Vitamin D in Depression and Suicidality

Grudet, Cécile

2022

Document Version:
Publisher's PDF, also known as Version of record

Vitamin D in Depression and Suicidality

CÉCILE GRUDET
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY

Vitamin D in Depression and Suicidality

Vitamin D in Depression and Suicidality

Cécile Grudet

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at conference room 12, Baravägen 1, Lund. Date 23rd of September 2022 at 1 pm.

Faculty opponent
Docent Ursula Werneke, Umeå University

Organization	Document name
LUND UNIVERSITY Faculty of Medicine, Department of Clinical Science Lund	DOCTORAL DISSERTATION **Date of issue:** 2022-09-23
Author(s) Cécile Grudet	Sponsoring organization

Title: Vitamin D in Depression and Suicidality

Abstract

Background and aims

The aim of this thesis is to explore the relationship between vitamin D and different aspects of depressive disorders and suicidality, as well as the relationship between vitamin D and inflammation in these conditions.

Material and methods

Study I: Patients (n=59), with diverse psychiatric diagnoses, were enrolled in the study after a suicide attempt. Seventeen non-suicidal patients with MDD and 14 healthy controls were also included. Interleukin (IL)-1β, IL-6, Tumor Necrosis Factor alpha (TNF)-α, vitamin D_2 ($25(OH)D_2$), and vitamin D_3 ($25(OH)D_3$) were analyzed.

Study II: Un-medicated, somatically healthy MDD subjects (n=48) and healthy controls (n=54) were enrolled in the study. IL-6, TNF-α, Neutrophil-to-Lymphocyte ratio (NLR), White Blood Cell count (WBC), vitamin D_2, and D_3 were analyzed.

Study III-IV: Patients with difficult-to-treat depression (Study III: n=202, Study IV: n=263), and healthy controls (Study III: n=46, Study IV: n=51) were recruited. Levels of vitamin D_2 and D_3 (Studies III and IV), and the inflammatory markers IL-6, TNF-α, CRP, IFN-gamma, IL-10, IL-8, IL-13, and IL-2 were analyzed (Study IV).

Results

Suicidal patients had significantly lower vitamin D levels than both non-suicidal depressed patients and healthy controls (Study I). There was a significant negative association between vitamin D and inflammatory markers in depressed patients, but not in controls (Studies I and II). Patients with 'difficult-to-treat' depression had significantly lower vitamin D levels than healthy controls, but vitamin D was not associated with any specific diagnosis or suicidality (Study III). Patients with difficult-to-treat depression had significantly higher IL-6 and IL-8 levels compared to healthy controls (Study IV), and an inflammatory depression subgroup was associated with more severe symptoms of sleeping problems, appetite disturbance, tiredness, and anhedonia as well as low vitamin D in combination with high IL6 and IL-8 levels.

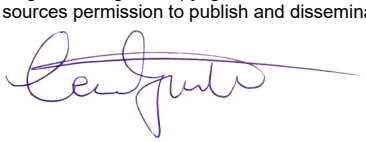
Conclusion

Our findings are consistent with previous studies of lower vitamin D levels in depression, which might play a role in the pathophysiology of the disorder. Low vitamin D and chronic low-grade inflammation may be part of the same subtype of depression, with a distinct symptom profile related to inflammation, but more research is needed before this can be established.

Key words Vitamin D, major depressive disorder, suicidality, inflammation

Classification system and/or index terms (if any)

Supplementary bibliographical information		**Language** English
ISSN 1652-8220		**ISBN** 978-91-8021-285-4
Recipient's notes	**Number of pages** 93	Price
	Security classification	

Signature  Date 2022-08-19

Vitamin D in Depression and Suicidality

Cécile Grudet

LUND
UNIVERSITY

Cover photo by Photographer @ jroberpg

Faculty of Medicine
Department of Psychiatry

ISBN: 978-91-8021-285-4
ISSN: 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2022

Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se

MADE IN SWEDEN

I dedicate this thesis to all people who face the challenges of long-lasting depressive symptoms in their lives.
You are strong and brave and deserve the best of support anyone can get!

Table of Contents

Abstract 11
List of papers 12
Preface 13
Populärvetenskaplig sammanfattning 15
Bakgrund 15
Material och metoder 16
Resultat 17
Slutsatser 17
Abbreviations 19
Definitions 21
Vitamin D 21
Psychiatry 22
Context of this thesis 23
Introduction 25
Major depressive disorder and suicidality 25
Major depressive disorder 26
Suicidality 26
An introduction to vitamin D 28
History 28
Metabolism 30
Mechanisms of action 32
Vitamin D deficiency 33
Extra-skeletal effects of vitamin D 37
Vitamin D in MDD and suicidality 41
Vitamin D in depression 41
Vitamin D in suicidality 42
Inflammation in depression and suicidality 43
Aim of the thesis 44

Materials and methods **45**
Summary of the study cohorts 45
Ethical considerations 45
Clinical setting and sample 47
Data collection, diagnostic assessment, and symptom severity ratings 49
Diagnostic interviews 51
Symptom rating scales 51
Biological sampling procedures and assays 53
Analytic procedure specifications 54
Statistical analyses **57**
Results **59**
Study I 59
Study II 61
Study III 62
Study IV 64
Discussion **67**
General limitations **73**
For the future **75**
Clinical relevance **77**
Acknowledgements **79**
References **83**

Abstract

Background and aims

The aim of this thesis is to explore the relationship between vitamin D and different aspects of depressive disorders and suicidality, as well as the relationship between vitamin D and inflammation in these conditions.

Material and methods

Study I: Patients (n=59), with diverse psychiatric diagnoses, were enrolled in the study after a suicide attempt. Seventeen non-suicidal patients with MDD and 14 healthy controls were also included. Interleukin (IL)-1β, IL-6, Tumor Necrosis Factor alpha (TNF)-α, vitamin D_2 ($25(OH)D_2$), and vitamin D_3 ($25(OH)D_3$) were analyzed.

Study II: Un-medicated, somatically healthy MDD subjects (n=48) and healthy controls (n=54) were enrolled in the study. IL-6, TNF-α, Neutrophil-to-Lymphocyte ratio (NLR), White Blood Cell count (WBC), vitamin D_2, and D_3 were analyzed.

Study III-IV: Patients with difficult-to-treat depression (n=202 Study III, n=263 Study IV), and 46 (Study III) and 51 (Study IV) controls were recruited. Levels of vitamin D_2 and D_3 (Studies III and IV), and the inflammatory markers IL-6, TNF-α, CRP, IFN-gamma, IL-10, IL-8, IL-13, and IL-2 were analyzed (Study IV).

Results

Suicidal patients had significantly lower vitamin D levels than both non-suicidal depressed patients and healthy controls (Study I). There was a significant negative association between vitamin D and inflammatory markers in depressed patients, but not in controls (Studies I and II). Patients with 'difficult-to-treat' depression had significantly lower vitamin D levels than healthy controls, but vitamin D was not associated with any specific diagnosis or suicidality (Study III). Patients with difficult-to-treat depression had significantly higher IL-6 and IL-8 levels compared to healthy controls (Study IV). An inflammatory depression subgroup was associated with more severe symptoms of sleeping problems, appetite disturbance, tiredness, and anhedonia as well as low vitamin D in combination with high IL6 and IL-8 levels.

Conclusion

Our findings are consistent with previous studies of lower vitamin D levels in depression, which might play a role in the pathophysiology of the disorder. Low vitamin D and chronic low-grade inflammation may be part of the same subtype of depression with a distinct symptom profile related to inflammation, but more research is needed before this can be established.

List of papers

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

Study I

Grudet, C., Malm, J., Westrin, A., & Brundin, L. (2014). Suicidal patients are deficient in vitamin D, associated with a pro-inflammatory status in the blood. *Psychoneuroendocrinology, 50*, 210-219. doi:10.1016/j.psyneuen.2014.08.016

Study II

Grudet, C., Wolkowitz, O. M., Mellon, S. H., Malm, J., Reus, V. I., Brundin, L., Nier, B. M., Dhabhar, F. S., Hough, C. M., Westrin Å., Lindqvist, D. (2020). Vitamin D and inflammation in major depressive disorder. *J Affect Disord, 267*, 33-41. doi:10.1016/j.jad.2020.01.168

Study III

Grudet, C., Lindqvist, D., Malm, J., Westrin, Å., & Ventorp, F. (2022). 25(OH)D levels are decreased in patients with difficult-to-treat depression. *Comprehensive Psychoneuroendocrinology, 10*, 100126. doi:10.1016/j.cpnec.2022.100126

Study IV – Manuscript (to be submitted shortly)

Suneson, K., Grudet, C., Ventorp, F., Malm, J., Asp, M., Westrin, Å., Lindqvist, D. An inflamed subtype of difficult-to-treat depression.

Preface

I became interested in vitamin D a long time ago due to my own difficulties enduring the long, dark winters in Sweden. Thus, since I suffer from seasonal affective disorder (SAD), I am naturally curious about everything that could be associated with a depressed mood during the winter, or with a depressed mood in general.

Therefore, in 2012, I attended a seminar about affective disorders where (my upcoming co-supervisor) associate Professor Lena Brundin discussed the possible connection between inflammation and depression, and what she said made much sense to me. Shortly after that, Professor Johan Malm (also one of my upcoming co-supervisors) held a class at medical school on the extra-skeletal effects of vitamin D in the body, highlighting vitamin D's connection to the immune system. Like most people, I had heard that vitamin D could be related to depression in some way, and instantly, during Johan Malms' class, it became obvious to me that *it could be the effect of vitamin D on the immune system that linked vitamin D to depression!*

Curious as I am, I was eager to explore my hypothesis, so I turned to professor Åsa Westrin (my upcoming, first, and main supervisor), whom I knew was involved in suicide research in Lund, and asked her if I could do "summer research" on vitamin D under her supervision. She generously took me under her wings and accepted me as a summer researcher and ultimately as a PhD student in 2015.

The road leading up to this thesis has been bumpy, but nevertheless focused on the possible connection between vitamin D and depression. At the beginning of my PhD studies, my somewhat naïve enthusiasm for doing research, combined with an attitude of unlimited possibilities, led me to wanting to explore every single idea I had regarding the relationship between vitamin D and psychiatric illness. Certainly, quite a few studies were discarded along the way, some of which only got to the point of a preliminary title, such as "Is there an association between vitamin D levels and 5-HIAA cerebrospinal fluid levels in psychiatric patients with the genetic makeup SS or SL alleles of the promoter region (5-HTTLPR) of the serotonin uptake transporter (5-HTT)?", or "Is mental fatigue in depressed patients associated with vitamin D levels?". Another discarded study, which I put significant work and effort into, was titled 'Is vitamin D levels in umbilical cord associated with suicide later in life?'. After giving me extensive freedom in my work, Åsa finally stepped in and guided me in my thesis so it became narrowed to focus solely on the relationship between vitamin D and inflammation in depression and suicidality. For this, I am very grateful.

Populärvetenskaplig sammanfattning

Bakgrund

Depression är en sjukdom som är så vanlig i Sverige idag att den klassas som en folksjukdom. Folkhälsomyndigheten har uppskattat att mer än var tredje kvinna och nästan var fjärde man kommer att uppleva en eller flera depression(er) under sin livstid. Depression orsakar stort lidande för den enskilde individen och påverkar också ofta även närstående. Att ha en psykisk sjukdom innebär även en stor riskfaktor för självmord och av alla psykiska sjukdomar sticker depression ut som den med störst risk för självmord. Trots att depression är en så pass vanlig sjukdom är dess bakomliggande orsaker inte helt kända. Man tror att orsaken består av en kombination av genetiska, biologiska, miljömässiga och psykologiska faktorer, som alla bidrar till en persons individuella sårbarhet för att utveckla depression. Sjukdomens komplexitet gör den svår att studera och det är inte troligt att en enskild förklaringsmodell kan förklara alla olika typer av depressionssjukdomar. Det är sannolikt så att det finns olika subgrupper inom den breda gruppen ”deprimerade”, med olika orsaker bakom utlösandet av depressionen, och därför är det inte heller troligt att samma typ av behandling kommer att fungera på alla. Idag är den vanligaste behandlingsformen för depression antidepressiva läkemedel. Det är dock en betydande andel deprimerade som inte svarar tillräckligt bra på antidepressiv behandling. Så många som 70 % av de som påbörjar en antidepressiv behandling blir inte helt friska med hjälp av denna, och i många fall kan depressionen övergå i en kronisk form. Behovet av ytterligare behandlingsmöjligheter är därför stort.

Under de senaste decennierna har den forskningsinriktning som försöker förstå de biologiska orsakerna bakom depression utvecklats med nya och förfinade metoder. Det långsiktiga målet med denna forskning är att bidra till utvecklandet av s. k. individanpassad vård, med en bättre förståelse för den enskilda individens underliggande sårbarhet och bidragande orsaker till depressionssjukdom. Med en individanpassad vård är förhoppningen att psykiatrin kommer att kunna erbjuda bättre och mer effektiva behandlingsformer i framtiden.

Inom den biologiska forskningen finns en forskningsgren som undersöker om inflammation kan ha ett samband med depressiv sjukdom. Många studier har visat att deprimerade och/eller suicidala individer, på gruppnivå, har högre inflammationsgrad i kroppen än psykiskt friska individer. En hypotes är att detta inte gäller alla typer av depressiv sjukdom, utan att det finns en subgrupp av

”inflammatorisk depression” med symptom såsom oförmåga att känna, trötthet, utmattning, förändrad aptit och minskat eller ökat sömnbehov. Det är allmänt känt att D-vitamin är viktigt för skeletthälsan, men vad många inte vet är att D-vitamin också har stor påverkan på flera andra system och funktioner i kroppen. En av de viktigaste effekterna D-vitamin har i kroppen, utöver skeletthälsan, är att den påverkar immunförsvaret på ett sätt som man förenklat kan säga är ’anti-inflammatoriskt’. Detta innebär att om du har låga nivåer av D-vitamin så är det möjligt att du har en högre inflammationsgrad i kroppen, vilket också är något som har visats i olika somatiska sjukdomar, som till exempel vissa cancerformer, autoimmuna sjukdomar, diabetes och hjärt- och kärlsjukdomar.

Med tanke på forskningsfältet som talar för att inflammation kan vara en bidragande orsak till depression och att låga D-vitaminnivåer har kopplats ihop med depression i många tidigare studier, vill vi undersöka hur inflammation är kopplat till D-vitamin hos deprimerade individer. Det övergripande syftet med denna avhandling var att undersöka kopplingen mellan D-vitamin och olika aspekter av depressiv sjukdom och suicidalitet, samt kopplingen mellan D-vitamin och inflammation vid dessa tillstånd.

Material och metoder

Alla studierna i avhandlingen var tvärsnittsstudier, dvs de bygger på att studera ett material vid ett enda tillfälle och vi kan därmed inte säga något om orsakssamband. Sammantaget ingick fyra olika kliniska patientgrupper i avhandlingen; en grupp patienter med olika psykiatriska diagnoser som nyligen gjort ett självmordsförsök (Studie I), två olika grupper med medelsvår-till-svår klinisk depression som inte nyligen hade gjort något självmordsförsök (Studie I; Studie II) och en grupp med svårbehandlad depression (Studie III; Studie IV). I alla studierna ingick också en kontrollgrupp med friska individer. Patientgrupperna i Studie I, III och IV kom främst från Lund med närområde och i Studie II kom deltagarna från San Fransisco, USA. Alla patienter var diagnosticerade enligt The Structured Clinical Interview for Axis-I Disorders (DSM-IV) och noggrant utredda vad gäller klinisk symptombild med bland annat olika självskattningsskalor.

Vitamin D och olika inflammatoriska markörer analyserades och jämfördes mellan studiegrupperna i de respektive studierna.

I studie IV delades patientgruppen in i två grupper baserad på närvaron av låggradig inflammation eller ej.

Resultat

I alla studier fanns en negativ koppling mellan D-vitamin och inflammation hos deprimerade patienter, dvs, låga D-vitaminnivåer var kopplade till högre inflammationsgrad, vilket inte sågs hos friska kontroller. Patienter med svårbehandlad depression hade lägre D-vitaminnivåer än friska kontroller, men inga skillnader sågs mellan patienter med olika typer av depressionsdiagnoser. Ingen av studierna visade någon koppling mellan D-vitamin och suicidalitet, förutom Studie I, där vi fann en skillnad i D-vitaminnivå mellan patienter som nyligen hade gjort ett suicidförsök och icke-suicidala deprimerade patienter och friska kontroller. I studie IV fann vi att ”inflammatorisk depression” var kopplat till såväl lågt vitamin D som vissa specifika symptom, såsom svårighet att känna känslor, trötthet, högre utmattning, förändrad aptit och sömnstörning.

Slutsatser

Resultaten i avhandlingen visar att det finns en koppling mellan låga nivåer av D-vitamin och depressiva syndrom. Mycket pekar också på att det finns en koppling mellan låga D-vitaminnivåer och ökad inflammation hos patienter med depressiva syndrom. Våra resultat kan tala för att lågt vitamin D och inflammation båda är del av en undergrupp av depression med vissa typer av symptom, men denna hypotes måste undersökas ytterligare innan man kan dra fasta slutsatser.

Abbreviations

MDD	Major depressive disorder
SI	Mild-to-moderate suicidal ideation/suicidal ideation
NSI	Non-(mild-to-moderate) suicidal ideation/non-suicidal ideation
hgSI	High-grade suicidal ideation
lgSI	Low-grade suicidal ideation
MINI	Mini International Neuropsychiatric Interview
SCID-I	Structured clinical interview for DSM-IV. Axis I Disorders
SCID-II	Structured clinical interview for DSM-IV. Axis II personality disorders
CPRS	Comprehensive Psychopathological Rating Scale
HDRS	Hamilton Depression Rating Scale
MADRS	Montgomery-Åsberg Depression Rating Scale
SUAS-S	Suicide Assessment Scale (self-reported version)
YPAS	Vigorous Activity Index Score
TNF-α	Tumor Necrosis Factor-alpha
IL	Interleukin
IFN-γ	Interferon-gamma
CRP	C-reactive protein
NLR	Neutrophil-to-Lymphocyte Ratio
WBC	White blood cell count
Th-1	T helper type 1 cell
Th-2	T helper type 2 cell
VDR	Vitamin D receptor
UVB	Ultraviolet B radiation
RCT	Randomized controlled trial
PCA	Principal component analysis
AHDH	Attention deficit hyperactivity disorder

Definitions

Vitamin D

Vitamin D

The sum of the two circulating biologically inert metabolites of vitamin D, i.e., $25(OH)D_2$ and $25(OH)D_3$, will be referred to as 'vitamin D' in the thesis. These are the metabolites we measure in the blood to assess vitamin D status.

Active vitamin D

The sum of the two active metabolites of vitamin D, i.e., $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$. These metabolites bind to the intracellular vitamin D receptor, predominantly in the nucleus of the target cells.

Deactivated vitamin D'/'inactive vitamin D

Vitamin D is catabolized in the kidneys to both $24,25(OH)_2D$ and $1,24,25(OH)_3D$. These two metabolites are biologically inert and excreted into the feces.

Vitamin D concentration in the blood

The mostly used units for measurement of vitamin D concentration in the blood are ng/mL and nmol/L, where 1 ng/mL equals approximately 2.5 nmol/L (2.496). The International System of Units (SI) for vitamin D is nmol/L, therefore, this unit is used in the thesis.

Psychiatry

Suicidality, suicidal ideation
No "gold standard" definition exists. It refers to a wide range of behaviors with suicidal intent, such as death by suicide and attempts at suicide, as well as suicide-related thinking, such as, "I have no reason to live."

Suicide attempt
In Study I, we used Beck et al.'s (1972) definition of suicide attempt: "Those situations in which a person has performed an actually or seemingly life-threatening behavior with the intent of jeopardizing his/her life or to give the appearance of such intent, but which has not resulted in death' (1).

Violent suicide attempt
Suicide attempts are classified as violent or non-violent based on the following criteria, as previously defined by Åsberg et al. (1976) and Paykel and Rassaby (1978): "Non-violent suicide attempts include drug overdoses and single wrists cuts or a combination of these. All other attempts were classified as violent" (e.g., hanging, drowning, suffocating, several deep cuts, poisoning, intentionally throwing themselves into traffic, etc.) (2, 3).

Repeaters
Individuals who made one or more suicide attempts before the index suicide attempt (i.e., the suicide attempt preceding inclusion in the study).

Difficult-to-treat depression
The patients included in Studies III and IV had previously been diagnosed with an affective disorder with insufficient treatment response, according to the outpatient psychiatrist, who was herein defined as having a "difficult-to-treat depression." An insufficient treatment response" was defined as not having achieved remission with previous and ongoing treatments during the current depressive episode.

Suicidal depression
A depressive episode in which the patient has attempted suicide. Suicidal depression is not a clinical diagnosis.

Inflamed depression
An inflamed depression is defined in Study IV as a depression subtype in which the patient expresses a low-grade inflammation in combination with specific depressive symptoms associated with an inflamed state in the body. There is currently no clinical consensus on how to define "inflamed depression," although a cut-off level of 3 mg/L on C-reactive protein (CRP) has been used in previous studies (4-8) and is a well-established indicator of chronic low-grade inflammation (9). The depressive symptoms associated with inflamed depression are, as interpreted by us, the inability to feel, lassitude, fatiguability, changes in appetite, and reduced or increased sleep.

Context of this thesis

Depression is known to have a complex etiology, including genetic, biological, environmental, and psychological factors. Thus, it is not possible that one hypothesis can explain all types of depression, or that one-size-fits-all treatments are a successful approach to treating depression. Most likely, there are subgroups among individuals with depressive symptoms where different combinations of susceptibility factors add up to outline an individual's proneness to developing a depressive disorder.

During the last decades, the field of biological psychiatry that investigates genetic, and other biological factors in relation to depression and suicidality, have expanded and developed new and more refined methods. The long-term goal of this research field is to better understand individual factors connected to the development, severity, and prognosis of psychiatric illnesses—ultimately leading to personalized treatment for depressive disorders. One branch of this research field investigates the role of inflammation in relation to different aspects of depression and suicidal behavior. Since vitamin D has a profound impact on the immune system (10-12), which can be briefly described as "anti-inflammatory," and low vitamin D levels have been suggested to be related to depression, it is highly relevant to investigate the relationship between vitamin D and inflammation in depressed and suicidal individuals.

By using clinical samples in this thesis, I investigated the relationship between vitamin D and inflammation in depressed individuals with different levels of suicidality. The main aim was to better understand the role of vitamin D in the development of depressive disorders and whether this role is more pronounced in certain subgroups of depression.

At the very beginning of my thesis work, there were no prior studies, to my knowledge, exploring the relationship between vitamin D and inflammation in depression/suicidality. Nor were there any studies investigating vitamin D in different subgroups of depressive disorders. Even now, at the end of my thesis, this research field is still limited. I hope that my contribution to this field of research will inspire future research to include the biological downstream effects of vitamin D, with the aim of expanding and ameliorating treatment options for depression and/or suicidality.

Introduction

Major depressive disorder and suicidality

Major depressive disorder (MDD) causes extensive morbidity and mortality worldwide and is a great risk factor for death by suicide. It has been estimated that 40–60% of those who die by suicide suffer from depression (13, 14). Nevertheless, the pathophysiology and somatic manifestations are not yet fully understood, and the complexity of the disease makes it difficult to study.

Today, according to the World Health Organization (WHO), approximately 400 million people are suffering from depression worldwide, and about 700, 000 individuals die by suicide each year (15). In Sweden, it is estimated that more than every third woman, and almost every fourth man, will develop one or more depressive episode(s) during their lifetime (16). According to the National Centre for Suicide Research and Prevention of Mental III-Health (NASP), around 9,000 suicide attempts are made in Sweden each year, and around 1,500 people die by suicide (17). Consequently, depression is classified as a public disease (18), and it causes enormous negative effects on a societal and economic level. Most importantly, though, the life of a depressed person is most often compromised on many levels, with profound suffering and many negative consequences emerging in relation to the illness. In its most severe form, the risk of a suicidal act is also present. Hence, there is an indispensable need for efficacious treatment options for depression. Sadly, only about 30% of depressed individuals reach full remission after the first line of treatment, and in many cases, depression may develop into a chronic illness (19, 20).

During the last decades, great efforts have been made to better understand the biological underpinnings of depression, with the long-term aim of developing personalized and more effective depression treatments (20-23). Several lines of evidence suggest a link between chronic low-grade inflammation and a subtype of depression (in this thesis, called “inflamed depression”) (24-28). Candidate mechanisms that have been explored in recent years include, but are not limited to, autoimmune disorders, infections, and genetic predisposition (29). Also, since vitamin D has a role in regulating the immune system, a deficiency may be an upstream cause of inflamed depression, but this has not been investigated previously.

Major depressive disorder

Major depressive disorder (MDD) is a clinically diagnosed mental disorder defined by a set of specific criteria. An MDD diagnosis, according to the Diagnostic and Statistical Manual of mental disorders (DSM) criteria (the classification system used in all studies included in the thesis), is defined by the presence of five or more specific core symptoms lasting for two continuous weeks (most of the day and nearly every day), which also causes significant distress or impairment to the individual (30). These depressive symptoms are depressed mood and/or markedly diminished interest or pleasure in almost all activities, significant weight loss/weight gain or decreased/increased appetite, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue/loss of energy, feelings of worthlessness or excessive inappropriate guilt, diminished ability to think/concentrate or indecisiveness, recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation or suicide attempt, or a specific plan for attempting suicide. Other depressive disorder diagnoses in the DSM are bipolar depression (with episodes of mania), dysthymic disorder (chronic low-grade depression), and depressive disorder not otherwise specified (aspects of depression but do not meet all criteria of an MDD).

Often, an MDD is an illness in which individuals experience more than one episode during a lifetime perspective (20). Findings from several large-scale studies have shown that most patients have recovered, or partially recovered within one year from the depressive episode (31). However, about 80% of patients diagnosed with a single MDD episode will later experience recurrent MDD episodes (32), and in 20–30% of cases, MDD will take a chronic illness course (33). The risk of developing depression is defined by a combination of unfavorable genetic and environmental factors, where genetic factors represent approximately 30–40% of the risk. It is also known that being female, having a family history of mental illness, having adverse childhood experiences (abuse, neglect, violence, etc.), and experiencing more recent stress factors are all risk factors for developing depression (23).

Suicidality

Suicidality refers to a wide range of behaviors with suicidal intent, such as death wishes, suicide thoughts, suicide plans, suicide attempts, and death by suicide. Suicide-related thinking, for example, “I have no reason to live,” is also included in the concept of suicidality. However, the construct “suicidality” does not have any exact definition and is therefore difficult to study. In accordance with depressive disorder, suicidality includes complex interactions between predisposing factors and environmental factors. Studies on suicide prevention have shown that predicting a suicide attempt/suicide is difficult (14). Nevertheless, several risk factors behind

suicide attempts or death by suicide have been investigated and identified, both at population-based levels and at individual levels (13). Many risk factors overlap with risk factors for developing depression, which is understandable because having depression (or other mental illnesses, schizophrenia-spectrum disorders, or substance use disorders) augments the risk of death by suicide. Thus, having a psychiatric diagnosis is a major risk factor for death by suicide (14, 34). However, the main risk factor for death by suicide is one or more previous suicide attempt(s) (34, 35). Certain personality traits have also been associated with elevated suicide risk, where impulsivity and aggression stand out in most models of suicide (14, 36). Contributing environmental risk factors include a lack of social support, economic difficulties, stressful life events, and access to lethal means (13, 34).

As noted before, it is extremely hard to predict a future attempt at suicide (34). To help clinicians, different structured suicide assessment instruments can be used to better understand the patient's present situation and state of mind (35, 36). One of these is the Suicide Assessment Scale (SUAS) (37), which has been used in some of the studies included in this thesis. However, the SUAS is, in several sites in Sweden, gradually being replaced by the Columbia-Suicide Severity Rating Scale (C-SSRS), suggested to be one of the best tools of its kind (38). It is a short questionnaire designed to work in most settings, research included, by any individuals who have received training in administrating the scale. In research settings, structured suicide assessment instruments are useful when, for example, the degree of suicidality or suicide risk is measured and associated with other studied factors. However, the division of patients into "high suicidal ideation" or "low suicide ideation" groups will never be perfect, and always be based on the researchers' choice of assessment instruments and cut-off levels.

An introduction to vitamin D

History

The history of vitamin D goes far back in time. The first substantial description of the disease "rickets" is dated as early as 1645 in a book written by Wistler, a medical doctor from England (39). Similar disorders had been described previously for centuries, but Wistler provided the very first, printed, clear description of a disease that caused poor mineralization and deformations of the skeleton, naming it "the rickets"—a disease we now know is caused by vitamin D deficiency. The disease became an epidemic during the 19th century, especially in England and Northern Europe, due to urbanization and deprived sunlight. The incidence among children in England was as high as 70–80% at the time, and this new disease was therefore called "English Disease" (40).

During the late 19th century and early 20th century, many biologic scientists were trying to understand the indispensable components of diets, and in 1913, the existence of vitamins was established by McCollum and Davis at the University of Wisconsin (41). In working with rat experiments, they discovered that cod-liver oil, together with butterfat, contained a water-soluble micronutrient that they called "vitamin A" and that this diet was able to cure an eye disease, xerophthalmia. Some years later, Sir Edward Mellanby, inspired by McCollum's work, was able to produce rickets in dogs, which he kept indoors for practical reasons and thus deprived of sunlight, and cured them with a cod-liver oil treatment. Mellanby was not aware that cod-liver oil also contained vitamin D; hence, he speculated that curing rickets was only another property of vitamin A.

McCullom, now aware of Mellanby's work, started to question whether it really was vitamin A in cod-liver oil that cured rickets. He managed to destroy the vitamin A activity in the cod-liver oil and discovered hereby that cod-liver oil could no longer cure xerophthalmia but was still able to cure rickets. McCollum then understood that cod-liver oil also contained another micronutrient, which he named "vitamin D" (40).

Today, vitamin D deficiency is a major global public health issue. It is estimated that 1 billion people are deficient worldwide, and that 50% of the population has insufficient levels (see Figure 2) (42). Nutritional rickets, which can easily be fully prevented by vitamin D supplements, continue to be a global health issue in Western countries.

Glisson examines a child with rickets as the mother looks on. Two more children with rickets play in the background, and bones deformed by rickets are hand on the wall. Glisson was a physician who, along with Wistler, was one of the first to clearly describe the disease (1650). Copyright: Free to use from the US National Library of Medicine digital collection.

Metabolism

Although once named vitamin D, it is not actually a vitamin but rather a fat-soluble steroid hormone, which comes in six forms and undergoes several metabolizing steps to form its active metabolite, 1α, 25-dihydroxyvitamin D ($1,25(OH)_2D$), also known as calcitriol (43) (see Figure 1). There are two forms of vitamin D: vitamin D_2 and vitamin D_3. Both vitamin D_2 and vitamin D_3 can be obtained through exogenous sources, such as varied diets and, in more significant amounts, vitamin D supplements and fortified food. Vitamin D_3 can also be produced in the skin. Metabolites from exogenous sources enter the bloodstream after intestinal absorption. However, the main source of vitamin D in our body is produced endogenously when the skin is exposed to ultraviolet B (UVB) radiation (wavelength 280-315 nm), and UVB rays penetrate the skin (44). Since only vitamin D_3 can be synthesized via the skin, and vitamin D_3 is the most important source of vitamin D in the body, I will focus solely on describing the endogenous vitamin D_3 metabolic pathway.

As previously mentioned, endogenous synthetization starts when UVB rays penetrate the skin, subsequently initiating a two-step reaction. First, there is a conversion of 7-dihydrocholesterol (7-DHC) in the skin, followed by thermal isomerization, which generates the end-product pre-vitamin D_3 (cholecalciferol). After entering the bloodstream, pre-vitamin D is rapidly hydroxylated mainly in the liver by 25-hydroxylase (CYP27A1) to form vitamin D (25[OH]D), also known as calcidiol, which is the biologically inert, circulating form of vitamin D. Second, and vitamin D-activating, conversion occurs in the kidneys by 1α-hydroxylase (CYP27B1), where most of the active form of vitamin D is synthesized, i.e., $1,25(OH)_2D$ (calcitriol) (42, 45). Both vitamin D and active vitamin D are predominantly bound to vitamin D-binding protein (DBP) when circulating in the blood. Notably, although the kidneys are the main source of circulating active vitamin D, the vitamin D-activating enzyme is present in numerous other cells and tissues as well, among which, cells related to the immune system, a fact of great importance to this thesis. Thus, by the extrarenal presence of the vitamin D-activating enzyme, these extrarenal sites can supply local needs for active vitamin D in a paracrine/autocrine manner, which will be described further in "mechanisms of action" (46) (see Figure 1).

Activated vitamin D is a potent hormone involved in the crucial regulation of blood calcium and phosphate levels in extracellular fluids by affecting intestinal absorption, renal excretion, and bone calcium turnover (43, 47). Since excessive calcium in the blood is potentially very harmful to the body by, for example, interfering with heart and brain function, vitamin D activation triggered by low calcium levels is under strict regulation. Calcium and phosphorus homeostasis, and the maintenance of bone substance, is thus regulated by a tightly controlled feedback loop between parathyroid hormone (PTH) and vitamin D, which is sometimes called

the “calcium-vitamin D-PTH axis.” PTH is stimulated and secreted upon a decrease in calcium levels in the blood, which in turn activates the synthesis of active vitamin D in the kidneys. Active vitamin D subsequently stimulates an increase in intestinal absorption of calcium and phosphorus. A continued hypocalcemia and exposure to increased PTH levels may also lead to calcium and phosphorus resorption from bone, as well as renal and intestinal calcium absorption, mediated by both PTH and active vitamin D. However, in the case of a rise in blood calcium levels, PTH secretion drops, consequently leading to decreased renal synthesizing of active vitamin D and decreased calcium mobilization.

As previously mentioned, active vitamin D can be catabolized in extrarenal tissues, generated by the expression of 24-hydroxylase. This is an important regulation mechanism of auto or paracrine signals originating from locally produced active vitamin D. The amount of active vitamin D synthesis and degradation in extrarenal tissues is under the control of local factors, such as cytokines and growth factors. However, the mechanisms behind this process are incompletely understood (48). The self-regulation of UVB-induced vitamin D production is generated by sun exposure (“tanning”), which in turn leads to an increase in epidermal melanin content. As melanin competes with 7-DHC to absorb UVB photons, the possibility of 7-DHC conversion to pre-vitamin D in the skin decreases. Adding to less availability of pre-vitamin D in the body, and thus less vitamin D originating from the skin, the associated photo-isomerization of excess pre-vitamin D (and vitamin D) into its inactive metabolites also occurs (44).

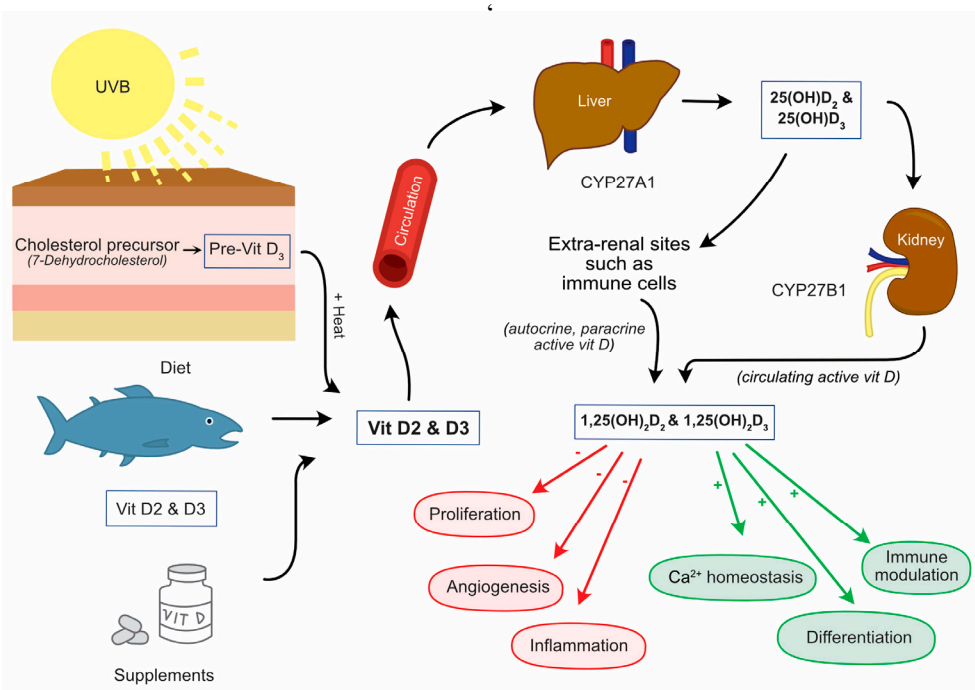


Figure 1. Vitamin D metabolism

Mechanisms of action

Since both vitamin D and active vitamin D are lipophilic molecules, they must be transported in the circulation bound to plasma proteins, predominantly DBP. After a release from DBP, vitamin D binds to intracellular vitamin D receptors (VDR), which are members of the superfamily of nuclear receptors for steroid hormones and are principally located in the nucleus of target cells. Through vitamin D responsive elements (VDREs), vitamin D acts as a transcription factor and alters the expression of vitamin D responsive genes by significantly enhancing or suppressing the rate of the gene transcription process (48). VDRs are present in almost all tissues and cell types and are thus located in most organs, for example, in the heart, skin, gonads, brain, prostate, and breasts. A genome-wide mapping of vitamin D receptor binding, conducted by Ramagopalan et al., showed that stimulation with active vitamin D regulates the expression of 229 genes directly, and more than 2,000 genes indirectly (49).

However, the genes affected by active vitamin D also have multiple functions beyond regulating calcium homeostasis in the body. These genes have been shown to be involved in, for example, cellular proliferation, differentiation, apoptosis,

angiogenesis, and modulation of the immune system (10, 11). Due to the presence of both VDR and the vitamin D-activating enzyme in numerous tissues and cells that are not associated with skeletal health, vitamin D can act in autocrine/paracrine ways, independently from the activation step occurring in the kidneys, and this will be of great importance to this thesis.

Vitamin D, like other steroid hormones, is also able to generate responses that are too rapid to include changes in gene expression. It is suggested that these rapid responses are mediated by cell surface receptors on the cell membrane and/or cytoplasm, where VDR is a candidate receptor, since several studies imply that the rapid actions of vitamin D require the presence of VDR as a mediator (48). The function of non-genomic actions remains uncertain in most cells; however, many studies have shown that non-genomic effects do not seem to be critical for vitamin D-mediated gene activation (49, 50). These non-genomic effects include, for example, increases in intracellular calcium levels and stimulation of intestinal calcium transport (51).

Vitamin D deficiency

Vitamin D deficiency and insufficiency are of pandemic proportions, with approximately 50% of the global population being deficient or having insufficient levels (42, 52, 53). However, it must be said that data about vitamin D levels are missing from numerous countries in the world, especially from countries in South America and Africa. Nevertheless, in a recent systemic review of vitamin D status worldwide, Palacios et al. (2015) concluded that low vitamin D status is a global issue in all ages, highlighting the problem to be most pronounced in the Middle East, particularly in girls and women (see Figure 2) (54). Even in countries with access to sun exposure all year round, deficiency has been shown to be common. In Australia, for example, 31% of the adult population were deficient (<50 nmol/L), and 73% had insufficient vitamin D levels (<75 nmol/L) (55). As for the situation in Sweden, large population-based studies are currently lacking. However, there is a reasonably up-to-date study from Gothenburg, Sweden (2017) examining seasonal variations in vitamin D levels in healthy individuals by measuring vitamin D levels of 40–60 participants every month for one year. In total, 540 blood donors were included in the study. They found that the mean serum levels over the year were insufficient: 60 nmol/L (ranging from 47 nmol/L in February to 82 nmol/L in July), and about 50% of the participants were vitamin D deficient (<50 nmol/L) during the winter months (56).

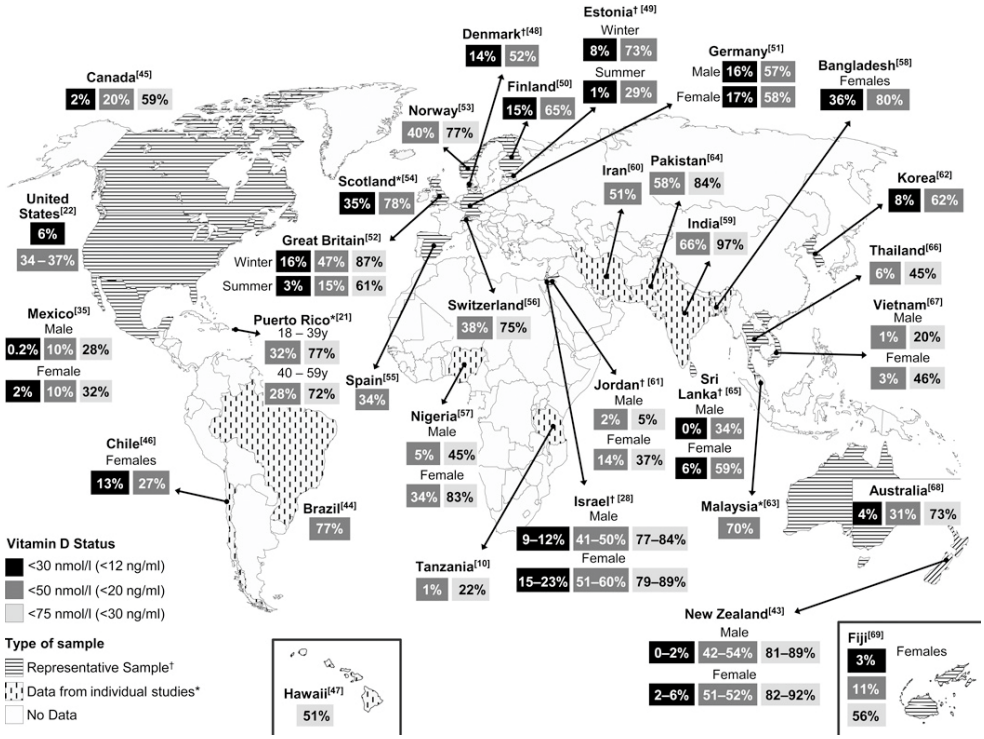


Figure 2. Prevalence of low vitamin D status in adults worldwide. (Palacios, copyright 2014, reproduced with permission)

Several factors contribute to the vast global deficiency, many of which are connected to our modern lifestyle with subsequent sun-avoiding behavior, which in turn impedes vitamin D synthesis (57). The knowledge of the connection between sun exposure and skin cancer has increased the use of sunscreen, sun-avoidant behavior, and the use of sun-protecting clothing. Other sun-related factors are industrialism, which has largely changed the working environment from outdoors to indoors, and the younger generation spending more and more time indoors, consequently at the expense of less outdoor physical activity. Also, there are several environmental factors hindering year-round vitamin D synthesis, such as latitude, season, and amount of air pollution. An important factor in northern countries is the high latitude (Lund, Sweden, 55.7°N), since the UVB availability above and below approximately 33° does not result in any significant vitamin D production during winter time (58). A study modeling UVB availability in nine European countries/regions, with latitudes ranging from 35 to 69°N, showed that all countries/regions had significant seasonality in UVB availability. However, the number of months in which UVB availability was too low for significant skin synthesis was 7-8 months in northern Europe, in contrast to being basically absent in the very south of Europe (59, 60). Lastly, personal

characteristics also affect a person's ability to produce vitamin D from sun exposure, i.e., skin pigmentation and age. Some groups of individuals have a particularly high risk of vitamin D deficiency for various reasons. One is the different genetic variants in deleterious alleles at three loci, confirmed by Wang et al., which more than doubled the odds of having insufficient vitamin D levels (<75 nmol/L) (61). Other individuals who are at higher risk for having low vitamin D levels are pregnant women, people with increased skin pigmentation, people with certain medical conditions, and obese individuals (see Figure 3). Therefore, an obese, pregnant, dark-skinned woman from a culture that includes whole-body clothing, who has a genetic variant susceptible to insufficient vitamin D levels, and who lives in a Nordic country, is most likely severely deficient in vitamin D. A 2016 study from Sweden indeed showed that 73% of 114 Somali women of reproductive age living in Sweden were severely deficient in vitamin D (<25 nmol/L) and none had levels above 75 nmol/L, which is considered at sufficient levels. Only 5% had vitamin D levels above 50 nmol/L (62).

It is essential to recognize that the definitions of vitamin D deficiency/sufficiency are, and have been under vivid debate for a long time, and there is no consensus yet regarding which levels are to be considered sufficient for optimal health (59). Most public nutrition guidelines emerge from thresholds primarily related to musculoskeletal health, where levels under 25-30 nmol/L are considered deficient and indicate increased risk of nutritional rickets or osteomalacia (60). In Sweden, the public guidelines of the National Food Administration recommend maintaining a vitamin D level > 50 nmol/L and defines a level of less than 50 nmol/L as below optimal levels (63). Adding to the complexity of the problem, there are also recommendations from non-governmental medical sources, such as the Endocrine Society (64), that conflict with the previously mentioned national guidelines. The Endocrine Society suggests that keeping a vitamin D level constantly above 75 nmol/L will provide the potential extra-skeletal benefits associated with vitamin D (26). Indeed, there are also studies implying a range between 100–150 nmol/L to be the most appropriate vitamin D level for maintaining optimal skeletal and extra-skeletal health (64-69). However, despite controverses regarding different vitamin D thresholds, the most frequently used cutoffs are <25 nmol/L = *severe deficiency*, <50 nmol/l = *deficiency*, 50-75 nmol/L = *suboptimal/insufficient levels*, and >75 nmol/L = *sufficient levels*. Therefore, these are the threshold levels used in this thesis (see Table 1). It is important, though, to point out that the exact threshold for optimal and safe vitamin D levels for skeletal and extra-skeletal health has not yet been established, and it is plausible that these thresholds would vary based on, for example, genetic variance, ethnicity, comorbidities, sex, age, and other factors (70).

It is nearly impossible to obtain sufficient vitamin D levels from dietary sources if one is not eating fatty fish in substantial amounts. The main source of vitamin D in the body comes from UVB exposure, as previously pointed out, which means

largely *unprotected* sun exposure. Although this exposure needs to be balanced with the increased risk of skin cancer, sensible sun exposure, together with the use of supplements, is the best way to obtain adequate vitamin D levels (59). A sensible UVB radiation exposure is dependent on the time of the day, latitude, season, and skin pigmentation; however, roughly, exposure of uncovered arms and legs for 5–30 minutes in the middle of the day, twice a week, is often sufficient (66). In the clinic, there are worries about possible vitamin D intoxication when using vitamin D supplements, since it can cause life-threatening hypercalcemia. Toxic levels of vitamin D are considered to be above 375 nmol/L. Though extremely rare, there are cases of vitamin D intoxication after taking vitamin D supplements. These have occurred due to manufacturing errors, sometimes up to 4,000 times the labeled concentration in supplements, or inadequate prescription by physicians (or inadequate self-administrated treatment), with doses that highly exceed the suggested recommendations (71). The upper tolerable limit of supplement doses, when administrated for a longer period, is 10,000 IU/day, according to several studies (72, 73). However, this upper limit is debated by experts, and Taylor et al. suggested the upper tolerable limit to be 4.000 IU/day to avoid toxicity (71).

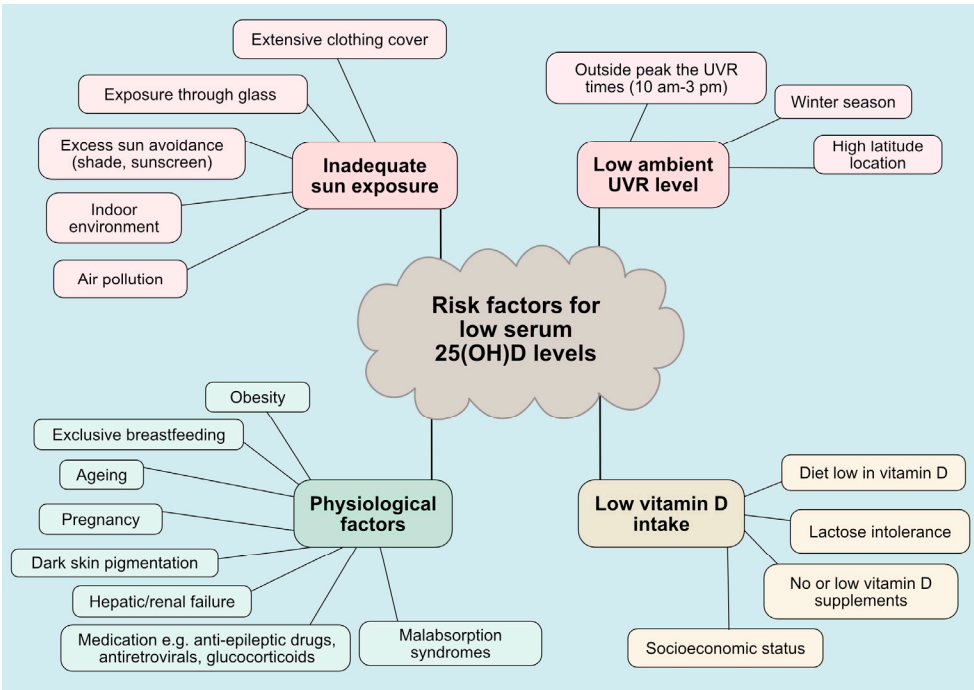


Figure 3. Risk factors of low vitamin D status

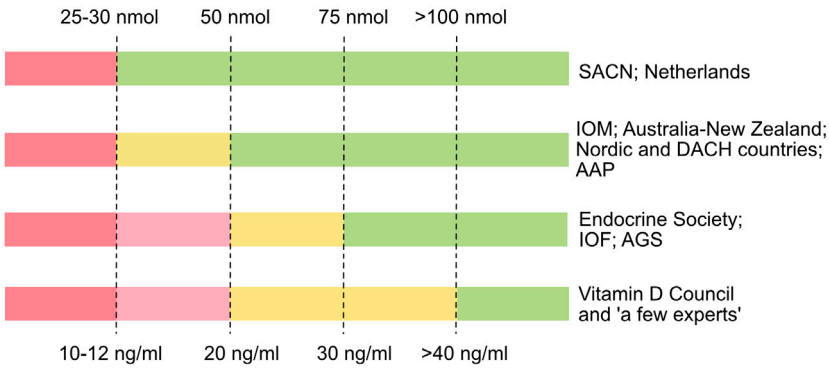


Figure 4. A schematic representation of vitamin D serum level interpretations in different agencies and countries. Color code: red = severe deficiency (danger), must be corrected without exception; orange = mild deficiency (modest concern), intervention is desirable; green = sufficient supply, no beneficial effects from additional supplementation. SACN, Scientific Advisory Committee on Nutrition; IOM, Institute of Medicine; DACH, Deutschland (Germany, Austria, and Confederation Helvetica (Switzerland); AAP, American Academy of Pediatrics; IOF, International Osteoporosis Foundation; AGS, American Geriatrics Society. © CT Sempos and N Binkley, 2020 (65).

Table 1.
The most used classifications of vitamin D status, which are also used in the thesis.

Vitamin D concentration a)	Classification
≤ 25 nmol/L	Severe deficiency
≤ 50 nmol/L	Deficiency
51 – 74 nmol/L	Suboptimal/insufficiency
≥ 75 nmol/L	Sufficient
> 375 nmol/L	Toxic

To convert nmol/L to ng/mL, divide nmol/L by 2,496.

Extra-skeletal effects of vitamin D

In general

As is well known, the main function of active vitamin D is to maintain tight calcium and phosphorus homeostasis in the circulation, which is vital for normal cellular physiology and skeletal health. However, over the last few decades, it has become apparent that vitamin D also exerts profound modulatory effects throughout the body that go beyond calcium regulation (74). The knowledge of vitamin D and its extra-skeletal effects has grown rather exponentially during the last decades, and we now know that vitamin D is also crucial for maintaining optimal somatic health. Convincing evidence from epidemiological, genetic, in vitro, case-control studies, prospective, retrospective studies, and meta-analyses suggest a link between vitamin D and reduced incidence/morbidity/mortality in diverse somatic illnesses, such as autoimmune diseases (75, 76), cardiovascular diseases (77), certain forms of cancer (78, 79), diabetes (76), allergic diseases, and decreased susceptibility to infectious

diseases (80). For example, extensive research has shown associations between vitamin D and respiratory tract diseases (81-83), where the most up-to-date research also suggests a relationship between vitamin D and SARS-CoV-2 (COVID-19) infection outcome (84). In most cases, the proposed relationship between vitamin D and different somatic illnesses is based on the immunoregulatory properties of vitamin D, which will be discussed in the next section (74, 85-88).

Of importance for this thesis, vitamin D is also suggested to have a significant impact on normal brain homeostasis (89). Abundant cells and areas in the brain express both VDR and the vitamin D-activating enzyme; thus, a local supply of activated vitamin D in the central nervous system (CNS) is possible, affecting cells in autocrine and paracrine ways (90). Many of these areas have previously been suggested to be involved in the pathophysiology of depression, e.g., the hippocampus, prefrontal cortex, substantia nigra, cingulate gyrus, and hypothalamus. Genetic, animal, and in vitro studies have shown, for example, both regulatory genomic and non-genomic effects of vitamin D in the brain, where vitamin D may have an impact on brain development, function, and maintenance (90-92). Both vitamin D and active vitamin D are able to cross the blood-brain barrier, and there are many ways by which vitamin D may influence, for example, the development and/or severeness of psychiatric illness, e.g., via its effects on catecholamine and serotonin biosynthesis and neurotransmission (93, 94), via its neuroprotective properties (90, 92) and, of highest interest for this thesis, via its immune-modulating effects within the brain as well as on a systemic level (95-97).

The effects of vitamin D on the immune system

First, it must be said that the immune system is an intricate system of interacting signaling and responses on many levels, and sometimes the same inflammatory marker can have seemingly opposite functions in the body. Therefore, the contribution of vitamin D to this complex system cannot be fully described within the framework of the thesis. Consequently, the descriptions herein will be provided to the reader in a simplified form and will focus on the most relevant mechanisms related to the studies included in the thesis.

Active vitamin D shows profound immunomodulatory effects on both innate and adaptive immune responses. VDRs are extensively expressed in key cells of the adaptive immune response, i.e., macrophages, dendritic cells (DCs), and activated T and B lymphocytes. Active vitamin D regulates the proliferation and function of macrophages and DCs, and since macrophages and DCs also possess the vitamin D-synthesizing enzyme, and the vitamin D-activating enzyme, it is possible for vitamin D to act locally in an immunological environment (10, 12). VDRs and the vitamin D-activating enzyme are also largely present in numerous epithelial cells and, thereby, together with cells of the adaptive immune response, vitamin D regulates, and enhances, the host defense via the innate immune response (50).

The immunomodulatory properties of vitamin D within the adaptive immune response can be briefly described as anti-inflammatory. Vitamin D inhibits the maturation of dendritic cells, which are important for antigen presentation, reduces T cell proliferation, and promotes a shift of T cell differentiation from the pro-inflammatory T-helper 1 (Th1) and Th17 cell response toward a T-helper 2 (Th2) and regulatory T cells (Tregs) cell response, the latter considered to alleviate a pro-inflammatory state in the body and, additionally, to be an important factor in autoimmune diseases (see Figure 5). These potent anti-inflammatory effects of vitamin D have been confirmed in several experimental in vitro and animal studies, and its immunosuppressive effects may prevent the harmful consequences of a prolonged inflammatory response (10, 48, 86, 95, 98).

Active vitamin D exerts its immunomodulatory effects primarily by regulating the expression of cytokines via its interaction with cells of the innate and adaptive immune systems. Cytokines include interferons (IFN), interleukins (ILs), chemokines, lymphokines, and tumor necrosis factor (TNF), and they are important both in health and disease. The pro-inflammatory Th1 and Th17 immune response mediates the production of cytokines, such as IFN-γ, TNF-α, IL-21, IL-6, IL-8, IL-12, IL-1β, IL-2, IL-23, and IL-17. As previously noted, the Th1 response is inhibited by vitamin D, thereby leading to lower levels of these pro-inflammatory cytokines. Also, vitamin D increases the Th2 cytokines IL-10, IL-4, and IL-5, with a subsequent enhancement of Th2 cell differentiation, which in turn increases the production of Treg cytokines. Treg-related cytokines are, among other immunological biomarkers, such as transforming growth factor-beta (TGF-β) and IL-10, suggested to stimulate a reduction in pro-inflammatory cytokines (10, 11, 80, 86, 98). Although promising results exist from experimental in vitro and animal studies, the immunomodulatory effects of vitamin D have been hard to demonstrate in humans, and few studies have confirmed the suppressive effects of vitamin D on Th1 cell response (80, 90).

One of vitamin D's critical immunomodulatory effects in the brain originates from microglia, the primary immune effector cell in the CNS, which interestingly comprises the vitamin D-activating enzyme. Rapid activation and response by different immune cells during infection, injury, or disease is crucial to protect the brain and bring the CNS environment back to a healthy condition. A prolonged inflammatory state—that is, chronic inflammation—could be detrimental to the CNS and has been associated with several neurological diseases (99). Notably, in animal studies, activated microglia have been shown to increase their expression of VDR and the vitamin D-activating enzyme upon an immunological challenge, with a subsequent enhancement of their receptiveness to vitamin D. Several studies have shown that activated microglia exposed to vitamin D and active vitamin D reduce their expression of pro-inflammatory cytokines and increase their expression of IL-10 (100). Thus, activated microglia have the possibility of promoting the previously mentioned switch from a harmful Th1 cell response to a less inflammatory Th2 cell

response. Altering the immune response via microglia suggests that vitamin D is a control mechanism for avoiding a prolonged inflammatory state in the CNS (101).

In my thesis, I investigated the relationship between vitamin D and the following inflammatory markers: TNF-α, IL-6, IL-1β, IL-2, IL-13, IL-8, IL-10, IFN-γ, and C-reactive protein (CRP). Also, in one of the included studies, we explored the relationship between vitamin D and the neutrophil-to-lymphocyte ratio (NLR) and white blood cell count (WBC), two markers considered to be inflammatory markers of systemic, low-grade inflammation (102).

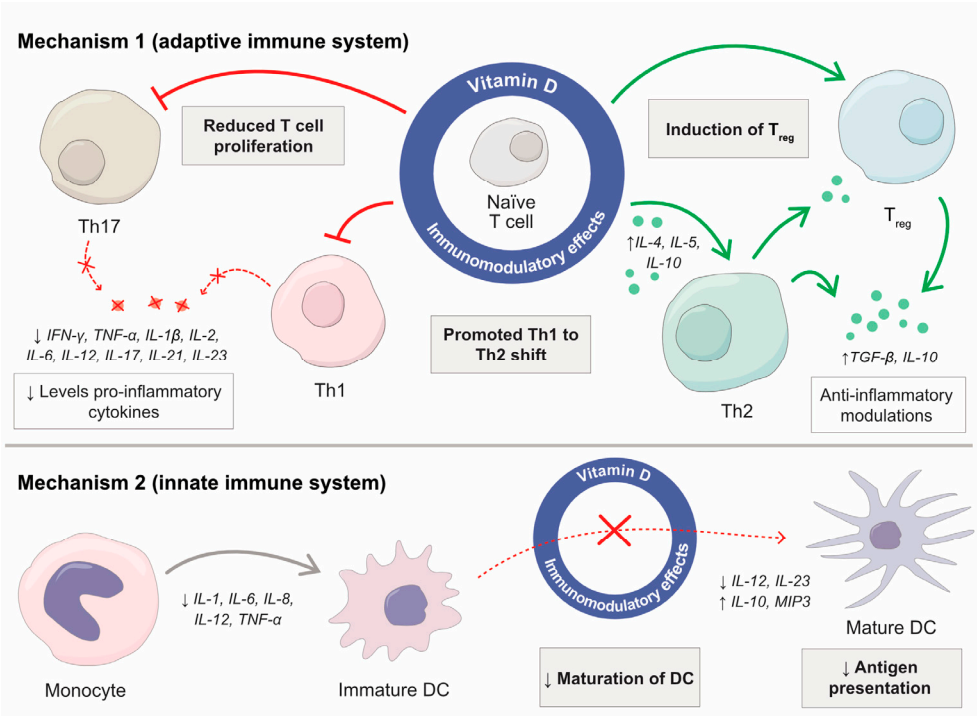


Figure 5. Effects of Vitamin D on the immune system.

Mechanism 1: Vitamin D promotes a shift of T cell differentiation from the pro-inflammatory Th1 and Th17 cell response toward a Th2 and Treg cell response, the latter considered to alleviate a pro-inflammatory state in the body.
Mechanism 2: Vitamin D inhibits the maturation of dendritic cells (DCs).

Vitamin D in MDD and suicidality

Vitamin D in depression

Research on vitamin D and depression has increased over the last 10–15 years. Most studies on vitamin D and psychiatric illness have used cross-sectional study designs; however, support for a relationship also comes from several systematic reviews, meta-analyses, and clinical trials. The main part of these studies relates to the association between vitamin D and MDD, psychotic disorders (predominately schizophrenia) (103, 104), neuropsychiatric disorders (attention deficit hyperactivity disorder [ADHD], autism) (104-106), and dementia (104). In cases of disorders strongly connected to neurocognitive function, animal studies have also frequently been used. In brief, the psychiatric disorders mentioned above are suggested to have some association with vitamin D, mainly via its impact on important brain functions and areas that subsequently lead to impaired neurocognition, such as memory functioning, executive function, processing speed, and attention, to name a few. Additionally, some studies imply that low prenatal levels of vitamin D increase the risk of developing schizophrenia and the cognitive impairments seen in ADHD.

Regarding vitamin D's relationship with MDD, the majority of previous studies, systematic reviews, and meta-analyses included show an association between low levels of vitamin D and depression (107-109), although there are also studies showing opposite results (110, 111), which might be due to methodological differences between studies (112). Some studies suggest that vitamin D is specifically associated with more severe depressive symptoms (i.e., associated with increased depression rating test scores) (113, 114), as in, for example, a large cohort study, the Netherlands Study of Depression and Anxiety (NESDA) (114). They investigated the association between vitamin D and depressive disorders (major depressive disorder and dysthymia), as well as the association between vitamin D and clinical depressive characteristics. In this study, low levels of vitamin D were associated with both the presence and severity of depressive disorders, suggesting that low levels of vitamin D might constitute an underlying biological vulnerability to depression. A special type of depressive disorder is seasonal affective disorder (SAD), which is characterized by symptoms of depression, anxiety, irritability, appetite changes, hypersomnia, and fatigue that occur during the winter months and diminish in the spring and summer. The incidence of SAD has been shown to increase with higher latitude (reduced sun exposure); therefore, SAD has also been suggested to be related to vitamin D (115). Also, there are studies suggesting that low levels of vitamin D may contribute to psychopathology only in certain types of depression, such as "suicidal depression" (116, 117) or "inflammatory depression" (117-120), and also that low vitamin D is relevant only at certain levels of deficiency (121).

Although there are promising results from epidemiological studies linking low vitamin D to depression, the possible benefits of vitamin D treatment for depression have not yet been demonstrated. There is presently a lack of well-designed randomized controlled trials (RCTs) (122), and it is therefore impossible to draw any conclusions regarding the potential causal effects of low vitamin D in relation to depression. Some RCTs have shown positive effects of vitamin D treatment on depressive symptoms, or the risk of developing depression over time (123, 124), however, overall, there is not yet sufficient evidence to recommend vitamin D supplements to treat or prevent depression (107, 125). In one recent, well-designed RCT, in which MDD patients with concurrent severe vitamin D deficiency were treated with vitamin D/placebo in addition to antidepressant treatment (escitalopram), the authors found that vitamin D levels did not correlate with subjective or objective depression ratings (126). Nonetheless, the patients who received placebo, and thus had uncorrected vitamin D levels, required significantly higher doses of escitalopram (mean difference, 4 mg/day) than those whose vitamin D levels were corrected. To the author's surprise, 25% in the placebo group had increased their levels from severely deficient to sufficient levels at the end of treatment, suggesting an intake of vitamin D supplements. In contrast, about 30% in the intervention group did not reach sufficient vitamin D levels at the end of treatment, suggesting that some of the patients did not take their subscribed capsules. Therefore, a post hoc analysis was conducted among study completers, showing that end-point escitalopram levels were significantly higher (by 4 mg/day) among patients with vitamin D deficiency compared to those with sufficient levels.

Vitamin D in suicidality

The research on vitamin D's relationship with suicidality has for a long time been limited. However, several studies have been published on the subject during the last three years, of which three are included in this thesis. (116-118, 127-134). Three recent studies have confirmed an association between vitamin D deficiency and increased suicide risk and/ suicidal ideation. One prospective study by Fond et al. (2019) investigated the possible association between severe vitamin D deficiency (<25 nmol/L) and suicide risk, along with other factors related to psychiatric illness, such as anxiety, psychosis, and depressive symptoms (129). The study sample comprised 251 schizophrenic outpatients, of which approximately 30% were severely deficient in vitamin D. They found that severe vitamin D deficiency was associated with suicide risk and several other factors, such as negative symptoms and antidepressant consumption. In another study, Gokalp et al. (2020) showed that adolescents patients (n=215), admitted to a pediatric hospital after a suicide attempt had significantly lower vitamin D levels than healthy individuals (n=200) (30 nmol/L and 50 nmol/L respectively) (128). Lastly, Kim et al. investigated whether the risk of having suicidal ideation was associated with vitamin D levels in healthy adults (n=157.211) (116). Using the vitamin D cut-off levels of <25 nmol/L to

define deficiency, >50 nmol/L as sufficient, and 25-50 nmol/L as insufficient, they found a higher risk of suicidal ideation in deficient individuals (<25 nmol/L) compared with sufficient individuals (>50 nmol/L). However, no significant difference in risk was seen between those with insufficient vitamin D levels and those with sufficient vitamin D levels.

Inflammation in depression and suicidality

Extensive research over the last few decades has supported a role for inflammation and a dysregulated immune system in the pathophysiology of depression and suicidal behavior (20, 135-141). Previous research has shown that major depression is associated with alterations in both the innate and adaptive immune system, including, for example, systemic immune activation and changes in the expression of inflammatory markers. These alterations are suggested to be related to a less favorable illness course and a poorer antidepressant response (142, 143). It has also been suggested that chronic low-grade inflammation could be particularly relevant in a subgroup of depressed individuals who express certain depressive symptoms and clinical characteristics (135, 144-146). The most frequently reported findings are that pro-inflammatory cytokines and acute-phase proteins are elevated in depressed and/or suicidal individuals, with the strongest evidence for IL-6, TNF-α, and CRP (29, 138). However, several other inflammatory markers have also repeatedly been associated with depression and/or suicidality, for example IL-1β and IL-8 (117, 139, 141, 147, 148).

It is not yet clear what causes the low-grade inflammation seen in some cases of depression. However, several upstream and downstream mechanisms have been suggested, including vitamin D deficiency. The potential role of vitamin D deficiency as a driver of inflammatory depression has been discussed by several other investigators (149, 150). As reviewed elsewhere, the connection between vitamin D, circulating pro-inflammatory cytokines, and inflammation may be important for the prevention and treatment of depression, and vitamin D supplementation has been discussed as a treatment option for mild to moderate depression via anti-inflammatory mechanisms (96). Despite several decades of research on the association between vitamin D and inflammation in somatic illnesses, fewer studies have investigated this relationship in depressed/suicidal individuals. The major aim of this thesis is to further investigate the relationship between vitamin D and different aspects of depressive disorders and suicidality, as well as the relationship between vitamin D and inflammation in these conditions.

Aim of the thesis

The overall aim of this thesis is to explore the relationship between vitamin D and different aspects of depressive disorders and suicidality, as well as the relationship between vitamin D and inflammation in these conditions.

Specific aims of the thesis

- To investigate associations between levels of vitamin D and depressive disorders;
- To investigate the relationship between vitamin D and inflammation in depressive disorders and suicidality; and
- To investigate the relationship between vitamin D and specific symptoms of depression: i) suicidality and ii) symptoms associated with "inflamed depression," such as inability to feel, lassitude, fatiguability, changes in appetite, and reduced or increased sleep.

Materials and methods

Summary of the study cohorts

Study I included two different patient cohorts and a healthy control group (n=14). The patient cohorts consisted of one group of recent suicide attempters with various Axis-I diagnoses (n=59), and the other group consisted of patients with moderate-to-severe clinical major depressive disorder without a recent suicide attempt (n=17, *"non-suicidal depressed patients"*).

Study II included patients with moderate clinical major depressive disorder (n=48) with different degrees of suicidal ideation ("mild-to-moderate suicidal ideation," n=17, and "no suicidal ideation," n=31), and a healthy control group (n=54).

Study III and IV included patients with "difficult-to-treat" depression (Study III: n=202, Study IV: n=263), i.e., most patients had a chronic illness course, to various degrees, despite previous or ongoing treatment. Thus, this sample was in accordance with the "real-life" heterogeneous population generally seeking specialized psychiatric care. Among the depressed patients, 100 had "high suicidal ideation," and 99 had "low suicidal ideation." Study III included a healthy control group of 41 individuals, and Study IV included 46 healthy controls.

Ethical considerations

All four studies included in the thesis were approved by an ethical review board in Sweden or in the United States (Study I: reference #479/2006 and #LU 82-01, Study II: protocol #10-00825, Studies III–IV: reference #2011/673), and also followed the principles expressed in the Declaration of Helsinki. The Declaration of Helsinki (151) is a statement of ethical principles for medical research involving human subjects and identifiable human materials and data. All patients gave their written informed consent before participating in the studies. They were all thoroughly informed about the possibility to stop participating in the study, and have their samples and records destroyed at any time, without any negative effects on their ongoing or future treatment. Healthy controls in Study I and participants in Study II received a small compensatory fee for participating in the study. In Studies III and IV, an amendment to the original ethical application was made and approved by the Regional Ethical Review Board in Lund (amendment # 2012/523), to authorize the

inclusion of vitamin D analysis in the previously approved biomarkers of the original ethical application.

Conducting research on psychiatrically vulnerable individuals requires great carefulness, and there are always risks involved. The risks must be considered in relation to the benefits of the intended research, both on an individual level and from a wider perspective. In the assessment of mental health, patients are subjected to sensitive questions about their lives and experiences, which might lead to distress. To avoid any worsening of symptoms during the study procedures, only experienced specialized psychiatrists or psychiatry residents trained in these types of interviews assessed the patients in the studies. Also, all assessments were made in person, and in cases of any prominent emotional reactions or marked discomfort during the study, the patient had access to the clinician-investigator who could assess and manage the situation. The participants were also told that they did not have to answer any question they did not want to, and that they could stop the activity at any time.

Admitting patients to a study is also a sensitive issue that must be considered. Patients may fear negative consequences related to their present or future health care if they decline to participate. They might be in a state of reduced judgment due to their illness or the traumatizing effects of a suicide attempt. Therefore, it is crucial that the research doctor is attentive to the patients' cognitive state and comprehension of the patient information, and that the patient is well informed about hers/his right to leave the study at any time, without any negative health care-related consequences.

Another important issue to consider is the stigma of mental disorders, which are highly present in society. It could possibly affect the participants negatively if any vulnerable, private information provided during the study would reach unauthorized persons. Therefore, careful handling of the study data is crucial. Also, all participants were anonymized by being given a study identification number in the included studies, and all results were presented on a group level to minimize any identification of individual study subjects. Also, all personal information, such as personal identification numbers, medical health information, and study data, were kept in a strongbox in a locked room, to which only the study investigators and a few other trusted administrative staff had access, and digital data were only kept in safe, encrypted, digital environments.

Clinical setting and sample

Table 2.
Overview of study populations in the thesis

	Patients (n)	Age, years (mean±SD)	Gender (females)	BMI, kg/m^2 (mean±SD)	Controls (n)	Age, years (mean±SD)	Gender (females)	BMI, kg/m^2 (mean±SD)
Study I a)	59	38 (14)	58%	26 (4)	14	33 (11)	50%	23 (3)
Study I b)	17	35 (11)	47%	25 (8)	14	33 (11)	50%	23 (3)
Study II	48	39 (15)	56%	26 (5)	54	38 (14)	61%	25 (5)
Study III	202	38 (14)	62%	26 (5)	41	35 (12)	73%	24 (4)
Study IV	263	38 (13)	67%	26 (5)	46	36 (13)	72%	24 (4)

a) The patient group "recent suicide attempters"
b) The patient group "non-suicidal depressed patients"
Abbreviations: BMI = body mass index, SD = standard deviation

Study I *(cross-sectional study)*

The first study in the thesis included patients from two different psychiatric cohorts (see Table 2); patients were enrolled between 2001–2003 and 2006–2008. The control group was included in the 2001–2003 study and was sex-and age-matched (± 5 years) with *Case II*. The original purpose of the 2001–2003 study (*Case II*) was to investigate the influence of exercise on factors possibly associated with depression (152). The original purpose of the 2006–2008 study (*Case I*) was to investigate inflammatory markers in suicide attempters (153). As shown in Table 2, there were no significant differences in body mass index (BMI) or age between the patient cohorts, but healthy controls were younger and had a lower BMI than the patients. None of the participants were on antibiotics or anti-inflammatory medications, which were considered potentially confounding. None of the patients or controls had any cardiovascular disorders, diabetes, or drug/alcohol abuse. The healthy controls were given a small compensatory fee for participating in the study in the form of a gift card. All participants gave their written informed consent before inclusion.

Cases I, "recent suicide attempters": Fifty-nine patients were enrolled in the study during 2006–2008 on admission to the medical intensive care unit at Lund University Hospital, or from a general psychiatric ward at the psychiatric clinic in Lund. Inclusion criteria were being over 18 years old and having attempted suicide within the last two weeks. The included patients had a variety of different psychiatric diagnoses, of which bipolar type II disorder and MDD were the most common. Approximately 50% of the suicide attempters had a personality disorder. The majority of the patients were treated with psychotropic drugs, and about 40% had somatic conditions.

Cases II, "non-suicidal depressed patients": Seventeen patients with moderate-to-severe MDD (9 men and eight women), without a recent suicide attempt, were

recruited from the psychiatric clinic of Lund University Hospital during 2001–2003. The depressed patients were not treated with any psychotropic drugs for at least one month before sample collection. About 40% had a somatic condition. Inclusion criteria were scoring ≥21 points on the Montgomery-Åsberg Depression Rating Scale (MADRS, presented later in the thesis), and exclusion criteria were pregnancy or any previous or present cardiovascular disease.

Control group: Healthy controls were randomly selected from the municipal population register in Lund in 2001–2003 and invited by letter. Fourteen physically, somatically, and mentally healthy control subjects participated in the study. Exclusion criteria were any previous or ongoing psychiatric condition, any significant somatic illness, pregnancy, or cardiovascular disease. None of the healthy controls were treated with psychotropic drugs.

Study II

Study II comprised a patient cohort and healthy controls recruited from the University of California San Francisco (UCSF). The study subjects were enrolled between 2011 and 2015 and were given a small compensatory fee for participation. Cases and controls were between 25–50 years old, matched on age (± 3 years), sex, and ethnicity, and they did not differ significantly in BMI (see Table 2). All participants gave their informed consent before inclusion.

All study participants were free of chronic illnesses, acute illnesses, infections, significant neurological conditions, or any other potential confounding medical conditions, assessed by history, physical examinations, and routine blood screening. The study participants were also free of medical, hormonal, or supplement treatments considered to be potentially confounding. None of the participants were taking vitamin D supplements above the U.S. recommended daily allowances (15 µg/day) for six weeks prior to study start, and all but one MDD participant stopped taking vitamin D supplements entirely for two weeks before study start.

Case: Forty-eight unmedicated patients with moderate MDD were recruited by clinical referral. Inclusion criteria for cases were: diagnosed with current MDD, without psychotic features, and scoring ≥17 on the 17-item version of the Hamilton Depression Rating Scale (HDRS-17, presented later in the thesis) (154). Exclusion criteria for MDD subjects were a suicide attempt or current suicidal intent within the past week, bipolar disorder, history of mania or hypomania, post-traumatic stress disorder (PTSD), or any eating disorder within one month of study participation. Subjects scoring 4 on the HDRS suicidality item, indicating a suicide attempt or current suicidal intent within the past week, were excluded from the study. Also, any history of psychosis that did not occur within the context of a past depressive episode was an exclusion criterion, as well as substance/alcohol abuse or dependence within six months of study entry.

Controls: Fifty-four healthy controls were recruited by flyers, bulletin board notices, Craigslist postings, and newspaper ads. Exclusion criteria were any previous or present psychiatric diagnosis according to the DSM-IV.

Study III and IV

The study sample in Studies III and IV was part of a cohort of the Genes Depression and Suicidality Study (GEN-DS) conducted by our research group under the direction of Professor Åsa Westrin (155). Patients were referred to the GEN-DS from four secondary psychiatric clinics in southern Sweden between 2012 and 2021. Data for Study III was collected between 2012–2020, and the data in Study IV originated from the full study sample; therefore, the sample size differs between the two studies, n=202 and n=263, respectively. The depressed patients were slightly older than the healthy controls and had a higher BMI (see Table 2).

Case: Inclusion criteria were ongoing depression with insufficient treatment response, according to the referring psychiatrist, and exclusion criteria were BMI >15, current liver disease, or pregnancy. All included patients had an outpatient psychiatrist but could also be referred to the study from inpatient care if their outpatient psychiatrist agreed to follow up.

Healthy controls: Forty-one healthy controls in Study III and 46 in Study IV were recruited through ads in social media and newspapers. Exclusion criteria for healthy controls were any previous or present psychiatric illness, psychotherapy, or treatment with psychotropic drugs, ongoing infection, severe or chronic somatic illness, immune-modulating drug therapy, pregnancy, or breastfeeding. Healthy controls received 500 SEK in compensation for participating in the study.

Data collection, diagnostic assessment, and symptom severity ratings

Study I

The study subjects underwent a structured interview by a specialist in psychiatry and were diagnosed according to the DSM-IV (*Cases I and II*), the Structured Clinical Interview for DSM-IV (SCID I) (*Case I*), and SCID II (*Cases I and II*). Depression symptom severity was rated using the MADRS (*Cases I and II*) and the Comprehensive Psychopathological Rating Scale (CPRS) (*Cases I*). All study participants underwent a general physical examination, which showed no evidence of ongoing infection or other unknown somatic conditions.

Case I: Patients were evaluated by a consulting psychiatrist shortly after a suicide attempt regarding diagnostics and risk of suicide. At the same time, information about the present study was given, both in oral and written forms. They were also

notified that they would be contacted within three weeks regarding an invitation to participate in the study. At invitation, patients were offered a renewed structured psychiatric evaluation, and, in conjunction, oral, and written information about the study was given a second time. Concurrently, patients who agreed to participate gave their written informed consent.

Case II: The research examination of the patients started within a week after recruitment. The day before the exercise test, the patients were evaluated using the CPRS, including a re-evaluation of MADRS, and SCID II.

Control group: The inclusion criteria for control subjects were no history of current mental or somatic disorders and good physical health. None of the healthy controls had any cardiovascular disease, diabetes, or drug/alcohol abuse. Controls were evaluated in person and assessed for a history of medical and psychiatric illness.

Study II

In Study II, the SCID I was used to establish all diagnoses. The interview was conducted in person and administrated by a Ph.D.-level clinical psychologist who had specific training in the use of this instrument. The diagnoses were verified by a board-certified psychiatrist after a thorough clinical evaluation. All participants underwent a routine physical examination, medical history assessment, and routine blood screening, including, for example, a complete chemistry panel, kidney and liver function, and protein, albumin, and complete blood count with differential count. On the day of the study visit, all subjects had to pass a urine toxicology screen for the use of drugs and a urine pregnancy test for persons with child-bearing potential. Physical activity was measured using the Yale Physical Activity Survey (YPAS) Vigorous Activity Index Score (156).

Case: Following the blood draw, the subjects underwent a depression severity rating using the HDRS-17. Two raters were present at the HDRS rating session and scored within one point of each other. If the rated score differed by more than one point, a consensus rating was determined by the two raters. For exploratory analyses, MDD subjects were categorized based on their HDRS suicidality item scores (item 3), which have a possible range of 0–4. Those with scores of 0 on item 3 (indicating the absence of suicidal ideation within the past week) were categorized as the "non-suicidal ideation" group (NSI; n = 31). Those with scores of 1–3 (indicating responses ranging from "feelings that life is not worth living" to "suicidal ideas or gestures" in the past week) were categorized as the "suicidal ideation" group (SI; n = 17).

Controls: Following the blood draw, healthy controls were evaluated with the SCID I interview to confirm the absence of previous or present Axis-I DSM-IV diagnoses.

Study III and IV

After inclusion in the GEN-DS study, patients were assessed by a board-certified psychiatrist or a resident in psychiatry under the supervision of a senior colleague. The patients were diagnosed according to the structured interview Mini International Neuropsychiatric Interview, version 6.0, (MINI 6.0), and the semi-structured interview SCID II. Psychiatric symptom severity was assessed using both expert and self-rating scales, such as CPRS, MADRS, and The Suicide Assessment Scale self-rated version (SUAS-S).

Diagnostic interviews

Structured and semi-structured interviews

MINI 6.0 (Studies III and IV)

The Mini International Neuropsychiatric Interview (157), version 6.0, is a short structured diagnostic interview used for screening the most common psychiatric disorders in the DSM-IV. It was developed by cooperating psychiatrists and clinicians to fill the gap between the costly thorough, academic, research-oriented interviews and the very brief screening tests used in the clinic. The aim was to develop a short but accurate structured psychiatric interview to be used in both research and clinical settings. MINI would also be easy to administer, have high sensitivity and specificity, and be compatible with international diagnostic criteria, which have also been confirmed in reliability and validation analyses.

SCID I (Study II)

The Structured Clinical Interview for DSM-IV Axis-I Disorders (158) is a diagnostic, semi-structured interview designed to be administrated by mental health professionals to determine major psychiatric disorders.

SCID II (Studies I, III, and IV)

The Structured Clinical Interview for DSM-IV Personality Disorders (159) is a semi-structured interview used to determine Axis-II disorders (personality disorders).

Symptom rating scales

HDRS-17 (Study II)

The Hamilton Depression Rating Scale (154) is a widely used clinician-administrated depression rating scale evaluating depression severity through 17 items based on symptoms experienced over the past week. Seventeen of these items

are scored on a five-point scale (0–4) and eight items on a three-point scale (0–2). Higher scores indicate greater depressive severity in all items. A total score <7 is generally accepted to be within the *normal range* (absence or remission of depression), a total score between 7–17 is considered *mild depression*, a total score between 18–24 represents *moderate depression,* and a total score ≥25 is considered *severe depression*.

CPRS (Studies III and IV)

The Comprehensive Psychopathological Rating Scale (160) was constructed to measure changes in a wide range of psychiatric symptoms over time. It consists of 65 items in total, of which 45 are reported by the patient, and 20 items are rated as observed by the interviewer. The rating range is between 0–3 points, with the possibility of rating half steps. The initial intention of the scale was to evaluate treatment response; therefore, the scale focuses mainly on assessing symptoms that are not related to psychological traits but are likely to be influenced by treatment. CPRS covers several aspects of psychopathology and can be used as a source from which specific symptom scales can be generated, as has been conducted in, for example, the MADRS or Brief Scale for Anxiety (BSA).

MADRS (Studies I, III, and IV)

The Montgomery-Åsberg Depression Rating Scale (161) is a 10-item subscale derived from the CPRS that focuses on depressive symptoms. It is often used by clinicians to assess the severity of depression in patients with a diagnosis of depression. The score range is between 0–6 points per item, as the half steps in CPRS have been transformed into full steps, thus resulting in a maximum total score of 60 points. Like CPRS, MADRS was designed to be sensitive to changes in depressive symptoms over time. A MADRS total score between 0–6 points is seen as *normal range/symptom absent*, 7–19 represents *mild depression*, 20-34 represents *moderate depression*, and >34 is considered *severe depression*.

SUAS-S (Study III)

The Suicide Assessment Scale is a clinician-administered rating scale developed by Stanley et al. in 1986 (162). The scale was later translated into Swedish and modified into a self-rated version of SUAS-S, which is the scale used in Study III (37, 163). Both the SUAS and SUAS-S comprise 20 items and combine questions on explicit suicidality with questions on psychiatric symptoms suggested to be indirectly related to suicidality: *affect* (items 1, 2, 9, 12, and 13), *bodily states* (items 3, 8, and 10), *emotional reactivity* (items 4, 5, and 14), and *control and coping* (items 6, 7, 11, and 15). Each item is rated on a five-point scale (0–4), with higher scores indicating increasing severity; thus, the maximum total SUAS/SUAS-S score is 80 points. The SUAS-S total score has been shown to correlate significantly with the total score of the expert interview version of the SUAS, and the only difference

between the two scales is that the SUAS-S contains defined scores. The final five questions in the SUAS (items 16–20) are direct questions on suicidal thoughts and behavior, i.e., *suicidal thoughts* (item 16), *purpose of suicide* (item 17), *wish to die* (item 18), *wish to live* (item 19), and *suicide plans* (item 20). Four of these five suicide items (items 16, 18, 19, and 20) were shown to predict a future suicide attempt with more accuracy than the SUAS total score (164).

Biological sampling procedures and assays

Study I

Blood samples from healthy controls/non-suicidal depressed patients were collected between 2001 and 2003 and from suicide attempters between 2006 and 2008. Blood samples from all subjects were collected between 7:30 and 8:00 in the morning, after a night of fasting. For non-suicidal depressed subjects and healthy controls, sampling occurred within a week of the physical and psychiatric examination. For recent suicide attempters, sampling occurred in conjunction with the suicide attempt. Samples were collected from all seasons throughout the year for the suicidal patients, and from all seasons, except for the summer, for the depressed non-suicidal patients and the healthy control group.

The same post-sampling conditions were applied to all samples. The blood was placed on ice and centrifuged (3000 rpm, at +4°C) within 1 h. All samples were frozen within 1 h after blood collection and stored at -80°C until biochemical measurements. Samples had never been thawed prior to the analyses, and the time of storage (4–11 years) was not likely to have had an impact on the quality of the analysis due to the relatively stable vitamin D molecule.

Study II

Plasma was collected into lavender ethylenediaminetetraacetic acid (EDTA) vacutainer tubes, and serum was collected into serum separator tubes. The tubes were stored at −80°C until assay. Blood samples were drawn between 8:00 a.m. and 11:00 a.m. after a night of fasting. Subjects were instructed to sit quietly and relax for 25–45 min before blood draw. Samples were collected from all seasons throughout the year. Stored serum samples were thawed once before vitamin D analysis, which has previously been shown not to affect the quality of the analysis (165). Study samples were collected between 2011 and 2015, and vitamin D analysis was conducted in 2019. The time of storage (4–8 years) is not likely to have had an impact on the quality of the analysis due to the relatively stable vitamin D molecule (165, 166).

Study III and IV

On the day of the study visit, screening of routine blood tests, including hs-C-reactive protein (CRP) assays, was conducted, and additional plasma was sampled and stored at -80°C until future analyses. Serum for vitamin D analysis was collected in serum separator tubes, protected from light by aluminum foil, and stored at −80◦C until assay. Patients were instructed to fast for four hours before the blood draw and to avoid nicotine use, as well as taking medications in the morning. Samples were collected during all seasons throughout the year. The time of storage (6 months−7 years) is not likely to have had an impact on the quality of the analysis due to the relatively stable vitamin D molecule (166).

Analytic procedure specifications

Vitamin D (I, II, III, IV)

The vitamin D analyses in all studies included in the thesis were conducted by the Department of Clinical Chemistry at Scania University Hospital using the same analysis apparatus and method, namely liquid chromatography-mass-spectrometry, model Sciex API 4000 LC/MS/MS (MA, USA). The coefficient of variation (CV) values were as follows in Studies I and II: for $25(OH)D_2$, 6.0% at 40 nmol/L and 5.0% at 120 nmol/L and for $25(OH)D_3$ 6.0% at 40 nmol/L and 4.0% at 120 nmol/L. Due to a change in the location of the vitamin D analysis (from Malmö to Lund), the CV values in Studies I and II differs slightly from those in Studies III and IV. CV values in Studies I–II and III–IV were as follows: for $25(OH)D_2$: 6.0% at 35 nmol/L and 5.0% at 114 nmol/L, and for $25(OH)D_3$: 8.0% at 33 nmol/L and 5.0% at 133 nmol/L, respectively.

According to clinical guidelines (167), in cases of a $25(OH)D_2$ level >10 nmol/L, the $25(OH)D_2$ level was added to the $25(OH)D_3$ level and accounted for as $25(OH)D_3$ in the statistical analysis, i.e., while describing vitamin D (25[OH]D) levels in the thesis, the vitamin D levels comprise the sum of $25(OH)D_2$ and $25(OH)D_3$. At most, 12% of the subjects had $25(OH)D_2$ levels >10 nmol/L (Study III, median=16.2 nmol/L). In Studies I, II, and IV, 0%, 4%, and 6% had $25(OH)D_2$ >10 nmol/L, respectively.

The cut-off levels used to define vitamin D status in all included studies were as follows: <25 nmol/L=severe deficiency, <50 nmol/L = deficiency, 50-75 nmol/L = suboptimal/insufficient, and ≥ 75 nmol/L = sufficient vitamin D levels. However, in Study II, levels <50 nmol/L are called low vitamin D levels.

White blood cell count and neutrophile-to-lymphocyte ratio (NLR) (II)

In Study II, we assessed the neutrophile-to-lymphocyte ratio (NLR), as well as white blood cell count (WBC), since they are considered to be inflammatory markers of

systemic, low-grade inflammation that had recently been related to vitamin D at the time of the study (168). The neutrophil-to-lymphocyte ratio was based on absolute neutrophil and lymphocyte counts.

Cytokines (I, II, IV)

Cytokines in Study I (IL-1β, IL-6 and TNF-α) were measured in plasma using ultra-sensitive electrochemiluminescence immunoassays according to the manufacturer's recommendations (MesoScale Discovery, UK). The assay was performed on a SECTOR Imager 6000 in the laboratory of the psychoimmunology unit at the Wallenberg Neuroscience Center in Lund, Sweden. All samples were analyzed in duplicates. The detection limits for IL-1β, IL-6, and TNF-α were 0.014 pg/mL, 0.050, and 0.075 pg/mL, respectively and the cytokine analyses were undertaken in 2008.

Cytokines in Study II (IL-6 and TNF-α) were quantified using a high-sensitivity multiplexed sandwich immunoassay concentration (Mesoscale Discovery, Gaithersburg, MD, USA). The sensitivity for each cytokine was IL-6 (0.07 pg/ml) & TNF-α (0.10 pg/ml). The intra-assay coefficients of variation (CV) were: IL-6 (4.4%) and TNF-α (4.2%). Cytokine assays were performed in the lab of Dr. Firdaus Dhabhar at Stanford University, USA.

In Study IV, cytokines (IFN-γ, TNF-α, IL-10, IL-6, and IL-8) were measured in plasma using a high-sensitivity electrochemiluminescence-based multiplex immunoassay (MesoScale Discovery, Gaithersburg, MD, USA), following the manufacturer's protocol. The analyses were performed at the Biomedical Center (BMC) in Lund, Sweden, by Dr. Filip Ventorp and the disserting PhD student Cécile Grudet. Average detection limit and CV values (duplicates, intraassay) for the cytokines were as follows: 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. The interassay CV value (control samples of IL-8) was 2.78. Since 36 samples (10.1%) of IL-10 were below the fit curve and considered Missing Not at Random, fill-in was used by multiple imputation using other cytokines as variables in the model (linear, 10 imputations, 0.00, and detection limit [0.082 pg/mL] as constraints).

CRP (IV)

Serum hs-CRP levels were analyzed in patients according to clinical standards at the Department of Clinical Immunology, Skåne University Hospital, Lund, Sweden. The limit of quantitation (LOQ) for hs-CRP was 0.60 mg/L. Hs-CRP was not available from the healthy controls.

Statistical analyses

All statistical analyses were performed using the Statistical Program for Social Sciences (SPSS). In Study III, the statistical program "R" Studio 2021.09.0 (R v 4.1) was also used. Figures were created using SPSS (Study I), the ggplot2 package v 3.3.5 (Study III), and Prism GraphPad in Studies II and IV. All statistical tests were 2-tailed with alpha level set at $p<0.05$.

For group-wise comparisons, one-way analysis of variance (ANOVA) or Student's t-tests were performed. In the case of non-normally distributed variables, data were transformed into normal distribution using the natural logarithms (ln) before statistical analysis, or non-parametric tests were used (Mann-Whitney U tests). Pearson's chi-square test was used to compare group differences in proportions, and in cases of small sample sizes, Fisher's exact test was used (Study I). For correlation analyses, we used Pearson's r, and the moderation analysis in Study II was performed using the PROCESS tool for SPSS. In Study III, binary logistic regression was used to model the probability of being a patient or control. In Study IV, a principal component analysis (PCA) was used to identify patterns of variation in certain inflammatory biomarkers and vitamin D.

Confounding factors

Several potential confounding factors are involved in studies on vitamin D, inflammation, and MDD. All confounding factors to adjust for were chosen *a priori* based on previous knowledge (*sex, age*, *smoking*, *BMI, sampling season*, *ethnicity,* and *somatic illness)*, and were adjusted for with appropriate methods, such as linear regression or analysis of covariance (ANCOVA).

Results

Study I

Our most salient findings in this study were i) that patients with a recent suicide attempt had significantly lower vitamin D levels than both depressed patients without suicide attempt and healthy controls (see Figure 6 and Table 3) and ii) that vitamin D was negatively associated with pro-inflammatory markers IL-1beta (suicide attempters, n=46; Pearson's r: −0.321, $p < 0.05$), and IL-6 (non-suicidal depressed patients, n=17; Pearson's r: −0.582, $p < 0.05$). Vitamin D levels were not associated with Axis-I diagnosis, the use of violent suicide attempt methods, or repeated suicide attempts (see Table 4 for specification of compared groups).

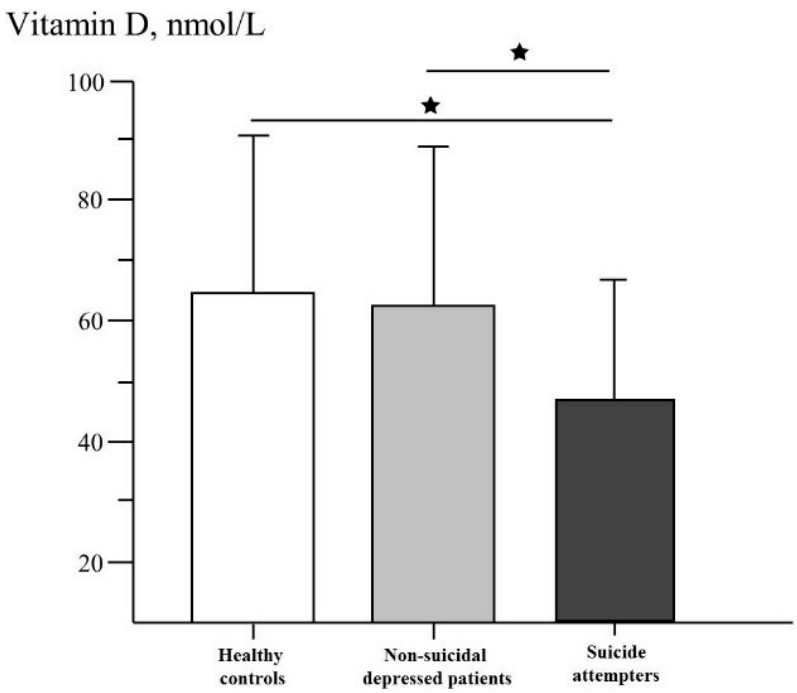


Figure 6. Uncorrected mean vitamin D levels for the three studied groups: healthy controls (n=14), non-suicidal depressed patients (n=17), and suicide attempters (n=59). The significant differences in the mean vitamin D levels between the suicidal patients and the healthy controls/non-suicidal depressed patients were analyzed statistically using a variable corrected for age and BMI (ANOVA with Bonferroni-Dunn's post-hoc tests) (*p<0.05). Error bars represent SD.

Table 3.
Vitamin D levels and percentage of participants below different vitamin D cut-off levels*

Variable	Healthy controls (n=14)	Non-suicidal depressed patients (n=17)	Suicide attempters (n=59)
Vitamin D (mean±SD), nmol/L	65 ± 26	62 ± 27	47 ± 20
% <25 nmol/L	0%	12%	7%
% <50 nmol/L	29%	29%	58%
% <75 nmol/L	71%	59%	92%

Table 4.
Vitamin D levels in different groupings of the 59 patients with a recent suicide attempt.

Group	(n)	Vitamin D levels (mean ± SD), nmol/L
Major depressive disorder	10	47 ± 18
Bipolar disorder	15	49 ± 19
Other	33	47 ± 22
Affective disorder [a)]	32	50 ± 20
Non-affective disorder [b)]	26	44 ± 20
Axis-II disorder	29	47 ± 22
No Axis-II disorder	30	47 ± 19
Repeater	38	49 ± 21
Non-repeater	20	42 ± 19
Violent suicide	6	45 ± 19
Non-violent suicide	52	47 ± 21

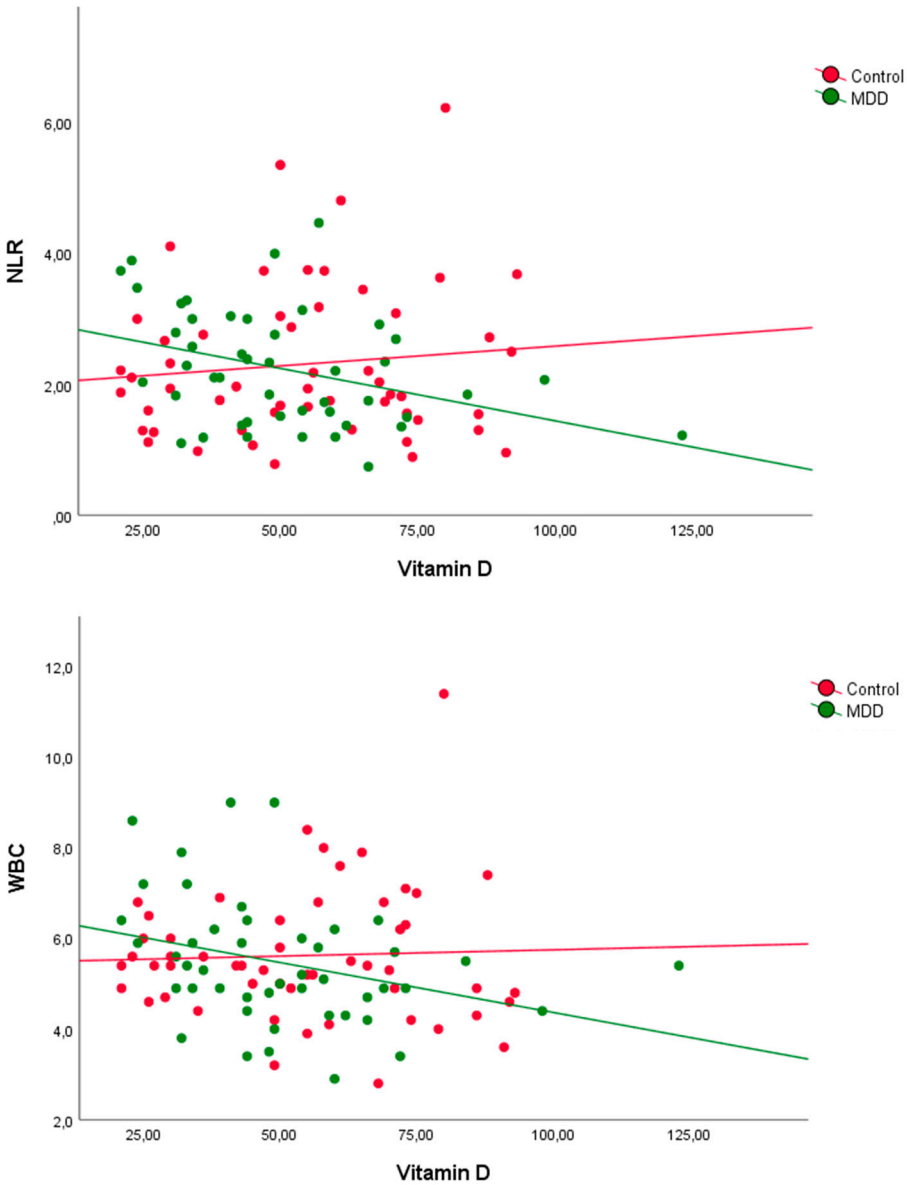
a) The affective disorder group contained patients with: major depressive disorder (n=10), bipolar type I and II disorder (n=15), and dysthymic disorder (n=4), and depressive disorder NOS (n=3).

b) The non-affective disorder group contains patients with schizoaffective disorder (n=2), psychiatic disorder NOS (n=1), anxiety disorder NOS (n=4), generelized anxiety disorder (n=1), alcohol dependence (n=6), substance dependence (n=2), adjustment disorder (n=7), and adjustment disorders with depressed mood (n=3).

There was no significant difference between vitamin D levels in the three main groups of psychiatric diagnosis, i.e., major depressive disorder (n = 10), bipolar disorder (n=15), and other psychiatric illnesses (n=28) (one-way ANOVA, ns) or between those with an affective disorder (n=31), and those without an affective disorder (n=22) (Mann-Whitney's U-test, ns). Likewise, there was no significant difference between subjects with an Axis-II disorder (n=27) compared to those without (n=26) (Student's T-test, ns), or when analyzing different aspects of suicidality by comparing suicide attempt repeaters (n=35) to non-repeaters (n=17), or those who had committed a violent suicide attempt (n=6) to those who had committed a non-violent suicide attempt (n=46) (Mann-Whitney's U-test, ns).

Study II

In Study II, our most salient findings were i) a significant negative association between vitamin D and inflammatory markers in MDD patients (see Figure 7), but not in healthy controls. The association was stronger in depressed individuals with suicidal ideation (SI-MDD) compared to those without (non-SI-MDD) and ii) no significant difference in vitamin D levels between MDD patients and healthy controls.



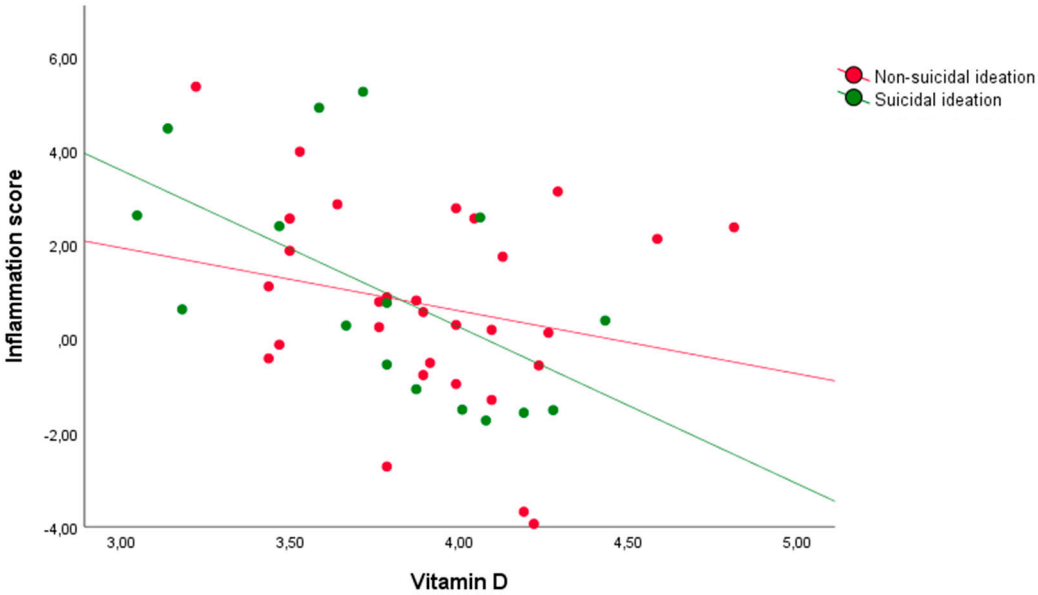


Figure 7. Correlation (Pearson's r) between vitamin D (25[OH]D) and different markers of inflammation in MDD subjects and healthy controls (A–B) and subjects with suicidal ideation and those without: A) neutrophil-to-lymphocyte ratio (NLR), B) white blood cell count (WBC), and C) inflammation composite score (IL-6 and TNF-α). There was a significant correlation between vitamin D and NLR (r=-0.39; p<0.01), and vitamin D and WBC (r=-0.36; p<0.05), and inflammatory composite score in SI subjects (r=-0.57; p<0.05).

Study III

The most important finding in Study III was that vitamin D levels differed significantly between patients with difficult-to-treat depression and healthy controls (see Figure 8). The odds of being depressed decreased by approximately 17% for every increase of 10 nmol/L in vitamin D (see Table 5). There were no significant correlations between 25(OH)D and symptom severity (MADRS, SUAS, and suicide composite score) in patients with a MDD single episode, MDD recurrent episodes, or chronic MDD (Spearman's rho, all $p > 0.26$).

There were no significant correlations between 25(OH)D and MADRS total score, SUAS total score, or suicide composite score in all patients (Spearman's rho, all $p>0.65$).

There were no significant correlations between 25(OH)D and symptom severity (MADRS, SUAS) and suicide composite score (suicidal thoughts, purpose of suicide, wish to die, wish to live, and to suicide plans) in patients with single episode depression, chronic MDD, or recurrent MDD episodes (Spearman's rho, all $p>0.26$). However, in patients with dysthymia, 25(OH)D correlated significantly and negatively with MADRS total score and SUAS-S total score, but not with suicide

composite score (Spearman's rho=-0.57, n=18, p=0.01 and -0.76, n=15, p<0.01, respectively).

Table 5.
Binary logistic regression, unadjusted and adjusted results, with MDD/controls as the dependent variable.

Variable	Unadjusted results			Adjusted results [a)]			Adjusted results [b)]		
	OR	*p*-value	R^2	OR	*p*-value	R^2	OR	*p*-value	R^2
25(OH)D	0.849	0.02*	0.036	0.840	<0.05*	0.230	0.828	0.02*	0.179
BMI	1.142	<0.01**	0.065	1.106	0.07		1.109	0.05*	
Smoking	0.314	0.06	0.031	0.289	0.06		N/A	N/A	
Season	0.986	0.07	0.024	1.420	0.38		N/A	N/A	
Age	1.017	0.22	0.011	1.003	0.82		N/A	N/A	
Sex	1.590	0.23	0.011	1.665	0.23		N/A	N/A	
Somatic illness	0.263	<0.01**	0.096	0.263	<0.01**		0.258	<0.01**	

a) Model I: All variables possibly affecting dependent variable; BMI, smoking, sampling season, age, sex, and somatic illness.
b) Model II: Only significant variables, or close to significant, are included in the model.
* Significant at the 0.05 level (2-tailed).
** Significant at the 0.01 level (2-tailed).
Acronyms: major depressive disorder (MDD), body mass index (BMI), odds ratio (OR)

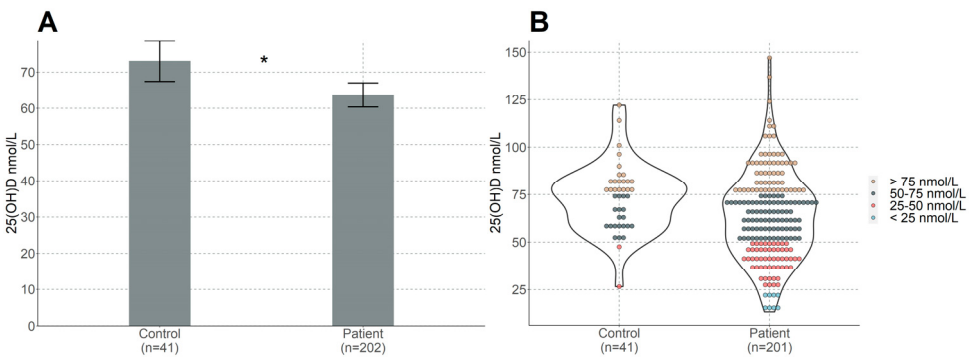


Figure 8. Comparison of vitamin D levels and distribution of vitamin D levels in MDD subjects and healthy controls.

(A): Bar graph showing mean levels of vitamin D in healthy controls and subjects with major depressive disorder (MDD). There were significantly lower levels of vitamin D in MDD subjects, which remained significant after adjusting for sex, age, smoking, sampling season, somatic illness, and body mass index (BMI) (ANCOVA, F = 4.89, p < 0.03). (B): Violin plot showing the distribution of vitamin D levels in healthy controls and major depressive disorder (MDD) subjects divided into groups based on different vitamin D cut-off levels. To gain greater visibility, one MDD subject with a vitamin D level of 174 nmol/L, was excluded in Figure B. However, this MDD subject was included in the statistical analysis. Error bars represent 95% CI. *Significant at the 0.05 level (2-tailed)

Study IV

In this study, we used a cut-off of hs-CRP 3 mg/L to define the "inflamed depression" subgroup, and depressive symptoms previously associated with inflamed depression (i.e., inability to feel, lassitude, fatiguability, changes in appetite, and reduced or increased sleep) were summarized into a composite inflammatory depression symptom score (infl-dep score). A PCA extracted a factor of high IL-6 and IL-8 levels together with low vitamin D levels (called the IL6-IL8-VitD component), which explained a significant amount of the variance in the infl-dep score (22.5% (see Figure 9).

Our most salient findings in Study IV were as follows: i) elevated levels of the pro-inflammatory markers IL-6 and IL-8 in all depressed patients compared to controls (see Table 6); ii) more severe inflammatory depressive symptoms in the "inflamed depression" patient group (CRP<3) compared to the uninflamed depression group (CRP>3), as well as higher scores on the IL6-IL8-VitD component (see Table 7 and Figure 10).

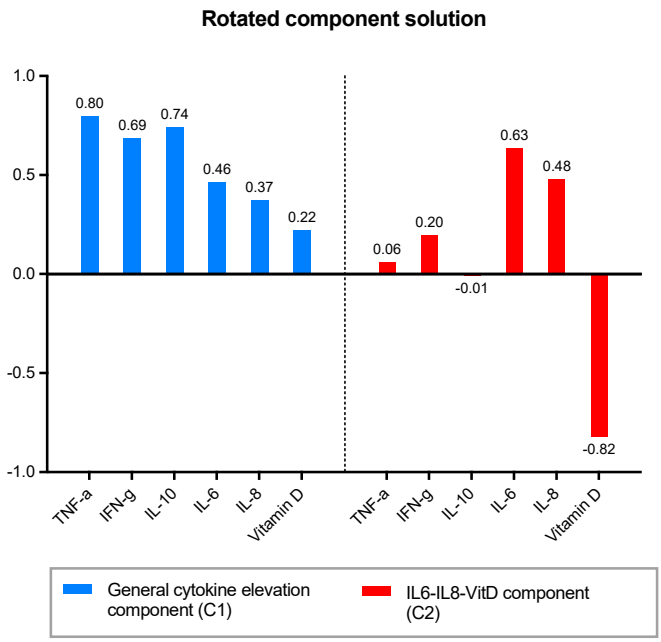


Figure 9. Rotated component solution extracted by principal component analysis. Rotation method: Varimax with Kaiser normalization. Rotation converged in three iterations. Ln-transformed z-scores were used for all included biomarkers (IFN-g, TNF-α, IL-10, IL-6, IL-8, and total vitamin D levels). A solution of two components was chosen, explaining 56.7% of the variance before and after rotation. The extracted components 1 (C1) and 2 (C2) explained 34.2% and 22.5% of the variance, respectively, after rotation. All of the chosen components had Eigenvalues >1. All cytokines except vitamin D loaded positively on C1. Vitamin D loaded negatively on C2. IL-6 and IL-8 loaded positively on both C1 and C2, although their loadings were higher on the latter factor.

Table 6.
Demographic characteristics and biomarkers in patients vs. controls

	PATIENTS	CONTROLS	p-value
PATIENTS, N	263	46	---
age, mean (SD)	37.9 (13.3)	36.0 (12.7)	0.4a
bmi, mean (SD)	26.3 (5.2)	23.8 (4.1)	<0.001**a
gender, female %	66.9	71.7	0.6b
smoking, everyday %	19.2	6.5	0.04*b
BIOMARKERS			
IFN-g (pg/mL), mean (SD)	5.53 (5.5)	6.25 (7.95)	0.97
TNF-α (pg/mL), mean (SD)	0.98 (0.4)	0.96 (0.47)	0.56
IL-6 (pg/mL), mean (SD)	0.73 (0.7)	0.40 (0.29)	0.001**
IL-8 (pg/mL), mean (SD)	2.91 (1.6)	2.21 (0.86)	0.001**
IL-10 (pg/mL), mean (SD)	0.29 (0.4)	0.24 (0.26)	0.26
CRP (mg/L), mean (SD)	2.08 (3.5)	---	

Raw data on biomarkers are presented, yet independent samples t-tests were performed for comparison with controls, using ln-transformed values to ensure normal distribution. Data was missing for patients on BMI (n=11), and smoking (both n=2). CRP was not available for the healthy controls. a) Mann-Whitney U-test, b) Pearson's chi-square test.
Abbreviations: Standard deviations (SD), Body Mass Index (BMI), Interferon-gamma (IFN-g), Tumor Necrosis Factor-alpha (TNF-α), interleukin (IL), and high-sensitive C-reactive protein (hs-CRP).
* Significant at the 0.05 level (2-tailed)
** Significant at the 0.01 level (2-tailed)

Table 7.
Demographic and clinical characteristics of patients with uninflamed and inflamed depression

	Uninflamed depression	Inflamed depression	p-value
PATIENTS, n	212	51	---
Age, mean (SD)	37.4 (13.2)	40.0 (14.0)	0.19[a]
BMI, mean (SD)	25.1 (4.2)	31.3 (5.7)	0.001**[a]
gender, female %	65.1	74.5	0.2[b]
smoking, everyday %	17.1	27.5	0.09[b]
MADRS, mean (SD)	19.6 (7.5)	21.0 (7.8)	0.19 [a]
SUAS, mean (SD)	32.6 (12.4)	32.8 (13.2)	0.94 [a]
INFL-DEP, mean (SD)	5.3 (2.3)	6.1 (2.2)	0.022*[a]

Characteristics of uninflamed vs. inflamed depression. Data were missing in the uninflamed depression group for BMI, n=9; smoking status, n=2; MADRS, n=9; SUAS, n = 10; Infl-Dep, n=9; and inflamed depression group for BMI, n=2; and SUAS, n = 1. Comparisons were carried out by Mann-Whitney U-test [a] or Pearson's chi-square [b].
Abbreviations: Standard deviations (SD), body mass index (BMI), interferon-gamma (IFN-γ, tumor necrosis factor-alpha (TNF-α), Interleukin (IL), C-reactive protein (CRP), Montgomery-Åsberg Depression Rating Scale (MADRS), inflammation-depression score (Infl-Dep), Suicide Assessment Scale (SUAS).
* Significant at the 0.05 level (2-tailed)
** Significant at the 0.01 level (2-tailed)

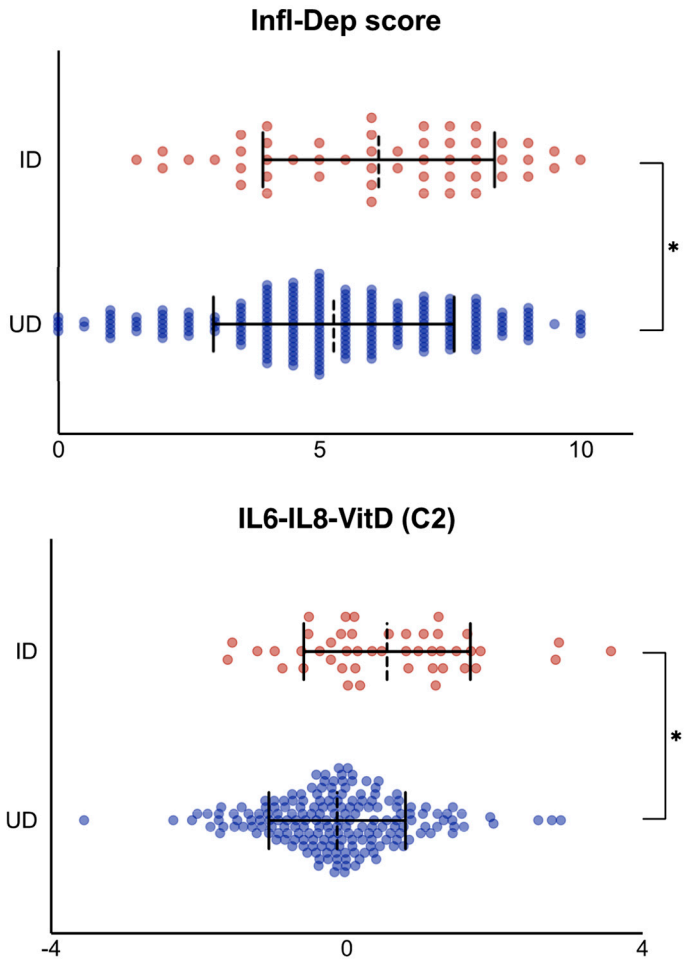


Figure 10. Significant differences between inflamed and uninflamed depression groups; Infl-Dep score (t l) and IL6-IL8-VitD component t r). Individual values were plotted together with the mean and standard deviation. Abbreviations: inflamed depression (ID) and uninflamed depression (UD). * Significant at the 0.05 level (2-tailed).

Discussion

The overall aim of this thesis was to explore the relationship between vitamin D levels and different aspects of depression and suicidality. So, is there actually a connection between vitamin D and these conditions, according to the results of the thesis?

Table 8. Summary of the four Studies included in this thesis

	Study I	**Study II**	**Study III**	**Study IV**
Design	Cross-sectional	Cross-sectional	Cross-sectional	Cross-sectional
Study period	2001–2008	2011–2015	2012–2021	2012–2021
Recruitment	Case I: Medical intensive care unit at Lund University Hospital Case II: Psychiatric Clinic, Lund University Hospital, Sweden	Clinical referrals to the University of California, San Francisco	Four secondary psychiatric clinics in the Scania region, southern Sweden	Four secondary psychiatric clinics in the Scania region, southern Sweden
Sample	Case I: "recent suicide attempters" (various Axis-I diagnoses) Case II: patients with clinical moderate-to-severe MDD	Outpatients with clinical mild-to-moderate MDD	Patients with "difficult-to-treat" depression	Patients with "difficult-to-treat" depression
Sample in main analysis (n)	Case I: 59 Case II: 17	48	202	263
Inflammatory markers	IL-6, TNF-α, IL-1β	IL-6, TNF-α, WBC, NLR	n/a	IL-6, TNF-α, CRP, IFN-γ, IL-10, IL-8, IL-13, IL-2
Main findings	Suicidal patients had significantly lower vitamin D levels than both non-suicidal depressed patients and healthy controls. Significant negative association between vitamin D and inflammatory markers in patients but not in controls.	Significant negative association between vitamin D and inflammatory markers in depressed patients, but not in controls.	Patients with "difficult-to-treat" depression had significantly lower vitamin D levels than the healthy controls. No significant differences in vitamin D levels between different groups of affective disorder diagnoses.	Significantly higher IL-6 and IL-8 levels in patients compared to controls. Patients with "inflamed depression" (CRP>3 mg/L) had more severe inflammatory depressive symptoms, and higher scores on a PCA extracted factor of increased IL-6 and IL-8 and lower vitamin D levels than patients in the "non-inflamed" depression group.

Vitamin D in depressive disorders (Studies I, II, and III)

The first question to answer is whether there is a difference in vitamin D levels between depressed individuals and mentally healthy individuals. This question was addressed in three of the four studies included in the thesis and yielded contradictory results. In Studies I and II, we did not find any significant difference in vitamin D levels between depressed subjects and healthy subjects, which was a bit surprising considering the previous literature (see Introduction). In Study III, however, a significant difference in vitamin D levels between depressed patients and healthy subjects was found, where patients with "difficult-to-treat" depression had significantly lower vitamin D levels than healthy individuals. The divergent results across these three studies are in line with similar inconsistencies within the field at large, though the main part of previous studies has shown lower vitamin D in depression. One explanation for our negative results in Studies I and II might be possible type II errors, i.e., a significant difference was present but could not be detected due to the small sample sizes (n=17 and n=48, respectively). In Study III, we had access to a significantly larger study cohort (n=202), and indeed, a significant difference between the depressed patient group and the healthy controls was found. This result is more in accordance with most of the previous literature, where an association between low vitamin D levels and depression has been suggested. I believe that these results are the most reliable among the three studies in the thesis related to the question of low vitamin D in depression, owing to the larger sample size. However, since Study III investigated a different type of depressed patient (i.e., difficult-to-treat), it might be that low vitamin D levels are more relevant in patients with persistent depressive symptoms, e.g., a chronic depression illness course or dysthymia, or possibly connected with treatment resistance. In all studies, we also looked at correlations between vitamin D levels and the severity of depressive symptoms (e.g., MADRS scores), but in general, no such significant relationships were found. However, when investigating the different diagnosis groups separately in Study III (*MDD single episode, chronic MDD, recurrent MDD, and dysthymia*), vitamin D was shown to correlate significantly and negatively with MADRS and SUAS total scores in patients with dysthymia. This has not previously been reported in other studies, but it might be a result of type 1 errors due to multiple comparisons and should therefore be interpreted with caution.

There are several important confounders to consider within the three studies and in studies on vitamin D and depression in general. Our main vitamin D source comes from sun exposure; thus, several symptoms of depression, e.g., isolation, and inactivity, might lead to sun-avoiding behavior, which could have affected our results. Also, a high BMI, age, female gender, and smoking are also associated with both lower vitamin D levels and depression. In the three studies, all results regarding vitamin D levels were adjusted for BMI, age, gender, and smoking. We did not have access to any data on the actual amount of sun exposure in any of the studies, which

is also lacking in most studies within the field due to the difficulties in assessing this factor. However, all results were adjusted for sampling season, which we consider to be a surrogate marker, though of lower reliability than the actual amount of sun exposure, of high vs. low possibilities of vitamin D production via the skin, and this did not change any of the results. It is important to note that the cross-sectional design of the three studies in the thesis does not allow any conclusions on causality, i.e., we are not able to determine if vitamin D is a direct biological cause of depression, or a consequence of other factors related to the depression itself.

In future studies on vitamin D and depression, my suggestion, based on the results of this thesis, is to study patients with long-lasting depressive symptoms or insufficient treatment responses *separately* from those without. To determine whether vitamin D supplementation might be of clinical value or not, well-designed RCT's and longitudinal studies must be implemented (125). In conclusion, together with the results from Study III and the previous literature, there is a moderate connection between low levels of vitamin D and depressive disorders.

The relationship between vitamin D and inflammation in depressive disorders and suicidality (Studies I, II, and IV)

The studies investigating the relationship between vitamin D and inflammation in this thesis consistently showed a significant association between low vitamin D levels and high inflammation in depressed patients, but not in healthy subjects. A variety of inflammatory markers have been investigated in these studies, and the included markers vary somewhat between studies. Most markers have shown a significant negative association with vitamin D, but some other inflammatory markers have not shown any significant correlation. For example, in Study I, IL-6 correlated significantly and negatively with vitamin D in depressed subjects, but no significant correlation was seen between vitamin D and IL-1β or TNF-α. In Study II, there were significant negative correlations between vitamin D and the inflammation composite score (IL-6 and TNF-α) in MDD patients and, additionally, to NLR and WBC (two markers previously associated with systemic, low-grade inflammation) (102), especially in MDD patients with SI. However, in Study IV, only IL-6 showed an association with vitamin D out of the investigated cytokines (IFN-g, TNF-α, IL-10, IL-8), and IL-6 correlated significantly and negatively with vitamin D in all patients. Hence, the most consistent finding across the three studies in the thesis is the negative correlation between vitamin D and cytokine IL-6. This is an interesting finding since, according to previous literature, IL-6 is one of the cytokines most consistently associated with depression, along with TNF-α and CRP (29, 138). To date, only a few studies have explored the relationship between vitamin D and inflammation in depressed patients, although a link between low vitamin D and inflammation has been known for decades. Moreover, despite the fact that the hypothesis of low vitamin D as a potential driver of the low-grade inflammation seen in some depressed individuals has been suggested before (150),

to the best of our knowledge, no previous studies have addressed this question in a psychiatric clinical sample prior to the publication of Study I. In a recent study that included a community sample (120), Dogan-Sander et al. sought to examine whether inflammation acts as a moderator or mediator of the association between vitamin D and depressive symptomatology. The study included more than 7,000 community-dwelling subjects. Depression symptomatology was measured by the self-rating scale Center of Epidemiologic Studies Depression Scale (CES-D), and the investigated inflammatory markers were IL-6, CRP, and WBC. First, vitamin D correlated negatively with all investigated inflammatory markers; however, after adding covariates to the model, the results only remained significant for IL-6 and WBC, which is in line with our results. Also, mediator models showed a direct effect of vitamin D on depressive symptom scores. Second, vitamin D had an indirect effect on depression symptomatology, partially mediated by inflammation, but this could only be seen for WBC, and none of the other inflammatory markers moderated the effect of vitamin D on depressive symptoms. It is interesting to see parts of our results being replicated, even in a study sample that differs significantly from ours.

In Study I, since we only saw decreased levels of vitamin D in patients who had recently attempted suicide but not in depressed patients, and since correlations between inflammatory markers and vitamin D were stronger in patients with SI in Study II, we hypothesized that low levels of vitamin D and increased inflammation could moderate depression to depression with significant SI. However, this hypothesis was refuted by the results from Study III and Study IV; instead, our results point to a connection between vitamin and inflamed depression.

While studying associations between vitamin D and IL-6 in depressed individuals, there is an important confounder to consider, namely, a high BMI. Obesity is associated with both lower vitamin D levels and increased inflammation. In all three studies of the thesis, patients showed higher BMI than healthy subjects, although not always significant. In the studies, none of the correlation analyses were adjusted for BMI; hence, there is a possibility that low vitamin D is not the driver of inflammation *per se*, but both factors may be related to a third factor, namely, BMI. On the other hand, there is a mechanistic link between low vitamin D and inflammation, as several experimental and clinical studies have shown that vitamin D decreases IL-6 levels through vitamin D-related mechanisms within the adaptive immune system (10, 12, 80). Interestingly, in a recent in vitro study, Nimitphong et al. showed that treatment with both active vitamin D and vitamin D ($25[OH]D_3$) inhibit inflammatory pathways in adipose tissue (169), and IL-6 was one of several inflammatory makers that decreased following vitamin D treatment. Similar results were shown by investigators prior to Nimitphong et al. (170, 171). In general, the positive anti-inflammatory effects of vitamin D have been hard to demonstrate in human studies, and results from RCT's in different somatic illnesses are inconsistent. However, in a recently published study, Sharifan et al. conducted a well-designed double-blinded RCT on the effect of vitamin D treatment on

inflammatory markers in obese individuals. They found that the counts of several blood cells linked to the immune system (NLR, PLR, and RDW to platelet ratio [RPR]) were significantly reduced in the vitamin D treatment group but not in the control group (172).

In summary, together with the results from Studies I, II, and IV, and previous literature, there are many indices linking low vitamin D levels to increased inflammation in patients with depressive syndromes, especially increased IL-6 levels. Since there are several different markers of inflammation, with different roles other than classical inflammation, future studies should consider other possible causes of the increased inflammatory markers seen in depressed individuals, such as obesity, bone remodeling, and response to muscular contractions, among others.

The relationship between vitamin D and specific symptoms of depression: i) "suicidal depression" (Studies I, II, and III)

The relationship between vitamin D and symptoms of suicidality was addressed in three of the four studies included in the thesis. In Study I, patients with a recent suicide attempt (diverse psychiatric diagnoses) had significantly lower vitamin D levels than both depressed patients without a recent suicide attempt and healthy controls. There is a possibility, though, that the lower vitamin D levels in the suicide attempt group are a consequence of a more severe psychiatric disorder (i.e., increased isolation), since there is a known connection between the severity of depression and the risk of a suicide attempt. Severely ill patients might also be less likely to engage in outdoor activities, and, as mentioned before, lack of sun exposure leads to lower vitamin D levels. Sadly, it is not possible to adjust our results for this factor. The results in Studies I and II point to an association between vitamin D and suicidality since we found a significant negative relationship between vitamin D and inflammatory markers in a cohort of MDD subjects and in subjects with mixed psychiatric diagnoses with a recent suicide attempt. In Study II, we also found a significant interaction effect between MDD status (i.e., SI or NSI) of the relationship between vitamin D and NLR and between vitamin D and WBC; hence it is possible that vitamin D and inflammation is especially linked to suicidality rather than depression. Our findings suggest that low vitamin D is associated with a pro-inflammatory state, frequently observed in depressed and suicidal individuals, and that this relationship between vitamin D and inflammation may be differentially regulated in MDD subjects with or without SI, suicidal individuals, and healthy controls.

However, in the first two studies, the study samples were small. In Study III, which was specifically designed to investigate possible connections between vitamin D and suicidality, all analyses were negative. Thus, in all, our results do not support that vitamin D plays a significant role in relation to different aspects of suicidality. It is difficult to investigate the mechanisms of suicidal behavior from depression separately, since these two conditions interact, and the outcome of suicidal thoughts

depends on a host of other external environmental factors. For example, a severe depression might come together with suicidal thoughts, but for some individuals, a suicide attempt is impossible due to considerations of relatives (sense of belonging) or inability to enact lethal self-injury, etc. (173). In future studies, it is therefore important to simultaneously consider many aspects of suicidal behavior and include patients with suicidal thoughts from mixed diagnostic groups.

The relationship between vitamin D and specific symptoms of depression: ii) "inflamed depression" (Study IV)

In Study IV, we used a different approach to identify individuals with long-lasting "difficult-to-treat" depression who might express an inflammatory depression subtype (inflamed depression) with specific symptom characteristics associated with inflammation, which in turn might be related to vitamin D. The results from Study IV are, as interpreted by me, of greater significance than the previous results presented herein, firstly due to the significantly larger sample sizes than in the other studies included in the thesis (Studies I and II), and secondly, due to the novelty of the results.

We found that patients with low-grade inflammation, the "inflamed depression" group (as defined by a hs-CRP value >3 mg/L), had significantly higher levels of the infl-dep score compared to the uninflamed patient group, i.e., patients with inflamed depression expressed more inflammatory depressive symptoms than the uninflamed patient group, but we did not find any between-group differences in overall severity of depressive symptoms. Most interestingly, a PCA showed an association between inflamed depression and higher IL-6, and IL-8 and low vitamin D levels (IL6-IL8-VitD component). Few studies to date have investigated the relationship between specific depressive symptoms and inflammation (25, 27, 28, 174), and our results are in line with previous reports of a link between peripheral hs-CRP levels and a specific depressive symptom profile, including, for example, inability to feel, lassitude, fatiguability, changes in appetite, and reduced or increased sleep. However, most of these previous studies used population-based cohorts (25, 27, 28), while we included clinically well-characterized patients in specialized psychiatric care who had long-lasting and more severe psychiatric symptoms.

The results point to a connection between low vitamin D levels, inflammation, and symptoms of depression related to an inflammatory state in the body, and this relationship has, to the best of our knowledge, never been studied before. Further studies investigating the relationship between low-grade inflammation and specific symptoms of depression should therefore also include vitamin D, together with traditional inflammatory markers, to deepen the knowledge of the connection between low levels of vitamin D, increased inflammation, and symptoms of inflamed depression.

General limitations

In this thesis, I aimed to better understand the connection between vitamin D and depressive symptomology and explored inflammation as a possible link between vitamin D in depressive disorders and different aspects of suicidality. All studies in the thesis were based on clinical samples, where most subjects had moderate-to severe psychiatric symptoms. Thus, generalizability in populations with milder depressive symptoms is limited.

The studies included in the thesis were of a cross-sectional design; therefore, no assumptions regarding causality can be drawn. We do not yet know if low vitamin D in patients with severe psychiatric symptoms (in this case, patients with a recent suicide attempt and patients with "difficult-to-treat" depression) is an antecedent risk factor, a correlate to the illness, or a consequence of lifestyle factors relating to the illness itself. Some studies have suggested that the inflammatory state seen in depressed and suicidal individuals *per se* causes low vitamin D levels (175, 176). However, longitudinal, and prospective studies are highly warranted to understand the potential causal role of vitamin D in depressive disorders and suicidality, including the complex relationship between vitamin D and inflammation in psychiatric illnesses.

For the future

The group of "depressed individuals" is highly heterogeneous; thus, I insist that we must move on from cross-sectional studies where vitamin D levels between depressed individuals and healthy controls are compared. It is time to go further, and deeper, and investigate up-stream and down-stream biological effects connected with vitamin D, as well as genetic predispositions to vitamin D deficiency, which subsequently might be connected to depression and/or suicidality. Therefore, due to the heterogeneity of the group "depressed individuals," one must start to look for subgroups within this large group that might also go beyond the borders of clinical diagnoses. Referring to the results in my thesis, one of these groups could be patients with inflamed depression. It is possible that some depressed individuals may benefit from vitamin D treatment alone or as an add-on treatment to conventional antidepressant treatment. Although speculative and a question for future research; there is a possibility that vitamin D supplementation might attenuate inflammation in MDD individuals with low vitamin D levels, which would be of importance since inflammation may underlie certain medical comorbidities in MDD and may foster antidepressant resistance (29, 177).

Another question to bring into the future is the vast differences in methodology between studies on vitamin D and depression (and sometimes non-relevant study designs) in current research, that makes it hard to draw reliable conclusions on the subject (112). For example, in a systemic review and meta-analysis of seven RCTs on the effect of vitamin D supplements on depressive symptoms, Shaffer et al. reported that five of the included studies either did not specifically recruit participants with depression or excluded those with elevated depressive symptoms, depressive disorder and/or current antidepressant use (122). Hence, these studies investigated the effect of vitamin D treatment on depressive symptoms in subjects who either had no depressive disorder or minimal non-clinically significant depressive symptoms. All five studies yielded negative results, while sub analyses of the two remaining studies, that recruited patients with clinically significant depressive symptoms and/or major depressive disorder, showed a significant (moderate) decrease in depressive symptoms after treatment. Other methodological problematic aspects within the field are diverse definitions of vitamin D deficiency/sufficiency, differences in defining and assessing depression/depressive symptoms, vitamin D levels upon inclusion or at end of treatment, treatment duration, and possibilities of adjusting for relevant confounders. Based on the

knowledge I have acquired during my thesis work, my suggestions for future research are to: i) study vitamin D deficient patients with severe depressive symptomatology, regardless of depressive disorder diagnosis ii) investigate upstream and downstream effects of vitamin D, that might be related to psychiatric illness, including a genetical approach while searching for underlying causes of possible disturbances in biological systems which are linked to vitamin D. By doing so, we might be able to identify possible subgroups of depressed individuals who may benefit from vitamin D treatment and hence, add one piece in the development of personalized medicine for depression.

Clinical relevance

To argue strongly for general vitamin D supplementation in depressed individuals, based on the studies in this thesis and current literature, is today premature. Nevertheless, it could be of interest to identify depressed individuals who are deficient in vitamin D, as it might point to a deeper depression severity, and work as, for example, a marker of isolation or a marker of a persistent illness course. Even if the evidence for a causal relationship between low vitamin D levels and depressive symptoms is not yet fully convincing, and treatment studies have shown inconsistent results to date, it is stated that vitamin D deficiency is overrepresented in psychiatric patients. Therefore, it is worth considering substituting depressed individuals up to at least sufficient vitamin D levels in cases where low vitamin D has been quantified, not to mention the known benefits of sufficient vitamin D levels for optimal somatic health, but also to avoid possible negative consequences on mental health.

Acknowledgements

Jag vill börja med att tacka **Åsa Westrin,** min första huvudhandledare som, genom sin generositet och stora intresse för suicidforskning, gav mig möjligheten att testa den hypotes jag så ivrigt och otåligt ville undersöka under mitt 'sommarforskande' 2012. Jag är innerligt tacksam över ditt omfattande stöd på olika sätt under hela min doktorandtid. Du trodde på mig, uppmuntrade min nyfikenhet och erbjöd mig att vara din doktorand. Utan allt du har bidragit med under de senaste 10 (!) åren hade jag aldrig kunnat disputera. Tack!

Daniel Lindqvist, min andra huvudhandledare, som tog vid under en period då jag tvivlade kring min forskning. Tack för att du ville bli min huvudhandledare och för allt du har lärt mig om inflammationens relation till depression och suicidalitet. Tack också för all värdefull input du har gett till min forskning och för att du sammanförde mig med Owen så att Studie II blev möjlig.

Ett stort tack också till **Johan Malm,** min bihandledare som stabilt har funnits med under hela resan. Tack för att du visade mig att D-vitamin har anti-inflammatoriska effekter i kroppen och därigenom bidrog till att jag fick för mig att det kanske var via inflammation som D-vitamin hängde ihop med depression, vilket i förlängningen ledde till avhandlingens tillblivelse. (Tack också för att du låter mig kolla mina D-vitaminnivåer titt som tätt…)

Tack **Lena Brundin,** min före detta bi-handledare, som också visade mig en viktig pusselbit till det som kom att bli min avhandling, nämligen att depression kan vara kopplat till inflammation. Tack för den inspiration din forskning har gett mig och för att du gjorde Studie I möjlig genom att så generöst dela med dig av patientmaterial till studien.

Tack **Filip Ventorp,** min bihandledare som kom in mot slutet av arbetet med avhandlingen. Att du ville vara med på denna resa och stötta mig i min forskning var a och o för att jag skulle återuppta doktorandstudierna efter Licentiatexamen. Din support, ditt stora intresse för biologisk psykiatrisk forskning och din inställning till forskning/doktorandstudier har hjälpt mig genom sista delen av avhandlingsarbetet. Det har känts väldigt tryggt att veta att jag har kunnat bolla alla de frågor jag haft, och även mina tvivel kring doktorandstudierna, med dig.

I also want to express my greatest gratitude to **Owen Wolkowitz,** whom I got the privilege to work with in Study II. Thank you for being so kind and generous, for

fruitful cooperation, and all your support in the work with Study II. I am looking forward to collaborate again with you in the future!

Jag vill också tacka **alla jag har stött på under min tid i forskningskorridoren vid psykiatrin i Lund**, som har inspirerat mig, hjälpt mig och uppmuntrat mig; professorer, forskare, doktorer, doktorander, sjuksköterskor, skötare och administrativ personal, alla har ni en del i att jag har nått ändra fram till disputationen. Jag vill särskilt lyfta fram, och ge ett extra stort tack till: **Lil Träskman-Benz**, **Sofie Westling**, **Mats Lindström**, **Anders Tingström**, **Agneta Öjehagen**, **Sara-Probert Lindström**, **Gustav Söderberg**, **Magnus Nilsson**, **Christina Thylander**, **Sofia Lundgren**, **Karin Mundt-Peterson Orre**, **Denise Bokander**, **Catharina Nygren**, **Linda Hansson**, **Lisette Jelivi**, **Brynja Gunnarsdottir**, **Anette Saltin** och **Åse Feltborg**.

Jag vill också tacka alla som har varit del av det omfattande arbetet med GEN-DS-studien för ett mycket givande samarbete: **Marie Asp**, **Amanda Holck**, **Johan Fernström**, **Livia Ambrus**, **Johan Ohlsson** och **Simon Ventorp**, och framför allt, **alla patienter** som så modigt och tålmodigt tog sig igenom många timmars utredning med ofantligt många självskattningsformulär. Ett särskilt stort tack vill jag också ge **Klara Sunesson** som, utöver att ha gjort ett fantastiskt bra arbete med Studie IV, har skapat flera av de bilder som finns med i min avhandling (Figure 1, 3 och 5).

Jag vill lyfta fram och tacka **Anders Håkansson** på Beroendecentrum i Malmö, som också har haft en avgörande del för att jag kunde avsluta mina doktorandstudier. Din stora flexibilitet vad gäller mitt arbete på beroendecentrum, under tiden jag har kombinerat min anställning på Region Skåne med doktorandstudier, har underlättat oerhört mycket för mig. Du har också pushat mig framåt i doktorandstudierna och motiverat mig till att disputera genom att peka på olika vägar som är möjliga att gå efter disputationen. Tack!

Jag vill också tacka de som har stöttat mig ekonomiskt under mina doktorandstudier: **Lunds universitet** för många 'doktorandmånader', **Förvaltning Psykiatri och Habiliterings** (interna forskningsmedel, Region Skåne), **stiftelsen Tornspiran**, **Stiftelsen Ellen och Henrik Sjöbrings Minnesfond** och **Stiftelsen O.M. Perssons Donationsfond**. Jag vill ge ett särskilt stort tack till **Fonden för psykisk hälsa** och **Suicide Zero**, för era generösa bidrag och uppmuntran av min forskning, samt för ert omfattande arbete med att minska stigmatiseringen av psykisk ohälsa och lyfta fram behovet av (mer) psykiatrisk forskning i Sverige.

Jag vill innerligt tacka min familj: **mamma, pappa, Johan** och **Murielle** och de nära vänner jag räknar till min familj: **Maria Nolvi, Marianne Eriksson** och **Hege Klausen,** för att jag alltid kan lita på att ni finns där för mig om jag behöver det, för att ni alltid tror på mig, är stolta över mig och hejar på mig, oavsett vad jag tar mig för.

Jag vill även tacka **min livspartner** och underbara mamma till Vidar. Du har förgyllt våra liv med din kärlek, humor, kreativitet, begåvning och stöd. Utan dig vid min sida hade det varit otroligt mycket svårare för mig att nå fram ända hit.

Till sist vill jag tacka dig**, Vidar**, min kloka son och största kärlek i livet**.** Du gav mig extra styrka, lugn och en push framåt inför slutarbetet med avhandlingen genom att säga *'mamma, det gör inget om du inte blir godkänd för du har jobbat jättemycket och jättebra med ditt arbete'*… Tack!

References

1. Beck A, Davis J, Frederick C, Perlin S, Schulman R, Wittlin R. Suicide Prevention in the 70s. Governmrnt Printing Office Washington. 1972.
2. Asberg M, Träskman L, Thorén P. 5-HIAA in the cerebrospinal fluid. A biochemical suicide predictor? Arch Gen Psychiatry. 1976;33(10):1193-7.
3. Paykel ES, Rassaby E. Classification of Suicide Attempters by Cluster Analysis. British Journal of Psychiatry. 1978;133(1):45-52.
4. Felger JC, Haroon E, Patel TA, Goldsmith DR, Wommack EC, Woolwine BJ, et al. What does plasma CRP tell us about peripheral and central inflammation in depression? Mol Psychiatry. 2020;25(6):1301-11.
5. Osimo EF, Baxter LJ, Lewis G, Jones PB, Khandaker GM. Prevalence of low-grade inflammation in depression: a systematic review and meta-analysis of CRP levels. Psychol Med. 2019;49(12):1958-70.
6. Shin YC, Jung CH, Kim HJ, Kim EJ, Lim SW. The associations among vitamin D deficiency, C-reactive protein, and depressive symptoms. J Psychosom Res. 2016;90:98-104.
7. Wium-Andersen MK, Ørsted DD, Nielsen SF, Nordestgaard BG. Elevated C-reactive protein levels, psychological distress, and depression in 73, 131 individuals. JAMA Psychiatry. 2013;70(2):176-84.
8. Foley É M, Parkinson JT, Kappelmann N, Khandaker GM. Clinical phenotypes of depressed patients with evidence of inflammation and somatic symptoms. Compr Psychoneuroendocrinol. 2021;8:100079.
9. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003;107(3):363-9.
10. Aranow C. Vitamin D and the immune system. J Investig Med. 2011;59(6):881-6.
11. Umar M, Sastry KS, Chouchane AI. Role of Vitamin D Beyond the Skeletal Function: A Review of the Molecular and Clinical Studies. Int J Mol Sci. 2018;19(6).
12. Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat Rev Immunol. 2008;8(9):685-98.
13. Hawton K, Casañas ICC, Haw C, Saunders K. Risk factors for suicide in individuals with depression: a systematic review. J Affect Disord. 2013;147(1-3):17-28.
14. Mann JJ, Apter A, Bertolote J, Beautrais A, Currier D, Haas A, et al. Suicide prevention strategies: a systematic review. Jama. 2005;294(16):2064-74.
15. World Health Organization W. Suicide worldwide in 2019: global health estimates. Geneva2021.

16. Socialstyrelsen. Vård vid depression och ångestsyndrom 2019, underlagsrapport. www.socialstyrelsen.se.
17. NASP. Nationellt Centrum för Suicidforskning och Prevention. https://ki.se/nasp.
18. Folkhälsomyndigheten(FHM). Om folksjukdomar. https://www.folkhalsomyndigheten.se/folkhalsorapportering-statistik/om-folksjukdomar/; 2021.
19. Kverno KS, Mangano E. Treatment-Resistant Depression: Approaches to Treatment. J Psychosoc Nurs Ment Health Serv. 2021;59(9):7-11.
20. Malhi GS, Mann JJ. Depression. Lancet. 2018;392(10161):2299-312.
21. Riese H, von Klipstein L, Schoevers RA, van der Veen DC, Servaas MN. Personalized ESM monitoring and feedback to support psychological treatment for depression: a pragmatic randomized controlled trial (Therap-i). BMC Psychiatry. 2021;21(1):143.
22. Willner P, Scheel-Krüger J, Belzung C. The neurobiology of depression and antidepressant action. Neurosci Biobehav Rev. 2013;37(10 Pt 1):2331-71.
23. Nemeroff CB. The State of Our Understanding of the Pathophysiology and Optimal Treatment of Depression: Glass Half Full or Half Empty? Am J Psychiatry. 2020;177(8):671-85.
24. Miller AH, Haroon E, Felger JC. Therapeutic Implications of Brain-Immune Interactions: Treatment in Translation. Neuropsychopharmacology. 2017;42(1):334-59.
25. Jokela M, Virtanen M, Batty GD, Kivimäki M. Inflammation and Specific Symptoms of Depression. JAMA Psychiatry. 2016;73(1):87-8.
26. Fried EI, von Stockert S, Haslbeck JMB, Lamers F, Schoevers RA, Penninx B. Using network analysis to examine links between individual depressive symptoms, inflammatory markers, and covariates. Psychol Med. 2020;50(16):2682-90.
27. Frank P, Jokela M, Batty GD, Cadar D, Steptoe A, Kivimäki M. Association Between Systemic Inflammation and Individual Symptoms of Depression: A Pooled Analysis of 15 Population-Based Cohort Studies. Am J Psychiatry. 2021;178(12):1107-18.
28. White J, Kivimäki M, Jokela M, Batty GD. Association of inflammation with specific symptoms of depression in a general population of older people: The English Longitudinal Study of Ageing. Brain Behav Immun. 2017;61:27-30.
29. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nature Reviews Immunology. 2016;16(1):22-34.
30. Diagnostic and statistical manual of mental disorders : DSM-IV: Fourth edition. Washington, DC : American Psychiatric Association, [1994] ©1994; 1994.
31. Richards D. Prevalence and clinical course of depression: A review. Clinical Psychology Review. 2011;31(7):1117-25.
32. Mueller TI, Leon AC, Keller MB, Solomon DA, Endicott J, Coryell W, et al. Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. Am J Psychiatry. 1999;156(7):1000-6.

33. Murphy JA, Byrne GJ. Prevalence and correlates of the proposed DSM-5 diagnosis of Chronic Depressive Disorder. J Affect Disord. 2012;139(2):172-80.
34. Hawton K, van Heeringen K. Suicide. The Lancet. 2009;373(9672):1372-81.
35. Probert-Lindström S, Öjehagen A, Ambrus L, Skogman Pavulans K, Berge J. Excess mortality by suicide in high-risk subgroups of suicide attempters: a prospective study of standardised mortality rates in suicide attempters examined at a medical emergency inpatient unit. BMJ Open. 2022;12(5):e054898.
36. Dumais A, Lesage AD, Alda M, Rouleau G, Dumont M, Chawky N, et al. Risk factors for suicide completion in major depression: a case-control study of impulsive and aggressive behaviors in men. Am J Psychiatry. 2005;162(11):2116-24.
37. Niméus A, Alsén M, Träskman-Bendz L. The suicide assessment scale: an instrument assessing suicide risk of suicide attempters. Eur Psychiatry. 2000;15(7):416-23.
38. Posner K, Brown GK, Stanley B, Brent DA, Yershova KV, Oquendo MA, et al. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. Am J Psychiatry. 2011;168(12):1266-77.
39. Whistler D. De morbopuerli anglorum, quem patrio ideiomate indigenase vocant “the rickets” (lugduni, batavorum 1645). Journal of the History of Medicine and Allied Sciences (1950) - As cited by Smerdon, GT, Daniel Whistler and the English Disease A translation and biographical note. 1645;5:397-415.
40. DeLuca HF. Vitamin D: Historical Overview. Vitam Horm. 2016;100:1-20.
41. McCollum EV, Davis M. THE NECESSITY OF CERTAIN LIPINS IN THE DIET DURING GROWTH. Journal of Biological Chemistry. 1913;15(1):167-75.
42. Matsui MS. Vitamin D Update. Current Dermatology Reports. 2020;9(4):323-30.
43. Battault S, Whiting SJ, Peltier SL, Sadrin S, Gerber G, Maixent JM. Vitamin D metabolism, functions and needs: from science to health claims. Eur J Nutr. 2013;52(2):429-41.
44. Neville JJ, Palmieri T, Young AR. Physical Determinants of Vitamin D Photosynthesis: A Review. JBMR Plus. 2021;5(1):e10460.
45. Pludowski P, Holick MF, Grant WB, Konstantynowicz J, Mascarenhas MR, Haq A, et al. Vitamin D supplementation guidelines. J Steroid Biochem Mol Biol. 2018;175:125-35.
46. Demer LL, Hsu JJ, Tintut Y. Steroid Hormone Vitamin D: Implications for Cardiovascular Disease. Circ Res. 2018;122(11):1576-85.
47. Gil Á, Plaza-Diaz J, Mesa MD. Vitamin D: Classic and Novel Actions. Ann Nutr Metab. 2018;72(2):87-95.
48. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. Am J Physiol Renal Physiol. 2005;289(1):F8-28.
49. Ramagopalan SV, Heger A, Berlanga AJ, Maugeri NJ, Lincoln MR, Burrell A, et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res. 2010;20(10):1352-60.

50. Trochoutsou AI, Kloukina V, Samitas K, Xanthou G. Vitamin-D in the Immune System: Genomic and Non-Genomic Actions. Mini Rev Med Chem. 2015;15(11):953-63.
51. Brown AJ, Dusso A, Slatopolsky E. Vitamin D. Am J Physiol. 1999;277(2):F157-75.
52. Cashman KD, Dowling KG, Škrabáková Z, Gonzalez-Gross M, Valtueña J, De Henauw S, et al. Vitamin D deficiency in Europe: pandemic? Am J Clin Nutr. 2016;103(4):1033-44.
53. Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Tmava Berisha A, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr. 2020;74(11):1498-513.
54. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? J Steroid Biochem Mol Biol. 2014;144 Pt A:138-45.
55. Daly RM, Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Sikaris KA, et al. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clin Endocrinol (Oxf). 2012;77(1):26-35.
56. Oleröd G, Hultén LM, Hammarsten O, Klingberg E. The variation in free 25-hydroxy vitamin D and vitamin D-binding protein with season and vitamin D status. Endocr Connect. 2017;6(2):111-20.
57. Wheeler BJ, Snoddy AME, Munns C, Simm P, Siafarikas A, Jefferies C. A Brief History of Nutritional Rickets. Frontiers in Endocrinology. 2019;10.
58. Wacker M, Holick MF. Sunlight and Vitamin D: A global perspective for health. Dermatoendocrinol. 2013;5(1):51-108.
59. Holick MF. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. Rev Endocr Metab Disord. 2017;18(2):153-65.
60. Cashman KD. Vitamin D Deficiency: Defining, Prevalence, Causes, and Strategies of Addressing. Calcif Tissue Int. 2020;106(1):14-29.
61. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010;376(9736):180-8.
62. Osmancevic A, Demeke T, Gillstedt M, Angesjö E, Sinclair H, Abd El-Gawad G, et al. Vitamin D treatment in Somali women living in Sweden - Two randomized, placebo-controlled studies. Clin Endocrinol (Oxf). 2016;85(4):535-43.
63. Livsmedelsverket. https://www.livsmedelsverket.se/livsmedel-och-innehall/naringsamne/vitaminer-och-antioxidanter/d-vitamin. Retrieved August 18, 2022.
64. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-30.
65. Sempos CT, Binkley N. 25-Hydroxyvitamin D assay standardisation and vitamin D guidelines paralysis. Public Health Nutr. 2020;23(7):1153-64.
66. Holick MF. Vitamin D Deficiency. New England Journal of Medicine. 2007;357(3):266-81.

67. Bikle DD. Vitamin D: Production, Metabolism and Mechanisms of Action. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc. Copyright © 2000-2022, MDText.com, Inc.; 2000.
68. Welsh J. Vitamin D and prevention of breast cancer. Acta Pharmacol Sin. 2007;28(9):1373-82.
69. Luxwolda MF, Kuipers RS, Kema IP, Janneke Dijck-Brouwer DA, Muskiet FAJ. Traditionally living populations in East Africa have a mean serum 25-hydroxyvitamin D concentration of 115 nmol/l. British Journal of Nutrition. 2012;108(9):1557-61.
70. Shaikh F, Baig S, Jamal Q. Do VDR Gene Polymorphisms Contribute to Breast Cancer? Asian Pac J Cancer Prev. 2016;17(2):479-83.
71. Taylor PN, Davies JS. A review of the growing risk of vitamin D toxicity from inappropriate practice. Br J Clin Pharmacol. 2018;84(6):1121-7.
72. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr. 1999;69(5):842-56.
73. Alshahrani F, Aljohani N. Vitamin D: deficiency, sufficiency and toxicity. Nutrients. 2013;5(9):3605-16.
74. Wacker M, Holick MF. Vitamin D - effects on skeletal and extraskeletal health and the need for supplementation. Nutrients. 2013;5(1):111-48.
75. Feige J, Moser T, Bieler L, Schwenker K, Hauer L, Sellner J. Vitamin D Supplementation in Multiple Sclerosis: A Critical Analysis of Potentials and Threats. Nutrients. 2020;12(3).
76. Mitri J, Pittas AG. Vitamin D and diabetes. Endocrinol Metab Clin North Am. 2014;43(1):205-32.
77. Latic N, Erben RG. Vitamin D and Cardiovascular Disease, with Emphasis on Hypertension, Atherosclerosis, and Heart Failure. Int J Mol Sci. 2020;21(18).
78. Zhang Y, Fang F, Tang J, Jia L, Feng Y, Xu P, et al. Association between vitamin D supplementation and mortality: systematic review and meta-analysis. Bmj. 2019;366:l4673.
79. Akutsu T, Kitamura H, Himeiwa S, Kitada S, Akasu T, Urashima M. Vitamin D and Cancer Survival: Does Vitamin D Supplementation Improve the Survival of Patients with Cancer? Curr Oncol Rep. 2020;22(6):62.
80. Sassi F, Tamone C, D'Amelio P. Vitamin D: Nutrient, Hormone, and Immunomodulator. Nutrients. 2018;10(11).
81. Zdrenghea MT, Makrinioti H, Bagacean C, Bush A, Johnston SL, Stanciu LA. Vitamin D modulation of innate immune responses to respiratory viral infections. Rev Med Virol. 2017;27(1).
82. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. Bmj. 2017;356:i6583.

83. Arshi S, Fallahpour M, Nabavi M, Bemanian MH, Javad-Mousavi SA, Nojomi M, et al. The effects of vitamin D supplementation on airway functions in mild to moderate persistent asthma. Ann Allergy Asthma Immunol. 2014;113(4):404-9.
84. Kumar R, Rathi H, Haq A, Wimalawansa SJ, Sharma A. Putative roles of vitamin D in modulating immune response and immunopathology associated with COVID-19. Virus Res. 2021;292:198235.
85. Caccamo D, Ricca S, Currò M, Ientile R. Health Risks of Hypovitaminosis D: A Review of New Molecular Insights. Int J Mol Sci. 2018;19(3).
86. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. Curr Opin Pharmacol. 2010;10(4):482-96.
87. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. Endocrinol Metab Clin North Am. 2010;39(2):365-79, table of contents.
88. Heaney RP. Vitamin D in health and disease. Clin J Am Soc Nephrol. 2008;3(5):1535-41.
89. Eyles DW, Burne TH, McGrath JJ. Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. Front Neuroendocrinol. 2013;34(1):47-64.
90. Harms LR, Burne TH, Eyles DW, McGrath JJ. Vitamin D and the brain. Best Pract Res Clin Endocrinol Metab. 2011;25(4):657-69.
91. Cui X, Gooch H, Petty A, McGrath JJ, Eyles D. Vitamin D and the brain: Genomic and non-genomic actions. Mol Cell Endocrinol. 2017;453:131-43.
92. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. Journal of chemical neuroanatomy. 2005;29(1):21-30.
93. Puchacz E, Stumpf WE, Stachowiak EK, Stachowiak MK. Vitamin D increases expression of the tyrosine hydroxylase gene in adrenal medullary cells. Brain research Molecular brain research. 1996;36(1):193-6.
94. Patrick RP, Ames BN. Vitamin D hormone regulates serotonin synthesis. Part 1: relevance for autism. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2014;28(6):2398-413.
95. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. J Steroid Biochem Mol Biol. 2005;97(1-2):93-101.
96. Antai-Otong D. Vitamin D: an anti-inflammatory treatment option for depression? Issues Ment Health Nurs. 2014;35(3):227-34.
97. Wichers M, Maes M. The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. Int J Neuropsychopharmacol. 2002;5(4):375-88.
98. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. Journal of immunology (Baltimore, Md : 1950). 2012;188(5):2127-35.
99. Pape K, Tamouza R, Leboyer M, Zipp F. Immunoneuropsychiatry - novel perspectives on brain disorders. Nat Rev Neurol. 2019;15(6):317-28.

100. Lee PW, Selhorst A, Lampe SG, Liu Y, Yang Y, Lovett-Racke AE. Neuron-Specific Vitamin D Signaling Attenuates Microglia Activation and CNS Autoimmunity. Front Neurol. 2020;11:19.
101. Boontanrart M, Hall SD, Spanier JA, Hayes CE, Olson JK. Vitamin D3 alters microglia immune activation by an IL-10 dependent SOCS3 mechanism. Journal of neuroimmunology. 2016;292:126-36.
102. Imtiaz F, Shafique K, Mirza SS, Ayoob Z, Vart P, Rao S. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. Int Arch Med. 2012;5(1):2.
103. Cui X, McGrath JJ, Burne THJ, Eyles DW. Vitamin D and schizophrenia: 20 years on. Mol Psychiatry. 2021;26(7):2708-20.
104. Roy NM, Al-Harthi L, Sampat N, Al-Mujaini R, Mahadevan S, Al Adawi S, et al. Impact of vitamin D on neurocognitive function in dementia, depression, schizophrenia and ADHD. Front Biosci (Landmark Ed). 2021;26(3):566-611.
105. Patrick RP, Ames BN. Vitamin D and the omega-3 fatty acids control serotonin synthesis and action, part 2: relevance for ADHD, bipolar disorder, schizophrenia, and impulsive behavior. Faseb j. 2015;29(6):2207-22.
106. Patrick RP, Ames BN. Vitamin D hormone regulates serotonin synthesis. Part 1: relevance for autism. The FASEB Journal. 2014;28(6):2398-413.
107. Menon V, Kar SK, Suthar N, Nebhinani N. Vitamin D and Depression: A Critical Appraisal of the Evidence and Future Directions. Indian J Psychol Med. 2020;42(1):11-21.
108. Ju SY, Lee YJ, Jeong SN. Serum 25-hydroxyvitamin D levels and the risk of depression: a systematic review and meta-analysis. J Nutr Health Aging. 2013;17(5):447-55.
109. Anglin RE, Samaan Z, Walter SD, McDonald SD. Vitamin D deficiency and depression in adults: systematic review and meta-analysis. Br J Psychiatry. 2013;202:100-7.
110. Zhao G, Ford ES, Li C, Balluz LS. No associations between serum concentrations of 25-hydroxyvitamin D and parathyroid hormone and depression among US adults. Br J Nutr. 2010;104(11):1696-702.
111. Dana-Alamdari L, Kheirouri S, Noorazar SG. Serum 25-Hydroxyvitamin D in Patients with Major Depressive Disorder. Iran J Public Health. 2015;44(5):690-7.
112. Spedding S. Vitamin D and depression: a systematic review and meta-analysis comparing studies with and without biological flaws. Nutrients. 2014;6(4):1501-18.
113. Hoogendijk WJ, Lips P, Dik MG, Deeg DJ, Beekman AT, Penninx BW. Depression is associated with decreased 25-hydroxyvitamin D and increased parathyroid hormone levels in older adults. Arch Gen Psychiatry. 2008;65(5):508-12.
114. Milaneschi Y, Hoogendijk W, Lips P, Heijboer AC, Schoevers R, van Hemert AM, et al. The association between low vitamin D and depressive disorders. Mol Psychiatry. 2014;19(4):444-51.
115. Bertone-Johnson ER. Vitamin D and the occurrence of depression: causal association or circumstantial evidence? Nutr Rev. 2009;67(8):481-92.

116. Kim SY, Jeon SW, Lim WJ, Oh KS, Shin DW, Cho SJ, et al. Vitamin D deficiency and suicidal ideation: A cross-sectional study of 157,211 healthy adults. J Psychosom Res. 2020;134:110125.
117. Grudet C, Malm J, Westrin A, Brundin L. Suicidal patients are deficient in vitamin D, associated with a pro-inflammatory status in the blood. Psychoneuroendocrinology. 2014;50:210-9.
118. Grudet C, Wolkowitz OM, Mellon SH, Malm J, Reus VI, Brundin L, et al. Vitamin D and inflammation in major depressive disorder. J Affect Disord. 2020;267:33-41.
119. He Y, Wu Z, Lan T, Wang Y, Tian Y, Chen X, et al. The 25(OH)D/VDR signaling may play a role in major depression. Biochem Biophys Res Commun. 2020;523(2):405-10.
120. Dogan-Sander E, Mergl R, Willenberg A, Baber R, Wirkner K, Riedel-Heller SG, et al. Inflammation and the Association of Vitamin D and Depressive Symptomatology. Nutrients. 2021;13(6).
121. Parker GB, Brotchie H, Graham RK. Vitamin D and depression. J Affect Disord. 2017;208:56-61.
122. Shaffer JA, Edmondson D, Wasson LT, Falzon L, Homma K, Ezeokoli N, et al. Vitamin D supplementation for depressive symptoms: a systematic review and meta-analysis of randomized controlled trials. Psychosom Med. 2014;76(3):190-6.
123. Sepidarkish M, Farsi F, Akbari-Fakhrabadi M, Namazi N, Almasi-Hashiani A, Maleki Hagiagha A, et al. The effect of vitamin D supplementation on oxidative stress parameters: A systematic review and meta-analysis of clinical trials. Pharmacol Res. 2019;139:141-52.
124. Mozaffari-Khosravi H, Nabizade L, Yassini-Ardakani SM, Hadinedoushan H, Barzegar K. The effect of 2 different single injections of high dose of vitamin D on improving the depression in depressed patients with vitamin D deficiency: a randomized clinical trial. J Clin Psychopharmacol. 2013;33(3):378-85.
125. Okereke OI, Reynolds CF, 3rd, Mischoulon D, Chang G, Vyas CM, Cook NR, et al. Effect of Long-term Vitamin D3 Supplementation vs Placebo on Risk of Depression or Clinically Relevant Depressive Symptoms and on Change in Mood Scores: A Randomized Clinical Trial. Jama. 2020;324(5):471-80.
126. Kumar PNS, Menon V, Andrade C. A randomized, double-blind, placebo-controlled, 12-week trial of vitamin D augmentation in major depressive disorder associated with vitamin D deficiency. J Affect Disord. 2022;314:143-9.
127. Grudet C, Lindqvist D, Malm J, Westrin Å, Ventorp F. 25(OH)D levels are decreased in patients with difficult-to-treat depression. Comprehensive Psychoneuroendocrinology. 2022;10:100126.
128. Gokalp G. The association between low vitamin D levels and suicide attempts in adolescents. Ann Clin Psychiatry. 2020;32(2):106-13.
129. Fond G, Faugere M, Faget-Agius C, Cermolacce M, Richieri R, Boyer L, et al. Hypovitaminosis D is associated with negative symptoms, suicide risk, agoraphobia, impaired functional remission, and antidepressant consumption in schizophrenia. Eur Arch Psychiatry Clin Neurosci. 2019;269(8):879-86.

130. Umhau JC, George DT, Heaney RP, Lewis MD, Ursano RJ, Heilig M, et al. Low vitamin D status and suicide: a case-control study of active duty military service members. PLoS One. 2013;8(1):e51543.
131. Wei YX, Liu BP, Qiu HM, Zhang JY, Wang XT, Jia CX. Effects of vitamin D-related gene polymorphisms on attempted suicide. Psychiatr Genet. 2021;31(6):230-8.
132. Tariq MM, Streeten EA, Smith HA, Sleemi A, Khabazghazvini B, Vaswani D, et al. Vitamin D: a potential role in reducing suicide risk? 2011;23(3):157-65.
133. Yagci I, Avci S. Biochemical predictors in presentations to the emergency department after a suicide attemp. Bratisl Lek Listy. 2021;122(3):224-9.
134. Calderón Espinoza I, Chavarria-Avila E, Pizano-Martinez O, Martínez-García EA, Armendariz-Borunda J, Marquez-Aguirre AL, et al. Suicide Risk in Rheumatoid Arthritis Patients is Associated With Suboptimal Vitamin D Levels. J Clin Rheumatol. 2022;28(3):137-42.
135. Raison CL, Miller AH. Is depression an inflammatory disorder? Curr Psychiatry Rep. 2011;13(6):467-75.
136. Kiecolt-Glaser JK, Derry HM, Fagundes CP. Inflammation: depression fans the flames and feasts on the heat. Am J Psychiatry. 2015;172(11):1075-91.
137. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008;9(1):46-56.
138. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. Biol Psychiatry. 2010;67(5):446-57.
139. Verduijn J, Milaneschi Y, Schoevers RA, van Hemert AM, Beekman AT, Penninx BW. Pathophysiology of major depressive disorder: mechanisms involved in etiology are not associated with clinical progression. Transl Psychiatry. 2015;5(9):e649.
140. Vasupanrajit A, Jirakran K, Tunvirachaisakul C, Solmi M, Maes M. Inflammation and nitro-oxidative stress in current suicidal attempts and current suicidal ideation: a systematic review and meta-analysis. Mol Psychiatry. 2022;27(3):1350-61.
141. Brundin L, Bryleva EY, Thirtamara Rajamani K. Role of Inflammation in Suicide: From Mechanisms to Treatment. Neuropsychopharmacology. 2017;42(1):271-83.
142. Beurel E, Toups M, Nemeroff CB. The Bidirectional Relationship of Depression and Inflammation: Double Trouble. Neuron. 2020;107(2):234-56.
143. Strawbridge R, Hodsoll J, Powell TR, Hotopf M, Hatch SL, Breen G, et al. Inflammatory profiles of severe treatment-resistant depression. J Affect Disord. 2019;246:42-51.
144. Gallagher D, Kiss A, Lanctot K, Herrmann N. Depression with inflammation: longitudinal analysis of a proposed depressive subtype in community dwelling older adults. Int J Geriatr Psychiatry. 2017;32(12):e18-e24.
145. Lucido MJ, Bekhbat M, Goldsmith DR, Treadway MT, Haroon E, Felger JC, et al. Aiding and Abetting Anhedonia: Impact of Inflammation on the Brain and Pharmacological Implications. Pharmacol Rev. 2021;73(3):1084-117.

146. Strawbridge R, Arnone D, Danese A, Papadopoulos A, Herane Vives A, Cleare AJ. Inflammation and clinical response to treatment in depression: A meta-analysis. Eur Neuropsychopharmacol. 2015;25(10):1532-43.
147. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med. 2009;71(2):171-86.
148. Tsai SJ. Role of interleukin 8 in depression and other psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2021;106:110173.
149. Rook GA. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: darwinian medicine and the 'hygiene' or 'old friends' hypothesis. Clin Exp Immunol. 2010;160(1):70-9.
150. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med. 2013;11:200.
151. Association WM. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013;310(20):2191-4.
152. Hallberg L, Janelidze S, Engstrom G, Wisén AG, Westrin A, Brundin L. Exercise-induced release of cytokines in patients with major depressive disorder. J Affect Disord. 2010;126(1-2):262-7.
153. Brundin L, Erhardt S, Bryleva EY, Achtyes ED, Postolache TT. The role of inflammation in suicidal behaviour. Acta Psychiatr Scand. 2015;132(3):192-203.
154. Hamilton M. A RATING SCALE FOR DEPRESSION. Journal of Neurology, Neurosurgery & Psychiatry. 1960;23(1):56-62.
155. Asp M, Lindqvist D, Fernström J, Ambrus L, Tuninger E, Reis M, et al. Recognition of personality disorder and anxiety disorder comorbidity in patients treated for depression in secondary psychiatric care. PLoS One. 2020;15(1):e0227364.
156. Dipietro L, Caspersen CJ, Ostfeld AM, Nadel ER. A survey for assessing physical activity among older adults. Med Sci Sports Exerc. 1993;25(5):628-42.
157. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59 Suppl 20:22-33;quiz 4-57.
158. First M B. Structured Clinical Interview for DSM-IV Axis I Disorders. Biometrics Research Department. 1997.
159. First MB, Gibbon M, Spitzer RL, Williams JB, Benjamin LS. Structured clinical interview for DSM-IV® axis ii personality disorders SCID-II: American Psychiatric Pub; 1997.
160. Asberg M, Schalling D. Construction of a new psychiatric rating instrument, the Comprehensive Psychopathological Rating Scale (CPRS). Prog Neuropsychopharmacol. 1979;3(4):405-12.
161. Asberg M, Montgomery SA, Perris C, Schalling D, Sedvall G. A comprehensive psychopathological rating scale. Acta Psychiatr Scand Suppl. 1978(271):5-27.

162. Stanley B, Träskman-Bendz L, Stanley M. The suicide assessment scale: a scale evaluating change in suicidal behavior. Psychopharmacol Bull. 1986;22(1):200-5.
163. Niméus A, Hjalmarsson Ståhlfors F, Sunnqvist C, Stanley B, Träskman-Bendz L. Evaluation of a modified interview version and of a self-rating version of the Suicide Assessment Scale. Eur Psychiatry. 2006;21(7):471-7.
164. Vang F. Brains, genes and environment of suicide attempters [Doctoral]. Lund, Sweden: Lund University; 2012.
165. Antoniucci DM, Black DM, Sellmeyer DE. Serum 25-hydroxyvitamin D is unaffected by multiple freeze-thaw cycles. Clin Chem. 2005;51(1):258-61.
166. Wielders JP, Wijnberg FA. Preanalytical stability of 25(OH)-vitamin D3 in human blood or serum at room temperature: solid as a rock. Clin Chem. 2009;55(8):1584-5.
167. Phinney KW, Tai SSC, Bedner M, Camara JE, Chia RRC, Sander LC, et al. Development of an Improved Standard Reference Material for Vitamin D Metabolites in Human Serum. Analytical Chemistry. 2017;89(9):4907-13.
168. Akbas EM, Gungor A, Ozcicek A, Akbas N, Askin S, Polat M. Vitamin D and inflammation: evaluation with neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio. Arch Med Sci. 2016;12(4):721-7.
169. Nimitphong H, Guo W, Holick MF, Fried SK, Lee MJ. Vitamin D Inhibits Adipokine Production and Inflammatory Signaling Through the Vitamin D Receptor in Human Adipocytes. Obesity (Silver Spring). 2021;29(3):562-8.
170. Wamberg L, Cullberg KB, Rejnmark L, Richelsen B, Pedersen SB. Investigations of the anti-inflammatory effects of vitamin D in adipose tissue: results from an in vitro study and a randomized controlled trial. Horm Metab Res. 2013;45(6):456-62.
171. Lorente-Cebrián S, Eriksson A, Dunlop T, Mejhert N, Dahlman I, Aström G, et al. Differential effects of 1α,25-dihydroxycholecalciferol on MCP-1 and adiponectin production in human white adipocytes. Eur J Nutr. 2012;51(3):335-42.
172. Sharifan P, Rashidmayvan M, Khorasanchi Z, Darroudi S, Heidari A, Hoseinpoor F, et al. Efficacy of low-fat milk and yogurt fortified with vitamin D3 on systemic inflammation in adults with abdominal obesity. Journal of Health, Population and Nutrition. 2022;41(1):8.
173. Van Orden KA, Witte TK, Cukrowicz KC, Braithwaite SR, Selby EA, Joiner TE, Jr. The interpersonal theory of suicide. Psychol Rev. 2010;117(2):575-600.
174. Fried EI, von Stockert S, Haslbeck JMB, Lamers F, Schoevers RA, Penninx BWJH. Using network analysis to examine links between individual depressive symptoms, inflammatory markers, and covariates. Psychol Med. 2020;50(16):2682-90.
175. Mangin M, Sinha R, Fincher K. Inflammation and vitamin D: the infection connection. Inflammation Research. 2014;63(10):803-19.
176. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol. 2014;2(1):76-89.
177. Haroon E, Daguanno AW, Woolwine BJ, Goldsmith DR, Baer WM, Wommack EC, et al. Antidepressant treatment resistance is associated with increased inflammatory markers in patients with major depressive disorder. Psychoneuroendocrinology. 2018;95:43-9.

Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences, Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2022:123
ISBN 978-91-8021-285-4
ISSN 1652-8220