

# Inflamed Depression - Origin, Essence, and Remedies

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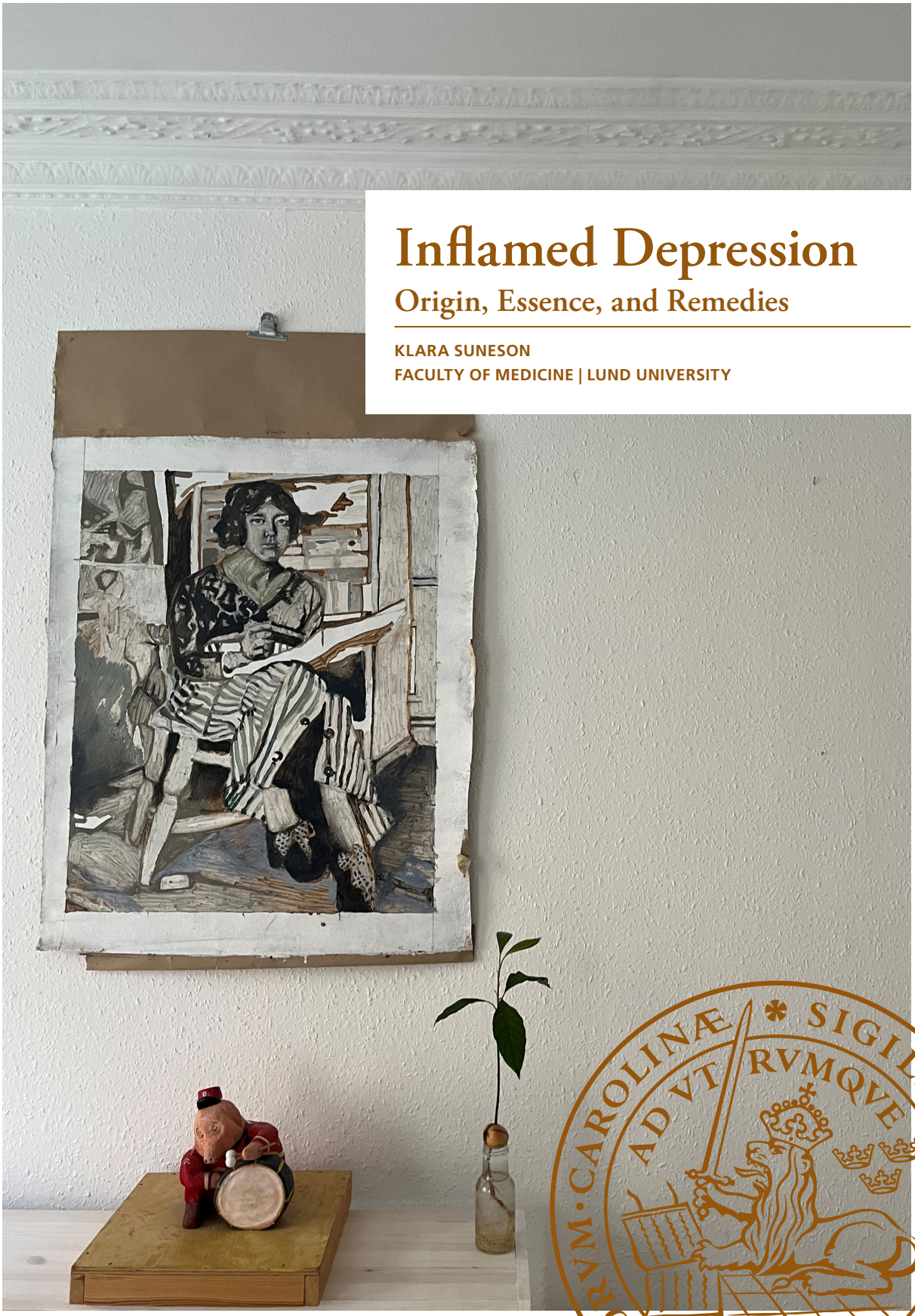

# Inflamed Depression

## Origin, Essence, and Remedies

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FACULTY OF MEDICINE | LUND UNIVERSITY

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**KLARA SUNESON** is part of the research unit for Biological and Precision Psychiatry (BAPP), Lund University, Sweden. Besides research, Klara is a medical doctor and a resident at the psychiatric clinic in Malmö, Region Skåne. Klara was born in 1990 and grew up in Jämtland, northern Sweden.

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Klara Suneson


LUND
UNIVERSITY

DOCTORAL DISSERTATION

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**Abstract**: *Background and aim*

Major Depressive Disorder (MDD) is an increasing global health problem with immense impact on quality of life and life expectancy. There is substantial heterogeneity within the diagnostic category of MDD, concerning symptom presentation, clinical characteristics, co-morbidities, course of illness and ultimately the plausible underlying biological mechanisms. Low-grade inflammation has been suggested as a cause, and maintaining factor, of depressive symptoms in some, but not all, patients with MDD. Increased understanding of how inflammation relates to depressive symptoms and response to treatment, including anti-inflammatory agents such as omega-3 fatty acid eicosapentaenoic acid (EPA), could pave the way for precision medicine in psychiatry. We hypothesized that a subgroup of MDD with signs of low-grade inflammation (inflamed depression phenotype) would display specific symptoms and other clinical and biochemical characteristics linked to inflammation when compared with 'non-inflamed' MDD. Furthermore we hypothesized that the inflamed depression phenotype would be associated with superior treatment response to EPA. Lastly, we investigated how peripheral inflammatory markers were related to kynurenine pathway (KP) metabolites, treatment response to selective serotonin reuptake inhibitors (SSRIs), glutamate metabolism and brain volumes in MDD.

*Materials and Methods*

Three cohorts were used for the papers included in this thesis. Firstly, in the Omega-3 study we applied a 'match/mismatch' study design and included 101 subjects with MDD. Subjects were stratified based on high sensitivity C-reactive protein (hs-CRP) into 'inflammation' and 'non-inflammation' groups. All participants received EPA supplementation added to stable antidepressant treatment for 8 weeks.

The second cohort was part of the Genes Depression and Suicidality (GEN-DS) study, including n=263 patients with difficult-to-treat depression and n=46 healthy controls. Patients underwent in-depth diagnostic interviews. Plasma samples were analysed for hs-CRP, a range of cytokines and vitamin D. Subjects were divided based on hs-CRP levels into 'inflamed' or 'uninflamed' depression. A principal component analysis including cytokines and vitamin D was conducted to assess patterns of variability among these variables and to better characterize the biosignature of inflamed depression.

The third cohort (part of the Cellular Aging and Neurobiology of Depression (CAN-D) study) consisted of 98 unmedicated patients with MDD that underwent a clinical assessment at baseline. A subset of these patients (n=48) was treated with SSRIs for 8 weeks. Inflammatory, metabolic and KP markers were measured in plasma during the study. Magnetic Resonance Imaging (MRI) assessment was carried out for hippocampal and amygdalar brain volumes (n=45) and MR spectroscopy for glutamate metabolism (glutamate+glutamine=Glx) in the anterior cingulate cortex (n=27).

*Results*

In the Omega-3 study, a greater antidepressant response was associated with baseline hs-CRP≥1 mg/L but not hs-CRP≥3 mg/L. In addition to a general antidepressant effect, EPA supplementation improved symptoms of fatigue, sleeping difficulties, and low energy.

In the GEN-DS study, the inflamed group (n=51, hs-CRP>3 mg/L) had significantly higher BMI and more severe symptoms of inflamed depression compared to the non-inflamed group (n=212, hs-CRP≤3 mg/L). The inflamed depression group was more associated with a biosignature of higher pro-inflammatory cytokines interleukin (IL)-6 and -8 plus lower vitamin D levels.

In the CAN-D study, several putatively neuroprotective ratios of the KP were significantly and negatively correlated with inflammatory markers and metabolic alterations (higher BMI and more insulin resistance). Neurotoxic ratios showed the opposite: generally positive correlations with inflammation and metabolic alterations. Responders to SSRIs showed a significant increase in neuroprotective ratio Kynurenic acid (Kyn-A)/3-Hydroxykynurenine (3-HK) during the study. Results from MRI showed positive baseline correlations between neuroprotective ratios and regional brain volumes, especially the amygdala.

*Conclusion*

In different cohorts and by use of different methodologies, the findings of this thesis highlight the importance to allow for biologically driven stratification of the multidimensionally heterogenous diagnosis of MDD. Inflammation seems to play a role in a subgroup of patients with MDD, with certain phenotypical traits involving clinical and biochemical characteristics. Inflammatory markers may be useful to predict treatment response to conventional (e.g. SSRIs) and non-conventional (e.g. EPA) interventions. However, there is still a need to optimize study methods, including delineation of subjects, to deepen our knowledge on the inflamed depression phenotype. Moreover, future studies are needed to further elucidate the clinical usefulness of the inflamed depression construct and its capacity to assist in the development of precision medicine in psychiatry.

**Key words:** Depression, Inflammation, Omega-3 fatty acids, Kynurenine Pathway, Phenotypes, Selective Serotonin Reuptake Inhibitors,

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# Inflamed Depression

## Origin, Essence, and Remedies

Klara Suneson


LUND
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Coverphoto by the author showing the wooden sculpture 'The Sad Circus Pig' by author's father Anders Suneson and a painting by the author picturing the Swedish artist Sigrid Hjertén who died in the sequelae of lobotomy performed at the Beckomberga hospital in 1948.



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*To Mum and Dad*

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Filip Ä, Simon, Sara (x2), Fernström, Jonas, Jeanette, L-O, Markus, Christina, Spyridon, Lisette, Brynja, Kolbrun, Åsa H, Denise, and so, so many more contributing to the development of psychiatric research in Skåne. Moreover, I would like to thank collaborators in the US for working with me on the last paper of this thesis. Thank you, Ryan, Owen, and Lena for trusting me to work with you and for making me feel close to you.

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# Table of Contents

# Abstract

*Background and aim*

Major Depressive Disorder (MDD) is an increasing global health problem with immense impact on quality of life and life expectancy. There is substantial heterogeneity within the diagnostic category of MDD, concerning symptom presentation, clinical characteristics, co-morbidities, course of illness and ultimately the plausible underlying biological mechanisms. Low-grade inflammation has been suggested as a cause, and maintaining factor, of depressive symptoms in some, but not all, patients with MDD. Increased understanding of how inflammation relates to depressive symptoms and response to treatment, including anti-inflammatory agents such as omega-3 fatty acid eicosapentaenoic acid (EPA), could pave the way for precision medicine in psychiatry. We hypothesized that a subgroup of MDD with signs of low-grade inflammation (inflamed depression phenotype) would display specific symptoms and other clinical and biochemical characteristics linked to inflammation when compared with 'non-inflamed' MDD. Furthermore we hypothesized that the inflamed depression phenotype would be associated with superior treatment response to EPA. Lastly, we investigated how peripheral inflammatory markers were related to kynurenine pathway (KP) metabolites, treatment response to selective serotonin reuptake inhibitors (SSRIs), glutamate metabolism and brain volumes in MDD.

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# Populärvetenskaplig sammanfattning

Depression är ett tillstånd som drabbar många människor över hela världen. Globala beräkningar uppskattar att ca 5 % av alla vuxna är drabbade varje år. Första linjens behandlingsalternativ har endast begränsad framgång. Depression skapar lidande främst för individen men också för dess omgivning och negativa hälsoekonomiska effekter för samhället. Depression kopplas även till ökad risk för kardiovaskulär sjukdom och för tidig död, bland annat som en stark riskfaktor för självmord. För att diagnosticera psykiatriska tillstånd, inklusive depression, används idag kriteriebaserad diagnostik där förekomst av olika symptom (objektivt eller subjektivt rapporterade av patient eller omgivning) vägleder klinikern som avgör om patienten uppfyller kriterier för diagnos. Den kriteriebaserad diagnostiken kom till för att särskilja patienter i väntan på att forskning skulle kartlägga de biologiska sjukdomsprocesser som orsakar och vidmakthåller lidandet. Dessvärre har majoriteten av forskning kring biologiska processer bakom psykiatriska diagnoser använt sig av de kriteriebaserade diagnoserna (till exempel egentlig depressiv episod) för att skilja sjuka från friska. Detta antas ha bidragit till att vi ännu idag har en begränsad förståelse för depressionens bakomliggande processer i kroppen. För depression finns idag nämligen inga objektiva biomarkörer varken för att avgöra diagnosen eller för att förutspå prognos och/eller behandlingsutfall. Det kan bero på att de som får diagnosen egentligen har drabbats av symptomen på grund av skilda biologiska sjukdomsmekanismer, alltså att patientgruppen är patofysiologiskt heterogen.

Under flera årtionden har inflammation fått ökad uppmärksamhet som en möjlig bakomliggande mekanism till depressiva symptom. Inflammation är ett samlingsbegrepp för de effekter immunförsvaret aktiverar när det detekterar ett hot, således är mekanismerna egentligen till för att skydda individen. Inflammation kan dock triggas av livsstilsfaktorer såsom låg fysisk aktivitet, stress, diet, övervikt och samsjuklighet med inflammatoriska sjukdomar. Det finns förändringar i beteendet kopplat till inflammation; man drar sig undan, sover mer, får ändrad aptit, tappar intresse och lust med mera. Detta kallas ’sickness behaviour’ och är beteendeförändringar hos individen som tros ha bevarats evolutionärt för att en individ som drabbats av till exempel en infektion ska spara energi. Sådana beteendeförändringar överlappar till viss del de förändringar som ses vid depression. Multipla studier har undersökt effekter av läkemedel och kosttillskott med antiinflammatoriska egenskaper, som omega-3 fettsyror, vid depression.

Sammantaget så har en koppling mellan inflammation och depression fått alltmer bekräftelse, men det tycks inte vara relevant för alla som uppfyller diagnoskriterier för egentlig depressiv episod (eller på engelska Major Depressive Disorder).

Denna avhandling syftar till att utforska om en andel av alla de som får diagnosen egentlig depressiv episod har låggradig inflammation som en del i sjukdomsbilden. I olika kohorter studerades låggradig inflammation, mätt genom blodprov. Dessa inflammationsmarkörer undersöktes i relation till olika biokemiska och kliniska markörer som föreslagits vara karaktäristiska för inflammatorisk depression, såsom metabola avvikelser. Vidare undersöktes om en inflammatorisk subgrupp (eller fenotyp) av depression i högre grad lider av symptom som tidigare kopplats till inflammation (överlappandes 'sickness behaviour').

Till hjälp för att utforska detta använde vi material från tre studier på deprimerade patienter. Två av studierna genomfördes i Skåne (Omega-3 och GEN-DS) med den tredje gjordes i USA (CAN-D). I Omega-3 studien som omfattade 101 deprimerade patienter undersökte vi om vi kunde skilja patienterna åt på förhand med hjälp av högkänslig mätning av snabbsänkan (C-reaktivt protein, CRP) för att skapa en inflammations- och en icke inflammationsgrupp. Hypotesen var att inflammationsgruppen skulle svara bättre på ett tillägg av omega-3 fettsyror under 8 veckor, jämfört med icke inflammationsgruppen. Ett uppmuntrande fynd från Omega-3 studien var att patienter med låggradig inflammation (CRP≥1 mg/L) svarade signifikant bättre på tillägg av omega-3 fettsyror än de med CRP<1 mg/L. Vi såg att de med CRP≥1 mg/L dels svarade bättre avseende total skattning av depressiva symptom, dels de symptom som kopplats till inflammation tidigare (trötthet, sömnstörning och en skala för "inflammatoriska depressiva symptom").

GEN-DS inkluderade patienter från specialistpsykiatrin med svårbehandlad depression. I den patientgrupp från GEN-DS som studerades i delarbete II kunde CRP>3 mg/L urskilja en "inflammationsgrupp" med signifikant högre skattning gällande symptom som tidigare kopplats till inflammation. Patienterna i denna grupp hade också högre BMI och en biologisk profil av ökade pro-inflammatoriska markörer och lägre D-vitaminvärde.

I CAN-D studerades en av de mekanismer som har föreslagits brygga mellan inflammation och depression, nämligen en inflammationsinducerad förskjutning av tryptofanmetabolismen. Flera studier har visat att inflammatoriska signalmolekyler kan aktivera en nedbrytningsbana där tryptofan (i stället för att brytas ned till neurotransmittorn serotonin) kan bilda ämnen som kan skada hjärnan och påverka dess signalering negativt. Vi såg i CAN-D att ration i blodet mellan neuroprotektiva (skyddande) nedbrytningsprodukter och neurotoxiska (skadliga) var kopplade till markörer för metabola förändringar och inflammation. Därtill visade det sig att ett neuroprotektivt/neurotoxiskt ratio ökade signifikant under behandling med antidepressiva läkemedel hos de som svarade på behandling jämfört med de som inte svarade.

Utifrån de studier som ingår i avhandlingen påvisas att inflammatorisk depression kan vara en subgrupp av depression som är relevant att urskilja. Det verkar helt enkelt som att depression kan vara inflammatoriskt betingat, men inte hos alla. För att detta ska bli kliniskt implementerat behövs dock ytterligare forskning. Förhoppningen är att denna avhandling ska bidra till fortsatt forskning avseende vilka biologiska mekanismer som faktiskt ligger bakom depression. Sannolikt behöver man i framtiden gå ifrån de nuvarande kriteriebaserade diagnoserna, för att förstå mer om den biologiska bakgrunden till det behandlingsbara mörker som drabbar mängder av människor världen över.

# List of Papers

*Paper I*

Suneson K, Ängeby F, Lindahl J, Söderberg G, Tjernberg J, Lindqvist D. Efficacy of eicosapentaenoic acid in inflammatory depression: study protocol for a match-mismatch trial. BMC Psychiatry. 2022 Dec 19;22(1):801. doi: 10.1186/s12888-022-04430-z. PMID: 36536364; PMCID: PMC9761617.

*Paper II*

Suneson K, Grudet C, Ventorp F, Malm J, Asp M, Westrin Å, Lindqvist D. An inflamed subtype of difficult-to-treat depression. Prog Neuropsychopharmacol Biol Psychiatry. 2023 Jul 13;125:110763. doi: 10.1016/j.pnpbp.2023.110763. Epub 2023 Apr 8. PMID: 37037323.

*Paper III*

Suneson K, Söderberg G, Lindahl J, Tjernberg J, Ståhl D, Ventorp S, Ängeby F, Lundblad K, Wolkowitz OM, Lindqvist D. Omega-3 fatty acids for inflamed depression – a match/mismatch study. *In review, Brain, Behavior, and Immunity, 2023*

*Paper IV*

Rampersaud R, Suneson K, Wu GWY, Reus VI, Lindqvist D, Ho TC, Meyerhoff D, Wolkowitz OM, Mellon SH, Brundin L. Neuroprotective kynurenine metabolites in major depressive disorder: associations with glutamate metabolism, brain volume, and antidepressant response. *Preliminary title, Manuscript*

# Author's contribution to the papers

*Paper I*

Substantial contributions to the conception and the design of the study, contributed to acquisition of data and wrote the original draft.

*Paper II*

Conceptualization, formal analysis, investigation, data curation, writing – original draft, visualization.

*Paper III*

Substantial contributions to the conception and the design of the study, contributed to acquisition of data, data curation, writing original draft, visualization.

*Paper IV*

Substantial contributions to conceptualization, formal analysis, investigation, data curation, writing original draft.

# Abbreviations

| | |
|---|---|
| MDD | Major Depressive Disorder |
| GEN-DS | Genes, Depression, and Suicidality |
| CAN-D | Cellular Aging and Neurobiology of Depression |
| DSM | Diagnostic and Statistical Manual of Mental Disorders |
| MAPK | Mitogen-activated protein kinase |
| SERT | Serotonin transporter |
| BH4 | Tetrahydrobiopterin |
| PUFA | Polyunsaturated Fatty Acid |
| EPA | Eicosapentaenoic Acid |
| DHA | Docosahexaenoic Acid |
| n-3, n-6 FA | Omega-3, omega-6 fatty acids |
| Hs-CRP | High Sensitivity C-reactive Protein |
| STAR*D | Sequenced Treatment Alternative to Relieve Depression |
| SSRI | Selective Serotonin Reuptake Inhibitor |
| SNRI | Serotonin and Norepinephrine Reuptake Inhibitor |
| ICD | International Classification of Diseases |
| WHO | World Health Organization |
| NIMH | National Institute of Mental Health |
| NESDA | Netherlands Study of Depression and Anxiety |
| RDoC | Research Domain Criteria |
| HPA | Hypothalamic-Pituitary-Adrenal |
| CSF | Cerebrospinal Fluid |
| RBC | Red blood cell |
| BMI | Body Mass Index |

| | |
|---|---|
| ID | Inflamed Depression |
| UD | Uninflamed Depression |
| IL | Interleukin |
| TNF | Tumour Necrosis Factor |
| IFN | Interferon |
| RA | Rheumatoid Arthritis |
| HCV | Hepatitis C |
| PET | Positron Emission Tomography |
| ACC | Anterior Cingulate Cortex |
| KP | Kynurenine Pathway |
| TRP | Tryptophan |
| Kyn | Kynurenine |
| IDO | Indoleamine 2,3 dioxygenase |
| TDO | Tryptophan-2,3-dioxygenase |
| Kyn-A | Kynurenic Acid |
| QA | Quinolinic Acid |
| 3-HK | 3-Hydroxykynurenine |
| NAD+ | Nnicotinamide adenine dinucleotide |
| KMO | Kynurenine 3-Monooxygenase |
| KAT | Kynurenine Aminotransferase |
| Glx | Glutamate + Glutamine levels |
| NMDA | N-methyl-D-aspartate |
| fMRI | Functional Magnetic Resonance Imaging (fMRI) |

# Introduction

*Depression* may refer to many things, e.g. a period of bad economy, a sunken area in the ground or a medical condition. When used in psychiatric terms, depression refers to a state seen in several psychiatric diagnoses as presented in the Diagnostic and Statistical Manual of Mental Disorders (DSM). Depression may allude to a depressive episode in bipolar disorder (type I or II), persistent depressive disorder (dysthymia) or a single or recurrent episode of unipolar major depressive disorder (MDD). This thesis will focus on the latter, MDD. However, MDD is far from a consistent syndrome, not least regarding symptomatology and treatment response [1]. This heterogeneity has prompted many investigations into the biological background of MDD which is central to the aims of this thesis.

## Major Depressive Disorder

The prevalence of MDD varies between studies but has overall been estimated at 5-6% during a 12-month period [2, 3]. This staggering prevalence is reflected in different global investigations on measurements of impact of illness, e.g. disability-adjusted life-years (DALYs). In 2019, MDD and dysthymia were the biggest contributors to DALYs among mental disorders and among the biggest causes of disability overall [4]. Notwithstanding the direct consequences of depressive symptoms, MDD is associated with an increased risk of cardiovascular disease, metabolic dysregulation, and overall mortality [5]. Furthermore, depressive syndromes are a major risk factor for death by suicide, the end-point for around 800 000 human lives globally each year [6].

Two major criteria-based systems are used to diagnose psychiatric illnesses including MDD: the DSM (published by the American Psychiatric Association) and the International Classification of Diseases (ICD, produced by the World Health Organization). A diagnosis of MDD requires, in the most recent DSM ($5^{th}$ edition) [7], the following criteria: at least two weeks of clinically significant distress or impairment in social, occupational or other important areas of functioning due to five, or more, of the following symptoms (and at least one of the symptoms depressed mood (1) or loss of interest or pleasure (2)):

(1) Depressed mood.

(2) Markedly diminished interest or pleasure in all, or almost all, activities (anhedonia).

(3) Significant change in weight or appetite (either decrease or increase, respectively).

(4) Insomnia or hypersomnia.

(5) Psychomotor retardation or agitation.

(6) Fatigue or loss of energy.

(7) Feelings of worthlessness or excessive or inappropriate guilt.

(8) Diminished ability to think or concentrate, or indecisiveness.

(9) Recurrent thoughts of death and/or suicidal ideations, plans or attempts.

It is also stated specifically for several of the symptoms above that they should be present nearly every day and/or most of the day. Some of the symptoms may be deemed present by either subjective reports and/or observation made by others. Moreover, the symptoms should not be better explained by other disorders (such as schizophrenia or bipolar disorder) or attributable to the effects of substance abuse or other medical conditions.

Even though the number of symptoms may seem rather modest, it is theoretically possible to combine the symptoms 1-9 above into thousands of unique symptom profiles of MDD, when taking the several 'or' into account [1]. The many ways to fulfil MDD criteria have also been shown in large clinical samples such as the study Sequenced Treatment Alternative to Relieve Depression (STAR*D). Among 3703 MDD patients, 1030 unique symptom profiles were found, whereas the most frequently identified profile was evident in only 1.8% of the sample [1]. Such heterogeneity leads to questioning of the MDD concept – if it covers diverse syndromes, lacks biological validity, and if the current classification aids to maintain low response rates to treatment and lack of progress in mapping underlying biological processes [1].

It is however not surprising that diagnostic categories of today, like MDD according to the DSM, does not seem to correlate to biological processes. The notion of depression has evolved from Hippocrates' theories about melancholia to Kraepelin's manic-depressive insanity to the operationalized DSM-criteria of today. While it has become clear that melancholia is not caused by an excess of black bile, little has been established regarding pathophysiology of depression up to this date. Thereby, the expert-consensus approach has probably been the best available option to classify mental disorders. However, research on the biological underpinnings of depressive symptoms may have been hampered by the ongoing tendency to search for biological correlates to the criteria-based diagnoses, including MDD.

## Heterogeneity in MDD; a Cause of Prolonged Suffering?

*Clinical and biological heterogeneity, lack of biomarkers*

As exemplified above, patients that fulfil MDD-criteria may present in numerous ways. Some sleep too little, are restless and lose weight whereas others might be slow, gain weight and sleep too much. In addition to disparate symptom profiles, MDD cohorts are also heterogenous concerning course of illness, co-morbidities (both somatic and psychiatric), treatment response, and more. Large studies have found that only about one-third of patients achieve remission with first-line antidepressant pharmacological treatment [8, 9], highlighting that *one size does not fit all*.

While other fields, such as oncology, have shown substantial progress over the last decades in the development of 'personalized' or 'precision' medicine, e.g. selection of treatment guided by objective measurements ('biomarkers') corresponding to pathophysiological processes underlying illness, psychiatry has not [10]. In the case of MDD, progress in development of precision medicine and implementation of biomarkers to clinical practice has presumably been hindered by the mentioned fact that the pathophysiology is still largely unknown and likely heterogeneous. While no etiological models have been completely established, multiple and potentially overlapping hypotheses on pathophysiology have been proposed. This particularly concerns alterations of monoaminergic neurotransmission, the hypothalamic-pituitary-adrenal (HPA) axis, neuroplasticity and neurogenesis as well as inflammation [3, 11]. Thereby, it is plausible that progress in personalized management of depressive illness demands that the MDD concept is challenged due to the mentioned inherent heterogeneity of this non-biologically but expert-consensus and symptom derived diagnosis. In turn, multidimensional approaches may be required to distinguish more homogeneous subtypes (phenotypes) of individuals that fulfil DSM criteria for MDD. In such approaches, both clinical *and* physiological features ought to be considered, including symptom profile, course of illness and treatment response as well as results of brain imaging modalities, laboratory tests and more [10, 12]. Hence, establishment of biomarkers that in harmony with clinical features could inform on the pathophysiological processes underlying depressive symptoms in an individual might be a crucial link to accelerate the development of personalized management of MDD [10, 11]. Yet, no biomarkers of MDD have been implemented in clinical practice, neither to distinguish the healthy from the ill nor to predict course of illness and treatment response [10, 13, 14] and the reproducibility of biomarker research in psychiatry has presumably been hampered by small samples and high risk of reporting bias [13, 15]. While some biomarkers have been repeatedly correlated to specific features of MDD, such as dexamethasone non-suppression in melancholic depression [16], the clinical utility and accessibility need to be considered. For example, proposed

biomarkers of inflammation may hold advantages due to being already implemented in other fields.

Altogether, the concept of MDD was never founded on biological correlates of depressive symptomatology, which is reflected in the current limited understanding of the illness and unsatisfactory treatment outcome. Increased understanding of the pathophysiological mechanisms is warranted to guide stratification of individuals diagnosed with MDD. Ultimately, biomarkers together with clinical features could aid clinicians in determining what intervention would more likely be efficacious in an individual, based on aetiology. Large studies have confirmed that depressed individuals with concurrent low-grade inflammation may be distinguished by easily accessible biomarkers and share clinical features including patterns of treatment response. Distinguishment based on inflammation therefore holds promise to fuel development of precision medicine in psychiatry.

# Inflammation and Depression

## History

For several decades, increased attention has been directed towards theories on how the immune system may be implicated in the pathophysiology of psychiatric disorders. At the same time, a better understanding has been achieved concerning the intricate functions of immune system components, such as cytokines, and their ability to interact with the nervous system and affect brain function [17, 18]. Simultaneously, research on behaviour and mood (including psychiatric illnesses) has gradually merged into neuroscience, which has paved the way for an integrative research area that some label 'psychoneuroimmunology' [19]. Early on, there was much focus on immunosuppression associated with depression, which was with time, and evidence, shifted towards a focus on immune activation [20]. In the beginning of the 90s, Smith conceptualized in The Macrophage Theory of Depression that secretion of cytokines could be an actual cause of depression [21]. This was in part based on accumulating data demonstrating that individuals who receive immune-activating interferon treatment frequently develop depressive symptoms [21]. Shortly after, a meta-analysis conducted by Herbert and Cohen demonstrated immune alterations, such as elevated white blood cell counts, in depressed patients compared to healthy controls [22]. Even though the exact mechanisms by which peripheral immune-activation may transduce into central alterations, manifesting as changes in mood and behaviour (i.e. depressive symptoms), have not been fully established, different models have been proposed [23-25]. The development of these models and studies that support them have substantially relied on the conceptualization of 'sickness behaviour' and its

immunological correlates in animal models, and has applied this reasoning to humans and depression [26]. Sickness behaviour is a term used for the symptoms oftentimes accompanying infections or other immune activations, e.g. anhedonia, lethargy, and appetite disturbance [27]. It has been argued that such primarily reward related behavioural and motivational deficits may have had evolutionary advantages as a protection for the individual, as energy expenditure can then be focused on battling the infection [27].

To this day, investigators have assessed the question if depression is an inflammatory illness or not, from a multitude of points-of-view. For example, by estimating levels of inflammatory markers in peripheral blood [28-30] or cerebrospinal fluid (CSF) [31] of depressed patients compared to healthy controls, or by assessing the prevalence of depression in patients with inflammatory conditions compared to the somatically healthy [24]. Other angles have examined whether inflammatory markers may predict [32], or change with, antidepressant treatment [33, 34]. As mentioned, an acceptance has sequentially been built up, noting that all patients diagnosed with MDD do not share the same underlying pathophysiological processes, and thus allowed the assumption that merely a subset of patients may be 'inflamed' [35, 36].

## Inflammation is a Broad Concept

The immune system is divided into two branches: the innate and the adaptive, by some referred to as the first and the second line of defence to immune challenges [37]. Cytokines are part of the first line of defence, the more rapid and non-specific innate immune system, as signal molecules in a cascade initiated by activated macrophages and dendritic cells. Activation of circulating macrophages (or in the brain microglia) and other myeloid immune cells of the innate system occurs by detection of specific molecular patterns (damage- or pathogen-associated, alias DAMPs or PAMPs respectively). DAMPs or PAMPs may respectively be triggered by e.g. damage to cells or threatening infectious pathogens. The role of cytokines (chemokines and interleukins (IL)), is to mobilize more immune cells, hence transducing a signal of threat and a need for defence. For example, cytokines such as IL-6 trigger release of acute phase reactants, e.g. C-reactive protein (CRP) from the liver. What follows is a presentation of antigens by dendritic cells to activate the second-line, adaptive, immune system. B and T lymphocytes of the adaptive system can react to the specific antigen presented and differentiate clonally to up-scale the adaptive response. A variety of cell types may be produced at this step; plasma cells (from B cells) that produce antibodies against the presented threat, T helper (Th) cells guide other immune cells, CD8+ cells are cytotoxic (cell killing) aimed at infected cells, and Treg cells that regulate the immune response. Parallelly, adaptive immune cells (B and T) may differentiate into memory cells that are ready for action if a future immune challenge presents with the same antigen.

Inflammation describes a set of intricate physiological changes aimed to protect against threats. Yet inflammation may become chronic or be activated on 'false' premises (as seen in autoimmune disorders) thereby causing damage and disease. Inflammation comes with accumulation of leukocytes, dilatation of blood vessels, and protein and fluid leakage. Classical presentation of acute inflammation, e.g. in the case of an infected wound are redness *(rubor)*, swelling (*tumor)*, pain (*dolor)*, heat (*calor)* and loss of function (*functio laesa)*. The occurrence of central inflammation, referred to as neuroinflammation, may stem from various triggers. Sun et al. exemplified this in a review on systemic inflammation as a cause of neuroinflammation, where viral infections, autoimmune diseases, and inflammation of peripheral organs (e.g. in colitis) were highlighted as potential causes of neuroinflammation, together with unhealthy eating habits, stress and metabolic dysregulations [25]. Activation of microglia is a central pathological feature of neuroinflammation occurring in response to signalling of immune components such as cytokines. One of two phenotypes of activated microglia, M1, produce pro-inflammatory cytokines, exert neurotoxic effects and enhance neuroinflammation [38]. The other activated phenotype M2, may be induced by anti-inflammatory cytokines (e.g. IL-10) and promote the opposite effects by battling neurotoxicity induced by M1 and through supporting neurogenesis and tissue repair [38]. Importantly, immune-activation of the brain (i.e. neuroinflammation) influence metabolism of serotonin precursor tryptophan (TRP) through modulation of the kynurenine pathway (KP) as well as glutamate metabolism [38], as will be described later. Moreover, neuroinflammation and pro-inflammatory cytokines have been repeatedly correlated to decreased neurogenesis in rodent models, especially of the hippocampus [38].

Whereas there is a plethora of potential markers to assess immune activation and inflammation, the complexity of these processes impedes distinct biochemical definitions of inflammation. However, it is widely agreed that inflammation can be defined as localized or systemic as well as acute or chronic. The term chronic is accompanied by the expressions low-grade or low-level inflammation. Clear definitions and directions for measurements of chronic low-grade inflammation are still lacking, yet in general the terms refer to a subclinical disorder associated with increases in inflammatory biomarkers and the risk of several diseases. In a growing part of the literature and in this thesis, low-grade inflammation is used to define systemic inflammation that is more persistent and subtle compared to acute inflammation [39]. The Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) recommend using high sensitivity (hs)-CRP as a measurement of low-grade inflammation corresponding to relative risk of cardiovascular disease, with set cut-offs for low, average and high risk (see **Table 1**) [40].

**Table 1. Recommendations on cardiovascular risk assessment based on C-reactive Protein (CRP) in Clinical and Public Health Practice**

| Relative Risk Category | Hs-CRP level |
|---|---|
| Low | <1 mg/L |
| Average | 1.0 to 3.0 mg/L |
| High | >3.0 mg/L |
| **General Recommendations** | |
| Obtain measurement twice, separated by two weeks | |
| Discard if >10 mg/L due to potential acute infection/inflammation | |

Summarised from the statement of inflammatory markers and cardiovascular disease, Centers for Disease Control and Prevention and the American Heart Association, 2003 [40]. Hs-CRP cutpoints for low, average and high risk approximately correspond to adult population tertiles of hs-CRP distribution. Abbreviations: high sensitivity C-Reactive Protein (hs-CRP).

## Transdiagnostic Aspects

It is essential to this thesis to make an early statement; the proposed association between systemic low-grade inflammation and altered mood, behaviour and cognition is not thought to be exclusive for depression. Symptoms of depression are also evident in other psychiatric illnesses, and so is inflammation [41, 42]. For example, an umbrella review including available meta-analyses on peripheral and non-genetical biomarkers showed that several inflammatory markers could be of transdiagnostic importance (covering MDD, bipolar disorder and schizophrenia). [13]. Moreover, treatments targeting immune dysregulation e.g. the monoclonal antibody Rituximab has been proven to be potentially beneficial for symptom reduction in patients with schizophrenia [43]. Of note, negative symptoms of schizophrenia which are conceptually overlapping with some depressive symptoms were improved. Based on the recognition that symptom dimensions and putatively related biological processes span over diagnostic categories, the National Institute of Mental Health (NIMH) launched the framework Research Domain Criteria (RDoC) in 2009 [44]. With the implementation of RDoC, the NIMH wanted to encourage research to focus on transdiagnostic symptom domains of human functioning, rather than discrete diagnostic categories founded on expert-consensus. As of October 2023, the RDoC matrix included six domains with corresponding constructs, subconstructs and units of analysis [45]. It was proposed that studies adhering to the RDoC framework could inform on potential underlying biological mechanisms, such as anhedonia seen among patients suffering from a wide range of symptom-based diagnoses, e.g. uni- or bipolar depression, schizophrenia, and anxiety disorders. Utilizing the RDoC approach, one could for example investigate the biological underpinnings of anhedonia irrespective of the underlying diagnosis, as an alteration of symptom dimension ‘positive valence’ with constructs 'reward responsiveness’, ‘reward learning’ and ‘reward valuation’. Peripheral pro-inflammatory markers could, as one example of biomarkers of interest, then be

assessed as one unit of analysis, and potentially be integrated into the matrix. The expert group behind the framework declared great expectations about RDoC to help counteract the heterogeneity of underlying neurobiological mechanisms yielded by current diagnostic systems. After more than a decade, the RDoC approach is still highly relevant [46]. While progress might have been slow by some means, both the remaining uncertainties in the neuroscientific understanding of human normal functioning (including mood and behaviour) and the complexity of mental disorders ought to be considered mitigating circumstances before judging the initiative as fruitless [46].

## Evidence Supporting a Connection Between Inflammation and MDD

As aforementioned, the research field investigating interactions between immunological alterations and MDD is steadily expanding. There are numerous ongoing lines of research that seek to advance our understanding of how inflammation may contribute to MDD pathophysiology. While not all the arguments, neither for nor against an association, can be presented in this thesis, some of the most important areas of consideration will be outlined in the following paragraphs.

*Immune Activation may Induce Depressive Symptoms*

In the exploration of associations between inflammation and altered mood and behaviour, several models have been applied in both animals and humans that actively induce immunological responses and allow for monitoring of subsequent behavioural alterations [47-49]. In rodents, behavioural changes that resemble depressive symptoms, e.g. increased time of immobile floating in the forced swim test, have been shown following administration of pro-inflammatory cytokines both centrally (IL-6 to the amygdala and hippocampus [50]) as well as peripherally (IL-1B intraperitoneally [51]). In exploration of more indirect activation of the immune response, models have utilized lipopolysaccharide (LPS) endotoxins, i.e. membrane components of gram-negative bacteria. Such models have been proposed to possess important qualities for investigation of associations between inflammation and depression and for development of new therapies and their targets [48]. In humans, it has been shown that endotoxin-induced inflammation correlates with depressive behaviours including social distancing [52] and fatigue [53]. However, these models have generally been applied in healthy volunteers and due to short-term effects on mood and behaviour from single LPS infusions (and tolerance development following repeated infusions), biological and behavioural changes have seldom been observed for more than a few hours. Mentioned factors may limit the interpretability of the findings with regards to the relationship between inflammation and MDD [48].

Effects from repeated administration of cytokines can however be observed among patients who receive immune-activating treatments, associated with relatively frequent development of depressive symptoms [21]. Specifically, treatment of hepatitis C (HCV) with interferon alpha (IFN-α) has been associated with depression development. While some state that this occurs in at least 20% of patients, incidence rates vary up to ≈50% [47, 54]. Hence, observation of changes in mood and behaviour following IFN-α and similar interventions has been proposed as suitable to model the relationship between inflammation and depression [47].

Based on this, several trials have investigated if antidepressant and/or anti-inflammatory agents may mitigate or even prevent the development of sickness behaviour and/or MDD following immune challenges. In rodents, behaviours resembling depressive symptoms following LPS have been shown preventable by antidepressant agents such as NMDA receptor antagonist ketamine [55] and anti-inflammatory compounds such as antibiotic minocycline [56]. In humans, results from one small placebo-controlled study indicated that the selective serotonin reuptake inhibitor (SSRI) paroxetine could effectively prevent the development of MDD among patients with malignant melanoma treated with high-dose IFN-α [57]. Interestingly, it was shown that illness trajectories and responsiveness to SSRIs differed between symptom clusters [58]. In general, neurovegetative symptoms overlapping sickness behaviour (e.g. sleep disturbance, lack of appetite, fatigue) appeared earlier in the treatment course and were less responsive to paroxetine treatment than later occurring symptoms such as depressed mood, anxiety and cognitive disturbance [58]. Several studies followed and in a later meta-analysis, the potential of antidepressant pre-treatment to prevent IFN-α associated depression (in HCV and malignant melanoma) was confirmed [59]. Due to potential side-effects from conventional antidepressants (e.g. SSRIs) combined with IFN-α treatment in often somatically fragile patients [60], interventions with more beneficial risk profiles have been sought for prevention of IFN-α associated depression. For instance, Su et al. reported that 2-weeks supplementation with omega-3 fatty acid eicosapentaenoic acid (EPA) before IFN-α therapy for HCV significantly reduced incidence rates of depression compared to placebo [61]. During the 24-week follow-up period, incident rates of MDD were 30% in the placebo group and 10% in the EPA group. Taken together, these observations from clinical (such as IFN-α therapy for HCV) and laboratory (such as LPS administration) settings endorse that immune activation can give rise to mood and behavioural alterations as seen in MDD.

*Depression in Autoimmune Disorders and Infections*

In further support of inflammation as a potential factor underpinning depressive symptoms, epidemiological studies have reported associations between MDD and somatic conditions with inflammation as a core mechanism of disease. It has been estimated that the risk of depression is increased among individuals with

autoimmune diseases, such as multiple sclerosis [62], autoimmune hepatitis [63] and rheumatoid arthritis (RA) [64, 65]. In a large, prospective and population-based study, Benros et al. showed marked increases in the risk of MDD among individuals with a prior hospital contact due to autoimmune disorders or infections [66]. Similarly, a nationwide population-based cohort with a follow-up time up to 14 years, Lu et al. found an increased risk for depression among patients with RA [67] and in the National Child Development Study a greater risk for depression was found after being diagnosed with any of the 23 autoimmune disorders assessed [68]. Consequently, several trials testing anti-inflammatory agents in autoimmune disorders have assessed depressive symptoms as a secondary outcome. In a systematic review and meta-analysis including samples of patients with various chronic inflammatory conditions, significant reductions of depressive symptoms were found with anti-cytokine treatment, particularly TNF-α inhibitors, compared to placebo [69]. Interestingly, antidepressant response was not correlated with change in physical illness but with baseline severity in depression ratings [69]. A subsequent mega-analysis including a wider range of immune modulating agents also showed significant antidepressant effects compared to placebo in patients with inflammatory (e.g. RA, psoriasis) or oncological disorders [70]. Again, the effects remained significant after adjustment for physical illness and the authors concluded that the effect sizes were comparable to those found in meta-analyses for SSRIs in MDD [70]. Taken together, it may be hypothesised that somatic conditions with inflammation as a core mechanism and related genetic predispositions may prime for a pro-inflammatory state, potentially triggering sickness behaviour and in some cases MDD [71].

*Inflammation and the Risk, Prognosis and Treatment Outcome of MDD*

Large community samples exploring correlations between peripheral inflammatory markers and depressive symptoms have reported mixed results and raised inevitable questions about both causality and clinical implications. Nonetheless, increased CRP has been found to predict subsequent depressive symptoms in a 12 year [72] and a 2-4 year follow-up on elderly patients [73]. One meta-analysis included longitudinal community-based studies and investigated how baseline CRP (8 studies, n=14832) and IL-6 (3 studies, n=3695) related to subsequent depressive symptoms [74]. Results indicated that elevated baseline CRP especially, but also IL-6, had a small, but significant, increasing effect on the risk for subsequent depressive symptoms [74]. The heterogenous study designs and inconsistent results across the limited number of studies investigating levels of inflammatory markers as prospective biomarkers for MDD development were summarized in a systematic review and meta-analysis by Kennis et al. [75]. When analysing the eight studies included, no significant predictive effects for MDD were found for CRP (five studies) and/or IL-1 and IL-6 (four studies) [75]. In a subsequent, large population-based study, elevation of CRP (≥3 mg/L) was however predictive of new onset MDD, defined as a PHQ-9 score >10 at follow-up after five years [76]. Yet, a

significant interaction was found with gender as elevated CRP predicted subsequent depression merely in men [76]. Interestingly, some studies have also looked at reversed and/or bidirectional correlations, whether depressive symptoms may predict subsequent elevated levels of inflammatory markers, with contradicting results [72, 77, 78]. Regardless of whether the association goes in both directions or not, there are substantial recognition of a longitudinal correlation indicating that inflammation could be considered a risk factor of MDD. Nevertheless, the inconsistency in results illustrate the need for further and more refined studies.

While the general response rates to first- and second-line treatments of depression are low [8], it has been proposed that systemic low-grade inflammation may be associated with less favourable response to conventional antidepressants [32, 79]. This is supported by studies showing elevations of inflammatory markers in treatment resistant cohorts compared to MDD in general [79], but also by studies showing elevations of inflammatory markers to be predictors of worse treatment outcome [32]. Furthermore, among patients with MDD, those categorised as treatment-resistant, but not treatment-responsive or unmedicated, were shown to have significantly elevated CRP-levels compared to healthy controls [79]. Indeed, in a cohort including 60 moderately depressed and treatment resistant patients, mean baseline hs-CRP level was >5 mg/L and 45% of participants had hs-CRP >3 mg/L [80]. However, even milder elevations of hs-CRP (>1 mg/L) have also been found correlated with antidepressant response in larger clinical trials such as Combining Medications to Enhance Depression Outcomes (CO-MED) [81] and Genome-Based Therapeutic Drugs for Depression (GENDEP) [82]. Unfavourable outcome after treatment with conventional antidepressants has been indicated in patients with CRP ≥1 mg/L overall [83] and specifically for SSRIs in comparison to serotonin and norepinephrine reuptake inhibitors (SNRIs) [84]. Several hypotheses have been put forward on how inflammation might modulate response to antidepressant treatment. Some have argued that antidepressants exert anti-inflammatory effects which might modulate the overall effects in patients with milder inflammatory activation whereas more pronounced or chronic inflammation would not be abated by antidepressants alone [32]. Others have postulated that inflammatory modulations of TRP metabolism along the KP and its interaction with serotonergic signalling may be in part explanatory of reduced response to SSRIs in relation to peripheral inflammation [79]. Hence, to address inflammation as a possible modulator of treatment response may substantially contribute to advancements in precision psychiatry.

### *Imaging Neuroinflammation in Depression and the Interaction with Glutamatergic Signalling*

The relationship between immune activation and depressive symptoms can be investigated by various methods including neuroimaging modalities. Reward-circuit hypoactivity (shown as decreased functional magnetic resonance imaging (fMRI) resting-state functional connectivity) among depressed patients is associated with

both higher plasma CRP and anhedonia [85]. By use of positron emission tomography (PET) tracing activated microglia, Setiawan et al. [86] demonstrated, in 20 MDD patients, that signs of neuroinflammation in the anterior cingular cortex (ACC) correlated positively with depression severity. Regardless of a relatively small sample size, this paper has been highly cited, illustrating the great hope for validated biomarkers of neuroinflammation in depression [11, 27]. Interestingly, typhoid vaccination with subsequent immune activation and mood alteration has been shown to correlate with increased activity in the ACC [87]. Also, decreased total volumes of the hippocampus and other areas influencing emotional processing have been repeatedly shown in MDD [88-90]. Such volume reductions have been hypothesised to associate with inflammation through HPA-axis dysregulation (surplus of glucocorticoids) and excitotoxicity by altered KP metabolism [38, 91, 92], which is described in more detail below. In support of this, Savitz and colleagues showed that a putatively neuroprotective ratio of KP metabolites (kynurenic acid (Kyn-A) over quinolinic acid (QA)) correlated positively with total volumes of hippocampus and amygdala in MDD [92]. Neuroimaging modalities can also inform on central measures of metabolism, for example by MR spectroscopy assessing combined glutamate and glutamine levels referred to as the Glx, a proxy marker of glutamate metabolism. By use of this modality, it has for instance been shown that patients receiving IFN-α treatment exhibits increased glutamate in the basal ganglia and dorsal ACC [93]. Interestingly, glutamate metabolism interacts with TRP metabolism along the KP, for example since Kyn-A is postulated to reduce release of glutamate and exert antagonism on the N-methyl-D-aspartate (NMDA) receptors [94]. Since pro-inflammatory cytokines can increase enzyme activity inducing the KP and alter its downstream effects, mechanisms of inflammation and glutamatergic signalling converge and therefore serve as a potential underlying mechanisms of inflamed depression [92, 95].

# Origin - Mechanistic Pathways, From Immune-activation to Depressive Symptoms and Back Again

While no causal underlying mechanisms linking immune activation to depressive symptoms have been established, some pathways have been more frequently explored. This includes an interplay with the stress response system, known to interact with processes modulating immunological and behavioural functioning. Moreover, alterations of monoaminergic neurotransmission in response to immune signalling, for example through dysregulation of TRP degradation along the KP with its downstream neuroactive metabolites. These and some other suggested links will be described below after a short introduction on how immune activation can be transmitted between the periphery and the brain.

## Bidirectional Immune Signalling Between Periphery and the Brain

Firstly, peripheral and central immune functioning interact bidirectionally [17]. Several reviews have summarized how cytokine signalling can transmit immune activation between the periphery and the brain and affect CNS functions influencing mood and behaviour [24, 27, 96]. Main routes of periphery-brain interactions are commonly divided into the 'neural', 'cellular/cell mediated', or 'humoral', pathways [27, 96]. Briefly, the 'neural' pathway describes e.g. the binding of cytokines to receptors on afferents of the vagus nerve that transmit the signal to the brain [96]. The neural pathway also includes efferent modulation, involving stress response, of the parasympathetic and sympathetic nervous systems (PNS and SNS respectively) [17, 27]. The 'cellular' or 'cell mediated' pathway describes signalling from and migration of activated immune cells from the periphery to the brain [27]. The 'humoral' pathway describes how cytokines may transduce immune response over the blood brain barrier (BBB) [24]. Various *in vitro* and *in vivo* models in animals and humans have been utilized to study how systemic inflammation can generate changes to BBB anatomy and function [97]. While it has been hypothesised that changes, e.g. increased permeability, of the BBB could be of importance for the association between MDD and systemic inflammation this has not been reliably proven in clinical samples of MDD [23] and has been contradicted by some [42, 98]. However, peripherally induced inflammation (either in the laboratory or clinical immune challenges) associates with both altered mood and behaviour (sickness behaviour, as previously described) and alterations of anatomy and function of the brain, especially in the hippocampus, amygdala, hypothalamus, and cortex [25].

## Stress Response

Psychological stress exposure (recent and/or in early life) is a well-established risk factor for MDD, and alterations of neurobiological processes underlying stress response has been repeatedly demonstrated in MDD [11, 99]. This could be mediated by an interplay between stress and immune-response regulation, that may differ depending on whether the stressor is acute or chronic [17, 100, 101]. Bidirectional links have been proposed between immune activation, the HPA axis and the SNS (the so called 'fight or flight' response) [17, 27]. Activation of the HPA axis and the SNS results in increased production of glucocorticoid cortisol and catecholamines epinephrine and norepinephrine. There is robust evidence for hypercortisolaemia in MDD [102]. Yet, cortisol is primarily an immunosuppressant [100], therefore stress-related hypercortisolaemia would theoretically promote an anti-inflammatory state. However, if stress is sustained, chronic alterations to the HPA axis are thought to render decays in stress-response with subsequent behavioural and mood-alterations that could ultimately accumulate to MDD [102]. This may be effected in part by pro-inflammatory cytokines [103], through

modulation of glucocorticoid receptors, leading to an actual desensitisation to circulating cortisol [17, 101, 102]. Furthermore, glucocorticoids have been shown to inhibit repair of neuronal damage, which in models of chronic stress has been proposed as a potential link to depression [17]. Hence, these stress-related mechanisms may serve as mediators in the association between inflammation and depression.

## Monoamine Transmission

The monoamine hypothesis (including the serotonin hypothesis) has been increasingly questioned as the primary pathophysiological alteration underlying depression. Yet, there are few doubts that serotonergic, dopaminergic, and noradrenergic neurotransmission play crucial roles in human mood, cognition, and behaviour. With the increased understanding of low-grade inflammation as a potential neurobiological mechanism underlying depressive symptoms, many studies and reviews have emerged on how inflammation may interact with monoaminergic neurotransmission [27]. This is consistent with findings on the potential of SSRIs to prevent IFN-α induced depression [101]. Indeed, variations in both IL-6 and serotonin transporter (SERT) genes have been associated with MDD following IFN-α treatment [104], whereas some have found this to be true only for IL-6 gene variations [105]. However, cytokines are known inducers of the intracellular signalling mechanisms through p38, a mitogen activated protein kinase (MAPK) pathway [101]. Such activations have been associated with altered serotonergic signalling through increased expression and function of SERT, both *in vitro* [106] and in animal models linking to behavioural alterations [107]. Moreover, cytokines have been repeatedly proposed to mediate the modulatory effects of dopaminergic neurotransmission on motivation [108, 109]. As mentioned, anhedonia and CRP have been shown to correlate with reduced functional connectivity in the reward circuit [85]. Capuron et al. used both a reward task during fMRI to assess striatal activity and PET to assess dopaminergic function in patients with HCV receiving IFN-α treatment or not [110]. Results showed both lower striatal activity and altered dopaminergic functioning in IFN-α treated subjects, associated with depressive symptoms including reduced motivation [110]. The exact mechanisms underlying the interplay between inflammation, depression and dopaminergic signalling are not clear, but may include reduced synthesis of dopamine. Dopamine synthesis is the result of enzymatic conversion steps that demand tetrahydrobiopterin (BH4) as co-factor (see **Figure 1**) [109]. Pro-inflammatory cytokines or oxidative stress may diminish the amount of available BH4 for critical steps in dopamine synthesis, resulting in decreased production. In patients treated with IFN-α negative correlations have been found between CSF levels of BH4 and pro-inflammatory cytokine IL-6 as well as fatigue, consistent of an association between inflammation, decreased dopaminergic neurotransmission

and depressive symptoms [111]. Taken together, mounting evidence points toward an interplay between inflammation, monoaminergic neurotransmission, and depressive symptomatology.

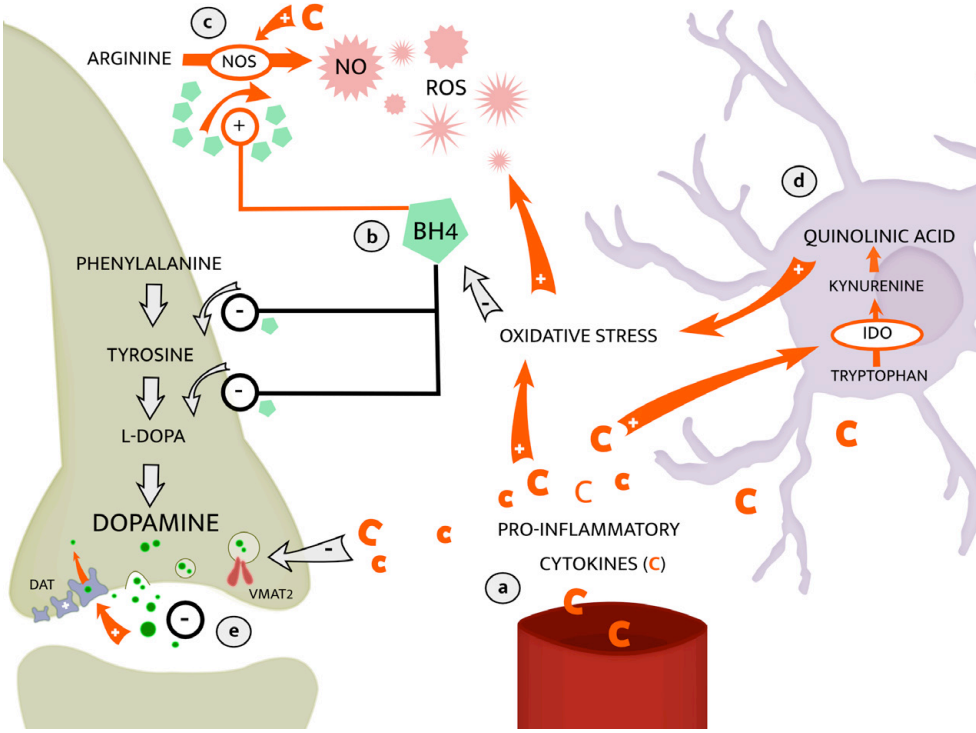


**Figure 1. Cross-talk between the immune- and dopaminergic system**
Cytokines from the peripheral circulation enter the central nervous system (CNS) (a). Cytokines may increase oxidative stress and reduce BH4, whereas less BH4 is available for synthesis of dopamine (b). As cytokines also activate NOS, oxidative stress is further enhanced and BH4 availability reduced (c). KP metabolites with predominantly neurotoxic properties are formed in a pro-inflammatory state in microglia (d). Cytokines decrease packaging of dopamine by VMAT2 to vesicles before release to pre-synaptic cleft, while DAT expression and re-uptake is increased. Abbreviations: nitric oxide synthase (NOS), nitric oxide (NO), reactive oxygen species (ROS), tetrahydrobiopterin (BH4), indoleamine 2,3-dioxygenase (IDO), vesicular monoamine transporter 2 (VMAT2), dopamine transporter (DAT). Previously published in a review by Suneson et al. in International Journal of Molecular Sciences [112].

## The Kynurenine Pathway

Altered degradation of the essential amino-acid TRP along the KP is as mentioned a presumable mediator of pro-inflammatory cytokine effects on neurotransmission [101, 109]. Several meta-analyses have shown altered peripheral levels of KP

metabolites in MDD compared to healthy controls [113, 114]. Moreover, levels of metabolite kynurenine (Kyn) in plasma have been shown to be inversely correlated with severity of depressive symptoms [115, 116]. TRP is the precursor of serotonin and melatonin but is predominantly converted into Kyn by enzymes indoleamine 2,3 dioxygenase (IDO) -1 or -2, or tryptophan-2,3-dioxygenase (TDO) in the first step of the KP [117]. IDO-1 may be upregulated by pro-inflammatory cytokines such as IFN-γ and TNF-α [91], as illustrated in **Figure 2**. The end product of KP is nicotinamide adenine dinucleotide (NAD+) that plays important roles in various parts of metabolism. Along the KP, several putatively neuroprotective or neurotoxic (by some referred to excitotoxic) intermediates are formed. Formation depends on the cell type as well as enzymatic activity. Inflammation is thought to affect several steps, mainly by facilitating the production of neurotoxic metabolites such as 3-hydroxykunurenine (3-HK) and QA [118, 119] by induction of kynurenine monooxygenase (KMO), the enzyme converting Kyn to 3-HK (see **Figure 2**). Moreover, KMO (neurotoxic branch) is found in macrophages, microglia and monocytes whereas degradation by enzyme kynurenine aminotransferase (KAT) converting Kyn to Kyn-A (neuroprotective branch) are found in astrocytes [120]. Several of the metabolites may affect glutamatergic metabolism: QA is an agonist of the NMDA glutamate receptor, has neurotoxic properties and can increase extracellular glutamate while Kyn-A exerts neuroprotective effects as an NMDA antagonist and reduce glutamate release to extracellular space [38, 119, 120]. Reduced levels of Kyn-A [114] and picolinic acid (PA) [121, 122] have been shown in MDD. However, rather than measurements of individual metabolites, focus has been drawn to the balance between KP metabolites, i.e. ratios, giving a presumably more dynamic view of neurotoxic vs. neuroprotective properties and their regulation of glutamatergic neurotransmission.

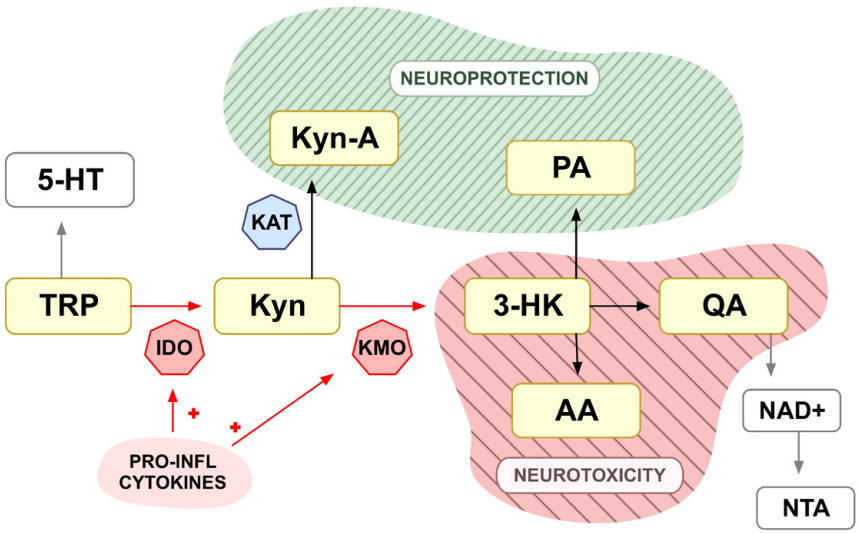


**Figure 2. Schematic overview of tryptophan degradation focusing on the Kynurenine Pathway. Metabolites of the Kynurenine Pathway (yellow boxes) divided by the putatively neuroprotective (green) or neurotoxic (red) effects.**
Abbreviations: Tryptophan (TRP), Serotonin (5-HT), Pro-infl (Proinflammatory), Indoleamine 2, 3 Dioxygenase (IDO), Kynurenine (Kyn), Kynurenine Aminotransferase (KAT), Kynurenic-Acid (Kyn-A), 3-Hydroxykynurenine (3-HK), Kynurenine 3-monooxygenase (KMO), Anthranilic Acid (AA), Picolinic Acid (PA), Quinolinic Acid (QA), Nicotinamide Adenine Dinucleotide (NAD+), Nicotinamide (NTA).

The accumulating evidence of associations between KP alterations, inflammation and MDD has endorsed studies exploring the correlations between changes in KP metabolites and disease course (including treatment response) in MDD. Also, it has been hypothesized that KP alterations are not evident overall in MDD, but merely in one or several subgroups [123]. Due to its prominent link to inflammatory processes, it has been suggested that altered KP metabolism may be a key mechanism of pathology among MDD patients with signs of low-grade inflammation and associated metabolic dysregulation (e.g. obesity and insulin resistance), as will be discussed later.

## Omega-6/Omega-3 Ratio

Even early reports suggesting inflammation as a potential pathophysiological mechanism of depression hypothesised that higher dietary intake of fish oils could, due to their anti-inflammatory properties, explain lower depression rates in some parts of the world [21]. Since then, more information has been revealed about the biological effects exerted by polyunsaturated fatty acids (PUFAs) omega-6 (n-6) and -3 (n-3). Specifically, their counteracting roles on the immune system; pro- and anti-inflammatory respectively, have become increasingly clear.

Briefly, all fatty acids consist of carboxylic acid and a hydrocarbon chain. Several means of fatty acid classification are applied today, yet most commonly and in this thesis, it is based on the number of double bounds between carbon molecules (saturations) in the hydrocarbon chain. PUFAs have more than one double bond, but are distinguished by the placement of their first, whereas n-3 PUFAs have a first double bond three carbon atoms away from their end (the omega carbon). Both n-3 and n-6 PUFAs are essential to obtain from the diet, since they are not synthesised de novo by humans [124, 125]. However, changes in diet over the last century have significantly shifted the ratio of n-3:n-6 PUFAs, exemplified by the so called Western diet for which the ratio is estimated to approach 1:16 [126]. The balance between n-3 and n-6 PUFAs has been attributed important roles in homeostasis, also in the central nervous system (CNS), with tight links to the immune system. As illustrated in **Figure 3**, n-3 and n-6 PUFAs are competitors for incorporation into phospholipid cell membranes [127]. Alterations to cell membrane constitution and thereby function may play an important role in the pathological processes underlying MDD [128]. Two of the most influential n-3 PUFAs in the human brain, with impact on e.g. fluidity of neuronal membranes, are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [124, 125]. A third dominating PUFA in the brain is n-6 PUFA arachidonic acid (AA) [125]. AA is a well-recognised precursor of immune modulators in the eicosanoid family including thromboxanes (TX), prostaglandins (PG) and leukotrienes (LT) [129]. Eicosanoids such as PG and TX may exert various effects on the immune system [130]. Yet, eicosanoids derived from AA are far more potently pro-inflammatory in their effects than those derived from EPA and DHA [124, 131]. Main interactions with a pro-inflammatory state are cytokine-induced cleavage of AA from the cell membrane by phospholipase A2 (PLA2) [129] (as illustrated in **Figure 3**). Several common anti-inflammatory treatments target and inhibit enzymes, such as cyclooxygenase (COX), that turns AA into eicosanoids [129]. Derived eicosanoids, like PGE2 may then promote inflammation by driving immune cells into expression of e.g. IL-6. The mentioned enzymes COX and LOX may also degrade n-3 PUFA EPA, leading to an accumulation of eicosanoids with generally anti-inflammatory properties [132]. Hence, and in a simplified manner, n-3 and n-6 PUFAs compete both for incorporation into cell membranes and for enzyme availability, ultimately leading to anti- and pro-inflammatory effects respectively [133, 134]. Moreover, direct

stimulation of n-3 PUFAs on immune cells, such as macrophages, may boost resolution of inflammation [134]. Several lines of pre-clinical evidence demonstrate that n-3 PUFAs reduce secretion of pro-inflammatory cytokines such as IL-6 and TNF-α from macrophages, e.g. by counteracting LPS-triggered upregulation of cytokine gene expression, as reviewed by Gutierrez et al. [134].

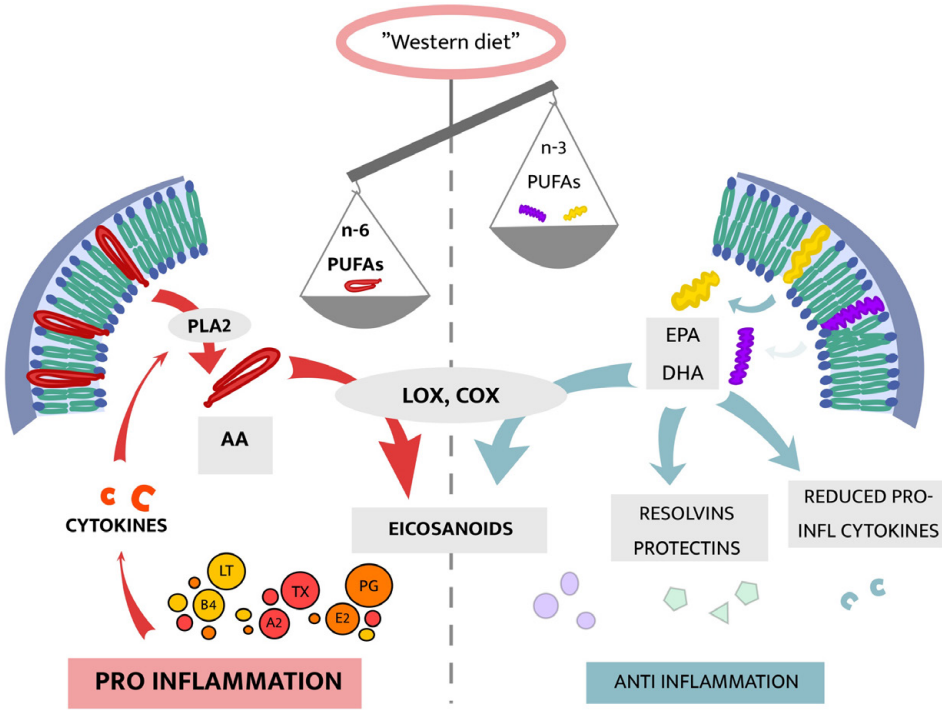


**Figure 3. Omega-3 and -6 balance in relation to inflammatory state. Enzymes LOX and COX are involved in various steps of PUFA metabolization.**
Abbreviations: Omega-6 (n-6), Omega-3 (n-3), Polyunsaturated Fatty Acids (PUFAs), Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA), Phospholipidase 2 (PLA2), Arachidonic Acid (AA), Lipoxygenase (LOX), Cyclooxygenase (COX), Prostaglandines (PG), Thromboxanes (TX), Leukotrienes (LT). This is an adjusted version of the figure published in the review by Suneson et al. in International Journal of Molecular Sciences [112]

Observational studies have confirmed associations between self-reported fish consumption and prevalence rates of MDD, yet with uncertainties regarding bias and causality [135, 136]. Except from dietary reports, several investigators have shown lower concentrations of n-3 and n-6 PUFAs in peripheral blood and/or in membranes of red blood cells (RBCs) in patients with MDD compared to controls [137]. A low dietary ratio, rather than n-3 PUFA intake per se, has been shown to increase the risk for depression [138] and depressive symptoms [139] in women. Accordingly, a low dietary n-3:n-6 PUFA ratio has been highlighted as a risk factor in the bidirectional relationships between low-grade inflammation and depression (bidirectional in the sense that depression could predispose for an unhealthy diet in some). The accumulating evidence has fuelled the interest in n-3 PUFA supplementation in the treatment of depression. The potential for n-3 PUFAs, and especially EPA, as a targeted intervention for inflamed depression will be presented later.

## Inflammation-associated Metabolic and Endocrinological Alterations

A bidirectional relationship between depression and obesity is well described; depression increases the risk of obesity and both prevalence of depression and treatment non-response is more pronounced in obese cohorts [140]. Diet and obesity may interact with the immune state by e.g. the described pro-inflammatory 'Western diet' with higher n-6:n-3 ratio. There are however several other mechanistic pathways that could explain interactions between obesity, low-grade inflammation, and depression. Of plausibly central importance is the immunological activity of adipose tissue, manifested by e.g. expression of pro-inflammatory cytokine IL-6 [141]. By some, obesity is even referred to as an inflammatory condition [140]. Insulin resistance [140], leptin and lipid profile dysregulation and genetical predisposition [142] are other biological pathways of mechanistic relevance for the link between immunometabolic alterations and depression. Investigations have been made into whether depression may share genetic architecture with specific lifestyle factors and BMI. In a large case-control study using data from the UK biobank, polygenic risk scores for MDD were shown to correlate with CRP levels, yet statistical significance did not remain after BMI and smoking were added to the model. Authors concluded that this pointed towards an impact from shared predisposition in habits of eating and smoking, rather than suggesting that autoimmune processes underpin MDD [143]. Indeed, in large GWAS, significant genetic correlations have been shown between depression and both BMI [144] and body fat, as well as waist-to-hip ratio [145]. Based on these findings, multiple associations between genetic predisposition for risk of depression and related behavioural traits seem potential. However, the associations between obesity, depression and inflammation are complex and relates to a wide range of other somatic consequences [5]. In this assumed 'vicious circle', adipose tissue produces

inflammatory cytokines, inflammation may trigger depressive symptoms which in turn predisposes for a sedentary lifestyle, leading to an increased risk for obesity and inflammation. In relation to this, some investigations have reported that correlations between inflammatory markers and depressive symptoms are diminished after adjustment for BMI [146, 147]. Others have shown no change of outcome after such adjustments. In line with a mediation theory, some researchers have presented the possibility that a subgroup of depressed patients may exhibit immunometabolic alterations, of potential importance in the pathophysiology, as discussed later. However, as previously argued, an inflammatory state might indeed trigger or maintain depressive symptoms, whereas these clinical characteristics may in fact be mediators in the link between inflammation and depression. Another example of a potential mediator, or confounder, in the relationship between inflammation and depression is vitamin D deficiency. Vitamin D is a steroid hormone with modulating effects on both immune system [148] and mental health [149]. Previous investigations have shown that lower levels of vitamin D is associated with increased inflammatory markers in depressed [150]. Whether these factors should be adjusted for or not when assessing the relation is debated and no 'gold standard' is established.

# Inflammation in a Subgroup of Patients with MDD

While several meta-analyses have shown that MDD cohorts have higher mean levels of some peripheral inflammatory markers, compared to non-depressed controls [30, 32, 151, 152], general effect sizes have been small and statistical heterogeneity large [142] and some have reported positive results only for a few of the investigated markers [152]. The lack of consistent confirmation of the associations between inflammation and MDD in all cases manifests that MDD is not a pathophysiological homogenous concept. Thereby, one possible explanation is that the inconsistency originates from the acknowledged heterogeneity of MDD whereas MDD does just not seem to be, in all cases, an inflammatory illness. However, existing evidence points toward inflammation as a mechanistic pathway of depression in a subgroup of patients diagnosed with MDD. Hence, 'inflamed depression' has been suggested as a phenotype of MDD. Phenotypes define individuals/organisms based on characteristics and biomarkers, stemming from genetic and environmental factors. For delineation of an 'inflamed depression' phenotype, different methods have been applied including division by inflammatory markers in plasma, symptomatology, and other characteristics (e.g. BMI, somatic comorbidities). Among biomarkers, stratification by hs-CRP levels has been among the most frequently applied to delineate low-grade inflammation in MDD [98]. For example, large community-samples [143] and meta-analyses [36] have shown that approximately 20-30% of patients with MDD show signs of low-grade inflammation, as indicated by plasma

CRP>3 mg/L [36]. The use of hs-CRP 1 or 3 mg/L as cut-offs is primarily adapted from the mentioned guidelines developed by the CDC and AHA [40], see **Table 1**. However, there is currently no consensus in how to best define the inflamed depression phenotype. Studying both biomarkers and other clinical characteristics could increase understanding of the inflamed depression phenotype and help accelerate development of target treatment.

## Target Treatment of Depression in Patients with Signs of Peripheral Inflammation

Among a growing number of studies testing anti-inflammatory compounds in the treatment of depression, only a few assess effects in relation to inflammation status (e.g. biomarker levels or phenotypical characteristics). In a meta-analysis published in 2019, Köhler-Forsberg et al. included 36 RCTs (almost 10 000 patients) testing anti-inflammatory pharmacological compounds as monotherapy or add-ons to conventional antidepressants [153]. Nonsteroidal anti-inflammatory drugs (NSAIDs) and cytokine-inhibitors were the most frequently tested compounds (13 and 9 studies respectively). Other included interventions were statins, minocycline, pioglitazone, and glucocorticoids. Overall, the anti-inflammatory compounds were more effective than placebo in reducing depressive symptoms and, when used as add-ons, more effective in terms of remission and response rates. However, authors rated the risk of bias as high among all included trials whereas smaller studies showed more effects compared to larger. Moreover, treatment duration was generally short, not guided by biomarkers and not discriminating subgroups/phenotypes more or less likely to benefit from the intervention.

Out of the trials included in the meta-analysis [153], some did however assess treatment response in relation to baseline levels of inflammatory markers [80, 154-156]. Among them, Raison et al., showed no general superiority of cytokine-inhibitor Infliximab compared to placebo in 60 treatment resistant depressed subjects [80]. However, post-hoc analyses revealed that patients with hs-CRP levels >5 mg/L at baseline were more likely responders to active treatment. Moreover, responders to Infliximab had significantly decreased CRP levels at the 12-week follow-up compared to placebo responders. Some subsequent trials have utilized enriched strategies for recruitment when investigating antidepressive effects of anti-inflammatory compounds. For example, Nettis et al. examined the effects of antibiotic minocycline as augmentation in a cohort with treatment resistant MDD and serum hs-CRP≥1 mg/L [157]. In the four-week placebo-controlled trial, minocycline was not found to be superior to placebo numerically (decrease in HAM-D 17) or dichotomously (partial response defined as reduction from baseline HAM-D 17 of at least 25%). However, when participants were grouped based on hs-CRP levels below or above/equal to 3 mg/L, minocycline treated with hs-CRP≥3 mg/L showed significantly larger decrease in HAM-D 17 and included more partial

responders than both minocycline treated with hs-CRP <3 mg/L and placebo treated participants regardless of hs-CRP levels. Despite the small groups (e.g. minocycline treated with CRP≥3 mg/L, n=6), the results encourage future studies to use peripheral biomarkers, such as CRP, to delineate MDD subjects based on inflammation status in the search for target interventions. In line with this, McIntyre et al. enriched a study on Infliximab for bipolar depression by including only patients with specific biochemical or phenotypic criteria, all previously shown to associate with a pro-inflammatory state [158], see **Table 5** in discussion. Even though there were no significant effect of Infliximab vs. placebo on depressive symptoms in the 12-week RCT (n=60), in secondary analyses, a subgroup that responded significantly on Infliximab was distinguished as patients reporting history of childhood maltreatment (physical abuse). In post-hoc analyses published separately; patients treated with Infliximab were shown to significantly improve in anhedonia ratings compared to placebo and the effect was moderated by baseline levels of plasma TNF-α and soluble TNF receptor 1 (sTNFR1) [159]. These results highlight the potential advantages of considering a wider range of inflammatory biomarkers, as well as phenotypic characteristics and symptomatology in the investigation of the association between inflammation and depression, including trials of target interventions. As a non-pharmacological compound, n-3 PUFAs have been tested for their efficacy in depression, however primarily without taking phenotypic characteristics of inflamed depression into account. Yet, some have indeed reported that n-3 PUFAs could be more efficacious in patients with MDD and signs of peripheral inflammation [160].

Taken together, future investigations of an inflamed phenotype of MDD calls for a multidimensional approach, including both inflammatory biomarkers and clinical characteristics such as symptomatology, co-morbidities, and treatment response that could ultimately be related to mechanistic pathways (e.g. altered KP metabolism). Moreover, potential target interventions need to be studied by more refined methods to consider the heterogeneity of MDD, e.g. by stratification of patients based on the presence or absence of biochemical and/or phenotypic characteristics of inflamed depression.

# Aims

This thesis has the overall aim to investigate biochemical and clinical characteristics, including treatment response, in relation to the proposed inflamed phenotype of depression.

Specific aims:

- To investigate if patients with MDD and elevated plasma CRP (indicating low-grade inflammation) respond better to add-on treatment with omega-3 fatty acid eicosapentaenoic acid (EPA) than patients with low CRP. Also, the used study design ('match/mismatch') will be evaluated for its suitability to address the issue of heterogeneity within MDD (Paper I and III).
- To delineate a subgroup within a cohort of patients with difficult-to-treat depression based on low-grade inflammation as measured by plasma CRP and investigate if this subgroup is more prone to exhibit a specific symptom profile and biosignature (pattern of alterations among associated biomarkers) (Paper II).
- To assess associations between plasma levels of inflammatory markers and metabolites of tryptophan degradation along the kynurenine pathway in unmedicated patients with MDD. Moreover, presumed neurotoxic vs. neuroprotective ratios of KP metabolites will be evaluated in relation to symptom profile, altered brain volumes, glutamate metabolism and response to SSRI treatment (Paper IV).

# Material and Methods

## Recruitment Procedures

### Paper I and III, the Omega-3 study

The clinical trial assessing EPA in inflamed depression is described in detail in paper I (study protocol) and III (first publication of results). The study will hereafter be referred to as Omega-3. Recruitment was carried out in the region of Skåne in southern Sweden between 2017 and 2023. Patients diagnosed with unipolar depressive episode (MDD according to the DSM 5$^{th}$ edition) with ongoing antidepressive pharmacotherapy were recruited through clinical referrals and ads in radio, newspaper, and social media. Inclusion and exclusion criteria are listed in **Table 2**.

**Table 2. Eligibility criteria for the Omega-3 study**

| **Inclusion criteria** |
|---|
| Age 18-80 years |
| Current MDD (according to DSM-5, symptom duration ≥4 weeks) |
| Antidepressive pharmacotherapy with stable doses for ≥ 6 weeks |
| Hamilton Depression Rating Scale 17 items (HAM-D17) score ≥15, |
| Clinical Global Severity (CGI) score ≥3 |
| Willingness to not make any significant changes to diet for the duration of the study |
| **Exclusion criteria** |
| Bipolar or psychotic disorder |
| Substance abuse (3 months before study) |
| Medical illness of serious and unstable character that could jeopardise interpretation of treatment outcome including response to treatment |
| Known allergy to omega-3 or fish |
| Ongoing infection |
| Dementia, mental retardation or other circumstances that could obstruct the individual's ability to make an informed decision regarding participation in the study and the study procedures |
| Ongoing electroconvulsive therapy (ECT) |
| Current participation in any other clinical study |
| Known bleeding disorder or treatment with anticoagulants |
| Suicide or homicide risk of severe degree according to judgment of study physician |
| Regular intake of n-3 PUFA supplementation (≥ 3 days in the month before screening) |
| Recent (last 4 weeks) start of psychotherapy or plan to start in the upcoming 8 weeks |
| Any ongoing regular medication that could interfere with biomarker analysis, e.g. non-steroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase-2 (COX-2) inhibitors, oral steroids, immunosuppressants, chemotherapy or interferon therapy |

Abbreviations: Major Depressive Disorder (MDD), the Diagnostic and Statistical Manual of Mental Disorders, 5 th version (DSM-5), omega-3 polyunsaturated fatty acids (n-3 PUFAs).

In case patients took occasional doses of e.g. NSAIDs such as Ibuprofen, they were asked not to do so 24 h before study visits and blood draws. A schematic overview of the study procedures is presented in **Figure 4.** At the baseline visit, a study physician conducted an assessment including the Mini International Neuropsychiatric Interview (MINI) 6.0 interview and the Hamilton Depression Rating Scale 17 items (HAM-D17) [161]. Treatment with omega-3 fatty acids was initiated after blood draw in proximity of the baseline visit. All participants received equal study product and were instructed to take four capsules per day for eight weeks. In total, the daily dose of the capsules contained 2,2 mg EPA, 400 mg DHA, 800 mg of other fatty acids and 10-24 mg tocopherol-rich extracts. Midsona AB provided the study product but did not have any influence on the study design or handling of data.

Patients were assessed at week 4 and week 8 (end of study). Primary outcome was change in total HAM-D17 scores between baseline and week 8. All patients conducted self-rating symptom scales at baseline, week 4 and week 8. These were

used to assess secondary outcome measures: Montgomery-Åsberg Depression Rating Scale (MADRS-S) [162], inflammatory depressive symptoms (defined as a Patient Health Questionnaire 9 (PHQ-9) [163] items #3 sleep problems, #4 lack of energy, #5 appetite-disturbance), Generalized Anxiety Disorder 7-item scale (GAD-7) [164], Snaith-Hamilton Pleasure Scale (SHAPS) [165], Insomnia Severity Index (ISI) [166], and Fatigue Severity Scale (FSS) [167].

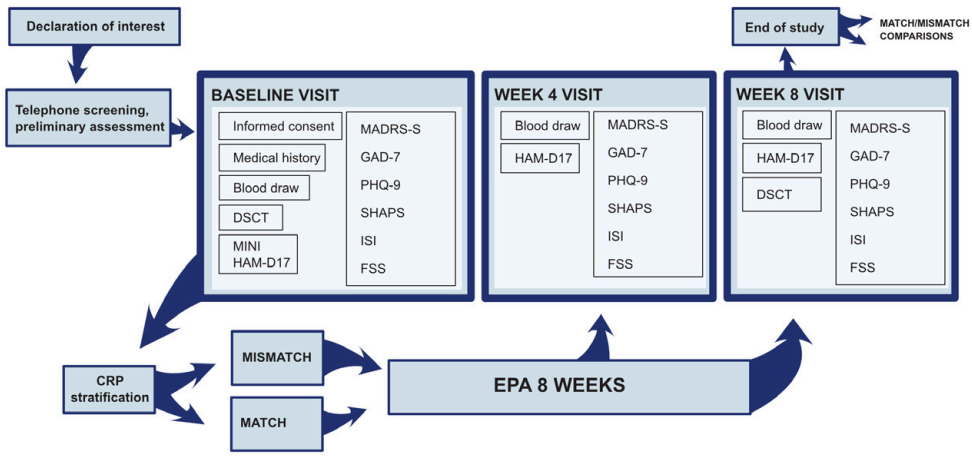


**Figure 4. Flow-chart of the Omega-3 study**
Abbreviations: C-Reactive Protein (CRP), Hamilton Depression Rating Scale 17-item (HAM-D17), Mini International Neuropsychiatric Interview (MINI), Digit Symbol Coding Test (DSCT), Generalized Anxiety Disorder 7-item scale (GAD-7), Patient Health Questionnaire-9 (PHQ-9), Montgomery Åsberg Depression Rating Scale (MADRS-S), Snaith Hamilton Pleasure Scale (SHAPS), Insomnia Severity Index (ISI), Fatigue Severity Scale (FSS), Eicosapentaenoic acid (EPA).

## Genes, Depression and Suicidality (GEN-DS) (Paper II)

The GEN-DS study included patients with depressive suffering and insufficient treatment response, thereby referred to as difficult-to-treat depression. Study procedures have been described in detail in previous publications [168]. Referrals to the study came from four secondary psychiatric clinics in the Skåne region of southern Sweden between 2012 and 2021. Exclusion criteria were defined as pregnancy, BMI <15 or liver failure. All patients met with a specialist in psychiatry or a resident in psychiatry supervised by a specialist to undergo a detailed clinical assessment including an in-depth interview plus several expert- and self-rated

symptom scales. The assessment included screening with MINI and the Structured Clinical Interview for DSM-IV Personality Disorders (SCID-II). Diagnoses were determined using the DSM 4th Edition (IV-TR). The Comprehensive Psychopathological Rating Scale (CPRS) [169] was used from which the 9-item expert rated MADRS was extracted. Among self-ratings in the assessment were Suicide Assessment Scale (SUAS).

A composite score ('Inflammation-Depression Symptoms') was calculated by adding scores of the CPRS items #5 Inability to feel, #14 Lassitude, #18 Reduced appetite, #19 Reduced sleep and #20 Increased sleep. The selection of these items was based on results from previous studies correlating peripheral inflammatory markers with individual depressive symptoms [146, 170-172], from which the most consistently inflammation-related symptoms were chosen (outlined in discussion and illustrated in **Table 6**). In the previous studies mentioned, different rating scales were used and therefore interpretation to corresponding CRPS items had to be carried out by consensus among collaborators in the current study. The cohort used for paper II is a subsample (n=263) of the whole recruited cohort. In this thesis, the study (paper II) will hereafter be referred to as GEN-DS.

*Healthy controls (paper II)*

Healthy controls were sought via ads in newspapers and social media. Compensation for participation (500 SEK) was offered. A total of 46 healthy controls were included for comparisons in paper II. Inclusion was based on an assessment carried out by a study physician at the psychiatric clinic in Lund, Sweden. History of or current psychiatric illness, treatment with psychopharmacological agents or psychotherapy was ruled out by the structured interview with the study physician. Further exclusion criteria were chronic or severe somatic illness, ongoing infection, immunomodulatory treatment, pregnancy, or breastfeeding. Participation also included a blood-draw within a couple of days from the study interview.

## CAN-D (Paper IV)

The study was conducted at University of California, San Francisco (UCSF), USA and included subjects recruited in the Cellular Aging and Neurobiology of Depression (CAN-D) study, https://clinicaltrials.gov/study/NCT00285935. The substudy included in this thesis (paper IV) will hereafter be referred to as CAN-D. All subjects were diagnosed with MDD and were free of psychotropic medications, including antidepressants, since at least 6 weeks prior to the study. Patients were recruited using clinical referrals, newspaper ads, flyers, Craigslist postings and bulletin board notices. MDD diagnosis was confirmed using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I) and a clinical interview with a specialist in psychiatry. HAM-D17 was used for evaluation of depression severity,

whereas a score of ≥17 was required for inclusion to the study. Exclusion criteria included: psychotic symptoms, bipolar disorder, history of psychosis, eating disorders, PTSD, substance use disorder 6 months before study, acute somatic illness including infections or chronic inflammatory disorder. None of the included subjects took psychotropic medications (e.g. antidepressants) or other interfering medications or had recently (within 6 weeks) been vaccinated. Sedatives or hypnotics with short-acting profile were accepted if taken for sleep ≤3 nights/week. Assessment also included urine toxicology screen at each study visit and a urine test for pregnancy among fertile women. The second phase of this study included an intervention (eight weeks of treatment with an SSRI) which a subset of participants took part in. At baseline and week 8, blood was drawn, and behavioural assessments were carried out. 69 healthy controls were recruited for baseline comparisons to MDD concerning characteristics and biomarkers.

# Ethical Considerations

All studies included in this thesis followed ethical principles as stated in the Declaration of Helsinki. All participants in the different studies were given oral and written information about study procedures and signed written informed consent before inclusion. Omega-3 was approved by the Regional Ethical Review Board in Lund with reference number 2017/150. Amendments to the initial study protocol for the omega-3 study were submitted and approved to improve procedures of recruitment and study management. The study was also monitored according to Good Clinical Practice guidelines by Clinical Studies Sweden, Forum South. Study participants were allowed to terminate their participation in the study at any time. Severe worsening of clinical symptoms including suicidality could call for study termination as initiated by study physician. Some investigators had, prior to study start, reported that n-3 PUFAs could increase bleeding tendency. Therefore, we excluded any patient prescribed anticoagulants or with known coagulation dysfunction. This, and other, potential risks with participation in the study (e.g. discomfort at blood draw or adverse events from study products) were carefully considered, yet the potential benefits (e.g. diagnostic assessments, potential improvement of depressive symptoms) were judged to outweigh risks. GEN-DS was approved by the Regional Ethical Review Board in Lund, reference number 2011/673. CAN-D was approved by the Institutional Review Board (IRB) of the University of California, San Francisco (UCSF).

# Biomarker analyses

## Omega-3 (Paper I and III)

In Omega-3, hs-CRP levels were determined before or in proximity of the baseline visit to stratify subjects into 'inflammation' and 'non-inflammation' groups. The hs-CRP analysis was conducted at the Laboratory Unit, Skåne University Hospital, using an automated particle-based immunoassay that was adjusted to the international reference preparation ERM-DA-474/IFCC. Limit for quantification was below 1 mg/L.

## GEN-DS (Paper II)

On the same day as (or in proximity of) the clinical assessment, patients had blood drawn in the morning (fasting 4 hours prior, instructed to not take medications or or use nicotine products the same morning). A set of routine tests were run including CRP. The rest of the blood samples were stored in the regional biobank (-80°C). Stored plasma was used to measure levels of cytokines and vitamin D in 266 patients who also had hs-CRP levels measured. Cytokines were assessed using the high sensitivity electrochemiluminescence based multiplex immunoassay (MesoScale Discovery, Gaithersburg, MD, USA) and performed on MESO QuickPlex SQ 120. The cytokines analysed were those of the MSD V-PLEX Proinflammatory Panel 1 (Human), including IFN-γ, IL-1b, -2, -4, -6, -8, -10, -12p70 and -13 as well as TNF-α. As the majority of samples fell below the detection limit for IL-2, IL-13, IL-1b, IL-12p70 and IL-4, the data pertaining to these cytokines was excluded from the analysis. Among cytokines, the average detection limit and coefficient of variation (% CV) values (duplicates, intraassay) for the cytokines were: IFN-γ: 0.99 pg/mL CV = 10.65, TNF-α: 0.17 pg/mL CV = 11.31, IL-10: 0.082 pg/mL CV = 21.47, IL-6: 0.10 pg/mL CV = 14.68 and IL-8: 0.046 pg/mL CV = 5.74. Interassay % CV value (control samples of IL-8) was 2.78. Imputation was carried out for IL-10 since 36 samples (10.1%) were below fit curve (Missing Not At Random). This was a multiple imputation that used other cytokines as model variables (linear, 10 imputation, 0.00 and detection limit 0.082 pg/mL as constraints).

The vitamin D analyses were carried out by liquid-chromatography-mass spectrometry using model Sciex API 4000 LC/MS/MS (MA, USA). The analytes were 25(OH)D2 and 25(OH)D3. Clinical guidelines were followed when 25(OH)D2 levels were >10 nmol/L, hence added to the 25(OH)D3 level in statistical analyses (Ref Phinney 2017). CV values were 25(OH)D2; 6,0% at 35 nmol/L and 5% at 114 nmol/L, and for 25(OH)D3; 8% at 33 nmol/L and 5% at 133 nmol/L. For both 25(OH)D2 and 25(OH)D3 the detection limit was 6 nmol/L.

### CAN-D (Paper IV)

To assess TRP, serotonin and KP-metabolites, metabolite quantification was carried out with reverse phase high performance liquid chromatography (HPLC) coupled to a triple quadrupole mass spectrometer (1290 Infinity II LC System, 6470 Triple quadrupole, Agilent Technologies, Santa Clara, CA). When running same plasma samples at all 10 days of analyses, inter-assay CV values for pooled plasma in the study were: TRP 2.50%, Kyn 3.36%, Kyn-A 3.50%, 3-HK 4.73%, QA 6.37%, PA 7.49%, nicotinamide (NTA) 0.87%, AA 7.58% and 5-HT 6.41% (same plasma sample ran all 10 days). Average intra-assay coefficients of variability (CV) for pooled plasma ran for each day: TRP 0.79%, Kyn 0.85%, Kyn-A 2.99%, 3-HK 4.52%, QA 2.68%, PA 3.73%, NTA 0.58%, AA 9.02% and 5-HT 2.22% (each day a pooled sample was created from that day's samples and ran throughout that run, this is the average CV of each days different QC). Lower limits of detections were found to be as follows: TRP 36.6 nM, Kyn 2.2 nM, Kyn-A 0.16 nM, 3-HK 0.29 nM, QA 4.15 nM, PA 0.63nM, NTA 0.98 nM, AA 0.98 nM and 5-HT 0.73 nM.

Inflammatory markers IL-6 and TNF-α were assessed in two different batches, using two different platforms. Method applied was a high sensitivity multiplexed sandwich immunoassay. Due to potential batch differences, values were z-transformed within each batch. Intra-assay CV values were for IL-6 and TNF-α were below 10%. Quest Laboratory (San Jose, California, USA) analyzed plasma samples to measure hs-CRP.

## Imaging in CAN-D (Paper IV)

In CAN-D, MR data were acquired on a 3T Siemens Vision system using a 24-channel transmit-receive head coil (Siemens, Erlangen, Germany). A Magnetization Prepared Rapid Gradient (TR/TE/TI = 2300/2.98/1000 ms, 9 degree flip angle, 1 mm x 1 mm x 1 mm resolution) plus a turbo spin-echo (TR/TE = 9000/91 ms, 150 degree flip angle, 0.9 mm x 0.9 mm x 5 mm resolution) sequence were used to obtain 3D sagittal T1-weighted and 2D axial T2-weighted anatomical MR images, respectively. The images were then displayed on the console for the prescription of the volumes-of-interest (VOIs). MR spectra were acquired from VOIs in the same scanning session. Placement of the MRS VOIs was carried out to maximize the inclusion of as gray matter (GM) as possible. After VOI-specific 3D shimming and optimization of water suppression, PRESS metabolite spectra were obtained from the dorsal ACC (35 mm x 25 mm x 20 mm, TR/TE = 1800/30 ms, 90 degree flip angle, 2000 Hz spectral bandwidth, 3:07 minutes acquisition time) and the left basal ganglia (30 mm x 30 mm x 15 mm, including the head of the caudate, lenticular nucleus, and anterior thalamus; acquisition parameters as above), each followed by the acquisition of a water spectrum at that same location, obtained with the water

suppression pulse turned off. In a large subset of the participants, semiLASER spectra and the corresponding water spectra were subsequently also acquired from the perigenual ACC (25 mm x 20 mm x 20 mm, TR/TE = 1800/40 ms, 90 degree flip angle, 2000 Hz spectral bandwidth, 4:05 minutes acquisition time). All available raw MRS data for indvidual participants and VOI were processed as previously described to yield absolute metabolite concentrations in institutional units [173-175].

Furthermore, GM volume estimates of the hippocampus, amygdala, and total intracranial volume (ICV) were calculated using the *recon-all* function (https://surfer.nmr.mgh.harvard.edu/fswiki/recon-all) from FreeSurfer v. 6.0 [176]. In line with procedures used in previous work [177, 178] volumes for each hemisphere we extracted and converted to z-scores. Any segmentations for volumes where z-scores ≥ |2.5| were visually examined. If segmentations were regarded as too poor of such statistical outliers these were removed from analyses.

# Statistics

## Omega-3 (Paper I and III)

A power calculation aiming to detect between-group (inflammation vs. non-inflammation) differences of total change in HAM-D17 scores of three points from baseline to week 8 resulted in the assumption that at least 45 patients were needed in each group, (power=0.80, alpha=0.05). After about 2/3 of patients were recruited, a statistician without involvement in study procedures recalculated sample size based on the standard deviation of HAM-D17 outcome, resulting in an estimation that a total of 96 subjects would be needed. The primary hypothesis and initial study design set hs-CRP≥3 mg/L as the cut-off for the inflammatory group. This approach resulted in an imbalance between-groups, with the non-inflammation group being substantially larger. This was discovered during the recruitment phase which led to the use of a secondary hs-CRP cut-off of ≥1mg/L in additional analyses. This was based both on previous studies using hs-CRP≥1mg/L to delineate mild-moderate inflammation in MDD [36, 157] and that the more equally sized groups were more consistent with the original power calculation specifying at least 45 patients in each group. Analyses were carried out using the Statistical Package for the Social Since (SPSS) 28 Windows and 29 Mac (IBM, Armonk, NY, USA) and SAS Enterprise Guide 8.3 (SAS Institute Inc., Cary, NC, USA). Since data was normally distributed, parametric tests were applied. For comparison of demographic data, we used Fisher's Exact Test for categorical variables and t-test for numerical variables. Linear mixed model repeated measures (MMRM) analyses were carried out assess the changes (baseline to follow-up at week 4 and week 8) between the groups in

primary and secondary outcome measures. In the MMRM models group (inflammation/non-inflammation), treatment week and the interaction between these were set as fixed effects. For all analyses, significance was defined as p-value ≤0.05. Response was defined as a ≥50% decrease in HAM-D17 from baseline to end of study and remission as a week 8 HAMD-17 score of ≤7. Effect sizes were calculated using Hedge's g.

## GEN-DS (Paper II)

To delineate subjects based on inflammatory status, we applied a hs-CRP cut-off of 3 mg/L (inflamed subjects hs-CRP>3 mg/L, uninflamed hs-CRP≤3 mg/L). To reduce the number of comparisons and identify shared patterns of variance in inflammatory markers and vitamin D among depressed subjects, we performed a principal component analysis (PCA). PCA included ln- and z-transformed individual values for IFN-γ, TNF-α, IL-6, -8, -10 and total vitamin D. Varimax rotation was applied in final analysis, yet an orthogonal rotation was also tested using direct-oblimin (oblique) method which rendered similar outcome. Sample size and average communalities (<0.6) resulted in selection of factors (components) based on the output scree plot (all factors pre inflection point chosen). Bartlett's method was chosen for extraction of component scores.

Non-normal distribution of variables as assessed by histograms and Q-Q plots was handled by ln-transformation when parametric tests were performed. For group-wise comparisons, Mann-Whitney U was used for rating scale outcomes. Biomarker levels were compared between groups using *t*-test after ln-transformation. To correct for multiple comparisons when the five inflammatory biomarkers were assessed between groups, the Bonferroni method was applied (p-value significance set at <0.01 (0.05/5)). Pearson's correlation method was used for all correlation analyses. The SPSS for Mac (SPSS version 27, IBM, Armonk, NY, USA) was used for all analyses.

## CAN-D (Paper IV)

Missing data for biomarkers (CRP, n=5, IL-6 and TNF-α. n=4) was handled by imputation using the mean values for each variable. Data was log transformed and parametric tests applied.

Partial Least Squares Discriminant Analysis (PLS-DA) [179] was used to explore correlations between different KP metabolites and depression status. Receiver Operating Curves (ROC) were used to explore the possibility to discriminate between MDD and HC using PLS-DA components and KP metabolite ratios. Pearson's correlations were applied for relationships between KP metabolite ratios and inflammatory markers as well as metabolic measurements (Homeostatic Model

Assessment for Insulin Resistance, HOMA-IR, CR score, allostatic load score, HbA1c, glucose and PSS). Inflammation status (low/high) and insulin resistance (low/high) was in this paper defined as median split for inflammatory markers (CRP, IL-6, TNF-α) and by cut-off 3 for HOMA-IR. The HOMA-IR cut-off was chosen to delineate severe insulin resistance, since a commonly used cut-off is 2.5 for insulin resistance overall [180], yet different cut-offs have been applied [181, 182]. Differences in levels of KP metabolite ratios between MDD and HC and between high/low MDD groups based on immunometabolic markers were assessed by independent samples *t*-test. Based on previous studies in the field, two symptom dimensions (atypical energy-related and melancholic) were calculated from the Inventory of Depressive Symptoms (IDS) [183]. The atypical energy-related symptom dimension was calculated as sum scores of items 'increased appetite', 'increased weight', 'hypersomnia', 'leaden paralysis' and 'low energy' (range 0-15). Melancholic symptom dimension consisted of sum scores on 8 items (range 0-24): 'diurnal variation', 'distinct quality of mood', 'excessive guilt', 'decreased appetite', 'early morning awakening', 'decreased weight', 'psychomotor agitation' and 'psychomotor retardation'. Associations between KP metabolite ratios and the symptom dimensions, measurements of brain volume and Glx were assessed in linear regression models (adjusted and unadjusted).

Treatment outcome (HAM-D17 score after 8 weeks vs. baseline) was assessed using a linear mixed model repeated measures (MMRM) testing for responder status and time effects but also interaction responder x time on change in metabolite ratios. Pearson's correlation was then applied to relate changes during the study in KP metabolite ratios to depression severity change.

# Review of Results

## Omega-3 (paper I and III)

101 subjects with at least one follow-up visit post-baseline were included in the intention-to-treat analyses. Overall, EPA supplementation was well-tolerated.

BMI was the only demographic characteristic that differed significantly between inflammation and non-inflammation groups whereas both hs-CRP≥3 mg/L (n=29) and hs-CRP≥1 mg/L (n=56) had higher mean BMI than hs-CRP<3 mg/L (n=72) and hs-CRP<1 mg/L (n=45) respectively (p<0.001).

The primary analyses, comparing CRP≥3 mg/L to hs-CRP<3 mg/L did not show any significant changes between the groups in primary and secondary outcomes from baseline to week 8 (all p>0.27). Instead, division by CRP below or above/equal to 1 mg/L revealed significant differences in several outcome measures between baseline and week 8: HAMD-17 (ES=0.45, p=0.03), Inflamed Depression Symptoms (ES=0.41, p=0.05), ISI (ES=0.42, p=0.04) and FSS (ES=0.44, p=0.03) (**Figure 5 A-D**).

Response (change in HAMD-17 with 50% or more from baseline to week 8) was evident in a total of 25 patients and among them, 18 reached remission (HAM-D17≤7 at week 8). There were significantly more responders and remitters in the hs-CRP≥1 mg/L group (38.5% and 28.8% respectively) compared to hs-CRP<1 mg/L (11.9% and 7.1% respectively, p=0.005 for response and p=0.009 for remission).

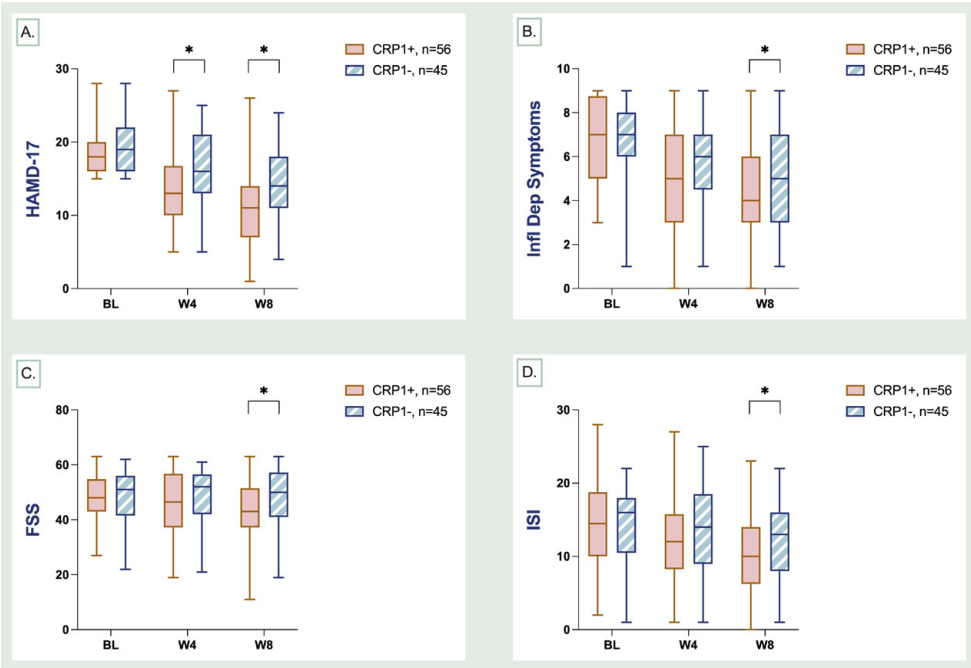


**Figure 5. Primary and secondary outcomes at baseline and after 4 and 8 weeks of treatment with omega-3 fatty acid eicosapentaenoic acid (EPA). 101 patients with MDD here stratified by hs-CRP above/equal to 1 mg/L (CRP1+) or below 1 mg/L (CRP1-) at baseline. A. HAMD-17. B. Composite score Infl Dep Symptoms. C. Fatigue Severity Scale. D. Insomnia Severity Index.**
Box and whiskers graph; boxes include second and third quartiles separated by median (solid line). Whiskers representing minimum to maximum. Missing data, all graphs: week 8 CRP1+ n=4, CRP1- n=3. Abbreviations: Baseline (BL), Week 4 (W4), Week 8 (W8), Hamilton Depression Rating Scale 17-items (HAMD-17), Inflamed Depression (Infl Dep), Fatigue Severity Scale (FSS), Insomnia Severity Index (ISI), high sensitivity C-Reactive Protein (hs-CRP), C-Reactive Protein ≥1 mg/L (CRP1+), C-Reactive Protein <1 mg/L (CRP 1-).

# GEN-DS (paper II)

In the final analysis, 263 patients with available measures of cytokines and CRP were included, out of which 238 also had levels of vitamin D. Depressed subjects had significantly higher mean BMI ($p<0.001$), were more likely to be smokers ($p=0.04$) and had elevated levels of IL-6 and IL-8 ($p=0.001$) compared to healthy controls (n=46). Recurrent MDD and dysthymia/chronic depression were the most common main diagnoses (41.8% and 29.7% respectively), while 10.6% did not fulfil criteria for a current affective disorder at the time of assessment. Co-morbidities

with other psychiatric diagnoses as well as somatic conditions were common, and the majority of patients were currently prescribed psychopharmacological agents.

Inflamed depression group (hs-CRP>3 mg/L, n=51) showed significantly higher mean BMI (31.3 compared to 25.1, p=0.001) and higher mean scores on the composite Inflamed Depression score (p=0.022) compared to uninflamed group (hs-CRP≤3 mg/L, n=212) as shown in **Figure 6A-B**. There was no significant difference between the groups concerning other characteristics assessed, or depression severity.

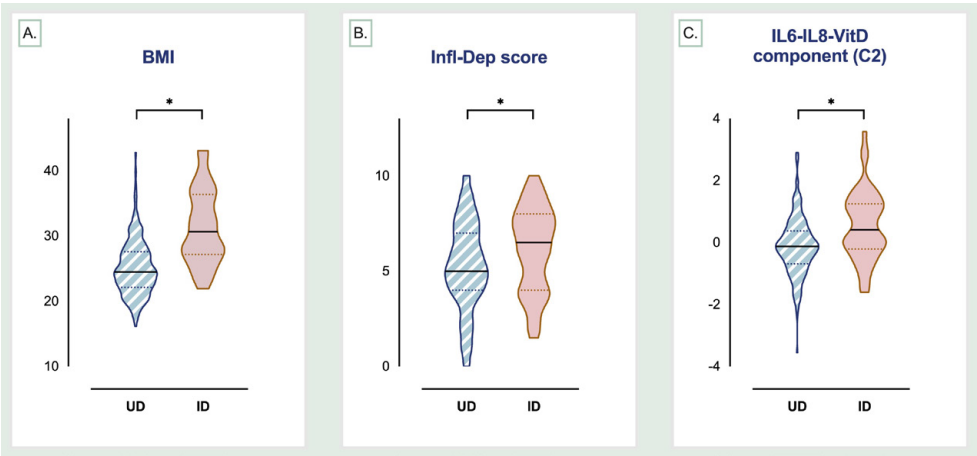


**Figure 6. Significant differences in outcome measure when 263 patients with difficult-to-treat depression were stratified by hs-CRP above 3 mg/L (Inflamed Depression) or equal to/below 3 mg/L (Uninflamed Depression). A. BMI, B. Infl-Dep score, and C. IL6-IL8-VitD component.**
Violin plots of distribution with median (solid line) and quartiles (dotted lines). Abbreviations: high sensitivity C-Reactive Protein (CRP), Uninflamed Depression (UD), Inflamed Depression (ID), Body Mass Index (BMI), Inflamed Depression Symptom score (Infl-Dep score), Interleukin 6, interleukin 8 and vitamin D component score 2 (IL6-IL8-Vit D C2). * Significant at the 0.05 level (2-tailed). BMI comparison, n=254; Infl-Dep score comparison, n=254; IL-6-IL8-VitD component comparison, n=238

The PCA included n=238 subjects. Two components were extracted; component 1 (C1) was interpreted as a general cytokine elevation component and component 2 (C2) was distinguished by positive loadings of IL-6 and -8 as well as negative loading of vitamin D levels (referred to as IL-6-IL-8-Vit D component). There was no significant difference in the general cytokine elevation (C1) component scores between inflamed and uninflamed subjects (t(60.8)=-1.1, p=0.27). As illustrated in **Figure 6C**, the IL-6-IL-8-Vit D component scores were significantly higher among inflamed subjects compared to uninflamed (t(62.0)=-3.6, p<0.001).

# CAN-D (Paper IV)

Participants with MDD had statistically significant higher mean BMI (p=0.02), were more likely former or current smokers (p=0.02) and reported lower household income (p=0.004) than healthy controls. None of the assessed KP metabolite ratios could significantly discriminate MDD from HC. Symptom dimensions (atypical energy-related or melancholic) in the MDD sample did not significantly associate with KP ratios.

Overall, metabolic dysfunction (higher HOMA-IR or BMI) and inflammation (higher CRP, IL-6 or TNF-α) correlated with increased activity of the KP, higher proxy markers of neurotoxicity and lower ratios of presumed neuroprotection, as illustrated in **Table 3** below.

**Table 3. Overview of baseline associations between immunometabolic markers (BMI, HOMA-IR, CRP, IL-6, TNF-α) and kynurenine pathway metabolite ratios among patients with MDD, total n=98.**

| KP metabolite ratio | Kyn/ TRP | Kyn-A/ Kyn | Kyn-A/ QA | Kyn-A/ 3-HK | 3-HK/ Kyn | QA/ Pic | QA/ TRP |
|---|---|---|---|---|---|---|---|
| **Effects*** | **Activation** | **Neuroprotection** | | | **Neurotoxicity** | | |
| **Age** | ↑ | ↓ | | | | ↑ | |
| *Metabolic dysregulation* | | | | | | | |
| **BMI[a]** | | | | | ↑ | | |
| **HOMA-IR[a]** | | | | ↓ | | | |
| **High HOMA-IR**[b]** | | | | ↓ | | | |
| *Inflammation* | | | | | | | |
| **CRP[a]** | | | | ↓ | | | |
| **IL-6[a]** | | | | ↓ | | | |
| **High CRP**[b]** | ↑ | | ↓ | ↓ | ↑ | | ↑ |
| **High TNF-α**[b]** | ↑ | | | | ↑ | ↑ | ↑ |
| **High IL-6**[b]** | | | | ↓ | ↑ | | |

Statistically significant [a]correlations or [b]group differences (all p<0.05) presented. Upward arrow: [a]positive correlation or [b]elevated in 'high' group compared to 'low' group. Downward arrow: [a]negative correlation or [b]decreased in 'high' group compared to 'low' group. *Proxy measures of: KP activation (beige), neuroprotective shift in KP metabolism (green), neurotoxic shift in KP metabolism (red). **Division into 'low' and 'high' groups based on: HOMA-IR <3 (low) vs. HOMA-IR ≥3 (high) and for inflammation median split of individual markers (CRP, TNF-α, IL-6). Statistics: [a]Correlations calculated using Pearson statistics, [b]Group differences calculated using independent samples *t*-test (exceptions: Kyn-A/Kyn, 3-HK/Kyn and QA/TRP group differences assessed by Wilcox rank sign test). Low/High division group sizes: HOMA-IR; low: n=83, high: n=15, CRP; low: n=50, high: n=48, TNF-α; low: n=49, high=49, IL-6; low: n=49, high: n=49. Abbreviations: Body Mass Index (BMI), Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), C-reactive Protein (CRP), Interleukin 6 (IL-6), Tumour Necrosis Factor Alpha (TNF-α), Major Depressive Disorder (MDD), Kynurenine Pathway (KP), Kynurenine (Kyn), Tryptophan (TRP), Kynurenic Acid (Kyn-A), Quinolinic Acid (QA), 3-Hydroxykynurenine (3-HK), Picolinic Acid (Pic).

The associations between higher inflammatory markers and KP-activation with neurotoxic branch predominance was further supported after division of the MDD cohort into 'low' and 'high' inflammation groups, see **Table 3**. Both 'high' CRP- and TNF-α groups showed significantly elevated Kyn/TRP (proxy marker of KP-activation). The 'high' CRP-group also showed lower Kyn-A/QA and Kyn-A/3-HK ($p=0.05$ and $p=0.004$), both presumed proxy markers of neuroprotective shift in KP-metabolism. Lastly, the 'high' TNF-α group showed increase of ratios QA/Pic ($p<0.001$) and 3-HK/Kyn ($p=0.002$), both suggested proxy markers of neurotoxic shift.

Several associations were found between KP metabolite ratios and regional brain volumes, as shown in **Table 4**. In general, brain volumes correlated positively with neuroprotective ratios and negatively with neurotoxic ratios. After adjustments for intracranial volume, correlations were significant between volume of the right hippocampus and QA/Pic (suggestive of more neurotoxicity, lower brain volume) plus correlations between right amygdala volume and three neuroprotective ratios (positive correlations), see **Table 4**. Glx in the ACC correlated significantly and positively with Kyn/TRP (proxy marker for activation of the KP) and neurotoxic ratio QA/TRP after adjustments for age, gender, and BMI.

**Table 4. A) Baseline kynurenine pathway metabolite ratio associations with baseline regional brain volumes and glutamate transmission. B) Kynurenine pathway metabolite ratio change during study in relation to SSRI treatment outcome, among patients with MDD.**

| KP metabolite ratio | Kyn/ TRP | Kyn-A/ Kyn | Kyn-A/ QA | Kyn-A/ 3-HK | 3-HK/ Kyn | QA/ Pic | QA/ TRP |
|---|---|---|---|---|---|---|---|
| **Effects*** | **Activation** | **Neuroprotection** | | | **Neurotoxicity** | | |
| **A)** *Brain volumes* | | | | | | | |
| **Left Hippocampus**[1] | ↑ | (↑) | (↑) | (↑) | | (↓) | |
| **Right Hippocampus**[1] | | (↑) | (↑) | (↑) | | ↓ | |
| **Left Amygdala**[1] | | | | | | (↓) | |
| **Right Amygdala**[1] | | ↑ | ↑ | ↑ | | (↓) | |
| *Glutamatergic transmission* | | | | | | | |
| **Glx ACC**[2] | ↑ | (↑) | | (↑) | | | ↑ |
| **B)** *SSRI treatment outcome* | | | | | | | |
| **↓ Δ HAM-D 17**[3] | | | Δ ↑ | Δ ↑ | | | |
| **Responders**[4] | | | | Δ ↑ | | | |

Statistically significant assocations (all p<0.05) presented. *Proxy measures of: KP activation (beige), neuroprotective shift in KP metabolism (green), neurotoxic shift in KP metabolism (red). (↑)/(↓): Significant positive/negative correlation in unadjusted model. ↑/↓: Positive/negative correlation significant in adjusted model ([1]Brain volumes: adjusted for intracranial volume, [2]Glx measurements: adjusted for age, gender, BMI). Δ: Delta change (week 8 - baseline). [3]Correlation between baseline to week 8 decrease in depression ratings (symptom improvement) and change in KP metabolite ratios. [4]Response defined as a decrease of ≥50% in HAMD-17 from baseline to week 8. Statistics: [1, 2]Linear regression. [3]Pearson correlation. [4]Linear mixed model repeated measures for group (Responder) x time interaction. Sample size:[1]n=45, [2]n=27, [3]n=48, [4]Non-Responders, n=29, Responders, n=19. Abbreviations: Selective Serotonin Reuptake Inhibitor (SSRI), Major Depressive Disorder (MDD), Kynurenine Pathway (KP), Kynurenine (Kyn), Tryptophan (TRP), Kynurenic Acid (Kyn-A), Quinolinic Acid (QA), 3-Hydroxykynurenine (3-HK), Picolinic Acid (Pic), Glutamatergic transmission (Glx), Anterior Cingulate Cortex (ACC), Hamilton Depression Rating Scale, 17 items (HAM-D 17).

49 of the participants with MDD were treated with SSRIs for 8 weeks. As shown in **Table 4**, change in depression severity (delta HAMD-17) correlated significantly with change in neuroprotective ratios Kyn-A/QA and Kyn-A/3-HK (indicating: more symptom reduction; more increase neuroprotection). Responders to treatment (but not non-responders) showed a significant increase (group x time interaction) from baseline to week 8 of neuroprotective ratio Kyn-A/3-HK ($F_{(1,46)}$=11.92, p=.001). Response did not significantly correlate with decrease in measured inflammatory markers.

# Discussion

## Inflamed depression phenotype

In all the included papers of this thesis, we stratified depressed individuals based on peripheral inflammatory markers. The foundation underlying the hypothesis of inflamed depression is described in the introduction (c.f. *the origin* of the thesis title). Despite methodological dissimilarities across the included studies, some features of subsamples with higher inflammatory markers were replicated across studies. We found that several clinical and biochemical characteristics (or *the essence*, as referred to in the title of this thesis) were associated with low-grade inflammation in the included studies, and these findings will be discussed below in relation to the previous literature. Moreover, the potential of this thesis, and related studies, to contribute to the expanding research field exploring more homogenous phenotypes of MDD based on pathophysiological processes will be discussed.

Inflammatory markers have been shown useful to subtype the increasing number of individuals fulfilling the DSM criteria for MDD, hence the pursuit of an inflamed depression phenotype has gained momentum [184, 185]. However, this field of research has evolved in disparate, yet partly overlapping, directions. Converging lines of evidence has led some researchers to investigate if there might be several inflamed subtypes, rather than one, assuming that it might be oversimplified to assess inflammation as controlled by an on/off switch. For example, Miller and Felger argued in a commentary on a publication by Lynall et al. [186] that evidence do not support the notion that there would be merely one immunophenotype to call inflamed depression [187]. Moreover, some have focused on the intersection of inflammation and metabolic dysregulation and presented this as the concept of immunometabolic depression (IMD), proposed as a 'dimensional profiler' rather than a binary subtype [142]. While uncertainties are evident regarding whether there is one or several immune-related phenotypes of depression, and the significance of an overlap with metabolic dysregulation, much promise has been attributed to delineation of patients with MDD and low-grade inflammation, not least for trials of immune-targeting interventions (*remedies*, as referred to in the title) [184].

## Origin and Essence - Clinical Characteristics

Several clinical features have been found to overlap between depression (risk, prevalence, prognosis) and inflammation, and have therefore been conceptualized as potential characteristics of the inflamed depression phenotype [12]. These features include age, gender, childhood trauma, metabolic parameters such as BMI, co-morbidities, genetical predisposition and more. However, there is currently no clear consensus on the exact set of such factors that predispose for the inflamed depression phenotype [184].

*Age*

In paper I-III (GEN-DS and Omega-3) no significant age differences were found between inflamed and non-inflamed subgroups. However, in paper IV (CAN-D) age correlated significantly with proxy markers of KP activation and a shift towards neurotoxicity. It should nonetheless be noted that no direct correlations were found between age, inflammation and MDD in paper IV. Instead, indirect associations were presented: older age and higher levels of inflammatory markers (CRP, IL-6, TNF-α) showed similar correlations to KP activation, lower neuroprotection, and higher neurotoxicity. These ambiguous results on age, inflammation and depression reflect the inconclusive results of the existing literature. Older age could theoretically be a characteristic of inflamed depression [188], as both human and animal studies have found older age to be correlated with a less efficient immune defence upon challenges [189]. Older age has also been proposed to associate with elevated inflammatory markers in depression and higher inflammatory markers have been correlated with an increased risk of reporting depressed mood among older adults [190]. In contrast, a meta-analysis on the long-term increased risk of depressive symptoms associated with elevated baseline IL-6 and CRP showed no significant differences based on age (under vs over 50 years) [74]. Moreover, a cross-sectional study stratifying MDD by high or low TNF-α and Kyn/TRP ratio did not present any significant age differences between groups, yet age was included as a covariate in all models [123]. These mentioned contradicting findings on older age as a clinical feature of inflamed depression supports the hypothesis that the inflamed phenotype covers several mechanistic pathways and is thereby in itself heterogenic (see **figure 7** for illustration of this reasoning).

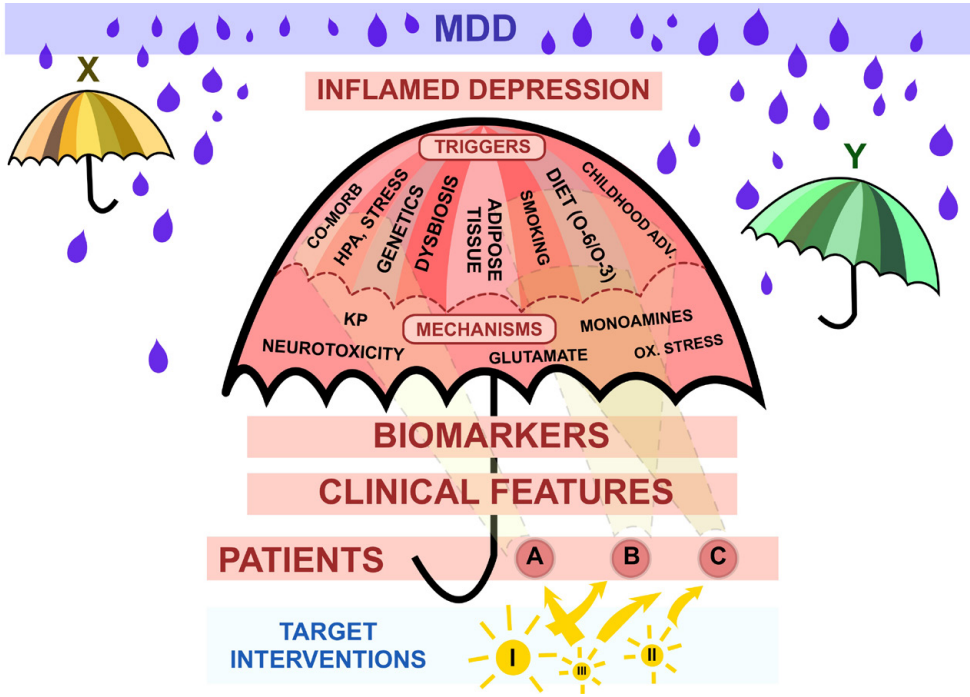


**Figure 7. Inflamed depression as an umbrella concept to delineate more homogenous subgroups of MDD and target interventions**
Low-grade inflammation can be triggered in various ways (see Triggers), which through mechanistic pathways (see Mechanisms) can alter mood and behavior that in some cases become manifested as depressive symptoms. Biomarkers and clinical features may enable the clinician to distinguish the mechanisms and triggers that underpin inflamed depression in individual patients (A, B and C). Thereby, target interventions (I, II and III) may be recommended based on a higher likelihood of response. Umbrellas X and Y represents other concepts that link pathophysiological processes with biomarkers and clinical features in patients with MDD. For example, X could represent melancholic depression with hyperactivity of the HPA axis with the biomarker non-suppression of dexamethasone suppression test, specific symptoms and target interventions (e.g. electroconvulsive therapy). Abbreviations: Major Depressive Disorder (MDD), Kynurenine Pathway (KP), Comorbidity (Co-morb), Hypothalamic-Pituitary Adrenal axis (HPA), Omega-6/omega-3 ratio (O-6/O-3), Childhood adversities (Childhood adv.).

The concept of inflamed depression holds potential for distinguishing a more uniform subset of MDD patients, but differences may exist within the phenotype regarding the origin of inflammation and the pathophysiological mechanisms affecting mood and behavior. Attempts to categorize inflamed depression based on single markers, such as increased hs-CRP in Omega-3 and GEN-DS, may capture individuals with diverse underlying mechanistic pathways, unrelated to age or any specific upstream factor and may obscure the effects of age on inflamed depression.

Instead, CAN-D supports involvement of age, possibly by focusing on a particular pathway (KP dysregulation) that may be more strongly associated with age as a

clinical feature than other mechanistic pathways. In sum, mechanistic pathways of inflamed depression likely differ between individuals, whereas elderly could be more prone to KP activation, and a neurotoxic shift associated with low-grade inflammation, rather than inflamed depression overall. Similar reasoning will recur in this discussion, leading to the hypothesis that inflamed depression could be useful as an umbrella concept including individuals with diverse mechanistic pathways underlying illness, as illustrated in **Figure 7**.

*Gender*

CAN-D did not investigate gender specific patterns of KP dysregulation in MDD. In both the Omega-3 and GEN-DS, there were no significant differences in gender between inflamed and non-inflamed subgroups. However, more women were recruited overall, especially in Omega-3 (87 women out of 101 recruited). Not only are women more likely to develop MDD during their lifetime [191], but they may also be more prone to the inflamed phenotype according to some previous reports. For example, healthy females were shown to be more prone to depressed mood correlating with increases in TNF-α and IL-6 levels compared to healthy men, during the hours after an infusion of low-dose endotoxin [52]. Yet, others have contradicted this, through reporting that males show both stronger associations between inflammatory markers and concurrent or subsequent depressive symptoms [76] and between IL-6 and depressed mood in an elderly cohort [190]. Even though we did not report any significant differences based on biological gender in the included studies, gender differences are proven in relation to immune system functioning and the prevalence of autoimmune disorders [192]. It is therefore reasonable to further study the influence of gender in respect to the inflamed depression phenotype.

*Metabolic dysregulation: BMI and insulin resistance*

BMI was assessed throughout the included studies since higher BMI is, as mentioned, a possible underlying factor in inflamed depression. This was indeed indicated in all included cohorts. In both the GEN-DS and the CAN-D, mean BMI was significantly higher in MDD overall, compared to healthy controls. Furthermore, in the GEN-DS and the Omega-3 cohorts, mean BMI was significantly higher in inflamed compared to non-inflamed depression. This was highly anticipated, based on the mentioned broad evidence on pro-inflammatory activity relating to adipose tissue and lifestyle factors relating to obesity and increased risk of MDD, such as low physical activity. Given the correlations between low-grade inflammation and higher BMI in depression (Omega-3 and GEN-DS), the need to further investigate the IMD concept is supported. In CAN-D, BMI correlated significantly with neurotoxic ratio 3-HK/Kyn. This is in line with previous findings of higher neurotoxic metabolites of the KP with higher BMI [121]. Yet again, results are inconclusive since e.g. Haroon et al. did not show significant differences in BMI

between groups based on high or low TNF-α and Kyn/TRP [123]. It is however well known that CRP is highly correlated with BMI (e.g. by adipose tissue secrete IL-6 which stimulates the liver to release CRP), hence using merely CRP as a biomarker to delineate inflamed depression (Omega-3, GEN-DS) could predominantly capture cases of inflamed depression where pro-inflammatory effects of adipose tissue is a key mechanism. The KP is activated by central pro-inflammatory cytokines, which makes it plausible that higher BMI may not be of central importance to inflamed depression where KP alteration is a key mechanistic pathway.

In CAN-D, insulin resistance was assessed by HOMA-IR to investigate the effects of altered metabolic parameters beyond BMI. As anticipated, the high HOMA-IR group had lower Kyn-A/3-HK (proxy marker of neuroprotection). This finding, indicative of neurotoxic predominance along the KP, connects insulin resistance with the pattern of KP effects shared by older age, higher BMI and elevated inflammatory markers. This is in line with the adjacent concept of IMD that has been evolving based on several studies, not least large investigations using the NESDA cohort [183]. In 2020, Milaneschi et al., collected the pieces and conceptualized IMD as a dimensional profiler of depression, characterised by increased inflammatory markers, metabolic dysregulation (e.g. obesity, higher insulin resistance and higher levels of leptin and lipids) and a specific symptom profile [142]. Also, as CAN-D presents similar effects on KP metabolism from with correlations to increased insulin resistance and higher BMI as well as higher inflammatory markers, further support of investigations into the IMD concept are offered. However, mechanistic pathways of IMD need to be clarified, not least to gain understanding as to whether this concept is equal to or should be included in the umbrella concept of inflamed depression.

*Other features*

There is a plethora of other characteristics, not explored in this thesis, that could theoretically predispose for inflamed depression. This includes early-life adversities such as childhood maltreatment that has been demonstrated as a risk factor for MDD overall [193, 194] and more severe illness trajectories [194]. A history of childhood trauma has also been associated with elevations of inflammatory markers later in life [195], albeit this has been debated as dependent on e.g. type of trauma and the individual markers of inflammation assessed [196]. Nonetheless, immune alterations have been postulated as mediators in the association between childhood adversity and MDD [197].

Physical activity is another factor that could influence inflammation in depression. In support of this, Frank et al. showed that among non-depressed individuals aged 50 or older, elevated baseline inflammatory markers (CRP and fibrinogen) were significantly associated with lower physical activity four years later [198]. On the other hand, subjects with lower physical activity at study start were at higher risk of more severe depressive symptoms at follow-up. In mediation analyses, the authors

showed that low physical activity was a partial, significant mediator of the relationship between elevated inflammatory markers at baseline and elevated depressive symptoms at follow-up [198]. Interestingly, low physical activity among patients with MDD has been hypothesized to be associated with the effects of inflammation on the dopamine system and more specifically reduced motivation and psychomotor slowing [184].

Co-morbidities, especially inflammatory illnesses, could be a phenotypical characteristic of inflamed depression due to potentially shared mechanistic pathways and a pro-inflammatory state. No structured examination of the impact of co-morbidites were carried out in the included papers. However, chronic inflammatory illness was an exclusion criterion in Omega-3 and CAN-D and sensitivity analyses were carried out in GEN-DS excluding patients with such co-morbidities. Yet again, one could question the methodology of excluding based on chronic inflammatory illness, since this approach will exclude those with a known risk factor of MDD as well as a pro-inflammatory state. Future studies need to carefully weigh the pros and cons of excluding these patients from studies examining inflamed depression.

As discussed later, the clinical features of an inflamed phenotype of depression might include less favourable outcome with conventional antidepressive treatment and specific symptomatology. Chamberlain et al. found that CRP was elevated in MDD and especially among subjects resistant to antidepressant treatment, compared to healthy controls [79]. The clinical phenotype found to be associated with higher CRP was characterised by e.g. higher BMI, reported childhood adversity and higher scores of vegetative symptoms (psychomotor slowing, sleep disturbance, difficulty getting started and difficulty working) [79]. This encourages a multidimensional approach in exploration of inflamed depression where clinical features, biochemical findings, and illness trajectories (including symptom presentation) are combined. Hence, study designs using merely one feature to delineate inflamed depression may be oversimplistic in their approach, which also applies for Omega-3, which will be discussed later.

An example of practical implementation of a multidimensional approach to identify a pro-inflammatory state in depression was demonstrated by McIntyre et al. in their study on TNF-α inhibitor Infliximab in the treatment of bipolar depression [158]. Patients with bipolar depression and with higher susceptibility of low-grade inflammation were recruited by adding as an eligibility criterion that at least one of the criteria listed in **Table 5** should be met.

**Table 5. Biochemical (green) and phenotypic (blue) inflammatory criteria in the 2019 paper by McIntyre et al [158].**

| Inflammatory Criteria for Eligibility | Clarification |
|---|---|
| CRP ≥5 mg/L | *Peripheral level* |
| Obesity and metabolic dysregulation | *Obesity based on waist circumference or BMI ≥30 and at least one of the following: elevated triglycerides/treatment for hypertriglyceridemia, decreased HDL cholesterol, hypertension/antihypertensive treatment* |
| Inflammatory Bowel Disorder (IBD) | *Ie. Ulcerative colitis or Crohns disease* |
| Diabetes type 1 or 2 | *According to levels of fasting plasma glucose or HbA1c, or antidiabetic treatment* |
| Rheumatic disorder | *Ie. rheumatoid arthritis or psoriasis* |
| Smoking | *Daily, more than half a package of cigarettes* |
| Migraine headaches | *According to International Headache Society Guidelines* |

Abbreviations: C-reactive protein (CRP), Body Mass Index (BMI), High-Density Lipoprotein (HDL), Hemoglobin A1c (HbA1c)

Interestingly, and as mentioned, the study by McIntyre et al. was negative concerning the effects of Infliximab on depressive symptoms overall [158] whereas post-hoc analyses revealed significant effects on anhedonia [159]. Hence, the criteria in **Table 5** were not specific enough to predict what dimensions of depression that would benefit from the anti-inflammatory intervention, but the addition of specific symptoms of anhedonia as secondary outcome measure demonstrated effect. This further supports that symptom presentation is to be considered a characteristic of inflamed depression that may give important clues in the development of target interventions.

*Symptomatology*

In none of the included studies of this thesis did overall severity of depressive symptoms relate significantly to biomarkers of inflammation or the KP. Namely, there were no significant differences in HAM-D17 or MADRS scores between the inflamed and the non-inflamed groups within either Omega-3 or GEN-DS. Likewise, in CAN-D, there were no significant correlations between baseline HAMD-17 score and the KP metabolite ratios. However, it is possible that the approach in CAN-D (to investigate correlations in the MDD cohort as a whole) obscured identification of any subgroup-specific associations, based on e.g. the results by Haroon et al. [123]. In their study, a cluster analysis identified a subgroup of MDD with both higher TNF-α and Kyn/TRP that exhibited increased overall depression severity (sum-score IDS) and anhedonia, compared to MDD with low TNF-α and Kyn/TRP [123]. Yet, in all studies included in this thesis, symptomatology was investigated beyond total sum-scores of depression rating scales by focusing on more specific dimensions of symptoms previously related to inflamed depression or IMD, see **Table 6**. To do so in Omega-3, secondary outcome

measures included self-ratings of fatigue, anhedonia, and sleep disturbances as well as a composite score of PHQ-9 called 'Inflamed Depression Symptoms' including lack of energy, appetite disturbance, and sleep problems. Importantly, the hs-CRP≥1 mg/L group improved significantly with EPA supplementation in terms of both fatigue and sleep disturbances as well as the composite score of Inflamed Depression Symptoms, compared to hs-CRP<1 mg/L. Consistent with these findings, we found that a similar composite score calculated in GEN-DS (referred to as Infl-Dep symptoms) (see **Table 6**), was significantly higher in the inflamed depression group compared to the uninflamed. Hence, both from the cross-sectional (GEN-DS) and longitudinal (Omega-3) point-of-view, our results support that taking specific dimensions of depressive symptoms into account, rather than total scores of depression severity, may be crucial to improve understanding of the inflamed depression phenotype.

In CAN-D however, symptom dimensions were investigated in relation to biomarkers in an attempt to replicate previous findings on IMD [142, 183]. Particularly, Lamers et al. showed that out of four assessed symptom dimensions, only the atypical energy-related was significantly correlated with elevated markers of inflammation and poorer metabolic health [183]. Interestingly, these symptoms overlap with vegetative symptoms/sickness behaviour linked to energy homeostasis, including hyperphagia and hypersomnia, weight gain, leaden paralysis, and fatigue. Hence, this dimension also overlaps with the composite scores of inflamed depression symptoms calculated in Omega-3 and GEN-DS **(Table 6)**. It was therefore hypothesised that the atypical energy-related symptom dimension would associate more strongly with KP activation and neurotoxic predominance compared to the melancholic dimension. However, there were no significant associations between KP metabolite ratios and either of the two symptom dimensions in CAN-D. Nonetheless, it is notable that the results on symptom dimensions in CAN-D are based on a cross-sectional design and included a much smaller sample than the longitudinal findings from the NESDA cohort [183]. Moreover, we did not investigate anhedonia as a specific symptom in CAN-D, nor the relation between symptom dimensions and immunometabolic markers, merely KP ratios. Therefore, the negative results from CAN-D pertaining to biomarker-symptom associations should be interpreted with caution, due to existing positive results based on larger samples and longitudinal approaches.

It does however stand clear, even in studies considering symptom ratings unrelated to inflammation or other biochemical features, that MDD is a heterogeneous diagnosis regarding symptom presentation which may relate to disease trajectories [199]. For example, using data from the STAR*D trial, Chekroud et al. applied a data-driven approach to identify three symptom clusters [200]. One of the symptom clusters identified in this large cohort was labelled atypical and included psychomotor slowing or agitation, hypersomnia, and suicidal ideation. In general, this cluster of symptoms was less responsive to antidepressant therapy [200]. These

and similar findings fuel the debate on whether the heterogeneous symptom presentation in MDD reflects diverse underlying mechanisms of pathology [201]. In the case of inflammation as one biological underpinning of MDD, different dimensions of depressive symptoms have indeed been shown to associate with inflammation, leading to the presumption that the inflamed depression phenotype is characterized by a specific symptom profile [12]. In turn, it has been hypothesised that such symptoms may be transdiagnostically more responsive to anti-inflammatory treatments [159, 185].

**Table 6. Summary of depressive symptoms proposed to correlate with peripheral inflammatory markers. Green: studies used for compilation of inflamed depressive symptoms composite score in the GEN-DS study (Paper II). Red: included items of inflamed depressive symptom composite score in the Omega-3 study (Paper I and III) and yellow: items in the atypical energy-related symptom dimension used in CAN-D study (Paper IV).**

| Author, publ. year | Thesis paper | Sample | Rating scale | Bio-markers | Rating scale items | Corresponding CPRS items |
|---|---|---|---|---|---|---|
| White, 2017 *[146]* | | General population, n=5909 | 8-item CES-D | CRP | 'Everything i did was an effort', 'Sleep was restless' and 'I could not get going' | #**14, 15, 19** |
| Jokela, 2016 *[170]* | | US national health and nutrition surveys, n=15071 | DSQ | CRP | 'Trouble sleeping or sleeping too much', 'Feeling tired or having little energy' and 'Poor appetite or overeating' | #**14, 15, 18, 19, 20** |
| Fried, 2020 *[171]* | | HC, current/previous /high risk for anxiety or depr. disorders, n=2321 | IDS-SR | CRP, IL-6, TNF-α | 'Sleep problems', 'Energy level', 'Appetite/weight changes', 'Aches and pain', 'Irritability'* | #4, **14, 15, 18, 19, 20,** 24, |
| Frank, 2021 *[172]* | | Population-based cohorts, n=56351 | CES-D, DSQ, GHQ, GDS | CRP, IL-6 | 'Changes in appetite', 'Felt everything was an effort', 'Could not get going/loss of energy', 'Sleep problems', 'Little interest in doing things/unmotivated' | **#5, 14, 15, 18, 19, 20** |
| Suneson et al. 2023 *[202]* | II | Difficult-to-treat depression, n=263 | CPRS | CRP, PCA cytokines + Vit D | **Composite score: #5 'Inability to feel', #14 'Lassitude', #15 'Fatiguability', #18 'Reduced appetite', #19 'Reduced sleep', #20 'Increased sleep'** | |
| Suneson et al. 2023, in review | I & III | MDD, n=101 | PHQ-9 | CRP | 'Lack of energy', 'Appetite disturbance', 'Sleep problems' | **#14, 15, 18, 19, 20** |
| Rampersaud, Suneson et al. Manuscript. | IV | MDD, n=98 | IDS, atypical energy-related dimension | CRP, IL-6, TNF-α, KP | 'Leaden paralysis', 'Low energy', 'Increased appetite', 'Increased weight', 'Hypersomnia' | **#14, 15, 20** |

Items used in composite score of GEN-DS (paper II) are written in bold. *Based on different models, with and without regularization, as proposed in discussion of article. Abbreviations: Healthy controls (HC), Comprehensive psychiatric Rating Scale (CPRS), Inventory of Depressive Symptoms – Self Rated (IDS-SR), Centre for Epidemiological Studies-Depression scale (CES-D), Depression Screening Questionnaire (DSQ), General Health Questionnaire (GHQ), Geriatric Depression Scale (GDS).

Some studies within the field have indeed investigated depressive symptoms as symptom clusters or dimensions, rather than the total scores of rating scales [201]. This stems from the recognition that when sum-scores (such as total scores of HAM-D17, IDS or MADRS) are used, there is an inherent risk that heterogeneity in the trajectories of specific symptoms are masked [199]. Hence, future studies investigating the biology of depression should consider symptom heterogeneity, either by approaching symptoms individually, in clusters, dimensions or by composite scores based on the previous literature. One of the most applied divisions of depressive symptoms into dimensions is the separation of psychological, cognitive and neurovegetative symptoms, of which the latter dimension (including sleep difficulties, low energy/fatigue and altered appetite) have been repeatedly associated with inflammation [201]. As previously mentioned, Capuron et al. showed that following administration of IFN-α, neurovegetative symptoms presented earlier and were less responsive to antidepressant treatment than depressed mood and cognitive symptoms [58]. These differences in symptom dimension trajectories can be interpreted as an indication there are disparate mechanisms underpinning each depression symptom dimension [58, 201]. Importantly, the neurovegetative symptom dimension overlaps with the concept of sickness behaviour as well as individual depressive symptoms most repeatedly associated with inflammation [172, 203]. Yet, to some extent, these symptoms also overlap with other approaches to characterise depressive symptomatology, such as the atypical specifier of MDD in the DSM, and as mentioned the atypical, energy-related dimension [183]. Further widening of the spectrum of depressive symptoms that overlap with sickness behaviour occurs, including e.g. decreased libido, pain sensitivity and irritability [47, 203] whereas some studies have focused particularly on anhedonia in relation to inflammation [123]. In a narrative review on associations between dimensions of depressive symptom and inflammatory markers, Majd et al. included 14 cross-sectional and 7 longitudinal studies in which they found that associations between inflammation and symptom severity were predominantly attenuated to non-significance after adjustments for covariates such as BMI, age and gender [201]. Overall, authors reported evidence for a positive association between neurovegetative symptom dimension (fatigue, altered appetite and sleep problems as well as psychomotor disturbances reported subjectively or by finger-tapping test) and inflammatory markers (especially CRP), even after adjustment for covariates and other symptom dimensions [201]. This review was followed by a study by Moriarity et al., where symptoms relating to inflammation were assessed for their influence on each other in a network perspective (meaning appreciation of the fact that depressive symptoms are to varying degrees related to each other), hence investigating variation in the structure of depressive symptoms as a function of CRP-levels [185]. In the study, a US community sample was divided by CRP≥3 mg/L (elevated) or <3 mg/L (non-elevated), reporting that the elevated CRP-group were overall older, had higher mean PHQ-9 ratings and included a higher percentage of females than the non-elevated group. Moreover, the elevated CRP group showed

stronger associations between symptoms, a finding previously shown in treatment resistant depression. Also, concentration difficulties and psychomotor alterations were shown to be more influential in symptom networks of the elevated CRP group. However, like Majd et al. [201], authors highlighted that psychomotor disturbances may include not only physical but also cognitive slowing whereas these findings may indeed capture the importance of cognitive symptoms in MDD [185]. Moreover, Moriarity et al. also showed that symptom-symptom associations were moderated by CRP levels, especially for symptoms of changes in appetite, psychomotor alterations, anhedonia and thoughts of death which is, at least for the first three, in accordance with many studies on specific symptoms positively correlated with CRP [185, 201].

Among studies investigating individual symptoms in relation to inflammation, Jokela et al. conducted a large cross-sectional survey study and found that CRP correlated with tiredness/lack of energy, sleep problems and changed appetite after adjusting for other symptoms of depression [170]. This was later replicated in a network analysis using the NESDA cohort, yet merely the correlations of CRP with energy level and sleep problems survived when a variety of covariates were included in the model (e.g. BMI, physical activity, smoking, chronic disease) [171]. The same study applied several other models to assess correlations between depressive symptoms, inflammatory markers (CRP, IL-6 and TNF-$\alpha$) and covariates. Authors concluded that their and previous reports demonstrate that sleep problems, energy level, appetite/weight changes, aches and pains and irritability are symptoms more probable to be associated with inflammatory markers [171]. Results from the two mentioned [170, 171] and two large population-based studies [146, 172], all correlating individual depressive symptoms with peripheral markers of inflammation, were reviewed for compilation of the inflamed depression composite score in study II of this thesis, as presented in **Table 6.**

Taken together, we applied different methods to test the hypothesis that dimensions of depressive symptoms, predominantly found in the overlap between atypical energy-related, neurovegetative and sickness mimicking symptoms, link more tightly to inflammation and hence may be more pronounced in the inflamed depression phenotype. Results are overall encouraging, yet preliminary not least due to the still unsettled notion of how to methodologically overcome heterogeneity in MDD. However, future studies should aspire to appreciate symptom heterogeneity and not close in on total sum-scores representing general severity of depressive symptoms. Hence, symptomatology holds promise as a marker of underlying pathophysiological processes (including inflammation) and response to interventions in MDD.

# Study designs and interventions to target inflamed depression

As aforementioned, the designs of all included papers of this thesis did, by different methods, involve division of their respective cohorts based on markers of peripheral inflammation. This approach stands out in relation to most existing studies within the field since the dominating methodology has historically been comparison of whole MDD cohorts, regardless of specific biological or clinical characteristics, versus healthy controls. Mixed results and a general disbelief that all depressed are inflamed have empowered the need for more elaborate study designs. Therefore, more sophisticated methods to conduct studies within the field could help overcome the obstacles of heterogeneity within MDD samples. One simplified view on what more elaborate study designs need to address is, again, to allow for the appreciation that *one size does not fit all*.

## Methods to delineate inflamed depression

In GEN-DS, division of the patient cohort was carried out by levels of CRP (inflammation vs non-inflammation) and then by comparison of the groups by means of related biomarkers as component scores from a factor analysis and clinical characteristics. In the cross-sectional design of GEN-DS, patients in the CRP>3 mg/L group were more likely to present with features (including symptoms) and related biochemical alterations previously proposed to be characteristic for the inflamed depression phenotype. Also, Omega-3 relied on peripheral CRP to delineate the cohort by inflammation status, while the CAN-D cohort was divided by IL-6, TNF-α and CRP. However, in CAN-D, high and low inflammation groups were created based on mean scores of the three inflammatory markers respectively and only mean differences in KP metabolite ratios were compared between the groups. In Omega-3 on the other hand, the cut-off for CRP at 1 mg/L rendered more positive results than a cut-off set at CRP 3 mg/L. Specifically, stratification of CRP≥1 mg/L identified a group that responded significantly better to EPA. This highlights the need to not only study what biomarkers to use but also their optimal cut-off to delineate inflamed depression as a clinically meaningful construct. One notable limitation in study design shared by GEN-DS and Omega-3 is that the separation into inflamed or uninflamed depression groups was carried out based on a single inflammatory marker (CRP) acquired predominantly at a single time point (some subjects in Omega-3 had more than one CRP taken for stratification) whereas the latter limitation is also true for CAN-D. The use of an inflammatory biomarker from one single time point limits interpretation, since immune activation and levels of inflammatory markers may vary significantly with e.g. time of day for blood sampling, recent stress or physical activity and inflammatory responses to mild infections that might not be reported or even experienced by the patient. In the case

of CRP, it is notable that for prediction of cardiovascular risks, recommendations are to obtain hs-CRP at two time-points separated by two weeks and to discard outliers as measurements of hs-CRP>10 mg/L due to possible obscuring acute processes (infections, inflammation) [40]. Future studies need to address this, since there are no *gold standard* to meaningfully distinguish the inflamed from the uninflamed among subjects fulfilling MDD criteria.

However, the utilization of factor analysis, PCA, in GEN-DS is one of the proposed methods to overcome the limitations related to the use of a single marker for stratification, and to avoid multiple comparisons. This was considered a purposeful approach since some of the individual cytokines as well as vitamin D levels are not decisively defined as either proinflammatory, omnipotently immune regulating or even anti-inflammatory. Through application of the PCA, the complexity of the immune system was recognised, as patterns of variance in the included variables were interpreted instead of raw or log-transformed individual values. The outcome, higher composite scores corresponding to higher IL-6 and IL-8 as well as low Vitamin D among the subgroup with CRP>3 mg/L indicates an interaction between these cytokines and immune regulating vitamin D. Therefore, it would be preferable if future studies would continue to both unveil the effects of specific inflammatory markers on mood and behaviour and address the intricate interplay among the immune modulating components. Moreover, both Omega-3 and CAN-D were designed as longitudinal follow-ups after 8 weeks of intervention. As of yet, biomarkers at week 8 have not been analysed in Omega-3. Also, in CAN-D, no follow-up data on inflammatory markers is presented. Hence, the study designs are of crucial importance when the aim is to explore pathophysiological processes underlying MDD. How to best narrow down heterogenous MDD cohorts is still unknown, yet several study designs including advanced statistical methods have been proposed and implicated in the sought of homogeneity.

## Innovative study designs

It has also been suggested that future studies on endotoxin-induced inflammation could illuminate the relationship between peripheral immune activation and depressive symptoms by applying the model in healthy controls as well as inflamed and uninflamed MDD [48]. Such trials could potentially aid in the delineation of patients more susceptible to benefit from interventions targeted at the inflamed depression phenotype [48]. Another proposed methodology is utilization of enriched study designs, meaning that study recruitment is weighted to include patients with some baseline characteristics that may improve the chance of identifying a specific phenotype, with an assumed better chance of treatment success. For inflamed depression, this has been done by e.g. McIntyre et al. when assessing effects of Infliximab [158] and Nettis et al. when testing antibiotic minocycline in patients with MDD and CRP>1 mg/L [157]. Moreover, some studies

have stratified MDD subjects making use of data-driven methods. Such techniques hold many potential advantages, not least when biomarker cut-offs and numbers of subgroups are not prespecified. As mentioned, it has been proposed that binary division of MDD samples (e.g. inflamed vs non-inflamed) or cytokines (pro- vs anti-inflammatory) might be oversimplified. When Lynall et al. [186], assessed 206 depressed individuals for several peripheral inflammatory markers, four subgroups of MDD were found, two of which were interpreted as inflamed. This highlights the fact that there might be different immunological patterns underlying inflamed depression [12, 187]. Additionally, data-driven approaches may be applied when assessing inflammatory markers, e.g. by factor analysis. Such approaches may aid in pairing markers based on variations between them, which may prove important since there are currently no determined reliable markers or cut-offs for inflamed depression. For example, factor analysis has been used to delineate inflammatory indexes in studies on the NESDA-cohort [203].

*Match/mismatch study design in the Omega-3 study (paper I and III)*

One study design highlighted for its potential to promote personalized medicine in the field has been called 'match/mismatch' [27, 204]. In such a design, patients are stratified a priori based on a biomarker representative of a biological alteration hypothesized to be shared by a phenotype that could respond better to the tested intervention. By doing so, the MDD phenotype (e.g. inflamed depression) cases could be separated from others that are potentially less responsive to the specific intervention. This design was tested in Omega-3 allowing for an 8-week assessment of response to an anti-inflammatory intervention (EPA) in match (inflamed) vs. mismatch (uninflamed) MDD subjects. However, the novelty of this study design came with several methodological issues, not least concerning recruitment. For example, one could question whether the power calculation should assume equal groups while the distribution of match/mismatch subjects are expected to be unequal based on previous large investigations of MDD cohorts [36]. Further insecurity arises in respect to the cut-off for match/mismatch when there are no established definitions of, in this case, inflamed depression based on hs-CRP. In Omega-3 we report group differences inflamed (match) vs. noninflamed (mismatch) MDD based on two separate hs-CRP cut-offs: 3 and 1 mg/L. This highlights the mentioned obstacles including insecurities regarding what biomarkers and cut-offs that are of importance to generally delineate inflamed depression and specifically to predict response to EPA.

Future studies are warranted to inform on clinical and biochemical characteristics of the inflamed depression phenotype, which could be beneficiary to the methodology of studies using the match/mismatch study design. Ideally, match/mismatch separation could be refined using both biochemical *and* clinical characteristics associated with the inflamed phenotype, in resemblance with the criteria used for enrichment by McIntyre et al (see **Table 5**) [158]. The

match/mismatch design does however hold advantages, not least by delineating a reference group (mismatch, 'non-phenotype' MDD) of patients predicted to not respond as well as 'match' to the tested intervention. This differs from enriched studies, where active intervention is generally compared to a placebo group (all patients have phenotypical features, since the sample is enriched). It is thereby reasonable that match/mismatch could better inform on the differences between phenotype and non-phenotype samples of MDD than the enriched studies. It is however worth discussing whether placebo groups could be of merit for future match/mismatch designs. Either in a three- (adding a match-placebo group) or four-armed design (i.e. match-active/match-placebo/mismatch-active/mismatch-placebo groups). A third arm (match-placebo group) could for example in the case of the Omega-3 study, have further elucidated the improvements seen with EPA-treatment in MDD with mildly/moderately elevated hs-CRP.

## Questioning causality and adjustments in studies exploring inflamed depression

Within the studies included in this thesis, analyses were overall not adjusted for the mentioned possible characteristics of the inflamed depression phenotype (BMI, age, gender etc). There was one exception though, when cytokine levels were compared between MDD overall and healthy controls in GEN-DS and brain imaging in CAN-D. In the mentioned analyses of GEN-DS, comparisons were adjusted for BMI, age, gender, and smoking to facilitate comparison with most previous reports on cytokine levels in MDD compared to healthy controls. Yet, the rationale for not adjusting other analyses was founded on the recognition of e.g. adipose tissue, age, gender differences and lifestyle factors as both possible causes and maintaining factors of inflammation in MDD. It was therefore hypothesised that adjustments for such potential mediators could suppress important pieces of knowledge in the multidimensional puzzle of inflamed depression.

Accordingly, except for division of heterogenous MDD cohorts into more homogenous subgroups/phenotypes, it is necessary to address the question of causality and directionality of e.g. the association between inflammation and depression. As mentioned, several studies have found elevations of inflammatory markers to be a risk factor of subsequent depressive symptoms. However, there is a need to further investigate the trajectories of the relationship with conflicting results on whether depressive symptoms may predict subsequent elevations of inflammatory markers. This would be theoretically conceivable, not least in relation to behavioural changes (sedentary lifestyle, sleep, and appetite alterations) seen in depression that could promote an inflammatory state and therefore enable bidirectionality in the association of inflammation and depression. This may result in the mentioned vicious circle where factors underlying inflammation trigger depression and related behavioural changes that in turn can generate increased

inflammation. For example, Pitharouli et al. used data from the UK biobank to report that CRP was elevated both among individuals with a lifetime history of depression and among those diagnosed with depression subsequent to the CRP measurement, which was evident even after adjustment for a wide range of potential confounders including BMI, age, sex, smoking, and childhood trauma [143]. On the contrary, in the Whitehall II cohort, there was no evidence that depressive symptoms predicted elevated inflammatory markers at follow-up [72]. Adjustments for health behaviours have attenuated results in some studies within the field. For instance, in a population with cardiac disease, depressive symptoms at baseline were positively correlated with subsequent elevations of hs-CRP and IL-6 [78]. However, after adjustments for health behaviours predictive of inflammation (physical activity, BMI, smoking) the association was no longer significant [78]. Similar reductions of the presumed association between depressive symptoms and subsequent CRP levels were found by Copeland et al. after adjustment for e.g. BMI and smoking in a younger population-based sample [77]. Hence, beyond the concern of temporality, it is likewise important to address the issue of whether to adjust for supposedly associated factors, such as obesity, diet, and co-morbidity with inflammatory conditions, when investigating inflamed depression phenotype.

The fact that adjustment for lifestyle factors may result in a weaker relationship between depressive symptomatology and inflammation could be interpreted in several ways; either BMI and other lifestyle factors are confounding factors (suggesting that elevated inflammatory markers are not truly related to depression), or these factors are integrated (mediators) in the association between inflammation and depression. In the study by Copeland et al., baseline CRP did not predict subsequent depression, but the opposite was found true especially for the total number of previous depressive episodes predicting later elevations in CRP-levels, even after mentioned adjustments [77]. Yet, many of these studies have used a single baseline blood-draw to assess inflammatory markers. On the contrary, data from the Whitehall II cohort showed that repeated elevations of IL-6 predicted greater likelihood of common mental disorders at a 10-year follow-up compared to single measurements of inflammation [205]. These findings were evident even after adjusting for e.g. obesity and acute inflammation. Findings do however need to be interpreted with caution since 'common mental disorders' were assessed by the self-rated GHQ thus not by an expert diagnosis of MDD.

It is a practically inescapable fact that when criteria-based diagnostic systems (i.e. DSM, ICD) guide inclusion to studies, heterogenous samples of MDD are obtained. To challenge this and detangle the patient cohorts, not only the study design per se can be optimized. Also, and as mentioned, the approach to assess severity and symptomatology calls for attention. Yet, the numerous rating scales, either expert or self-rated, available to assess depressive symptoms may ease or obstruct increased understanding of specific features related to MDD phenotypes. In the case of inflamed depression or IMD, this has been discussed as a potential obstacle [142].

For example, disturbances of sleep and appetite may, according to some rating scales, be collapsed into 'sleeping difficulties' or 'change in appetite'. Hence, a depressed subject eating or sleeping too much may rate or be rated the same as someone who sleeps too little and lack appetite. Concerning research on specific links between inflammation and depressive symptoms (individually or as dimensions/clusters), Majd et al. highlighted some directions for future research including the potential gain of applying both subjective and objective measures when assessing sleep, anhedonia, fatigue and other symptoms of depression [201]. Hence, using both symptom ratings and behavioural tasks (e.g. for anhedonia; effort-expenditure for Reward Task, for sleep; polysomnography) could further deepen the understanding of the associations between inflammation and MDD symptomatology.

## Remedies - Target Interventions

It has been hypothesised that the inflamed depression phenotype might respond less favourably to conventional antidepressants [79]. Responsiveness to previous antidepressant treatment was not categorically assessed in Omega-3 and GEN-DS. However, both these cohorts included patients based on treatment-resistance to some extent. Firstly, as an inclusion criterion for Omega-3, it was stated that patients should have been taking antidepressant medication at a stable dose for at least 6 weeks, yet still suffer from depressive symptoms (HAM-D17$\geq$ 15). In the GEN-DS, referrals to the study came from second-line psychiatric clinics and patients were considered to suffer from difficult-to-treat depression. In some resemblance, CAN-D assessed chronicity as a measure of self-reported lifetime number of months depressed. However, chronicity in CAN-D only correlated significantly with age (in an anticipated positive association), and none of the investigated KP metabolite ratios.

CAN-D did on the other hand offer data on unmedicated patients and their response to initiated treatment with SSRIs during an 8-week follow-up. It was therefore very encouraging that responders to SSRI treatment showed a significant increase in the neuroprotective KP ratio Kyn-A/3-HK as compared to non-responders. In accordance, change in HAM-D17 correlated significantly and positively with change in several neuroprotective ratios (Kyn-A over 3-HK, Kyn and AA). None of the inflammatory markers decreased significantly in responders compared to non-responders. However, the findings of increased neuroprotective ratios in SSRI responders were consistent with some of the previous literature. For example, preclinical evidence suggest that SSRIs may increase neuroprotective ratio Kyn-A/3-HK in astroglia cells [206]. Altered KP metabolism in response to antidepressant treatment has also been shown in clinical studies. For example, KP activity as assessed by Kyn/Melatonin and 3-HK/Melatonin has been shown to decrease significantly with sertraline treatment in MDD, yet specifically in

responders [207]. Moreover, Myint et al. found that the neuroprotective ratio Kyn-A/Kyn increased significantly during 6 weeks of treatment with antidepressants in patients suffering from a first-time episode of MDD as compared to patients with recurrent MDD [208]. In accordance, Halaris et al. found several significant correlations between baseline levels of pro-inflammatory cytokines and putatively neurotoxic metabolites of the KP (e.g. IL-1 correlated with 3-HK) et al. in a 12-week study of escitalopram treatment in MDD [209]. Overall, the results implied a reduction of neurotoxicity, inflammation, and depression severity with escitalopram treatment. This was manifested as significantly increased neuroprotective ratio Kyn-A/QA along with decreased neurotoxic metabolites (QA, 3-HK) while several pro-inflammatory markers decreased at a trend level to week 8, yet changes were overall attenuated at the study end [209]. Similarly, Sun et al. reported that the suggested neuroprotective Kyn-A/3-HK ratio increased significantly among MDD patients who reached remission with escitalopram after 8 weeks of treatment [115]. Authors did not assess inflammatory markers, yet hypothesised that their found association between a better response to escitalopram and lower baseline ratios of Kyn/TRP and QA/TRP could correspond to lower inflammation-induced activation of the KP [115]. Taken together, our finding, in light of previous results, imply an immune-mediated shift of the KP towards neurotoxicity in MDD that could be restored, in favour of neuroprotective metabolites, with successful antidepressant treatment [115]. This highlights that inflamed depression could in fact be better understood as an umbrella concept, since less favourable response to SSRIs has been proposed a phenotypical characteristic of inflamed depression whereas the KP as one mechanistic pathway seem to be modifiable with SSRIs. Yet, mentioned studies have in general assessed response as decrease in sum-scores of depressive symptoms whereas it is still possible that dysregulation and restoration of KP metabolism may be more accurately reflected in a change in specific symptom dimensions. If however, inflamed depression is an umbrella concept, this allows for prediction of treatment response based on mechanistic pathways where some may respond to SSRIs while some need other targeted interventions, either as monotherapy or add-on to conventional antidepressants.

## Omega-3

As mentioned, in the Omega-3 study, patients were initially divided by hs-CRP above or below 3 mg/L which resulted in no significant differences between the groups concerning response to add-on treatment with 2,2 g EPA per day for 8 weeks. Instead, hs-CRP $\geq$ 1 mg/L did identify a subgroup (54.5%) of MDD that responded significantly better to EPA supplementation, based on both primary (HAM-D17 reduction) and secondary (change in ratings of depressive symptoms previously associated with inflammation including fatigue and sleep problems) outcome measures.

Apparently, only a limited number of previous studies have taken inflammatory status into account when exploring the antidepressant effect of n-3 PUFAs, and the results have in general been vague. For example, two Cochrane reviews and meta-analyses have been published on n-3 PUFAs in the treatment of depression, with none of them considering the potentially superior effect in a subgroup of inflamed depression [210, 211]. The most recent Cochrane publication on n-3 PUFAs and MDD included 28 RCTs constituting 1944 participants [211]. With low overall quality of evidence, the effect sizes for n-3 PUFAs in MDD were small-to-modest. Other meta-analyses have considered potential factors underlying more beneficial antidepressant effects of n-3 PUFAs. For example, both Mocking et al. and Liao et al. showed that higher EPA doses and augmentation strategy, rather than monotherapy, may be predictive of a superior antidepressant response of n-3 PUFA supplementation in depression [212, 213]. Moreover, Rapaport et al., included 155 patients with MDD and HAM-D17 score ≥15 in an eight-week study randomizing patients to monotherapy with either EPA- or DHA-enriched n-3 PUFAs or placebo [160]. Overall treatment effects were not significant between the three groups. However, higher baseline inflammatory markers predicted a separation between intervention and placebo. Specifically, EPA supplementation was significantly more efficacious than placebo in subjects with any baseline inflammatory marker indicative of high inflammation (elevated CRP, IL-6, IL-1 receptor antagonist (IL-1ra), leptin or decreased adiponectin) and the effects of EPA compared to placebo were even more pronounced in patients with ≥2 high inflammatory markers. These results indicate that n-3 PUFAs, and especially EPA, could be more efficacious in inflamed depression. Still, the optimal methods to delineate subjects more likely to benefit from EPA are still unknown and more multidimensional approaches may be needed, than using merely one biomarker of inflammation as in the Omega-3 study. Future studies could benefit from involving networks of the mentioned phenotypical characteristics of the inflamed phenotype, not least immunometabolic dysregulations and information on dietary habits based on the relationship between dietary omega-6/omega-3 ratio, obesity, and inflammation.

The Omega-3 study served another important purpose in relation to the aims of this thesis, namely, to test if match/mismatch is a feasible study design to investigate response to interventions presumably more efficacious in biologically derived subtypes of MDD. The outcome was overall promising, yet with some precautions. Importantly, a match/mismatch study design demands careful consideration in respect to the chosen biomarker/-s and the cut-off for stratification. Selection of the hs-CRP cut-offs in Omega-3 was guided by recommendations from the American Heart Association (AHA) on cardiovascular risks [40]. Translation of the AHA guidelines directly to delineation of a phenotype of MDD is indeed somewhat arbitrary. Secondary analyses using the omega-3 sample may be able to clarify what features (clinical and biochemical) that could have been used for stratification of those most responsive to EPA. Then, future studies are needed to replicate the

findings in larger samples. Hence, the match/mismatch design was feasible, even though the still limited understanding of the inflamed depression concept and the integrated unsettled business of optimal delineation of subjects blurs the picture.

# Conclusions

This thesis highlights the heterogeneity of MDD according to the DSM. Using three different cohorts, peripheral markers of inflammation and the mechanistic pathway of KP activation and neurotoxicity were shown to correlate with metabolic dysregulation, symptomatology and response to both EPA and SSRIs. Results support the hypothesis that inflammation plays a role in a depression phenotype rather than among all patients with MDD. In sum, further research is needed to replicate the findings of our and related studies. However, study methodology of future studies needs to be calibrated to allow for identification of phenotypes of MDD. We believe that the match/mismatch study design utilized in the Omega-3 study may be advantageous for future studies seeking to define phenotypes of MDD and test target interventions.

# Clinical implications

The combined experiences and outcomes of this thesis supports the notion that the inflamed depression phenotype could be a clinically meaningful construct. Yet, much needs to be clarified before patients presenting with depressive symptoms can be offered targeted treatments based on biomarkers of underlying pathophysiological processes. What this thesis does, however, is to highlight that some characteristics (e.g. a specific symptom profile and metabolic dysregulation) tend to recur in patients with increased inflammatory biomarkers. Moreover, these patients may be more likely to improve with omega-3 supplementation. One could speculate that these findings, if replicated in independent cohorts, could endorse a point-of-view where lifestyle advice and anti-inflammatory interventions may be more effective than conventional antidepressants in patients with e.g. higher BMI, unhealthy diet (high omega-6/omega-3 ratio), low physical activity and inflammatory co-morbidities. If such reasonings are reliably proven they may be valuable both to individuals suffering from MDD but also relevant from a public health perspective as lifestyle advices in the prevention of MDD.

# Future aspects

Investigations of the inflamed depression phenotype are presented from different points of view in this thesis. Due to inconsistencies in existing literature and diverging lines of research (IMD, several immunophenotypes) one could hypothesise that inflamed depression is in fact an umbrella concept. This is further supported by the diversity of mechanistic pathways that have been shown to link inflammation with depressive symptoms. Together with study designs that do not take the heterogeneity of MDD into account, such reasoning could in part explain the mixed results of trials testing anti-inflammatory compounds as treatment of depression. Appreciation of inflamed depression as an umbrella concept calls for refinement of future study designs, where the intrinsic complexity of the immune system and range of mechanistic pathways are considered. This further opens for transdiagnostic approaches, in line with e.g. the RDoC initiative. A range of symptoms overlapping between sickness behaviour and MDD could also be evident in several other psychiatric disorders (e.g schizophrenia, bipolar disorder). Whether there are inflamed phenotypes among those other DSM-based diagnostic categories is still to be revealed. It is also uncertain whether such plausible phenotypes, e.g. an inflamed phenotype of bipolar disorder, present with different characteristics including response to targeted treatments than an inflamed phenotype in MDD or if features are shared. In sum, the field of biological and precision psychiatry is growing, and the steady accumulation of more refined studies does in fact make the future look bright.

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