aim of the study was to characterize biomarkers and disease progression in mild dementia focusing on Alzheimer's disease (AD) and, in particular, DLB. Our study included 155 patients recruited from specialist clinics of neurology and old age psychiatry in Hordaland and Rogaland County who had available blood samples for metabolite measurements. These participants were recruited from 2005 to 2007. Dementia was defined according to the Diagnostic and Statistical Manual of Mental disorders, version four (DSM-IV). Patients were diagnosed with mild dementia using the following criteria: a Mini-Mental State Examination (MMSE) test score equal to or above 20 or a Clinical Dementia Rating (CDR) no higher than one. Patients with dementia due to Parkinson's disease were also included. As DLB and Parkinson's disease dementia have similar pathophysiology, they were classified together as Lewy body dementia (LBD). [96]

The study applied the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) criteria to diagnose AD, [97] and the revised consensus criteria for DLB (2005). [98] Consensus meetings regarding the diagnosis were held at baseline and after five years, using all available information. For the subgroup of patients who

consented to an autopsy, the diagnosis was revised following the neuropathological examination, as described previously. [99] A physician interviewed the patient alongside a caregiver, reviewed electronic health records, and performed a clinical and neurological examination.

At baseline, cognitive function and dementia severity were assessed using the MMSE, CDR, and a standardized neuropsychological test battery, which have been described previously. [96] The MMSE and the Neuropsychiatric Inventory (NPI) were assessed at baseline and annually until death or dropout. However, most patients reached a zero score on the MMSE after five years of follow-up. Information regarding neuropsychiatric symptoms becomes challenging to evaluate in end-stage dementia. Thus, we censored the data after the fifth annual examination to avoid floor effects. [96]

Study III

To investigate changes in kynurenines with human aging, we included data from four independent cohorts: two with repeated plasma measurements of kynurenines, the HUSK cohort of community-dwelling persons, and a cohort of CSF donors with repeated CSF measures in a subgroup.

Melbourne Collaborative Cohort Study

The Melbourne Collaborative Cohort Study (MCCS) was started in the 1990s to prospectively investigate the impact of diet and lifestyle on the development of cancer and other non-communicable diseases. [100] Study participants were primarily identified using electoral enrollment (compulsory) and phone directories. Advertisements and community announcements were used to recruit non-citizens. [100] The participants received an invitational letter and were sampled from 1990 to 1994 using the following criteria: Australian born residents and Greek-born or Italian-born migrants aged 40-69 years old at recruitment. Migrants were overrepresented to increase genetic variation and extend the number of lifestyle exposures. At the start of the study, participants filled out questionnaires concerning lifestyle (alcohol consumption, diet, physical activity, and smoking), demographics, and self-reported medical history.

Altogether, 67% of the participants provided fasting blood samples, while 33% donated non-fasting blood samples. Most of the measurements and blood samples gathered at baseline were repeated for 26 824 participants during the first follow-up conducted from 2003 to 2007. A subset of 970 participants with repeated measurements of the kynurenines in plasma (baseline, and follow-up after a median of eleven years) were included. All study participants provided informed consent according to the Declaration of Helsinki. [100] Vital status was ascertained through linkage of the cohort to the Victorian Registry of Births Deaths and Marriages through the Victorian Cancer Registry and the National Death Index through the Australian Institute of Health and Welfare.

Hordaland Health Study

Community-dwelling older adults (n = 3136) aged 71-74 years old with non-fasting plasma samples and mortality data from HUSK were included. [94] In addition, a subset of 1691 participants who underwent cognitive testing with available non-fasting plasma samples were included for the construction of a frailty index (Please see METHODS; Study I p. 33 for the full description of the cohort).

Western Norway B Vitamin Intervention Trial

The Western Norway B Vitamin Intervention Trial (WENBIT) is a prospective, double-blind, placebo-controlled secondary prevention study investigating the clinical effects of B vitamin intervention. [101] The study included patients who underwent coronary angiography for suspected coronary artery disease. The 3090 study participants were recruited at Haukeland University Hospital, Norway (January 2000 – April 2004), and Stavanger University Hospital, Norway (September 2000 – April 2004). [101] All participants underwent a routine clinical interview and examination before coronary angiography at the study baseline. Exclusion criteria were unavailability for follow-up, participation in other trials, known alcohol abuse, cancer, or severe mental illness. The participants were randomized to one of four arms: 1) vitamin B_6, 2) vitamin B_{12}/folate, 3) B_{12}/folate/B_6, and 4) placebo supplementations. Participants from the placebo group (n = 604) with repeated non-fasting plasma

samples were included in the analyses. Follow-up visits were scheduled at one month, one year, and a final study visit. As the aim of the current study was to investigate age-related changes in kynurenines, the one-month follow-up was excluded. The mean time to the final study visit was three years after baseline for the placebo group. Thus, repeated measurements of Trp, the kynurenines, and CRP were available from baseline, one-year, and three-year follow-up. Each visit involved an interview, a clinical examination, and blood sampling. Participants unable to attend visits underwent a telephone interview or answered a questionnaire sent by mail. [101]

Elective Surgery Cohort

The elective surgery cohort on CSF from Cognitively Normal Persons (COGNORM) is a collaborative study between Oslo University Hospital and Diakonhjemmet Hospital, Oslo, aiming to assess CSF and magnetic resonance imaging (MRI) biomarkers in cognitively healthy persons. [102] The participants were scheduled for elective surgery (gynecological, orthopedic, or urological) and CSF was sampled at the onset of spinal anesthesia. [102] It included 172 participants (≥65 years) during 2012 and 2013. [103] The term "cognitively healthy" was defined as scoring >27 points on the MMSE and not being referred to a memory clinic. Patients were also tested using a neuropsychological test battery. Based on this, they were still defined as cognitively healthy if the MMSE was ≤ 27 points and just one other test score was more than 1.5 standard deviations (SD) outside the age, education, and sex-based normative value. Exclusion criteria were a history of stroke with sequela and other neurodegenerative diseases affecting cognitive function. In the current study we included 109 patients who had available CSF and serum samples at baseline, and 33 patients volunteered to provide a second CSF sample four years after baseline. [102]

ETHICS

Study I

The HUSK study was approved by The Regional Committee for Medical and Health Research Ethics (REK approval no.: 2016/2208). Participants provided written consent to participate in the study.

Study II

The DemVest study was approved by The Regional Committee for Medical and Health Research Ethics (REK approval no.: 2010/33). Participants provided written consent after a detailed explanation of the study procedures were explained in the presence of a caregiver.

Study III

HUSK (REK approval no.: 2016/2208), WENBIT (REK approval no.: 2013/2022), and COGNORM (REK approval no.: 2011/2052) were approved by The Regional Committee for Medical and Health Research Ethics in Norway. The MCCS was approved by the Human Research Ethics Committee of the Cancer Council Victoria.

PSYCHOMETRICS AND CLINICAL SCORING SYSTEMS

Study I: Cognitive Performance and Depressive Symptoms

Ceiling effects were identified in both a brief version of the MMSE and Block-Design (***Study I:*** *Supplementary Figure 1*). This implies that cognitive function was not accurately measured in participants who reached the ceiling effects. Further, the Trail Making Test A displayed a log-normal distribution with a bimodal trend. These three cognitive tests were therefore considered unsuitable as measurements of variation in normal cognitive function.

Three cognitive tests were normally distributed (***Study I****: Supplementary Figure 2*), indicating an appropriate difficulty level with a centralized mean, and were selected to describe cognitive function in community-dwelling older adults: The Controlled Word Association Test (COWAT), Digit Symbol Test (DST), and Kendrick Object Learning Test (KOLT). The COWAT is considered a measure of language, memory, and executive function. The test encourages participants to write as many words as possible, beginning with a given letter, in 60 seconds. [104] The DST is considered to measure executive function. To perform the DST, the participant is given a single sheet of paper and an assignment to match symbols to numbers according to a key located on the top of the page. [105] The participant copies the symbol into spaces below a row of numbers. The number of correct symbols within the allowed time, usually 90 to 120 seconds, constitutes the score. [106] Lastly, KOLT measures immediate recall and requires participants to observe picture charts before telling the examiner what they observed. [107] KOLT is considered valid and reliable in a broad range of older persons: community-dwelling, depressed patients, patients with dementia, and institutionalized elderly. [108]

Depression is independently associated both with cognitive function and with kynurenine concentrations, and may therefore act as a confounder in the relation between the two.[109] We assessed depressive symptoms using the HADS, a screening tool for mood disorders. [110] HADS includes fourteen statements on emotions and feelings, with seven items in each of the subscales on anxiety and depression. This

scoring system ranges from zero to three, indicating probable absence, possible presence, and the probable presence of an anxiety or mood disorder. The maximum score is 42, with a higher score indicating probable/possible mood disorder. [110] We defined a score of ≥ 8 as indicative of mild depressive symptoms, in accordance with Stern et al[110]. The sensitivity and specificity for HADS are both approximately 0.80. It performs reasonably well in assessing symptom severity and caseness for depression and anxiety disorders in somatic, psychiatric, and primary care patients and in the general population. [111]

Study II: Longitudinal Evaluation of Cognitive Performance

The MMSE has a maximum score of 30, a minimum score of zero, and consists of multiple questions grouped into seven categories. These include orientation to time, orientation to place, registration of three words, attention and calculation, recall of three words, language, and visual construction. These are summed to give a global cognitive score. [112] The MMSE's simplicity is an advantage in longitudinal studies of dementia, as patients are typically unable to perform more complex neuropsychological tests as the disease progresses. The annual decline in MMSE score in patients with dementia is around three points, and a two to four points reduction is considered a reliable change. [113] Antemortem cognitive impairment measured by the MMSE is consistently associated with amyloid-beta (Aβ) plaques, neurofibrillary tangles and a decreased number of synapses. [114] The MMSE demonstrates satisfactory reliability and construct validity and displays high levels of sensitivity for moderate-to-severe cognitive impairment. However, the MMSE is less sensitive in detecting patients with mild cognitive impairment. [112]

Study II: Longitudinal Assessment of Neuropsychiatric Symptoms

The NPI evaluates twelve neuropsychiatric symptoms that may occur in dementia: delusions, hallucinations, agitation, anxiety, dysphoria, apathy, euphoria, disinhibition, irritability, aberrant motor behavior, disturbances of sleep, and appetite disturbances. A caregiver familiar with the patient is presented with descriptions fitting the

symptoms and asked if they are present. If present, the caregiver is asked to rate the severity and frequency of each neuropsychiatric symptom using a standardized questionnaire. A combined score for each symptom is calculated by multiplying the frequency by severity. The total score is calculated by adding all the domain scores together. [115] The NPI is considered valid and reliable with regards to concurrent validity, intra- and interrater reliability, test-retest reliability, and internal consistency. [116]

Study III: Frailty Index

To investigate the potential relationship between frailty and the kynurenines, we followed the stepwise procedure to construct a frailty index described by Rockwood et al[117]. Thirty-eight health conditions/deficits (binary, ordinal, and continuous variables) were included from 2152 HUSK participants who underwent cognitive testing. The number of deficits was divided by the total number of possible deficits, resulting in a frailty index score from zero to one. However, there were missing data for several participants. We excluded participants with missing information on six or more conditions and adjusted the denominator to available health deficits. Thus, the frailty analyses included measurements from 1691 participants in HUSK with information on more than 32 health deficits for each participant. Of these, 327 participants had all available data (i.e. no missing data) for the potential 38 deficits registered, 633 had one deficit missing, 329 had two deficits missing, and 190 had three or more missing deficits. The frailty index is well validated. [118] Several studies have found that this frailty index has a higher predictive ability for adverse outcomes than other frailty scores, both in hospital and community settings. [119] The total frailty index score, rather than individual health deficits, is typically the most predictive of adverse outcomes. An upper limit of deficit accumulation measured by the frailty index is estimated to be around 0.67, beyond which survival is unlikely. [119]

BLOOD SAMPLES AND METABOLIC BIOMARKERS

Measurements of Trp, the kynurenines, and inflammatory markers were performed in five cohorts (see Table 4). Trp, the kynurenines, PLP, and neopterin were measured using liquid chromatography-tandem mass spectrometry. [120] CRP was measured using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. [121] The samples were stored at -80°C in all five cohorts. KTR was defined as Kyn (µM)/Trp (µM) * 100. The limit of detection for the kynurenines and neopterin ranged from 0.5 nmol/L to 7 nmol/L, while the limit of detection for Trp was 0.4 µmol/L. Within-day and between-day coefficients of variation were 5.7-16.9% and 3.0–9.5 %, respectively. For CRP, the limit of detection was 0.2 µg/L, and within- and between-day coefficients of variation were 5.5–8.4 % and 7.0–11.7 %, respectively. The biochemical analyses of biomarkers included in this thesis were performed at the Bevital AS laboratory (Bergen, Norway, http://bevital.no).

Table 4. Biomarker Measurements in Five Cohorts.

Cohort (Study)	Population	Fasting status	Fluid
HUSK (I, III)	Community-dwelling	Non-fasting	Plasma
DemVest (II)	Mild dementia	Fasting	Serum
MCCS[a] (III)	Community-dwelling	Fasting / non-fasting	Plasma
WENBIT (III)	Stable angina pectoris	Non-fasting	Plasma
COGNORM[b] (III)	Elective surgery	Fasting/ non-fasting	Serum / CSF

Abbreviations: COGNORM, Elective Surgery Cohort; CSF, cerebrospinal fluid; DemVest, Dementia Study of Western Norway; HUSK, Hordaland Health Study; MCCS, Melbourne Collaborative Cohort Study; WENBIT, Western Norway B Vitamin Intervention Trial.

[a] In the MCCS, 67% of the participants provided fasting blood samples at baseline, with 90% fasting samples at follow-up.
[b] In COGNORM, 109 participants donated CSF samples per-operatively during elective surgery, and of these, 33 volunteered to provide a second CSF sample after four years. The participants were fasting at baseline and non-fasting at follow-up.

STATISTICAL METHODS

In all studies, metabolite concentrations are reported as median concentrations, and the variance is reported using the interquartile range, as the metabolites did not follow a normal distribution. Univariate analyses were conducted using the Mann-Whitney U-test and Spearman's Rho (R_s) correlation coefficients. Multivariable analyses were conducted following both statistical transformations to obtain normality and scaling, the latter by standardization (i.e. z-scores where all variables have a mean of zero and a SD of one). The mean following log-transformation corresponds to the geometric mean.

Hypothesis Testing and Multiple Comparisons

Adjustment for multiple comparisons aims to maintain the nominal alpha, thus reducing false-positive findings (type I errors), which increases as a function of the number of hypotheses tested. There are several methods that can achieve this aim. In **study I**, we applied the Bonferroni correction. This method's main disadvantage is that it can lead to a higher rate of type II errors. [122] However, due to the relatively large sample size in **study I**, occurrence of type II errors was not a significant concern. In **study II**, we used a method based on the false-discovery rate [123] adapted to correlated predictors, as is the case with the kynurenine metabolites. In **study II**, in contrast to **study I**, the sample size was relatively small. We thus wanted to avoid the inflation of the type II error rate typically associated with using the Bonferroni correction. In **study III**, generally, we could assess the same hypotheses (that kynurenine metabolites are associated with aging) across independent populations. As the probability of finding two significant p-values in independent studies is much lower, we did not adjust for multiple comparisons in **study III**.

Study I

To find optimal statistical transformations of metabolite concentrations to a normal distribution, we applied Tukey's ladder of powers.[124] Cognitive tests are frequently correlated with each other, as was the case for COWAT, DST, and KOLT. In order to identify whether the kynurenines were associated with one of these tests, adjusted for the between-test associations, we applied multi-outcome regression, namely Zellner's seemingly unrelated regression (SUR). [125] Linear regression assumes that the outcomes are independent. In the SUR model, the Breusch-Pagan test was highly significant, confirming dependent outcomes and the appropriateness of a SUR model. A two-step estimation procedure was applied. We could not formulate a hypothesis specifying which metabolite is associated with which cognitive test. Accordingly, we tested the joint significance of the association between each metabolite and the three cognitive outcomes. These were executive function (DST), language (COWAT), and memory (KOLT). Specifically, the joint significance was tested using the Wald test on a composite linear hypothesis of association between the metabolite and the three cognitive tests. The joint significance ($\alpha = 0.05$) threshold was then adjusted for the number of hypotheses tested, according to the Bonferroni method. [122] We first estimated an unadjusted model, followed by adjustment for the *a priori* identified confounders: age, sex, body mass index, education (in years), estimated glomerular filtration rate (GFR), current smoking, diabetes, previous myocardial infarction, prior stroke, and PLP. Further, we evaluated whether metabolites were associated with depressive symptoms on HADS (score ≥ 8), [110] anti-depressant use [126], non-steroidal anti-inflammatory drugs use, [127] and CRP. [128] We included any significant association into the SUR model.

Study II

The metabolite concentrations were transformed to a normal distribution following Tukey's ladder of powers.[124] We used a joint model to estimate the association between, Trp and the kynurenines, and the dependent variables, MMSE score and the NPI-total score, adjusted for covariates. The MMSE and the NPI were assessed at

baseline and annually for five years. There were occasional delays, and some patients were followed for six years so the data were unbalanced. MMSE test scores were transformed using the square root of errors transformation; √(30-MMSE). Following this transformation, higher values indicated poorer cognitive performance. The MMSE reached a ceiling-effect. We therefore implemented right censoring using a linear mixed-effects Tobit model. This model included random intercepts and slopes.

The NPI-total score was fitted using a negative binomial random-intercept model, selected as per the Bayesian information criterion. [129] Likely related to the considerable variation in neuropsychiatric symptoms over time, random slopes could not be fitted. The MMSE and NPI-total models were linked in a joint-model by allowing their respective random effects to be correlated, using a generalized structural equation model framework (Stata 15 package "gsem"). The metabolites were measured at baseline only. Due to strong correlations between metabolites, risking collinearity, each metabolite was entered as a predictor in separate joint models with MMSE and NPI as outcomes. Covariates included years in study (time), age, age*time interaction, sex, AD vs. LBD, AD vs. LBD*time interaction, current smoking, GFR, and PLP in the MMSE sub-model. Compared to **study I**, we included only the most important confounders as sample size limited the possibility for more complex models. The same covariates were used in the NPI-total sub-model, excluding the non-significant age*time interaction. In brief, each metabolite in the joint-models predicted MMSE and NPI, adjusted for the association between these two outcomes, and covariates. The potential presence of non-linearity was evaluated using orthogonal polynomials of the transformed metabolite concentrations. Post-hoc, we re-estimated the above model stratified by diagnosis. We applied logistic mixed-effects models with random intercepts to estimate associations between metabolites and individual items of the NPI. The items were classified as present or not present, and covariates were included as described above for the sub-model with the NPI-total score as the outcome. Effect sizes are reported as fixed effects (FE), referred to as estimates in **study II**. All study findings were adjusted for multiple comparisons, using the tail-area

based false discovery rate due to dependency, and adjusted p-values are reported (Q-values, or Q). This was done separately for post-hoc tests (R package: fdrtool).

Study III

Trp and the kynurenine metabolites were outcomes in random intercept models (as MCCS had two repeated measurements) or random coefficient models (WENBIT had three repeated measurements). We estimated a model with baseline age and time (in study) as the only predictors. Time was a category (0 for baseline, 1 for follow-up) in the MCCS, and change per year was calculated by dividing the effect sizes by 11 due to a median follow-up time of 11 years. In WENBIT, the exact time of follow-up occurred at a median of one and three years, and due to variations, time was entered as a continuous variable.

These analyses provided partially standardized fixed effects on the log-scale where a one-year change in years in study or age gave a one SD change in the standardized log-transformed outcome. Further, we reported fully standardized fixed effects, calculated by multiplying the partially standardized coefficients by the SD of the predictors. The effect sizes have a similar interpretation as correlation coefficients.

The survival analyses in MCCS and HUSK were performed using Cox regression, after determining that the models were in line with the assumption of proportional hazards. The analyses were adjusted for the covariates age, sex, and GFR. The baseline for survival analysis in the MCCS was after the last biomarker assessment at a median of 11 years, as endpoints were available only after this time-point. Investigation of associations between Trp, the kynurenines, and CRP and the frailty index (HUSK) were performed using a standardized linear regression model, adjusted for age and sex (as GFR was included in the frailty index). For the purpose of log-transformation, the frailty index was multiplied by 100, and a constant of one was added prior to log-transformation and subsequent scaling to a z-score.

The sample size in COGNORM prevented the assumption of a normal distribution on the lognormal scale. Therefore, we estimated correlation coefficients between age

and the kynurenine metabolites in serum and CSF using non-parametric Spearman's Rho. Further, we explored if the correlation coefficients between age and the kynurenines in sera were equal to the correlation coefficients between age and the kynurenines in CSF, adjusted for the metabolites' correlation between each other. [130] The Wilcoxon signed-rank test was used to compare metabolite concentrations between baseline and follow-up in the subgroups with repeated CSF measurements.

RESULTS

STUDY I

Characteristics of the Study Participants

Community-dwelling older adults (n = 2174) (55% women) from HUSK, with cognitive test scores and available blood samples were included in the study. The mean (SD) scores for the cognitive tests were: COWAT, 15 (5.5); DST, 10 (4.2); and KOLT, 35 (8.1). *Please see **Study I:** Results, p. 158, and Table 1, p. 159.*

The Kynurenine Pathway and Cognitive Performance

Higher plasma concentrations of KTR and neopterin were associated with reduced cognitive performance in the domains of memory (KOLT) and language (COWAT) (Figure 3 on the next page. *From **Study I**: Figure 2, p. 158*). This association was not present for executive function (DST). Of the two identified predictors, KTR was most associated with cognitive performance (*please see **Study I**: Table 2, p. 159*). In addition to adjusting for the *a priori* identified covariates age, sex, body mass index, education, GFR, current smoking, diabetes, previous myocardial infarction and stroke, and PLP, we evaluated other potential confounders. The kynurenines were not associated with depression as defined by a HADS score on the depression subscale ≥ 8, or the use of anti-depressants. The kynurenines were, however, associated with CRP levels and the use of NSAIDs (***Study I**: Table 3, p. 160*). However, adjustment for CRP or NSAIDs use did not attenuate the associations between KTR, Kyn, neopterin and cognitive outcomes (***Study I**: Table 4, p. 160*).

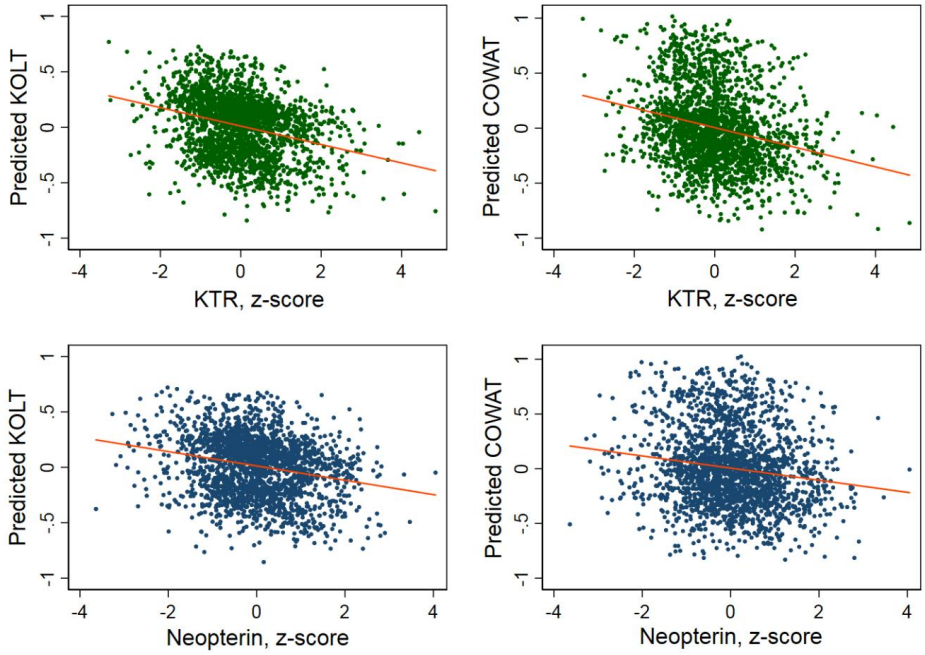

Figure 3. Cognitive Tests and Markers of Immune Activation. Predicted results from Zellner's seemingly unrelated regression, adjusted for age, sex, body mass index, educational level, estimated glomerular filtration rate, current smoking, diabetes, hypertension, previous myocardial infarction, prior stroke, and pyridoxal 5' phosphate as covariates.

Abbreviations: COWAT, Controlled Oral Word Association Test; KOLT, Kendrick Object Learning Test; KTR, kynurenine to tryptophan ratio.

Figure 3 in the thesis corresponds to *Figure 2 in* ***Study II.***

STUDY II

Characteristics of the Study Participants

Patients with dementia (n = 155, 90 AD, 65 LBD, 56.1% women) were included from DemVest. At baseline, the patients had a mean MMSE score of 23.7, a mean education of 9.7 years, and 20% indicated current smoking (***Study II****: Table 1, p. 4*).

Non-Linear Kynurenine-MMSE Association in Mild Dementia

Serum Kyn concentrations measured at the beginning of the study displayed a non-linear association with the average MMSE score over the five annual follow-ups. Specifically, the first polynomial of Kyn (FE -0.023 (*referred to as estimate in* ***Study II***), Q > 0.05) did not show an association with cognitive function, whereas the second polynomial of Kyn did (FE 0.10, Q = 0.046), showing a significant quadratic association (Figure 4 on the next page. *From* ***Study II****: Figure 2, p. 6*). Kyn was not associated with the rate of cognitive decline in patients suffering from mild dementia. None of the other kynurenine metabolites were associated with cognitive performance (***Study II****: Table 2, p. 5*). There were no significant differences in this association when the analyses were stratified according to a diagnosis of AD or LBD (data not shown).

Kynurenines and Neuropsychiatric Symptoms

We first estimated the associations between kynurenines and the NPI-total score. Higher serum kynurenic acid to kynurenine ratio (KKR) was not associated with neuropsychiatric symptoms at baseline (FE -0.050, Q > 0.05). However, higher KKR was associated with an increase in the NPI total score per year (FE 0.063, Q = 0.045), see Figure 5 on the page following the next page (p. 52)*, from* ***Study II****: Figure 3, p. 6*. KA and XA also displayed a trend of being associated with increasing neuropsychiatric symptoms over time but were not significant following adjustment for multiple comparisons. Post-hoc, we estimated the associations between individual NPI items and the kynurenines. KKR was associated with increasing hallucinations

over time and KA was associated with more hallucinations overall without affecting the longitudinal development of hallucinations (both Q < 0.05) (***Study II****: Figure 4, p. 7*). There were no significant differences in these associations between LBD and AD (data not shown).

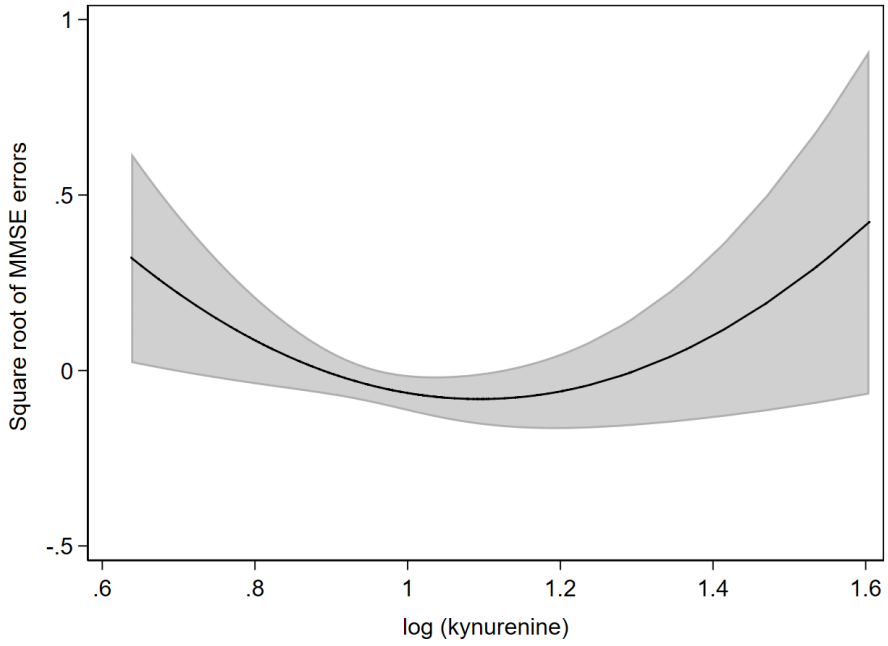

Figure 4. Non-Linear Association Between MMSE Test Scores and Serum Kynurenine. Kynurenine concentrations around the geometric mean was not associated with MMSE, whereas high or low serum concentrations were associated with more MMSE-errors. The model was estimated as a joint model together with a model for the NPI-total score (see statistics). Of note, a constant of one was added to kynurenine prior to logarithmic transformation, shifting the log (mean) from 0.55 to 1.02.

Abbreviations: MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.
Figure 4 in the thesis corresponds to *Figure 2 in* ***Study II.***

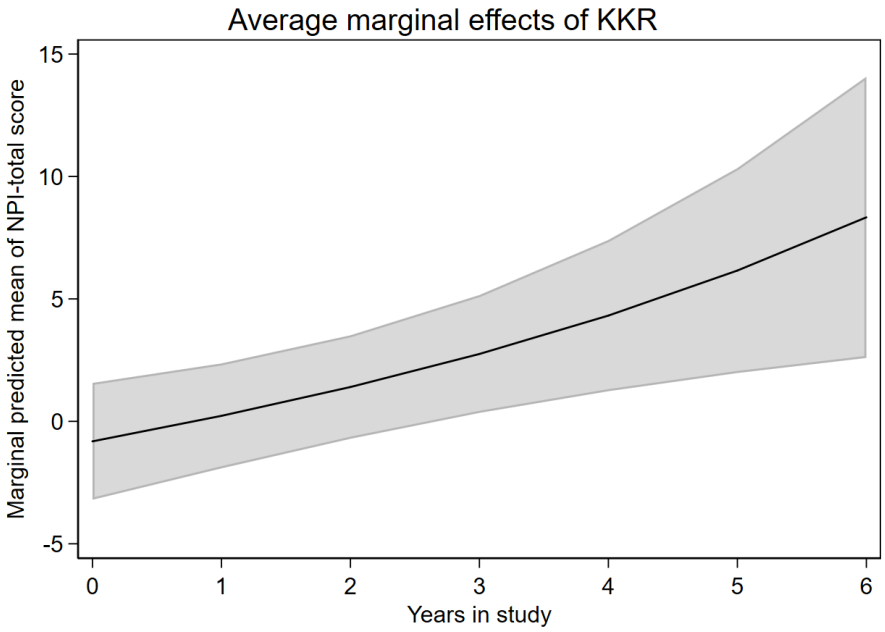

Figure 5. The Kynurenic Acid to Kynurenine Ratio and Neuropsychiatric Symptoms. The graph shows how a change in one standard deviation of the transformed and standardized levels of KKR (the reciprocal of $1/\sqrt{(KKR)}$) was associated with an increase in neuropsychiatric symptoms over time, using a negative binomial random-intercept model linked to the MMSE model (i.e., a joint model), adjusted for age, sex, current smoking, estimated glomerular filtration rate, and PLP in the model for NPI-total score.

Abbreviations: KKR, kynurenic acid to kynurenine ratio; MMSE, Mini-Mental State Examination; NPI, neuropsychiatric inventory; PLP, pyridoxal 5'-phosphate.
Figure 5 in the thesis corresponds to *Figure 3 in* ***Study II***.

STUDY III

Characteristics of the Study Participants

The characteristics of the study participants in the four independent cohorts are summarized below and in ***Study III****: Table 1, p. 30*.

Melbourne Collaborative Cohort Study

Community-dwelling participants (n = 970, 32% women) with a mean (SD) baseline age of 57.6 (7.9) years were included in the study. Plasma concentrations of Trp, the kynurenines, and CRP were measured at baseline and at follow-up after a median of eleven years. Mortality data were recorded until 16 years after the follow-up.

Western Norway B Vitamin Intervention Trial

Patients with stable angina pectoris (n = 604, 22.9% women) with a mean (SD) age of 61.9 (9.7) years at baseline were included in this study. Trp, the kynurenines, and CRP were measured at baseline and at two follow-up visits after a median of one and three years.

Hordaland Health Study

Community-dwelling older adults aged 71-74 years old (n = 3161, 44.3 % women) who had donated blood samples were included in this study. Mortality data were recorded until 17 years after baseline. A subset of 2152 older adults underwent cognitive testing, of which 1691 participants had sufficient data to compute a frailty index.

Elective Surgery Cohort

Patients undergoing elective surgery (n = 109, 46% women) with a mean (SD) age of 73.3 (6.8) years provided CSF and serum samples and were included in the analyses. A subset of patients (n = 33) voluntarily donated CSF at a follow-up after four years.

Associations Between Kynurenines and Age

The associations between age, time and metabolites were characterized by FE from a multilevel model where metabolites were outcomes with age and time as independent variables. The estimated FE represents a one SD change in log-transformed plasma biomarker concentrations per year of age or time in study.

In MCCS, a study on community-dwelling persons, the strongest associations with age were for QA, KTR, and Kyn. The estimated associations with age were weaker for HK, CRP, KA, and AA. In contrast, Trp was inversely associated with age. Please see Figure 6 on the next page. *From **Study III**: Figure 2, p. 25*.

In WENBIT, a study on patients with stable angina, KTR, QA, and Kyn displayed the strongest associations with age. There were weaker associations between age, HK, KA, and AA. Again, Trp was inversely associated with age. Please see Figure 7 on the page after the next page (p. 56). *From **Study III**: Figure 3, p. 27*.

Changes in Kynurenine Concentrations Over Time

Samples in MCCS were taken at baseline and again after a median of 11 years. Figure 6 (on the next page) summarizes changes in the kynurenine metabolites with age, and over time in MCCS. We identified an increase in plasma KTR and QA over time, with smaller increases in Kyn, HK, KA, and HAA. Trp and anthranilic acid (AA) decreased over time.

Samples in WENBIT were taken at a median of one and three years after baseline. Figure 7 (on the page after the next page (p. 56)) summarizes changes in the kynurenine metabolites with age, and over time in WENBIT. Here, plasma QA and KTR increased. However, QA was measured only at baseline and after one year. Kyn, HK, KA, AA, HAA, and XA increased to a lesser extent over time, whereas Trp decreased. By comparison, CRP showed minor associations with age and inconsistent changes over time in MCCS and WENBIT. Standardized fixed effects, which can be interpreted similarly to correlation coefficients, can be found in ***Study III**: Supplementary Table 2, p. 36*.

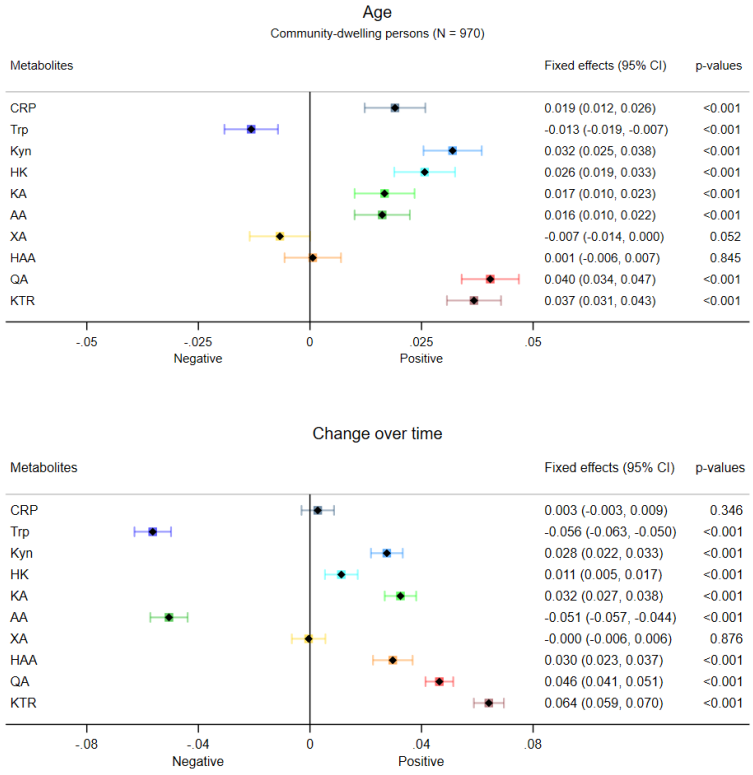

Figure 6. Metabolites of the Kynurenine Pathway Change with Age (upper panel), and Over Time (lower panel) in Community-Dwelling Persons (MCCS study). The mean age of MCCS participants were 57.6 years at baseline, with follow-up after 11 years. Log-transformed and standardized metabolites (with CRP as a reference) were entered as outcomes in linear mixed-effects models with baseline age and time as predictors. In the model, time was a categorical variable, and the effect sizes for time were divided by 11 to yield change per year. The fixed effects reflect a one standard deviation change in the standardized, log-transformed metabolite concentration per year of chronological age or per year in the study. Of note, the x-axes for the fixed effects on the upper and lower panels are on different scales.

Abbreviations: AA, anthranilic acid; CRP, C-reactive protein; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine to tryptophan ratio; Kyn, kynurenine; MCCS, Melbourne Collaborative Cohort Study; p, p-value; QA, quinolinic acid; Trp, tryptophan; XA, xanthurenic acid; 95% C.I., 95% confidence interval.

Figure 6 in the thesis corresponds to *Figure 2 in* ***Study III.***

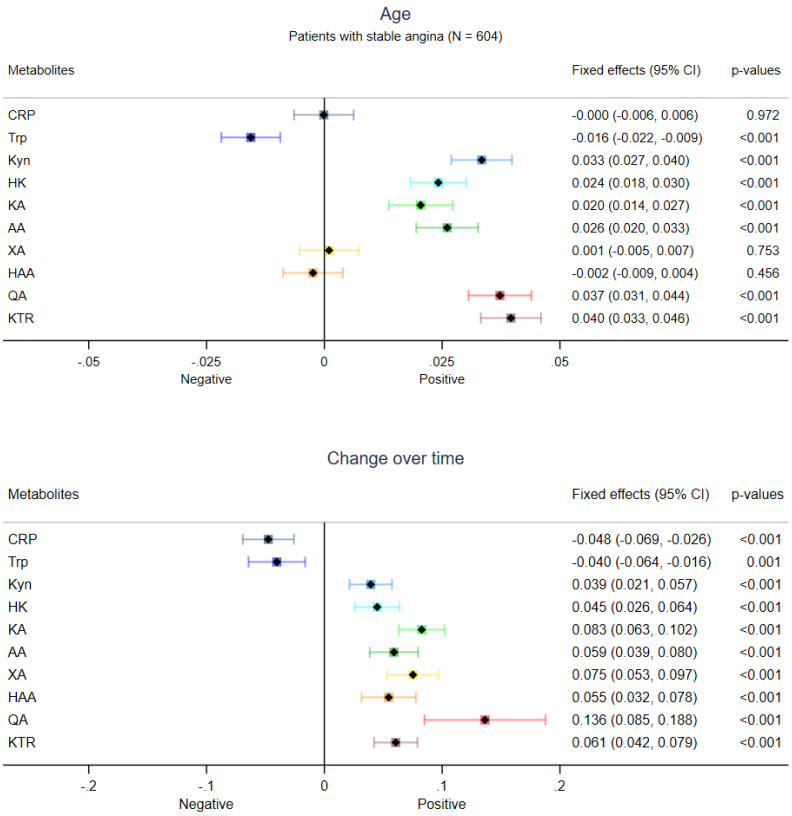

Figure 7. Metabolites of the Kynurenine Pathway Change with Age (upper panel), and Over Time (lower panel) in Patients with Stable Angina Pectoris (WENBIT study). The mean age of the patients was 61.9 years at baseline, and they were followed up after a median of one and three years. Metabolites (and CRP as a reference) were log-transformed, standardized and entered as outcomes in linear mixed-effects models with age at the study baseline and time (years in study) as predictors. The fixed effects reflect a one standard deviation change in the standardized, log-transformed metabolite per year of chronological age or per year in study. Of note, the x-axes for the fixed effects on the upper and lower panels are on different scales.

Abbreviations: AA, anthranilic acid; CRP, C-reactive protein; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine to tryptophan ratio; Kyn, kynurenine; p, p-value; QA, quinolinic acid; Trp, tryptophan; WENBIT, Western Norway B Vitamin Intervention Trial; XA, xanthurenic acid; 95% C.I., 95% confidence interval.

Figure 7 in the thesis corresponds to *Figure 3 in* ***Study III***.

Metabolite Concentrations in Persons Aged 71 to 74 Years in HUSK

Plasma Trp was lower in persons aged 74 years old compared to those aged 71 years. In contrast, HK, KTR, QA, and CRP were higher, although CRP did not reach statistical significance. HK was also higher in persons aged 73 years compared to those aged 71 years. QA was also higher in persons aged 72 or 73 years compared to those aged 71 years (***Study III****: Table 2, p. 31*).

Aging and Kynurenine Pathway Metabolites in the Cerebrospinal Fluid

In the 109 participants from COGNORM, statistical analyses were conducted using R_s to estimate correlation coefficients. The metabolites in serum correlated moderately (HK, KA, Trp) to strongly (QA, PIC, Kyn) with the corresponding CSF-metabolites. HK and PIC were not correlated with age in either serum or CSF. Trp was significantly, inversely correlated with age in the serum (R_s -0.27) and positively but non-significantly in the CSF (R_s 0.14). Kyn correlated significantly with age in serum (0.24) but more so in the CSF (0.39). KA correlated non-significantly with age in serum (R_s 0.15) but significantly in the CSF (R_s 0.32), whereas AA was more correlated with age in serum (R_s 0.29) than in the CSF (R_s 0.23). Finally, QA was most strongly correlated to age in serum (R_s 0.37) and in the CSF (R_s 0.55). The correlation between metabolites and age was significantly stronger in the CSF than in serum for QA and Kyn (Table 5A on the next page). Compared to the first age-quartile, CSF-QA concentrations doubled in the fourth quartile (Table 5B on the next page). In the subgroup of 33 participants with a second lumbar puncture after four years, Kyn (median 51.4 nmol/L at baseline, 57.7 after four years, $p < 0.001$) and QA (median 31.2 nmol/L at baseline, 42.6 after four years, $p < 0.001$) increased, whereas HK increased marginally (median 4.42 nmol/L at baseline, 4.91 after four years, $p = 0.038$). There was no significant change in Trp, KA, AA, and PIC (Table 5C on the next page). Table 5 corresponds to ***Study III****: Table 3, p. 32.*

Table 5A. Serum and Cerebrospinal Fluid Correlations with Age for Tryptophan and Kynurenines in 109 Cognitively Healthy Persons Undergoing Elective Surgery

	Correlations between metabolites[a]	**Correlations between metabolites and age[a]**		**Difference in serum vs. CSF age-correlations[b]**
	Serum vs. CSF	Serum	CSF	Null-hypothesis of equivalence
Trp	0.26*	-0.27*	0.14	.001*
Kyn	0.68**	0.24*	0.39**	.039*
HK	0.40**	0.17	0.17	.999
KA	0.28*	0.15	0.32**	.136
AA	0.36**	0.29*	0.23*	.574
PIC	0.70**	-0.11	0.03	.067
QA	0.78**	0.37**	0.55**	.001*

Table 5B. Quinolinic acid concentrations in nmol/L according to age-quartiles

	Serum Median (IQR)	**CSF** Median (IQR)	% in CSF vs. Serum Median (IQR)
64-68	373 (158)	29.4 (10.1)	7.95 (2.62)
69-71	398 (267)	33.0 (20.3)	8.74 (2.64)
72-77	429 (135)	42.7 (16.1)	8.75 (4.28)
78-91	647 (723)	65.9 (65.1)	10.6 (3.52)

Table 5C. Change in cerebrospinal fluid kynurenines over four years (n = 33)[c]

	Baseline Median (IQR)	**Four-year follow-up** Median (IQR)	p[d]
Trp	2.64 (0.7)	2.64 (0.6)	.774
Kyn	51.4 (14.2)	57.7 (29.9)	<.001**
HK	4.42 (2.27)	4.91 (2.45)	.038*
KA	2.47 (1.33)	2.25 (1.13)	.816
AA	9.48 (7.18)	10.9 (2.88)	.788
PIC	19.2 (8.2)	18.9 (7.4)	.469
QA	31.2 (20.3)	42.6 (24.7)	<.001**

Note: CSF concentrations of HAA and XA were below the limit of detection and thus omitted from this analysis.

Abbreviations: AA, anthranilic acid; CSF, cerebrospinal fluid; HK, 3-hydroxykynurenine; IQR, interquartile range; KA, kynurenic acid; Kyn, kynurenine; p, p-value; PIC, picolinic acid; QA, quinolinic acid; Trp, tryptophan.

[a] Spearman's Rho correlation coefficients.

[b] Equivalence of correlation coefficients in serum and CSF samples in paired samples, $p < 0.05$ indicates a significant difference. [130]

[c] Trp, HK, KA, AA, and PIC in µmol/L, Kyn and QA in nmol/L.

[d] Wilcoxon signed rank test for paired samples.

* $p < 0.05$; ** $p < 0.001$.

Table 5A, 5B, and 5C in the thesis corresponds to *Table 3A, 3B, and 3C in* ***Study III***.

Kynurenines Concentrations and Frailty in HUSK

Using the frailty index score as the outcome, plasma CRP showed the strongest association with frailty. Significant associations with frailty were identified for several kynurenines adjusted for age, sex, and CRP: QA (β 0.11, $p < 0.001$), Kyn (β 0.10, $p < 0.001$), KTR (β 0.10, $p < 0.001$), HAA (β 0.08, $p<0.001$), KA (β 0.08, $p = 0.002$), and HK (β 0.07, $p = 0.007$). However, following adjustment for age, sex and KTR, CRP was still more strongly associated with frailty (β 0.15, $p < 0.001$). *Please see **Study III**: Table 4, p. 33.*

Kynurenines as Predictors of All-Cause Mortality

Following adjustment for age, sex, GFR and plasma CRP, increased Trp concentrations were associated with lower all-cause mortality, whereas increased QA and KTR concentrations were associated with higher all-cause mortality in MCCS and HUSK. After adjusting for age, sex, GFR and KTR, increased CRP concentrations were also associated with increased all-cause mortality in both cohorts (Table 6 on the next page. *From **Study III:** Table 5, p. 34*).

Table 6. Associations of Kynurenines, and CRP with All-Cause Mortality[a]

Metabolites	Cohort[b]	HR	95% CI	p	HR	95% CI	p
		Unadjusted for KTR[c]			Adjusted for KTR[c]		
CRP	HUSK	1.10	[1.05, 1.16]	<.001**	1.06	[1.00, 1.11]	.039*
	MCCS	1.21	[1.09, 1.34]	.001*	1.20	[1.09, 1.34]	<.001**
		Unadjusted for CRP[c]			Adjusted for CRP[c]		
Trp	HUSK	0.90	[0.85, 0.95]	<.001**	0.91	[0.86, 0.96]	<.001**
	MCCS	0.86	[0.78, 0.96]	.006*	0.87	[0.78, 0.97]	.006*
Kyn	HUSK	1.11	[1.05, 1.18]	<.001**	1.09	[1.03, 1.16]	.004*
	MCCS	1.06	[0.92, 1.24]	.356	1.01	[0.87, 1.20]	.534
HK	HUSK	1.13	[1.07, 1.19]	<.001**	1.10	[1.05, 1.17]	<.001**
	MCCS	1.09	[0.96, 1.24]	.172	1.05	[0.93, 1.20]	.424
KA	HUSK	1.05	[0.98, 1.11]	.140	1.05	[0.99, 1.11]	.138
	MCCS	1.06	[0.94, 1.18]	.322	1.05	[0.94, 1.18]	.368
AA	HUSK	1.10	[1.04, 1.16]	<.001**	1.09	[1.03, 1.15]	.001*
	MCCS	1.12	[0.99 1.26]	.060	1.11	[0.98, 1.25]	.102
XA	HUSK	0.94	[0.89, 0.99]	.019*	0.95	[0.90, 1.00]	.044*
	MCCS	0.96	[0.86, 1.06]	.407	0.96	[0.86, 1.06]	.405
HAA	HUSK	0.95	[0.91, 1.01]	.100	0.95	[0.90, 1.00]	.046*
	MCCS	1.10	[0.97, 1.24]	.645	1.05	[0.93, 1.20]	.086
PIC	HUSK	1.00	[0.95, 1.05]	.926	1.00	[0.95, 1.06]	.897
	MCCS	1.08	[0.96, 1.21]	.187	1.06	[0.95, 1.19]	.300
QA	HUSK	1.15	[1.09, 1.22]	<.001**	1.14	[1.07, 1.20]	<.001**
	MCCS	1.30	[1.14, 1.48]	<.001**	1.25	[1.09, 1.43]	.002*
KTR	HUSK	1.23	[1.16, 1.30]	<.001**	1.21	[1.14, 1.28]	<.001**
	MCCS	1.15	[1.02, 1.30]	.022*	1.13	[1.01, 1.34]	.043*

Note: Effect sizes indicate the hazard ratio associated with a one standard deviation increase in the log-transformed metabolite concentrations.

Abbreviations: AA, anthranilic acid; 95% CI, 95% confidence interval; CRP, C-reactive protein; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine to tryptophan ratio; Kyn, kynurenine; PIC, picolinic acid; QA, quinolinic acid; Trp, tryptophan; XA, xanthurenic acid.

[a] Cox proportional hazard analysis with age, sex, and estimated glomerular filtration rate as covariates in all models, with or without adjustment for CRP for the kynurenines, or KTR for CRP.

[b] HUSK, Hordaland Health Study (n = 3161); MCCS, Melbourne Collaborative Cohort Study (n = 970).

[c] CRP adjusted for KTR. Trp, Kyn, HK, KA, AA, XA, HAA, PIC, QA, and KTR adjusted for CRP.

* $p < 0.05$; ** $p < 0.001$.

Table 6 in the thesis corresponds to *Table 5 in* ***Study III.***

DISCUSSION

PRINCIPAL FINDINGS

In this thesis, we aimed to assess whether the kynurenines were associated with cognitive test performance in community-dwelling persons (**study I**), five year cognitive and neuropsychiatric prognosis in mild dementia (**study II**), and aging (**study III**). In **study I**, higher plasma concentrations of KTR and neopterin were associated with reduced cognitive performance. Kyn, however, had a non-linear quadratic association with cognitive performance. In **study II**, investigating AD and LBD patients with mild dementia, we also found a non-linear quadratic association between serum Kyn and cognitive performance. However, Kyn was not associated with the rate of cognitive decline. Our findings in **studies I** and **II** suggest that homeostatic Kyn concentrations around the geometric mean may benefit cognitive function where both high and low concentrations were associated with poorer cognitive performance. Patients with mild dementia who had a higher serum KKR had increasing neuropsychiatric symptoms over five years. **Study III** focused on repeated measurements of the kynurenines in plasma, serum and CSF. In two longitudinal cohorts, plasma Kyn, KTR, HK, KA, and QA were positively associated with age and displayed consistent longitudinal changes over time. In contrast, plasma Trp was negatively associated with age and consistently decreased over time. Kyn, HK, KA, HAA, QA, KTR, and CRP were all associated with frailty in HUSK. Higher QA, KTR, and CRP concentrations were independently associated with higher all-cause mortality in both MCCS and HUSK. In contrast, higher Trp concentrations were associated with lower mortality. Kyn, KA and QA correlated more strongly with age in the CSF compared to serum. Kyn and QA consistently increased in the CSF over four years. Overall, our results indicate that the kynurenine pathway is significantly altered by aging, has a complex relationship with cognitive performance, and relates to the risk of neuropsychiatric symptoms in mild dementia. The immunomodulatory and neuroactive properties of the kynurenines highlight the importance of investigating whether the kynurenines are actors or bystanders in aging and age-related disorders.

SYSTEMIC AND CEREBROSPINAL FLUID CONCENTRATIONS

An important question is whether peripheral concentrations of the kynurenines reflect brain concentrations, as **studies I** and **II** focused on plasma and serum measurements. In **study III**, we found a strong correlation between QA in serum versus CSF (R_s, 0.78) and a moderate-to-strong correlation between Kyn in serum versus CSF (R_s, 0.68) among patients undergoing elective surgery. For HK, this correlation was 0.40, for KA 0.28, for AA 0.38, and for PIC 0.70 (HAA and XA concentrations in CSF were below the limit of detection when analyzed using liquid chromatography-tandem mass spectrometry). The results are in line with previously identified serum-CSF correlations for kynurenines in inflammatory- [131] and neurodegenerative disease. [132] We also found a weak correlation between serum and CSF Trp (Rho, 0.26), similar to findings in patients with multiple sclerosis. [133] Trp, Kyn, and HK are transported across the BBB, while KA and QA must be synthesized in the brain, mainly within astrocytes and microglia, respectively (see the introduction, p. 24). [10] Thus, according to results from **study III** and previous reports, the correlations between serum and CSF are strong for Kyn, PIC and QA, but are weak to moderate for other kynurenines.

THE KYNURENINE PATHWAY AND COGNITIVE FUNCTION

Cognitive Performance in Community-Dwelling Older Adults

In **study I**, we aimed to investigate associations between kynurenine metabolites in plasma and cognitive performance in community-dwelling older adults. We found that higher concentrations of KTR and neopterin were associated with lower cognitive test performance. Specifically, higher KTR and neopterin were associated with lower test performance on the two cognitive tests KOLT and COWAT. KOLT is designed to assess memory performance and dementia status among non-institutionalized elderly. [134] COWAT has been used to assess the cognitive domains of language and executive function. However, there is no clear consensus on which interpretation is correct, as cognitive domains are not mutually exclusive constructs. [104] Previous studies in patients without neurodegenerative disease have focused on patients with

end-stage renal disease [54] (n = 27) and patients undergoing cardiac bypass and thoracic surgery (n = 56). [53] Both of these studies found that higher concentrations of Kyn, KA, KTR, and neopterin were associated with reduced cognitive function. Even though the results are similar to those in our study, the presence of chronic inflammation in these sick individuals and somewhat different cognitive test protocols limit the comparability with our study. [53,54] Our findings indicate that associations between the kynurenines, neopterin and cognition are not only present in patients with relatively severe disease but also in community-dwelling older adults.

Immune Activation as a Potential Confounder

Clinical and experimental studies have investigated relationships between inflammation and cognitive function. Inflammation activates the kynurenine pathway and is thus a potential confounder in our study (for details, see also introduction p. 22-23 and 30). Peripheral pro-inflammatory mediators, such as tumor necrosis factor-α, interleukin-6, and CRP, are associated with reduced cognitive performance in healthy persons. [135-139] Although **study I** lacked comprehensive immune profiling, CRP did not confound the identified associations. The main activator of the kynurenine pathway, IFN-γ, activates both the rate-limiting enzyme, IDO, and GTP cyclohydrolase I catalyzing the synthesis of neopterin in monocytes. IFN-γ knockout mice show improvements in neurogenesis, synaptic plasticity, and cognitive performance. [140] Thus, higher concentrations of the kynurenines could be indirect markers of underlying immune activation.

Previous Experimental Studies on Cognition

Experimental researchers have previously investigated whether there is a link between changes in Trp, the kynurenines, and cognitive function. One study investigating acute Trp depletion identified subsequent impairment in episodic memory among mice. [141] In an animal model of sepsis, IDO knockout mice did not show inflammation-induced cognitive impairment after lipopolysaccharide injection. [5] Knockout or

pharmacological inhibition of KAT II, which catalyzes the conversion of Kyn to KA, improved both spatial discrimination and contextual memory in rats. [142]
Further, studies of rats indicate that neurons within the hippocampus, striatum, and neocortex are sensitive to higher QA concentrations. [49] These experimental studies suggest that under certain conditions, manipulation of the kynurenine pathway can alter cognitive function. However, it is not known whether the kynurenines are involved in normal cognitive function.

Summary of Study I

In **study I**, we identified associations between the kynurenines and cognitive performance, in agreement with previous studies, and identified a non-linear quadratic association between the key metabolite Kyn and cognitive performance. This indicates that both high and low Kyn concentrations are associated with poor cognitive performance, whereas concentrations around the geometric mean (i.e., the mean on the log-scale) are associated with better cognitive performance. Although some circulating kynurenines are moderately-to-strongly correlated with CSF concentrations, including the CSF of healthy persons would add significantly to our work. However, due to the risk of lumbar puncture, such studies are not easy to perform. In addition, our results were only adjusted for CRP, so underlying immune activation is a potentially unidentified confounder. Further experimental studies are needed to establish whether the kynurenine pathway plays a role in cognitive function under physiological conditions.

THE KYNURENINE PATHWAY IN MILD DEMENTIA

Kynurenines and Cognitive Performance

In **study II,** we first aimed to determine associations between serum concentrations of the kynurenines and cognitive performance over five years. We found that Kyn had a non-linear relationship with cognitive function where both low and high levels were associated with reduced cognitive function in patients with mild dementia diagnosed

with AD or LBD (**study II**). A similar non-linear relationship was also seen in community-dwelling older adults (**study I**). A previous cross-sectional study identified that higher QA was associated with lower cognitive function in elderly patients with AD. [60] Kyn concentrations are tightly regulated [9] and higher Kyn concentrations may reflect an inflammatory state and increased IDO-activity. [143] Low substrate availability of Kyn may lead to reduced production of downstream neuroactive kynurenines such as KA and QA, and of NAD^+. [144] Although it is not clear from our data why the association between Kyn and cognitive performance was non-linear, Kyn concentrations around the geometric mean may reflect more homeostatic conditions.

Animal Models and Cell Studies on Neurodegeneration

The kynurenine pathway has been studied in animal models and cell studies of neurodegenerative disease. In AD knockin mice, cerebral injection of the IDO inhibitor coptisine resulted in less neuronal loss, reduced amyloid plaque formation, and improved cognition, suggesting that IDO activation could be detrimental in AD. [4] Experimental studies on astrocytes, microglia, and hippocampal neurons have found increased immunoreactivity for IDO and QA in the brains of AD patients post-mortem. [145] Studies on cell cultures of human neurons suggest that higher concentrations of QA lead to increased tau phosphorylation. [49] In both human neurons and mouse models, elevated Aβ induces the expression of enzymes of the kynurenine pathway, likely by increasing IFN-γ. [146] Thus, findings from both animal models and human cell studies suggest on the one hand that amyloid deposits can activate the kynurenine pathway, and on the other hand, that increased activity of the kynurenine pathway may promote disease progression.

Kynurenines and Neuropsychiatric Symptoms

In **study II**, we further aimed to determine associations between serum concentrations of the kynurenines and neuropsychiatric symptoms over five years. The primary outcome was the total score from the twelve domains of the NPI with individual items assessed in post-hoc analysis. [115] Neuropsychiatric symptoms are frequent in dementia

and include hallucinations, delusions, aggression, disinhibition, depression, anxiety, and aberrant motor behavior. [65] We found that higher serum KKR was associated with increasing neuropsychiatric symptoms over five years in patients with mild dementia. A higher KKR reflects a relatively higher KA compared to Kyn, which may suggest higher enzyme activity of KATs. [9] Investigating individual domains, KKR was associated with increasing hallucinations over five years, whereas higher KA was associated with more hallucinations overall without affecting the rate of change. We have not identified any previous studies investigating neuropsychiatric symptoms and the kynurenine pathway in dementia.

Kynurenic Acid and Previous Studies on Psychotic Disorders

Previous studies have found associations between the kynurenine pathway and major psychiatric disorders, including schizophrenia and bipolar disorder. [52] Compared to healthy volunteers, patients with schizophrenia display higher KA concentrations in the CSF [147] and the prefrontal cortex following neuropathological examination. [148] Similarly, patients with bipolar disorder with psychotic symptoms have elevated CSF-KA, [52] with increased Kyn and KA in the anterior cingulate cortex on post-mortem examination. [149] Notably, KMO expression is lower in patients with schizophrenia and bipolar disorder with psychotic symptoms, potentially contributing to increased conversion of Kyn to KA. [52] Thus, our findings highlighting the potential relevance of the relationship between Kyn and KA for neuropsychiatric symptoms in mild dementia are in line with findings from studies on patients with psychosis from major psychiatric disorders.

Several studies have addressed whether KA can be mechanistically linked to psychosis. KA is a regulator of dopaminergic and glutamatergic neurotransmission and an antagonist of the NMDAR, the latter a feature with the potential to trigger psychosis. [150] Furthermore, KA is an agonist for the AhR and an antagonist for the α7nAChR, linked to schizophrenia. [151,152] An increase in KA may decrease the levels of the neurotransmitters glutamate, [153] dopamine, [154] and acetylcholine. [155] The diverse effects of higher KA concentrations have been proposed to induce psychotic

symptoms and impair executive functioning. [10] Taken together, research investigating how KA affects neurotransmitter systems suggests that the metabolite may be of pathophysiological relevance to psychosis although there is as of yet no evidence from clinical trials in humans.

Summary of Study II

Study II was limited by lack of CSF measurements and lacked measures on pro-inflammatory activation, a potential confounder. Overall, our results suggest that Kyn has a complex relationship with cognition but is not associated with the rate of cognitive decline. Further, our findings indicate that higher KA and KKR, potentially related to KAT activity, could be biomarkers of an increased risk of neuropsychiatric symptoms in mild dementia.

THE KYNURENINE PATHWAY AND HUMAN AGING

Associations Between Circulating Kynurenines and Age

In **study III**, we aimed to assess the relationship between the kynurenines and age using cross-sectional and longitudinal measurements in serum, plasma and CSF. Trp concentrations in plasma were inversely associated with age, whilst Kyn, QA, and KTR were positively associated with age in two independent cohorts (MCCS and WENBIT) with repeated measurements of kynurenines. These associations with age were consistent with changes in the same metabolites over time. Similarly, community-dwelling older adults 74-years old had lower plasma Trp and higher HK, QA, and KTR compared to those 71 years of age (**study III**). Our findings corroborate findings from previous cross-sectional studies investigating associations between the kynurenine pathway and human aging. Generally, these studies identified lower Trp with older age, in different populations. [72-74,76-78] For example, Capuron et al[78] included non-institutionalized participants aged 65 years and older, whilst Collino et al[76] enrolled centenarians and their off-spring. Further, studies investigating serum or plasma concentrations of the kynurenines found positive correlations between age and

Kyn, [75,78] KTR, [75,78,91], HK, KA, and AA. [75] Collectively, these clinical findings across diverse populations suggest that aging is associated with increased systemic activity of the kynurenine pathway. The observations suggest that Trp is converted to downstream kynurenines with the most notable increases in Kyn and QA.

Aging rats displayed an increase in Kyn, KA, PIC, and QA with age both peripherally and in the brain, accompanied by increased IDO and decreased QPRT gene expression in the liver and brain. [156] QPRT is involved in the *de novo* synthesis of NAD^+, which is reduced in aging and neurodegenerative disorders. [157] Inhibition of QA degradation, as observed in aging rats, could serve as a potential explanation for the relative abundance of QA, compared to other kynurenine metabolites, with aging (**study III**).

The kynurenine pathway is activated by pro-inflammatory cytokines, which increase with aging. Trp typically decreases with inflammation, as IDO catalyzes the formation of Kyn and downstream kynurenines. [2] IFN-γ stimulation of human monocytes ex-vivo results in increased concentrations of QA and KTR. [158] Aged macrophages have higher IDO-activity and produce more cytokines. [32] This suggests that increased pro-inflammatory activation associated with aging, or inflammaging, could contribute to increased conversion of Trp to kynurenines. This could in turn affect the immune system. Furthermore, higher IDO expression in breast cancer has been linked to immune evasion and poor outcomes. [159] A lack of Trp and an increase in Kyn concentrations likely suppress $CD8^+$ and $CD4^+$ T-cells via amino acid sensing signals and the AhR. [2,160-162] $CD4^+$ and $CD8^+$ $CD28^-$ T-cells, increase with age, whilst T_{regs} are depleted. [163] The immunomodulatory effects of low Trp and high Kyn could thus serve to constrain inflammaging. [2] However, our study cannot determine whether activation of the kynurenine pathway in human aging is harmful or adaptive.

The kynurenine pathway is involved in energy metabolism through the conversion of its end-product QA to NAD^+ in a reaction catalyzed by QPRT. Declining levels of NAD^+ is observed across multiple species with aging, and this phenotype of aging is associated with age-related diseases. [164] Mice display declining intracellular NAD^+

concentrations in multiple organs, including brain, liver, muscle, pancreas, adipose tissue, and skin. An age-dependent reduction of NAD^+ has also been identified in *C. elegans* and aged human tissue. [164] This apparent discrepancy where QA increases with age in our study while NAD^+ decreases with age [157] could be explained by reduced QPRT activity, which has been identified in an animal study of aging. [156] Accordingly, it is plausible that both the well-known reduction in micronutrient intake observed with aging [165] and reduced conversion of QA contributes to age-related NAD^+ deficiency, [157] a topic for future studies.

Aging and Kynurenines in the Cerebrospinal Fluid

We further aimed to assess associations between kynurenines in CSF and age using data from COGNORM. Kyn and QA displayed stronger correlations with age in CSF compared to serum. Kyn and QA, and marginally HK, also increased in the CSF over time. CSF-QA was most strongly correlated with age, doubling in the fourth versus the first age quartile, and increased the most over time. There was no significant change in Trp, KA, AA, and PIC in the CSF over time, and CSF concentrations of HAA and XA were below the limit of detection. Our findings correspond with previous cross-sectional studies reporting a correlation between age and CSF-QA, and between age and CSF-KA. [74,80] Trp correlated inversely with age in serum, but positively in the CSF, in line with previous studies demonstrating an increase in Trp in the CSF with aging. [10,74] Our study, comparing serum and CSF correlations with age, suggest that altered activity of the kynurenine pathway may be more profound in the brain than systemically.

An increase of QA in the CSF of aging individuals could be of consequence. In the brain, QA is mainly generated from microglia or migrating monocytes. [10] QA is linked to NMDAR-dependent neurotoxicity, oxidative stress, and inhibition of mitochondrial function. [50] Significantly, patients with neurodegenerative and psychiatric disorders such as schizophrenia and bipolar disorder have increased concentrations of kynurenines in the CSF and brain tissue. [12] Thus, increased activity of the kynurenine pathway with age may lead to adverse outcomes. However, it is not

known at which brain concentrations QA becomes clinically relevant to adverse outcomes. Consequently, whether QA is a relevant pathophysiological factor in age-related, neurological, and psychiatric disorders needs to be further investigated.

Frailty and the Kynurenine Pathway

We further aimed to assess associations between plasma Trp, the kynurenines, and CRP with frailty in HUSK. Frailty is an important hallmark of pathological aging, [166] defined as: "a state of vulnerability to adverse outcomes". One method to measure frailty is by a frailty index. Its principle is to count health deficits on the basis that the more health deficits a person has, the frailer they are. [117] Across multiple studies, frailty is significantly associated with poor health outcomes and a shorter lifespan. [167] We constructed a frailty index based on available data on health deficits in HUSK from community-dwelling older adults aged 71-74 years, as previously described (see Methods, Study III: Frailty Index, p. 42). [117] Among the kynurenines, plasma QA was most strongly associated with frailty, followed by Kyn and KTR. However, CRP was more strongly associated with frailty than any of the kynurenine metabolites. HK, KA, and HAA were also significant but less strongly related to frailty. These findings are largely consistent with the kynurenines most associated with aging in HUSK, MCCS, and WENBIT (**Study III**). Our results are also in line with previous findings on kynurenines and frailty, [168,169] and underscore a consistent association between QA and frailty. Westbrook et al[168] found higher Kyn, HK, KA, QA, and KTR, and lower Trp in frail persons above 70 years of age compared to younger individuals. Similarly, in a study by Marcos-Pérez et al[169] of older adults above 65, frailty was associated with higher KTR and neopterin concentrations and reduced Trp concentrations. Our results and those of previous studies suggest that the kynurenines are associated with frailty, although we did not find lower Trp with increasing frailty as in previous studies.

Experimental studies have also found associations between inflammation, the kynurenine pathway, and frailty. In older IL-10 knockin mice, higher levels of KTR and reduced concentrations of Trp were associated with reduced function and loss of integrity of neuromuscular junctions. [168] Further, aged mice injected with an IDO-

inhibitor showed an increase in the size of muscle fibers and muscle strength. [170] Taken together, a potential role in sarcopenia in experimental studies, a hallmark of frail individuals, [171] adds support for a role of the kynurenine pathway in frailty.

Mortality and the Kynurenine Pathway

Lastly, we aimed to assess associations between Trp, the kynurenines, and CRP with mortality. In comparison to the other kynurenines, plasma QA and KTR displayed relatively stronger associations with mortality consistent in both MCCS and HUSK, which were independent of age, sex, renal function, and CRP. Higher plasma Trp concentrations were associated with enhanced survival in both cohorts. The associations between Kyn, HK, AA, KTR, and CRP with mortality in HUSK have been published previously. [172] Increased KTR has been associated with coronary events, cancer, frailty, and mortality in nonagenarians. [2] The kynurenine biomarkers most associated with aging (i.e., KTR and QA) were also the most associated with mortality.

Experimental studies have identified potential links between Trp, the kynurenines, and longevity. In *C. elegans* and *D. melanogaster,* depletion or loss of function of TDO results in increased longevity. [173,174] TDO-2 knockout in *C. elegans* increased lifespan by 15%. [173] In a study encompassing 26 mammalian species, KTR was negatively correlated with longevity. [175]

Summary of Study III

Overall, our results are in line with previous findings indicating altered kynurenine pathway activity with age. Trp decreases with age, accompanied by an increase of downstream kynurenines. Our study expands upon previous studies and show that these age-related changes are consistent with changes over time in MCCS and WENBIT for Trp, Kyn, HK, QA, and KTR, a critical finding to identify an aging biomarker. [87] Further, decreased Trp and increased KTR and QA are seen with minor differences in age. Higher Trp concentrations were associated with lower all-cause mortality, whereas higher QA and KTR concentrations were associated with higher

all-cause mortality. Compared to other kynurenines, QA displayed the strongest association with age and changed the most over time. Kyn and the potentially neurotoxic QA were more strongly correlated with age in the CSF than in serum, and increased in the CSF over a period of four years. It is unknown at which concentrations QA becomes clinically relevant for adverse outcomes in the brain. Still, it appears the aging brain could be exposed to a disproportionate increase in the excitotoxic QA.

FUTURE DIRECTIONS

The kynurenine pathway is connected to multiple physiological systems. Future studies of the kynurenine pathway in aging and age-related diseases should include multiple sample types, such as plasma, urine, feces, CSF, and immune cells. This would help provide a more complete picture. It is currently not clear whether the kynurenines are simply markers of immune activation or mediators between inflammatory activation and immune function, and potentially cognitive and psychiatric outcomes by way of their neuroactivity. In humans, studies including more detailed immune profiling with longitudinal, repeated measurements would be highly informative. Similarly, comprehensive metabolic profiling, including the NAD^+ metabolome would provide information on the relationship between QA and NAD^+, which is implicated in aging and neurodegenerative diseases. [164]

The role, if any, of the kynurenine pathway in normal cognitive function needs to be further investigated in otherwise healthy animals using experimental manipulation more reflective of the physiological condition rather than genetic knockout of enzymes. KAT inhibitors are being considered for clinical trials in schizophrenia [176] and if proven effective, this could be considered for neuropsychiatric symptoms in dementia, as there are few, if any, available and effective treatments for patients with dementia and neuropsychiatric symptoms. [177]

There are indeed numerous ways of intervening therapeutically to target enzymes of the kynurenine pathway, each with its challenges. The depletion of Trp with aging suppresses T-cell proliferation, and theoretically, replenishing Trp could improve the immune response. However, higher substrate availability may also lead to an increase in downstream neuroactive and immunomodulatory metabolites. [9] Analogues of neuroprotective kynurenines or KMO inhibitors aim to skew the balance of kynurenines towards neuroprotection and are considered to hold therapeutic potential. However, it remains unclear whether KA displays clinically relevant neuroprotective effects. [12] Further, as selective NMDAR knockout is an animal model of schizophrenia, caution should be exercised in considering an NMDAR antagonist for

clinical trials investigating psychiatric disorders or neuropsychiatric symptoms in dementia. KMO inhibition, like IDO inhibitors, may lead to an excessive reduction of QA, which functions as a precursor of NAD^+. Accordingly, concomitant NAD^+ supplementation might be pertinent. [11] KMO inhibition may elevate peripheral Kyn levels, which can be converted to QA in the brain after crossing the BBB. Therefore, an efficient KMO inhibitor would require sufficient brain penetrance for therapeutic efficacy. [12] This could be avoided with an IDO inhibitor, which inhibits the formation of Kyn, the main precursor of brain kynurenines. IDO inhibitors have been investigated in cancer research, and may potentiate the efficacy of chemotherapy, and promote tumor regression, but these mechanisms remain unclear. [178] In addition, clinical and experimental studies suggest KAT inhibitors to reduce brain KA concentrations as a new therapeutic approach for cognitive and psychotic disorders. However, no randomized trials have been conducted to date. [52] All told, further research investigating the kynurenine pathway has the potential to result in novel and more effective treatments for multiple diseases. However, due to the kynurenines' complex relationship with numerous biological pathways and organ systems, this is likely not without risk.

STRENGHTS AND LIMITATIONS

The research included in this thesis has several strengths. We included five independent cohorts from different populations, with cross-sectional and longitudinal data. Several of the cohort studies had relatively large sample sizes (MCCS, HUSK, WENBIT), which increase the statistical power of our analyses. We performed comprehensive, targeted metabolomic profiling of the kynurenines in serum, plasma, and CSF using a centralized laboratory for all analyses. The HUSK study had a relatively high response rate among the participants, and they were of similar age (71-74 years old), which limits the impact of age itself on metabolites and cognition (**study I**). Further, the DemVest study had a longitudinal design with annual follow-up examinations until death, combined with a low dropout rate amongst the patients who survived five years of follow-up (**study II**). In addition, the COGNORM performed repeated CSF sampling of patients who underwent elective surgery (**study III**).

This thesis also has several limitations. General limitations include a lack of ability to identify causal relationships, common to all observational studies. Multilevel models assume that missing data follow the missing at random assumption. This means that persons with missing data can be characterized by observed data rather than unobserved data. This statistical assumption is not easily testable and may bias the results. The frailty analysis was performed under the assumption that the data were missing completely at random, increasing the risk of bias (**study III**). The relatively small sample size in **study II** is associated with a higher risk of bias compared to **studies I** and **III** as the normality assumptions are less likely to hold. The kynurenine metabolites are intercorrelated. It is therefore difficult to ascertain whether associations represent single metabolites or more broad activities in the metabolic pathway. Statistical transformation assures that the assumption of normality, a prerequisite of most multivariable models, is not violated but unfortunately complicates the interpretation of effect sizes, as the statistical relationships become non-linear.

This thesis included five independent cohorts with comprehensive kynurenine metabolite measurements. However, the sampling was non-standardized and metabolite differences between cohorts may relate to variation in follow-up times, blood sampling, and time to censoring. As Trp is an essential amino acid, non-fasting blood samples is a limitation in DemVest, HUSK, WENBIT and among a proportion of MCCS samples. CSF measurements in **studies I** and **II** would have been informative, as only Trp, Kyn, and HK are transported across the BBB. The absence of a suitable control group is a further limitation in **study II.**

There are limitations related to the assessment of cognitive function, depression, and neuropsychiatric symptoms in this thesis. When measuring cognitive function, cognitive domains are not mutually exclusive, which can make interpretation of cognitive test results challenging, as one test may measure multiple cognitive domains such as language, memory, and executive function (**studies I** and **II**). Further, the HADS is not a diagnostic test for depression (**study I**). In addition, we used antidepressants as a surrogate marker for depressive symptoms, but these medications have several other indications, such as treatment of anxiety and sleep disturbances. Patients with major depression are also less likely to participate in studies (**study I**).

Finally, at present there is no clear consensus on how to best measure frailty, but the frailty index has been validated in several studies. [117] In comparison, previous studies [168,169] have measured frailty using the validated frailty screening tool by Fried et al[179], which consists of grip strength, walking speed, and questions concerning fatigue, weight loss, and physical activity. It is not clear whether the use of the Fried screening tool or an alternative frailty scale would have changed the associations we observed in **study III**.

CONCLUSIONS

1. Plasma KTR and neopterin were associated with lower cognitive function in the domains of memory and language in community-dwelling older adults.
2. There were no associations between serum kynurenine pathway metabolites at baseline and cognitive prognosis in patients with mild dementia.
3. The relationship between Kyn and cognition was non-linear in community-dwelling persons and patients with mild dementia. Kyn concentrations around the geometric mean were associated with better cognitive performance whereas low and high concentrations were associated with poorer cognitive performance.
4. Higher KKR was associated with more neuropsychiatric symptoms over time in patients with mild dementia.
5. Kyn, HK, KA, and most notably QA and KTR were positively associated with age and increased over time, whereas Trp was inversely associated with age and decreased over time.
6. Kyn and the potentially neurotoxic QA were more strongly correlated with age in the CSF than in serum.
7. Several kynurenines, most notably QA, Kyn, and KTR were associated with frailty. Higher Trp concentrations were associated with lower all-cause mortality, whereas higher QA and KTR concentrations were associated with higher all-cause mortality in two independent cohorts.
8. Kynurenines were better biomarkers of chronological age compared to CRP and were about equivalently associated with frailty and mortality.

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Full-length Article

The kynurenine pathway and cognitive performance in community-dwelling older adults. The Hordaland Health Study

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ABSTRACT

Introduction: Tryptophan, its downstream metabolites in the kynurenine pathway and neopterin have been associated with inflammation and dementia. We aimed to study the associations between plasma levels of these metabolites and cognitive function in community-dwelling, older adults.

Methods: This cross-sectional study included 2174 participants aged 70–72 years of the community-based Hordaland Health Study. Tryptophan, kynurenine, neopterin and eight downstream kynurenines were measured in plasma. Kendrick Object Learning Test (KOLT), Digit Symbol Test (DST) and the Controlled Oral Word Association Test (COWAT) were all outcomes in standardized Zellner's regression. The Wald test of a composite linear hypothesis of an association with each metabolite was adjusted by the Bonferroni method. Age, body mass index, C-reactive protein, depressive symptoms, diabetes, education, glomerular filtration rate, hypertension, previous myocardial infarction, prior stroke, pyridoxal 5′phosphate, sex and smoking were considered as potential confounders.

Results: Higher levels of the kynurenine-to-tryptophan ratio (KTR) and neopterin were significantly associated with poorer, overall cognitive performance ($p < 0.002$). Specifically, KTR was negatively associated with KOLT ($\beta -0.08$, $p = 0.001$) and COWAT ($\beta -0.08$, $p = 0.001$), but not with DST ($\beta -0.03$, $p = 0.160$). This pattern was also seen for neopterin (KOLT: $\beta -0.07$; $p = 0.001$; COWAT: $\beta -0.06$, $p = 0.010$; DST: $\beta -0.01$, $p = 0.800$). The associations were not confounded by the examined variables. No significant associations were found between the eight downstream kynurenines and cognition.

Conclusion: Higher KTR and neopterin levels, biomarkers of cellular immune activation, were associated with reduced cognitive performance, implying an association between the innate immune system, memory, and language.

1. Introduction

Tryptophan (TRP), an essential amino acid, is degraded primarily through the kynurenine pathway (KP, Fig. 1) which generates metabolites collectively referred to as the kynurenines (Chen and Guillemin, 2009). TRP and the kynurenines have been related to cognitive impairment (Baran et al., 1999), cardiovascular disease (Sulo et al., 2013; Zuo et al., 2016), renal function (Theofylaktopoulou et al., 2013), inflammation, obesity, diabetes and psychiatric disorders (Cervenka et al., 2017). However, a relationship between the kynurenines and

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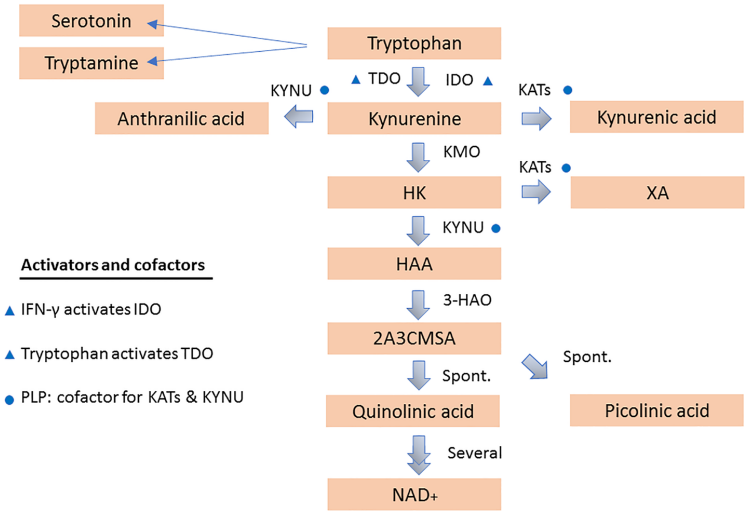

Fig. 1. The kynurenine pathway. TDO and IDO converts tryptophan to kynurenine. HK is converted to 3-hydroxyanthranilic acid (HAA) by kynureninase (KYNU), and subsequently to quinolinic acid (QA), catalyzed by quinolinate phosphoribosyl transferase. QA is converted to nicotinamide adenosine dinucleotide (NAD), the final product of the pathway. Anthranilic acid is produced from KYN by KYNU. Kynurenine aminotransferases (KATs) generate KA from KYN and xanthurenic acid (XA) from HK. Picolinic acid is produced by spontaneous conversion of HAA. Both KYNU and KATs have pyridoxal 5′-phosphate (PLP) as a cofactor (Chen and Guillemin, 2009). HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; 3-HAO, 3-hydroxyanthranilic acid 3, 4-dioxygenase; IDO, indoleamine 2, 3-dioxygenase; KATs, kynurenine aminotransferases; KMO, kynurenine monooxygenase; KTR, kynurenine-tryptophan ratio; NAD^+, nicotine adenine dinucleotide; Spont, spontaneous; TDO, tryptophan 2, 3-dioxygenase.

cognitive function in a community-dwelling cohort has not been established.

Reduced levels of circulating TRP and several kynurenines have been found in persons with dementia compared to controls (Giil et al., 2017), while elevated levels of anthranilic acid (AA), a derivative of kynurenine (KYN), has been linked to dementia in a prospective study (Chouraki et al., 2017). In cognitively healthy persons, elevated levels of inflammatory mediators are linked to poor cognitive performance (Smith et al., 2012). The kynurenines are closely linked to the innate immune system and have immune regulatory actions (Hwu et al., 2000). During an inflammatory state, cytokines stimulate the activity and expression of indoleamine 2, 3-dioxygenase (IDO), which converts TRP to KYN, mostly in monocytes. This leads to reduced TRP and an increase in downstream kynurenines, especially KYN (Capuron et al., 2011). The most important activator of IDO is interferon-γ (IFN-γ), which also activates GTP-cyclohydrolase I (GTP-CH), the rate-limiting enzyme in the biosynthesis of neopterin, which is a pteridine produced by monocytes during inflammation (Wirleitner et al., 2002). Increased levels of neopterin have been linked to dementia (Parker et al., 2013).

IDO is also expressed in the brain and may have importance in the relationship between systemic inflammation and cognitive impairment (Comim et al., 2017). Inflammation activates IDO and may increase levels of neurotoxic kynurenine metabolites in the brain with potential harmful effects on the hippocampus (Lim et al., 2013; Schwarcz and Kohler, 1983). Further, TRP and KYN pass the blood-brain barrier (BBB) (Fukui et al., 1991; Smith et al., 1987) and are key substrates for the brain's synthesis of serotonin and kynurenines (Chen and Guillemin, 2009).

Our aim was to study the relationship between circulating levels of TRP, kynurenines, and neopterin with cognitive test performance in a community-based cohort of adults aged 70–72 years, The Hordaland Health Study (HUSK).

2. Methods

2.1. Study participants

Study participants were included from HUSK, conducted in Hordaland County, Western Norway (http://husk-en.w.uib.no). Details of the recruitment procedures in both the main study and the cognitive sub-study have been described previously (Nurk et al., 2007). Briefly, from the source cohort, 2841 participants born in 1925–27, living in Bergen and three surrounding municipalities, were invited by letter to participate in HUSK during 1997 to 1999. Of these, 2197 participants (77.3%) were included in the sub-study on cognitive function and of these, 2174 had available blood samples and were included in the present study. The presence of disease was not an exclusion criterion in this population-based cohort. The self-reported prevalence of hypertension was 32.8%, previous myocardial infarction 10.6%, diabetes 6.7%, and prior stroke 4.7% (Table 1). The Regional Committee for Medical and Health Research Ethics approved the study protocol (REC number: 2016/2208) and all participants provided signed informed consent.

2.2. Measurement of metabolites

Non-fasting blood samples were collected at baseline, and aliquots of EDTA plasma samples were stored at −80 °C until analysis. TRP, eight kynurenines (KYN, AA, kynurenic acid (KA), 3-hydroxykynurenine (HK), 3-hydroxyanthranilic acid (HAA), xanthurenic acid (XA), picolinic acid (PIC), quinolinic acid (QA)), pyridoxal 5′ phosphate (PLP), neopterin and cotinine were measured using liquid chromatography-tandem mass spectrometry (Midttun et al., 2009). In general, the kynurenine metabolites remain stable under long-term cryopreservation. TRP, KYN, KA, XA, PIC and QA remain stable. Under non-optimal preanalytical handling or storage, HK and HAA may decrease, while AA may increase (Hustad et al., 2012). However, all these three markers were within their normal concentration range in our study (Midttun et al., 2017). The ratio between kynurenine and tryptophan was calculated as KYN (μM)/TRP (μM) * 100. The limit of detection was 0.4 μmol/L for TRP, while for neopterin and the kynurenines, limits of detection ranged from 0.5 nmol/L to 7 nmol/L. Within-day and between-day coefficients of variation were 3.0–9.5% and 5.7–16.9%, respectively.

Plasma high-sensitivity C-reactive protein (CRP) level was determined using an immune-MALDI (matrix-assisted laser desorption/ionization) mass spectrometry method (Meyer and Ueland, 2014). For CRP, the limit of detection was 0.2 μg/L, and within-day and between-day coefficients of variation were 5.5–8.4% and 7.0–11.7%, respectively. All biochemical analyses were performed in the laboratory of Bevital AS (http://bevital.no).

Table 1
Demographic and clinical characteristics.

Variable	Statistic
Demographics and general health	
Age, years, median [range]	71 [70–72]
Women, %	55.2
Education, %	
< 7 years of Primary School	7.3
7–10 years of Primary School	31.6
1–2 years of High School	30.2
3 years of High School	11.9
College/University	19.0
Diabetes, %	6.7
Current smoking[a], %	17.8
eGFR, mL/min/1.73 m^2, mean [SD]	71.7 [15.7]
BMI, mean [SD]	26.1 [3.9]
Hypertension, %	32.8
Stroke, %	4.7
MI, %	10.6
Depressive symptoms, %	8.8
Antidepressants[b], %	4.3
NSAIDs[c], %	5.9
Cognitive test scores	
KOLT score, mean [SD]	35 [8.1]
COWAT score, mean [SD]	15 [5.5]
DST score mean [SD]	10 [4.2]
Metabolite levels	
TRP, μmol/L, median [IQR]	67.8 [17.5]
KYN, μmol/L, median [IQR]	1.72 [0.50]
KA, nmol/L, median [IQR]	54.8 [25.2]
AA, nmol/L, median [IQR]	16.0 [7.20]
XA, nmol/L, median [IQR]	16.4 [10.0]
HK, nmol/L, median [IQR]	36.1 [15.5]
HAA, nmol/L, median [IQR]	35.0 [17.0]
PIC, nmol/L, median [IQR]	49.4 [28.1]
QA, nmol/L, median [IQR]	462 [2 2 6]
Neopt, nmol/L, median [IQR]	8.70 [3.30]
PLP, nmol/L, median [IQR]	49.1 [44.5]
KTR, μmol/L/ μmol/L*100, median [IQR]	2.50 [0.70]
KA/QA, nmol/L/nmol/l*100, median [IQR]	11.87 [5.55]

[a] Plasma cotinine level ≥ 10 nmol/l.
[b] ATC-classification system, NX5 and N06A: selective serotonin and norepinephrine reuptake inhibitors. Tricyclic and tetracyclic antidepressants.
[c] ATC-registry groups M01A and N02B. AA, anthranilic acid; BMI, body mass index; COWAT, Controlled Oral Word Association Test; DST, Digit Symbol Test; eGFR, estimated glomerular filtration rate; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; IQR, inter-quartile range; KA, kynurenic acid; KA/QA, kynurenic acid-quinolinic acid ratio; KOLT, Kendrick Object Learning test; KTR, kynurenine-tryptophan ratio; KYN, kynurenine; MI, previous myocardial infarction; Neopt, neopterin; NSAIDs, non-steroidal anti-inflammatory drugs; PIC, piconilic acid; PLP, pyridoxal 5′phosphate; QA, quinolinic acid; TRP, tryptophan; XA, xanthurenic acid.

2.3. Testing of cognitive function

We identified ceiling effects in both a brief version of the Mini-Mental Status Examination and Block-Design (supplementary materials, Figure S1). This implies that the true level of cognitive function has not been accurately measured in the participants who reached the ceiling effects. Further, the Trail Making Test A displayed a log-normal distribution with a bimodal trend. These cognitive tests were therefore considered unsuitable as measurements of cognitive function.

The following normally distributed tests, which indicates an appropriate difficulty level with a centralized mean, were selected to describe cognitive function: Kendrick Object Learning Test (KOLT), the Controlled Oral Word Association Test (COWAT), and the Digit Symbol Test (DST) (Nurk et al., 2007). Briefly, KOLT measures immediate recall and requires participants to observe picture charts, before telling the examiner what they observed (Kendrick, 1985). DST evaluates executive function and is performed by completing a coding table that consists of the numbers 1–9 and symbols. Participants are instructed to fill in blank squares with the symbol that is paired with the digit displayed above the square (Wechsler, 1981). Lastly, COWAT encourages participants to write as many words as possible beginning with a given letter in 60 s and is considered a measure of language, memory, and executive function (Benton A, 1989). Thus, the cognitive domains of memory, language, and executive function were examined in this study.

2.4. Potential confounders

Age, gender and educational attainment (Ngandu et al., 2007) were adjusted for. Cardiovascular disease (Zuo et al., 2016), diabetes (Stone and Darlington, 2002) and stroke (Darlington et al., 2007) have been associated with both kynurenines and cognitive performance (Biessels et al., 2008; Stampfer, 2006; Tatemichi et al., 1994). Similarly, a high body mass index (BMI) is associated with higher levels of kynurenines (Mangge et al., 2014) and poor cognition (Cournot et al., 2006). PLP is a coenzyme in the kynurenine pathway and associated with inflammation and cognitive function (Kennedy, 2016). Renal function determines kynurenine levels (Pawlak et al., 2002) and poor renal function is associated with cognitive dysfunction (Seliger et al., 2004). The same applies to smoking (Anstey et al., 2007). Thus, estimated glomerular filtration rate (Modification of Diet in Renal Disease equation) (Levey et al., 2006) and current smoking (plasma cotinine ≥ 10 nmol/L) were adjusted for in our analyses (Seccareccia et al., 2003).

Kynurenine levels are higher in major depression and TRP levels is lower (Myint et al., 2007). Furthermore, depression is associated with poor cognitive performance (Biringer et al., 2005). We aimed to establish whether kynurenines were associated with depressive symptoms in this population-based sample. To this purpose, we applied the Hospital Anxiety and Depression Scale (HADS) and defined a score of ≥8 as an indicator of mild depressive symptoms, in accordance with Stern et al. (Stern, 2014). HADS questionnaires with one or two missing answers on the items examining depressive symptoms were imputed as the mode of the other answers (N = 122). A total of 234 participants who underwent cognitive testing did not answer the HADS questionnaire. To further characterize if TRP and kynurenines were related to depressive symptoms in this study, we used antidepressant agents as a surrogate marker. Anti-depressive medications were categorized according to the 1997 ATC-classification system and included all agents under N06A (selective serotonin reuptake inhibitors, tricyclic and tetracyclic antidepressants) and NX5 (selective norepinephrine reuptake inhibitors). Further, we investigated if the use of non-steroidal anti-inflammatory drugs (NSAIDs) were associated with kynurenine levels. If so, these would be included in the multivariate models. NSAIDs were defined by the 1997 ATC-registry groups M01A and N02B.

Finally, CRP is of special interest, as it is one of the most frequent measures of inflammation reported as a negative determinant of cognitive performance. Innate immune activation is expected to increase both CRP levels, and the levels of several kynurenines downstream of TRP (Kuo et al., 2005; Zuo et al., 2016). We thus evaluated associations between TRP, kynurenines and CRP, and adjusted any significant findings for CRP levels.

2.5. Statistics

Prior to multivariable analysis, metabolites were transformed according to Tukey's ladder of Powers (Tukey, 1977). The purpose was to linearize relationships by achieving approximately normal distributions, as assessed by histograms and quantile-quantile plots. KYN was transformed by an inverse transformation, QA and KTR as the inverse of the square root and KA, XA, PIC, the KA/QA ratio, PLP and neopterin by log transformations. CRP was transformed by a Box-Cox transformation (Box and Cox, 1964). To compare effect sizes across the scales that arose from the use of transformations, all continuous covariates and

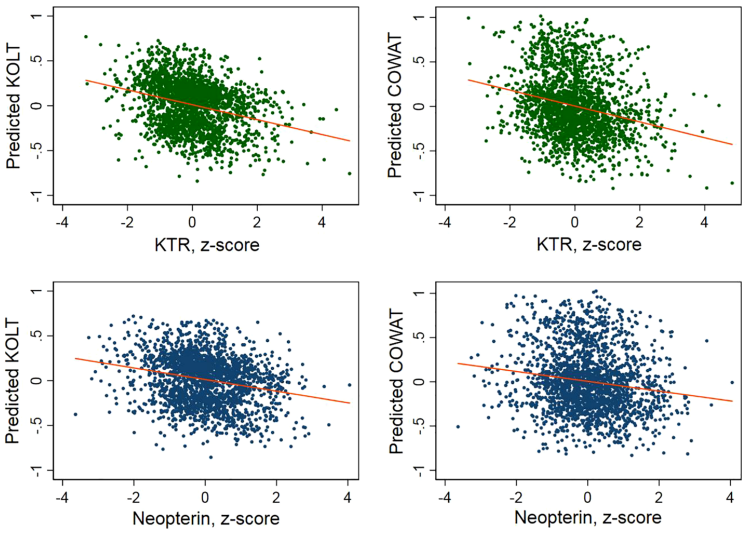

Fig. 2. Cognitive tests and markers of immune activation. Predicted results from Zellner's regression, adjusted for age, sex, body mass index, educational level, estimated glomerular filtration rate, current smoking, diabetes, hypertension, previous myocardial infarction, prior stroke, and pyridoxal 5′ phosphate as covariates. COWAT, Controlled Oral Word Association Test; KOLT, Kendrick Object Learning Test; KTR, kynurenine-tryptophan ratio.

outcomes were scaled to z-scores. Logistic- and linear regressions were used to determine associations between potential confounders, kynurenines and outcomes.

The cognitive tests were positively correlated (supplementary materials, Figure S2) and potentially not independent outcome variables. We first identified a highly significant Breusch-Pagan test (Breusch and Pagan, 1979), which indicates correlated residuals from separate linear regressions. Therefore, we decided to assess the associations between all cognitive tests and each metabolite by Zellner's seemingly unrelated regression (SUR), which estimates a set of *m* linear regressions with correlated error terms (Jahanshad et al., 2015). We used the two-step estimation procedure. After initial analysis with age, gender, body mass index, education (in years), GFR, current smoking, diabetes, previous myocardial infarction, prior stroke and PLP, additional confounders associated with TRP, kynurenines or neopterin, were adjusted.

It is impractical to formulate a hypothesis about which exact cognitive test is related to which metabolite. Therefore, we tested the joint significance of the association between each metabolite and "cognition", represented by the three cognitive outcomes in SUR. In order to test the joint significance, we applied the Wald test on a composite linear hypothesis (Cameron, 2009), composed of the three hypotheses of association between the metabolite and the three cognitive tests. The joint significance ($\alpha = 0.05$) threshold was adjusted for the number of hypotheses tested, according to the Bonferroni method (Chen et al., 2017). All statistical analyses were conducted using Stata (version 15, Stata Corp, College Station, Texas, USA).

3. Results

3.1. Participant characteristics

A total of 2174 participants (55.2% women), aged 70–72 years with cognitive tests and available blood samples are included in the analysis. The mean scores and standard deviations (SD) of KOLT, DST, and COWAT were 35 (SD: 8.1), 10 (SD: 4.2) and 15 (SD: 5.5), respectively. KOLT, DST, and COWAT were approximately normally distributed within the population (supplementary materials, Figure S2). Nineteen percent of the study participants went to college or university (Table 1). There were no major differences in the plasma concentrations of kynurenines between the subgroups completing cognitive testing and the HADS questionnaire.

3.2. Kynurenines and cognitive performance

KTR and neopterin were significantly inversely associated with cognitive performance measured by KOLT (memory) and COWAT (language) (Fig. 2), while no such associations were seen with DST (executive function) (Table 2). KTR showed the strongest association with cognitive performance (Table 2). Further, PLP was significantly associated with DST (β 0.069, p = 0.001), but did not act as a confounder.

3.3. Kynurenines and potential confounders

3.3.1. Depressive symptoms and antidepressant agents

TRP, KTR, the kynurenines, the KA/QA ratio and neopterin were not associated with depressive symptoms or antidepressant agents (Table 3). Thus, depression was not considered as a potential confounder for the relationship between kynurenines and cognition.

3.3.2. Non-steroidal anti-inflammatory drugs

Six percent of the participants reported use of NSAIDs (Table 1). NSAIDs showed an association with TRP, KYN and KTR (Table 3), but did not confound our results (Table 4).

3.3.3. C-reactive protein

CRP was associated with KYN, HK, HAA, QA, KTR, the KA/QA ratio and neopterin (Table 3). After adjusting the SUR model for CRP, it did not act as a confounder (Table 4).

4. Discussion

We studied cognition in relation to neopterin, tryptophan, and the kynurenines in a community sample of older adults and found that elevated levels of both KTR and neopterin were associated with lower performance in the cognitive domains of memory and language. KTR showed the strongest association.

Our study included 2174 persons (55.2% women) aged 70–72 years recruited from a population of home-dwelling older adults. In comparison, other studies that have investigated the relationship between the kynurenines, neopterin and cognitive function, have been based on small patient groups with specific diseases. Higher levels of neopterin and kynurenines were related to lower cognitive performance post-

Table 2
Cognitive performance and individual metabolites (N = 2174).[a]

	KOLT Memory			COWAT Language			DST Executive function			Wald test[d]	
Association of each metabolite with three cognitive tests											
	β	SE	p	β	SE	p	β	SE	p	X^2	p[c]
TRP	0.047	0.022	0.03	0.043	0.022	0.05	0.050	0.021	0.02	9.2	0.027
KYN[b]	-0.021	0.024	0.4	-0.030	0.024	0.2	0.026	0.023	0.3	4.6	0.2
KYN2[b]	-0.061	0.021	0.003	-0.021	0.020	0.3	-0.044	0.020	0.03	11.3	0.01*
KA	0.022	0.026	0.4	-0.011	0.025	0.7	0.034	0.024	0.2	3.1	0.38
AA	0.003	0.022	0.9	0.014	0.021	0.5	0.003	0.021	0.9	0.42	0.94
XA	0.049	0.023	0.03	0.024	0.023	0.3	0.047	0.022	0.03	7.3	0.06
HK	-0.002	0.242	0.9	-0.009	0.024	0.7	0.026	0.023	0.3	1.8	0.6
HAA	0.032	0.023	0.2	0.013	0.023	0.6	0.043	0.022	0.05	4.7	0.2
PIC	0.031	0.022	0.2	0.005	0.021	0.2	0.005	0.021	0.8	2.0	0.6
QA	-0.007	0.024	0.8	-0.050	0.024	0.04	0.034	0.023	0.1	9.0	0.03
KTR	-0.084	0.024	0.001	-0.077	0.024	0.001	-0.032	0.023	0.16	17.7	< 0.001*
Neopt	-0.074	0.023	0.001	-0.056	0.022	0.01	-0.007	0.022	0.8	14.6	0.002*
KA/QA	0.022	0.021	0.3	0.028	0.021	0.2	-0.003	0.021	0.9	2.8	0.43
Association between KTR and three cognitive tests, with covariates											
	β	SE	p	β	SE	p	β	SE	p	X^2	p
Age											
71	-0.024	0.050	0.6	0.072	0.049	0.1	-0.040	0.048	0.4	4.3	0.2
72	-0.050	0.050	0.3	0.072	0.049	0.1	-0.062	0.048	0.2	6.8	0.1
Female	0.408	0.044	< 0.001	0.072	0.043	0.1	0.085	0.042	0.05	85.6	< 0.001
GFR	-0.061	0.024	0.01	-0.067	0.024	0.005	-0.033	0.023	0.2	11.5	0.01
Edu	0.150	0.022	< 0.001	0.340	0.022	< 0.001	0.402	0.021	< 0.001	486.7	< 0.001
Smoke	-0.021	0.050	0.7	-0.016	0.049	0.8	-0.061	0.048	0.2	1.8	0.6
Dia	-0.103	0.086	0.2	-0.206	0.084	0.01	-0.090	0.082	0.3	6.8	0.08
BMI	-0.001	0.006	0.8	-0.005	0.005	0.4	0.001	0.005	0.8	0.92	0.8
HT	-0.045	0.046	0.3	-0.014	0.045	0.8	-0.049	0.044	0.3	1.5	0.7
MI	-0.123	0.069	0.07	-0.051	0.067	0.4	-0.022	0.066	0.7	3.4	0.3
Stroke	-0.246	0.098	0.01	-0.208	0.097	0.03	-0.091	0.095	0.3	8.7	0.03
PLP	-0.003	0.022	0.9	0.032	0.021	0.1	0.069	0.021	0.001	13.1	0.005
KTR	-0.084	0.024	0.001	-0.077	0.024	0.001	-0.032	0.023	0.16	17.7	< 0.001*

[a] Zellner's seemingly unrelated regression, estimated for each metabolite with age, BMI, dia, edu, GFR, MI, sex, smoking, hypertension, MI, stroke and PLP as covariates.
[b] The association between KOLT and KYN was non-linear. A second degree orthogonal polynomial gave a good fit.
[c] The significance threshold for 12 tests is 0.0042, according to the Bonferroni method, indicated by *.
[d] Test of the joint significance of the association between each metabolite and three cognitive outcomes. AA, anthranilic acid; BMI, body mass index; COWAT, Controlled Oral Word Association Test; Dia, diabetes; DST, Digit Symbol Test; Edu, education; GFR, glomerular filtration rate; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; HT, hypertension; KA, kynurenic acid; KA/QA, kynurenic acid-quinolinic acid ratio; KOLT, Kendrick Object Learning Test; KYN, kynurenine; KYN2, 2nd degree orthogonal polynomial of KYN; KTR, kynurenine-tryptophan ratio; MI, previous myocardial infarction; Neopt, neopterin; p, p-value; PIC, picolinic acid; PLP, pyridoxal 5′phosphate; QA, quinolinic acid; SE, standard error; Smoker, current smoking; Stroke, prior stroke; TRP, tryptophan; X^2, chi-squared; XA, xanthurenic acid; β, standardized regression coefficient.

operatively amongst patients who had undergone cardiac bypass surgery (N = 28, mean age of 60.2 years, 11% women), and major non-cardiac thoracic surgery (N = 28, mean age of 67.6 years, 32% women) (Forrest et al., 2011). Additionally, a study of patients with stage IV renal failure (N = 27, mean age of 76.4 years, 33% women), suggested that rising levels of neopterin and KYN were associated with lower cognitive performance (Karu et al., 2016). Further, neopterin was associated with progression of cognitive deficits in patients with Alzheimer's disease (Blasko et al., 2007; Leblhuber et al., 1999). Previous studies have shown that acute TRP depletion may impair episodic memory, and suggest a role of the serotonergic system in cognitive function (Mendelsohn et al., 2009). Our results support that the kynurenine pathway may be relevant for cognitive function.

There is evidence of an association between peripheral pro-inflammatory mediators, such as tumor necrosis factor-α, Il-6, and CRP, and reduced cognitive performance in healthy humans (Economos et al., 2013; Schram et al., 2007; Teunissen et al., 2003; Wichmann et al., 2014; Yaffe et al., 2003). Therefore, the kynurenines could rather be indirect markers of their underlying activators, which are mainly related to inflammation. We did not identify confounding from CRP in our data, but a more comprehensive assessment of inflammation would have been informative.

In this study, nine percent of the participants had depressive symptoms (HADS score > = 8), but we found no association with TRP or kynurenine levels. Although an association between kynurenines and major depression has been described, our study is not comparable and does not generalize to major depressive disorder. First, HADS is not a diagnostic test of depression (Cosco et al., 2012; Myint et al., 2007). Second, patients with major depression are less likely to participate as study volunteers (Hughes-Morley et al., 2015). Finally, participants with depressive symptoms and patients using antidepressants likely represent a heterogeneous group, as antidepressant agents have broad indications, for example for treating anxiety and sleeping disturbances in the elderly (Noordam et al., 2015). Here, depressive symptoms were mainly of interest as potential confounders.

PLP, the active form of vitamin B6, was associated with DST but did not act as a confounder in our study. Our findings are in line with studies indicating both a detrimental effect on cognition from PLP deficiency. Vitamin B6 is actively transported over the BBB and is a rate-limiting cofactor in the synthesis of neurotransmitters such as dopamine and serotonin (Kennedy, 2016). Circulating PLP levels are lower in individuals with inflammation compared to healthy subjects (Ueland et al., 2017), and has been proposed to contribute to cognitive decline (Kennedy, 2016). Intervention studies administering vitamin B6

Table 3
Evaluating potential confounders. Association with exposure [a].

	Depressive symptoms [b]		Anti-depressants [b]		NSAIDs [b]		C-reactive protein [c]	
	OR	p	OR	p	OR	p	β	p
TRP	1.01	0.9	0.91	0.4	0.65	< 0.001	-0.02	0.3
KYN	0.94	0.5	1.16	0.3	0.74	0.003	0.19	< 0.001
KA	0.88	0.2	0.89	0.4	0.96	0.7	0.04	0.2
AA	0.89	0.2	0.98	0.9	1.2	0.1	0.06	0.003
XA	0.93	0.4	0.80	0.1	0.93	0.4	-0.04	0.05
HK	1.10	0.3	1.06	0.7	1.00	0.9	0.17	< 0.001
HAA	0.97	0.7	1.11	0.4	0.93	0.5	0.15	< 0.001
PIC	0.98	0.8	0.87	0.2	1.02	0.9	-0.02	0.3
QA	0.98	0.8	1.07	0.6	1.02	0.82	0.24	< 0.001
KTR	0.94	0.5	1.27	0.1	1.29	0.01	1.21	< 0.001
Neopt	1.09	0.3	1.20	0.1	1.03	0.74	0.18	< 0.001
KA/QA	0.94	0.4	0.88	0.3	0.95	0.6	-0.15	< 0.001

Note. 252/2869 had depressive symptoms, 141/3319 used anti-depressants, and 196/3319 used NSAIDs.
[a] All models adjusted for age, body mass index, current smoking, diabetes, educational level, glomerular filtration rate, hypertension, previous myocardial infarction, prior stroke, pyridoxal 5′ phosphate and sex.
[b] Logistic regression.
[c] Linear regression. AA, anthranilic acid; HAA, hydroxyanthranilic acid; HK, hydroxykynurenine; KA, kynurenic acid; KA/QA, kynurenic acid-quinonilic acid ratio; KTR, kynurenine-tryptophan ratio; KYN, kynurenine; Neopt, neopterin; OR, odds ratio; p, p-value; PIC, picolinic acid; QA, quinonilic acid; TRP, tryptophan; X^2, chi-squared; XA, xanthurenic acid; β, standardized regression coefficient.

Table 4
Cognitive performance and individual metabolites. Adjusted models (N = 2174).[a]

	KOLT Memory			COWAT Language			DST Executive function			Wald test[b]	
Model 1: Unadjusted model											
	β	SE	p	β	SE	p	β	SE	p	X^2	P
KTR	-0.084	0.024	0.001	-0.077	0.024	0.001	-0.032	0.023	0.2	17.7	< 0.001
Neopt	-0.074	0.023	0.001	-0.056	0.022	0.01	-0.007	0.022	0.8	14.6	0.002
Model 2: Adjustment for C-reactive protein											
KTR	-0.078	0.025	0.002	-0.081	0.025	0.001	-0.040	0.024	0.1	16.1	0.001
Neopt	-0.071	0.024	0.003	-0.052	0.023	0.02	-0.011	0.022	0.6	11.7	0.008
Model 3: Adjustment for non-steroidal anti-inflammatory drugs											
KTR	-0.082	0.024	0.001	-0.079	0.024	0.001	-0.032	0.023	0.2	17.8	< 0.001
Neopt	-0.074	0.022	0.001	-0.055	0.022	0.01	-0.007	0.022	0.7	14.5	0.002

Note. 196 out of 3319 participants used non-steroidal anti-inflammatory drugs.
[a] Zellner's seemingly unrelated regression, estimated for each metabolite with age, sex, body mass index, educational level, glomerular filtration rate, current smoking, diabetes, hypertension, previous myocardial infarction, prior stroke, pyridoxal 5′ phosphate as covariates and either CRP (Model 2) or NSAIDs (Model 3).
[b] Test of the joint significance of association between each metabolite and all three cognitive outcomes. Neopt, neopterin; KTR, kynurenine-tryptophan ratio; p, p-value; SE, standard error; X^2, chi-squared; β, standardized regression coefficient.

supplementation for age-related memory decline has shown some encouraging trends (Deijen et al., 1992). However, given the involvement of PLP in a wide range of biological processes, it remains to be determined if PLP plays an active role in cognitive performance.

Our rationale for using the KTR was that it provides a better measure of IDO activity than the individual metabolites, particularly when KTR correlates with inflammatory markers, such as neopterin (Schrocksnadel et al., 2006). The rate-limiting enzymes in kynurenine and neopterin biosynthesis, IDO and GTP-CH respectively, are both induced by IFN-γ. The absence of IFN-γ is associated with improvements in neurogenesis, synaptic plasticity, and performance in hippocampus-dependent tasks in mice (Monteiro et al., 2016). Experimental studies have implicated IDO in inflammation-associated cognitive dysfunction (Chen and Guillemin, 2009; Comim et al., 2017; Heisler and O'Connor, 2015; Yu et al., 2015).

Experimental studies support a neuroexcitatory role of QA in the brain, and a neuroprotective role of KA. The hippocampus has been reported to be particularly susceptible to the neurotoxic effects of QA (Schwarcz and Kohler, 1983). KA, on the other hand, is considered neuroprotective (Leib et al., 1996). We did not find evidence to support that the ratio between these metabolites (KA/QA) in peripheral blood was related to cognitive function. However, QA and KA cross the BBB poorly (Fukui et al., 1991), and therefore measurement in the cerebrospinal fluid will be needed to settle this issue.

An important question in studies such as ours is to what extent, if any, peripheral inflammation relates to neuroinflammation. Blood-borne cytokines can enter the brain by transport systems at the BBB (Varatharaj and Galea, 2017) and immune cells enter the brain under physiological conditions, though at a much lower rate than in other organs (Takeshita and Ransohoff, 2012). TRP and KYN are themselves transported to the brain and are precursors of both brain serotonin (Young and Leyton, 2002) and kynurenines (Chen and Guillemin, 2009). In microglia, KYN is a precursor for QA, which could activate the N-methyl-D-aspartate receptor (NMDAR) (Ganong and Cotman, 1986). Thus, high plasma KTR may be related to cognitive function as a marker of inflammation, serotonin depletion and NMDAR activation in the brain, but this must be investigated in future studies.

Strengths of this study include a large sample size of 2174 persons, a

relatively high response rate among the participants, and similar age of the participants (70–72 years), which limits the impact of age itself on metabolites and cognition. The main limitations are the constraints of cross-sectional studies. This involves difficulties in ascertaining the direction of associations between predictors and outcomes and the potential for unmeasured confounders. Cognitive domains are not mutually exclusive, which can make interpretation challenging (Malek-Ahmadi et al., 2011). Further, non-fasting blood samples is a limitation, and measurements of the kynurenines in the cerebrospinal fluid would have been informative. Longitudinal studies are needed to further delineate these associations.

In summary, we found that KTR and neopterin, biomarkers of cellular immune activation, were associated with a lower cognitive function in the domains of memory and language in a sample of community-dwelling older adults. The findings add support for a role of the innate immune system in cognitive function.

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Appendix A. Supplementary data

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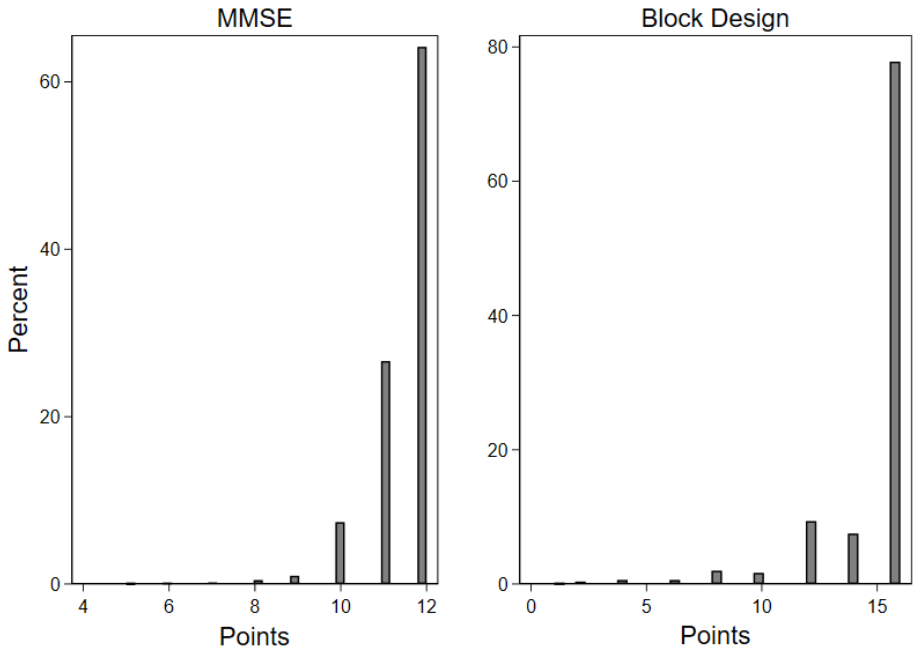
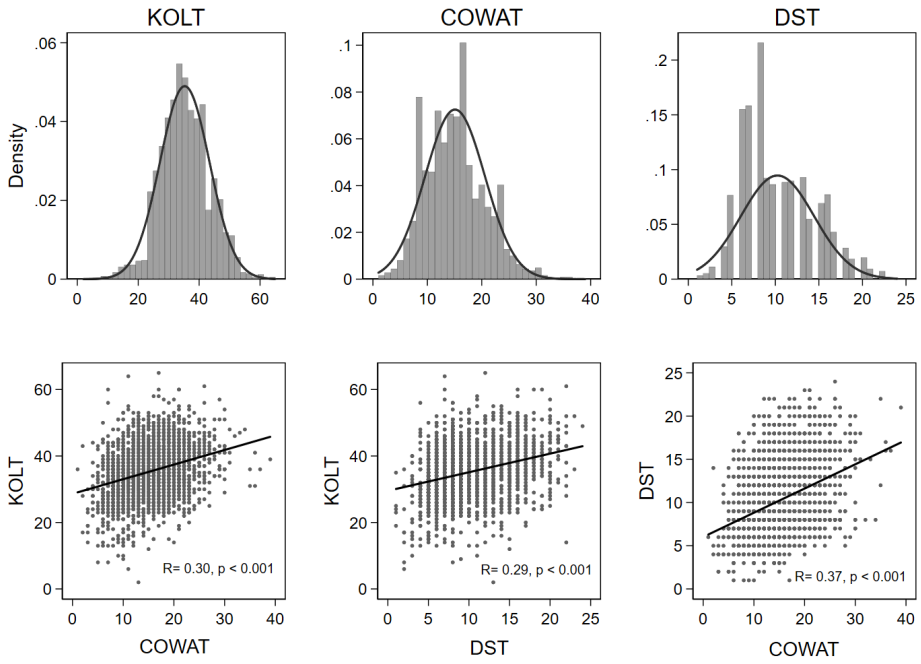
Supplementary Figure 1. Ceiling effects in MMSE and Block Design



The 12-point MMSE displayed considerable ceiling effects. 90.3 % scored ≥ 11 points; of these, 26.5% scored 11 and 63.8% scored 12 points. 87.7% of these participants also scored 16 out of 16 points on Block Design (97.7% scored 12 points or more). The ceiling effects were increased amongst participants with higher education. 2152 participants completed the Mini-Mental Status Examination, and 2168 participants completed the Block Design Test. MMSE, Mini-Mental Status Examination.

Supplementary Figure 2. Distributions of KOLT, COWAT and DST



First row = histograms, second row = bivariate distributions with regression lines. COWAT, Controlled Oral Word Association Test; DST, Digit Symbol Test; KOLT, Kendrick Object Learning Test; p, p-value; R, Pearson's correlation coefficient.

Kynurenines, Neuropsychiatric Symptoms, and Cognitive Prognosis in Patients with Mild Dementia

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ABSTRACT

INTRODUCTION: Circulating tryptophan (Trp) and its downstream metabolites, the kynurenines, are potentially neuroactive. Consequently, they could be associated with neuropsychiatric symptoms and cognitive prognosis in patients with dementia.

OBJECTIVE: The objective of this study was to assess associations between circulating kynurenines, cognitive prognosis, and neuropsychiatric symptoms.

METHODS: We measured baseline serum Trp, neopterin, pyridoxal 5′-phosphate (PLP), and 9 kynurenines in 155 patients with mild dementia (90 with Alzheimer's disease, 65 with Lewy body dementia). The ratios between kynurenine and Trp and kynurenic acid (KA) to kynurenine (KKR) were calculated. The Mini-Mental State Examination (MMSE) and the Neuropsychiatric Inventory (NPI) were administered at baseline and annually over 5 years. Associations between baseline metabolite concentrations with MMSE and the NPI total score were assessed using a generalized structural equation model (mixed-effects multiprocess model), adjusted for age, sex, current smoking, glomerular filtration rate, and PLP. Post hoc associations between KKRs and individual NPI items were assessed using logistic mixed-effects models. False discovery rate (0.05)–adjusted *P* values (*Q* values) are reported.

RESULTS: Kynurenine had a nonlinear quadratic relationship with the intercept of the MMSE scores over 5 years ($Q < 0.05$), but not with the slope of MMSE decline. Kynurenine was associated with a higher NPI total score over time ($Q < 0.001$). Post hoc, both KKR and KA were associated with more hallucinations ($Q < 0.05$).

CONCLUSIONS: Kynurenine has a complex relationship with cognition, where both low and high levels were associated with poor cognitive performance. A higher KKR indicated risk for neuropsychiatric symptoms, especially hallucinations.

KEYWORDS: Kynurenines, hallucinations, neuropsychiatric symptoms, kynurenic acid, kynurenine, Alzheimer's disease, Lewy body dementia

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Introduction

The essential amino acid tryptophan (Trp) is degraded through the kynurenine pathway (Figure 1), giving rise to metabolites referred to as kynurenines.[1] The kynurenine pathway is most highly expressed not only in liver and monocytes[2] but also in muscle, brain, and intestine.[3] The kynurenines and the rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO) of the kynurenine pathway have been implicated in experimental cognitive dysfunction in mice,[4–7] and kynurenines are lower in Alzheimer's disease (AD) compared with healthy controls.[8]

Tryptophan 2,3-dioxygenase (TDO) and IDO generate kynurenine (Kyn) from Trp,[9] which gives rise to downstream metabolites that have shown neuroprotective (kynurenic acid [KA])[10] and neurotoxic properties (quinolinic acid [QA]).[11] Both KA and QA act as antagonist and agonist, respectively, at the *N*-methyl-D-aspartate receptor (NMDAR), suggesting a potential role of kynurenines in relation to signal transduction pathways related to cognitive dysfunction.[12] The key enzymes IDO and kynurenine 3-monooxygenase (KMO) are induced by pro-inflammatory cytokines. KMO converts Kyn

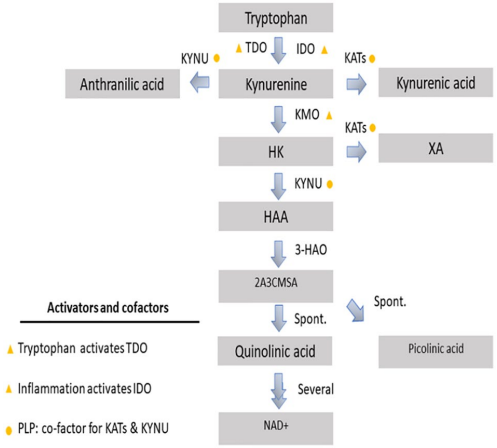

Figure 1. The kynurenine pathway. TDO and IDO convert tryptophan to kynurenine (Kyn). HK (3-hydroxykynurenine) is converted to 3-hydroxyanthranilic acid (HAA) by kynureninase (KYNU), and subsequently to quinolinic acid (QA), catalyzed by quinolinate phosphoribosyl transferase. QA is converted to nicotinamide adenosine dinucleotide (NAD^+), the final product of the pathway. Anthranilic acid (AA) is produced from Kyn by KYNU. Kynurenine aminotransferases (KATs) generate KA from Kyn and xanthurenic acid (XA) from HK. Picolinic acid (PIC) is produced by spontaneous conversion of HAA. Both KYNU and KATs have pyridoxal 5′-phosphate (PLP) as a cofactor.[9] IDO indicates indoleamine 2,3-dioxygenase; TDO, tryptophan 2, 3-dioxygenase; KMO, kynurenine 3-monooxygenase; KATs, kynurenine aminotransferases; 3-HAO, 3-hydroxyanthranilic acid 3, 4-dioxygenase; Spont., spontaneous; NAD^+, nicotine adenine dinucleotide; HK, 3-hydroxykynurenine; HAA, 3-hydroxyanthranilic acid.

to 3-hydroxykynurenine (HK).[13] Interferon gamma (IFN-γ) is the most potent activator.[2] Higher circulating levels of kynurenine metabolites are associated with depression[14] and elevated postmortem brain levels of kynurenines, and relevant enzymes have been observed in patients with psychotic and mood disorders.[15-19] Cerebrospinal fluid (CSF) levels of KA were not significantly altered in patients with dementia with Lewy bodies (DLB) compared with controls.[20] The kynurenine-to-tryptophan ratio (KTR) and neopterin, which are biomarkers of cellular immune activation, have been associated with reduced cognitive performance in community-dwelling older adults.[21]

We aimed to assess whether the levels of circulating kynurenines at baseline predicted cognitive prognosis and neuropsychiatric symptoms over 5 years in patients with AD and Lewy body dementia (LBD).

Methods

Study participants

The Dementia Study of Western Norway (DemVest) is a multicenter longitudinal cohort study with annual follow-up until death. The study recruited 155 participants from specialist clinics of neurology and old-age psychiatry situated in the Norwegian counties Hordaland and Rogaland with available blood samples in a biobank. Participant recruitment during 2005 to 2007 relied on fulfillment of the inclusion criteria: patients diagnosed with mild dementia for the first time and a minimal Mini-Mental State Examination (MMSE) score of 20.[22] Thereafter, selective recruitment of patients with either DLB or Parkinson disease with dementia (PDD) was undertaken. Thus, the latter 2 patient groups are overrepresented in the study. Due to similar pathologies, DLB and PDD were classified together as LBD.

Independently, 2 physicians experienced in the diagnostic workup of dementia made a clinical diagnosis using the NINCDS-ADRDA criteria for AD (National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association)[23] and the revised consensus criteria for DLB (2005).[24] A detailed study protocol has been published.[22] Briefly, a physician interviewed the patient together with a caregiver who provided complementary information. Medical history was also obtained from electronic records and a clinical neurological examination was performed. In addition to a global cognitive assessment of cognition by the MMSE, and dementia severity using Clinical Dementia Rating, patients were assessed with a standardized neuropsychological test battery. In situations with diagnostic uncertainty, physicians discussed each case until consensus. In addition, after 5 years, 3 specialists in geriatrics and psychiatry revised the diagnoses in consensus meetings. All patients were followed longitudinally with annual assessments with MMSE and the Neuropsychiatric Inventory (NPI), mostly until death. Due to the progressive nature of dementia, most patients followed over time will reach a point where they score 0 on the MMSE on each consecutive follow-up. This is called the floor effect. At this point, the MMSE can no longer measure further disease progression, and for a statistical model, it will look as if disease progression has stopped. Furthermore, variance will be reduced at follow-ups with many 0 scores. This will result in the introduction of a range of statistical biases, which are not easily compensated for, especially if a substantial proportion of patients reach a floor or ceiling effect.[25] Therefore, a decision was made to censor the study on biomarkers after the fifth follow-up.

Postmortem studies from the full DemVest study (56 autopsies) found that the concordance rate for a clinical diagnosis compared with a pathological diagnosis was 83% for AD and 80% for LBD.[26]

Some data during follow-up were missing. Most were observed in an intermittent pattern, meaning that the patient missed one appointment and later returned to the study. The proportion of missing measurements that was not due to death was small. The MMSE and NPI were assessed at the same visit and thus had largely corresponding missing measurements. Accordingly, missing measurements for the MMSE are listed. For the MMSE, there were no missing measurements at baseline, 6 missing measurements at the first follow-up, 11 at the second follow-up, 9 at the third follow-up, 6 at the fourth

follow-up, and 8 at the fifth follow-up. During the study period, several patients died prior to their planned follow-up. At the second follow-up, 15 patients had died, 34 at the third, 55 at the fourth, and 78 at the fifth follow-up.

The Mini-Mental State Examination

The MMSE has maximum score of 30 and a minimum of 0 and consists of a variety of questions, grouped into 7 categories representing different cognitive domains. The categories are orientation to time, orientation to place, registration of 3 words, attention and calculation, recall of 3 words, language, and visual construction.[27] A decline of 2 to 4 points is considered a reliable change,[28] and about 3 points is also the expected annual decline.[29]

The Neuropsychiatric Inventory

The NPI evaluates 12 neuropsychiatric symptoms common in dementia: delusions, hallucinations, agitation, apathy, dysphoria, anxiety, irritability, euphoria, disinhibition, motor disturbances, and sleep- and appetite disturbances. A caregiver familiar with the patient rates the severity and frequency of each neuropsychiatric symptom using a standardized questionnaire. A combined score for each symptom is calculated by multiplying the frequency by severity. The total score is determined by adding all the domain scores together.[30] We used the NPI total score to limit the number of outcomes.

Measurement of metabolic biomarkers

Baseline levels of Trp, anthranilic acid (AA), 3-hydroxyanthranilic acid, HK, KA, Kyn, picolinic acid, QA, xanthurenic acid (XA), pyridoxal 5′-phosphate (PLP), and neopterin were measured using liquid chromatography-tandem mass spectrometry in serum samples, collected between 2005 and 2009, and stored at −80°C until analysis in 2018. The ratio between Trp and Kyn (KTR) was defined as Kyn (μM)/Trp (μM)*100 and the kynurenic acid-to-kynurenine ratio (KKR) was estimated. The limit of detection for neopterin and the kynurenines ranged from 0.5 to 7 nmol/L, whereas the limit of detection for Trp was 0.4 μmol/L. Within-day and between-day coefficients of variation were 5.7% to 16.9% and 3.0% to 9.5%, respectively. The biochemical analyses were performed at the laboratory of Bevital AS (Bergen, Norway; http://bevital.no). We did not detect any significant correlations between metabolite levels and storage time using Spearman rank order correlations (data not shown).

Statistics

Univariate differences between AD and LBD were assessed using *t* tests, Pearson χ^2, and Mann-Whitney *U* tests for normal, categorical, and skewed variables, respectively. Metabolite concentrations were transformed to approximate normality using Tukey's ladder of powers.[31] A constant of one was added prior to logarithmic transformation for all metabolites with a minimum concentration below 1 to avoid an uneven spread of the data after logarithmic transformations. Associations between cognitive deterioration and neuropsychiatric symptoms over 5 years and baseline metabolite levels were examined in a multiprocess model or joint model. Of note, although patients underwent 5 annual follow-up examinations, there were occasional delays, and some patients were followed for 6 years. The MMSE test scores were transformed by the square root of errors, $\sqrt{}$(30-MMSE), thereby higher values indicate poorer performance. The MMSE raw scores were right skewed toward higher scores, which is problematic in statistics, as transformations typically work best to obtain normality with left-skewed data. Thus, the reciprocal of MMSE (30 – MMSE) was calculated to obtain a right-skewed distribution of the number of errors committed by patients on the MMSE (an MMSE score of 24 is 30 – 24 = 6 errors). After this, the square root transformation of the MMSE errors resulted in an approximately normal distribution as assessed by quantile-quantile plots and histograms.[32] However, 37 measurements of the transformed MMSE test scores reached a ceiling effect. Thus, right censoring was implemented using a linear mixed-effects Tobit model with random intercepts and slopes.

The NPI total score was best fitted using a negative binomial random intercept model, according to the Bayesian information criterion. Random slopes could not be fitted, likely due to considerable individual deviation from a linear slope. The MMSE and NPI total models were linked by correlated random effects, implemented using a generalized structural equation model framework (Stata 15 package "gsem"). Each metabolite measured at baseline was entered in a separate multiprocess model, with years in study (time), age, age*time interaction, sex, AD vs LBD, AD vs LBD*time interaction, current smoking, glomerular filtration rate, and PLP as independent variables in the MMSE model. The independent variables were the same in the NPI total model, without a nonsignificant age*time interaction. Nonlinearity was checked using orthogonal polynomials of the transformed metabolite levels.

Post hoc, we compared the multiprocess models stratified by diagnosis. We further assessed the association between metabolite concentrations and the presence of individual NPI items (domain score $\geq$1) using a logistic random intercept model with time, age, sex, AD vs LBD, AD vs LBD*time interaction, current smoking, glomerular filtration rate, and PLP as independent variables. Finally, all study findings were adjusted for multiple comparisons, using the tail area–based false discovery rate (FDR) due to dependency, and adjusted *P* values are reported (*Q* values or *Q*). This was done separately for post hoc tests (R package: fdrtool).[33,34] The statistical analyses, besides FDR correction, were conducted in Stata (version 15; StataCorp, College Station, TX, USA).

Table 1. Participant demographics of the Dementia Study of Western Norway and serum metabolite concentrations at baseline.

CLINICAL CHARACTERISTICS	DEMENTIA (N = 155)	AD (N = 90)	LBD (N = 65)	*P* VALUE
Age, y, mean (SD)	75.1 (7.31)	75.1 (7.8)	75.1 (6.3)	.694[a]
Education, y, mean (SD)	9.7 (3.0)	9.7 (3.1)	9.6 (2.8)	.738[a]
Female, %	56.1	67.8	40.0	.001[b]*
Lewy body disease, %	42.3			
Current smokers, %	20.0	23.3	15.4	.222[b]
MMSE, score, mean (SD)	23.7 (2.8)	23.6 (2.5)	23.8 (3.1)	.597[a]
GFR[c], mean (SD)	79.2 (20.4)	79.2 (22.4)	80.7 (25.4)	.459[a]
METABOLITES				
Trp[d,e]	66.2 (22.4)	66.0 (22.8)	66.6 (15.5)	.547[f]
Kyn[d,e]	1.74 (0.67)	1.74 (0.58)	1.74 (0.73)	.582[f]
HK[e,g]	50.0 (33.7)	48.0 (28.8)	54.4 (34.3)	.033[f]*
KA[e,g]	51.1 (24.4)	51.5 (21.7)	50.2 (25.8)	.772[f]
XA[e,g]	12.3 (9.0)	12.5 (8.9)	12.3 (9.9)	.558[f]
AA[e,g]	21.7 (10.7)	20.1 (11.5)	22.5 (8.6)	.539[f]
HAA[e,g]	36.1 (16.5)	35.2 (16.2)	39.2 (16.3)	.360[f]
PIC[e,g]	35.9 (22.4)	33.0 (18.0)	38.1 (26.5)	.143[f]
QA[e,g]	474 (312)	465 (312)	481 (309)	.736[f]
KTR[e]	2.59 (1.06)	2.49 (1.02)	2.69 (0.96)	.244[f]
KKR[e]	7.97 (0.43)	8.00 (0.40)	7.95 (0.41)	.357[f]
Neopt[e,g]	19.7 (14.0)	18.7 (11.3)	20.6 (15.3)	.276[f]
PLP[e,g]	31.6 (33.9)	34.0 (33.7)	29.6 (24.9)	.021[f]*

Abbreviations: AA, anthranilic acid; AD, Alzheimer's disease; GFR, glomerular filtration rate; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine-to-tryptophan ratio; Kyn, kynurenine; LBD, Lewy body dementia; MMSE, Mini-Mental State Examination; Neopt, neopterin; PIC, picolinic acid; PLP, pyridoxal 5´-phosphate; Trp, tryptophan; QA, quinolinic acid; XA, xanthurenic acid.
[a]Student *t* test.
[b]Pearson χ^2 test.
[c]Metabolite levels in median and interquartile range.
[d]Micromoles per liter.
[e]Milliliters per minute per 1.73 m^2 surface area.
[f]Mann-Whitney *U* test.
[g]Nanomoles per liter.
*$P < .05$.

Ethics

The Regional Committee for Medical and Health Research Ethics approved the study protocol and a notification of change relating to biomarker analyses (REC number: 2010/633). All participants provided signed informed consent at baseline after a detailed explanation of the procedures.

Results

Study participants

The study included 155 patients (56% women) with dementia (90 AD, 65 LBD). The baseline mean MMSE score was 23.7 and mean educational level was 9.7 years. A total of 20% of the patients were current smokers at baseline (Table 1).

Kynurenines and cognitive performance

Kynurenine measured at baseline had a significant nonlinear, quadratic, relationship with the average MMSE score over the 5 follow-up examinations (Table 2, Figure 2), but not with the rate of change. Using orthogonal polynomials, the first polynomial of kynurenine, representing a linear relationship, was not significant (estimate [Est.] –0.023, $Q = 0.840$), whereas the second, representing a nonlinear relationship, was significant (Est. 0.10, $Q = 0.035$).

Table 2. Associations between serum kynurenines and neopterin at baseline and 5-year prognosis in dementia.[a]

COGNITIVE PERFORMANCE (MMSE)					NEUROPSYCHIATRIC SYMPTOMS (NPI TOTAL SCORE)				
	EST.	SE	*P* VALUE	*Q*		EST.	SE	*P* VALUE	*Q*
Trp	0.059	0.044	.185		Trp	0.010	0.055	.852	
Kyn	−0.023	0.052	.656		Kyn	−0.036	0.065	.569	
Kyn2	0.102	0.030	.006*	.046*					
AA	−0.080	0.048	.096		AA	−0.097	0.058	.099	
KA	0.072	0.057	.209		KA	−0.049	0.087	.575	
					KA*T	0.051	0.022	.021*	.080
HK	−0.099	0.060	.099		HK	−0.005	0.077	.950	
XA	0.017	0.050	.728		XA	−0.101	0.077	.190	
					XA*T	0.051	0.021	.017	.075
HAA	−0.004	0.048	.932		HAA	0.027	0.058	.636	
QA	−0.014	0.052	.795		QA	−0.087	0.063	.170	
PIC	0.011	0.045	.808		PIC	0.022	0.057	.701	
Neopt	0.023	0.050	.647		Neopt	−0.086	0.060	.149	
KTR	0.023	0.051	.648		KTR	−0.074	0.064	.247	
KKR	0.092	0.046	.046	.133	KKR	−0.050	0.074	.501	
					KKR*T	0.063	0.021	.003	.045*

Abbreviations: AA, anthranilic acid; Est., estimate; GFR, glomerular filtration rate; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KKR, kynurenic acid-to-kynurenine ratio; KTR, kynurenine-to-tryptophan ratio; Kyn, kynurenine; Kyn2, second degree orthogonal polynomial of Kyn; MMSE, Mini-Mental State Examination; Neopt, neopterin; NPI, Neuropsychiatric Inventory; PIC, picolinic acid; PLP, pyridoxal 5′-phosphate; SE, standard error; Trp, tryptophan; *Q*, *Q* value; QA, quinolinic acid; XA, xanthurenic acid.

[a]Generalized structural equation model linking 2 mixed models by their random effects: Model 1: Tobit mixed-effects model with MMSE as the outcome, measured at baseline and for 5 consecutive years. Model includes random intercepts and slopes. MMSE transformed to $\sqrt{(30-\text{MMSE})}$. Model 2: Negative binomial mixed-effects model with NPI total (sum of items 1 through 10) measured at baseline and for 5 consecutive years. Model includes random intercepts. Link: Random intercepts and slopes of MMSE correlated with random intercepts of NPI total. Covariates: Time, age (also *time for MMSE), sex, Lewy body dementia vs Alzheimer disease (also *time), current smoking, GFR, and PLP as independent variables.

*$P < .05$ or $Q < 0.05$.

Kynurenines and neuropsychiatric symptoms

The kynurenic acid-to-kynurenine ratio was positively associated with the rate of change per year in neuropsychiatric symptoms, specifically with more neuropsychiatric symptoms over time ($Q = 0.045$; see Figure 3). There was a trend for KA and XA to also be positively associated with more neuropsychiatric symptoms over time, but these findings were not significant after adjustment for multiple comparisons (Table 2).

Post hoc analyses

Differences in prognostic associations of kynurenines between AD and LBD. The associations between the kynurenines, cognitive prognosis, and neuropsychiatric symptoms over 5 years did not differ between AD and LBD after corrections for multiple comparisons (no significant interaction by clinical diagnosis [AD versus LBD]; Supplementary Table 1, and Supplementary Figure 1).

Individual neuropsychiatric symptoms. The kynurenic acid-to-kynurenine ratio was significantly associated with an increasing probability of hallucinations over time (odds ratios in Figure 4 indicate increased odds per year), whereas KA was significantly associated with more hallucinations, on average, over 5 years ($Q < 0.001$) with no change over time. There were several other observed trends. Of note, KA, KKR, and XA displayed trends for increasing agitation over time. Kyn, AA, QA, neopterin, and KTR showed trends for association with reduced average test scores on the item for irritability, whereas Trp, Kyn, HK, and neopterin displayed trends for association with higher average probabilities of experiencing apathy (Figure 4).

Discussion

In this study, Kyn had a nonlinear relationship with the participants' average MMSE test performance over 5 years. This relationship suggests that both low and high levels of Kyn are associated with poorer MMSE test performance, as compared

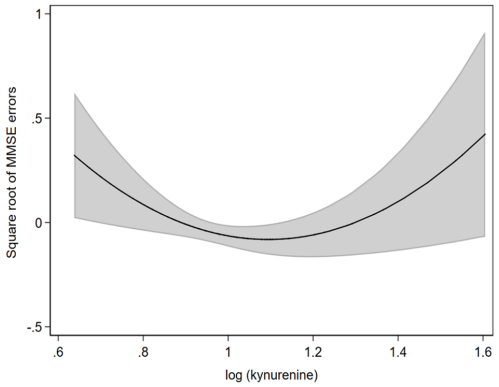

Figure 2. Nonlinear association between MMSE and kynurenine. Low levels of kynurenine are associated with more errors on the MMSE on average (intercept). At mean kynurenine levels, there is no association with MMSE, whereas high or low serum concentrations are associated with more average MMSE errors. The model was estimated as a multiprocess model together with a model for the NPI total score (see statistics). Of note, a constant of 1 was added to kynurenine prior to logarithmic transformation to avoid an uneven spread below and above a kynurenine level of 1, shifting the log (mean) from 0.55 to 1.02. MMSE indicates Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.

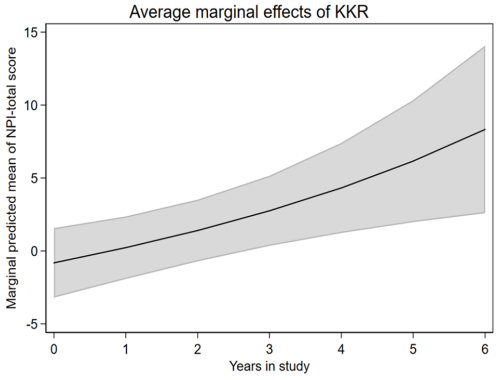

Figure 3. Kynurenic acid-to-kynurenine ratio and neuropsychiatric symptoms. The graph shows how a change in 1 standard deviation of the transformed and standardized levels of KKR, the reciprocal of $1/\sqrt{(KKR)}$ is associated with an increase in neuropsychiatric symptoms over time, using a negative binomial random intercept model, adjusted for age, sex, current smoking, glomerular filtration rate, and PLP in the model for MMSE. KKR indicates kynurenic acid-to-kynurenine ratio; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.

with values around the mean. Kynurenine was not associated with the rate of MMSE decline over time. A higher KKR was significantly associated with increasing neuropsychiatric symptoms over time. In post hoc analyses, we found that KKR and KA were significantly associated with more hallucinations. The associations between the kynurenines, cognitive prognosis, and neuropsychiatric symptoms over 5 years did not differ between patients with AD or LBD. However, several trends were observed, which should be investigated in a study with statistical power for subgroup analyses.

Kynurenine showed a nonlinear association with average MMSE score over 5 years (Figure 2), but not with the rate of change. Previously, we observed a similar nonlinear trend between Kyn and cognitive function in a cohort of community-dwelling older adults.[21] This may suggest that a homeostatic level of Kyn around the mean value can be beneficial for cognitive function. One might speculate that the lack of association between kynurenines and the rate of cognitive decline suggests that circulating kynurenines are not related to strong drivers of cognitive deterioration, such as synaptic loss[35] and tau pathology.[36] Availability of precursors of neuroactive kynurenines which are linked to both nicotinamide adenosine dinucleotide (NAD^+) metabolism[9] and low-grade inflammation,[13] could lead to cognitive differences that are stable throughout the disease course. Circulating Kyn, which crosses the blood-brain-barrier (BBB), may affect kynurenines in the brain, as both TDO and IDO converting Trp to Kyn have low activity in the brain.[9] Furthermore, Kyn is induced by pro-inflammatory cytokines, but notably also gives rise to metabolites that suppress inflammation, indicating a complex relationship.[37] There is ample evidence that IDO activation has a negative impact on cognitive function in rodent models[4-7] and can exacerbate AD pathology in amyloid knock-in mice.[5] However, it is less clear how IDO activity outside the brain relates to cognitive function. Peripheral interferon alpha may increase both blood and CSF levels of Kyn.[38] Kynurenine could be a marker of IFN-γ activity, but neopterin and KTR, which are more strongly related to IFN-γ induction,[39,40] were not associated with cognitive function in older humans.[21] Whereas high Kyn levels may signify inflammation,[13] low levels may limit the availability of a key precursor of neuroactive kynurenines and perhaps NAD^+.[9] Deficiency of kynurenines may explain poor outcomes with low Kyn levels, by decreasing levels of NAD^+ leading to neuronal degeneration in dementia. Reduced availability of NAD^+ may impair the activity of the NAD^+-dependent enzymes, such as the sirtuins, resulting in accumulation of amyloid-beta plaques and tau tangles.[41]

A higher KKR was significantly associated with more neuropsychiatric symptoms over time. A similar association was found in post hoc analysis, suggesting that KKR was related to hallucinations with a similar trend for delusions and disinhibition, indicative of psychotic symptoms. Kynurenic acid was significantly associated with hallucinations independent of time in post hoc analysis, with a similar trend for agitation. The NMDAR antagonism, a function of KA, is a known trigger of psychosis,[42] and KA is increased in the brain[19] and CSF of patients with schizophrenia,[43] making this finding intriguing. Increased KA levels, indicating higher kynurenine aminotransferase (KAT) activity, may produce symptoms of

Figure 4. Post hoc: neuropsychiatric symptoms and metabolites. The bubble diagram shows associations between individual neuropsychiatric symptoms over 5 years and metabolites assessed by logistic random intercept models. The KKR was significantly associated with an increasing probability of hallucinations over time, whereas KA was significantly associated with more hallucinations, on average, over 5 years. The analyses were adjusted for using the Benjamini-Hockberg procedure with a false discovery rate of 0.05, and *Q* values, representing adjusted *P* values, were estimated. The bubble sizes are proportional to $-\log^{10}$ *P* values. Odds ratios (ORs) are depicted inside bubbles with thin dark borders representing significant *P* values and thick dark borders representing significant *Q* values. Light blue coloring represents an OR of <1, whereas pink represents an OR >1. Odds ratios are stratified by color transparency as 0% (OR: 0.60-0.69/1.75-2.00), 20% (OR: 0.70-0.79/1.50-1.74), 40% (OR: 0.80-0.89/1.25-1.49), 60% (OR: 0.90-0.99, 1.00-1.24). AA indicates anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KA*T, kynurenic acid interaction with time; KKR, kynurenic acid-to-kynurenine ratio; KKR*T, kynurenic acid-to-kynurenine ratio interaction with time; KTR, kynurenine-to-tryptophan ratio; Kyn, kynurenine; Neopt, neopterin; OR, odds ratio; PIC, picolinic acid; Trp, tryptophan; QA, quinolinic acid; XA, xanthurenic acid; XA*T, xanthurenic acid interaction with time.

schizophrenia in experimental animals.[44] In addition, mice with genomic deletion of the KAT II enzyme show improved cognitive function.[45] Furthermore, KA may lead to decreased levels of the neurotransmitters glutamate,[46] dopamine,[47] and acetylcholine,[48] and KA has been linked to elevated dopaminergic activity in the brain.[49] KKR might reflect the activity of peripheral KATs in the periphery. Notably, KATs also generate XA, which was associated at trend toward more neuropsychiatric symptoms over time, specifically, agitation in post hoc analyses. However, contrary to Kyn, KA does not cross the BBB, but is formed in the brain from Kyn catalyzed by KATs.[50] Accordingly, follow-up studies measuring CSF kynurenines would be highly informative. In addition, KA is an agonist for the aryl hydrocarbon receptor[51,52] and is an antagonist of α7 nicotinic acetylcholine receptors (α7nAChR), both implicated in schizophrenia.[53,54]

There were several nonsignificant associations in post hoc analyses indicating that in particular AA and QA, but also KTR, Kyn, and neopterin, could be associated with less irritability and motor disturbances. It is interesting that increased concentrations of many of these metabolites may indicate metabolic flux away from KA. Reduced activity of KMO, linked to higher KA,[55] has been shown in schizophrenia.[56]

Our study suggests that increased circulating KA and KKR, potentially related to KAT activity, could be biomarkers of an increased risk of neuropsychiatric symptoms in dementia. Furthermore, several direct and indirect effects of kynurenines on neurotransmitter receptors[51-54] suggest the possibility of a potential role in the pathogenesis of such symptoms. There are several important regulators of the kynurenine pathway in the periphery, such as IFN-γ[40] and interleukin 1β (IL-1β).[57] Furthermore, IL-1β can affect the activity of KAT.[57] Thus, both clinical and experimental studies are needed to confirm and elaborate on our findings.

Strengths of the study include its longitudinal design with annual follow-up examinations until death, a low dropout rate among the participants and centralized laboratory analyses of all metabolites. The main limitations are a relatively small sample size, use of nonfasting blood samples, lack of longitudinal measurements of kynurenines, and KKR might not accurately reflect KAT activity. Furthermore, we could

not conclude that the associations with cognition are confined to patients with dementia, due to the absence of an age-matched longitudinal control group. Our previous study on community-dwelling older adults indeed found a similar association between Kyn and cognitive function.[21] Kynurenines in the brain may mostly be derived from circulating kynurenines with Kyn as the main precursor.[3] Still, synthesis of the potentially neuroprotective KA is confined to astrocytes, whereas the potentially neurotoxic QA is synthesized in microglia.[3] Thus, our assessment of kynurenines in dementia is incomplete without measurements of CSF and/or brain samples.

In summary, circulating Kyn concentrations around the mean level may be beneficial for cognitive function in patients with dementia. Serum Kyn concentrations which diverge from the mean in either direction (higher or lower) may be associated with poorer global cognitive function. We observed an association of KA and KKR with neuropsychiatric symptoms, which adds to existing literature suggesting a role of kynurenines in mental health.[3]

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Author Contributions

All authors have approved of the final manuscript to be published and agrees to be accountable for all aspects of the work. S-EHS contributed to the planning of the study, performed statistical analyses and interpretation of the results, and was responsible for drafting the manuscript. JEN was involved in planning of the study, preparing an analytic protocol, and interpretation of the results. JEN critically revised the manuscript. DA is the principal investigator of DemVest and was involved in the conception of the study, interpretation of the results, and critically revised the manuscript. JL was involved in the planning of the study, organized the biological samples, contributed to the interpretation of measurements and results, and revised the manuscript. PMU was involved in the planning of the study, measurements and quality control of metabolic biomarkers, interpretation and critically revised the manuscript. ØM performed measurements and quality control of metabolic biomarkers in sera, participated in the interpretation of the results, and critically revised the manuscript. AM was involved in measurements and quality control of metabolic biomarkers in sera, interpretation of the results and critically revised the manuscript. GST was involved in the planning of the study, statistical analyses, participated in the interpretation of the results, and critically revised the manuscript. LMG was involved in the conception of the study, assessed and checked all statistical analyses and their presentation, interpreted the results and critically revised the manuscript.

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Supplemental Material

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