Diss. ETH No. 28642

The role of nutrients in paediatric and perinatal depression: investigating n-3 polyunsaturated fatty acids, iodine and iron

A dissertation submitted to attain the degree of

DOCTOR OF SCIENCES of ETH ZURICH

(Dr. sc. ETH Zurich)

presented by

Ester Osuna

Msc in Health Science and Technology ETH Zurich

born 8th September 1993

citizen of Winterthur, ZH, Switzerland and Spain

accepted on the recommendation of

Prof. Dr. med. Michael B. Zimmermann, Examiner

Dr. Jeannine Baumgartner, Co-Examiner
Dr. Isabelle Herter-Aeberli, Co-Examiner
Dr. Jane Pei-Chen Chang, Co-Examiner

2022

Only in the darkness can you see the stars
- Martin Luther King Jr.

Acknowledgements

I am grateful to Prof. Dr. med. Michael Zimmermann for giving me the opportunity to perform my PhD at the Human Nutrition Laboratory, for the freedom to conduct my research project and guidance when asked for.

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My sincerest thanks to Dr. Jeannine Baumgartner and Dr. Isabelle Herter-Aeberli for your excellent supervision, valuable scientific inputs, and endless patience.

⋆⋆⋆

A special thank to Dr. med. Gregor Berger for your enthusiasm, motivation and making me feel appreciated. The devotion to your patients is inspiring.

⋆⋆⋆

Many thanks to Dr. Isabelle Häberling, Dr. Sophie Emery, Dr. Mona Albermann, Dr. Noemi Baumgartner for your patience in introducing me to psychological assessment methods, for always picking up the phone whenever I had a little question, and for your seeming endless energy and persevering when working with your patients.

⋆⋆⋆

I would like to thank Srikanthan Pakeerathan (Pakee) and the Hersberger Lab for your support and many laughters while working in the laboratory.

⋆⋆⋆

A special thanks to Fiona Stifter and Tiffany Lau for their audio recording support and designing of the Omega-3 Flyer

⋆⋆⋆

The financial contribution from the Ebnet Stiftung is gratefully acknowledged.

⋆⋆⋆

I wish to extend a particular thanks to all the mothers and adolescents who participated in our studies. Without you none of this science would have happened.

⋆⋆⋆

I am very grateful to Silvia Probst, Olivia Wunderlin, Sascha Pleus, Amelie Zacher, and Diego Salinas for their priceless energy and engagement during the recruitment of study participants.

⋆⋆⋆

A cordial thanks to all my collegues at the Laboratory of Human Nutrition. Christophe Zeder, Adam Krzystek, Timo Christ, and Verena Jaeger - the lab and me we cannot deprive you. Ambra Giorgetti and Hanna Von Siebenthal thank you for your friendship, tears, laughter and many teas we shared.

⋆⋆⋆

Thanks to Daniela, Belinda, Martina, and Philippe for all your love and your wonderful friendship.

⋆⋆⋆

Finally, I want to thank my parents Ruth & José, and my entire family Pablo, Jael, Rafael, Susi, Chloey and Aurelio. Thank you for all your support, encouragement, and love. Os quiero mucho. And Jan my private consultant, you don't annoy me too much.

Contents

Abbreviations

5-HT	Serotonin
AA	Arachidonic acid
ACTH	Adrenocorticotropic hormone
AGP	α-1-acid-glycoprotein
ALA	α-linoleic acid, 18:3n-3
APR	Acute phase reactants
ATP	Adenosine triphosphate
BBB	Blood-Brain-Barrier
BDNF	Brain-derived neurotrophic factor
BIS	Body iron stores
BMI	Body mass index
CDRS-R	Children's Depression Rating Scale-Revised
CHD	Coronary heart disease
CNS	Central nervous system
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
DA	Dopamine
D-A-CH	Germany-Austria-Switzerland region
DCYTB	Duodenal cytochrome b
DHA	Docosahexaenoic acid
DIT	Diiodotyrosine
DMT1	Divalent metal ion transporter 1
DPA	Docosapenaenoic acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
EFSA	European food safety authority
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
EPDS	Edinburgh Postnatal Depression Scale
ER	Endoplasmic reticulum
FA	Fatty acids
FAO	Food and Agriculture Organization
Fe	Iron
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric iron
FFQ	Food frequency questionnaire
GC	Glucocorticoids
GC/MS	Gas chromatography/ Mass spectometry
GLA	γ-linoleic acid, 18:3n-6
GR	Glucocorticoid receptors
Hb	Haemoglobin
HIC	High-income countries
HCP1	Heme iron transporter 1
HPA	Hypothalamic-pituitary-adrenal
HPT	Hypothalamic-pituitary-thyroid
H_2O_2	Hydrogen peroxide
ID	Iron deficiency
IDA	Iron deficiency anaemia
IDD	Iodine deficiency disorders
I-FABP	Intestinal fatty acid binding protein
I^-	Iodide
I_2	Elemental iodine
IO_3^-	Iodate
IDO1	Indoleamine 2,3-deoxygenase
IFN-γ	Interferon-γ
IQR	Interquartile range

K-SADS	Kiddie-Schedule for Affective Disorders and Schizophrenia
LA	Linoleic acid
LMIC	Low- and middle-income countries
MAO	Monoamine oxidase inhibitors
MDD	Major depressive disorder
M.I.N.I. KID	Mini-International Neuropsychiatric Interview for Children and Adolescents
MIT	Monoiodotyrosine
MUFA	Monounsaturated fatty acids
MS	Multiple sclerosis
n-3 index	Omega-3 index
NE	Norepinephrine
NEFA	Non-esterified fatty acids
NFκB	Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
NIS	Sodium/iodide symporter
NMDA	N-methyl-D-aspartate
NuPED	Nutrition during pregnancy and early development
OR	Odds ratio
PCA	Principal component analysis
IO_4^-	Periodate
PFC	Prefrontal cortex
pMDD	Paediatric major depressive disorder
PPAR	Peroxisome proliferator-activated receptors
PSS	Perceived stress scale
PVN	Paraventricular nucleus
PUFA	Polyunsaturated fatty acids
RBC	Red blood cell
RCT	Randomized control trial
SD	Standard deviation
SF	Serum ferritin
SFA	Saturated fatty acids
SSRI	Selective serotonin reuptake inhibitors
sTfR	Soluble transferrin receptor
T3	Triiodothyronine
T4	Thyroxine
TAG	Triacylglycerols
Tf	Transferrin
Tg	Thyroglobulin
TH	Thyroid hormones
TKP	Tryptophan-kynurenine pathway
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor-α
TPO	Thyroperoxidase
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone
UIC	Urinary iodine concentration
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organisation
YLD	Years lived with disability

Abstract

Background Depression is a leading cause of disability worldwide, and its multifactorial aetiology has been linked to nutrient deficiencies. n-3 Polyunsaturated fatty acids (PUFA), iodine, and iron might be involved in different theories behind the pathophysiology of depression by (1) alterations in neurochemical processes, (2) their role in aberrant brain tissue volume, connectivity, and neurogenesis, (3) their involvement in the Hypothalamic-pituitary-adrenal (HPA) axis, and (4) through their actions on the immune system. This link between nutrition and depression is intriguing in adolescents and during pregnancy since individuals are at life stages of increased nutrient requirements and at risk for developing depressive disorders.

PhD objective This PhD thesis aimed to investigate associations of n-3 PUFA, iodine, and iron status with Paediatric major depressive disorder (pMDD) and perinatal depression.

Methods Two research studies formed part of this PhD project. The first study, the Nutrition during pregnancy and early development (NuPED) study, followed 242 South African women throughout pregnancy up to 12 months postpartum. Red blood cell (RBC) Fatty acids (FA) status was assessed at $<$18 weeks gestation, and the women's depression was assessed at different time points during pregnancy and postpartum. The second study comprised a matched case-control study in 95 Swiss adolescents with and 95 without pMDD. The Children's Depression Rating Scale-Revised (CDRS-R) was used to assess depression. RBC's total FA composition was analysed, and n-3 PUFA intake was assessed using a focused Food frequency questionnaire (FFQ). Iodine status was assessed by Urinary iodine concentration (UIC), and thyroid status was assessed by Thyroid stimulating hormone (TSH) and Thyroxine (T4). Serum ferritin (SF) and Soluble transferrin receptor (sTfR) served as measures of iron status. Also, the history of Iron deficiency (ID) diagnosis and treatment was assessed with a self-reported questionnaire. Finally, inflammation was assessed by measuring C-reactive protein (CRP) and α-1-acid-glycoprotein (AGP), and intestinal permeability by measuring Intestinal fatty acid binding protein (I-FABP).

Results Among South African women, higher RBC Docosahexaenoic acid (DHA) and an Omega-3 index (n-3 index) early in pregnancy were associated with lower odds for depression at 12 months postpartum. Furthermore, higher n-6/n-3 PUFA and Arachidonic acid (AA)/Eicosapentaenoic acid (EPA) ratios at $<$18 weeks gestation were associated with higher odds for depression at 12 months postpartum. In Swiss adolescents with and without pMDD, higher RBC EPA and DHA were associated with

lower odds for depression. Also, an n-3 index >6% was associated with lower odds for depression. In the case-control study, the median UIC did not differ between cases and controls and indicated adequate iodine nutrition. The prevalence of hypothyroxinaemia (low T4 and normal TSH concentrations) was higher among cases compared to controls (15% vs 4%). Furthermore, no difference in SF levels among cases and controls could be observed. However, the proportion of adolescents taking iron supplementation at study inclusion was higher among cases (16%) compared to controls (4%).

Conclusions Overall, results from both studies suggest lower odds for depression (pMDD and perinatal depression) with higher n-3 PUFA status. Also, the results suggest the observed increased prevalence of hypothyroxinaemia among adolescents with pMDD to be unrelated to iodine status. Whether the lack of SF differences among cases and controls and associations with pMDD were masked by iron treatment remains unclear. Finally, this thesis' findings provide a rationale to further investigate n-3 PUFA and iron in the context of depression within the fields of the immune system and inflammation having a possible impact on neurochemical processes and brain plasticity, structure, and neurogenesis. Also, this thesis provides a rationale to further investigate the link between the Hypothalamic-pituitary-thyroid (HPT) and HPA axis possibly involved in depression through neuroendocrine processes.

Zusammenfassung

Hintergrund Depressionen sind weltweit eine der Hauptursachen für Arbeitsunfähigkeit, und deren multifaktorielle Ätiologie wurde mit Nährstoffmängeln in Verbindung gebracht. Es gibt verschiedene Theorien, welche die Ätiologie von Depressionen zu Erklären versuchen. n-3 mehrfach ungesättigte Fettsäuren (PUFA), Jod und Eisen können durch ihre Wirkungsweisen in (1) Veränderungen in neurochemischen Prozessen, (2) ihrer Rolle bei abweichendem Gehirngewebevolumen, Konnektivität und Neurogenese, (3) ihrer Beteiligung in der Hypothalamus-Hypophysen-Nebennieren-Achse (HPA) und (4) durch ihre Wirkungen auf das Immunsystem auf die Pathophysiologie von Depressionen wirken. Dieser Zusammenhang zwischen Ernährung und Depression ist besonders für Jugendliche und während der Schwangerschaft interessant, da sich diese Personen in Lebensstadien mit erhöhtem Nährstoffbedarf und einem erhöhten Risiko für das Entwickeln von depressiven Störungen befinden.

Zielsetzung Das Ziel dieser Dissertation war es, Assoziationen von n-3 PUFA, Jod, und Eisenstatus bei pädiatrischer Majorer Depression (pMDD) und perinatal Depression zu untersuchen.

Methoden Dieses PhD Projekt umfasste zwei Forschungsstudien. Die erste Studie, die Ernährung während der Schwangerschaft und frühe Entwicklung (NuPED) Studie, begleitete 242 südafrikanische Frauen während der Schwangerschaft bis zu 12 Monate nach der Geburt. Bei diesen Frauen wurden Fettsäuren (FA) aus roten Blutkörperchen (RBC) bis zur 18. Schwangerschaftswoche untersucht und es wurden Depressionen zu verschiedenen Zeitpunkten während der Schwangerschaft und nach der Geburt erfasst. Die zweite Studie umfasste eine gematchte Fall-Kontroll-Studie mit je 95 Schweizer Jugendlichen mit und ohne pMDD. Der Children's Depression Rating Scale-Revised (CDRS-R) wurde verwendet, um Depressionen bei den Jugendlichen zu erfassen. Die RBC FA-Zusammensetzung wurde analysiert und die Einnahme von n-3 PUFA wurde anhand eines fokussierten Fragebogens (FFQ) erfasst. Der Jodstatus wurde anhand der Jodkonzentration im Urin (UIC) und des Schilddrüsenstatus beurteilt. Der Schilddrüsenstatus wurde durch Schilddrüsen-stimulierendes Hormon (TSH) und Thyroxin (T4) bestimmt. Serum Ferritin (SF) und löslicher Transferrinrezeptor (sTfR) dienten als Mass für den Eisenstatus. Ausserdem wurde die Vorgeschichte zur Diagnose und Behandlung von Eisenmangel (ID) durch ein Selbstauskunftsfragebogen erfasst. Schließlich wurden die Entzündungsparameter C-reactive Protein (CRP) und alpha-1-Säure-Glykoprotein (AGP), sowie die intestinale Permeabilität durch Messung des intestinalen Fettsäurebindungsproteins (I-FABP) erfasst.

Ergebnisse Unter südafrikanischen Frauen wurden höhere RBC Docosahexaensäure (DHA) und ein Omega-3-Index (n-3-Index) in der Frühschwangerschaft mit niedrigeren Wahrscheinlichkeiten für Depressionen 12 Monate nach der Geburt assoziiert. Zudem wurden höhere n-6/n-3 PUFA und Arachidonsäure (AA)/Eicosapentaensäure (EPA) Verhältnisse in der Frühschwangerschaft mit einer höheren Wahrscheinlichkeit für Depressionen 12 Monate nach der Geburt verbunden. Bei Schweizer Jugendlichen mit und ohne pMDD waren höhere RBC EPA und DHA mit einer geringeren Wahrscheinlichkeit für Depressionen assoziiert. Auch ein n-3-Index $>$6% war mit geringeren Wahrscheinlichkeit für Depressionen verbunden. In der Fall-Kontroll-Studie underschied sich der Median UIC nicht zwischen Fällen und Kontrollen und zeigte eine angemessene Jodernährung an. Die Prävalenz von Hypothyroxinämie (niedrige T4- und normale TSH-Konzentrationen) war höher unter den Fällen im Vergleich zu den Kontrollen (15% vs. 4%). Ausserdem wurde kein Unterschied in SF-Konzentrationen zwischen Fällen und Kontrollen beobachtet. Allerdings war der Anteil der Jugendlichen, welche bei Studieneinschluss Eisensupplemente einnahmen, höher unter den Fällen (16%) im Vergleich zu den Kontrollen (4%).

Schlussfolgerungen Insgesamt deuten die Ergebnisse beider Studien auf eine niedrigere Wahrscheinlichkeiten für Depressionen mit höherem n-3-PUFA-Status hin (pMDD und perinatale Depression). Zudem deutet die beobachtete erhöhte Prävalenz von Hypothyroxinämie bei Jugendlichen auf einen Zusammenhang zwischen Schilddrüsenfunktion und pMDD hin. Dieser scheint allerdings nicht mit dem Jodstatus in Zusammenhang zu stehen. Es bleibt unklar, ob das Fehlen von SF Unterschiede bei den Fällen und Kontrollen aufgrund nicht vorhandener Assoziationen zwischen tieferem Eisenstatus und pMDD bedingt ist oder durch Eisenbehandlung maskiert wurde. Zusammenfassend geben die Ergebnisse dieser Arbeit eine Grundlage für weitere Untersuchungen von n-3 PUFA und Eisen im Zusammenhang mit Depressionen im Bereich des Immunsystems und Entzündung mit einem möglichen Einfluss auf neurochemische Prozesse und Plastizität, Struktur und Neurogenese im Gehirn. Zudem gibt diese Dissertation eine Grundlage, um die Verbindung zwischen Hypothalamus-Hypophysen-Schilddrüse (HPT) Achse und der Hypothalamus-Hypophysen-Nebennierenrinden (HPA) Achse zu untersuchen, welche möglicherweise durch neuroendokrine Prozesse an der Pathophysiologie von Depressionen beteiligt sind.

Resumen

Trasfondo La depresión es una de las principales causas de incapacidad laboral en el mundo y su etiología multifactorial, se pudo relacionar con determinadas deficiencias nutricionales. Existen varias teorías que quieren explicar las causas de las depresiones. Ácidos grasos n-3 poliinsaturados (PUFA), el yodo y el hierro pueden causar fisiopatologías de la depresión por su modo de acción (1) por alteraciones en los procesos neuroquímicos, (2) por su papel en las anomalías de volumen del tejido cerebral, la conectividad y la neurogénesis, (3) por su implicación en las interacciones del eje hipotálamo-hipofisario-suprarrenal (HPA), y (4) por efecto sobre el sistema inmunológico. Esta relación entre la nutrición y la depresión es sobre todo interesante en los adolescentes y embarazadas. Son personas que se encuentran en una etapa de la vida en la que la alimentación es muy importante y pueden corren el riesgo de desarrollar trastornos depresivos.

Objetivo del doctorado Esta tesis doctoral tuvo como objetivo, investigar cual podría ser la relación entre n-3 PUFA, yodo y hierro con el trastorno depresivo mayor en pediatría (pMDD) y la depresión psicológica perinatal.

Métodos Este proyecto de doctorado se apoya en dos estudios de investigación. El primer estudio, Nutrición durante el embarazo y desarrollo temprano (NuPED), siguió a 242 mujeres surafricanas durante el embarazo y en los 12 meses posteriores al parto. A estas mujeres se les midió el ácido graso (FA) de los glóbulos rojos (RBC) en las primeras 18 semanas de gestación, y se las detecto depresión en diferentes momentos del embarazo y en el periodo del posparto. El segundo estudio, se llevó a cabo en Suiza con 95 adolescentes con pMDD y 95 adolescentes sin pMDD, utilizando el formulario "Children's Depression Rating Scale-Revised" (CDRS-R) para evaluar la depresión de los jóvenes. Se analizó la relación de FA en los RBC y se controló la toma de n-3 PUFA utilizando un cuestionario enfocado en la frecuencia de toma de alimentos (FFQ). El yodo se midió mediante la concentración de yodo en la orina (UIC) y el estado de la tiroides. Este se evaluó mediante la hormona que estimula la tiroides (TSH) y tiroxina (T4). Para medir el estado del hierro se tomaron los valores de la ferritina sérica (SF) y el receptor de transferrina soluble (sTfR). El historial de diagnóstico y tratamiento de deficiencia de hierro (ID) se evaluó con un cuestionario. Finalmente, se evaluaron los marcadores de inflamación como son, las proteínas C-reactiva (CRP) y alfa-1-glucoproteína (AGP) y la permeabilidad intestinal midiendo la proteína transportadora de ácidos grasos intestinales (I-FABP).

Resultados Entre las mujeres surafricanas, se asociaron menores probabilidades de depresión a los 12 meses del posparto, cuando tenían un RBC ácido docosahexaenoico (DHA) y un índice omega-3 (índice n-3) más alto al principio del embarazo. Además, se asociaron mayores probabilidades de depresión a los 12 meses del posparto, cuando los valores de n-6/n-3 PUFA y ácido araquidónico (AA)/ácido eicosapentaenoico (EPA) eran más altos en las primeras 18 semanas de gestación. En los adolescentes suizos - con y sin pMDD -, con RBC EPA y DHA más altos se asociaron menores probabilidades de depresión. También un índice n-3 $>$6 % se asoció con menores probabilidades de depresión. En el estudio de casos y controles, la media de UIC no difirió entre casos y controles e indicó una nutrición adecuada con yodo. La prevalencia de hipotiroxinemia (concentraciones bajas de T4 y normales de TSH) fue mayor entre los casos que entre los controles (15 % vs 4 %). Además, no se pudo observar ninguna diferencia en los niveles de SF entre casos y controles. Sin embargo, la proporción de adolescentes que tomaban suplementos de hierro en el momento de ser incluidos en el estudio, fue en comparación mayor entre los casos (16 %) que entre los controles (4 %).

Conclusiones En general, los resultados de ambos estudios sugieren que con un mayor estado de n-3 PUFA las probabilidades de depresión disminuyen (pMDD y depresión perinatal). Además, los resultados sugieren, que el aumento de la prevalencia de hipotiroxinemia observado en los adolescentes tiene una correlación entre la tiroides y el pMDD pero que no depende del estado del yodo. Aún no está claro, si la falta de diferencias de SF, entre casos y controles se debe a la falta de asociaciones del estado del hierro y el pMDD o si los resultados fueron enmascaradas por el tratamiento con hierro. En resumen, los resultados de esta tesis son importantes para ver que se podría seguir investigando más a fondo los n-3 PUFA y el hierro en el contexto de la depresión y sistema inmunológico y la inflamación con su posible impacto en los procesos neuroquímicos y plasticidad, estructura y neurogénesis del cerebro. Además, esta tesis proporciona una base para investigar más a fondo el vínculo entre los ejes hipotálamo-hipófisis-tiroides (HPT) y HPA que posiblemente estén implicados en la depresión a través de procesos neuroendocrinos.

1 Introduction

Over the past decades, depression has developed into the third leading cause of disability worldwide [1, 2]. Depressive disorders, often long-lasting and with severe intensity, are a serious global health issue with a sizeable socioeconomic impact [1]. For instance, affected people experience difficulties performing at work, school, and in the family [1]. However, there is still insufficient evidence for safe and efficacious treatment options [3] despite anti-depressant treatment being a very active field of research. One promising way of investigation is understanding the pathophysiology underlying depressive disorders, especially for further developing treatment options for individuals with treatment-resistant depression.

The aetiology of depressive disorders is multifactorial and complex. Different theories behind the pathophysiology of depression include (1) alterations in neurochemical processes (e.g. neurotransmission) [4, 5], (2) aberrant brain tissue volume, connectivity, and neurogenesis in depressed patients [6, 7], (3) the involvement of the Hypothalamic-pituitary-adrenal (HPA) axis [8], and (4) the immune system, particularly inflammation [9]. Over the last thirty years, evidence has emerged that the modifiable habitual diet, and specifically deficiencies in certain nutrients, might be relevant to the aetiology of Major depressive disorder (MDD) [10]. This link between depression and nutrition is intriguing in adolescents and during pregnancy since these individuals are at a life stage of increased body growth, neuronal development (of the foetus in case of pregnancy) and nutrient requirements. Therefore, this PhD thesis focuses on vulnerable population groups, namely adolescents and pregnant women.

This PhD thesis investigates n-3 Polyunsaturated fatty acids (PUFA), iodine, and iron in the context of depressive disorders in adolescents and pregnant women. All three nutrients play crucial roles in neuronal development and maintenance, the endocrine system, or inflammation, either due to their biochemical properties or as co-factor for hormones and enzymes [11–13], and were thus proposed to be involved in the aetiology of depressive disorders.

The overall aim of this PhD project was to investigate associations between the named three nutrients and paediatric as well as perinatal depressive disorders. Two different research studies form part of the PhD project: a data analysis of a prospective cohort study following 250 South African women from pregnancy to birth and their infants up to 12 months of age (Nutrition during pregnancy and early development (NuPED) study); and a case-control study with 200 Swiss children and adolescents with and without Paediatric major depressive disorder (pMDD).

This thesis is structured in three parts, starting with a review of the literature providing an introduction and overview of the state of the art in the field of depressive disorders and its associations with n-3 PUFA, iodine, and iron.

The second part of the thesis comprises research results presented in 4 manuscripts. **Manuscript 1** presents and discusses results from the NuPED study, where the objective was to investigate associations of n-3 PUFA status during early pregnancy with perinatal depression among South African women. **Manuscripts 2-4** display and elaborate results from the case-control study investigating associations of nutrients with depression in Swiss adolescents. **Manuscript 2** investigated associations of n-3 PUFA status and intake with pMDD among Swiss adolescents. **Manuscript 3** determined associations of iodine status and thyroid functions with pMDD and perceived stress in Swiss adolescents. **Manuscript 4** assessed the history of Iron deficiency (ID) diagnosis and treatment, and further investigated associations between iron status and pMDD in the context of inflammation and intestinal permeability among Swiss adolescents.

Next, the findings from these four manuscripts are summarised and brought together. Finally, open questions are addressed and suggestions for future research are provided.

2 Background

2.1 Depression - a multifactorial disease

Depression is the third leading cause of disability worldwide [1, 2] with an estimated prevalence of 3% among all age groups and sexes [14]. Overall, depressive disorders contribute 14% to all-age Years lived with disability (YLD) just after low back pain (18%) and headache disorders (15%) [2]. Within 17 years, between 1990 and 2007, these numbers on all-age YLD attributed to depressive disorders increased by 33% [2]. Especially when long-lasting and with moderate or severe intensity, depressive disorders can become a serious health issue with significant socioeconomic consequences. The risk for every individual to experience a depressive episode at least once in their lifetime is around 20% [15]. The onset of depression follows complex interactions of social, psychological, and biological factors [1]. Four broad dimensions describe these risk factors for depression [16–18].:

1 Socio-demographic factors such as gender (e.g. sociocultural roles, norms, and social identities)[19, 20], maritial status [21–23], age [20, 24], educational level [20, 25–28], and unemployment [29];
2 Physical disease factors such as chronicity and length of a disease [30–33] of a variety of diseases such as e.g. rheumatoid arthritis [34, 35], or cancer [36];
3 Hereditary factors, such as history of depression within biological family members [37], where the heritability of depression is estimated to be around 30-45% [38] and so far cannot be attributed to a single gene;
4 Adverse childhood experiences such as emotional abuse, sexual and physical neglect during childhood [39–41].

Depressive disorders are thus multifactorial, complex, and heterogeneous diseases, often not recognised even by professionals [42]. Furthermore, patients with diagnosed depression often respond suboptimally to therapy, not least as a result of the overall poor evidence-base for safe and efficacious treatment options [3]. Therefore, understanding the pathophysiology of depressive disorders is crucial for the development of treatment options. Different models are contributing to understanding the pathophysiology of depressive disorders [4, 9, 43, 44]. However, no single model or mechanism can satisfactorily explain all aspects of the disease. Moreover, even if one model could describe the pathophysiology of a patient's depressive episode, this pathophysiology within one person can differ in different episodes [45].

Over the last years, increasing evidence from research suggests a role of diet and nutrients in the aetiology and symptom management of MDD [44, 46]. Adolescents show poorer eating habits concerning diet quality compared to adults [47]. Also, adolescents are in a developmental stage of increased neuronal plasticity [48], where they have higher nutrient requirements for healthy body growth and maintenance compared to adults. Besides childhood and adolescence, pregnancy and lactation are periods where nutrient requirements rapidly increase. Inadequate nutrient intake during pregnancy is a risk factor for the mother's health and her child, e.g. by increasing the risk for pre-mature birth [49], or poorer offspring outcomes [50]. Therefore, the link between depression and modifiable nutritional risk factors is particularly interesting in children and adolescents, as well as during pregnancy and lactation. Focusing on the most vulnerable groups to nutrient status due to rapid physiological changes can probably provide better insight into associations between depression and modifiable single nutrients than studies in adults. The following subsections will provide insight into pMDD, as well as into perinatal depression and different theories on the aetiology of depressive disorders.

2.1.1 Paediatric major depressive disorder

The most frequent onset of depression is during childhood and adolescence between the age of 10 to 18 years [42]. Globally, one in seven children and adolescents experience a mental disorder [51]. Within this age group, mental disorders account for 13% to the global burden of disease, with depression, anxiety and behavioural disorders being the leading causes of illness and disability [51]. In Switzerland, the estimated prevalence of depressive symptoms among adolescents is 10% [52]. Early-onset depression is a risk factor for chronic and recurrent forms of depressive disorders in adulthood [53, 54]. Depression during childhood and adolescence can impair physical and mental health, lead to poor educational, work and social functioning, and increase the rate of smoking, substance abuse, eating disorders, and obesity [51, 55].

Diagnostic criteria and tools for paediatric major depressive disorder

Health care professionals in the United States and internationally use the Diagnostic and Statistical Manual of Mental Disorders (DSM) as a guide to diagnose mental disorders [56]. It contains descriptions, symptoms and other criteria for diagnosis of mental disorders. Table 2.1 shows the criteria for diagnosis of pMDD according to the DSM-5 manual [56]. The major challenge is the diagnosis itself. Experts estimate that only around 50% of pMDD cases are being diagnosed [57]. One possible explanation for this under-diagnosis could be that children and adolescents display different signs of depression than adults, such as e.g. differences in the verbalisation of thoughts and feelings [58].

Table 2.1: **Diagnostic criteria for (paediatric) major depressive disorder ((p)MDD) according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) 5**. Major depressive disorder is categorised within the DSM-5 as a depressive disorder and diagnosed when five or more of the following A criteria (at least one includes A1 or A2) are met. This table is based on [56].

	Criteria [1] [2]
A1	Depressed mood - indicated by subjective report or observation by others (in children and adolescents, can be irritable mood).
A2	Loss of interest or pleasure in almost all activities - indicated by subjective report or observation by others.
A3	Significant (more than 5 percent in a month) unintentional weight loss/gain or decrease/increase in appetite *(in children, failure to make expected weight gains)*.
A4	Sleep disturbance (insomnia or hypersomnia).
A5	Psychomotor changes (agitation or retardation) severe enough to be observable by others.
A6	Tiredness, fatigue, or low energy, or decreased efficiency with which routine tasks are completed.
A7	A sense of worthlessness or excessive, inappropriate, or delusional guilt (not merely self-reproach or guilt about being sick).
A8	Impaired ability to think, concentrate, or make decisions - indicated by subjective report or observation by others.
A9	Recurrent thoughts of death (not just fear of dying), suicidal ideation, or suicide attempts.
○	The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
○	The symptoms are not due to direct physiological effects of a substance (e.g. drug abuse, a prescribed medication's side effects) or a medical condition (e.g. hyperthyroidism).
○	There has never been a manic episode or hypomanic episode.
○	Major depressive episode (MDE) is not better explained by schizophrenia spectrum or other psychotic disorders.

[1] The symptom must either be new or must have clearly worsened compared with the person's pre-episode status and must persist most of the day, daily, for at least two weeks in a row. Exclude symptoms that are clearly due to a general medical condition, mood-incongruet delusions, or mood in-congruent hallucinations.

[2] Symptom must persist most of the day, daily, for at least 2 weeks in a row, *excluding A3 and A9*.

To identify and diagnose depression within research settings, Children's Depression Rating Scale-Revised (CDRS-R) is often used to quantify children's and adolescent's depression [59, 60]. The CDRS-R is a semi-structured and validated [60] clinical interview. It comprises 17 depressive symptoms, with 14 items being assessed and rated by the interviewer on a 7- or 5-point Likert scale, and three non-verbal items such as depressed facial affect rated by the interviewer only. Based on the individual ratings, a total score ranging from 17 to 113 is calculated, where higher scores indicate worse depressive symptoms compared to lower scores. The CDRS-R was used to score adolescents' depression within this PhD project.

2.1.2 Perinatal depressive disorder

Perinatal depression comprises major and minor depressive episodes during pregnancy (prenatally) and in the first year after delivery (postnatally) [61]. The global prevalence of perinatal depression is estimated to be around 12% [62]. However, there are significant differences in the prevalence of perinatal depression between High-income countries (HIC) with approximately 9% [62] compared to prevalence rates in Low- and middle-income countries (LMIC), where estimated prevalence rates reach up to 50% [61–63]. Women experiencing perinatal depression often find it difficult to perform everyday tasks, fail to seek perinatal care, have poor dietary intake and are at risk for self-harm or suicide [64, 65]. Also, perinatal depression is associated with poorer childhood outcomes such as behavioural problems and impaired cognitive function [50, 63, 66–68]. Therefore, perinatal depression can have profound health implications for the mother and her child.

Diagnostic criteria and tools for perinatal depression

Similarly as described in section 2.1.1, diagnosis of perinatal depression often follows the DSM manual for diagnosis of mental disorders [56]. Table 2.1 shows diagnostic criteria for MDD according to the DSM-5 manual [56].

In research settings, the validated interviewer-administered Edinburgh Postnatal Depression Scale (EPDS) is often used to quantify women's depression [69]. The EPDS is a 10-item scale assessing depressive symptoms experienced in the past seven days [70]. The items are rated on a 0 to 3-point Likert scale, resulting in total scores between 0 to 30, where higher scores indicate worse depressive symptoms compared to lower scores. This PhD project used the EPDS to quantify perinatal depression among women.

2.1.3 Theories on the aetiology of depressive disorders

The literature on the biochemical pathophysiology underlying depression is vast. This section will provide an overview of different theories behind the aetiology of depressive disorders within four significant fields of brain health: (1) neurochemical processes; (2) plasticity, structure and neurogenesis; (3) neuroendocrine processes; and (4) immune system and inflammation. These fields follow an integrated framework from Dean and Keshavan [4].

Neurochemical processes

There are multiple theories involving neurotransmitters such as monoamines and glutamatergic metabolites in the aetiology of depression. A major hypothesis on the underlying pathophysiology of depression has been the monoamine hypothesis, suggesting that depression is caused by decreased monoamine levels such as Serotonin (5-HT), Norepinephrine (NE), and Dopamine (DA) in the Central nervous system (CNS) [4]. Therefore, anti-depressant drug development in the past century focused on increasing circulating levels of mentioned monoamines [71]. Figure 2.1 shows a general way of action of monoaminergic neurotransmission in the synaptic cleft and neuronal targets of anti-depressant drugs. However, the lack of clinical efficacy with monoaminergic anti-depressant drugs such as Monoamine oxidase inhibitors (MAO) or Selective serotonin reuptake inhibitors (SSRI) suggests that "simple" depleted concentrations of monoamines do not cause depression [72]. Monoaminergic neurotransmission instead might play a modulatory role influencing other biological systems such as intracellular signalling or other neurotransmitter and neuropeptide systems [72].

The glutamate hypothesis of depression suggests that decreased levels of glutamatergic metabolites and altered glutamatergic neurotransmission are associated with depression [5]. Similarly to the mechanistic pathway of monoaminergic anti-depressant drugs, N-methyl-D-aspartate (NMDA) (ionotropic glutamate receptor) antagonists such as e.g. ketamine are robustly associated with a decrease in depressive symptoms in depressed patients by increasing circulating levels of glutamatergic metabolites [5, 71, 73]. The hypothesis says that glutamatergic neurotransmission drives depression via the inflammation-induced up-regulation of the Tryptophan-kynurenine pathway (TKP) (see Fig. 2.2) by disturbing the balance between neurotoxic and neuroprotective metabolites [74]. The essential amino acid tryptophan serves as a precursor for both the kynurenine metabolism and the 5-Hydroxytryptophan-Serotonin pathway [74]. Therefore, the activation of the kynurenine pathway was initially suggested to drive depressive symptoms by depleting brain 5-HT [74]. However, increasing evidence suggests that TKP is involved in depression via glutamatergic neurotransmission [75]. Figure 2.2 shows the TKP, where the essential amino acid tryptophan is degraded to several neuroactive compounds such as kynurenic acid, 3-hydroxykynurenine, and quinolinic

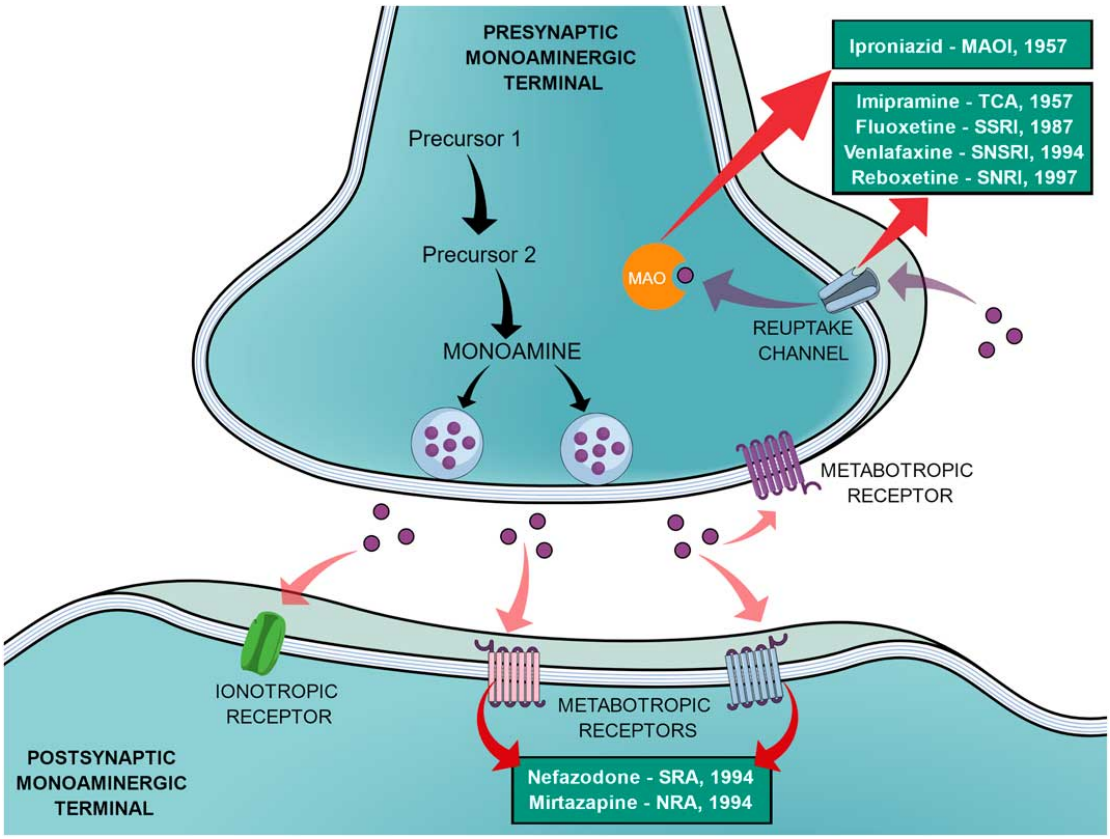

Figure 2.1: **General way of action of monoaminergic neurotransmission in the synaptic cleft**. *Black arrows*: general synthesis process of monoamines until storage in synaptic vesicles, purple dots representing monoamines; *Pink arrows*: Main neuronal targets of monoamines; *Purple arrows*: Re-uptake mechanism of monoamines leading to degradation by the Monoamine oxidase inhibitors (MAO); *Red arrows*: Main targets of classical monoaminergic anti-depressant drugs; *Green boxes*: Main drugs and classes of anti-depressant drugs related to each target and the year the drug reached the market. This figure is taken from [71].

acid [75]. These TKP downstream metabolites target glutamatergic neurotransmitter receptors [75]. They are also associated with the exertion of neurotoxic effects, e.g. by activating NMDA receptors or enhancing free radical production [76–78]. The ratio tryptophan/kynurenine is regulated through the activity of Indoleamine 2,3-deoxygenase (IDO1) driven by pro-inflammatory cytokines such as type 1 interferons like Interferon-γ (IFN-γ) [76]. However, none of the previously described mechanisms fully describe the aetiology of depressive disorders.

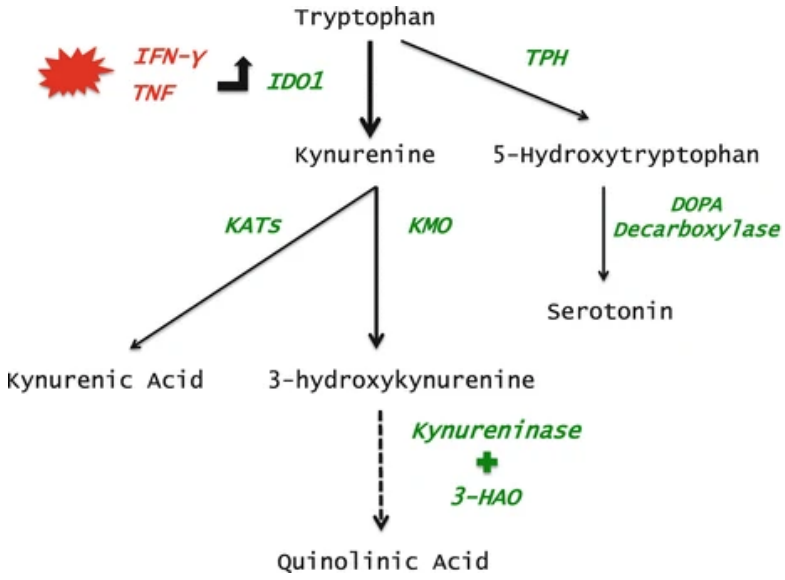

Figure 2.2: **Simplification of kynurenine metabolism pathway**. IFN-γ: Interferon-γ; IDO1: indoleamine 2,3-deoxygenase; TNF: Tumour necrosis factor; KMO: Kynurenine monooxygenase; KAT: Kynurenine aminotransferase. This figure is taken from [74].

Plasticity, structure and neurogenesis

There is strong evidence for altered brain tissue volume, connectivity and neurogenesis in depressed patients. For instance, firm and consistent evidence from brain-imaging studies of depressed patients suggests decreased volume of cortical and limbic brain regions such as the Prefrontal cortex (PFC) and the hippocampus, which control emotion, mood, and cognition [6, 7]. Also, reduced connectivity between the hippocampus, the PFC, and other brain regions, reported in functional imaging studies, indicate complex disruption of brain regions in depressive patients [79, 80]. Further, studies report decreased synapses, levels of glutamate receptor subtypes, pre-synaptic neurotransmitter vesicle-associated proteins, and postsynaptic structural and functional proteins in post-mortem forebrain tissue of depressed patients [81–84]. Finally, human post-mortem imaging, non-human animal research, and in-vivo quantitative magnetic resonance imaging provide evidence for an altered myelin composition in MDD patients [85–87].

These structural abnormalities in depressed patients can partially be explained by physiological alterations contributing to the "healthy" brain's plasticity during learning and adaptation by rapidly creating and eliminating synapses and other functional circuits. Brain-derived neurotrophic factor (BDNF) is a neurotrophin promoting survival of existing neurons and growth and differentiation of new neurons, and synapses [88]. Studies in adult depressed patients report reduced levels of peripheral serum BDNF [89]. However, these decreased peripheral BDNF levels increase significantly after anti-depressant treatment [90]. This data supports the hypothesis that neuroplastic changes are associated with improving depression. Also, mitochondrial dysfunction has been associated with depression [91]. Mitochondria are the organelles of major Adenosine triphosphate (ATP) production in the eukaryotic cell and are found particularly densely in dendrites, and synapses [92]. Figure 2.3 shows a conceptual summary of stimuli causing mitochondrial dysfunction, leading from structural and functional changes in brain architecture to depression [91]. Finally, epigenetic events, especially histone acetylation and methylation, altering chromatin structure and gene expression contribute to structural and functional changes in multiple brain regions involved in the pathophysiology of depression [93–96].

To conclude, altered brain architecture in depressed patients could be explained by underlying mechanisms influencing neurogenesis and plasticity, such as decreased levels of BDNF, mitochondrial dysfunction and epigenetic changes. Neuroendocrine and inflammatory processes influencing neuronal architecture and plasticity will be introduced in the following sections since these processes can be seen as separate theories on the aetiology of depression due to their broader field of action.

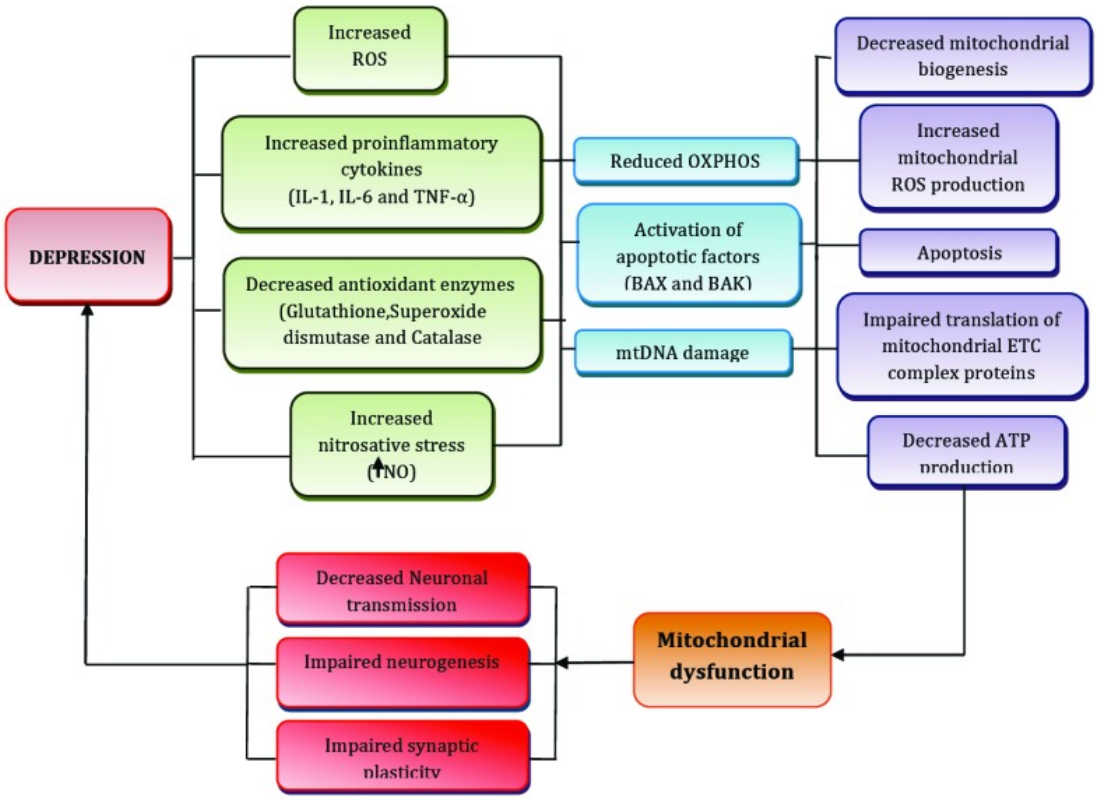

Figure 2.3: **Exposure to different stimuli causing mitochondrial dysfunction, structural and functional changes in brain architecture, and finally leading to depression**. IL-1: Interleukin-1; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor-alpha OXPHOS: oxidative phosphorylation; BCL-2-associated X protein; BAK: BCL-2 antagonist/killer; mtDNA: mitochondrial DNA; ROS: reactive oxygen species; ETC: electron transport chain; ATP: adenosine triphosphate. This figure is taken from [91].

Neuroendocrine processes

Depression is often referred to as a stress-related disorder with robust evidence of the HPA axis being involved in the pathophysiology of MDD [8]. Figure 2.4 shows a graphical illustration of the HPA axis. Neurons in the Paraventricular nucleus (PVN) of the hypothalamus release Corticotropin-releasing hormone (CRH), which in turn activates the secretion of Adrenocorticotropic hormone (ACTH) from the pituitary. ACTH then induces the release of Glucocorticoids (GC) such as, e.g. cortisol in the adrenal glands. These GC regulate the HPA axis via a negative feedback loop mediated by Glucocorticoid receptors (GR). These GR are located in the hippocampus, the PVN, and the anterior pituitary gland and inhibit the further release of CRH [97]. The body releases monoamines as a response to environmental stress, which then activate the HPA axis initiating the so-called "fight-or-flight" reaction and secretion of GC. Thus, GC increase the cardio-vascular tone, the respiratory rate, and generally inhibit vegetative functions such as feeding, digestion, growth, reproduction, and immunity to improve an individual's chance of survival [98]. In MDD, GR seem to have impaired sensitivity leading to reduced negative feedback mechanisms of GC. This impaired GR sensitivity results in central hypersecretion of CRH and increased production of GC leading to a chronic "fight-or-flight" hormonal state within MDD patients [99, 100].

To conclude, solid and vast preclinical and clinical data confirm the dysregulation of the HPA axis in stress-related mental disorders such as MDD. Nevertheless, no anti-depressant drugs or treatments targeting specific components of the HPA axis have been approved for application in clinics so far [8]. Meanwhile, there is evidence that childhood trauma might lead to increased sensitivity of the HPA axis with increased responses to stressors later in life [101, 102]. Furthermore, glucocorticoid resistance has also been shown in the context of chronic inflammation, exposure to infectious agents, and chronic stress [103].

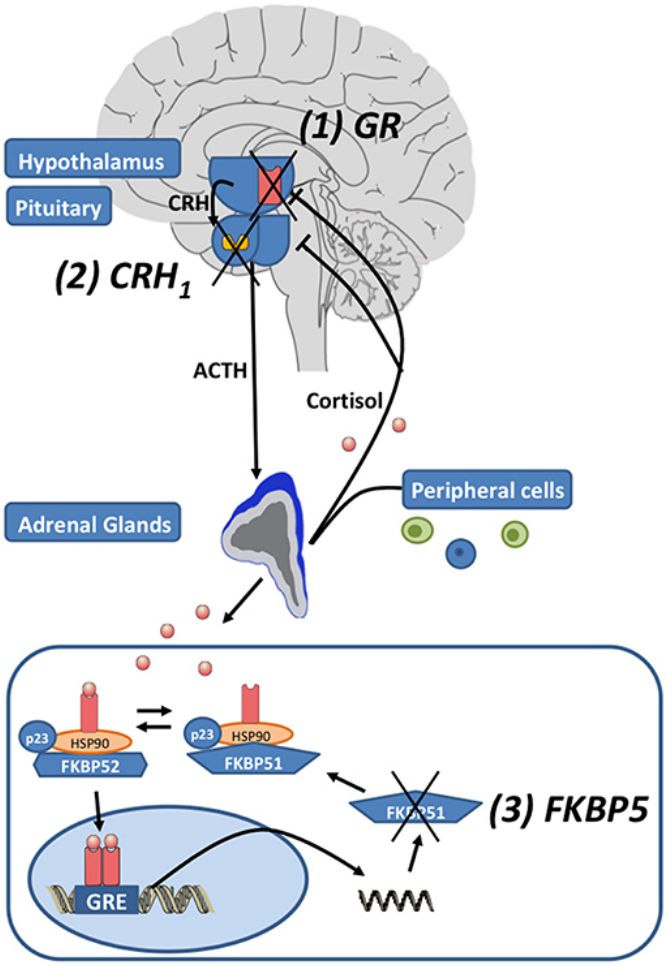

Figure 2.4: **Hypothalamic-pituitary-adrenal (HPA) axis**. CRH: Corticotropin-releasing hormone; ACTH: Adrenocorticotropic hormone; GR: Glucocorticoid receptors; FKBP51/FKBP52: co-chaperones involved folding of proteins; GRE: Glucocorticoid response elements; Drugs regulating the function of the HPA axis target: (1) the GR, (2) the CRH_1 receptors, and (3) FKBP51/FKBP52. This figure is taken from [8].

Immune system and inflammation

Inflammation has been linked to depression through changes in the Blood-Brain-Barrier (BBB) and changes in the brain parenchyma, leading to structural and functional changes in the hippocampus (Fig. 2.5) [9]. For instance, increased levels of systemic Tumour necrosis factor-α (TNF-α) have been associated with lower levels of tight junction proteins in the BBB, and higher depression-like behaviour in mice [104]. However, the disrupted BBB integrity was reversed after administration of anti-inflammatory treatment and mice recovered from depression-like behaviour [104]. Similarly, increased CNS inflammation due to neurological disorders such as Multiple sclerosis (MS) also seems to disrupt the BBB, allowing easier entry of cytokines and immune cells into the brain [105, 106]. Increased inflammatory markers in the brain have been associated with decreased hippocampal neurogenesis, and anxiety levels in mice [107]. These changes in brain structure could be driven by inflammation which (1) induces the production of neurotoxic downstream products of the kynurenine pathway, evoking glutamate release from microglia [74], and (2) causes impairment of long-term potentiation in hippocampal neurons by type 1 interferons acting through the interferon receptor chain 1 pathway [108]. Overall, inflammation-induced disruptions of the BBB and structural changes within the CNS have been shown to react to anti-inflammatory treatment by changes in white matter structure, global brain connectivity, and functional activation, all of which is linked to depression [9].

To summarise, different theories try to explain the underlying mechanisms in the pathophysiology of depressive disorders. So far, no theory can explain depression's aetiology to the full extent. This fact highlights that, most probably, the aetiology of depressive disorders is a complex interplay between the previously introduced different mechanisms. The role of n-3 PUFA, iodine and iron on these mechanisms is very diverse for each nutrient. The following three sections will introduce these nutrients and elaborate on their role in the aetiology of depression.

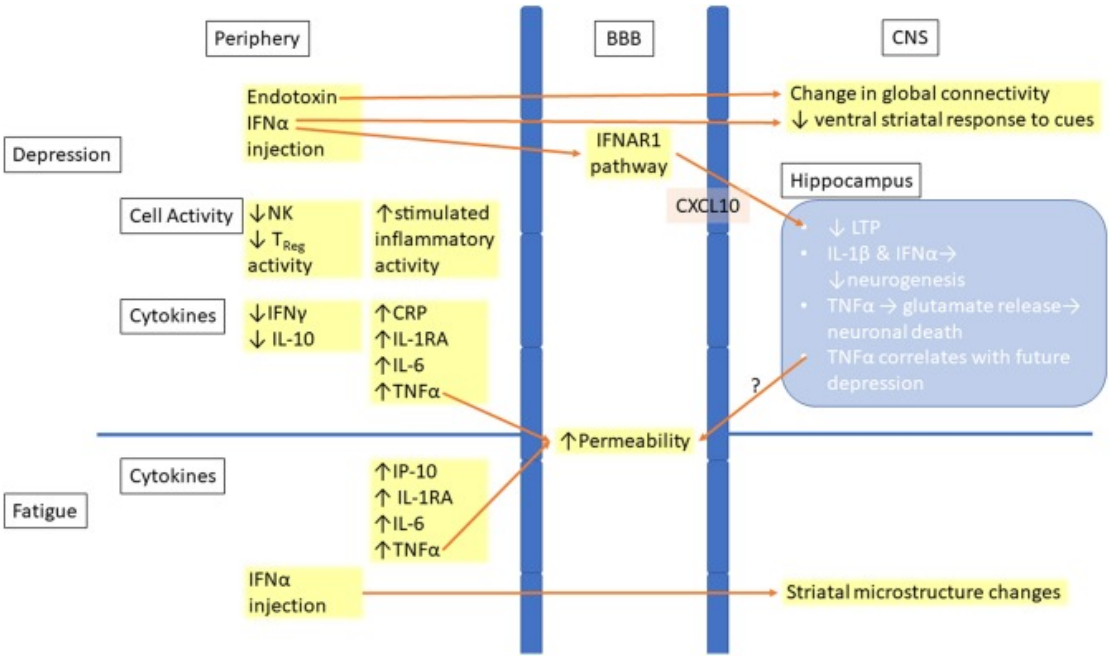

Figure 2.5: **Links between peripheral inflammation and changes in the Central nervous system (CNS) in depression and fatigue**. Increased inflammation in the periphery and the CNS leads to increased Blood-Brain-Barrier (BBB) permeability, allowing easier entry of inflammatory molecules or immune cells into the CNS. Inflammatory signalling in the CNS leads to structural and functional changes in the brain, e.g. in the hippocampus, associated with depression. BBB: Blood- brain-barrier; CNS: central nervous system; CRP: C-reactive protein; IFN: Interferon; IFNAR1: Interferon-alpha/beta receptor alpha chain; IL: Interleukin; IP-10: Interferon gamma-induced protein 10; TNF: Tumor necrosis factor; NK: Natural killer cell; T_{reg}: Regulatory T cell; LTP: Long-term potentiation. This figure is taken from [9].

2.2 n-3 polyunsaturated fatty acids and depressive disorders

Omega (n)-3 PUFA form a class within the heterogeneous group of lipids - molecules formed by carbon atoms and double bonds [109]. Lipids range from simple short carbon chains to complex molecules such as Triacylglycerols (TAG), phospholipids, and sterols. The number of carbon atoms, double bonds, the branching of the hydrocarbon chain, and the position and the orientation of double bonds of lipids does differ within each class. Still, the heterogeneous group of lipids shares common properties such as being soluble in organic solvents and being hydrophobic [109].

The nomenclature of Fatty acids (FA) is based on their chemical structure: hydrocarbon chains of varying lengths, the presence of double bonds (degree of unsaturation), a carboxyl group and a methyl group at each end. Usually, the numbering of carbon atoms is from the carboxyl group to the methyl group. The methyl group, the "end" of the carbon chain, is often referred to as Ω or n. Short-chain FA comprise less than 6 carbons, medium-chain FA have 6-12 carbons, and more than 12 carbons characterise long-chain FA. Further, FA can have several degrees of unsaturations varying from no double bond (Saturated fatty acids (SFA)), one double bond (Monounsaturated fatty acids (MUFA)), to two or more double bonds (PUFA). The position and orientation of the double bonds add complexity to the structure of FA, which determines the FA's biophysical properties. Figure 2.6 displays examples of structure and naming of selected FA.

The human diet shapes the composition of FA in the human body. The role of FA in human metabolism is vast, from maintaining body temperature, being key constituents of cell membranes, to their role in chemical messaging within the body [110–112]. However, the focus of this section will be on n-3 PUFA and their role in human metabolism, especially their role in the pathophysiology of depressive disorders.

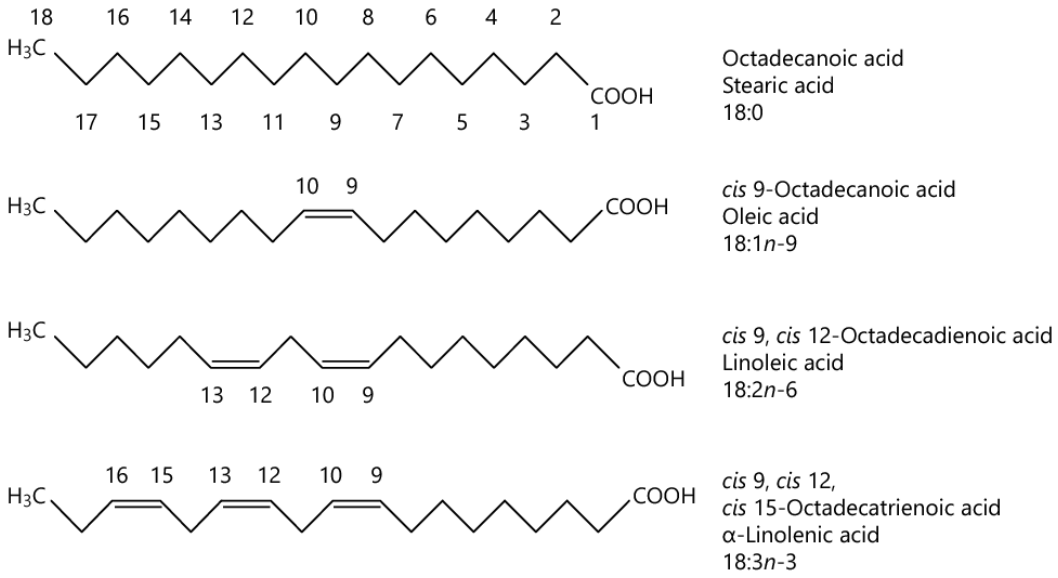

Figure 2.6: **Structure and nomenclature of selected 18-carbon Fatty acids (FA)**. Carbons are numbered from the carboxylic acid end of the FA. This figure is taken from [109].

2.2.1 n-3 polyunsaturated fatty acid metabolism

n-3 polyunsaturated fatty acid biosynthesis

FA are synthesised by sequentially adding carbon atoms, with palmitic acid generally considered the main product of FA synthesis [109]. In eukaryotic cells, longer FA are formed from palmitic acid by seven elongases [113]. These elongases differ in selectivity for chain length, and level of unsaturation [113]. This desaturation process is highly conserved in bacteria, yeasts, algae, higher plants, protozoa, and animals [109]. However, there are essential differences in the specificity of desaturases expressed in animal and plant tissue with implications for human dietary FA requirements. Namely, Δ12- and Δ15-desaturases, which introduce double bonds beyond carbon 9, are lacking in mammals; thus, they cannot synthesise Linoleic acid (LA) and α-linoleic acid, 18:3n-3 (ALA). Nevertheless, LA, ALA, and their metabolites are essential to mammal cells and must therefore be consumed in the diet.

Once consumed through the diet, LA and ALA are converted to longer-chain metabolites, as shown in Figure 2.7. This pathway of desaturation and elongation in mammals mainly occurs in the Endoplasmic reticulum (ER) within the liver [109]. As shown in Figure 2.7, the substrates LA, ALA and oleic acid compete for the rate-limiting Δ6-desaturase enzyme. It follows a competition between their respective n-6, n-3, and n-9 families for the desaturation-elongation pathway. The affinity of the Δ6-desaturase

for its substrate is highest for ALA, followed by LA, and then oleic acid [114]. Nevertheless, since LA is most abundant in human diets, the n-6 PUFA metabolism is quantitatively more important than the n-3 PUFA metabolism, which would be the preferred substrate for the Δ6-desaturase [115]. In conditions of insufficient dietary LA and ALA intake, the oleic acid pathway is enhanced, resulting in an accumulation of mead acid which indicates a dietary essential FA deficiency. Besides nutritional status, the activity of Δ6- and Δ5-desaturases is regulated by hormones, and feedback inhibition by end products [109]. Also, genetic variants in the FADS1 and FADS2 gene clusters have been associated with differences in n-6, and n-3 metabolism [116].

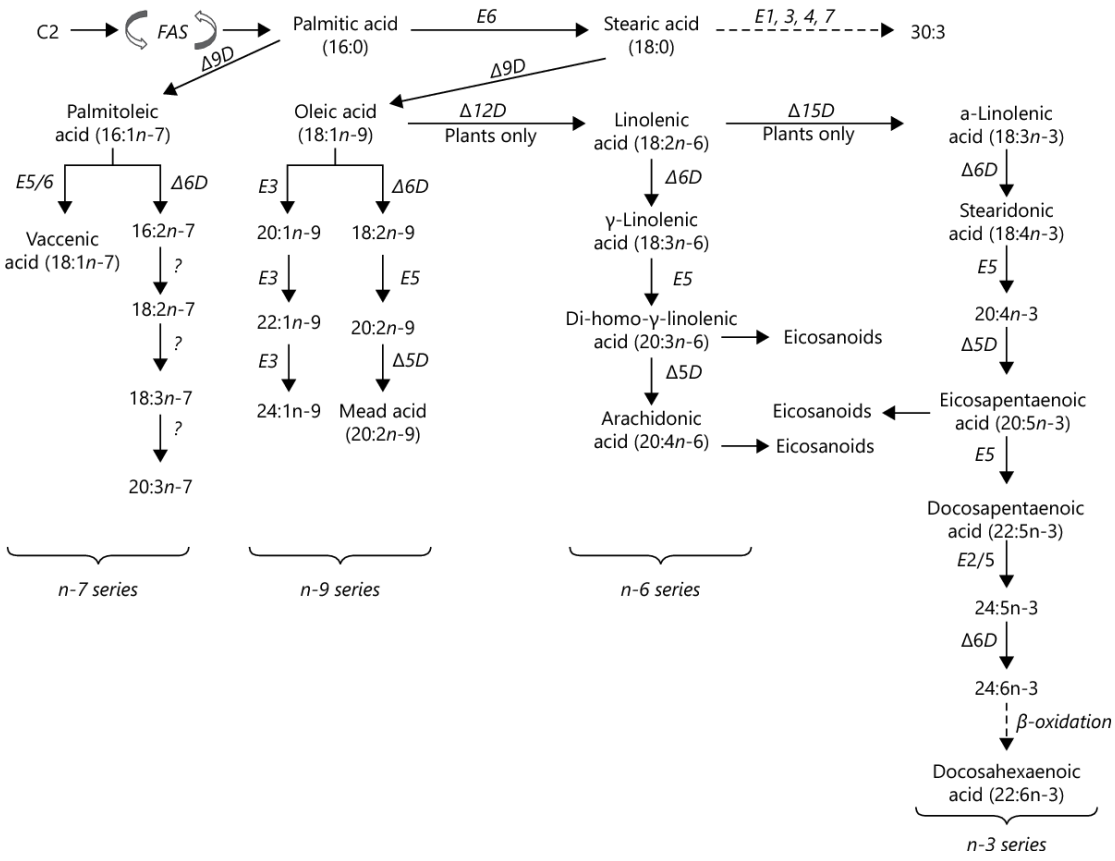

Figure 2.7: **Biosynthesis of Saturated fatty acids (SFA), Monounsaturated fatty acids (MUFA), and Polyunsaturated fatty acids (PUFA)**. C2: Acetyl CoA; E1-7: elongases 1-7, FAS: fatty acid synthase; Δ5-15D, Δ5-15: desaturases. This figure is taken from [109].

Fatty acid absorption, excretion and transport within the body

The main form of human dietary lipid is TAG. In brief, TAG have a glycerol core, to which FA and other groups are attached mainly through ester bonds [109]. Before TAG can be absorbed across enterocyte membranes, they must be hydrolysed to their constituent FA. This process is mainly driven by the pancreatic lipase [117]. Once hydrolysed, the short- and medium-chain FA are absorbed by enterocytes primarily in the jejunum and directly released into the portal blood stream [117]. On the other hand, long-chain FA are re-esterified into TAG and phospholipids and then packed into lipoproteins called chylomicrons within the enterocyte. Next, chylomicrons are secreted into the lymphatic circulation and enter the bloodstream bypassing the liver via a thoracic lymphatic duct [109]. In healthy conditions, approximately 95% of ingested lipids are absorbed, and the rest is excreted from the body as faeces [118]. The transport of the characteristically hydrophobic lipids within the predominantly aqueous body is done by two mechanisms. First is the transport as Non-esterified fatty acids (NEFA), where FA are non-covalently bound to albumin, and second is the transport of complex lipids in lipoproteins [117].

The roles and mechanism of action of fatty acids in the healthy body

FA have diverse functions in the human body; their principal roles are as an energy source, and membrane constituents [109]. Table 2.2 shows a summary of the known functions of n-3 PUFA.

Table 2.2: **Summary of the physiological roles of n-3 Polyunsaturated fatty acids (PUFA).** This table is taken and adapted from [119].

Physiologcial roles of n-3 polyunsaturated fatty acids
Regulation of blood pressure
Regulation of platelet function
Regulation of blood coagulation
Regulation of plasma triglyceride concentrations
Regulation of vascular function
Regulation of cardiac rhythm
Regulation of inflammation
Regulation of immune function
Regulation of bone turnover
Regulation of insulin sensitivity
Regulation of tumour cell growth
Regulation of visual signalling
Structural component of brain and central nervous system

n-3 PUFA act on the mentioned physiological functions by influencing cell and tissue behaviour. The two ways of action can be summarised as follows: (1) action via surface or intracellular FA receptors or sensors; and (2) action via changes in the composition of cell membrane phospholipids [120].

(1) Action via surface or intracellular FA receptors or sensors can be observed e.g. in n-3 PUFA's involvement in gene transcription. n-3 PUFA have been observed to mediate gene transcription factors such as Peroxisome proliferator-activated receptors (PPAR) and Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NFκB). PPAR is activated by the noncovalent binding of n-3 PUFA and regulates adipocyte differentiation, and inflammation [121–125]. Similarly, n-3 PUFA act inhibitory on inflammatory processes through the binding on G-protein coupled surface receptors and inhibiting NFκB [126, 127].

(2) Action via changes in cell membrane composition of n-3 PUFA phospholipid composition can influence cell function. Figure 2.8 shows a summary of how membrane phospholipid composition can influence cellular function via alterations in the physical properties of the cell membrane. Membrane phospholipids maintain membrane fluidity [128] and influence lipid raft formation [129], subdomains of cell membranes where many proteins are involved in signal transduction [130].

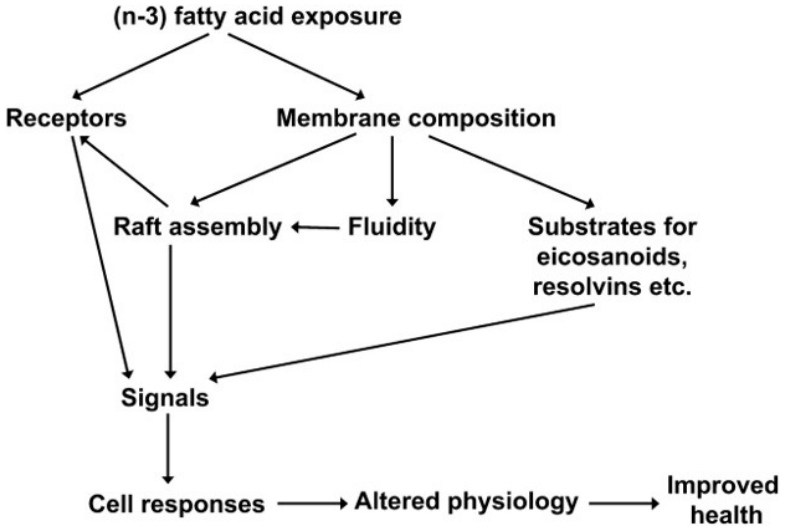

Figure 2.8: **Overview of the mechanisms by which n-3 Polyunsaturated fatty acids (PUFA) influence cell function**. This figure is taken from [120].

2.2.2 Fatty acid intake requirements, assessment of fatty acid status, and global n-3 polyunsaturated fatty acid status

Fatty acids intake requirements and dietary sources

Adequate n-3 PUFA intake has been investigated within the context of various conditions such as Coronary heart disease (CHD), inflammation and immune response, ageing or cancers. However, according to the Food and Agriculture Organization (FAO)'s definition for the strength of evidence, there is no probable or convincing evidence for significant effects of dietary fats on health conditions other than body weight (overweight and obesity) [131]. Energy balance has been identified as critical for maintaining healthy body weight and ensuring optimal nutrient intake. Table 2.3 shows requirements of total fat and different FA groups for children, adolescents and adults based on the recommendations of the FAO [131].

Table 2.3: Recommended dietary intakes for total fat and Fatty acids (FA) intake for children and adolescents (2-18 years) and adults (>18 years). This table is taken and adapted from [131].

Fat / Fatty acid	Age group	% of total Energy or mg [1]
Total fat	2-18 yrs	25-35% E
	Adults	20-35%E
SFA	2-18 yrs	8% E [2]
	Adults	10%E
MUFA	2-18 yrs	total fat [%E] - SFA [%E] - PUFA [%E] - TFA [%E]
	Adults	total fat [%E] - SFA [%E] - PUFA [%E] - TFA [%E]
Total PUFA	2-18 yrs	11%E
	Adults	6-11%E [3]
EPA + DHA	6-9 yrs	200-250 mg
	≥10 yrs	0.25-2[4]g/day or 0.5-2%E
	Adults	0.25-2[4]g/day or 0.5-2%E
TFA	2-18 yrs	<1%E
	Adults	<1%E

SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; TFA: Trans fatty acids; ALA: α-linoleic acid; LA: linoleic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; E: Energy

[1] Based on the acceptable macronutrient distribution range (AMDR)

[2] Children from families with evidence of familiar dyslipidemia (high LDL cholesterol) should receive lower SFA but nod reduced total fat intake

[3] LA + ALA + EPA + DHA

[4] For secondary prevention of coronary heart disease

Dietary fat is present in plant and animal tissues. Sources of n-3 and n-6 PUFA are summarised in Table 2.4. LA and ALA are present in all dietary fats and contribute to major proportions in most vegetable oils [132]. Arachidonic acid (AA) is mainly present in meat, dairy products, and eggs, and in small amounts in algae and other aquatic plants [133–135]. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are primarily produced in marine environments and are available to humans through fatty marine fish such as mackerel, salmon, sardine, herring and smelt, or algae oil [134, 136, 137].

Table 2.4: Dietary food sources for selected n-3 and n-6 Polyunsaturated fatty acids (PUFA). This table is taken and adapted from [131].

Fatty acid	N minus abbreviation	Typical sources
n-3 PUFA		
α-linoleic acid	18:3n-3 ALA	Flaxseed oil, perilla oil, canola oil, soybean oil
Eicosapentaenoic acid	20:5n-3 EPA	Marine sources, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
Docosapentaenoic acid	22:5n-3 DPA	Marine sources, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
Docosahexaenoic acid	22:6n-3 DHA	Marine sources, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
n-6 PUFA		
Linoleic acid	18:2n-6 LA	Most vegetable oils
Arachidonic acid	20:4n-6 AA	animal fats, liver, egg lipids, fish

PUFA: Polyunsaturated fatty acids

Assessment of fatty acid status

FA can be measured in various specimens such as serum in the form of free FA, components of erythrocyte membranes, adipose tissue from various sites or circulating triglycerides, phospholipids, or cholesterol esters [138]. Proportions of FA in chylomicrons reflect the shortest-term markers of fat intake, namely dietary fat entering the enterohepatic circulation directly after a meal [138]. Triglycerides in serum or plasma measures reflect dietary intakes of the past few hours, while serum or plasma levels of individual FA can reflect intake over the last few days or meals [138]. However, one preferred specimen for reflecting long-term dietary fat intake is Red blood cell (RBC) membranes and platelets since they reflect aggregated intake over the lifespan of erythrocytes (approx. 120 days).

Measuring the proportions of individual FA usually involves Gas chromatography/ Mass spectometry (GC/MS). Generally, the procedures involve separation, identification and quantitation stages [138]. Separation is usually performed with thin layer chromatography, followed by identification of peaks based on relative retention times and equivalent chain lengths [138]. Finally, weight quantitation of specific FA depend upon addition of internal standards of known weights [138].

FA composition is usually reported as % of total FA or as ratios. This is, significant intakes of a specific FA can decrease the relative percentage of another FA even though its intake is unaltered [138]. Consequently, percentages of individual FA contributing to the profile of all FA are rather interpreted than the absolute amount of the FA. Also, profile percentages tend to have lower variability compared to absolute concentrations [139]. Additionally, n-3 PUFA concentrations without context are more difficult to interpret since they could result from another underlying condition such as general lipemia [139]. Therefore, blood biomarkers of n-3 PUFA status are typically relative percentages or ratios [140–142]. Below, the most common n-3 PUFA indicators are introduced and explained.

Omega-3 index (n-3 index) is expressed as EPA + DHA as a percentage of total RBC FA [143]. Since its introduction in 2004 to classify risk for CHD [140] it has found a wide application in clinics and other health-related fields such as, e.g. nutritional psychiatry. So far, there are cut-off points for CHD risk with an n-3 index $<4\%$ being a high-risk, 4%-8% being an intermediate risk, and $>8\%$ a low-risk [140].

AA/EPA ratio is often used in the context of inflammation. While, EPA is a key anti-inflammatory/anti-aggregatory n-3 PUFA [144], AA is a precursor to numerous pro-inflammatory/pro-aggregatory mediators [145]. EPA competes with AA for incorporation into inflammatory cell membranes and for enzymes of eicosanoid synthesis (Fig. 2.7) [146]. To conclude, the AA/EPA ratio can be used as a marker of chronic inflammation, with higher levels representing higher levels of inflammation [142].

Dietary FA patterns are alternatives for presenting FA data which, in contrast to single fatty FA status indicators, do consider the interdependencies of FA and, therefore, can give insight into FA metabolism. The use of dietary FA patterns is not widespread but increasing [50, 147]. Factor analysis based on Principal component analysis (PCA) is applied to reduce the data to a more manageable size while retaining as much information as possible [148].

n-6/n-3 PUFA ratio has been used widespread, considering n-6 PUFA being pro-inflammatory "bad" fatty acids and n-3 PUFA being anti-inflammatory "good" fatty acids [149]. However, concerns about this ratio have become more frequent since it has some flaws [131, 150, 151]. For instance, as proven in recent years, some n-6 metabolites are pro- and others anti-inflammatory [152]. Also, there is lacking evidence that reducing n-6 PUFA intake and improving the n-6/n-3 PUFA ratio will reduce the

risk for CHD, whereas increasing n-3 PUFA intake will [150, 151]. Experts, therefore, are concerned that focusing on the ratio could distract from the more critical issue of increasing n-3 PUFA intake [151]. Therefore alternative approaches to expressing PUFA status were suggested in the field, such as the n-3 index, AA/EPA ratio or dietary FA patterns.

Global n-3 polyunsaturated fatty acid status

Low dietary n-3 PUFA status, especially EPA and DHA, has been associated with various health conditions such as CHD, inflammation, cancers, ageing, brain development, cognitive decline, preterm birth and offspring outcome, and depressive disorders [131, 140, 153–156]. Figure 2.9 displays global blood levels of the n-3 index based on a systematic review from 2016 [153] with cut-off points based on n-3 index risk levels for CHD [140]. Generally, low and very low blood levels of EPA + DHA are observed in different regions around the globe.

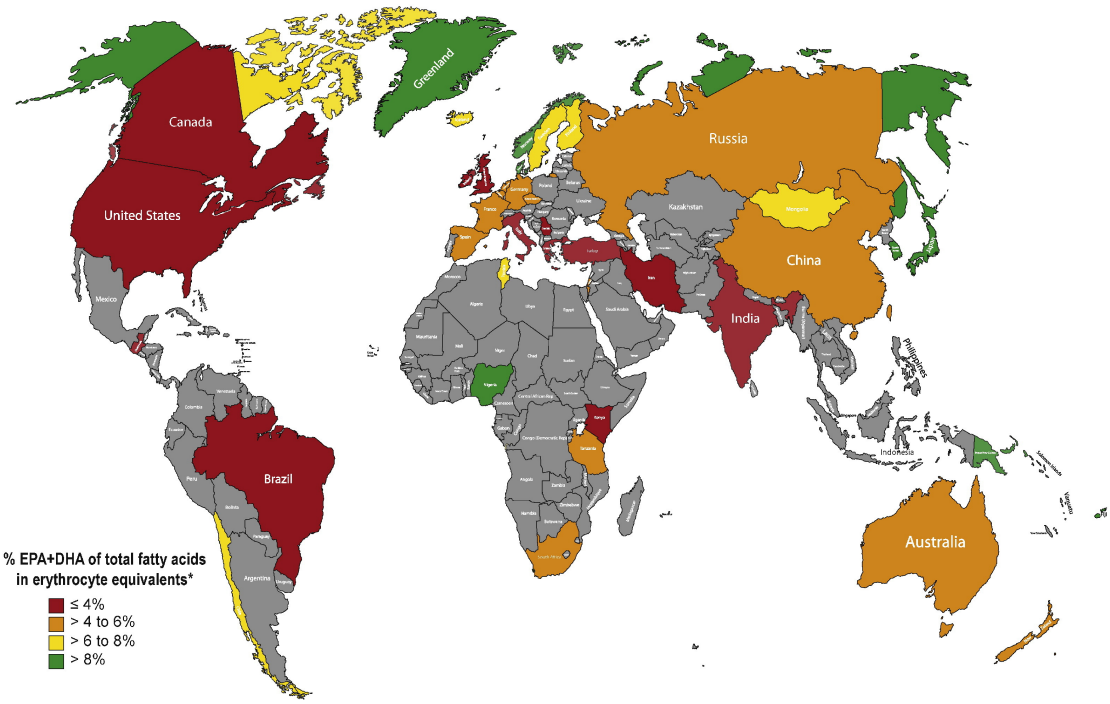

Figure 2.9: **Global blood levels of the Omega-3 index (n-3 index) (% of Eicosapentaenoic acid (EPA) + Docosahexaenoic acid (DHA))**. With fatty acid composition data from plasma total lipids, plasma phospholipids and whole blood were assigned to categorical ranges that were estimated as equivalent to erythrocyte categories. This figure is taken from [153].

To conclude, individual n-3 PUFA status is shaped by their diet. n-3 PUFA have multiple roles in an individual's metabolism, such as involvement in gene transcription and influence on cell function [120]. Also, low levels of n-3 PUFA have been associated with health conditions such as inflammation, brain development, and cognitive decline [154–156]. The following section will discuss n-3 PUFA's role in neuronal development and depressive disorders.

2.2.3 Fatty acids and depressive disorders

n-3 polyunsaturated fatty acids and neuronal development

Figure 2.10 shows the composition of the mammalian brain, which is mainly comprised of SFA, MUFA, and PUFA [155]. Within the n-3 PUFA, DHA is the most predominant FA contributing with 10-20% to total brain FA composition. The ratio of SFA, MUFA, and PUFA observed in post-mortem human brains seems to be conserved among other mammals such as monkeys, rats, and mice [157–159].

The highest accumulation of DHA is observed in astrocytes, mitochondria, synaptic vesicles involved in neurotransmitter release, brain microvessels, the retina, and in oligodendrocytes forming myelin [160–162]. This accumulation is mainly driven by dietary n-3 PUFA intake, specifically preformed DHA [163, 164], and is significantly higher in the frontal cortex compared to other brain regions [157]. Also, reduction of dietary n-3 PUFA seems to affect brain regions differently, with the pituitary gland, the frontal cortex, and the striatum being the most affected regions with up to 40% reduction of DHA content [157]. This accumulation within the brain happens predominantly during periods of rapid brain growth in humans between the third trimester of pregnancy to the third postnatal year [165, 166]. While evidence on inadequate n-3 PUFA intake during pregnancy and lactation might lead to aberrant development and function of the offspring's brain, data on whether consumption of n-3 PUFA throughout the life span is vital to maintaining brain health is more scarce. However, cerebral cortex FA concentrations change from early childhood through late adulthood [167], and maybe play a crucial role in ageing, neurodegenerative and psychiatric disorders [168–170].

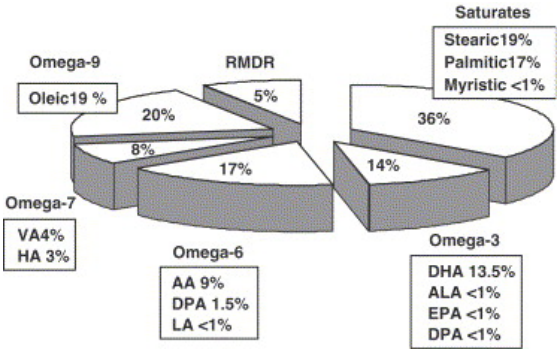

Figure 2.10: **Fatty acids (FA) compositoin of human postmortem prefrontal cortex (Brodman Area 10)** from healthy male (n=15) and female (n=15) subjects aged 29-45 years residing in the US at time of death. FA composition was determined by gas chromatography. AA: Arachidonic acid; ALA: α-linoleic acid; DHA: Docosahexaenoic acid; DPA: Docosapentaenoic acid; EPA: Eicosapentaenoic acid; LA: Linoleic acid; HA: Heptadecenoic acid; VA: Vaccenic acid; RMDR: Remainder. This figure is taken from [155].

Possible mechanisms linking n-3 polyunsaturated fatty acids to depression

In the past thirty years, evidence emerged linking lower n-3 PUFA status and insufficient n-3 PUFA intake with higher risk for MDD [10]. The link between n-3 PUFA and MDD was first suggested after seeing lower n-3 PUFA status and a reduction of dietary n-3 PUFA intake being associated with an increase in depressive symptoms [156, 171–173]. Nevertheless, despite n-3 PUFA being linked to MDD's pathophysiology and the first suggestions of evidence-based treatment guidelines using n-3 PUFA for psychiatric disorders [11], there is inconsistent data on treatment efficacy of n-3 PUFA in MDD [174]. Therefore, understanding the underlying mechanisms behind n-3 PUFA's involvement in the pathophysiology of MDD might allow for tailored treatment options, especially for treatment-resistant MDD patients.

n-3 PUFA play a crucial role in neuronal development as (1) the building block for membranes and (2), in conditions of insufficient n-3 PUFA availability, causing changes in myelination, neurogenesis, synaptogenesis, brain connectivity, and neurotransmitter turnover [11]. Since there are links between changes in brain structure and function and depression (described in section 2.1.3), n-3 PUFA's role in the pathophysiology might be partly explained by their ability to modulate the brain's functional properties, such as neurotransmitter signalling, and modifications of synaptic membrane and receptor properties via lipid rafts in cell membranes [120, 175].

Inadequate n-3 PUFA availability during critical periods in early brain development lead to irreversible altered dopaminergic function [168]. Deficits in dopaminergic function probably caused by dysregulation of afferent circuits, has been observed in MDD patients [176]. Furthermore, n-3 PUFA have anti-inflammatory, and immunomodulatory properties [144], and might be involved through modulation of inflammatory processes in the pathophysiology of depression. A dietary n-3 PUFA deficiency could increase the vulnerability to inflammation associated disrupted BBB integrity ultimately associated with depression (the inflammatory theory behind depression is described in section 2.1.3). Such an association between a dietary n-3 PUFA deficiency and increased vulnerability to inflammation-induced brain alterations has been observed among mice in the context of spatial memory impairment [177]. Also, there is increasing interest in n-3 PUFA's role in the gut-brain axis, which seems to be involved in the pathophysiology of depression [178–180]. n-3 PUFA could influence the gut microbiome by (1) modulating the type and abundance of gut microbes; (2) altering levels of pro-inflammatory mediators, and (3) regulating levels of SFA [181].

2.3 Iodine and depressive disorders

Iodine belongs to the group of halogens and its most common forms on earth are Iodide (I^-), Elemental iodine (I_2), Iodate (IO_3^-), and Periodate (IO_4^-) [182]. The most abundant forms, iodate, iodide, and elemental iodine are naturally present in surface soils and seawater. However, 68% of iodine is found in the ocean sediment [182]. Iodine is emitted from oceans into the atmosphere, precipitated with rainfall, and incorporated into the soil. An illustration of this cycle is shown in Figure 2.11.

Iodine is a trace nutrient essential to human health and needs to be consumed through diet [183]. Inside the human body, it is incorporated into the Thyroid hormones (TH), Thyroxine (T4), and Triiodothyronine (T3) [184]. These TH control various physiological processes such as embryological growth, physical and mental development, metabolic rate, and heat production [184]. Inadequate iodine nutrition is associated with impaired cognitive development and growth [12, 185]. Moreover, severe nutritional Iodine deficiency disorders (IDD) during pregnancy are responsible for most cases of mental retardation worldwide [186, 187]. The most visible effect of nutritional IDD is goitre - a condition where thyroid cells diffuse and homogeneously enlarge [184]. This section will provide insights into iodine physiology and the link between iodine and depressive disorders.

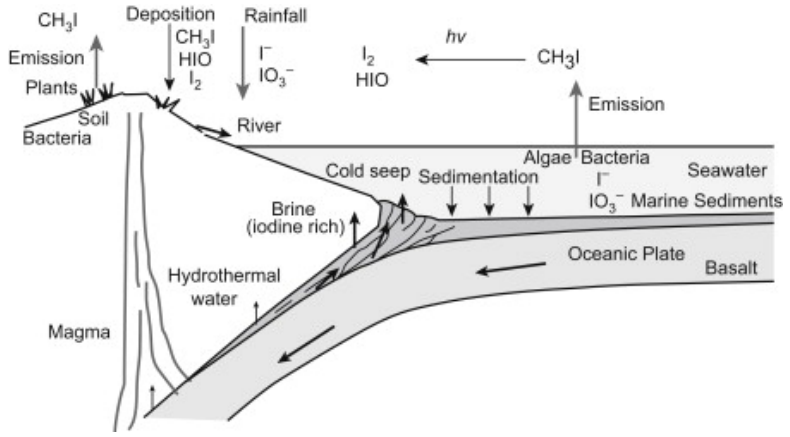

Figure 2.11: **Illustration of the global iodine cycle**. I^-: iodide, I_2: elemental iodine; IO_3^-: iodate; CH_3I: Iodoform; HIO: Hypoiodous acid; This figure is taken from [182].

2.3.1 Iodine physiology

Iodine absorption and excretion

The absorption of iodine mainly occurs in the stomach and duodenum with a very efficient absorption rate of approximately 90% in healthy individuals [183, 188]. Most of the ingested iodine is in the form of Iodide (I^-) ions, which are then actively absorbed by the Sodium/iodide symporter (NIS) located on the apical site of enterocytes [188, 189]. Iodine in the body is almost homogeneously distributed within the extracellular fluid. It is cleared from the body circulation by the thyroid gland and kidney (Figure 2.12). Usually, the body of a healthy adult contains 15-20 mg of iodine located at 70-80% in the thyroid [190]. While renal clearance is constant, thyroid clearance depends on iodine intake: in iodine sufficient conditions, $\leq$10% of iodine is absorbed and metabolised by the thyroid [191]. Thus, after clearance, approximately 90% of absorbed iodine is ultimately excreted in the urine, and only a little amount in the faeces [183].

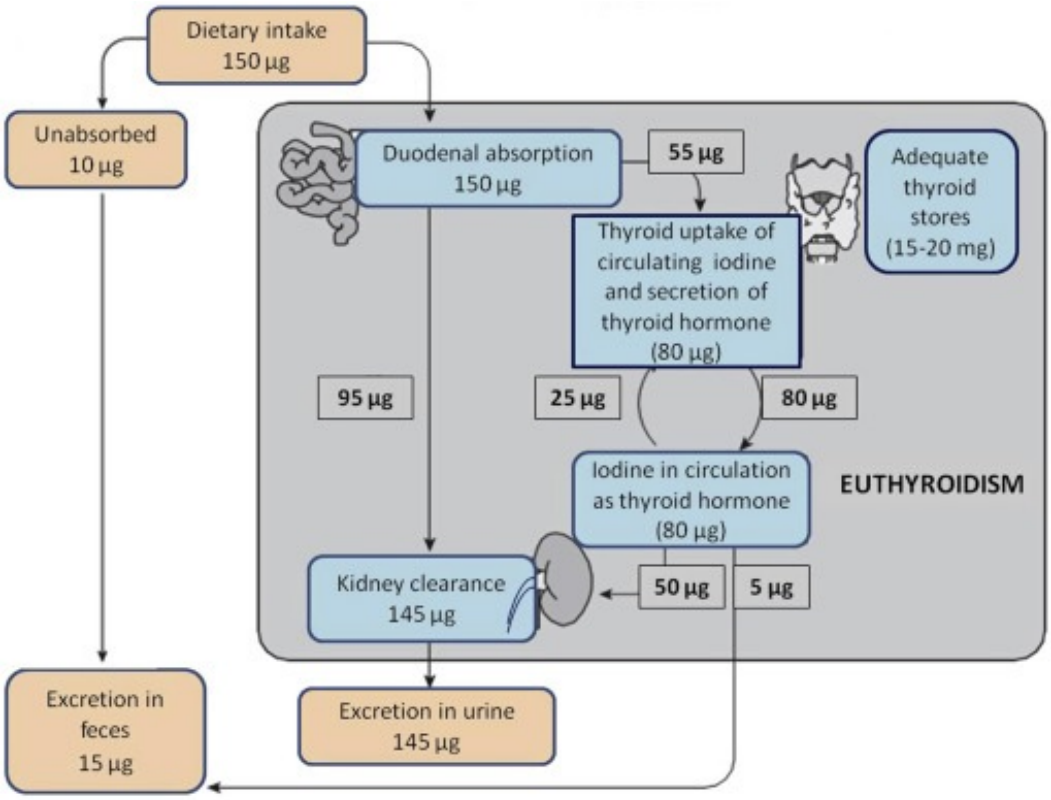

Figure 2.12: **Illustration of the absorption, clearance, and excretion of dietary iodine**. Euthyroidism: healthy state with regular function of the thyroid gland. This figure is adapted and taken from [183].

The role of iodine in the healthy body

Iodine is an essential component of the TH, T4, and T3. The structure of T4 and T3 is displayed in Figure 2.13. These TH control various physiological processes such as embryological growth, physical and mental development, metabolic rate, and heat production [184].

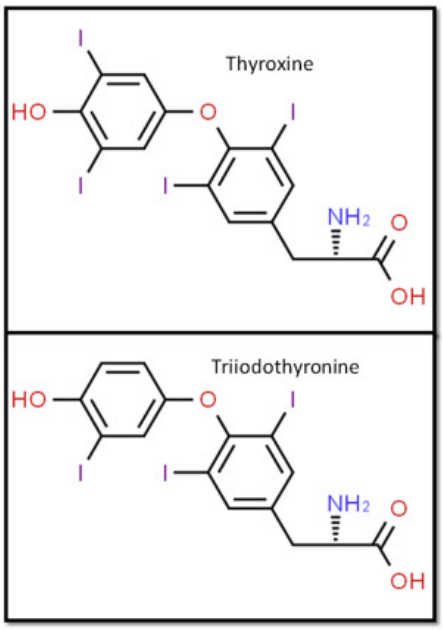

Figure 2.13: **Illustration of the structure of Thyroid hormones (TH) Thyroxine (T4) and Triiodothyronine (T3)**. Iodine is an essential component of TH. This figure is taken from [183].

TH are produced in the thyrocytes of the thyroid gland. In this case, NIS are located at the basolateral membrane of the thyrocytes and import iodide from the bloodstream. At the apical site of the thyrocyte, iodide is oxidised by the Thyroperoxidase (TPO) and Hydrogen peroxide (H_2O_2), and next covalently bound to tyrosyl residues on the glycoprotein Thyroglobulin (Tg) [183]. Tg is a storage protein for iodine within the thyroid. Together with the oxidised iodide, it produces the precursors of thyroid hormones Monoiodotyrosine (MIT) and Diiodotyrosine (DIT). In a next step, the TPO catalyses coupling of the phenyl groups of MIT and DIT to form the TH: Linking two DIT molecules produces T4, while linkage of an MIT and DIT produces T3 [183]. Figure 2.14 displays a graphical illustration of this process. Thus, the structure of T4 and T3 is identical, except T3 lacks one iodine atom compared to T4.

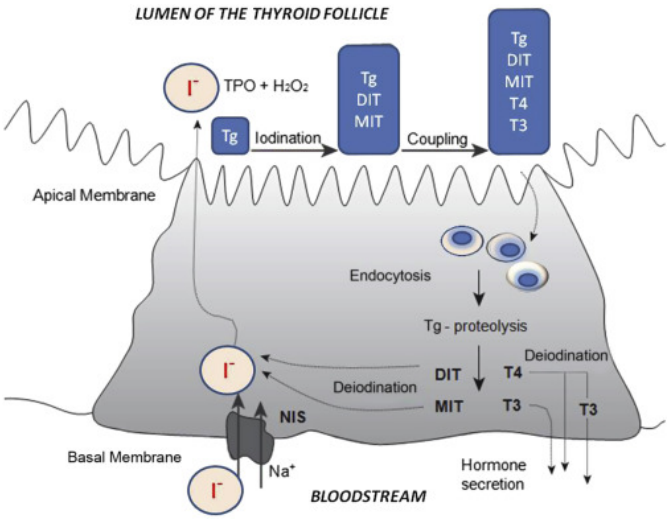

Figure 2.14: **Illustration of a cell in the thyroid gland for the production of Thyroid hormones (TH)**. I^-: Iodide; NIS: sodium/iodide symporter, TG: Thyroglobulin; TPO: Thyroperoxidase; MIT: Monoiodotyrosine; DIT: Diiodotyrosine; T4: Thyroxine; T3: triiodothyronine. This figure is taken from [183].

The Hypothalamic-Pituitary-Thyroid (HPT) axis

The production of TH T3 and T4 is regulated through a negative feedback loop of the Hypothalamic-pituitary-thyroid (HPT) axis. The hypothalamus is the highest integration centre of vegetative functions such as regulating body temperature, drinking and eating behaviour [192]. Within the hypothalamus, the hypothalamic PVN produces Thyrotropin-releasing hormone (TRH). Next, TRH stimulates the synthesis of Thyroid stimulating hormone (TSH) within the adenohypophysis, a part of the pituitary gland [193]. Finally, TSH triggers the thyroid gland's secretion of T3 and T4 into the bloodstream. While these TH evoke their functions in peripheral cells, they also inhibit further production of TRH and TSH through negative feedback. Figure 2.15 shows a graphical illustration of the HPT axis.

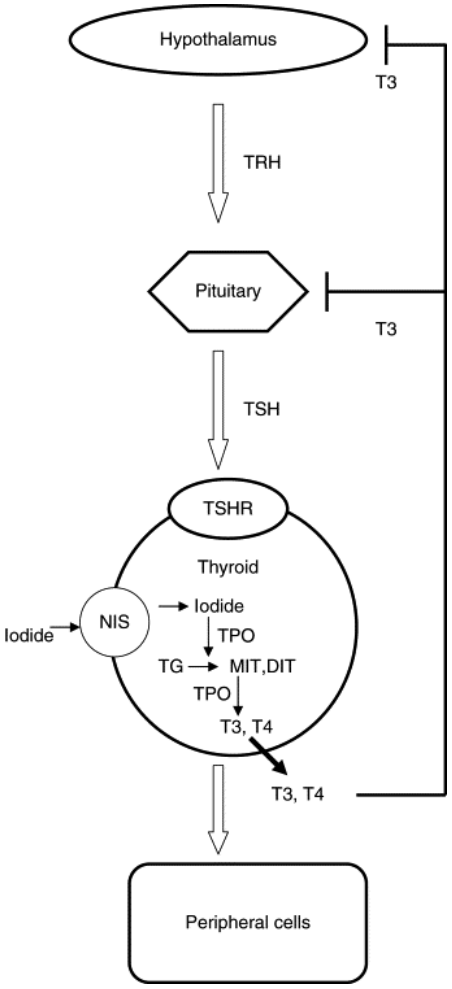

Figure 2.15: **Illustration of the Hypothalamic-pituitary-thyroid (HPT) axis**. Both Triiodothyronine (T3) and Thyroxine (T4) are involved in the negative feedback loop. However, due to simplicity in this figure, only T3 is displayed to be part of the inhibition. TSHR: TSH receptor; NIS: sodium iodide symporter; TPO: thyroid peroxidase; TG: thyroglobulin; MIT: mono-iodotyrosyl; DOT: di-iodotyrosyl. $\downarrow$ indicates enhancement; $\dashv$ indicates inhibition. This figure is taken from [193].

2.3.2 Iodine intake requirements and assessment of iodine status

Iodine intake recommendations and salt iodization programs

Iodine is available to humans through food, crops and drinking water growing on iodine-rich sources and seawater. Dietary iodine intake needs to meet the requirements to sustain a healthy thyroid function. These iodine requirements vary depending on age, sex, body weight, and physiological state [194, 195]. Table 2.5 shows dietary reference intakes for iodine in different age and life-stage groups according to the World Health Organisation (WHO) recommendations [194].

With naturally occurring geological processes such as glaciation, flooding and erosion, the surface soils can leach, reducing the nutritionally available iodine through crops [184]. Mountainous areas and areas of frequent flooding are most affected by iodine-deficient soils. Therefore, due to their geographical and climate conditions, inland areas in central and eastern Europe, the Midwestern Region of North America, and central Asia and Africa are iodine deficient [184]. Hence, populations living in these areas are at higher risk for IDD unless iodine is added to foods (e.g. salt iodisation) or introduced by food produced outside iodine-deficient areas. The most commonly used methods to correct iodine deficiency in populations are iodised oil and iodised salt [191]. Thus, the most significant fortified sources of iodine are bread, dairy products, and foods of marine origin [184, 196, 197], typically providing between 3-80μg of iodine per serving [184, 196, 197].

Table 2.5: Iodine intake (μg/day) recommendations per age group. Based on the World Health Organisation (WHO), United Nations International Children's Emergency Fund (UNICEF), and International Council for the Control of Iodine Deficiency Disorders (ICCIDD) reference values [194]

Age or population group	Iodine intake (μg/day)
0-5 years	90
6-12 years	120
>12 years and adults	150
Pregnant and lactating women	250

Assessment of iodine status

Iodine status within individuals and populations is assessed with various methods. The most commonly used are Urinary iodine concentration (UIC), blood concentrations of TSH, T4, T3, and Tg, as well as measurement of thyroid size [184].

UIC is a reliable marker for very recent iodine intake. As mentioned in section 2.3.1 most of ingested iodine is excreted in the urine. As for individuals these excretions can vary daily, while levelling out on a population level [194]. Table 2.6 shows WHO reference values for the population assessment of iodine status by UIC for adults and children.

Table 2.6: Criteria for assessing iodine nutrition based on median Urinary iodine concentration (UIC) in school aged children and adults, but not pregnant women [198]

Median urinary iodine (UIC) (μg/L) [1]	Iodine intake	Iodine status
$<$20	Insufficient	Severe iodine deficiency
20-49	Insufficient	Moderate iodine deficiency
50-99	Insufficient	Mild iodine deficiency
100-199	Adequate	Adequate iodine nutrition
200-299	Above requirements	May pose a slight risk of more than adequate iodine intake in these populations
$\geq$300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)

[1] Recommendations for school-aged children ($\geq$6 years) and adults (but not pregnant women)

Thyroid size is traditionally determined by inspection, palpation, and ultrasonography [194]. It is an often-used measure of IDD severity and the long-term impact of iodine control programs. The thyroid size changes with iodine intake: in iodine-deficient conditions, the thyroid enlarges and shrinks in iodine-sufficient conditions. A goitre is a thyroid gland which is enlarged. More precisely, the gland will be considered goitrous when each of the two gland lobes has a greater volume than the terminal phalanges of the thumb of the person examined [194]. A goitre is an adaptation of the thyroid gland to maximise iodine intake by proliferation (hyperplasia) of the cells through elevated TSH levels [199].

Blood constituents such as TSH, T4, T3, and Tg are measured in dried blood spots on filter paper, or serum samples [194]. While T4 and T3 concentrations in serum samples might reflect accurately the individual's thyroid function [200], measuring these parameters is usually not recommended for population-wide monitoring of iodine nutrition due to higher expenses and more laborious effort [194]. TSH has been shown to be a insensitive marker for iodine status, since concentrations may rise slightly in iodine-deficiency conditions but commonly remain within reference ranges [200]. Tg has been shown to be a sensitive marker for decreased and increased iodine intake on a population level but lacks diagnostic specificity on an individual level [200].

Global prevalence of nutritional iodine deficiency disorders

The research on the treatment of endemic goitre reaches as far back as the ancient Greeks, which used marine sponges to treat swollen glands [201]. Later, Italian physicians first reported using a sponge and dried seawater to treat goitre [201]. In 1922, the first public health program using iodised salt was implemented in Switzerland [201]. Nowadays, nutritional IDD are rarer and known to be preventable [194]. In 1993, the WHO and UNICEF recommended universal salt iodisation as the primary strategy to eliminate IDD [202]. Since then, many countries have introduced mandatory and voluntary salt iodisation by legislation, resulting in 88% of the global population using iodised salt by the year 2020 [203]. Moreover, the number of countries with adequate iodine intake has nearly doubled between the years 2003 and 2020 from 67 to 118 [203]. Figure 2.16 shows a map of global iodine intake based on school-aged children data from 2021 of the Iodine Global Network [204].

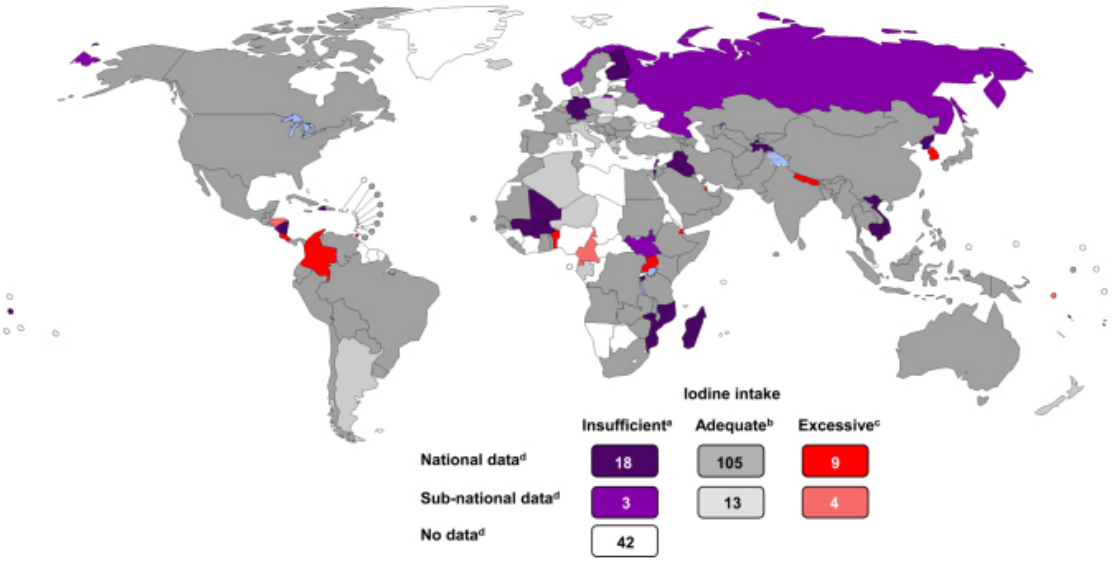

Figure 2.16: **Estimated global iodine intake in 194 World Health Organisation (WHO) Member states in 2020 based on national median Urinary iodine concentration (UIC) in school aged children**. Data is taken from studies conducted from 2005-2020. [a]Median UIC: $<$100 μg/L; [b]Median UIC: 100-299 μg/L; [c]Median UIC: $\geq$300 μg/L; [d]Number of countries. This picture is taken from [204].

Diseases of the Hypothalamic-Pituitary-Thyroid axis

Nowadays, nutritional IDD are less frequent. Nevertheless, when it comes to diseases of the HPT axis, any part of the axis can be dysfunctional, resulting in the exact clinical representations caused by nutritional IDD. One can distinguish between congenital disorders, traumatic lesions of brain parts and disorders induced through inadequate iodine intake [183, 193]. Clinically, these disorders often have identical symptoms despite their so diverse aetiology. The following section focuses on the clinical presentation of HPT axis disorders such as hypo- and hyperthyroidism and describes the current state of research on their aetiologies.

Clinical or overt hypothyroidism is characterised by abnormally decreased levels of T4 and T3, combined with elevated TSH levels [205]. In the pre-clinical form, subclinical hypothyroidism is characterised by normal T4 and T3 levels with elevated TSH levels [205]. In the past, the most common cause of hypothyroidism has been iodine deficiency. Iodine deficiency still affects many people in LMIC in Africa, and South Asia [203]. On the contrary, in HIC, hypothyroidism is most often caused by an autoimmune disease causing inflammation in the thyroid tissue (Hashimoto's thyroiditis), faulty embryogenesis, or a reduction of functioning thyroid tissue due to surgery, cancer, and radiation damage [193]. Overall, most cases of hypothyroidism in iodine-sufficient conditions are non-goitrous [193]. However, the focus of this thesis will lie on hypothyroidism caused by nutritional iodine deficiency.

The major impact of hypothyroidism due to nutritional iodine deficiency is impaired neuronal development, particularly early in life. In the foetal brain, inadequate thyroid hormone impairs myelination, cell migration, differentiation, and maturation [12]. Therefore, the most vulnerable groups to IDD are pregnant and lactating women, newborns, and children [206]. Symptoms of hypothyroidism are often vague and non-specific, including fatigue, cold intolerance, constipation, dry skin, and hair loss [205].

Isolated or transient hypothyroxinaemia is characterised by low T4 levels and normal TSH levels [207]. Up to this point, it has predominantly been described during pregnancy with iodine deficiency [207]. Hypothyroxinaemia does not resolve after iodine supplementation, and it can also be found in iodine-sufficient areas [208]. Consequently, isolated hypothyroxinaemia is considered multifactorial pathophysiology of the HPT axis [208, 209]. Potential risk factors for hypothyroxinaemia among women of child-bearing age include vitamin D deficiency, insulin resistance, increased Body mass index (BMI), and abnormal lipid profile [210].

There is evidence, that hypothyroxinaemia might be associated with poor neurobehavioural outcomes of the offspring, but there is no indication of adverse events during pregnancy [209]. The poor neurobehavioural outcome of the offspring can include lowering the non-verbal IQ, brain morphology, more internalisation and widespread behavioural problems [211, 212].

Clinical or overt hyperthyroidism is characterised by elevated T4, and T3 values and low TSH values [213]. The pre-clinical form subclinical hyperthyroidism is characterised by normal T4, and T3 values and low TSH levels [213]. Hyperthyroidism can be caused by nutritional iodine excess, an autoimmune disorder known as Grave's disease, thyroiditis, tumours, toxic multinodular goitre, and large amounts of T4 received through medications or dietary supplements [213]. Common signs and symptoms are nervousness, increased appetite, insomnia, and weight loss [213].

Hyperthyroidism has been associated with cardiovascular complications [214, 215], and adverse outcomes during pregnancy for both the mother and her child [216].

To conclude, iodine needs to be consumed through diet. It is a crucial component of TH which regulate physiological processes such as neuronal development, metabolic rate, and heat production [183]. Nutritional iodine deficiency has been associated with impaired neuronal development and cognitive retardation [12]. The following section will discuss iodine's role in neuronal development and depressive disorders.

2.3.3 The Hypothalamic-pituitary-thyroid axis and depressive disorders

Iodine, thyroid hormones and neuronal development

Iodine acts as an integral component of TH in neurological development. These TH act on migration and differentiation of neuronal cells, synaptogenesis, and myelination [217]. These actions are mediated through nuclear thyroid hormone receptors and regulation of gene expression [217]. Figure 2.17 shows critical time points during gestation which require TH action for foetal neurological development. The most critical period for neuronal development is from the first trimester of pregnancy to the third year after birth, the periods of most significant brain growth [185, 187]. The foetal thyroid gland, however, develops in the second trimester of pregnancy, and before that time, the only possible source of TH is the maternal thyroid [217]. Therefore, maternal TH is transported across the placenta [217]. Severe nutritional iodine deficiency causing maternal and foetal hypothyroidism is the most common cause of mental retardation worldwide [186, 187]. To conclude, TH are of fundamental importance for brain development during gestation, in the first years of life, and for the healthy functioning of the adult brain [218]. Therefore, TH could be potential targets for therapeutic intervention in brain-health-related diseases such as depressive disorders.

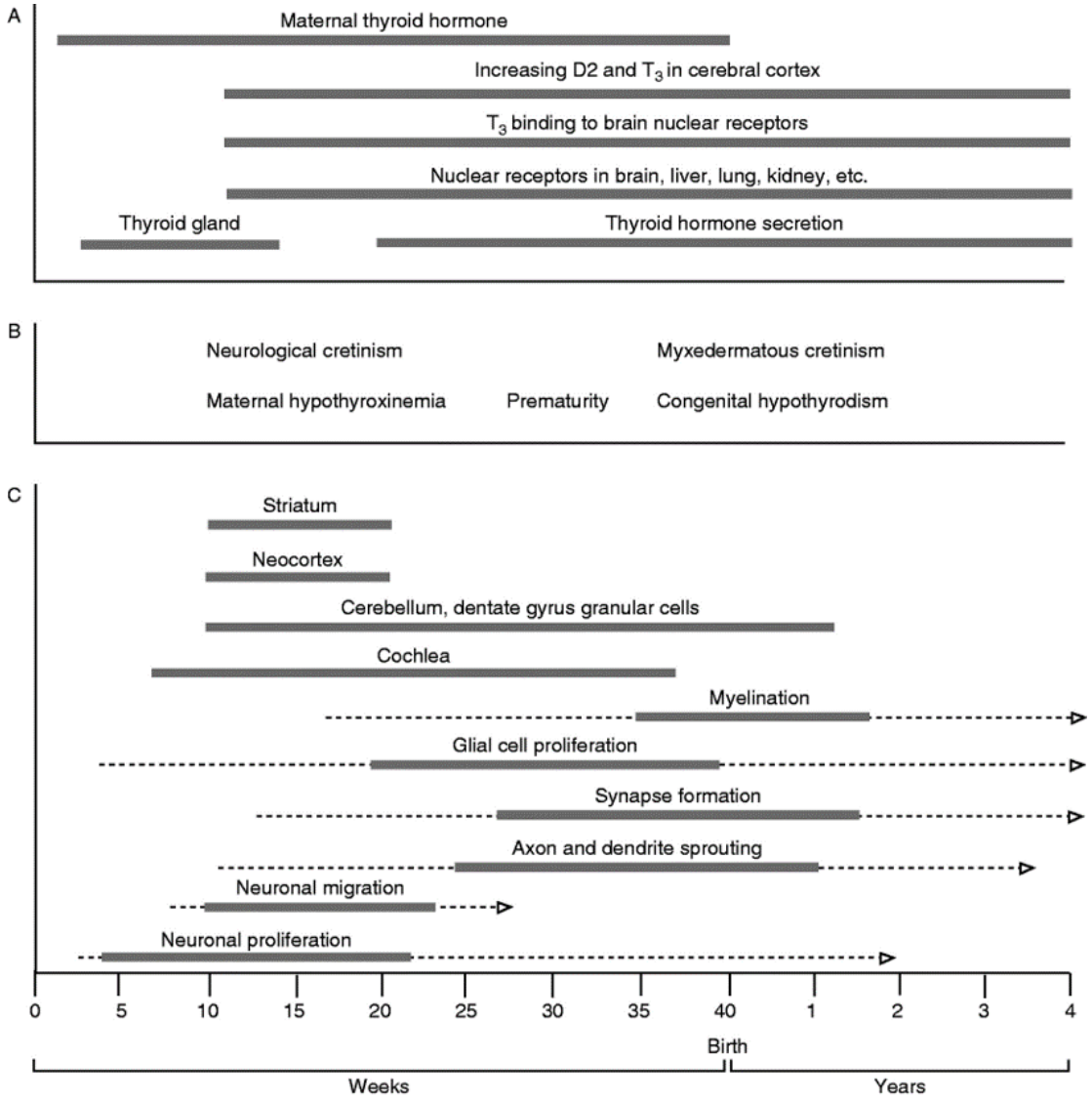

Figure 2.17: **A summary of Thyroid hormones (TH)'s role in human foetal and postnatal brain development**. (A) The development of foetal thyroid function and expression of TH receptors and deiodinase enzymes during gestation and postnatally early years. (B) Critical time points during gestation requiring TH action for foetal neurological development. Neurological abnormalities are observed when maternal or foetal hypothyroidism is present during gestation. (C) Rodent studies, where time-specific actions of TH on precise neurological and auditory structures could be observed. This figure is taken from [219] and originally adapted from [217].

Possible mechanisms linking iodine and thyroid hormones to depression

The link between altered thyroid functions and psychiatric symptoms reaches as far as 200 years back, when increased incidence of "nervous affections" were reported in thyroid disorders [220]. Particularly the associations between hypothyroidism and depression have consistently been reported for more than 100 years [205]. Hyperthyroidism presents a broad range of psychiatric symptoms such as dysphoria, anxiety, irritability, and concentration impairment. Nevertheless, it is in conditions of hypothyroidism where alterations in cognition and depression are most frequently observed [205, 220]. It is well established that symptoms of depression during overt hypothyroidism generally resolve after the euthyroid status is restored [221].

The relationship between subclinical hypothyroidism and depression has been increasingly investigated in recent years resulting in inconsistent evidence. For instance, a meta-analysis of 21 studies reported a 2.5 times higher risk for depression in adults with subclinical hypothyroidism, particularly in an elderly population, compared to euthyroid adults [222]. Also, a meta-analysis of 17 publications found associations between mild thyroid dysfunction and depression aomng adult patients ($<$60 years) [223]. On the other hand, the same meta-analysis in 17 publications found no significant association of subclinical hypothyroidism and depression within older patients ($\geq$60 years old) [223]. Therefore, whether restoring healthy thyroid functions in subclinical hypothyroidism can ameliorate depressive symptoms needs further investigation.

The mechanisms underlying the interaction between thyroid function and depression are still unclear [220]. One possible mechanism of aberrant HPT axis functioning in depressive disorders is linked to the HPA axis (for the HPA axis, see section 2.1.3). The most important relationship could be that hypercortisolemia inhibits TRH secretion, controlling TSH release in the PVN [224, 225]. However, recent studies fail to confirm the role of elevated cortisol levels in the HPT axis dysregulation in depression among adults and adolescents [226, 227]. Another intriguing hypothesis connects dysfunctions of the HPT axis with aberrant DA function in depressed patients [228]. In some patients, a TRH compensatory mechanism can be observed, where TRH secretion is increased, leading to a reduced TSH, to correct for the reduced central 5-HT activity [229]. This mechanism is suggested based on pre-clinical studies, which show that TRH increases DA synthesis and release in the nucleus accumbens and the striatum [230, 231]. However, recent data on this link between the HPT axis and DA system show unaltered hypothalamic DA function in depressed patients with HPT dysregulation. On the contrary, in patients with regular HPT activity, decreased hypothalamic DA-receptor function was identified [228]. Therefore, the hypothesis was suggested that the TRH compensatory mechanism seen among depressed patients requires functional integrity of the hypothalamic DA system [228]. Thus, a potential therapeutic option in treatment-resistant depression would be applying DA agonist agents to stimulate TRH-mediated compensatory production of 5-HT [228].

2.4 Iron and depressive disorders

Iron (Fe) is the fourth most abundant element on the earth's surface and, like many other nutrients, follows a geochemical cycle [232]. Iron presents itself in different oxidation states ranging from -2 to +6 [233]. However, the most abundant and biologically relevant forms are Ferrous iron (Fe^{2+}) and Ferric iron (Fe^{3+}) [232]. Iron is essential to human health through complex interactions between iron homeostasis, oxygen supply, and cellular energy metabolism [234]. For instance, iron-sulfur clusters play a crucial role in electron transfer in CI, CII, and CIII complexes involved in universal ATP production, which provides energy for many processes in living cells [235]. Iron is available to humans mainly through food growing on soils containing high iron concentrations, such as dark green vegetables, cereals, and potatoes. Other sources of iron are animal products such as meat, fish, egg yolks, and fortified food products [233].

The process of iron uptake in humans is usually described with terms such as absorption, utilisation and bioavailability. In this thesis, iron absorption refers to iron uptake from the gut lumen to the bloodstream. Iron utilisation describes the uptake of circulating iron for the body's metabolic processes. Bioavailability is the combined measure of absorption and utilisation of dietary iron, expressed as a percentage or fraction of the total iron intake [233]. The following section will introduce iron metabolism and its link to depression, starting with an overview of iron physiology, followed by methods of iron status assessment, and finally, iron's role in neuronal development and depressive disorders.

2.4.1 Iron physiology

Cellular and systemic iron homeostasis

Figure 2.18 illustrates the systemic use of iron in the human body. Iron concentration in human bodies is exclusively regulated by iron absorption in the duodenum, as humans do not actively excrete iron [236]. Further, biologically available iron is scarce and humans, therefore, efficiently conserve and recycle iron within the body [237].

Dietary iron is found in two forms: non-heme and heme iron. Heme is a protein in different natural derivates and possesses a central iron ion. As an electron transferring co-factor of cytochromes, heme plays an essential role in aerobic, and anaerobic respiration [238]. Cellular non-heme iron uptake is mediated by the Divalent metal ion transporter 1 (DMT1) [239]. Therefore, most dietary iron (Fe^{3+}) must be reduced to Fe^{2+} for example, by ferrireductases such as Duodenal cytochrome b (DCYTB) located at the apical membrane of duodenal cells or by other reducing agents such as ascorbic acid [236, 240]. On the other hand, cellular heme iron uptake is mediated by the Heme iron transporter 1 (HCP1) [241]. Once iron enters the enterocyte, it can be

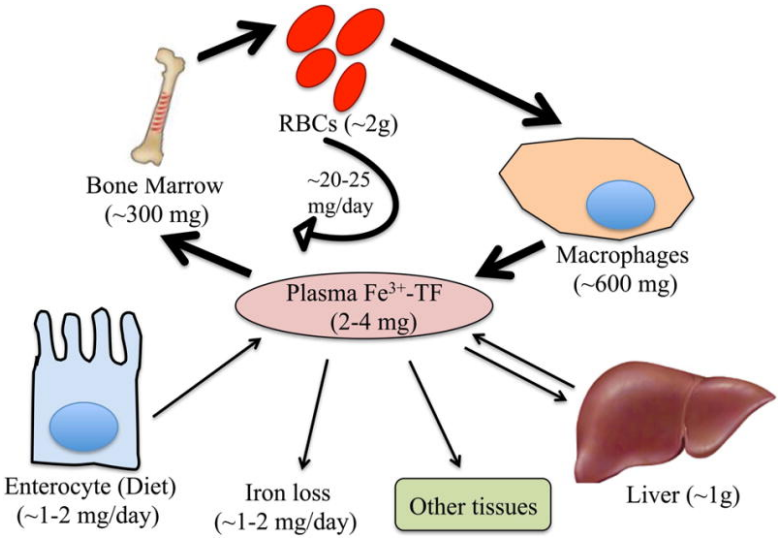

Figure 2.18: **Illustration of the systemic iron homeostasis**. RBC: Red blood cells; Fe^{3+}: ferric iron; TF: transferrin. This figure is taken from [237].

used for intrinsic metabolic processes, storage, or for iron export into the blood [236, 237]. Cellular iron storage occurs in enterocytes in the form of ferritin [242]. Cellular iron export across the basolateral membrane is mediated by the transport protein ferroportin 1, and the iron oxidase hephaestin [243, 244]. The main form of iron in the bloodstream is bound to Transferrin (Tf), which can carry up to two iron molecules [237]. Figure 2.19 shows an overview of cellular iron homeostasis processes from iron uptake and storage to iron export.

The three primary sources of systemic iron are (1) dietary absorption in the duodenum, (2) the release of recycled iron from macrophages and (3) the release of stored ferritin in hepatocytes [246]. Systemic iron homeostasis is regulated through various mechanisms. For instance,ID or hypoxia stimulate the duodenal expression of DMT1, DCYTB, and ferroportin and enhance intestinal absorption [240, 247]. However, the primary regulator of systemic iron homeostasis is hepcidin, a peptide hormone that inhibits iron entry into plasma [246]. Hepcidin is secreted from hepatocyte cells [248] and is overexpressed under iron overloaded conditions [249]. Once synthesised, hepcidin will bind to ferroportin and internalise and degrade the transport protein, which will lead to a decreased export of cellular iron [250]. The iron concentration in the body is therefore regulated by a homeostatic loop with iron regulating the secretion of hepcidin, which in turn controls the concentration of ferroportin on cell membranes and cellular iron export [250]. Since iron is not actively excreted, it is removed from the body via intestinal secretions by the mucosa of the gastrointestinal, respiratory and genito-urinary tracts via sweat, urine, semen, and menstrual blood [233].

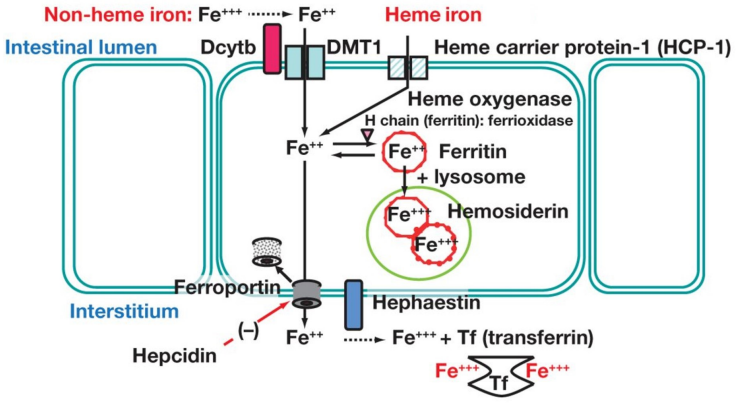

Figure 2.19: **Illustration of the cellular iron homeostasis**. Dcytb: Duodenal cytochrome b; DMT1: Divalent metal ion transporter 1; Tf: Transferrin. This figure is taken and adapted from [245].

The role of iron in the healthy body

Iron is essential to cellular energy metabolism through various mechanisms. Firstly, it affects cellular oxygen consumption and is a component of the significant oxygen transporter haemoglobin [234]. Secondly, iron is involved in mitochondrial energy production through iron-sulfur clusters in electron transfer complexes CI, CII, and CIII [235].

Since iron plays a vital role in producing ATP, the universal energy metabolite, iron is utilised by bacteria and pathogens for growth. Consequently, the human body controls iron fluxes in response to bacterial infection [251]. In response to inflammation, Acute phase reactants (APR) serum concentrations change to battle the pathogen. However, APR cause adverse effects such as fever, lethargy, or anaemia of chronic disease [252, 253]. Hepcidin and ferritin are positive APR and therefore up-regulated during inflammation [252, 253]. As described in section 2.4.1, hepcidin inhibits iron absorption and transport by binding to ferroportin in intestinal mucosal cells and macrophages. Ferritin sequesters iron for storage in cells. By up-regulating hepcidin and ferritin, the body reduces freely available iron for pathogens [253]. In contrast, transferrin is a negative APR which is therefore down-regulated in case of inflammation [252, 253]. Consequently, macrophages' internalisation of Tf also reduces available iron.

Anaemia of inflammation is usually mild to moderately severe anaemia that develops under infection, or inflammatory disease [254]. Under this condition, serum iron is low despite adequate systemic iron stores. In cases of chronic inflammatory disease, the red blood cells usually are mildly decreased in size, and haemoglobin content [254].

2.4.2 Iron intake requirements and assessment of iron status

Iron intake recommendations and food fortification programs

As described previously, there are two forms of dietary iron: heme and non-heme iron. Heme-iron, mainly present in meat, poultry, and fish, is estimated to contribute $\geq$40% to total iron absorption [255, 256]. In contrast, non-heme iron includes ferritin and is present in animal and plant foods such as liver and legume seeds [233]. Typically it is worse absorbed compared to heme-iron [257]. Further, both the diet composition and the iron status influence the bioavailability of iron [256, 257]. However, the daily iron intake needs to meet the iron requirements to sustain healthy body functioning. Table 2.5 shows dietary reference values for iron based on the European food safety authority (EFSA)'s and Germany-Austria-Switzerland region (D-A-CH) recommendations [233, 258].

Table 2.7: Iron intake recommendations by sex and age group. Based on the European food safety authority (EFSA) and Germany-Austria-Switzerland region (D-A-CH) reference values [233, 258]

	Females			Males		
Age population group	Average requirement (mg/day) EFSA	Population Reference Intake (mg/day) EFSA	Individual intake (mg/day) D-A-CH	Average requirement (mg/day) EFSA	Population Reference Intake (mg/day) EFSA	Individual intake (mg/day) D-A-CH
7-11 months	8	11	8	8	11	8
1-6 years	5	7	8	5	7	8
12-17 years	7	13	15	8	11	12
$\geq$18 years			15	6	11	10
Premenopausal [1]	7	16				
Postmenopausal	6	11				
Pregnant women			30			
Lactating women			20			

[1] Including pregnant and lactating women

Assessment of iron status

Individual and population-wide iron status can be assessed straightforward using serum ferritin cut-off values. However, by measuring Soluble transferrin receptor (sTfR) and haemoglobin concentrations, additional information about the severity of the nutritional ID can be acquired.

Serum ferritin concentration is positively correlated with the total iron stored in the body [259]. However, since ferritin is an APR, interpreting ferritin values is difficult in areas of widespread infection or inflammation, e.g. due to malaria or other infectious diseases. Table 2.8 shows ferritin cut-off concentrations indicating depleted iron stores or iron overload in the absence and presence of inflammation.

Table 2.8: World Health Organisation (WHO) serum ferritin cut-off values for diagnosis of iron status [259]

	Serum ferritin μg/L			
	<5 years of age		≥5 years of age	
	Female	Male	Female	Male
Depleted iron stores	<12	<12	<15	<15
Depleted iron stores in presence of infection	<30	<30	-	-
Severe risk of iron overload			>150	>200

sTfR reflects the intensity of erythropoiesis and demand for iron as it is mainly derived from developing RBC [260]. Mostly, sTfR is measured with and commercially available Enzyme-linked immunosorbent assay (ELISA) which provides cut-off values provided by the manufacturer.

Measurement of iron status of populations in places with widespread infection it is recommended to assess the combination of serum ferritin values and sTfR concentrations [260]. Table 2.9 shows the interpretation of serum ferritin and sTfR in population surveys according to WHO guidelines [261]

Table 2.9: World Health Organisation (WHO) interpretation of serum ferritin and Soluble transferrin receptor (sTfR) concentration in population surveys based on [261].

Percentage of serum ferritin values below threshold [1]	Percentage of serum transferrin receptor values above cut-off values [2]	Interpretation
<20[3]	<10	Iron deficiency is not prevalent
<20[3]	≥10	Iron deficiency is prevalent; inflammation is prevalent
≥20[4]	≥10	Iron deficiency is prevalent
≥20[4]	<10	Iron deficiency is prevalent

[1] Apply cut-off values by age group as described in Table 2.8 [259].
[2] Apply cut-off values recommended by manufacturer of assay until international reference standard is available
[3] <30% for pregnant women
[4] ≥30% for pregnant women

Since iron status indicators alone are usually difficult to interpret, they are typically used in combination with other parameters such as anaemia [262]. Anaemia is most commonly caused by nutritional ID. Thus it is often used as an indirect indicator for iron status [263].

Anaemia is a condition where the body's physiological oxygen needs are insufficient due to a reduced number of RBC and their oxygen-carrying capacities [263]. Anaemia is most commonly caused by ID. However, there are other known causes such as nutritional vitamin A, B_{12} deficiency, chronic inflammation, parasitic infections, and inherited disorders such as, e.g. disorders affecting haemoglobin synthesis or erythropoiesis [263]. Anaemia is quantified using haemoglobin concentration. However, haemoglobin concentrations alone cannot be used to diagnose ID. Nevertheless, haemoglobin concentrations can provide information about the severity of the ID, and together with other iron status parameters, they are important health status indicators [263]. Table 2.10 shows haemoglobin cut-off values to diagnose anaemia at sea level based on WHO reference values [263]. These reference values are set at sea level since an elevation above sea level is known to increase haemoglobin concentrations [263].

Table 2.10: World Health Organisation (WHO) cut-off values for haemoglobin to diagnose anaemia at sea level based on [263]

		Anaemia [1]		
Population	Non-Anaemia [1]	Mild [2]	Moderate	Severe
Children 6-59 months of age	110 or higher	100-109	70-99	lower than 70
Children 5-11 years of age	115 or higher	110-114	80-109	lower than 80
Children 12-14 years of age	120 or higher	110-119	80-109	lower than 80
Non-pregnant women ($\geq$15 years of age)	120 or higher	110-119	80-109	lower than 80
Pregnant women	110 or higher	100-109	70-99	lower than 70
Men ($\geq$15 years of age)	130 or higher	110-129	80-109	lower than 80

[1] Haemoglobin in g/L

[2] "Mild" is a misomer: iron deficiency is already advanced by the time anaemia is detected. The deficiency has consequences even when no anaemia is clinically apparent.

Global prevalence of nutritional iron deficiency

Nutritional ID has long been and is still considered to be one of the most prevalent forms of malnutrition [264]. Still, there is few data available on the prevalence of ID itself [262]. Therefore anaemia as an indirect indicator for nutritional ID is typically used to estimate the prevalence of nutritional ID [263]. However, for developing strategies and programs to control anaemia, it is important to consider the fact, that anaemia can also be caused by other factors as described in section 2.4.2 [262]. Nevertheless, in this thesis anaemia will be used as indirect indicator for ID. Due to iron's role in energy metabolism, the most vulnerable groups to ID are children under the age of 5 years, women of reproductive age, pregnant and lactating women [265]. These population groups show rapid growth, menstrual blood losses and higher nutritional iron requirements from the foetus [262]. Figure 2.20 shows WHO global estimates for anaemia prevalence in infants and children aged 6-59 months [266].

Worldwide, 42% of children (<5 years of age) and 33% of women of reproductive age were anaemic [264], while around 43% of cases in children and around 50% of cases in women are estimated to be caused by ID [266].

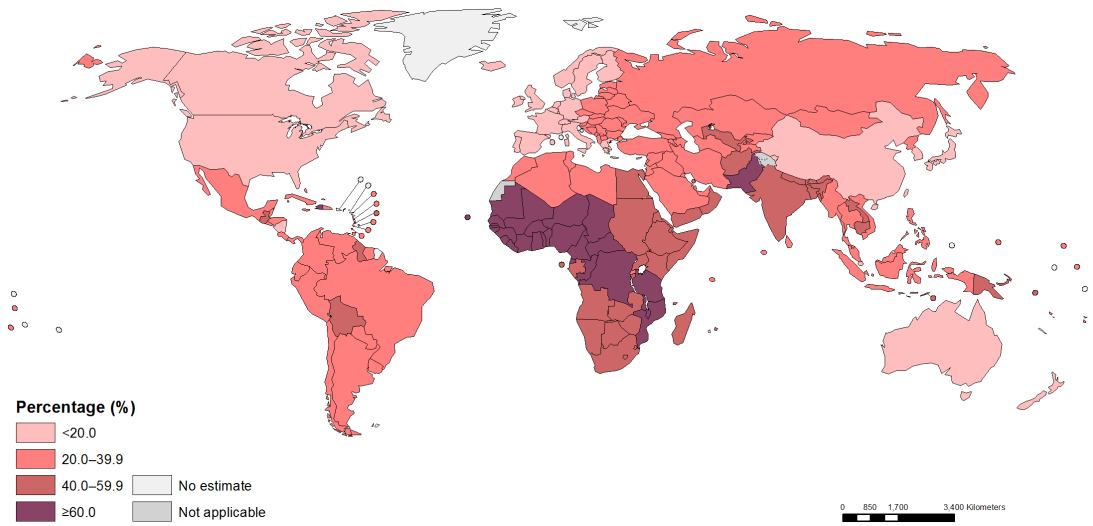

Figure 2.20: **Global estimates of anaemia prevalence in infants and children aged 6-59 months in 2011**. This figure is taken from [266].

Three main strategies are applied in public health measures to overcome nutritional ID: (1) dietary diversification, (2) food fortification, (3) and supplementation [267]. While supplementation is the most rapid approach to reverse ID, fortification and dietary diversification are more sustainable in terms of long-term solutions.

To conclude, iron is essential for oxygen transport, and energy metabolism in human health [13, 234]. Therefore, iron needs to be consumed through the diet. Nutritional ID is the most common cause of anaemia [263], and ID has been associated with aberrant neuronal development in the last trimester of pregnancy, and early life [268]. The following section will discuss iron's role in neuronal development and depressive disorders.

2.4.3 Iron and depressive disorders

Iron deficiency and neuronal development

As a critical co-factor in electron transfer reaction of cellular respiration, gene regulation, binding and transport of oxygen, and regulation of cell growth and differentiation, iron plays a significant role in brain development [268]. Most of the current knowledge about the role of iron in neuronal development is based on human and animal studies. Iron is spatially unevenly distributed among different brain tissues [269], with most brain iron located in the basal ganglia, substantia nigra, and deep cerebellar nuclei [270]. During gestation, the foetus stores about 250 mg of iron in the body [236]. The infant then draws from these iron stores while breastfed since breast milk only provides 0.15 mg/day iron of daily iron requirements of about 0.55 mg/day [236]. The transport of iron across the BBB to the brain is mediated by Tf [271]. ID during the foetal/early postnatal period does affect neurological development, and functioning by alterations in brain energy metabolism [13]. These alterations in brain energy metabolism could lead to the ID's observed consequences on neuronal development, such as decreased myelin formation and alterations in neurotransmitter metabolism [268, 269].

Iron is a crucial co-factor of the cytochromes involved in the electron transfer chain for the production of ATP [13]. Therefore, there is evidence that early life ID disrupts the brain energy metabolism through enzyme-driven mitochondrial dysfunction [13]. Figure 2.21 illustrates early life ID leading to persistent mitochondrial dysfunction in the adult brain. Even if there is a partial reduction of the neurological alterations due to early-life ID after iron repletion, deficits in the neurological energy metabolism do persist [13]. These changes in the brain energy metabolism can influence neuronal architecture and the brain's neurophysiologic capacity (plasticity). The following paragraphs will give an overview of the processes in simplified neuronal architecture and plasticity having detrimental consequences on neuronal development, which have been independently observed with early-life ID.

Simplified neuronal architecture due to lack of reduced energy availability has been observed in the context of myelination. Oligodendrocytes are the predominantly iron-containing cells in the brain and the primary myelinating cells within the CNS [269]. Several studies in the rat model of ID show decreased myelin proteins after dietary iron restriction during gestation and the early postnatal period [272, 273]. Nevertheless, oligodendrocyte alterations in the structure and neuronal physiology of myelination do persist in adulthood despite later supplementation of iron [274]. Further, studies indicate that ID distorts dendrites and their branching pattern, especially in the hippocampus, resulting in decreased hippocampal volume [275–277].

Neurophysiologic capacity such as the ability to generate action potentials is also affected by the disruption of energy metabolism due to ID [278]. Furthermore, foetal ID disrupts the maturation of the synaptic function in the development of rat hippocampus [279]. Synaptic pruning and maturation, in turn, are crucial for neurotransmission. In the same way, iron is essential for several enzymes involved in neurotransmitter synthesis [280].

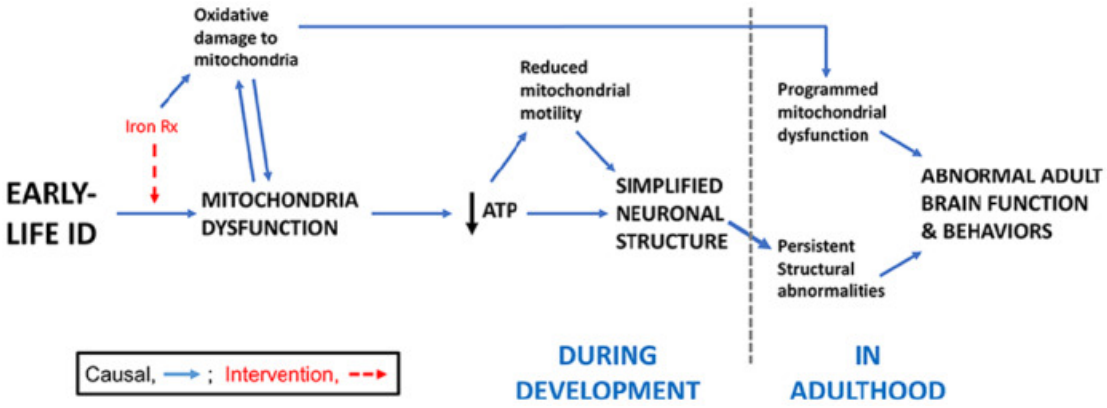

Figure 2.21: **Conceptual model of energy metabolism and long-term neurobehavioural dysfunction**. Iron Rx: Iron repletion. ATP: Adenosine triphosphate. ID: Iron deficiency. This figure is taken from [13].

The roles of iron in the adult brain are discussed with increasing interest in the context of neurophysiological mechanisms, cognition, and social behaviour [281]. Disruption of iron homeostasis during adolescence and adulthood could nudge the onset and progression of psychosocial disorders such as depression [281].

Possible mechanisms linking iron to depression

For children and adolescents, the most common neurological sign of ID include poor school performance, decreased cognitive abilities and behavioural problems [282, 283]. These are also symptoms associated with hypomyelination and depressive symptoms [56, 271]. However, there is inconsistent literature on the association between lower levels of circulating ferritin values with increased risk for depression [284–287].

One significant role of iron in the mechanism of depressive disorders is associated with mitochondrial dysfunction and resulting structural and functional changes observed in depressed patients (see section 2.1.3 for mitochondrial dysfunction in depressive disorders) [13]. Further, there is evidence that ID acts on dopamine-dependent pathways by altering DA frontal-striatal circuits [288, 289]. However, most of the research concerning ID and depression is in line with the "critical period hypothesis", suggest-

ing that the absence of adequate nutrients in critical developmental periods results in irreversible and permanent structural deficits even with nutrient supplementation after that period [281, 290]. Nevertheless, a recent prospective cohort study of low- and middle-socioeconomic status in adolescents concluded that interventions reducing infant psychosocial stress were more likely to prevent neurocognitive deficits in adolescence than interventions reducing infant ID [291]. Nevertheless, a better understanding of iron's role in depressive disorders could advance options for treatment-resistant depression patients.

This chapter introduced different theories on the aetiology of depressive disorders. These theories can broadly be summarised within the fields of (1) neurochemical processes, (2) plasticity, structure and neurogenesis, (3) neuroendocrine processes, and (4) immune system and inflammation. The nutrients n-3 PUFA, iodine and iron, are linked to these fields through their diverse roles within the human body and its metabolism. In the next part of this PhD thesis, 4 manuscripts will present results and discuss associations of the named single nutrients with perinatal and pMDD.

3 Manuscript One

Higher n-3 polyunsaturated fatty acid status during early pregnancy is associated with lower risk for depression at 12 months postpartum: The NuPED study

Ester Osuna[1], Elizabeth A Symington[2], Linda Malan[3], Cristian Ricci[4], Lizelle Zandberg[3], Cornelius M Smuts[3], Jeannine Baumgartner[1,3,5]

[1]Laboratory of Human Nutrition, Institute of Food, Nutrition and Health, ETH Zurich, Switzerland.
[2]Department of Life and Consumer Sciences, University of South Africa, Johannesburg, South Africa.
[3]Centre of Excellence for Nutrition, North-West University, Potchefstroom, South Africa.
[4]Africa Unit for transdisciplinary Health Research (AUTHER), North-West University, Potchefstroom, South Africa.
[5]Department of Nutritional Sciences, King's College London, London, United Kingdom.

Funding: This study has been funded by the National Research Foundation of South Africa, the South African Medical Research Council, the University of South Africa's Research Department through the Academic Qualification Improvement Programme.

This manuscript has been submitted to *Prostaglandins, Leukotrienes & Essential Fatty Acids (PLEFA)*

Highlights

- ⋆ Higher red blood cell (RBC) DHA and n-3 index were associated with lower odds for depression at 12 months postpartum.
- ⋆ Higher n-6/n-3 PUFA and AA/EPA ratios at early pregnancy were associated with higher odds for depression at 12 months postpartum.
- ⋆ Women with higher RBC n-3 PUFA status during early pregnancy may be at lower risk for depression at 12 months postpartum.

Keywords

Perinatal depression, pregnancy, n-3 polyunsaturated fatty acids, low- and middle-income countries (LMIC), Edinburgh postnatal depression scale (EPDS), n-3 Index, AA/EPA ratio, n-6/n-3 ratio, fatty acid patterns

Summary

Perinatal depression can negatively affect the health of the mother and her offspring. N-3 polyunsaturated fatty acids (PUFA) may play a role in the aetiology of depression. Therefore, we investigated the association of n-3 PUFA status during early pregnancy with perinatal depression among women living in urban Johannesburg, South Africa. For this prospective analysis, we analysed red blood cell (RBC) total phospholipid fatty acid (FA) composition (% of total FA) of 242 pregnant women at $<$18 weeks' gestation. We used the Edinburgh Postnatal Depression Scale (EPDS) to identify women at risk for depression (EPDS score $\geq$9) at $<$18, 22 and 36 weeks' gestation, and at 6 and 12 months postpartum. RBC EPA status was negatively (β=-0.22, p$<$0.05), and the AA/EPA ratio positively (β=0.24, p$<$0.05) associated with EPDS scores at 12 months postpartum. Higher RBC DHA and n-3 index were further associated with lower odds (OR=0.56 [0.32–0.91]; OR=0.63 [0.39–0.94]), while higher n-6/n-3 PUFA and AA/EPA ratios early in pregnancy were associated with higher odds for depression at 12 months postpartum ((OR=2.34 [1.12–4.97]; OR=1.02 [1.00–1.05]). Our results suggest that women with a higher RBC n-3 PUFA status during early pregnancy may be at lower risk for depression at 12 months postpartum.

3.1 Introduction

Perinatal depression refers to major and minor depressive episodes during pregnancy (prenatally) and the first year after delivery (postnatally) [61]. Estimated prevalence rates for perinatal depression in high-income countries (HIC) are approximately 9% [62]. In contrast, estimated prevalence rates in low- and middle-income countries (LMIC) reach up to 50% [61–63]. Perinatal depression may result in women finding it difficult to perform everyday tasks, failure to seek perinatal care, poor dietary intake, and self-harm or suicide [64, 65]. Furthermore, perinatal depression has been associated with poorer childhood outcomes such as behavioural problems, attentional deficits and impaired cognitive function [50, 63, 66–68]. Thus, perinatal depression can have serious health implications for both the mother and her child.

Even though the aetiology of depressive disorders remains unclear, it is likely multifactorial. There is a growing body of research indicating a link between depressive symptoms and modifiable nutritional risk factors, especially poor n-3 polyunsaturated fatty acid (PUFA) status [44, 64, 292–295]. Some of the putative mechanisms underlying the observed associations between n-3 PUFA status and mental health involve their role in neurogenesis and neuroplasticity, the tryptophan-kynurenine metabolism and the gut-microbiota-brain axis [44]. Furthermore, inflammatory processes may play a major role in the development of depression [9, 296, 297]. The n-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are precursors for the production of anti-inflammatory and inflammation-resolving mediators [144]. A low n-3 PUFA status might be particularly detrimental during the perinatal period when women are at increased risk of depression relapse or newly diagnosed depression. However, data on the associations between maternal n-3 PUFA status and perinatal depression are limited and inconsistent [64, 298, 299].

The n-3 PUFA status of an individual can be assessed by measuring the fatty acid (FA) composition of red blood cell (RBC) membranes and expressed either as individual n-3 PUFA (in percentage of total FA) or as the sum of EPA + DHA (also called the "omega (n)-3 index"). Globally, low and very low levels of n-3 PUFA status (n-3 index $\leq$6% and $\leq$4%, respectively) have been observed in many high-income populations, while data from LMIC are still scarce [153]. Factors such as diet, supplementation, sex, age, and body-mass-index (BMI) have been associated with n-3 PUFA status [300, 301]. Low conversion rates of the essential n-3 PUFA precursor alpha-linolenic acid (ALA) to EPA and DHA contribute to a low n-3 PUFA status in populations with a low intake of preformed EPA and DHA, e.g. from fish or supplements. Conversion rates can be further affected by variations in genes coding for desaturase and elongase enzymes. During pregnancy and lactation, requirements of n-3 PUFA are increased because the supply to the foetus mainly depends on the mother's intake [49, 302]. Therefore, pregnant and lactating women are at increased risk of inadequate n-3 PUFA intake and status compared to other population groups.

In South Africa, the estimated prevalence of postnatal depression is around 20-30% [63]. The nutritional status of pregnant women in South Africa is poorly described. However, a previous South African study in women of reproductive age indicated that the intake of n-3 PUFA is below recommendations in both rural and urban areas [303]. Therefore, the aim of this study was to investigate the association of n-3 PUFA status during early pregnancy with perinatal depression among women living in urban Johannesburg, South Africa. Thereby, we described the n-3 PUFA status as single RBC n-3 PUFA, the n-3 index, and the n-6/n-3 PUFA ratio. We further determined RBC FA patterns by Principal Component Analysis (PCA) to overcome the limitations of other n-3 PUFA status indicators not considering the mutual dependence of FA and the influence of change in proportion of one FA on the rest of the FA in RBC membranes.

3.2 Methods and materials

3.2.1 Study design and participants

This study formed part of the Nutrition during Pregnancy and Early Development (NuPED) study, a prospective cohort study conducted in Johannesburg, South Africa. The NuPED protocol has been published previously [304]. The NuPED study was approved by the Health Research Ethics Committee (HREC) of the North-West University (NWU), South Africa (NWU-00186-15-A1 and NWU-00049-16-A1) as well as the University of Witwatersrand, Johannesburg (M150968 and M161045). In brief, generally healthy pregnant women were recruited from primary healthcare clinics in Johannesburg between March 2016 and November 2017. The women were followed throughout pregnancy to birth and up to 12 months postpartum at Rahima Moosa Mother and Child Hospital (RMMCH) in Johannesburg, South Africa until June 2018. Data were collected at early pregnancy ($<$18 weeks of gestation), mid-pregnancy ($\pm$22 weeks), late pregnancy ($\pm$36 weeks), and at 6 weeks, 6 months, and 12 months postpartum.

Women were eligible for inclusion if they were aged 18-39 years, $<$18 weeks of gestation with singleton pregnancies, proficient in local languages, born in South Africa or neighbouring countries, and if they have been residing in Johannesburg for at least 12 months. Women were excluded if they reported using illicit drugs, were smoking, or had been diagnosed with a non-communicable disease such as diabetes, renal disease, high cholesterol, and hypertension; an infectious disease such as tuberculosis and hepatitis; or a serious illness such as cancer, lupus or psychosis. Due to South Africa's high prevalence of HIV infection (36%) [305], women living with HIV were included in the study to be a better representation of the general South African population.

3.2.2 Outcome measurements

The primary outcome measure was perinatal depression at six time points: early pregnancy (<18 weeks of gestation), mid-pregnancy (±22 weeks), late pregnancy (±36 weeks), and at 6 weeks, 6 months, and 12 months postpartum. Depression was assessed using the interviewer-administered Edinburgh Postnatal Depression Scale (EPDS). The EPDS is a 10-item scale assessing depressive symptoms experienced in the past 7 days [70], which has been validated for assessing perinatal depression in African settings, including South Africa [69]. To classify women as being at risk of depression, we used an EPDS cut-off score of ≥9 (out of a maximum score of 30), which was shown to have a high sensitivity for detecting perinatal depression in African contexts [69].

3.2.3 Exposure measurements

The primary exposure measure was maternal n-3 PUFA status assessed at <18 weeks of gestation by analysing the FA composition of RBC total phospholipids and expressed as single FA (ALA, DHA and EPA), the n-6/n-3 PUFA ratio, and the n-3 index. Further exposure variables were RBC FA patterns derived by conducting principal component analysis (PCA).

Maternal venous blood for the analysis of RBC FA composition was drawn into EDTA-coated evacuated tubes (Vacuette®, Greiner Bio-One) and was processed within 1 hour. During processing, RBC were separated from plasma by centrifugation at 2000 g for 10 minutes and were washed twice with normal saline solution. Aliquots were stored on site at -20°C for a maximum of 14 days until transportation for final storage at -80°C until analysis.

RBC total phospholipids FA analysis was carried out at the North-West University Centre of Excellence for Nutrition using Gas Chromatography-Mass Spectrometry (GC-MS) as described previously [306]. Lipids were extracted from RBC samples with chloroform: methanol (2:1, vol:vol) by using a modification of the method of Folch [307]. Lipid extracts were concentrated using nitrogen gas, and thin-layer chromatography (silica gel 60 plates without fluorescent indicator, 10 x 20 cm; Merck) was used to separate neutral lipids from the phospholipids. Phospholipids were eluted using petroleum ether: diethyl ether (peroxide free): acetic acid (90:30:1, vol:vol:vol) and a pinch of UV fluorescent indicator 2.5-Bis(5-tert-butyl-benzoxazol-2-yl) thiophene. The lipid fraction containing the phospholipids was visualized under long-wave UV light, removed from the thin-layer chromatography plate and trans-methylated with methanol: sulphuric acid (95:5, vol:vol) at 70°C for 2 hours. This led to the formation of fatty acid methyl esters (FAME). The FAME were extracted using hexane and water. The organic layer was aspirated, evaporated, redissolved in hexane, and analysed by gas chromatography-electron ionisation mass spectrometry. All solvents used during

the extraction procedure contained 0.01% butylated hydroxytoluene. Samples were analysed on an Agilent Technologies 7000 GC/MS Triple quad system comprising an Agilent 7890A gas chromatograph equipped with an Agilent G7001B triple quad mass spectrometer (Agilent Technologies). The gas chromatography separation of FAME was carried out on an HP-88 capillary column (100m x 0.25 mm x 0.20 μm; Agilent Technologies) by using helium as the carrier gas at a flow rate of 2.2 mL/min. Initial inlet temperature was held at 70°C for 0.02 min, after which it was ramped to 270°C at 500°C/min. The mass spectrometer source was maintained at a temperature of 230°C. A sample volume of 1 μL was injected and a split ratio of 80:1 for RBC samples was used. The oven temperature was maintained at 50°C for 1 min, then ramped to 170°C at 30°C/min, then from 170°C to 215°C at 2°C/min, after which it was ramped to 230°C at 4°C/min. The temperature was then held isothermally at 230°C for 7 min. The total analysis time was 38.25 min. Mass spectrometry with 70 eV electron-ionisation was carried out in multiple-reaction monitoring mode, with at least two transitions per compound. The FAME were quantified using MassHunter Quantitative Analysis software (Version B.05.02, Agilent Technologies). FAME peaks were identified and calibrated against a standard reference mixture of 33 FAME (Nu-Check-Prep) and two single FAME standards (Larodan Fine Chemicals AB). Relative percentages of FAs were calculated by expressing the concentration of a given FAME as a percentage of the total concentration of all FAME identified in the sample [306].

3.2.4 Covariates

All the covariates were assessed at the first visit (<18 weeks gestation).

Maternal socio-economic and -demographic data were collected during a structured interview, and included date and country of birth, marital status, ethnicity, educational level, and living standards measure (LSM) scores. The LSM score was developed by the South African Audience Reference Foundation (SAARF) and is widely used in South Africa to describe the socio-economic status of the population [308, 309].

Anthropometric measurements, such as height and weight, were obtained using standardised methods from the International Society for the Advancement of Kinanthropometry [310]. All measurements were conducted twice and recorded to the nearest 0.05 kg of weight and 0.1 cm for height.

Medical files were inspected to obtain data on maternal HIV status and sex of the baby. During analyses, women were considered HIV positive irrespective of date of HIV contraction (prior to or during pregnancy).

3.2.5 Statistical methods

Data were tested for outliers and normality by means of Q-Q plots, histograms and Shapiro-Wilk test. Outliers were inspected and checked for unrealistic values, typing errors, and quantitation errors. Normally distributed data and non-normally distributed data were expressed as mean ($\pm$SD) and as medians (interquartile range (IQR)), respectively. We used multivariate linear regression analysis to assess associations of different FA status indicators with EPDS scores as continuous outcome variable, and logistic regression analysis to assess associations of FA status indicators with perinatal depression defined as EPDS score $\geq$9 (dichotomous variable). Thereby we applied two models: In the basic model 1, age of the mother and gestational age were included as covariates; in model 2, age of the mother, gestational age, BMI, HIV status, and the LSM score were included as covariates. All statistical tests were two tailed and type-I error rate was set at α =0.05. Raw data were captured in Microsoft Access (Microsoft Corporation, Washington, USA) and 20% of all captured data were randomly checked for correctness. Data processing and statistical analysis of data were performed using R Version 3.6.0 [311]. R packages used for PCA were: 'corpcor', 'GPA rotation', 'psych'.

A factor analysis based on PCA of the correlation matrix with orthogonal rotation (varimax) was conducted on the 29 phospholipid FA to explore maternal FA patterns. To correct for skewness, we applied a Blom transformation on the data [312]. The Kaiser-Meyer-Olkin (KMO) measure verified the sampling adequacy for the analysis (KMO = 0.62; 'mediocre' according to Kaiser, 1974 [148]), and all KMO values for individual variables were >0.3. An initial analysis was run to obtain eigenvalues for each component in the data. Four components had eigenvalues over Kaiser's criterion of 1 and in combination explained 46.9% of the variance. The scree plot was slightly ambiguous and showed inflexions that would justify retaining both four and five components. Given the convergence of the scree plot and Kaiser's criterion on four components, four components were retained in the final analysis. Factor loadings, which describe how strongly each individual FA contributes to each FA pattern, were calculated and are presented in Table 3.3. On the basis of high factor loadings ($\geq$|0.40|) for the respective fatty acids (Table 3.3), we named these patterns: 1) 'high saturated FA' pattern; 2) 'Low DHA and n-6 PUFA, and high trans FA' pattern; 3) 'high ALA, EPA, n-3 DPA and n-6 DGLA'; and 4) 'high n-3 PUFA' pattern. Each woman had an individual score on each of the fatty acid patterns.

3.3 Results

3.3.1 Participant characteristics and prevalence of perinatal depression

A flow-chart of the pregnant women included and followed-up in the NuPED study and the availability of data for analysis in this study is shown in Figure 3.1. A total of 250 women were enrolled into the NuPED study, but FA data were available for 242 women only and therefore included in the analysis. Of these, EPDS data were available from 238, 224, 191, 50, 84, and 89 women at enrolment, mid-pregnancy ($\pm$22 weeks), late pregnancy ($\pm$36 weeks), and at 6 weeks, 6 months, and 12 months postpartum, respectively.

Baseline characteristics of study participants are presented in Table 3.1. We found a median age of 28 years and a median BMI of 26.5 kg/m^2. Most study participants lived in medium living standards according to the LSM. Finally, around one quarter of study participants were HIV positive.

3.3.2 Perinatal depression prevalence scores

Figure 3.2 shows the prevalence of having an EPDS score indicative for perinatal depression (cut-off $\geq$9) in the women at the pre- and postnatal follow-ups. The prevalence ranged from 17% to 26%, with the highest prevalence at 6 months postpartum (26%) and the lowest prevalence at $\pm$36 weeks of gestation (17%). The median (IQR) EPDS scores were 5 (2, 8) at $<$18 weeks gestation, 3 (1, 6) at 22 weeks gestation, 3 (0, 6) at 36 weeks gestation, 4.5 (0.25, 7.75) at 6 weeks postpartum, 5 (2, 9) at 6 months postpartum, and 4 (1, 8) at 12 months postpartum. We did not find a significant difference in the medians of the pre- and post-delivery visits. Only 4% (n = 9) of the women who showed symptoms of depression (cut-off $\geq$9) at $<$18 weeks gestation were also depressed at 12 months postpartum. When considering all antenatal timepoints, 5% (n = 12) of women experienced depression both antenatally and at 12 months postpartum. As for postpartum depression, 3% (n = 8) of women experiencing depression at 6 months postpartum also experienced depression at 12 months postpartum.

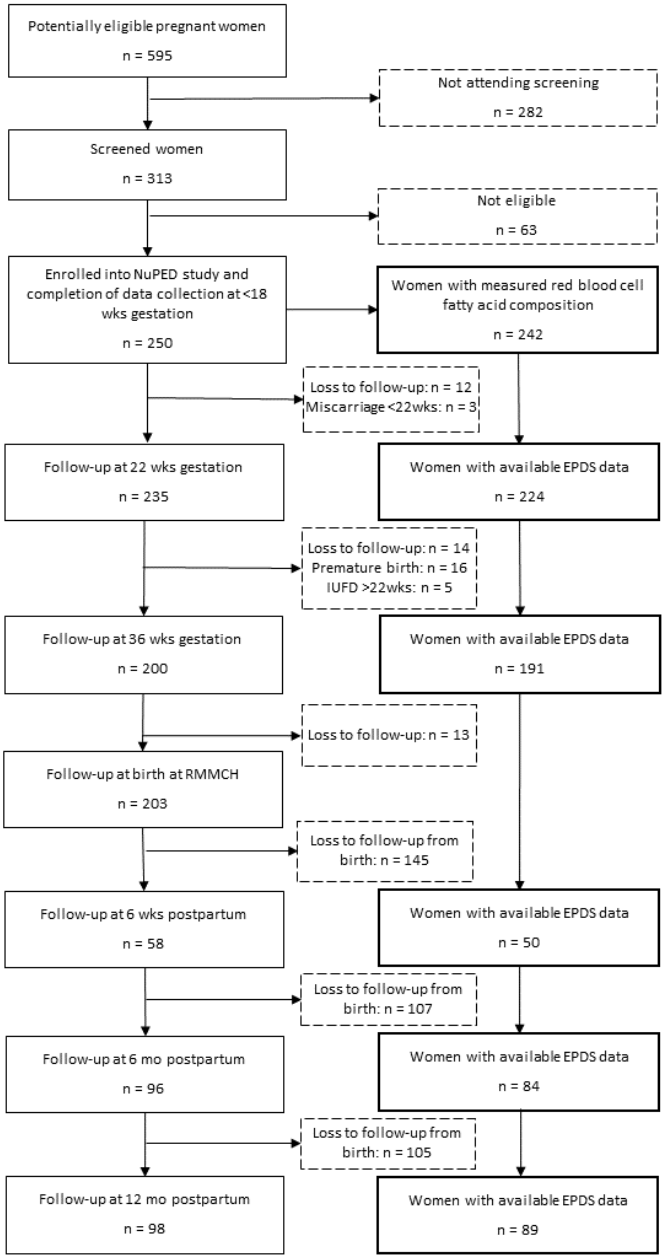

Figure 3.1: Flow-chart of pregnant women included and followed up in the NuPED study and in the prospective analysis of this study (boxes in bold). EPDS: Edinburgh Postnatal Depression Scale; IUFD: Intrauterine foetal death; RMMCH: Rahima Moosa Mother and Child Hospital

Table 3.1: Characteristics of the pregnant women at enrolment (<18 weeks of gestation).

Maternal characteristics	n	
Age (years)	242	28.0 (24.0, 32.0)
Gestational age (weeks)	242	14.3 (12.1, 16.1)
BMI (km/m^2)	241	26.5 (23.2, 30.7)
Underweight (<18.5 km/m^2)		8 (3)
Normal weight (18.5-24.9 km/m^2)		82 (34)
Overweight (25-29.9 km/m^2)		81 (34)
Obese (≥30 km/m^2)		70 (29)
Ethnicity	241	
Black African		211 (87)
Mixed ancestry		28 (12)
Indian		1 (0.4)
White		1 (0.4)
Country of birth	233	
South Africa		169 (70)
Zimbabwe		57 (24)
Lesotho		4 (2)
Swaziland		3 (1)
Living Standards Measure (LSM)	242	7.0 (6.0, 8.0)
Low (LSM 1-4)		17 (7)
Medium (LSM 5-7)		141 (58)
High (LSM 8-10)		84 (35)
Maritial Status	241	
Unmarried / single		96 (40)
Married		67 (30)
Living together		55 (23)
Traditional marriage		21 (9)
Divorced/Separated		2 (1)
Highest level of education	241	
Primary school or less		7 (3)
Grade 8-10		37 (15)
Grade 11-12		140 (58)
Tertiary post school education		57 (24)
Parity	242	
Nulliparous		69 (29)
Primiparous		86 (36)
Biparous		67 (28)
Multiparous		20 (8)
HIV status	242	
Negative		179 (74)
Positive		63 (26)
EPDS score	238	5(2, 8)
EPDS score <9		179 (74)
EPDS score ≥9		59 (24)
Inflammatory status	242	
No inflammation		92 (38)
Inflammation		150 (62)

Values are displayed as median (interquartile range) for continuous variables and n (%) for categorical variables. AGP: alpha-1 glycoprotein; BMI: body mass index; CRP: C-reactive protein; EPDS: Edinburgh Postnatal Depression Scale
Inflammation was defined as CRP >5mg/L and/or AGP >1g/L

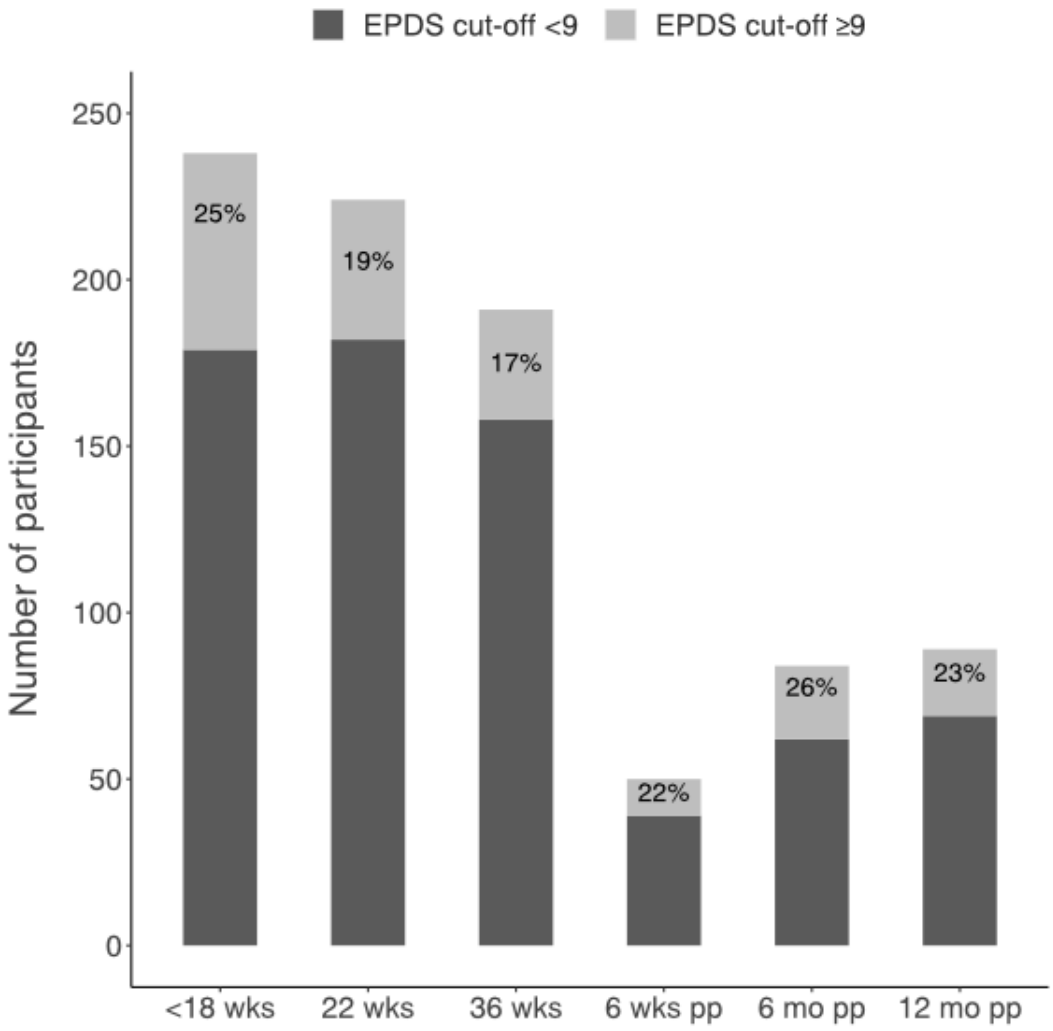

Figure 3.2: Prevalence of women with EPDS score indicative for perinatal depression along study course. <18 weeks of gestation; ±22 weeks; ±36 weeks; 6 weeks postpartum; 6 months postpartum; 12 months postpartum

3.3.3 Maternal red blood cell fatty acid composition and patterns

Table 3.2 shows the RBC phospholipid FA composition of the pregnant women at <18 weeks of gestation. Mean RBC phospholipid EPA and DHA was 0.29 ± 0.15% and 5.28 ± 1.15%, respectively. The mean n-3 index (sum of EPA + DHA) was 5.92 ± 1.39%, and 55% and 5% of women had an n-3 index indicative of a low (<6%) and very low (<4%) n-3 PUFA status, respectively. The mean n-6/n-3 PUFA ratio was 4.53 ± 0.94.

With the four retained components from PCA, we identified four major RBC FA patterns shown in Table 3.3: *high saturated FA* (Pattern 1); *low DHA and n-6 PUFA, and high trans FA* (Pattern 2); *high ALA, EPA, n-3 DPA and n-6 GLA* (Pattern 3); and *high n-3 PUFA* (Pattern 4).

3.3.4 Association of indices of n-3 PUFA status and FA patterns with perinatal depression

Table 3.4 shows a summary of results from the conducted multivariate linear regression models on the associations of different FA status indicators at <18 weeks gestation with EPDS scores at 12 months postpartum. In the fully adjusted model 2, RBC EPA at <18 weeks gestation was negatively associated with EPDS scores at 12 months postpartum (β=-0.22; p=0.040). Furthermore, RBC FA pattern 4 (high n-3 PUFA) at <18 weeks gestation was negatively associated with EPDS scores at 12 months postpartum (β=-0.23; p=0.025). We found a positive association of the AA/EPA ratio at <18 weeks gestation with EPDS scores at 12 months postpartum (β=0.24; p=0.022).

Table 3.5 displays a summary of results from the conducted logistic regression models on the associations of different FA status indicators at <18 weeks gestation with depression (EPDS score ≥9) at 12 months postpartum. In the fully adjusted model 2, higher RBC DHA and n-3 index levels at <18 weeks' gestation were associated with lower odds for depression at 12 months postpartum (OR=0.56 [0.32-0.91]; p=0.030). Likewise, we found that women with higher scores for the RBC FA pattern 3 (high ALA, EPA, n-3 DPA and n-6 GLA) at <18 weeks gestation had lower odds for depression (EPDS ≥9) at 12 months postpartum (OR=0.47 [0.22-0.92]; p=0.038). On the contrary, higher n-6/n-3 PUFA and AA/EPA ratios at <18 weeks' gestation were associated with higher odds for depression at 12 months postpartum (OR=2.34 [1.12-4.97]; p=0.016; and OR=1.02 [1.00-1.05]; p=0.023, respectively).

None of the indices of n-3 PUFA status and FA patterns at early pregnancy were associated with depression at the prenatal visits or at 5 weeks and 6 months postpartum (results not shown).

Table 3.2: Red blood cell total phospholipid fatty acid composition of 242 pregnant women at study enrolment (<18 weeks gestation).

	Fatty acid	% of total FA[1]
SFA		
C14:0	Myristic acid	0.19 ± 0.10
C16:0	Palmitic acid	21.5 ± 2.76
C18:0	Stearic acid	16.3 ± 1.62
C20:0	Arachidic acid	0.29 ± 0.07
C22:0	Behenic	1.43 ± 0.39
C24:0	Lignoceric	5.11 ± 1.31
MUFA		
C16:1n-7	Palmitoleic acid	0.23 ± 0.09
C18:1n-7	Cis-vaccenic acid	0.93 ± 0.15
C18:1n-7t	Trans-vaccenic acid	0.07 ± 0.11
C18:1n-9	Oleic acid	9.06 ± 1.13
C18:1n-9t	Elaidic acid	0.11 ± 0.23
C20:1n-9	Gondoic acid	0.19 ± 0.04
C22:1n-9	Erucic acid	0.00 ± 0.00
C24:1n-9	Nervonic acid	4.33 ±1.16
n-3 PUFA		
C18:3n-3	Alpha-linolenic acid (ALA)	0.08 ± 0.04
C18:4n-3	Stearidonic acid	0.00 ± 0.00
C20:3n-3	Eicosatrienoic acid	0.00 ± 0.00
C20:5n-3	Eicosapentaenoic acid (EPA)	0.29 ± 0.15
C22:3n-3	Docosatrienoic acid	0.01 ± 0.01
C22:5n-3	Docosapentaenoic acid	1.85 ± 0.39
C22:6n-3	Docosahexaenoic acid (DHA)	5.28 ± 1.15
n-6 PUFA		
C18:2n-6	Linoleic acid	11.4 ± 1.70
C18:3n-6	Gamma-linolenic acid	0.03 ± 0.02
C20:2n-6	Eicosadienoic acid	0.40 ± 0.11
C20:3n-6	Dihomo-gamma-linolenic acid	1.25 ± 0.39
C20:4n-6	Arachidonic acid (AA)	14.4 ± 1.74
C22:4n-6	Adrenic acid	4.32 ± 0.75
C22:5n-6	Osbond acid	1.05 ± 0.34
n-9 PUFA		
C20:3n-9	Mead acid	0.00 ± 0.00

[1] Mean ±SD.

Table 3.3: Summary of Principal Component Analysis (PCA) factor loadings for four maternal fatty acid patterns after conduction of a PCA on 29 red blood cell phospholipid fatty acids with orthogonal rotation (varimax).

	Phospholipid fatty acid	Pattern 1 [1]	Pattern 2 [2]	Pattern 3 [3]	Pattern 4 [4]
SFA					
C14:0	Myristic acid	0.09	**0.73**	0.34	-0.17
C16:0	Palmitic acid	-0.26	**0.83**	-0.08	-0.17
C18:0	Stearic acid	-0.09	0.08	**-0.62**	0.16
C20:0	Arachidic acid	**0.85**	0.20	0.06	0.01
C22:0	Behenic	**0.89**	0.09	-0.01	0.00
C24:0	Lignoceric	**0.89**	0.03	-0.09	0.03
MUFA					
C16:1n-7	Palmitoleic acid	-0.09	0.37	0.38	-0.15
C18:1n-7	Cis-vaccenic acid	-0.15	0.02	0.21	-0.09
C18:1n-7t	Trans-vaccenic acid	0.22	**0.53**	-0.15	0.33
C18:1n-9	Oleic acid	**-0.40**	0.14	0.18	-0.07
C18:1n-9t	Elaidic acid	0.14	**0.59**	-0.20	0.22
C20:1n-9	Gondoic acid	**0.63**	0.15	0.33	-0.11
C22:1n-9	Erucic acid	0.02	-0.37	-0.28	**0.42**
C24:1n-9	Nervonic acid	**0.79**	0.00	-0.03	-0.08
n-3 PUFA					
C18:3n-3	Alpha-linolenic acid (ALA)	0.00	-0.07	**0.69**	0.17
C18:4n-3	Stearidonic acid	-0.17	-0.14	-0.22	**0.51**
C20:3n-3	Eicosatrienoic acid	0.10	0.19	0.00	**0.58**
C20:5n-3	Eicosapentaenoic acid (EPA)	-0.04	-0.02	**0.46**	**0.59**
C22:3n-3	Docosatrienoic acid	-0.03	-0.01	-0.04	**0.49**
C22:5n-3	Docosapentaenoic acid	0.04	-0.33	**0.45**	**0.41**
C22:6n-3	Docosahexaenoic acid (DHA)	-0.20	**-0.47**	0.19	**0.51**
n-6 PUFA					
C18:2n-6	Linoleic acid	-0.26	**-0.48**	0.14	-0.08
C18:3n-6	Gamma-linolenic acid	-0.07	-0.04	**0.56**	0.18
C20:2n-6	Eicosadienoic acid	**0.61**	-0.25	0.38	-0.11
C20:3n-6	Dihomo-gamma-linolenic acid	0.15	-0.05	**0.60**	-0.15
C20:4n-6	Arachidonic acid (AA)	**-0.45**	**-0.51**	0.06	0.01
C22:4n-6	Adrenic acid	0.20	**-0.54**	0.01	-0.17
C22:5n-6	Osbond acid	0.09	-0.27	0.14	**-0.40**
n-9 PUFA					
C20:3n-9	Mead acid	0.24	0.22	0.18	**0.51**
Eigenvalues		4.54	3.58	2.85	2.62
% of variance		33	26	21	19

Factor loadings $\geq$ |**0.40**| appear in bold. KMO = 0.62 and Bartlett's test of sphericity $\mathcal{X}^2(406)$ = 3'653, p <0.001, indicate sufficiently large correlations for PCA.

[1] Pattern 1: High saturated FA

[2] Pattern 2: Low DHA and n-6 PUFA, and high trans FA

[3] Pattern 3: High ALA, EPA, n-3 DPA and n-6 DGLA

[4] Pattern 4: High n-3 PUFA

Table 3.4: Multivariate linear regression models assessing associations of different fatty acid status indicators at <18 weeks gestation with Edinburgh Postnatal Depression Scale scores at 12 month postpartum

	Model 1[1]						Model 2 [2]					
FA Status Indicator	ΔR^2	*B*	*95% CI*		β_i	*P*	ΔR^2	*B*	*95% CI*		β_i	*P*
			Lower	*Upper*					*Lower*	*Upper*		
DHA	0.10	-0.42	-1.25	0.41	-0.11	0.32	0.17	-0.64	-1.50	0.22	-0.16	0.14
EPA	0.11	-5.83	-13.1	1.40	-0.16	0.11	0.19	-7.66	-15.0	-0.34	-0.22	**0.040**
n-3 Index	0.10	-0.33	-1.02	0.35	-0.10	0.34	0.17	-0.53	-1.24	0.18	-0.16	0.14
n-6/n-3 ratio	0.11	0.86	-0.31	2.03	0.15	0.15	0.18	1.15	-0.06	2.36	0.20	0.06
AA/EPA ratio	0.12	0.03	-0.01	0.07	0.17	0.09	0.20	0.04	0.01	0.08	0.24	**0.022**
Pattern 1 [3]	0.09	-0.30	-1.24	0.64	-0.07	0.53	0.15	-0.27	-1.22	0.68	-0.06	0.57
Pattern 2 [4]	0.10	-0.61	-1.66	0.44	-0.12	0.25	0.16	-0.61	-1.68	0.45	-0.12	0.26
Pattern 3 [5]	0.10	-0.67	-1.89	0.54	-0.11	0.27	0.16	-0.68	-1.89	0.55	-0.12	0.26
Pattern 4 [6]	0.12	-1.04	-2.14	0.05	-0.19	0.06	0.20	-1.26	-2.37	-0.16	-0.23	**0.025**

[1] Model 1: "FA status indicator" + age of the mother + gestational age.
[2] Model 2: "FA status indicator" + age of the mother + gestational age + BMI at visit 1 + HIV status + LSM score
[3] Pattern 1: High saturated FA
[4] Pattern 2: Low DHA and n-6 PUFA, and high trans FA
[5] Pattern 3: High ALA, EPA, n-3 DPA and n-6 GLA
[6] Pattern 4: High n-3 PUFA

3.4 Discussion

This prospective cohort study conducted in Johannesburg, South Africa, followed 242 pregnant women throughout pregnancy up to 12 months postpartum. We found various associations of different FA status indicators early in pregnancy (<18 weeks of gestation) with perinatal depression at 12 months postpartum. In the multivariate linear regression models, RBC EPA levels were negatively, and the RBC AA/EPA ratio positively associated with EPDS scores at 12 months postpartum. In the multivariate logistic regression models, higher RBC DHA and n-3 index were associated with lower odds for depression, while higher n-6/n-3 PUFA and AA/EPA ratios early in pregnancy were associated with higher odds for depression at 12 months postpartum. We further identified four major RBC FA patterns by conducting a PCA: *high saturated FA* (Pattern 1); *low DHA and n-6 PUFA, and high trans FA* (Pattern 2); *high ALA, EPA, n-3 DPA and n-6 GLA* (Pattern 3); and *high n-3 PUFA* (Pattern 4). Consistently with the associations observed for the single RBC n-3 PUFA and ratios, higher scores for pattern 4 and pattern 3 were associated with lower EPDS scores and lower odds for depression, respectively. However, we found no significant associations between the various FA status indicators and depression at any of the prenatal or other postnatal time points.

Table 3.5: Multivariate logistic regression models assessing associations of different fatty acid status indicators at <18 weeks gestation with Edinburgh Postnatal Depression Scale scores with cut-off ≥9 at12 month postpartum

	Model 1 [1]				Model 2 [2]			
FA status Indicator	Odds ratio	*95% CI OR*		*P*	Odds ratio	*95% CI OR*		*P*
		Lower	*Upper*			*Lower*	*Upper*	
DHA	0.61	0.37	0.95	**0.038**	0.56	0.32	0.91	**0.030**
EPA	0.03	0.00	1.46	0.10	0.01	0.00	1.13	0.09
n-3 Index	0.67	0.44	0.97	**0.045**	0.63	0.39	0.94	**0.034**
n-6/n-3 ratio	2.19	1.19	4.13	**0.016**	2.34	1.12	4.97	**0.016**
AA/EPA ratio	1.02	1.00	1.04	**0.050**	1.02	1.00	1.05	**0.023**
Pattern 1 [3]	0.78	0.48	1.26	0.32	0.73	0.44	1.22	0.23
Pattern 2 [4]	0.86	0.49	1.48	0.60	0.86	0.47	1.55	0.63
Pattern 3 [5]	0.50	0.25	0.95	**0.042**	0.47	0.22	0.92	**0.038**
Pattern 4 [6]	0.66	0.35	1.17	0.17	0.66	0.34	1.19	0.19

[1] Model 1: "FA status indicator" + age of the mother + gestational age.
[2] Model 2: "FA status indicator" + age of the mother + gestational age + BMI at visit 1 + HIV status + LSM score
[3] Pattern 1: High saturated FA
[4] Pattern 2: Low DHA and n-6 PUFA, and high trans FA
[5] Pattern 3: High ALA, EPA, n-3 DPA and n-6 GLA
[6] Pattern 4: High n-3 PUFA

In our cohort of generally healthy pregnant women, we observed relatively high and constant prevalence rates of depression of around 22% during the pre- and postnatal period (ranging from 17 to 26%). These rates are comparable to the estimated prevalence of perinatal depression in LMIC from recent reviews [62, 66]. The high prevalence of depression in women from LMIC has been attributed to low socioeconomic status, lack of social support, low maternal educational attainment, and sexual abuse [66, 313]. However, to our knowledge, this is the first study to investigate whether there is a link between maternal n-3 PUFA status and perinatal depression in a LMIC.

In this study, we observed that women with a higher n-3 PUFA status during early pregnancy have a lower risk for postnatal depression at 12 months postpartum. This is in agreement with a recent prospective cohort study in 72 Belgian women, which reported a low n-3 PUFA status and high n-6/n-3 PUFA ratio during early pregnancy to be associated with an increased risk for postnatal depression at 12 months postpartum [292]. Nonetheless, literature on protective associations of a higher n-3 PUFA status with a lower risk for perinatal depression remains inconsistent [64, 298, 299]. A recent longitudinal case-control study including 54 Swiss pregnant women with diagnosed anxiety disorder, major depressive disorder, or a mixed anxiety-depression disorder, and 40 healthy controls found lower n-3 PUFA levels and higher n-6/n-3 PUFA ratios to be associated with prenatal and postnatal depression [314]. However, the trends for postnatal depression disappeared when adjusting for prenatal depression [314]. In the current study, the associations between the n-3 PUFA indices and depression at 12 months postpartum remained significant when adjusting for prenatal depression (data not shown). A prospective cohort study (n=2'663) in the South West of England showed no robust association of EPA or DHA with postnatal depression at 8 weeks postpartum [315]. In contrast, in a community-based prospective cohort study in Norway (n=43), a low n-3 index in late pregnancy was associated with higher depression scores at 3 months postpartum [294]. Overall, the time point of assessing postpartum depression in these studies differed, ranging from 1 week up to 3 months. Few studies have assessed depression up to 12 months postpartum. Still, symptoms of postnatal depression can occur at any time within the first 21 months postpartum [316]. We only found associations of FA status with depression at 12 months postpartum but no other time points. Furthermore, only 3% of women experiencing depression at 6 months postpartum also experienced depression at 12 months postpartum. This finding may indicate a different aetiology of depression at 12 months than at 6 months postpartum.

There are various mechanisms that may explain the protective role of n-3 PUFA in the aetiology of depression. The most widely researched one is related to EPA and DHA being pre-cursors for the synthesis of anti-inflammatory and inflammation-resolving lipid mediators, while molecules derived from n-6 PUFA mainly exhibit pro-inflammatory properties [154]. Inflammation is a major risk factor for depression [9]. Evidence from animal models demonstrates that an imbalance in the n-6/n-3 PUFA ratio in

phospholipid membranes in favour of n-6 PUFA can lead to an exaggerated immune response to an inflammatory stimulus, such as stress, and compromise the resolution of inflammation [317]. Thus, higher n-6/n-3 PUFA and AA/EPA ratios in RBC membranes have been associated with higher levels of inflammation, as well as postpartum depression [144, 318]. The prevalence of inflammation in our study was high, with 60% of women having elevated C-reactive protein (CRP) or alpha-1 glycoprotein (AGP) concentrations at <18 weeks of gestation (CRP >5 mg/L or AGP <1 g/L). This high prevalence may be related to the high prevalence of overweight and obesity, and infectious disease, such as HIV, in the study population. Thus, our results support the hypothesis of n-3 PUFA, and the balance of n-3 to n-6 PUFA playing a role in the aetiology of depression via their immune-modulatory properties [9, 180].

In this study, we used various indicators to reflect the n-3 PUFA status of pregnant women. There are limited data on blood levels of PUFA in African women. However, the mean n-3 index in pregnant women participating in the NuPED study is comparable to previously reported n-3 index levels in 101 male and female South African fisher people, where the n-3 index in erythrocyte equivalents was found to be between 4-6% [153, 319]. In contrast to the n-3 index target range for cardiometabolic health of 8-11%, there is no established n-3 index target range for pregnant and lactating women. However, increasing evidence suggests that achieving an n-3 index ≥8% during pregnancy might reduce the risk of premature birth and is therefore desirable also during pregnancy [320].

In addition to the single n-3 PUFA, the n-3 index and the PUFA ratios, we identified four major RBC FA patterns by conducting a PCA. PCA is used to reduce the data set to a more manageable size while retaining as much of the original information as possible [148]. In this study, we used this technique to identify groups of women with similar RBC FA compositions, so called patterns. These patterns, in comparison to single FA status indicators, do consider the interdependencies of FA and therefore can give insight into FA metabolism. The patterns derived in our sample of pregnant women are similar to the FA patterns detected in a sample of Dutch pregnant women from the Generation R Study at early pregnancy [50], specifically the high saturated FA (Pattern 1) and high n-3 PUFA (Pattern 4) pattern. However, the Dutch study did not determine associations between these patterns and perinatal depression, instead they suggest that FA patterns during pregnancy may affect the offspring's body composition and cardiometabolic health [50]. To our knowledge, this is the first study to show that women with higher scores for a RBC FA pattern characterised by high levels of n-3 PUFA at early pregnancy have a lower risk for developing depression at 12 months postpartum.

A major limitation of this study is the high loss to follow-up during the postnatal period resulting in a relatively small sample size and a potential bias. Participants experiencing a depressive episode might be more prone to drop out of extra-daily activities compared to non-depressed participants. However, the main reason for the high loss to follow-up is that many women in this urban setting moved back to their home towns after giving birth to get support by family members. Furthermore, we did not observe a significant decrease in the prevalence of depression during the course of the study. Therefore, the prevalence of depression observed at the postnatal visits may still be representative of the prevalence in the study population. Strengths of the current study include the prospective design and the long follow-up period until 12 months postpartum, as well as the analysis of n-3 PUFA status by measuring RBC total phospholipid FA composition and expressing it using various indices. Unfortunately, we did not measure RBC total phospholipid FA composition at 6 and 12 months postpartum. However, we assume that the n-3 PUFA status determined during early pregnancy is most representative for habitual dietary intake and is therefore strongly correlated with the n-3 PUFA status during the postpartum period. Major changes in dietary intake during pregnancy and lactation are not expected in this setting due to poor dietary counselling, limited financial means and neglectable seasonal impact on food intake in this urban setting [321].

3.5 Conclusion

To conclude, our results indicate that a higher RBC n-3 PUFA status during early pregnancy may lower the risk for perinatal depression at 12 months postpartum. This finding agrees with a previous study in Belgian women showing that a low n-3 PUFA status, alone and combined with a high n-6 status, during early pregnancy is associated with an increased risk for postnatal depression at 12 months postpartum [292]. However, evidence on the protective associations of n-3 PUFA status on perinatal depression is inconsistent and the time point of assessing postnatal depression varied from 1 week, a few months up to 12 months postpartum. Therefore, our results suggest a new perspective on postpartum depression, indicating that depression occurring at 12 months postpartum might be particularly sensitive to PUFA status in early pregnancy, which is likely to reflect habitual intake. Potential future studies should explore biochemical mechanisms underlying these associations.

Conflict of interest

All authors declare that they have no conflict of interest.

Funding

The NuPED study was supported in part by the National Research Foundation of South Africa, Unique Grant 99374 (EAS) (www.nrf.ac.za/) and the South African Medical Research Council under a Self-Initiated Research Grant (CMS) (www.mrc.ac.za/). The NuPED work has further received financial support from the University of South Africa's Research Department through the Academic Qualification Improvement Programme (EAS) (www.unisa.ac.za). EO received a PhD fellowship from the Ebnet Foundation, Teufen, Switzerland.

Acknowledgements

We thank the following people for their contribution to the NuPED study: Prof A Coovadia, Dr A Wise, Prof H Lombaard, Dr E Loock, Dr R Adams, Dr R Strehlau, Prof M Faber, Dr O Sotunde, Dr M Rothman, Dr L Siziba, Mrs C Cooke, the sonographers and nurses of Rahima Moosa Mother and Child Hospital, the nurses at the primary healthcare clinics, the fieldworkers and the study participants.

Author contributions

Conceptualization of current analysis: EO, JB, CR. Conceptualization and execution of NuPED study: EAS, LM, LZ, CMS, JB. Data collection as part of NuPED study: EAS. Data analysis: EO, CR, JB. Writing – original draft: EO, JB. Writing – review & editing: EO, EAS, LM, CR, LZ, CMS, JB

4 Manuscript Two

Associations of n-3 polyunsaturated fatty acid status and intake with depression in Swiss adolescents with and without diagnosed paediatric major depressive disorder: a case-control study

Ester Osuna[1], Isabelle Herter-Aeberli[1], Silvia Probst[1], Sophie Emery[2], Mona Albermann[2], Noemi Baumgartner[2], Cristian Ricci[3], Klaus Schmeck[4], Susanne Walitza[2], Michael Strumberger[4], Michael B. Zimmermann[1], Isabelle Häberling[2], Gregor Berger[2], Jeannine Baumgartner[1,5], on behalf of the Omega-3 study team

[1]Laboratory of Human Nutrition, Institute of Food, Nutrition and Health, ETH Zurich, Switzerland.
[2]Clinics for Child and Adolescent Psychiatry, University Hospital Zurich, Switzerland.
[3]Africa Unit for Transdisciplinary Health Research (AUTHER), North-West University, Potchefstroom, South Africa.
[4]Research Department of Child and Adolescent Psychiatry, Psychiatric University Hospitals Basel, University of Basel, Switzerland.
[5]Department of Nutritional Sciences, King's College London, London, United Kingdom.

Funding: This study has been supported by the Swiss National Science Foundation, and the Ebnet Foundation, Teufen, Switzerland.

This is a manuscript draft approved by all co-authors to be included into this PhD thesis

Abstract

Background: The age of onset of depression, a leading cause of disability worldwide, is often during adolescence. The aetiology of depression is poorly understood and likely multifactorial. Previous observational studies suggest a link between n-3 Polyunsaturated fatty acids (PUFA) intake, status, and depression in adults, but studies in adolescents are scarce.

Objective: We determined associations of n-3 PUFA status and intake with Paediatric major depressive disorder (pMDD) among Swiss adolescents.

Methods: We conducted a matched case-control study in 95 adolescents with diagnosed pMDD and 95 healthy controls aged 13 to <18 years. We analysed Red blood cell (RBC) Fatty acids (FA) composition (% of total FA). n-3 PUFA intake was assessed using a focused food frequency questionnaire and pMDD was assessed with the Children's Depression Rating Scale-Revised (CDRS-R).

Results: Mean RBC Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) were lower in cases than controls (EPA: 0.41 $\pm$ 0.11 vs 0.46 $\pm$ 0.12, p<0.001; DHA: 4.07 $\pm$ 1.04 vs 4.73 $\pm$ 1.04, p<0.001). Subsequently, the mean RBC n-3 index was lower (4.51$\pm$1.10 vs 5.20$\pm$1.11, p<0.001) and the n-6/n-3 PUFA ratio higher (5.51$\pm$1.25 vs 4.96$\pm$1.08, p<0.001) in cases than controls. Using logistic regression, adolescents with a higher n-3 index had lower odds for depression (OR=0.57 [0.41-0.77], p<0.001). In contrast, the n-6/n-3 ratio was associated with higher odds for depression (OR=1.47 [1.12-1.97], p=0.007). Higher RBC EPA and DHA were associated with lower odds for depression (EPA: OR=0.02 [0.00-0.32], p=0.007; DHA: OR=0.54 [0.38-0.73], p<0.001). However, intake of α-linoleic acid, 18:3n-3 (ALA), EPA and DHA did not differ between cases and controls.

Conclusion: Our results suggest that a higher RBC n-3 PUFA status during adolescence may be associated with a lower risk for pMDD, and that the lower n-3 PUFA status in adolescents with pMDD compared to healthy counterparts are not explained by differences in n-3 PUFA intake.

4.1 Introduction

Depression is a leading cause of disability worldwide [51], affecting an estimated 300 million people [322]. An early onset of depression is a risk factor for chronic and recurrent forms of depression in adulthood [53]. Furthermore, Paediatric major depressive disorder (pMDD) is one of the most common psychiatric disorders during childhood and adolescence, with an estimated 5% of adolescents in Europe being affected [323]. However, pMDD often remains undiagnosed and therefore untreated [42]. Depression during adolescence has been associated with poor educational, work, and social functioning as well as an increased rate of smoking, substance abuse, eating disorders, and obesity [55].

The aetiology of pMDD remains unclear, but is most likely multifactorial [4], involving biological, genetic, psychosocial, and environmental factors. Alterations in brain tissue volume, connectivity, monoamine levels, glutamatergic metabolites, and neurogenesis are often reported in depressed compared to non-depressed individuals [5–7, 79]. Alterations in factors promoting plasticity within the brain have further been observed among depressed individuals. Brain-derived neurotrophic factor (BDNF) is such a factor promoting survival, differentiation and growth of neurons [88, 324]. Previous studies report low peripheral BDNF concentrations in depressed patients, which rise after anti-depressant treatment [89, 90]. Increasing evidence suggests that nutrition plays a role in the aetiology of depressive disorders [44], especially n-3 Polyunsaturated fatty acids (PUFA) [174]. Of particular interest are the long-chain n-3 PUFA Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA), which play important roles in myelination, neurogenesis, brain connectivity, neurotransmitter turnover, and inflammatory processes, all involved in the aetiology of depression [11, 44, 144, 325].

Globally, a low n-3 index ($<$4% EPA + DHA in Red blood cell (RBC) membranes) has been observed in many high-income countries [153], and several studies have linked a low n-3 PUFA status to depression [326]. A previous study has recommended an n-3 index of $>$6% for healthy cognitive development in children and adolescents [327]. As for other biomarkers related to depressive disorders, most of these studies have been conducted in adults. However, the results from studies in adults cannot be generalized to adolescents [328]. Adolescents often have a poorer overall diet quality compared to adults [47, 329]. Furthermore, adolescents are at a developmental stage of increased neuronal plasticity and nutrient requirements [48]. Therefore, the relationship of depression with n-3 PUFA intake and status is of special interest among this vulnerable group.

In Switzerland, the estimated prevalence of depression among adolescents is 10% [52]. To date, the nutritional status of Swiss children and adolescents is poorly described. Therefore, the aim of this study was to determine the associations of n-3 PUFA status and intake with depression in adolescents with and without diagnosed pMDD. We determined n-3 PUFA status by measuring the Fatty acids (FA) composition of RBC membranes and expressing it as single RBC n-3 PUFA (in percentage of total FA), the n-3 index (sum of RBC EPA + DHA), the n-6/n-3 PUFA ratio, and RBC FA patterns (determined by Principal component analysis (PCA)). We further measured peripheral serum BDNF concentrations and explored whether the associations between n-3 PUFA status and depression are mediated by alterations in peripheral BDNF. We hypothesized that adolescents with pMDD are having lower n-3 PUFA status compared to healthy controls, and that these differences are at least partly related to differences in n-3 PUFA intake.

4.2 Participants and Methods

4.2.1 Study design

This is an observational case-control study in 13–17-year-old adolescents with diagnosed pMDD and healthy controls, matched in a 1:1 ratio according to sex, age group (13 to <16 and 16 to <18 years), and education. Sample size calculation was performed with G*Power V3.1.9.2. A power calculation was applied to a logistic model with depression (diagnosed using the Children's Depression Rating Scale-Revised (CDRS-R)) as a dichotomous outcome variable in a model with 10 covariates (with a residual (R^2 =0.2) and one Standard deviation (SD) increase of the continuous predictor generated an Odds ratio (OR) of 1.5 and 2. We determined that a sample size of 200 individuals with a 1:1 matching case-control ratio is sufficient (power >80%, β $\geq$20%) to detect associations of medium to large effect sizes for a type-I error of 5% ($\alpha = 0.05$). These results seemed robust to a drop out of up to 10%. We aimed to include 100 cases and 100 controls, with equal representation of sex, age groups, and education in cases and controls. In the first age group (13 to <16 years), we intended to include 50 adolescents (25 male and 25 females). In the second age group 16 to <18 years of age, the aim was to recruit 52 adolescents (26 males and 26 females). All adolescents in the younger age group were considered to attend lower secondary school level (mandatory school years in Switzerland). However, the adolescents in the older age group were further divided based on their higher secondary school educational level (n=26 from each level): 1) vocational education (apprenticeship), and 2) baccalaureate / vocational baccalaureate. For this case-control analysis, we recruited controls according to this recruitment strategy and then randomly selected cases to match the controls.

The study was approved by the ethics committee of the Canton of Zurich (BASEC-Nr. 2019-00717) and registered at www.ClinicalTrials.gov (NCT04158869). The study was approved as an add-on study to the investigator-initiated clinical trial (SNSF 33IC30_166826, BASEC-Nr. 2016-02116). All caregivers and adolescents $\geq$14 years of age gave their written informed consent, and adolescents $<$14 years of age gave their oral assent before any research-related assessments were conducted.

4.2.2 Participants

Control group

Healthy controls were recruited by and assessed at the Laboratory of Human Nutrition at ETH Zurich, Switzerland. Healthy female and male controls were recruited from the Canton of Zurich and surrounding German-speaking Cantons of Switzerland from September 2019 until December 2020. Recruitment of controls was done via schools, leisure time clubs, and social media. Inclusion criteria for controls were no present nor past primary diagnosed psychiatric disorder according to the Mini-International Neuropsychiatric Interview for Children and Adolescents (M.I.N.I. KID) [330]; no use of chronic medication; and being 13 to $<$18 years of age. Controls were not eligible if they took n-3 PUFA supplements (providing $>$600 mg EPA/DHA) for more than four weeks within the last six months; if they reported pre-existing neurological or medical conditions likely to be a risk factor for developing depressive symptoms; or if they were unable to follow the study procedures, for example due to language barriers. After providing consent and being enrolled into the study, participants electronically completed questionnaires on REDCap® (Research Electronic Data Capture) within the two weeks prior to the physical data assessment at ETH Zurich.

pMDD group

Cases were participants of the Omega-3 Fatty Acid Paediatric Depression Trial (Omega-3 pMDD) under the lead of the Psychiatric University Hospital Zurich. The Omega-3 pMDD protocol has been published previously [331]. The cases for this case-control analysis were female and male adolescents with diagnosed pMDD, randomly selected from the participants of the Omega-3 pMDD study to match the controls. Seven in- and outpatient services in five German speaking Cantons of Switzerland (study centres) recruited participants for the Omega-3 pMDD study. Adolescents were either informed by their clinician in one of the study centres about the study or they contacted the study team on their own initiative, after seeing the poster or receiving a flyer. The recruitment took place from May 2017 until June 2021. Inclusion criteria were a main diagnosis of Major depressive disorder (MDD) according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria [332] of at least moderate severity defined by a CDRS-R total score of $\geq$40 [333]; and for teenagers at the age

of 13 to <18 years. Adolescents were not eligible if they fulfilled diagnostic criteria for an eating disorder within the last 6 months, or a lifetime diagnosis of schizophrenia, bipolar affective disorder, substance use dependency, mental retardation, or pervasive development disorder. In addition, adolescents were not eligible to participate if they had pre-existing neurological or medical conditions likely to be responsible for their depressive symptoms. Furthermore, adolescents were not eligible if they were taking an n-3 PUFA supplement (>600mg combined EPA/DHA) within the last 6 months; or if their families were unable to follow the study procedures, for example, due to language barriers. After obtaining consent, the screening interview was conducted with the adolescents in presence of a parent to assess inclusion and exclusion criteria. The presence of MDD was assessed according to the Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS) [334]. Further, the severity of depression was assessed using the CDRS-R interview. Within a few days, the lead-in interview took place, during which the adolescents' symptoms and severity of depression were again assessed with the CDRS-R. At this point, participants were ready to start the lead-in phase of the intervention study, which is not part of this analysis. For this case-control study, only data (biological samples and CDRS-R scores) from the lead-in interview was used.

4.2.3 Data collection

The study procedures were aligned as much as possible between the controls and cases.

Assessment of anthropometry and socio-demographic information

For the controls and the cases, weight (to the nearest 0.1 kg) and height (to the nearest 0.5 cm) was measured at the end of the physical data assessment. Body mass index (BMI) (as kg/m2) for age z-scores were calculated using the R package "anthroplus" which is provided by the World Health Organisation (WHO) and uses children' and adolescents' growth reference data [335]. Further, BMI categories (underweight, normal weight, overweight, and obese) for adolescents were also based on WHO reference data for the corresponding age group. Adolescents with a z-score at -1 SD coincide with adult underweight (BMI <18), adolescents with a z-score +1 SD coincide with adult overweight (BMI = 25) and adolescents with a z-score +2 SD with adult obesity (BMI = 30). Socio-economic and -demographic data were assessed by self-reporting questionnaires which the participants were asked to complete together with one parent.

Assessment of depression

Depression was assessed using the CDRS-R. The CDRS-R is a semi-structured clinical interview instrument and takes 15-20 minutes to administer. In research settings, it is one of the most frequently used rating scales for measuring the severity of depression and the change in depressive symptoms in children and adolescents [60]. The validity of the scale has been established for children [59] and adolescents [60]. The interview can be conducted with the child, parents, and/or teacher, and allows for a comprehensive assessment. The interview covers 17 symptom areas which are rated on a 5 or 7 point Likert rating scale. The domains include social withdrawal, sleep disturbance, excessive fatigue, suicidal ideation etc. These domains are aligned with the DSM-IV criteria for childhood depression [336]. Participants are asked about information on 14 items. Additional three non-verbal symptoms are rated only by the interviewer including depressed facial affect, and speech velocity. The interviewers were trained to conduct the interview. For this study, a score of $\geq$40 was used as a cut-off criteria for pMDD [337] and scores were taken from the interview conducted with the adolescent.

n-3 PUFA intake

Assessment of n-3 PUFA intake was done with a self-reported focused Food frequency questionnaire (FFQ). This FFQ has been previously validated in a sample of Swiss adults [338]. The FFQ assesses the quantity and frequency of fish and seafood, walnuts, rapeseed oil, linseed and linseed oil, chia seeds, and margarine intake over the past six months. The FFQ therefore allows a quantitative estimation of EPA and DHA intake (in mg/day).

Biochemical analysis

Blood samples were collected by a study nurse. These were collected into EDTA coated and serum tubes (for controls: BD Vacutainer, for cases: Sarstedt). Within 15 minutes, the plasma was separated from RBC by centrifugation at 2110 RCF (2113 x g) for 10 min at 4°C. The tubes containing RBC were stored for a maximum of one week at 4°C until further processing. The RBC were washed with 0.9%-NaCl solution and afterwards stored at -80°C in Eppendorf tubes treated with 1%-BHT solution until analysis. Serum tubes were let stand for 60 minutes for clotting. Afterwards, the serum tubes were centrifuged, and the serum was then stored at -80°C until further analysis.

The FA in RBC membranes were measured by gas chromatography coupled to a tandem mass spectrometer (GC-MS/MS, Thermo Scientific TSQ8000, Waltham, MA, USA), based on a previously published method [339, 340]. Briefly, the FA in the RBC were extracted with a mixture of MeOH and Dichlormethane and the organic phase

was derivatized by the addition of acetyl chloride. The resulting methyl ester FA were purified by a liquid-liquid extraction with hexane. Thereafter, the samples were injected in the GC-MS/MS system and were recorded by means of selective reaction monitoring (SRM). The quantity of each FA was expressed as a percentage of total RBC FA. We expressed the n-3 PUFA status of the participants as single RBC n-3 PUFA (in percentage of total FA), the n-3 index (sum of RBC EPA + DHA), the n-6/n-3 PUFA ratio and RBC FA patterns (determined PCA, described below). A low n-3 index was defined as $<6\%$ and a very low n-3 index was defined as $<4\%$ [140].

Peripheral blood serum mature BDNF concentrations were measured using a commercially available Enzyme-linked immunosorbent assay (ELISA) assay (CE-Marked BDNF Rapid, BEK-2211-1P-CE, ELISA Kit, Biosensins).

4.2.4 Data management and statistical analyses

For the controls, study data were captured either electronically using REDCap® or on paper and later entered the REDCap® system. The data were entered by the person doing the assessment and later double checked for entry errors by a second person. REDCap® is an electronic capture tool hosted at ETH Zurich and is a secure, web-based software platform designed to support data capture for research studies [341, 342]. For the cases, data were assessed on paper and then entered the electronical data capture tool secuTRIAL by two individual persons. Afterwards, entered data were checked for entry errors by a third person. After matching the cases to the controls, data from the matched cases were added to the REDCap® database.

Data processing and statistical analysis of data were performed using R Version 3.6.0 [311]. Data were tested for outliers and normality by means of Q-Q plots, histograms, and Shapiro-Wilk test. Normally distributed data and non-normally distributed data were expressed as mean ($\pm$SD) and as medians (Interquartile range (IQR)), respectively. Chi-square tests were used to assess significant differences when the expected cell count was ≥ 5, and Fisher's exact test when the expected cell count was <5. The Wilcoxon rank sum test was applied for comparing not normally distributed continuous variables and t-tests for normally distributed data. The Kruskal-Wallis rank sum test was used to determine differences between not normally distributed continuous variables with >2 levels. For calculating these differences and producing tables, the R package "gtsummary" was used. This R package has been shown to produce reproducible summary tables within R [343]. For correlations between two non-normally distributed variables, the Spearman's correlation coefficient was calculated. To assess associations of different FA status indicators with depression (CDRS-R score ≥ 40), multivariate logistic regression analysis was used. In the statistical model, the matching criteria sex, age, and education, and BMI-for-age z-scores were included as covariates.

A factor analysis based on PCA of the correlation matrix with orthogonal rotation (varimax) was conducted on the 27 RBC measured FA to explore adolescent RBC FA patterns. Blom transformation was applied to the data to correct for skewness [312]. The Kaiser-Meyer-Olkin measure verified the sampling adequacy for the analysis (KMO = 0.51 ('mediocre' according to Kaiser, 1974 [148])), and all except one KMO values for individual items were >0.3. Bartlett's test of sphericity, $X^2(351) = 2'826$, p<0.001, indicated that correlations between items were sufficiently large for PCA. An initial analysis was run to obtain eigenvalues for each component in the data. Five components had eigenvalues over Kaiser's criterion of one and in combination explained 56.4% of the variance. The scree plot was slightly ambiguous and showed inflexions that would justify retaining four, five and six components. Given the convergence of the scree plot and Kaiser's criterion on five components, five components were retained in the final analysis. Each adolescent had an individual score on each of the five components, so called FA patterns. These individual scores were used in the logistic regression models. R packages used for PCA were: 'corpcor', 'GPA rotation', 'psych'. Factor loadings describing the strength of individual FA contributing to each FA pattern (PCA component) are displayed in Table 4.4. High factor loadings ($\geq|0.40|$) for the respective fatty acids were used to name these patterns: low Monounsaturated fatty acids (MUFA), high n-6 PUFA and high Docosapenaenoic acid (DPA) (Pattern 1); high Saturated fatty acids (SFA), oleic acid, DHA and γ-linoleic acid, 18:3n-6 (GLA) (Pattern 2); High SFA, nervonic acid and α-linoleic acid, 18:3n-3 (ALA) (Pattern 3); High SFA but low behenic acid, high elaidic acid and high EPA (Pattern 4); and low palmitic acid, high gondoic acid and high n-6 PUFA (Pattern 5).

4.3 Results

A total of 98 controls were enrolled into the study. Thereof, two participants dropped out; one decided not to provide a blood sample and one voluntarily dropped out after the screening interview. For the participants of the Omega-3 pMDD study, a total of 173 participants completed the study and were therefore eligible as cases for the case-control study. One control could not be matched to a case according to the matching criteria. Finally, 95 cases and 95 controls were matched according to sex, age group and education, resulting in a total of 190 participants for this case-control analysis. A detailed flow diagram is shown in Figure 4.1. For four adolescent pairs in the age group 16 to <18 years, matching between cases and controls was done only according to sex and age group, as there was no match by education.

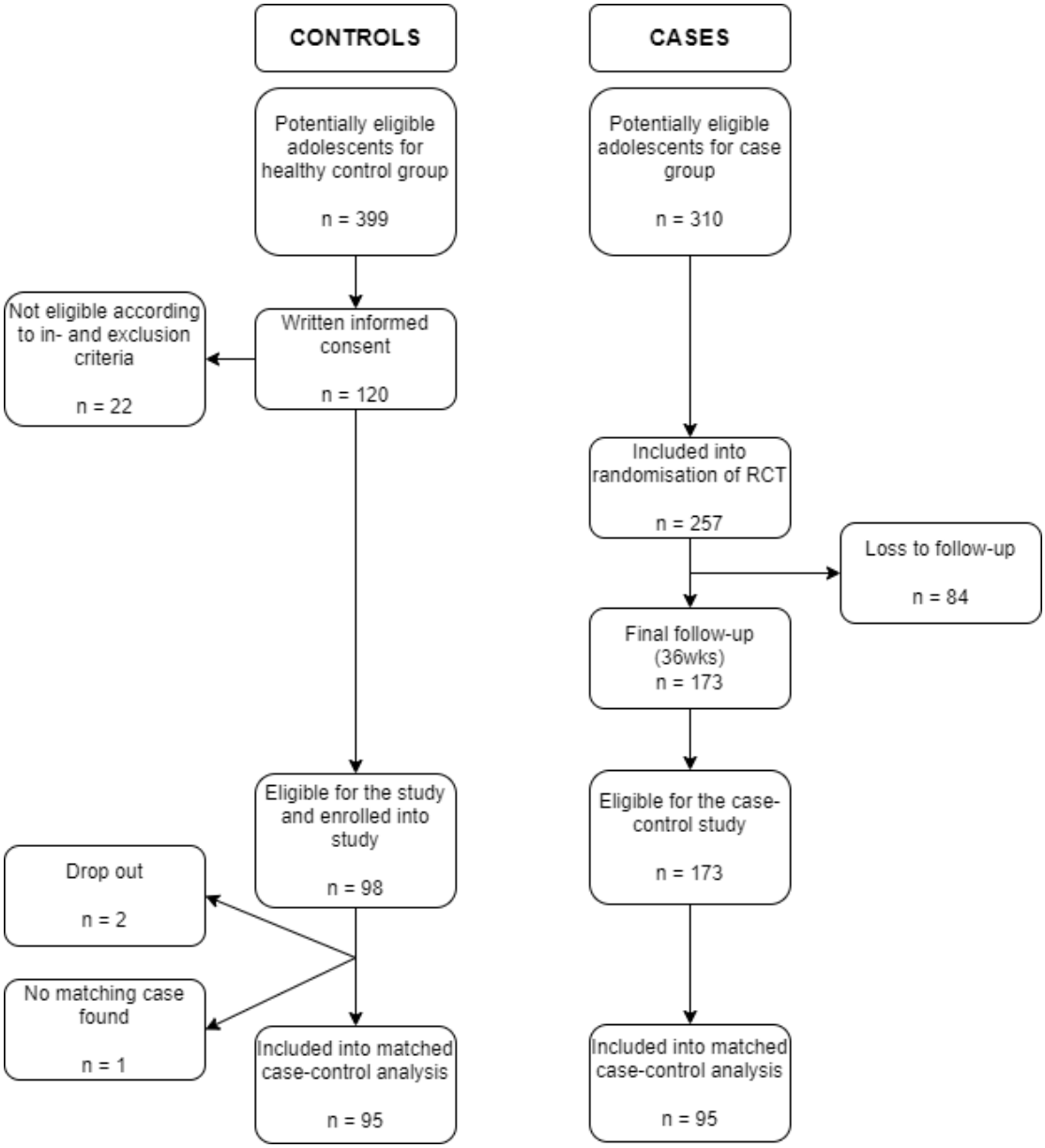

Figure 4.1: Flow-chart of participants included into this case-control study: RCT: Randomised Control Trial

Detailed participant characteristics are presented in Table 4.1. There were no significant differences in age, sex, and education between cases and controls, indicating that matching was successful. Furthermore, the BMI-for-age z-scores. were comparable between the groups, while CDRS-R scores were significantly higher among the cases compared to the controls ($p<0.001$). Slightly more adolescents among the cases were of East-Asian descent compared to controls ($p=0.051$).

Data on RBC FA composition was available for 189 participants, with one data point missing in the cases (missing RBC sample). Table 4.2 shows the RBC FA composition of the adolescents with pMDD and the healthy controls. RBC FA composition differed significantly between cases and controls, with differences among all types of FA. Mean RBC EPA and DHA (in % of total FA) were lower among cases than controls (EPA: 0.41 ± 0.11 vs 0.46 ± 0.12, $p<0.001$; DHA: 4.07 ± 1.04 vs 4.73 ± 1.04, $p<0.001$). Subsequently, the n-3 index was also lower among cases than controls (4.48 ± 1.09 vs 5.20 ± 1.11, $p<0.001$). The proportion of adolescents with a very low n-3 index ($<4\%$) was significantly higher among cases (32%) than controls (8%) ($p<0.001$), as was the proportion of adolescents with a low n-3 index ($<6\%$) (82 vs. 72%, $p=0.043$). Also, mean RBC Arachidonic acid (AA) was lower among cases compared to controls (13.5 ± 1.38 vs 14.2 ± 1.01, $p<0.001$). However, the n-6/n-3 PUFA ratio was significantly higher in cases than controls (5.53 ± 1.24 vs 4.96 ± 1.08, $p<0.001$).

The intake of n-3 PUFAs (mg/day) and consumption frequency of n-3 PUFA-rich sources, assessed with the focused FFQ, are shown in Table 4.3. Intake of ALA, EPA and DHA did not differ between cases and controls ($p>0.3$). Nonetheless, EPA and DHA intake correlated with RBC EPA and DHA status, respectively (EPA: $\rho=0.26$, $p<0.001$; DHA: $\rho=0.44$, $p<0.001$). We further found no correlation between ALA intake and RBC EPA or DHA status ($p>0.4$). The frequency of consumption of n-3 PUFA-rich foods and oils did not differ significantly between cases and controls, except for margarine: The proportion of cases who consumed margarine more than once per week was larger than the proportion of controls who reported the same (27% vs 12%, $p=0.025$). For adolescents consuming fish, the n-3 index was significantly higher with increasing frequency of consumption among both cases and controls. In the cases, the mean n-3 index was 4.69 ± 0.82, 5.29 ± 1.06 and 6.36 ± 1.16 for adolescents who reported to consume fish $<$1x per month, 1-3x per month and $>$1x per week, respectively ($p<0.001$). In the controls, the mean n-3 index was 3.84 ± 0.75, 4.72 ± 1.10, and 5.16 ± 1.04 for fish consumption $<$1x per month, 1-3x per month, and $>$1x per week, respectively ($p<0.001$). No significant differences in n-3 index by intake frequencies could be found for other n-3 PUFA-rich food and oil sources.

Table 4.1: Characteristics of Swiss adolescents with (n=95) and without (n=95) Paediatric major depressive disorder (pMDD)

	Overall	Cases	Controls	p-value
Age	16.1 (14.9, 17.1)	16.1 (14.9, 17.2)	16.0 (14.9, 17.1)	0.8[2]
Sex				>0.9[3]
Female	110 (58%)	55 (58%)	55 (58%)	
Male	80 (42%)	40 (42%)	40 (42%)	
CDRS-R score	36 (18, 56)	56 (50, 62)	18 (17, 20)	**<0.001**[2]
BMI-for-age z-score[1]	0.20 ± 1.03	0.25 ± 1.05	0.16 ± 1.02	0.4[2]
BMI category[1]				0.5[4]
Underweight	7 (4%)	2 (2%)	5 (5%)	
Normal weight	138 (76%)	65 (75%)	73 (77%)	
Overweight	20 (11%)	12 (14%)	8 (8%)	
Obese	17 (9%)	8 (9%)	9 (10%)	
Ethnicity				0.051[4]
European	174 (92%)	87 (92%)	87 (92%)	
East-Asian	5 (3%)	5 (5%)	0 (0%)	
Indian-Asian	2 (1%)	0 (0%)	2 (2%)	
Middle East	2 (1%)	1 (1%)	1 (1%)	
Not declared	7 (4%)	2 (2%)	5 (5%)	
Swiss education				0.8[3]
Lower secondary school (Mandatory school)	104 (55%)	54 (57%)	50 (53%)	
Upper secondary school				
Vocational education	28 (15%)	14 (15%)	14 (15%)	
Baccalaureat/ vocational baccalaureat	58 (31%)	27 (28%)	31 (33%)	

Median IQR; mean ±SD

CDRS-R: Children's Depression Rating Scale-Revised

[1] Data on Body mass index (BMI) was not available for all participants (n_{cases}= 87; $n_{controls}$=95), BMI-for-age z-scores and BMI categories were defined according to World Health Organisation (WHO) reference data (Adolescents with a z-score at -1 SD coincide with adult underweight (BMI <18), adolescents with a z-score +1 SD coincide with adult overweight (BMI = 25) and adolescents with a z-score +2 SD with adult obesity (BMI = 30))

[2] Wilcoxon rank sum test

[3] Pearson's Chi-squared test

[4] Fisher's exact test

Table 4.2: Red blood cell (RBC) Fatty acids (FA) composition of Swiss adolescents with (n=94) and without (n=95) diagnosed Paediatric major depressive disorder (pMDD)

	RBC fatty acid	Overall	Cases	Controls	p-value[1]
	(% of total fatty acids)				
Saturated fatty acids (SFA)					
C14:0	Myristic acid	0.44 ± 0.10	0.44 ± 0.11	0.43 ± 0.09	0.9
C16:0	Palmitic acid	22.6 ± 1.36	22.7 ± 1.73	22.5 ± 0.83	**0.003**
C18:0	Stearic acid	15.4 ± 0.93	15.6 ± 1.07	15.1 ± 0.70	**0.01**
C20:0	Arachidic acid	0.34 ± 0.05	0.33 ± 0.04	0.34 ± 0.06	0.12
C22:0	Behenic	1.24 ± 0.17	1.18 ± 0.13	1.30 ± 0.17	<**0.001**
C24:0	Lignoceric	3.48 ± 0.42	3.37 ± 0.43	3.58 ± 0.39	<**0.001**
C26:0	Cerotic acid	0.24 ± 0.05	0.24 ± 0.05	0.25 ± 0.05	0.14
Monounsaturated fatty acids (MUFA)					
C16:1n-7	Palmitoleic acid	0.24 ± 0.09	0.25 ± 0.10	0.23 ± 0.07	0.094
C18:1n-7	Cis-vaccenic acid	0.79 ± 0.11	0.81 ± 0.12	0.76 ± 0.09	**0.005**
C18:1n-9	Oleic acid	14.1 ± 1.33	14.7 ± 1.39	13.5 ± 0.99	<**0.001**
C20:1n-9	Gondoic acid	0.26 ± 0.08	0.26 ± 0.04	0.25 ± 0.10	<**0.001**
C24:1n-9	Nervonic acid	3.99 ± 0.43	4.08 ± 0.47	3.90 ± 0.36	**0.009**
Trans fatty acids (TFA)					
C16:1n-7t	Trans-palmitoleic acid	0.03 ± 0.04	0.04 ± 0.05	0.03 ± 0.01	<**0.001**
C18:1n-7t	Trans-vaccenic acid	0.21 ± 0.07	0.23 ± 0.08	0.19 ± 0.07	<**0.001**
C18:1n-9t	Elaidic acid	0.06 ± 0.06	0.11 ± 0.06	0.01 ± 0.01	<**0.001**
C18:2n-6t	Trans-linoleic acid	0.11 ± 0.04	0.11 ± 0.04	0.11 ± 0.03	0.4
n-3 Polyunsaturated fatty acids (PUFA)					
C18:3n-3	alpha-linolenic acid	0.09 ± 0.03	0.09 ± 0.04	0.09 ± 0.03	0.5
C20:5n-3	Eicosapentaenoic acid	0.43 ± 0.12	0.41 ± 0.11	0.46 ± 0.12	<**0.001**
C22:5n-3	Docosapentaenoic acid	1.14 ± 0.18	1.08 ± 0.17	1.19 ± 0.17	<**0.001**
C22:6n-3	Docosahexaenoic acid	4.41 ± 1.09	4.07 ± 1.04	4.73 ± 1.04	<**0.001**
n-6 Polyunsaturated fatty acids (PUFA)					
C18:2n-6	Linoleic acid	9.60 ± 1.03	9.61 ± 1.16	9.59 ± 0.88	>0.9
C18:3n-6	gamma-linolenic acid	0.05 ± 0.02	0.05 ± 0.03	0.04 ± 0.01	<**0.001**
C20:2n-6	Eicosadienoic acid	0.17 ± 0.04	0.17 ± 0.04	0.16 ± 0.04	**0.009**
C20:3n-6	Dihomo-gamma-linolenic acid	1.94 ± 0.44	1.89 ± 0.42	1.98 ± 0.45	0.15
C20:4n-6	Arachidonic acid	13.8 ± 1.25	13.5 ± 1.38	14.2 ± 1.01	<**0.001**
C22:4n-6	Adrenic acid	3.69 ± 0.66	3.60 ± 0.69	3.78 ± 0.62	0.063
C22:5n-6	Osbond acid	1.22 ± 0.24	1.15 ± 0.23	1.29 ± 0.23	<**0.001**
Fatty acid status indicators					
	n-3 Index	4.84 ± 1.16	4.48 ± 1.09	5.20 ± 1.11	<**0.001**
	n-6/n-3 PUFA ratio	5.25 ± 1.19	5.53 ± 1.24	4.96 ± 1.08	<**0.001**
	AA/EPA ratio	34.0 ± 9.00	35.0 ± 9.00	33.0 ± 10.0	0.062

Mean ±SD; AA: Arachidonic acid; EPA: Eicosapentaenoic acid

[1] Wilcoxon rank sum test

Table 4.3: Intake of n-3 Polyunsaturated fatty acids (PUFA) and consumption frequency of n-3 PUFA-rich dietary sources in Swiss adolescents with (n=95) and without (n=95) Paediatric major depressive disorder (pMDD)

Characteristic	Overall	Cases	Controls	p-value
n-3 Polyunsaturated fatty acid (PUFA) intake (mg/day)				
C18:3n-3 ALA	419 ± 668	418 ± 677	420 ± 663	0.7[2]
C20:5n-3 EPA	22 ± 39	24 ± 38	20 ± 41	0.4[2]
C22:6n-3 DHA	40 ± 66	44 ± 64	37 ± 67	0.3[2]
Total intake of EPA and DHA	62 ± 104	68 ± 101	56 ± 107	0.3[2]
Consumption frequencies				
Fish				0.4[3]
<1x per month	71 (37%)	35 (37%)	36 (38%)	
1-3x per month	88 (46%)	41 (43%)	47 (49%)	
>1x per week	31 (16%)	19 (20%)	12 (13%)	
Walnuts				0.092[3]
<1x per month	103 (54%)	59 (62%)	44 (46%)	
1-3x per month	56 (29%)	23 (24%)	33 (35%)	
>1x per week	31 (16%)	13 (14%)	18 (19%)	
Rapeseedoil[1]				>0.9[3]
<1x per month	75 (40%)	38 (40%)	37 (39%)	
1-3x per month	35 (19%)	17 (18%)	18 (19%)	
>1x per week	79 (42%)	39 (41%)	40 (42%)	
Linseeds				0.2[4]
<1x per month	151 (79%)	80 (84%)	71 (75%)	
1-3x per month	24 (13%)	10 (11%)	14 (15%)	
>1x per week	15 (7.9%)	5 (5.3%)	10 (11%)	
Linseed oil[1]				0.6[4]
<1x per month	180 (95%)	91 (97%)	89 (94%)	
1-3x per month	8 (4.2%)	3 (3.2%)	5 (5.3%)	
>1x per week	1 (0.5%)	0 (0%)	1 (1.1%)	
Chia seeds				0.4[4]
<1x per month	151 (79%)	76 (80%)	75 (79%)	
1-3x per month	27 (14%)	15 (16%)	12 (13%)	
>1x per week	12 (6.3%)	4 (4.2%)	8 (8.4%)	
Margarine[1]				**0.025[3]**
<1x per month	134 (71%)	59 (63%)	75 (79%)	
1-3x per month	19 (10%)	10 (11%)	9 (9.5%)	
>1x per week	36 (19%)	25 (27%)	11 (12%)	

Mean ±SD; n(%); ALA: alpha-linoleic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid

[1] Data on Rapeseed oil, linseed oil and margarine intake was not available for all participants (n=189)

[2] Wilcoxon rank sum test

[3] Pearson's Chi-squared test

[4] Fisher's exact test

Table 4.4 shows the factor loadings for the five identified RBC FA patterns. Based on high factor loadings ($\geq|0.40|$) for the respective FA, we named these patterns: low MUFA, high n-6 PUFA and high DPA (Pattern 1); high SFA, oleic acid, DHA and GLA (Pattern 2); High SFA, nervonic acid and ALA (Pattern 3); High SFA but low behenic acid, high elaidic acid and high EPA (Pattern 4); and low palmitic acid, high gondoic acid and high n-6 PUFA (Pattern 5).

A summary of the conducted multivariate logistic regression models on the associations of different n-3 PUFA status indicators and RBC FA patterns with pMDD is shown in Table 4.5. Higher RBC EPA, DPA, and DHA were associated with lower odds for depression (OR=0.02 [0.00-0.32], p=0.007; OR=0.02 [0.00-0.12], p<0.001; and OR=0.54 [0.38-0.73], p<0.001, respectively). Also, a higher n-3 index was associated with lower odds for depression (OR=0.55 [0.40-0.74], p<0.001). A n-3 index >6% was associated with lower odds for depression (OR=0.42[0.17-0.94], p=0.039), while a n-3 index of >8% showed no association with pMDD (OR=1.16[0.04-30.7], p>0.9). Furthermore, a higher n-6/n-3 PUFA ratio was associated with higher odds for depression (OR=1.52[1.16-2.04], p=0.004). Within the FA patterns, we found lower odds for depression with higher scores for Pattern 1 (low MUFA, high n-6 PUFA, high DPA) and 5 (low palmitic acid, high gondoic acid and high n-6 PUFA) (OR=0.34[0.22-0.49], p<0.001; and OR=0.56[0.39-0.78], p<0.001, respectively). Higher scores for Pattern 3 (high SFA, nervonic acid and ALA) were associated with higher odds for depression (OR=4.34[2.83-7.07], p<0.001). Finally, no associations were found between n-3 PUFA intake and pMDD (all, p>0.4).

We measured serum BDNF concentrations to explore whether the associations between n-3 PUFA status and pMDD are mediated by alterations in BDNF. However, we found no significant difference in median (IQR) BDNF (ng/mL) between cases and controls (19.04 [8.43, 76.66] ng/mL vs 19.20 [1.51, 38.09] ng/mL, respectively, p=0.9). We also found no association between BDNF concentrations and pMDD when using multivariate logistic regression, adjusting for sex, age, level of education and BMI-for-age z-scores (data not shown).

Table 4.4: Factor loadings of individual red blood cell fatty acids for five fatty acid patterns determined using Principal component analysis (PCA) in Swiss adolescents with (n=94) and without (n=95) Paediatric major depressive disorder (pMDD)

	Fatty acid	Pattern 1[1]	Pattern 2[2]	Pattern 3[3]	Pattern 4[4]	Pattern 5[5]
Saturated fatty acids (SFA)						
C14:0	Myristic acid	-0.01	**0.62**	0.14	**0.41**	-0.12
C16:0	Palmitic acid	0.01	0.05	0.07	0.12	**-0.76**
C18:0	Stearic acid	-0.11	0.38	0.19	**0.63**	0.01
C20:0	Arachidic acid	0.18	0.12	**0.72**	0.01	-0.06
C22:0	Behenic	-0.04	-0.29	0.17	**-0.65**	0.05
C24:0	Lignoceric	**-0.63**	0.07	0.17	0.23	0.11
C26:0	Cerotic acid	0.01	**0.64**	0.47	-0.15	-0.08
Monounsaturated fatty acids (MUFA)						
C16:1n-7	Palmitoleic acid	**-0.78**	-0.11	0.07	-0.10	-0.17
C18:1n-7	Cis-vaccenic acid	**-0.48**	0.16	-0.21	-0.11	-0.07
C18:1n-9	Oleic acid	**-0.45**	**0.56**	-0.09	0.23	0.13
C20:1n-9	Gondoic acid	0.15	0.34	0.15	-0.12	**0.68**
C24:1n-9	Nervonic acid	**-0.50**	-0.10	**0.47**	0.03	0.39
Trans fatty acids (TFA)						
C16:1n-7t	Trans-palmitoleic acid	**-0.40**	-0.03	**0.63**	-0.21	-0.15
C18:1n-7t	Trans-vaccenic acid	0.00	**0.68**	0.11	0.23	-0.05
C18:1n-9t	Elaidic acid	-0.31	0.12	-0.03	**0.50**	-0.13
C18:2n-6t	Trans-linoleic acid	0.10	-0.34	0.09	0.28	**0.58**
n-3 Polyunsaturated fatty acids (PUFA)						
C18:3n-3	alpha-linolenic acid	-0.07	-0.04	**0.70**	0.21	0.17
C20:5n-3	Eicosapentaenoic acid	0.06	-0.04	0.12	**0.62**	-0.01
C22:5n-3	Docosapentaenoic acid	**0.52**	-0.22	-0.34	-0.06	0.21
C22:6n-3	Docosahexaenoic acid	0.09	**0.78**	-0.25	0.01	0.05
n-6 Polyunsaturated fatty acids (PUFA)						
C18:2n-6	Linoleic acid	**0.49**	0.27	-0.03	0.01	**0.61**
C18:3n-6	gamma-linolenic acid	0.22	**0.64**	-0.13	0.05	0.15
C20:2n-6	Eicosadienoic acid	0.19	-0.19	-0.08	**0.62**	**0.46**
C20:3n-6	Dihomo-gamma-linolenic acid	**0.46**	0.35	-0.14	-0.08	0.07
C20:4n-6	Arachidonic acid	**0.70**	0.15	0.23	0.01	**0.44**
C22:4n-6	Adrenic acid	0.13	-0.34	**0.54**	0.04	0.11
C22:5n-6	Osbond acid	**0.67**	0.26	0.39	0.05	0.03
	Eigenvalues	3.82	3.63	2.79	2.44	2.55
	% of variance	25	24	18	16	17

Factor loading $\geq$|**0.40**| appear in bold. KMO = 0.51 and Bartlett's test of sphericity $X^2(351)$ =2826, p<0001, indicated sufficiently large correlations for PCA.

[1] Pattern 1: Low MUFA high n-6 PUFA and high docosapenaenoic acid (DPA)

[2] Pattern 2: High SFA, oleic acid, docosahexaenoic acid (DHA) and gamma-linoleic acid (GLA)

[3] Pattern 3: High SFA, nervonic acid and alpha-linoleic acid (ALA)

[4] Pattern 4: High SFA but low behenic, high elaidic acid and high eicosapentaenoic acid (EPA)

[5] Pattern 5: Low palmitic acid, high gondoic acid and high n-6 PUFA

Table 4.5: Multivariate logistic regression models assessing associations of fatty acid status indicators with (n=94) and without (n=95) Paediatric major depressive disorder (pMDD)

	Odds ratio (OR)	95% CI OR lower	95% CI OR upper	p-value
Single n-3 PUFA				
C18:2n-6 ALA	0.41	0.00	3044	0.8
C20:5n-3 EPA	0.02	0.00	0.32	**0.007**
C22:5n-3 DPA	0.02	0.00	0.12	<**0.001**
C22:6n-3 DHA	0.54	0.38	0.73	<**0.001**
n-3 PUFA status indicators				
n-6/n-3 PUFA ratio	1.52	1.16	2.04	**0.004**
AA/EPA ratio	1.02	0.99	1.05	0.2
n-3 Index	0.55	0.40	0.74	<**0.001**
n-3 Index >6%	0.42	0.17	0.94	**0.039**
n-3 Index >8%	1.16	0.04	30.7	>0.9
Fatty acid patterns				
Pattern 1	0.34	0.22	0.49	<**0.001**
Pattern 2	0.82	0.60	1.11	0.2
Pattern 3	4.34	2.83	7.07	<**0.001**
Pattern 4	0.74	0.53	1.02	0.070
Pattern 5	0.56	0.39	0.78	<**0.001**

PUFA: Polyunsaturated fatty acids; ALA: alpha-linoleic acid; EPA: Eicosapentaenoic acid; DPA: Docosapentaenoic acid; DHA: Docosahexaenoic acid; AA: Arachidonic acid; CI: Confidence interval

The dependent variable was the diagnosis of depression (CDRS-R ≥40). The independent variables were fatty acid status indicators. All models were controlled for sex, age, education, and BMI-for-age z-scores.

Pattern 1: Low MUFA high n-6 PUFA and high DPA

Pattern 2: High SFA, oleic acid, DHA and gamma-linoleic acid (GLA)

Pattern 3: High SFA, nervonic acid and ALA

Pattern 4: High SFA but low behenic, high elaidic acid and high EPA

Pattern 5: Low palmitic acid, high gondoic acid and high n-6 PUFA

4.4 Discussion

In this matched case-control study in Swiss adolescents with and without pMDD, higher RBC n-3 PUFA status was associated with lower odds for depression. In agreement with our hypothesis, adolescents with diagnosed pMDD had lower RBC EPA, DHA, and a lower n-3 index, but a higher n-6/n-3 PUFA ratio, compared to healthy controls matched for age, sex, and education. However, in contrast to our hypothesis, we found no differences in n-3 PUFA intake between cases and controls, indicating that the observed differences in n-3 PUFA status might be explained by other factors.

Our results confirm previous studies that observed associations between n-3 PUFA status (based on different indices) and depressive symptoms in adults [174, 344]. However, studies in adolescents are limited. To our knowledge, only one previous case-control study has been conducted in U.S. adolescents with a diagnosis of depression (n =150) and healthy controls (n=161), which also found higher RBC DHA and the n-3 index to be associated with lower odds for depression [345]. In contrast, a recent cross-sectional study in 252 Dutch adolescents in lower general secondary education found no associations of whole blood DHA, EPA, and the n-3 index with depression scores [346]. However, this study did not include adolescents with a clinical diagnosis of depression. Further, there is inconsistency in the associations observed between lower n-3 PUFA status and depressive symptom severity among depressive patients [347, 348]. To conclude, our results contribute towards the currently limited evidence for n-3 PUFA being associated with pMDD.

In this case-control study, there was no significant difference in EPA and DHA intake between adolescents with and without pMDD. Also, no associations between n-3 PUFA intake and pMDD were found when adjusting for potential confounders. This finding contrasts the results of a previous Japanese study, which detected higher intake of fish, EPA and DHA to be associated with lower prevalence of depressive symptoms in male but not female adolescents [349]. Nevertheless, EPA and DHA intake was associated with EPA and DHA status. While there was no significant difference in consumption frequency of fish between cases and controls, the n-3 index was significantly higher with increasing fish consumption in both groups. Thus, a higher n-3 index can partially be explained by higher intakes of n-3 PUFA rich food sources such as fish. Nevertheless, other factors must be contributing to the lower n-3 PUFA status observed in cases compared to controls in our study. One such factor could be differences in conversion rates of the n-3 PUFA pre-cursor ALA to EPA and DHA, which can be affected by polymorphisms in genes coding for desaturase and elongase enzymes [350], hormone levels, sex, age, BMI, alcohol consumption, and by smoking [300]. Metabolic differences, as opposed to intake differences, may also explain lower RBC AA observed in cases than controls, which is converted from the n-6 PUFA pre-cursor Linoleic acid (LA) by the same desaturase and elongase enzymes. Furthermore, EPA, DHA, and AA are pre-cursors for lipid mediators involved in peripheral and brain

inflammatory processes [144, 351]. These single FA being lower among adolescents with pMDD compared to those without could indicate a depletion of EPA, DHA, and AA within an inflammatory state. Such an increased inflammatory state has been associated with depression before [9].

Overall, 81% of participating Swiss adolescents had a low n-3 index (n-3 index <6%) and 20% of Swiss adolescents had a very low n-3 index (n-3 index <4%). For children and adolescents, a n-3 index of >6% has been recommended in the context of a healthy cognitive development [327]. In this study, an n-3 index >6% was associated with lower odds for depression. In contrast, no associations between an n-3 index >8% and depression could be found. This finding suggests, that in the context of the prevention of depression, an n-3 index >8% might not be more beneficial compared to an n-3 index >6%. Therefore, an n-3 index cut-off of >6% might not only be useful in the context of healthy cognitive development but also in the prevention of depression. The low n-3 index observed in these Swiss adolescents is within the range of previously reported n-3 index values in European adult populations (>4% and ≤6%) [153]. Even more, our results indicate the urge for public health measures to improving n-3 PUFA status. This, especially among vulnerable populations such as children and adolescents, who might be at a higher risk for depression in conditions of insufficient n-3 PUFA status and intake.

We measured peripheral BDNF concentrations to investigate whether BDNF mediated the associations between n-3 PUFA status and depression. However, against our hypothesis, we did not find lower peripheral BDNF concentrations in cases compared to controls. These findings are aligned with previous results in adolescents, where no significant differences in peripheral BDNF concentrations were shown in depressed adolescents (n=83) and healthy controls (n=52) [328]. In adults, however, lower peripheral BDNF concentrations have been consistently associated with depression [89, 90, 352, 353]. Discrepancies in the associations between BDNF levels and depression between adults and adolescents highlight, that results from adults might not be generalizable to adolescents, probably due to neurophysiological differences. Thus, as opposed to adults, BDNF levels might not be a reliable biomarker for depression [328].

We identified five RBC FA patterns in our study population, which we determined using factor analysis based on PCA. PCA reduces a large data set to a more manageable size while retaining as much of the original information as possible. The factors we retained, so called patterns, group adolescents with similar RBC FA compositions. The participants then receive an individual score for each retained pattern, indicating to what extend the patterns best explain the individual's FA status. These patterns, in comparison to other FA status indicators, do consider interdependencies of FA and therefore give insight into the FA metabolism. However, none of the identified FA patterns could clearly be described as exclusively rich in n-3 PUFA. Nevertheless, we found lower odds for depression with higher factor scores for patterns 1 (low MUFA

high n-6 PUFA and high DPA) and 5 (low palmitic acid, high gondoic acid and high n-6 PUFA). Further, we found higher odds for depression with higher scores for pattern 3 (High SFA, nervonic acid and ALA). Associations between these patterns and pMDD indicate that most probably the balance between n-3 PUFA and other FA might play a role within the aetiology of pMDD.

Our study has several strengths and limitations. The first strength of this study is the carefully matched study participants. Also, a further strength was the inclusion of the cases based on a clinical diagnosis of pMDD, enrolling only participants with moderate to severe depressive symptoms. Further, we assessed the adolescent depression score with an interviewer administered interview, which also included non-verbal items. Despite the strengths of our matching, the observational nature of this study design doesn't allow to draw conclusions whether the observed associations are causal. A further limitation is that we used a focused FFQ, which only assesses n-3 PUFA intake and not n-6 PUFA or the intake of other macro- and micronutrients that might affect n-3 PUFA status. However, as underlined by the identified FA patterns, not only n-3 PUFA may play a role in the pathophysiology of depression but also other FA. It therefore is important to assess at least n-6 PUFA intake together with n-3 PUFA intake. Furthermore, the use of an FFQ for the quantitative assessment can be potentially affected by recall bias and reverse causation. Also, the limited number of food items included may have led to an underestimation of n-3 PUFA intake, especially of ALA. And even though the questionnaire we used was validated for Switzerland and showed an acceptable agreement between intake and status [338], the validation was done in adults only.

In conclusion, the results of this study consistently demonstrate lower n-3 PUFA status to be associated with pMDD. However, the differences in n-3 PUFA status between cases and controls could not be explained by differences in n-3 PUFA intake. This finding undermines the importance to further investigate the mechanisms underlying the relationship between n-3 PUFA nutrition and paediatric depression, such as the role of n-3 PUFA in preventing or resolving depression-associated inflammation. Overall, the high proportion of participants with a low and very low n-3 index is of concern, highlighting the need for public health measures to improve-3 PUFA status during adolescence.

Conflict of Interest

All authors declare that they have no conflict of interest.

Funding

The Omega-3 pMDD study was supported by the Swiss National Science Foundation. EO received a PhD fellowship from the Ebnet Foundation, Teufen, Switzerland.

The omega-3 study team

The Omega-3 Study Team contributed with implementation of the design with following roles: Sponsor-investigator of the trial is GB. Chief investigators are SW and KS. IH is study coordinator. Principal investigators and research psychologist from the clinical sites are as follows: Principal Investigator Zurich: SW; Research psychologists: Noemi Baumgartner, Sophie Emery, Mona Albermann, and Kristin Nalani (Department of Child and Adolescent Psychiatry, University Hospital of Zurich); Principal Investigator Basel: KS; Investigators and research psychologists: Oliver Pick, Alain Di Gallo, and Michael Strumberger (Department of Child and Adolescent Psychiatry, Psychiatric University Hospitals Basel); Principal Investigator Basel-Stadt: Brigitte Contin; Investigator: Stefan Müller (Child and Adolescent Psychiatric Services Baselland); Principal Investigator: Silke Bachmann; Investigators: Lars Wöckel, and Simone Heitzer (Clienia Littenheid); Principal Investigator: Bruno Rhiner; Investigators: Amir Yamini (Child and Adolescent Psychiatric Services Thurgau); Principal Investigator: Suzanne Erb; Investigators: Michael Schmid (Child and Adolescent Psychiatric Services St. Gallen); Principal Investigator: Ulrich Müller-Knapp; Investigator: Ioannis Christodoulakis (Klinik Sonnenhof). UH and Burkhardt Seifert (retired) are statistical consultants. Renate Drechsler is head of the neuropsychology department and Edna Grünblatt head of the department for translational molecular psychiatry (Department of Child and Adolescent Psychiatry, University Hospital of Zurich). Martin Hersberger is head of the division of Clinical Chemistry and Biochemistry at the University Children's Hospital Zürichand his PhD student Ivan Hartling of the division of Clinical Chemistry and Biochemistry WHO will analyse the bioactive lipids; Romuald Brunner (University of Heidelberg), Jürgen Drewe (University of Basel), and Julia Braun (Epidemiology, Biostatistics, and Prevention Institute, University of Zürich) are members of the Data Monitoring Committee. Jenny Peterson, Clinical Trials Pharmacy (Kantonsapotheke) Zürich, responsible for the packaging, handling, and quality of the study medication.

Acknowledgements

We would like to give our sincerest thank the following people for their valuable contribution to this case-control study: all the adolescents participating in this study; for their support in laboratory analyses: S Pakeerathan, T Christ, A Krzystek, C

Zeder; for their support in study management and data assessment: A Zacher, D Salinas, S Pleus, O Wunderlin, Msc students PUK; and all the study nurses for their contributions.

Author contributions

Conceptualization of current case-control analysis: EO, IHA, JB. Conceptualization and execution of Omega-3 pMDD study: IH, GB, KS, UH, SW, Omega-3 study team. Recruitment of controls (including data collection): EO, AZ, DS, SP, SP, OW. Data analysis: EO, IHA, JB, CR. Writing of original draft: EO, IHA, JB. Writing (review & editing): EO, IHA, SP, SE, MA, NB, CR, KS, SW, MS, MBZ, IH, GB, JB

5 Manuscript Three

Higher prevalence of hypothyroxinaemia unrelated to iodine status in Swiss adolescents with paediatric major depressive disorder compared to healthy controls: a matched case-control study

Ester Osuna[1], Jeannine Baumgartner[1,2], Sophie Emery[3], Mona Albermann[3], Noemi Baumgartner[3], Klaus Schmeck[4], Susanne Walitza[3], Michael Strumberger[4], Michael B. Zimmermann[1], Isabelle Häberling[3], Gregor Berger[3], Isabelle Herter-Aeberli[1], on behalf of the Omega-3 study team

[1]Laboratory of Human Nutrition, Institute of Food, Nutrition and Health, ETH Zurich, Switzerland.
[2]Department of Nutritional Sciences, King's College London, London, United Kingdom.
[3]Clinics for Child and Adolescent Psychiatry, University Hospital Zurich, Switzerland.
[4]Research Department of Child and Adolescent Psychiatry, Psychiatric University Hospitals Basel, University of Basel, Switzerland.

Funding: This study has been supported by the Swiss National Science Foundation, and the Ebnet Foundation, Teufen, Switzerland.

This is a manuscript draft approved by all co-authors to be included into this PhD thesis

Abstract

Background: Globally, over 300 million people are affected by depression. The aetiology of depression is multifactorial and has been associated with subclinical hypothyroidism and altered Hypothalamic-pituitary-adrenal (HPA) functioning in adults. It is unknown whether alterations in thyroid function are also present in adolescents with Paediatric major depressive disorder (pMDD) and are related to iodine nutrition.

Objective: We aimed to determine associations of iodine status and thyroid function with pMDD and stress among Swiss adolescents.

Methods: In this case-control study, we included 95 adolescents with diagnosed pMDD and 95 healthy controls aged 13-17 years, matched for age, sex and education. We assessed depression using the Children's Depression Rating Scale-Revised (CDRS-R) and perceived stress using the Perceived stress scale (PSS). We determined iodine status by measuring Urinary iodine concentration (UIC), and thyroid status by measuring Thyroid stimulating hormone (TSH) and Thyroxine (T4) in serum.

Results: Median Interquartile range (IQR) UIC did not differ between cases (121 (87, 174) μg/l) and controls (114 (66, 183) μg/L, p=0.3), and indicated adequate iodine nutrition. However, we found significantly lower TSH and T4 concentrations in cases compared to controls (TSH: 1.36 (0.91, 2.00) mIU/L vs 1.50 (1.18, 2.06) mIU/L, respectively, p=0.039; T4: 14.7(12.9, 16.9) pmol/L vs 15.7(14.3, 17.2) pmol/L, respectively, p=0.004). Furthermore, the prevalence of hypothyroxinaemia (characterized by low T4 and normal TSH levels) was higher among cases than controls (15% vs 4%, p=0.004).

Conclusion: We found a higher prevalence of hypothyroxinaemia in adolescents with diagnosed pMDD compared to healthy controls. Iodine nutrition was adequate and did not differ between adolescents with and without pMDD. Therefore, our results suggest a link between hypothyroxinaemia and pMDD unrelated to iodine status. Potential risk factors for hypothyroxinaemia should further be investigated in the context of lipid profiles, iron status, and antidepressant treatment.

5.1 Introduction

Over 300 million people worldwide are affected by depression [322], which makes depression a leading cause of disability worldwide [51]. Depression during adolescence is associated with poor educational, work, and social functioning as well as an increased rate of smoking, substance abuse, eating disorders, and obesity [55]. Also, an early onset of depression is a risk factor for chronic and recurrent forms of depression in adulthood [53]. Nevertheless, Paediatric major depressive disorder (pMDD) often remains undiagnosed and therefore untreated [42]. Experts estimate the prevalence of pMDD in Europe to be around 5% [323], making it one of the most common psychiatric disorders during childhood and adolescence. To develop effective strategies to prevent pMDD or delay its progression it is important to better understand its aetiology.

Iodine deficiency results in inadequate production of thyroid hormones since iodine is an essential component of the thyroid hormones Thyroxine (T4) and Triiodothyronine (T3). In the foetus, inadequate thyroid hormones as a result of inadequate maternal iodine intake impairs myelination, cell migration, differentiation and maturation in the brain [12, 187]. The aetiology of depression remains unclear, but it is most likely multifactorial [4]. For instance, there is a large body of evidence indicating that the Hypothalamic-pituitary-thyroid (HPT) axis is altered in various psychiatric disorders such as bipolar disorder, schizophrenia, anxiety disorders and depression [220, 354, 355]. Nevertheless, these previous studies and meta-analyses fail to consistently establish a link between pathological thyroid functions and depressive disorders [222, 223]. Potential mechanisms of the HPT axis being involved in the aetiology of depression include, reduced availability of thyroid hormones implicating decreased myelination and synaptic plasticity within the brain [218]. Both, decreased myelination and synaptic plasticity have been associated with depression before [81, 85]. Next, there is evidence that factors such as age and sex might play a role in the link between thyroid disfunction and depression [222, 223].

To date, most of the studies linking pathological thyroid function, such as subclinical hypothyroidism, with depressive disorders have been conducted in adults. However, findings from studies in adults cannot be generalized to adolescents due to differences in developmental stage and nutrient requirements, as well as poorer diet quality in this age group [47, 329]. Therefore, it seems important to investigate the link between iodine nutrition, the HPT axis and depression among adolescents to gain further insights into the aetiology of depression.

There is evidence from animal models and human studies that an altered Hypothalamic-pituitary-adrenal (HPA) axis might affect the production of Thyrotropin-releasing hormone (TRH) [224, 225]. Environmental stress is known to increase sensitivity to the HPA axis and enhances response to subsequent stressors [8, 102]. Furthermore, there is robust evidence for the HPA axis being implicated in the pathophysiology of

Major depressive disorder (MDD) and other stress-related diseases by alterations of the endocrine system in adults [8, 356]. Nevertheless, there is still a lack of evidence for a relationship between the HPA and HPT axis in depressed adolescents [227].

In Switzerland, around 10% of adolescents experience depressive symptoms [52]. Moreover, even though Switzerland has a model iodized salt program, and school aged children were shown to have adequate iodine intakes [357], national studies report poor iodine nutrition in several population groups, including adults [358, 359], lactating women and infants [360]. Thus, the aim of this study was to determine associations between iodine status, thyroid function, and depressive symptoms in adolescents with and without diagnosed pMDD. We further explored whether these associations are sex specific. Finally, we aimed to investigate the relationship between iodine status, thyroid function parameters and perceived stress and its link to pMDD among adolescents. Therefore, we assessed iodine status by measuring Urinary iodine concentration (UIC) and thyroid function by measuring serum T4 and Thyroid stimulating hormone (TSH) concentrations. We hypothesized adolescents with pMDD having a higher prevalence of hypothyroidism or subclinical hypothyroidism compared to healthy adolescents without pMDD, and that these differences are related to differences in iodine nutrition. Further, we hypothesized stress being associated with thyroid hormone concentrations and pMDD.

5.2 Participants and Methods

5.2.1 Study design

This study is an observational case-control study in adolescents with diagnosed pMDD and healthy controls aged 13–17 years. The cases and controls were matched according to sex, age group (13 to <16 and 16 to <18 years) and education in a 1:1 ratio. To calculate the sample size G*Power V3.1.9.2 was used. A power calculation was applied to a logistic model where the Children's Depression Rating Scale-Revised (CDRS-R) score was coded as a dichotomous variable in a model with 10 covariates (with a residual (R^2 =0.2). In this model, one Standard deviation (SD) increase of the continuous predictor generated an Odds ratio (OR) of 1.5 and 2. According to these power calculations a sample size of 200 individuals with a 1:1 matching ratio between cases and controls was sufficient (power >80%, $\beta \geq$20%) to detect medium to large effect sizes for a type-I error of 5% ($\alpha = 0.05$). Up to a 10% drop out rate, these results seemed robust. To have a balanced study population we aimed to include 100 cases and 100 controls, with equal representation of sex, age groups, and education in cases and controls. In the first age group of 13 to <16 years of age, the aim was to recruit 50 female and male participants (25 each) in lower secondary school. In the second age group of 16 to <18 years, the aim was to recruit 52 female and male

participants: 13 female and male participants each for (1) vocational education and (2) baccalaureat / vocational baccalaureat at higher secondary school. The cases were selected to match the recruited controls.

The ethics committee of the Canton of Zurich approved this study (BASEC-Nr. 2019-00717) and it was registered at www.ClinicalTrials.gov (NCT04158869). This study is an add-on study to the investigator-initiated clinical trial (SNSF 33IC30_166826, BASEC-Nr. 2016-02116). Caregivers and adolescents $\geq$14 years of age consented to this study in written form, and adolescents $<$14 years of age consented orally before any research-related assessments were conducted.

5.2.2 Participants and procedures

Control group

The control group was recruited and assessed by a study team of the Laboratory of Human Nutrition at ETH Zurich, Switzerland. From September 2019 until December 2020 healthy female and male controls were recruited from the canton of Zurich and surrounding German-speaking cantons of Switzerland. Recruiting of controls took place in schools, leisure time clubs, and via social media. Inclusion criteria for controls were age of 13 to $<$18 years; no present nor past primary diagnosed psychiatric disorder according to the Mini-International Neuropsychiatric Interview for Children and Adolescents (M.I.N.I. KID) [330]; and no use of chronic medication. Controls were not eligible if they were unable to follow the study procedures, for example due to language barriers; if they took n-3 Polyunsaturated fatty acids (PUFA) supplements (providing $>$600 mg combined Eicosapentaenoic acid (EPA)/Docosahexaenoic acid (DHA)) for more than four weeks within the last six months; and if they reported pre-existing neurological or medical conditions likely to result in the development of depressive symptoms. After consenting and enrolling into the study, participants electronically completed questionnaires on REDCap® (Research Electronic Data Capture) within two weeks prior to the physical data assessment at ETH Zurich.

pMDD group

The cases in this study were participants of the Omega-3 Fatty Acid Paediatric Depression Trial (Omega-3 pMDD) under the lead of the Psychiatric University Hospital Zurich, which were randomly selected to match the controls. The protocol of the Omega-3 pMDD study has been published previously [331]. The recruitment of participants took place in seven different in- and outpatient services in five German speaking cantons of Switzerland from May 2017 until June 2021. The adolescents were either informed about the study by their clinicians in one of the participating centres or contacted the study team on their own initiative after seeing posters of flyers. The

inclusion criteria for the cases were for teenagers aged between 13 to <18 years; and a main diagnosis of MDD according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria [332] of at least moderate severity defined by a CDRS-R [333] total score of ≥40. Cases were not eligible if they fulfilled diagnostic criteria for an eating disorder within the last 6 months, or a lifetime diagnosis of schizophrenia, bipolar affective disorder, substance use dependency, mental retardation, or pervasive development disorder. Further, cases were not eligible if they had pre-existing neurological or medical conditions likely causing their depressive symptoms; if they were taking n-3 PUFA supplementation (>600mg combined EPA/DHA) within the last 6 months; or if their families were unable to follow the study procedures, for example, due to language barriers. After consenting to the study, the screening interview was conducted with the adolescents in the presence of a parent. The inclusion and exclusion criteria were assessed with the Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS) [334] for assessing the presence of MDD and the CDRS-R for assessing the severity of the depression. Within a few days, the adolescent's depression score was assessed again with the CDRS-R in the context of the lead-in interview. At this point, participants started the lead-in phase of the intervention study which is not part of this analysis. For this case-control study, only data (biological samples and CDRS-R scores) from the lead-in interview was used.

5.2.3 Data collection

This study tried to align the procedures between cases and controls as much as possible.

Assessment of anthropometry and socio-demographic information

For the cases and the controls weight (to the nearest 0.1 kg) and height (to the nearest 0.5 cm) was measured at the end of the physical data assessment. The Body mass index (BMI) was calculated using the individual's weight in kilograms (kg) divided the individual's height in meters (m) squared (kg/m2). BMI-for-age z-scores were calculated using the R package "anthroplus" provided by the World Health Organisation (WHO) [335]. Further, the data on BMI was then categorized into four BMI categories of underweight, normal weight, overweight, and obese according to the WHO's age-dependent reference values [361]. Adolescents with a z-score at -1 SD coincide with adult underweight (BMI <18), adolescents with a z-score +1 SD coincide with adult overweight (BMI = 25) and adolescents with a z-score +2 with adult obesity (BMI = 30). Socio-demographic and -economic data was assessed using self-reported questionnaires which the participants were asked to fill out together with one parent.

Assessment of depression and perceived stress

The CDRS-R was used to assess depression. The CDRS-R is a semi-structured clinical interview which takes 15-20 minutes to administer. It is one of the most frequently used rating scales for measuring severity of depression and change in depressive symptoms in children and adolescents [60]. The validity of the scale has been shown for adolescents [60] and children [59]. By providing the possibility to conduct the interview with the child, the parents, and/or teacher the interview allows a comprehensive assessment of the severity of the child's or adolescent's depression. The interview covers 17 depressive symptoms rated on a 5 to 7 point Likert scale. The domains of depressive symptoms are aligned with the DSM-IV criteria for childhood depression [336] and cover suicidal ideation, social withdrawal, sleep disturbance, excessive fatigue, etc. Individuals are asked about information on 14 items and three non-verbal symptoms are assessed only by the interviewer (depressed facial affect, hypoactivity, and speech velocity). The interviewers were trained to conduct the interview. The individual ratings are summed up to a total score ranging from 17 to 113 with a score of $\geq$40 being used as a cut-off for pMDD [337].

Data on perceived stress were assessed with a self-reporting Perceived stress scale (PSS) [362]. This questionnaire is structured in 10 items which are rated on a 0-to-4-point Likert scale with total scores ranging from 0 to 40. Higher scores indicate that the individual perceives its momentary situation as more stressful. The validity of the scale's German version has been shown in a German female and male population in the age range from 14 to 90 years [363].

Biochemical analysis

Participants were asked to collect a spot urine sample (Urin-Monovette®, Sarstedt) voluntarily to assess the UIC as a marker for iodine status. No standardized collection timepoint was used. The UIC was measured using the Pino modification of the Sandell-Kolthoff reaction [364]. UIC in spot urine samples were measured by one single person at the Laboratory for Human Nutrition, which successfully participates in the EQUIP network (US Centers for Disease Control and Prevention, Atlanta, GA). All urine samples were measured in duplicate and re-analysed when difference in absorbance was >5% for UIC >150 μg/L, >10% for UIC 50-150 μg/L, and >15% for UIC <50 μg/L. Control urine samples were added to every plate for external quality control. The reference range for adequate iodine nutrition was set according to WHO reference values for UIC between 100-199 μg/L [198].

Venous blood was drawn into serum tubes (for controls: BD Vacutainer, for cases: Sarstedt) was let stand for 60 minutes to allow clotting. Afterwards, the serum tubes were centrifuged, and the serum aliquoted and stored at -80°C until further analysis. The thyroid parameters T4 and TSH were measured in serum by electrochemilumin-

escence (Cobas e411, Roche Elecsys) at the Children's Hospital Zurich, Switzerland. The reference range for peripheral T4 in the age group of >11 to <20 years of age was defined as T4 values between 12.6 pmol/L to 21 pmol/L [365]. The reference range for peripheral TSH values in the age group of >11 to <20 years of age was defined as TSH values between 0.51 mIU/L to 4.3 mIU/L [366]. Values below or higher than the reference range were classified accordingly. Clinical or overt hypothyroidism was characterized as T4<12.6 pmol/L and TSH>4.3 mIU/L. Subclinical hypothyroidism was characterized as normal T4 and TSH>4.3 mIU/L. Clinical or overt hyperthyroidism was defined by T4>21 pmol/L and TSH<0.51 mIU/L. Subclinical hyperthyroidism was characterized by normal T4 and TSH<0.51 mIU/L. Hypothyroxinaemia was characterized by T4<12.6 pmol/L and normal TSH values.

5.2.4 Data management and statistical methods

Data capture for the controls was done either electronically on REDCap® or on paper and retroactively entered to the REDCap® system. When captured on paper, the person assessing the data entered it to the system and later the data was checked for entry errors by a second member of the study team. REDCap® is a secure, web-based software platform and electronic data capture tool designed for research studies which is hosted at ETH Zurich [341, 342]. For the cases, data was assessed on paper and entered to the electronical data capture tool secuTRIAL by two individual persons. Data entry was checked by a third person for entry errors. After completing the matching between the cases and the controls, all data was combined and managed using REDCap®. Data processing and statistical analysis of data were performed using R Version 3.6.0 [311]. By means of Q-Q plots, histograms, and Shapiro-Wilk test data were tested for outliers and normality. Normally distributed data and non-normally distributed data were expressed as mean $\pm$SD and as medians (Interquartile range (IQR)), respectively. Wilcoxon rank sum test was applied to test not normally distributed continuous data and t-test for normally distributed data. Chi-square tests were applied to assess significant differences when the expected cell counts was $\geq$5, and Fisher's exact test when the expected cell count was <5. For producing tables and calculating these differences, the R package "gtsummary" was used. This R package has been shown to produce reproducible summary tables within R [343]. Further, to assess associations of depression (CDRS-R score $\geq$40) with different thyroid function indicators, multivariate logistic regression analysis was used. In the statistical model, matching criteria sex, age, education, and BMI-for-age z-scores were included as covariates. To assess associations between the PSS score and thyroid function indicators, multivariate linear regression analysis was used. The matching criteria sex, age, education, and BMI-for-age z-scores were included as covariates into the statistical model.

5.3 Results

After the recruitment process, 98 controls were enrolled into this case-control study. Thereof, two individuals dropped out: one voluntarily after the screening interview, and one by not providing a blood sample. For the selection of the cases from the Omega-3 pMDD study, a total of 173 participants completed the intervention study and were therefore eligible as cases for the case-control study. One control could not be matched to a case according to the matching criteria. Therefore, a total of 95 controls were matched to 95 cases according to sex, age group (13 to <16 and 16 to <18), and education. The final sample size for this case-control analysis was n=190. A detailed flow chart of the study inclusion can be seen in Figure 5.1. For four adolescent pairs in the age group 16 to <18 years of age the matching was done only according to sex and age group, since matching by education was not possible.

The detailed participant characteristics are presented in Table 5.1. The matching between cases and controls was successful, which was shown by no significant differences in age, sex, and education. Also, there were no significant differences between cases and controls in respect to BMI-for-age z-scores. On the other hand, significantly higher CDRS-R and PSS scores could be observed among cases compared to controls (both p<0.001). The proportion of adolescents with an East-Asian descent was slightly higher among the cases compared to the controls (p=0.051).

A detailed description of iodine and thyroid status parameters is displayed in Table 5.2. Urine samples were not available for 27 participants, since collection was not compulsory. Blood samples for TSH and T4 analysis were not available for three of the study participants, this was due to lack of sufficient sample to conduct all the analyses. The median (IQR) UIC for the entire study population was 119 (75, 178) μg/L, with no significant difference between groups (p=0.3). However, TSH and T4 values were significantly lower among cases compared to controls (TSH: 1.36(0.91, 2.00) mIU/L vs 1.50(1.18, 2.06) mIU/L, respectively, p=0.039; and T4: 14.7(12.9, 16.9) pmol/L vs 15.7(14.3, 17.2) pmol/L, respectively, p=0.004). There was a significantly higher prevalence of hypothyroxinaemia (normal TSH and low T4) among cases compared to controls of 15% vs 4% (p=0.004). In Table 5.3 details on iodine and thyroid status parameters by sex are displayed. Significantly lower T4 values could be observed among female cases compared to female controls (14.4(12.6, 15.3) pmol/L vs 15.1(14.2, 16.6) pmol/L, respectively, p=0.004), but no difference was observed among males. Also, a significantly higher prevalence of hypothyroxinaemia could be observed among female cases compared to female controls with 21% vs 4%, respectively (p=0.006), which was not observed among males.

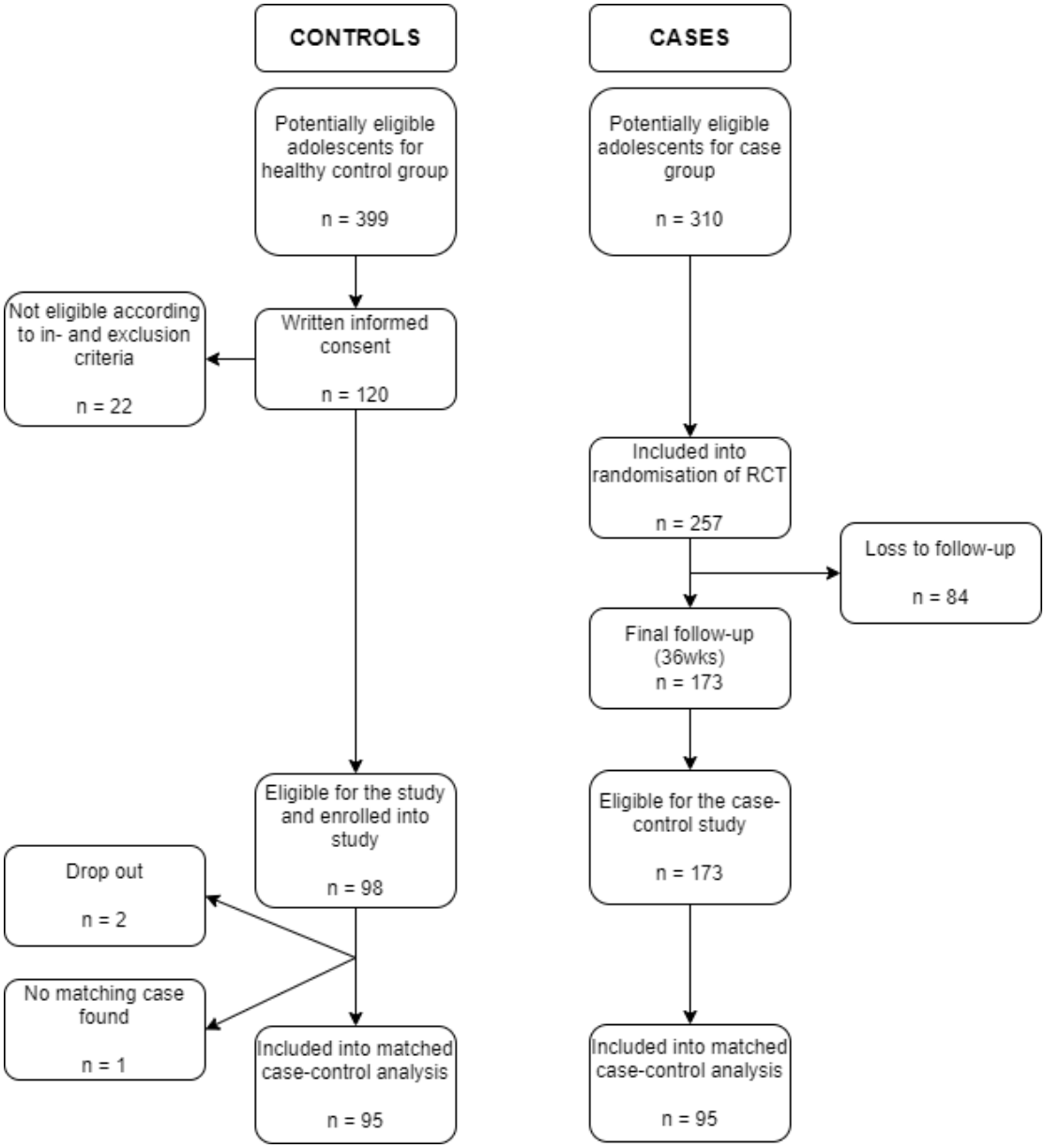

Figure 5.1: Flow chart of this case-control study's included adolescents with and without diagnosed Paediatric major depressive disorder (pMDD): RCT: Randomised Control Trial

Table 5.1: Characteristics of Swiss adolescents with (n=95) and without (n=95) Paediatric major depressive disorder (pMDD)

	Overall	Cases	Controls	p-value
Age	16.1 (14.9, 17.1)	16.1 (14.9, 17.2)	16.0 (14.9, 17.1)	0.8[2]
Sex				>0.9[3]
Female	110 (58%)	55 (58%)	55 (58%)	
Male	80 (42%)	40 (42%)	40 (42%)	
CDRS-R score	36 (18, 56)	56 (50, 62)	18 (17, 20)	**<0.001**[2]
PSS score[+]	17 (11, 25)	25 (20, 28)	11 (7,15)	**<0.001**[2]
BMI-for-age z-score[1]	0.20 ± 1.03	0.25 ± 1.05	0.16 ± 1.02	0.4[2]
BMI category[1]				0.5[4]
Underweight	7 (4%)	2 (2%)	5 (5%)	
Normal weight	138 (76%)	65 (75%)	73 (77%)	
Overweight	20 (11%)	12 (14%)	8 (8%)	
Obese	17 (9%)	8 (9%)	9 (10%)	
Ethnicity				0.051[4]
European	174 (92%)	87 (92%)	87 (92%)	
East-Asian	5 (3%)	5 (5%)	0 (0%)	
Indian-Asian	2 (1%)	0 (0%)	2 (2%)	
Middle East	2 (1%)	1 (1%)	1 (1%)	
Not declared	7 (4%)	2 (2%)	5 (5%)	
Swiss education				0.8[3]
Lower secondary school (Mandatory school)	104 (55%)	54 (57%)	50 (53%)	
Upper secondary school				
Vocational education	28 (15%)	14 (15%)	14 (15%)	
Baccalaureat/ vocational baccalaureat	58 (31%)	27 (28%)	31 (33%)	

Median (IQR); mean ±SD

CDRS-R: Children's Depression Rating Scale-Revised

[+] Data on Perceived stress scale (PSS) score was not available for all participants (n_{cases}=93; $n_{controls}$=95)

[1] Data on Body mass index (BMI) was not available for all participants (n_{cases}= 87; $n_{controls}$=95), BMI-for-age z-scores and BMI categories were defined according to World Health Organisation (WHO) reference data (Adolescents with a z-score at -1 SD coincide with adult underweight (BMI <18), adolescents with a z-score +1 SD coincide with adult overweight (BMI = 25) and adolescents with a z-score +2 SD with adult obesity (BMI = 30))

[2] Wilcoxon rank sum test

[3] Pearson's Chi-squared test

[4] Fisher's exact test

Table 5.2: Summary of iodine status and thyroid function parameters in a sample of adolescents with and without diagnosed Paediatric major depressive disorder (pMDD)

	Overall	n_{cases}	Cases	$n_{controls}$	Controls	p-value
UIC (μg/L)	119 (75, 178)	74	121 (87, 174)	89	114 (66, 183)	0.3[5]
TSH (mIU/L)	1.45 (1.02, 2.01)	93	1.36 (0.91, 2.00)	94	1.50 (1.18, 2.06)	**0.039**[5]
T4 (pmol/L)	15.1 (13.8, 17.0)	93	14.7 (12.9, 16.9)	95	15.7 (14.3, 17.2)	**0.004**[5]
Thyroid functions		92		94		**0.004**[6]
Normal thyroid function[1]	163 (88%)		76 (83%)		87 (93%)	
Subclinical hyperthyroidism[2]	2 (1%)		2 (2%)		0 (0%)	
Hypothyroxinaemia[3]	18 (10%)		14 (15%)		4 (4%)	
Hyperthyroxinaemia[4]	3 (2%)		0 (0%)		3 (3%)	

Median IQR; n(%)
UIC: urinary iodine concentration; TSH: thyroid stimulating hormone; T4: thyroxine
[1] Normal thyroid function in adolescents >11 and <20 years classified as TSH between 0.51-4.3 mIU/L and T4 between 12.6-21 pmol/L
[2] Subclinical Hyperthyroidism defined as normal T4 levels and TSH<0.51 mIU/L
[3] Hypothyroxinaemia defined as normal TSH levels and T4<12.6 pmol/L
[4] Hyperthyroxinaemia defined as normal TSH levels and T4>21 pmol/L
[5] Wilcoxon rank sum test
[6] Fisher's exact test

A summary of conducted multivariate logistic regression models assessing associations of iodine and thyroid status indicators as well as perceived stress with pMDD is shown in Table 5.4. There was no significant association between UIC and pMDD (p=0.3). However, higher TSH concentrations were associated with lower odds for depression (OR=0.59[0.6-0.93], p=0.026). Also, higher T4 concentrations were associated with lower odds for depression (OR=0.81[0.70-0.92], p=0.002). Further, higher PSS scores were associated with higher odds for depression (OR=1.42[1.30-1.59], p<0.001). Table 5.5 shows a summary of multivariate linear regression models assessing associations of iodine and thyroid status indicators with perceived stress. No significant associations could be found between iodine or thyroid status indicators and perceived stress. However, there was a trend for T4 values being negatively associated with PSS scores (beta=-0.49 (-1.00, 0.03), R2=0.095, p=0.065).

Table 5.3: Summary of iodine status and thyroid function parameters by sex in a sample of adolescents with diagnosed Paediatric major depressive disorder (pMDD) and without pMDD

	Female					Male				
Characteristic	n_{cases}	Cases	$n_{controls}$	Controls	p-value	n_{cases}	Cases	$n_{controls}$	Controls	p-value
UIC (µg/L)	44	126 (89, 174)	52	107 (51,183)	0.2[5]	30	119 (79, 170)	37	128 (75, 179)	>0.9[5]
TSH (mIU/L)	53	1.20 (0.94,1.90)	55	1.42 (1.02, 1.90)	0.2[5]	40	1.45 (0.87, 2.09)	39	1.79 (1.39, 2.20)	0.1[5]
T4 (pmol/L)	52	14.4 (12.6, 15.3)	55	15.1 (14.2, 16.6)	**0.004**[5]	40	15.5 (13.8, 17.6)	40	16.3 (14.9, 18.0)	0.2[5]
Thyroid functions	52		55		**0.006**[6]	40		39		0.2[6]
Normal thyroid function [1]		41 (79%)		53 (96%)			35 (88%)		34 (87%)	
Subclinical hyperthyroidism[2]		0 (0%)		0 (0%)			2 (5%)		0 (0%)	
Hypothyroxinaemia[3]		11 (21%)		2 (4%)			3 (8%)		2 (5 %)	
Hyperthyroxinaemia[4]		0 (0%)		0 (0%)			0 (0%)		3 (8%)	

Median (IQR); n(%)

UIC: urinary iodine concentration; TSH: thyroid stimulating hormone; T4: thyroxine

[1] Normal thyroid function in adolescents >11 and <20 years classified as TSH between 0.51-4.3 mIU/L and T4 between 12.6-21 pmol/L

[2] Subclinical Hyperthyroidism defined as normal T4 levels and TSH<0.51 mIU/L

[3] Hypothyroxinaemia defined as normal TSH levels and T4<12.6 pmol/L

[4] Hyperthyroxinaemia defined as normal TSH levels and T4>21 pmol/L

[5] Wilcoxon rank sum test

[6] Fisher's exact test

Table 5.4: Multivariate logistic regression models assessing associations of iodine and thyroid status indicators as well as perceived stress with Paediatric major depressive disorder (pMDD) in Swiss adolescents (n=186)

	Odds ratio (OR)	Lower 95% CI OR	Upper 95% CI OR	p-value
UIC	1.00	1.00	1.01	0.3
TSH	0.59	0.36	0.93	**0.026**
T4	0.81	0.70	0.92	**0.002**
PSS	1.42	1.30	1.59	$<$**0.001**

UIC: urinary iodine concentration; TSH: thyroid stimulating hormone; T4: thyroxine; CI: confidence interval; PSS: perceived stress scale
The dependent variable was the diagnosis of depression (CDRS-R $\geq$40). The independent variables were iodine/thyroid status indicators and perceived stress. All models were controlled for sex, age, education, and BMI-for-age z-scores.

Table 5.5: Multivariate linear regression models assessing associations of iodine and thyroid status indicators with Perceived stress scale (PSS) in Swiss adolescents (n=186)

	R^2	Adjusted R^2	Beta	Std. Error Beta	Standardised beta	p-value
UIC	0.094	0.058	0.01	0.01	0.06	0.5
TSH	0.090	0.057	-1.57	0.94	-0.13	0.1
T4	0.095	0.063	-0.49	0.27	-0.14	0.065

UIC: urinary iodine concentration; TSH: thyroid stimulating hormone; T4: thyroxine;
The dependent variable was the perceived stress. The independent variables were iodine/thyroid status indicators. All models were controlled for sex, age, education, and BMI-for-age z-scores.

5.4 Discussion

In this case-control study, we found an adequate iodine status and no significant difference in UIC between Swiss adolescents with and without pMDD. However, we found a significantly higher prevalence of isolated hypothyroxinaemia among cases (14%) compared to controls (4%), which mainly occurred in female adolescents. Further, we found significantly lower TSH and T4 levels among cases compared to controls.

In this study, we found a higher prevalence of hypothyroxinaemia among depressed adolescents compared to healthy controls. Hypothyroxinaemia is a condition characterized by normal TSH values and low T4 values, and has been described predominantly in iodine deficiency during pregnancy [207]. In this study, the UIC did not differ significantly between cases and controls. Furthermore, UIC for both groups was within the recommended UIC reference range for adequate iodine nutrition of 100-199 μg/L among adolescents [198]. This finding suggests appropriate iodine nutrition among the adolescents. Therefore, the observed hypothyroxinaemia might be caused by other factors rather than through low iodine intake. Among Polish women of childbearing age, vitamin D deficiency, insulin resistance, increased BMI, and an abnormal lipid profile were associated with increased odds for hypothyroxinaemia [210]. Among Chinese pregnant and non-pregnant women, hypothyroxinaemia was higher among women with iron deficiency compared to women without iron deficiency [367]. Interactions between iron and iodine have been shown in rats, where iron deficiency anaemia reduced the activity of Thyroperoxidase (TPO), a heme-containing enzyme catalysing the first two steps of thyroid hormone synthesis [368]. Finally, among Chinese adult MDD patients, the use of mirtazapine, an antidepressant involving adrenoceptors and 5-hydoxytryptamine receptors, was associated with an increased risk for hypothyroxinaemia [369]. To conclude, the increased prevalence of hypothyroxinaemia among depressed adolescents compared to healthy controls could be explained by several factors, including interactions with other nutrients such as fatty acids, iron, or antidepressant treatment. Still, to confirm possible interactions between nutrients or medication, further research is needed.

Stress hormones known as glucocorticoids and primarily cortisol are known to increase during stress situations [370]. In this study, we used the adolescent's perceived stress as an approximation to biological parameters describing the HPA axis regulating stress responses. Within our adolescent study population, higher perceived stress scores were associated with higher odds for depression. Further, although not significant, a negative trend between T4 levels and PSS scores could be observed. This finding supports the hypothesis of a link between the HPT and the HPA axis being involved in the aetiology of depression. However, this finding should be confirmed by investigating biological parameters of the HPA axis such as cortisol.

This study has several strengths and limitations. First, our study population consists of well-matched participants with and without pMDD. Also, for cases only individuals with a clinical diagnosis of pMDD of moderate to severe depressive symptoms were enrolled into the study. Next, we assessed the adolescent depression score using an interviewer administered assessment which includes non-verbal items, allowing a comprehensive assessment of the severity of the adolescent's depression. Despite the strength of our study design, due to its observational nature, no causal conclusions can be drawn. Further, one limitation of this study was that the iodine status of the participants was determined based on only one single spot urine sample. According to previous results from our group, to calculate the UIC on an individual level with a 20% precision, at least ten spot urine samples are needed [371]. Therefore, results from the regression analyses including spot UIC may need to be interpreted with caution. Nevertheless, the WHO states that casual single urine samples can provide adequate assessment of iodine nutrition on a population level [194]. Therefore, the combination of UIC measurements with T4 and TSH concentrations within this population is valuable as it helps interpreting the origin of the observed thyroid dysfunctions.

In conclusion, we found a higher prevalence of hypothyroxinaemia in a depressed adolescent population compared to matched healthy controls. Also, our results suggest a link between hypothyroxinaemia and pMDD unrelated to iodine status. Potential risk factors for hypothyroxinaemia should further be investigated in the context of lipid profiles, iron status, and antidepressant treatment.

Conflict of Interest

All authors declare that they have no conflict of interest.

Funding

The Omega-3 pMDD study was supported by the Swiss National Science Foundation. EO received a PhD fellowship from the Ebnet Foundation, Teufen, Switzerland.

The omega-3 study team

The Omega-3 Study Team contributed with implementation of the design with following roles: Sponsor-investigator of the trial is GB. Chief investigators are SW and KS. IH is study coordinator. Principal investigators and research psychologist from the clinical sites are as follows: Principal Investigator Zurich: SW; Research psychologists: Noemi Baumgartner, Sophie Emery, Mona Albermann, and Kristin Nalani (Depart-

ment of Child and Adolescent Psychiatry, University Hospital of Zurich); Principal Investigator Basel: KS; Investigators and research psychologists: Oliver Pick, Alain Di Gallo, and Michael Strumberger (Department of Child and Adolescent Psychiatry, Psychiatric University Hospitals Basel); Principal Investigator Basel-Stadt: Brigitte Contin; Investigator: Stefan Müller (Child and Adolescent Psychiatric Services Basel-land); Principal Investigator: Silke Bachmann; Investigators: Lars Wöckel, and Simone Heitzer (Clienia Littenheid); Principal Investigator: Bruno Rhiner; Investigators: Amir Yamini (Child and Adolescent Psychiatric Services Thurgau); Principal Investigator: Suzanne Erb; Investigators: Michael Schmid (Child and Adolescent Psychiatric Services St. Gallen); Principal Investigator: Ulrich Müller-Knapp; Investigator: Ioannis Christodoulakis (Klinik Sonnenhof). UH and Burkhardt Seifert (retired) are statistical consultants. Renate Drechsler is head of the neuropsychology department and Edna Grünblatt head of the department for translational molecular psychiatry (Department of Child and Adolescent Psychiatry, University Hospital of Zurich). Martin Hersberger is head of the division of Clinical Chemistry and Biochemistry at the University Children's Hospital Zürich and his PhD student Ivan Hartling of the division of Clinical Chemistry and Biochemistry who will analyse the bioactive lipids; Romuald Brunner (University of Heidelberg), Jürgen Drewe (University of Basel), and Julia Braun (Epidemiology, Biostatistics, and Prevention Institute, University of Zürich) are members of the Data Monitoring Committee. Jenny Peterson, Clinical Trials Pharmacy (Kantonsapotheke) Zürich, responsible for the packaging, handling, and quality of the study medication.

Acknowledgements

We would like to give our sincerest thank the following people for their valuable contribution to this case-control study: all the adolescents participating in this study; for their support in laboratory analyses: M Volleberg, T Christ, A Krzystek, C Zeder; for their support in study management and data assessment: A Zacher, D Salinas, S Pleus, S Probst, O Wunderlin, Msc students PUK; and all the study nurses for their contribution.

Author contributions

Conceptualization of current case-control analysis: EO, IHA, JB. Conceptualization and execution of Omega-3 pMDD study: IH, GB, KS, UH, SW, Omega-3 study team. Recruitment of controls (including data collection): EO, AZ, DS, SP, SP, OW. Data analysis: EO, IHA, JB. Writing of original draft: EO, IHA, JB. Writing (review & editing): EO, IHA, SE, MA, NB, KS, SW, MS, MBZ, IH, GB, JB

6 Manuscript Four

Iron status in Swiss adolescents with paediatric major depressive disorder and healthy controls: a matched case-control study

Ester Osuna[1], Jeannine Baumgartner[1,2], Olivia Wunderlin[1], Sophie Emery[3], Mona Albermann[3], Noemi Baumgartner[3], Klaus Schmeck[4], Susanne Walitza[3], Michael Strumberger[4], Michael B. Zimmermann[1], Isabelle Häberling[3], Gregor Berger[3], Isabelle Herter-Aeberli[1], on behalf of the Omega-3 study team

[1]Laboratory of Human Nutrition, Institute of Food, Nutrition and Health, ETH Zurich, Switzerland.
[2]Department of Nutritional Sciences, King's College London, London, United Kingdom.
[3]Clinics for Child and Adolescent Psychiatry, University Hospital Zurich, Switzerland.
[4]Research Department of Child and Adolescent Psychiatry, Psychiatric University Hospitals Basel, University of Basel, Switzerland.

Funding: This study has been supported by the Swiss National Science Foundation, and the Ebnet Foundation, Teufen, Switzerland.

This is a manuscript draft approved by all co-authors to be included into this PhD thesis

Abstract

Background: Depression, a leading cause of disability worldwide, has been associated with lower iron status in adults and adolescents. Depression has further been associated with low-grade inflammation and impaired intestinal permeability. Whether the lower iron status in depressed individuals is related to these factors is not known. On the other hand, depression-associated inflammation may mask Iron deficiency (ID).

Objective: We determined the associations between iron status parameters and Paediatric major depressive disorder (pMDD) in the context of inflammation and intestinal permeability among Swiss adolescents. We further assessed the history of ID diagnosis and treatment.

Methods: This is a matched (for age, sex, and education) case-control study in 95 adolescents with diagnosed pMDD and 95 healthy controls aged 13-17 years. We used the Children's Depression Rating Scale-Revised (CDRS-R) for assessing depression. We measured iron status (Serum ferritin (SF), Soluble transferrin receptor (sTfR), Body iron stores (BIS)) and inflammation (C-reactive protein (CRP), α-1-acid-glycoprotein (AGP)). Furthermore, we assessed intestinal permeability by measuring Intestinal fatty acid binding protein (I-FABP). We assessed history of ID diagnosis and treatment with a self-reported questionnaire.

Results: SF concentrations did not differ between adolescents with pMDD (median (Interquartile range (IQR)) SF: 31.2 (20.2, 57.0) μg/L) and controls (32.5 (22.6, 48.3) μg/L, p=0.4). There was a trend for higher BIS among cases compared to controls (BIS: 5.73$\pm$3.31 mg/kg body weight vs BIS: 5.00$\pm$2.92 mg/kg body weight, p=0.083). sTfR concentrations were significantly lower among cases compared to controls (4.50 (4.00, 5.50) mg/L vs 5.20 (4.75, 6.10) mg/L, p$<$0.001), indicative of higher iron status. CRP, AGP and I-FABP concentrations were higher among cases compared to controls (CRP: 0.16 (0.03, 0.43) mg/L vs 0.04 (0.02, 0.30) mg/L, p=0.003; AGP: 0.57 (0.44, 0.70) g/L vs 0.52 (0.41, 0.67) g/L, p=0.024); I-FABP: 307 (17, 515) pg/mL; 232 (163, 357) pg/mL, p=0.047). There was a significant positive correlation between sTfR and AGP (ρ=0.15, p=0.044) and a positive trend between sTfR and CRP (ρ=0.13, p=0.073) in cases and controls combined. Of the cases, 44% reported ever having been diagnosed with ID, compared to 26% among the controls (p=0.02). Finally, 16% of cases took iron supplements at study inclusion compared to 4% among controls.

Conclusion: We did not observe differences in ferritin concentrations between adolescents with and without pMDD. However, sTfR concentrations were lower and BIS higher among cases compared to controls, indicative of better iron status in adolescents with depression. This may be explained by the higher proportion of depressed adolescents with a previous ID diagnosis and taking iron supplements at the time of assessment. Cases had significantly higher systemic inflammation and intestinal per-

meability compared to controls. However, whether this is related to the higher rate of ID diagnosis and iron treatment in adolescents with depression remains unclear.

6.1 Introduction

Depression, a leading cause of disability [51], affects an estimated 300 million people worldwide [322]. It is estimated that 5% of adolescents in Europe are affected by Paediatric major depressive disorder (pMDD), one of the most common psychiatric disorders during childhood and adolescence [323]. An early onset of depression is a risk factor for chronic and recurrent forms of depression in adulthood [53]. Furthermore, pMDD is associated with poor educational, work, and social functioning as well as an increased rate of smoking, substance abuse, eating disorders, and obesity [55]. However, pMDD often remains undiagnosed and therefore untreated [42]. Furthermore, the aetiology of pMDD is poorly understood and believed to be multifactorial [4]. Thus, a better understanding of the development as well as effective strategies to prevent pMDD or inhibit its progression are needed.

Iron deficiency (ID) persists to be one of the most prevalent forms of malnutrition [264] and a major cause of anaemia. Worldwide in 2016, 42% of children (<5 years of age) and 33% of women of reproductive age were anaemic [264]. The World Health Organisation (WHO) estimates that 43% of anaemia cases in children 6-59 months old and 50% in women are due to ID [266]. In children and adolescents, ID has been associated with poor school performance, decreased cognitive abilities, and behavioural problems [282, 283]. Iron, a key co-factor in the electron transfer reaction of cellular respiration and component of the oxygen transporter haemoglobin [234], is known to play a crucial role in neurodevelopment by being responsible for gene regulation, and regulation of cell growth and differentiation [235, 372]. Furthermore, iron is an important co-factor for the enzymes responsible for myelination of neurons and for the synthesis of dopamine and serotonin [373]. Therefore, since many of these processes are ongoing during adolescence, inadequate iron status during this period could negatively affect brain myelination, synaptogenesis, and brain connectivity, all of which have been associated with depression previously [6, 79, 85]. Nevertheless, the aetiology of pMDD remains unclear with increasing evidence pointing towards the importance of the intake of nutrients such as iron in the pathophysiology of Major depressive disorder (MDD) [44, 374]. For instance, previous studies did associate low iron status with depression, while ruling out evidence for a genetic relationship between circulating iron and measures of depression [284, 286, 375–377].

Depression has been associated with inflammation and altered immune response [9]. Inflammation can decrease intestinal iron absorption by upregulating the synthesis of the iron-regulating hormone hepcidin [378]. Furthermore, emerging evidence indicates that depression is being associated with increased intestinal permeability [379–381],

which may be cause by a gut microbiota dysbiosis and which may trigger low grade systemic inflammation. Thus, depression-associated inflammation and increased intestinal permeability may contribute to the development of ID in depressed individuals, which could potentially aggravate disease severity.

On the other hand, the iron status biomarker ferritin is also an Acute phase reactants (APR), responding to inflammation by rapid up-regulation [382]. Therefore, the interpretation of this iron status parameter is difficult in populations of widespread infection or inflammation. Thus, inflammatory markers such as C-reactive protein (CRP) and α-1-acid-glycoprotein (AGP) need to be considered when evaluating iron status in depressed patients.

In Switzerland, surveys suggest the prevalence of depressive symptoms to be around 10% [52]. Data on iron status of Swiss adolescents is scarce. However, studies in Swiss adults report an ID prevalence among women (34$\pm$8 years, n=46) of 50% and Iron deficiency anaemia (IDA) prevalence of 15% [383]. Recently, daily intake of iron in young Swiss adults (18-34 years) was determined in a dietary survey, and was estimated to be 10.3 mg/day for men and 9.0 mg/day for women [384]. These intakes are below the Germany-Austria-Switzerland region (D-A-CH) recommended intake values for males aged 19-51 years of 10 mg/day and for 19-51 year old women of 15 mg/day [258]. Thus, the aim of this study was to determine the association between iron status and pMDD in Swiss adolescents. Considering the potential bi-directional relationship between depression and ID, we further explored the associations of systemic inflammation and intestinal permeability with depression. We hypothesized that adolescents with pMDD would have a lower iron status compared to adolescents without pMDD. We further hypothesized that adolescents with pMDD would have increased levels of inflammation and impaired gut permeability, and that biomarkers of inflammation and gut permeability are correlated with biomarkers of iron status. We further assessed the history of ID diagnosis and treatment, considering that adolescents seeking medical care with depressive symptoms might get diagnosed with ID as part of routine care.

6.2 Participants and Methods

6.2.1 Study design

This study is an observational matched case-control study in 13–17-year-old adolescents with diagnosed pMDD and healthy controls. The adolescents were matched in a 1:1 ratio according to sex, age group (13 to <16 and 16 to <18 years) and education. The sample size calculation for this study was performed with G*Power V3.1.9.2. A logistic model, where the Children's Depression Rating Scale-Revised (CDRS-R) score was coded as a dichotomous variable in a model with 10 covariates (residual R^2=0.2) and one Standard deviation (SD) increase of the continuous predictor generated an Odds ratio (OR) of 1.5 and 2, was used as the base for a power calculation. According to these power calculations, to detect medium to large effect sizes for a type-I error of 5% ($\alpha = 0.05$) a sample size of 200 individuals with a 1:1 matching case-control ratio was sufficient (power >80%, $\beta \geq$20%). Up to a drop-out rate of 10%, these results seemed robust. To have a balanced study population group, we aimed to include 100 cases and 100 controls, with equal representation of sex, age groups, and education in cases and controls. In the first age group (13 to <16 years, the aim was to include 25 female and 25 male participants between attending lower secondary school level. In the second age group 16 to <18 years, we aimed to recruit 13 female and 13 male participants attending higher secondary school doing vocational education; and 13 female and 13 male participants attending higher secondary school doing the baccalaureat / vocational baccalaureat. The cases were then selected to match the recruited controls.

The ethics committee of the Canton of Zurich approved this study (BASEC-Nr. 2019-00717) and the study was registered at www.ClinicalTrials.gov (NCT04158869). The study was approved as an add-on study to the investigator-initiated clinical trial (SNSF 33IC30_166826, BASEC-Nr. 2016-02116). Written informed consent was obtained from all the caregivers and adolescents ≥14 years of age, and adolescents <14 years of age gave their oral assent before any research-related assessments were conducted.

6.2.2 Participants and procedures

Control group

The Laboratory of Human Nutrition at ETH Zurich, Switzerland, recruited the healthy controls for this study. From the canton of Zurich and surrounding German-speaking cantons of Switzerland, healthy female and male controls were recruited from September 2019 until December 2020. Schools, leisure time clubs, and social media were sites for recruiting the controls. Inclusion criteria for controls were no present nor past primary diagnosed psychiatric disorder according to the Mini-International Neuropsychiatric Interview for Children and Adolescents (M.I.N.I. KID) [330]; the age of

13 to <18 years; and no use of chronic medication. Adolescents were not eligible as controls if they reported pre-existing neurological or medical conditions likely to be a risk factor for developing depressive symptoms; if they took n-3 Polyunsaturated fatty acids (PUFA) supplements (providing >600 mg combined Eicosapentaenoic acid (EPA)/Docosahexaenoic acid (DHA)) for more than four weeks within the last six months; or if they were unable to follow the study procedures, for example due to language barriers. Once consent was given and the participants enrolled into the study, they electronically completed questionnaires on REDCap® (Research Electronic Data Capture) within two weeks prior to the physical data assessment at ETH Zurich.

pMDD group

The cases in this study were randomly selected from the participants of the Omega-3 Fatty Acid Paediatric Depression Trial (Omega-3 pMDD) under the lead of the Psychiatric University Hospital Zurich to match the controls. The Omega-3 pMDD protocol has been published before[331]. The recruitment for the omega-3 pMDD study took place at seven different in- and outpatient service centres in five German speaking cantons of Switzerland from May 2017 until June 2021. The information about the study reached the adolescents either via their clinician or via posters and flyers in one of the participating centres. When seeing the study on their own initiative, individuals contacted the study team themselves without the involvement of a clinician. Inclusion criteria for teenagers were age of 13 to <18 years; and a main diagnosis of MDD according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria [332] of at least moderate severity defined by a CDRS-R total score of ≥40 [333]. Adolescents were not eligible if they had a lifetime diagnosis of schizophrenia, bipolar affective disorder, substance use dependency, mental retardation, or pervasive development disorder; or if they fulfilled the diagnostic criteria for an eating disorder within the last 6 months. Also, cases were not eligible if they had pre-existing neurological or medical conditions, which were likely to cause their depressive symptoms. Furthermore, if cases were taking n-3 PUFA supplements (>600mg combined EPA/DHA) within the last 6 months; or if they or their families were unable to follow the study procedures, for example, due to language barriers, they were not eligible for the study. After consenting to the study, the screening interview was conducted with the adolescents in presence of a parent. In this screening interview, inclusion and exclusion criteria were assessed using the Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS) [334] for assessing the presence of MDD and the CDRS-R for assessing the severity of the depression. Within a few days, the lead-in interview took place, where the adolescent's depression score was assessed anew with the CDRS-R. At this point, participants started the lead-in phase of the intervention study which does not form part of this analysis. For this case-control study, only data (biological samples and CDRS-R scores) from the lead-in interview was used.

6.2.3 Data collection

The study team made every possible effort to align the study procedures between controls and cases as much as possible.

Assessment of anthropometry and socio-demographic information

For the cases and the controls weight (to the nearest 0.1 kg) and height (to the nearest 0.5 cm) was measured. Thereof, the Body mass index (BMI) was calculated as body weight in kilograms (kg) over the person's height in meters (m) squared (BMI=kg/m2). Further, BMI-for-age z-scores were calculated using the R package "anthroplus" provided by the WHO [335]. This data on BMI was then age-dependently categorized into four categories underweight, normal weight, overweight and obese according to the WHO's reference values [361]. Adolescents with a z-score at -1 SD coincide with adult underweight (BMI $<$18), adolescents with a z-score $+$1 SD coincide with adult overweight (BMI $=$ 25) and adolescents with a z-score $+$2 SD with adult obesity (BMI $=$ 30). Socio-economic and demographic data was assessed by self-reporting questionnaires which the participants were asked to fill out together with one parent.

Assessment of depression

We used the CDRS-R to assess adolescent's depression [333]. The CDRS-R, a semi-structured clinical interview which takes 15-20 minutes to administer, is one of the most frequently used rating scales for measuring the severity of depression and the change in depressive symptoms in children and adolescents with depression [60]. The validity of the scale has been shown for children [59] and adolescents [60]. The interview allows a comprehensive assessment by providing the possibility of conducting it with the child, the parents, and/or teacher. It covers 17 depressive symptom areas which are rated on 5 to 7 point Likert rating scales. The depressive symptom domains are aligned with the DSM-IV criteria for childhood depression [336] and include sleep disturbance, excessive fatigue, suicidal ideation, social withdrawal, etc. Participants are asked about information on 14 items. Further, three non-verbal symptoms, such as depressed facial affect, are rated only by the interviewer. The interviewers were trained to conduct the interview. Based on the individual ratings, a total score is calculated, which ranges between 17 and 113. For this study, scores from the interview conducted with the adolescent were used and a score of $\geq$40 was used as cut-off criteria for pMDD [337].

Questionnaire on iron deficiency history, iron treatment and iron supplementation

Participants were asked to fill out a questionnaire on the history of ID diagnosis, ID treatment, and iron supplementation. This questionnaire assessed time point of ID treatment/supplementation up to one year before study inclusion, based on the estimated effect duration of iron supplementation of one year [385]. For cases and controls, this questionnaire was introduced after recruitment and data assessment had already started. Therefore, for some participants this questionnaire was answered retrospectively to the inclusion and completion of the study.

Biochemical analysis

Blood samples were collected into EDTA coated tubes and serum tubes (for controls: BD Vacutainer, for cases: Sarstedt). For controls, Haemoglobin (Hb) was measured in whole blood using a Sysmex XE_5000 analyzer (Sysmex Corporation). Serum tubes were let stand for 60 minutes to allow clotting. Afterwards, the serum tubes were centrifuged, and the serum was then stored at -80°C until further analysis.

We measured iron status as serum Serum ferritin (SF) and Soluble transferrin receptor (sTfR) and inflammation as CRP and AGP using a multiplex immunoassay [386]. SF values were adjusted for inflammation using the BRINDA method using the R-package "BRINDA", applying "other" population groups as reference [382]. ID was defined as inflammation adjusted SF values $<$15 μg/L and/or sTfR $>$8.3 mg/L. Anaemia was defined according to the age and sex dependent WHO cut-off values for Hb [263]. For participants between 12 and 14 years of age and female participants 15 years of age and above, anaemia was defined as Hb $<$120 g/L. For male participants 15 years of age and above, anaemia was defined as Hb $<$130 g/L. IDA was defined as the combination of ID with anaemia. CRP $>$5mg/L and/or AGP $>$1g/L concentrations were defined to indicate inflammation [387]. Intestinal fatty acid binding protein (I-FABP) concentrations were measured using a commercially availableEnzyme-linked immunosorbent assay (ELISA) (Hycult Biotech, Uden, The Netherlands).

6.2.4 Data management and statistical methods

For the controls, the data capture was done either electronically using REDCap® or on paper and later entered to the REDCap® system. When captured on paper, the data was entered by the assessing person and later double checked for entry errors by a second member of the study team. REDCap® is an electronic data capture tool hosted at ETH Zurich and provides a secure, web-based software platform designed for supporting data capture in research studies [341, 342]. For the cases, data was assessed on paper and then entered to the electronical data capture tool secuTRIAL

by two individual persons. Afterwards, every data entry was checked by a third person for entry errors. Once cases and controls were matched, study data was managed using REDCap®.

Data processing and statistical analysis of data were performed using R Version 3.6.0 [311]. To test for outliers and normality Q-Q plots, histograms, and the Shapiro-Wilk test were used. Normally distributed data and non-normally distributed data were expressed as mean ($\pm$SD) and as medians (Interquartile range (IQR)), respectively. For testing not normally distributed continuous data, Wilcoxon rank sum test was applied, and t-test for normally distributed data. Chi-square tests were applied to assess significant differences when the expected cell counts was $\geq$5, and Fisher's exact test when the expected cell count was $<$5. For producing tables and calculating these differences, the R package "gtsummary" was used. This R package has been shown to produce reproducible summary tables within R [343]. For correlations between two non-normally distributed variables, Spearman's correlation coefficient was calculated. Further, to assess associations of different iron status parameters with depression (CDRS-score $\geq$40), multivariate logistic regression analysis was applied. In these models we used the matching criteria sex, age, education, and BMI-for-age z-scores as covariates.

6.3 Results

A total of 98 controls were enrolled into this case-control study after the recruitment process. Thereof, two individuals dropped out, either by not providing a blood sample or voluntarily after the screening interview. A total of 173 participants completed the Omega-3 pMDD study and were eligible as cases for this case-control study. For one control no match within the cases could be found. Consequently, a total of 95 controls were matched to 95 cases (total n=190) according to sex, age group and education. A detailed overview of the study inclusion process is given in Figure 6.1. For four adolescent pairs in the age group of 16 to $<$18 years of age, the matching was done according to sex and age group since there was no match by education.

Table 6.1 provides a detailed overview of the study participant's characteristics. The successful matching between cases and controls is shown by no significant difference in age, sex, and education. Furthermore, BMI-for-age z-scores were comparable between the groups. CDRS-R scores were significantly higher among the cases compared to the controls ($p<0.001$). A slightly higher number of adolescents among the cases had an East-Asian descent compared to controls ($p=0.051$).

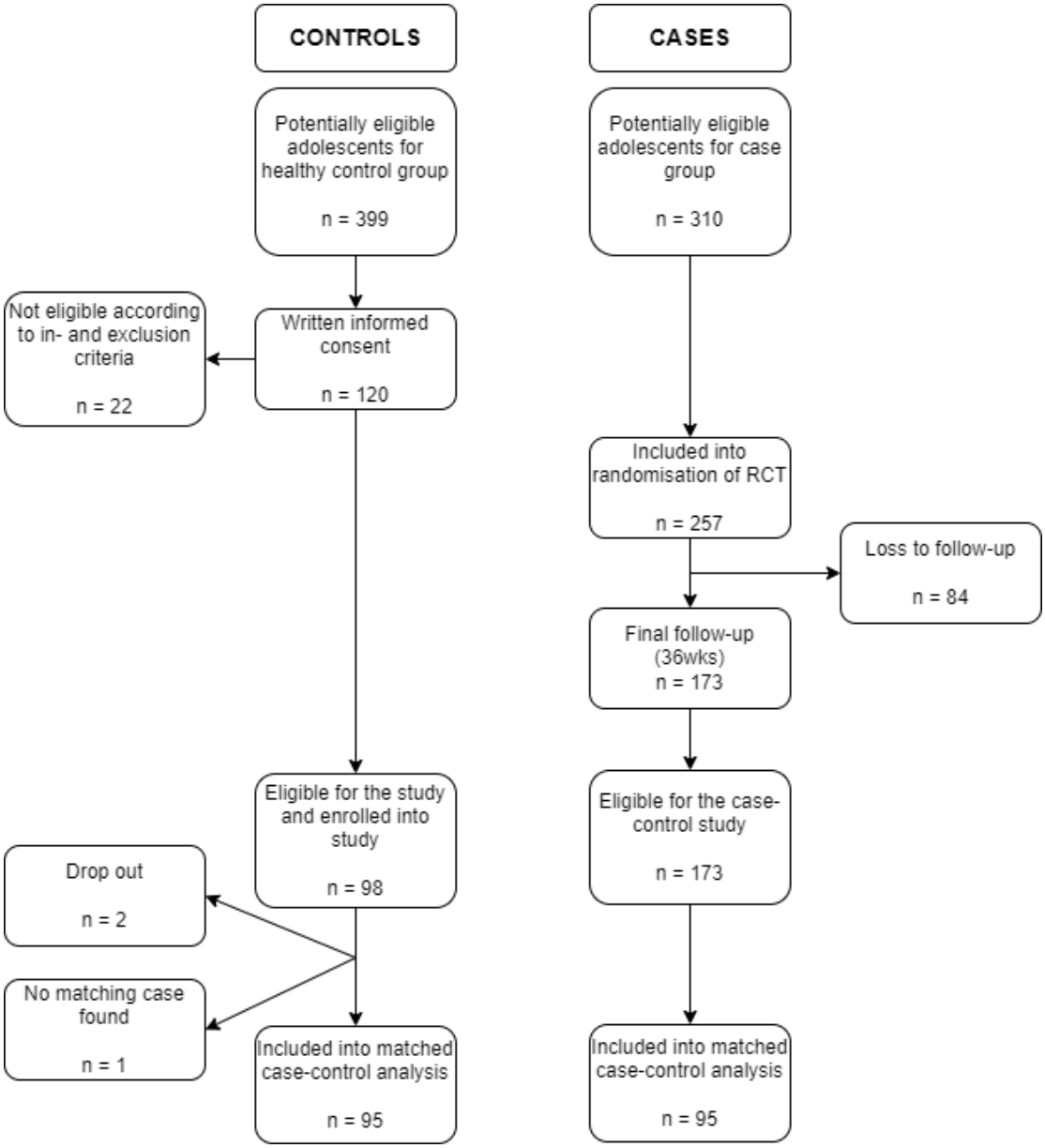

Figure 6.1: Flow chart of adolescents with and without diagnosed Paediatric major depressive disorder (pMDD) included in the analysis of this case-control study: RCT: Randomised Control Trial

Table 6.1: Characteristics of Swiss adolescents with (n=95) and without (n=95) Paediatric major depressive disorder (pMDD)

	Overall	Cases	Controls	p-value
Age	16.1 (14.9, 17.1)	16.1 (14.9, 17.2)	16.0 (14.9, 17.1)	0.8[2]
Sex				>0.9[3]
Female	110 (58%)	55 (58%)	55 (58%)	
Male	80 (42%)	40 (42%)	40 (42%)	
CDRS-R score	36 (18, 56)	56 (50, 62)	18 (17, 20)	**<0.001[2]**
BMI-for-age z-score[1]	0.20 ± 1.03	0.25 ± 1.05	0.16 ± 1.02	0.4[2]
BMI category[1]				0.5[4]
Underweight	7 (4%)	2 (2%)	5 (5%)	
Normal weight	138 (76%)	65 (75%)	73 (77%)	
Overweight	20 (11%)	12 (14%)	8 (8%)	
Obese	17 (9%)	8 (9%)	9 (10%)	
Ethnicity				0.051[4]
European	174 (92%)	87 (92%)	87 (92%)	
East-Asian	5 (3%)	5 (5%)	0 (0%)	
Indian-Asian	2 (1%)	0 (0%)	2 (2%)	
Middle East	2 (1%)	1 (1%)	1 (1%)	
Not declared	7 (4%)	2 (2%)	5 (5%)	
Swiss education				0.8[3]
Lower secondary school (Mandatory school)	104 (55%)	54 (57%)	50 (53%)	
Upper secondary school				
Vocational education	28 (15%)	14 (15%)	14 (15%)	
Baccalaureat/ vocational baccalaureat	58 (31%)	27 (28%)	31 (33%)	

Median (IQR); mean ±SD

CDRS-R: Children's Depression Rating Scale-Revised

[1] Data on Body mass index (BMI) was not available for all participants (n_{cases}= 87; $n_{controls}$=95), BMI-for-age z-scores and BMI categories were defined according to World Health Organisation (WHO) reference data (Adolescents with a z-score at -1 SD coincide with adult underweight (BMI <18), adolescents with a z-score +1 SD coincide with adult overweight (BMI = 25) and adolescents with a z-score +2 SD with adult obesity (BMI = 30))

[2] Wilcoxon rank sum test

[3] Pearson's Chi-squared test

[4] Fisher's exact test

A detailed description of the iron and inflammatory parameters is given in Table 6.2. There was no significant difference in SF concentrations between cases and controls (median (IQR): 31.2 (20.2, 57.0) μg/L; 32.5 (22.6, 48.3) μg/L, p=0.4). There was a trend for higher Body iron stores (BIS) among the cases compared to the controls (mean $\pm$SD: cases: 5.73$\pm$3.31 mg/kg; controls: 5.00$\pm$2.92 mg/kg, p=0.083). However, sTfR concentrations were significantly lower among cases compared to controls (4.50 (4.00, 5.50) mg/L; 5.20 (4.75, 6.10) mg/L, p<0.001). The inflammatory markers CRP, AGP, and I-FABP were significantly higher among cases compared to controls (p=0.003; p=0.024; and p=0.047). There was no significant correlations between SF or BIS and CRP, AGP, or I-FABP in the combined sample of adolescents with and without pMDD (all p>0.3) or within the groups separately (all p>0.4). There was a significant positive correlation between sTfR and AGP (ρ=0.15, p=0.044) and a positive trend between sTfR and CRP (ρ=0.13, p=0.073) in the combined sample of adolescents with and without pMDD. For the groups separately, there was a positive correlation between sTfR and CRP among the cases (ρ=0.31, p=0.002) but not among the controls (p>0.3). Also, there was a positive correlation between sTfR and AGP among the cases (ρ=0.24, p=0.017), and a positive trend between sTfR and AGP among the controls (ρ=0.18, p=0.077). No correlations between sTfR and I-FABP in the combined samples of adolescents with and without pMDD (p=0.5) nor within the groups separately (both p>0.4) could be observed. The prevalence of ID among cases (19%) and controls (18%) was comparable (p=0.7).

Data from the self-reported questionnaire on history of ID diagnosis and iron treatment is displayed in Table 6.3. The questionnaire was returned by 61 cases and 92 controls, which resulted in a response rate of 64% among cases and 97% among controls. Significantly more cases have been ever diagnosed with ID compared to controls (44% vs 26%, p=0.02). Further, the prevalence of participants being under iron treatment at study inclusion was higher for the cases with 16% compared to controls with 4% (p = 0.2).

A summary of the multivariate logistic regression models on the associations of iron status parameters and inflammatory markers with pMDD is displayed in Table 6.4. Higher sTfR concentrations were associated with lower odds for depression (OR=0.68 [0.53-0.86], p=0.002). Higher BIS were associated with higher odds for depression (OR=1.12 [1.00-1.25], p=0.045). Finally, higher AGP concentrations were associated with higher odds for depression (OR=6.94 [1.59-34.00], p=0.013).

Table 6.2: Summary of iron and inflammatory parameters for adolescents with (n=95) and without (n=95) Paediatric major depressive disorder (pMDD)

Characteristic	Overall	Cases	Controls	p-value
SF [μg/L]	35.6 (21.4, 52.8)	37.2 (20.2, 57.0)	32.5 (22.6, 48.3)	0.4[2]
Adjusted SF [μg/L]	32.5 (20.0, 46.2)	32.7 (19.1, 53.1)	30.8 (21.1, 45.0)	0.6[2]
sTfR [mg/L]	4.99 (4.25, 5.76)	4.50 (4.00, 5.50)	5.20 (4.75, 6.10)	**<0.001**[2]
Adjusted sTfR [mg/L]	4.72 (4.05, 5.46)	4.20 (3.79, 5.00)	4.94 (4.48, 5.92)	**<0.001**[2]
Body Iron Stores (BIS) [mg/kg]	5.36 $\pm$ 3.13	5.73 $\pm$3.31	5.00 $\pm$ 2.92	0.083[2]
Haemoglobin [g/L]$^{+}$	140.7 $\pm$ 12.5	140.6 $\pm$ 13.8	140.8 $\pm$ 11.2	0.8[2]
CRP [mg/L]	0.10 (0.02, 0.35)	0.16 (0.03, 0.43)	0.04 (0.02, 0.30)	**0.003**[2]
AGP [g/L]	0.57 (0.44, 0.70)	0.60 (0.47, 0.74)	0.52 (0.41, 0.67)	**0.024**[2]
I-FABP [pg/mL]	267 (168, 448)	307 (17, 515)	232 (163, 357)	**0.047**[2]
Iron deficiency (ID)$^{\&}$				0.7[3]
No ID	153 (81%)	76 (80%)	77 (81%)	
Present ID	37 (19%)	19 (20%)	18 (19%)	
Anaemia (A)$^{+\%}$				0.3[4]
No A	179 (96%)	86 (93%)	93 (98%)	
Mild A	6 (3%)	4 (4%)	2 (2%)	
Moderate A	2 (1%)	2 (2%)	0 (0%)	
Iron deficiency anaemia (IDA)$^{+}$				0.4[4]
No IDA	182 (97%)	89 (96%)	94 (99%)	
IDA	5 (3%)	4 (4%)	1 (1%)	
Inflammatory status$^{\circ}$				0.4[3]
No Inflammation	175 (92%)	86 (91%)	89 (94%)	
Inflammation	15 (8%)	9 (10%)	6 (6%)	

Median (IQR); mean $\pm$SD; n(%)

AGP: alpha-1-acid-glycoprotein; CRP: C-reative protein; ID: iron deficiency; IDA: iron deficiency anaemia; I-FABP: Intestinal fatty acid binding protein; SD: standard deviation; SF: Serum ferritin; sTfR: soluble transferrin receptor

$^{+}$ Data on haemoglobin was not available for all participants (n_{cases}=92; $n_{controls}$=95)

$^{\&}$ ID was defined as adjusted SF <15 μg/L and/or elevated sTfR >8.3 mg/L

$^{\%}$ Anaemia was defined according to age and sex specific World Health Organisation (WHO) cut-off values (displayed in section 6.2.3)

$^{\circ}$ Inflammation was defined as CRP >5mg/L and/or AGP >1g/L

[2] Wilcoxon rank sum test

[3] Pearson's Chi-squared test

[4] Fisher's exact test

Table 6.3: Data on Iron deficiency (ID) diagnosis and iron treatment in adolescents with (n=61) and without (n=92) Paediatric major depressive disorder (pMDD) based on a self-reported questionnaire assessing this information up to one year before study inclusion

Characteristic	Overall	Cases	Controls	p-value
Ever diagnosed ID				**0.02**[1]
No	102 (67%)	34 (56%)	68 (74%)	
Yes	51 (33%)	27 (44%)	24 (26%)	
Treatment of ID				0.6[2]
No diagnosis of ID	102 (67%)	34 (56%)	68 (74%)	
Diagnosis of ID, no treatment	3 (2%)	2 (3%)	1 (1%)	
Diagnosis of ID, treatment with infusion	6 (4%)	2 (3%)	4 (4%)	
Diagnosis of ID, treatment with tablets/sirup	40 (26%)	21 (34%)	19 (21%)	
Diagnosis of ID, treatment else	2 (1%)	2 (3%)	0 (0%)	
Supplementation with iron				0.2[2]
No intake of iron	105 (69%)	37 (61%)	68 (74%)	
Yes, at inclusion	14 (9%)	10 (16%)	4 (4%)	
Yes, <6 months before incl.	11 (7%)	5 (8%)	6 (7%)	
Yes, approx. 1 year before incl.	5 (3%)	2 (3%)	3 (3%)	
Yes, >1 year before incl.	15 (10%)	6 (10%)	9 (10%)	
Yes, can't remember when	3 (2%)	1 (2%)	2 (2%)	
Duration of iron supplementation				0.7[2]
No intake of iron	109 (71%)	38 (62%)	71 (77%)	
<1 month	9 (6%)	4 (7%)	5 (5%)	
approx. 1 month	4 (3%)	1 (2%)	3 (3%)	
approx. 3 months	17 (11%)	11 (18%)	6 (7%)	
>3 months	8 (5%)	4 (7%)	4 (4%)	
Can't remember	6 (4%)	3 (5%)	3 (3%)	
Number of iron infusions				>0.9[2]
No iron infusions	145 (94%)	57 (93%)	88 (96%)	
1	3 (2%)	2 (3%)	1 (1%)	
2	2 (1%)	1 (2%)	1 (1%)	
3	1 (1%)	0 (0%)	1 (1%)	
>3	1 (1%)	0 (0%)	1 (1%)	
Can't remember	1 (1%)	1 (2%)	0 (0%)	

n (%) ; ID: Iron deficiency

[1] Pearson's Chi-squared test

[2] Fisher's exact test

Table 6.4: Multivariate logistic regression models assessing associations of different iron status and inflammation parameters with Paediatric major depressive disorder (pMDD) based on scores of the Children's Depression Rating Scale-Revised (CDRS-R)

	Odds ratio (OR)	95% CI OR lower	95% CI OR upper	p-value
Iron status parameters				
SF	1.01	1.00	1.02	0.066
sTfR	0.68	0.53	0.86	**0.002**
BIS	1.12	1.00	1.25	**0.045**
Inflammation parameters				
CRP	0.97	0.83	1.10	0.6
AGP	6.94	1.59	34.0	**0.013**
I-FABP	1.00	1.00	1.00	0.7

BIS: Body iron stores; SF: serum ferritin; sTfR: soluble transferrin receptor; AGP: alpha-1-acid-glycoprotein; CRP: C-reactive protein; I-FABP: Intestinal fatty acid binding protein; CI: Confidence Interval; OR: Odds ratio
The dependent variable was the diagnosis of depression (CDRS-R $\geq$40). The independent variables were iron status indicators or inflammation parameters. All models were controlled for sex, age, education, and BMI-for-age z-scores.

6.4 Discussion

This well controlled, matched case-control study in Swiss adolescents with and without pMDD did not confirm our hypothesis of depressed adolescents having a lower iron status than their healthy counterparts. While there was no difference in SF concentrations and BIS between cases and controls, cases had significantly lower sTfR concentrations compared to controls, indicating that adolescents with pMDD have a better iron status. We found significantly higher concentrations of the inflammatory markers CRP and AGP, as well as the marker of intestinal permeability I-FABP in cases compared to controls, confirming our hypothesis of more prevalent inflammation among pMDD compared to controls. However, only AGP correlated with sTfR concentrations. Furthermore, compared to the controls, a significantly higher proportion of cases was ever diagnosed with ID and was taking iron supplements at the timepoint of assessment. This may explain the better iron status observed in cases compared to controls.

Previous studies have found low iron status to be associated with depression [284, 376, 377, 388], but overall the evidence is limited and ambiguous [287, 389]. Specifically lower SF concentrations have been associated with depression in adult Iranian students [284], in English elderly persons (>65 years of age) [376], and in middle-age male Japanese workers [377]. On the other hand, lower SF concentrations in depressed populations could not be confirmed in elderly people (65-83 years) in Germany [287] or in Chinese women after giving birth [389]. Also, for adolescents there is limited and inconsistent data on associations between iron status parameters and depressive disorders. In Mexican adolescents, female participants (n=403) aged 12-20 years with ID had greater odds for being "likely depressed" (OR=2.01) or "highly likely depressed" (OR=2.80) [388]. In New Zealand higher BIS in males were associated with greater depressive symptoms while this could not be shown in females [390]. On the other hand, adolescents with heavy menstrual bleeding and ID or IDA in Michigan, US, were not more likely to be depressed in comparison with adolescents without ID or IDA [391]. Similarly, no associations between internalizing (depression) problems in 6 year old Spanish children and iron status could be found [392]. In this case-control study, we found significantly lower sTfR concentrations in cases compared to controls. Elevated sTfR concentrations in absence of other conditions causing erythroid hyperplasia, usually indicates ID [393]. Also, there was a trend for higher BIS stores among the cases compared to the controls and higher odds for depression with higher BIS. Therefore, against our hypothesis, our results point towards a slightly better iron status among cases compared to controls. Furthermore, we found no difference in iron status based on SF concentrations and the prevalence of ID was around 19% in both groups. However, the better iron status in depressed adolescents compared to health controls based on sTfR and BIS, and the lack of difference between cases and controls based on SF might be attributed to the differences in ID diagnosis

history and treatment.

Symptoms of ID and depression can manifest in similar ways such as fatigue, poor school performance, decreased cognitive abilities, and behavioural problems [282, 283]. We therefore investigated the history of ID diagnosis as well as iron treatment prior to and at study inclusion. Indeed, we found that adolescents with pMDD were four times as likely to be under iron treatment at study inclusion compared to healthy controls. Furthermore, the prevalence of ever being diagnosed with ID was markedly higher in cases (44%) compared to controls (26%). Effects of iron treatment/supplementation are detectable up to one year after administration [385]. Thus, even though the response rate to the questionnaire was only 64% in cases compared to 97% in controls, these findings indicate that ID in cases may have been masked by recent or ongoing iron treatment and may also explain the better iron status based on sTfR. Thus, future research investigating associations between iron status and depression should assess and control for iron treatment/supplementation up to one year before data assessment.

There are two ways in which depression-associated inflammation is of interest in the current analysis. On one hand, measurements of iron status, particularly SF, are prone to bias due to inflammation. SF is an APR, and therefore its concentration is elevated in response to inflammation [252], which may mask ID. On the other hand, inflammation can decrease intestinal iron absorption, mediated by an upregulation of hepcidin, and therefore increase the risk for ID. We have thus determined the inflammatory markers CRP, AGP as well as I-FABP (marker of intestinal permeability) to better understand the interrelations between iron status, depression, and inflammation. Our results support the inflammatory theory of depression [9]. Although the cases did not show clinically significant signs of infection, their CRP and AGP concentrations were significantly elevated in comparison to those of controls, indicating a state of subclinical systemic inflammation. Also, higher AGP concentrations were associated with higher odds for pMDD. However, this increased inflammatory state did not seem to influence SF or BIS measures in a significant way, as these parameters did not correlate with CRP, AGP or I-FABP. In contrast, we found correlations between sTfR and AGP and CRP concentrations. In addition to the markers of systemic inflammation, also I-FABP, an indicator of intestinal permeability, was significantly higher among the cases compared to the controls, indicating reduced gut integrity. This is in line with previous data where elevated I-FABP concentrations were found among Spanish adult MDD patients [394] and adult MDD patients in the US [395]. Thus, taken together, our data might support the evidence for stress-induced fragilization of the Blood-Brain-Barrier (BBB) and gut barriers [180], finally leading to increased inflammatory markers within the Central nervous system (CNS), which has ultimately been linked to depression [9].

Our study has several strengths and limitations. An important strength of this study is our thoroughly matched case-control study design. Also, the inclusion of the cases was based on a clinical diagnosis of pMDD of moderate to severe depressive symptoms. Next, we assessed the severity of the depressive symptoms with a comprehensive interviewer administered assessment which included non-verbal items. However, despite the strengths of the study design, due to its observational nature it does not allow to draw causal conclusions. A major limitation of this study is the limited response rate to the questionnaire on the history of ID diagnosis and iron treatment/supplementation among the cases. This can be explained by the way the data were collected: Our questionnaire on the history of ID diagnosis and treatment was not part of the original Omega-3 pMDD intervention trial and was only introduced retrospectively for a large proportion of the cases as well as some of the controls. Some of the cases were asked to complete the questionnaire up to four years after inclusion into the study, which led to a low response rate and potential misreporting. Nevertheless, we found cases to be significantly more likely to be under iron treatment/supplementation at study inclusion compared to healthy controls. However, we were not able to determine whether previous iron treatment was a confounder in our models determining the associations between iron status and depression.

To conclude, our findings do not support the hypothesis of depression being linked to lower iron status. However, as iron supplementation was more prevalent among cases compared to controls, we cannot rule out that this may have influenced our findings. On the other hand, our data confirms the inflammatory theory of depression, showing both increased systemic inflammation and intestinal permeability among cases compared to controls. Future studies investigating the associations of iron status and depression, should consider the higher likeliness for iron treatment in depressed individuals.

Conflict of Interest

All authors declare that they have no conflict of interest.

Funding

The Omega-3 pMDD study was supported by the Swiss National Science Foundation. EO received a PhD fellowship from the Ebnet Foundation, Teufen, Switzerland.

The omega-3 study team

The Omega-3 Study Team contributed with implementation of the design with following roles: Sponsor-investigator of the trial is GB. Chief investigators are SW and KS. IH is study coordinator. Principal investigators and research psychologist from the clinical sites are as follows: Principal Investigator Zurich: SW; Research psychologists: Noemi Baumgartner, Sophie Emery, Mona Albermann, and Kristin Nalani (Department of Child and Adolescent Psychiatry, University Hospital of Zurich); Principal Investigator Basel: KS; Investigators and research psychologists: Oliver Pick, Alain Di Gallo, and Michael Strumberger (Department of Child and Adolescent Psychiatry, Psychiatric University Hospitals Basel); Principal Investigator Basel-Stadt: Brigitte Contin; Investigator: Stefan Müller (Child and Adolescent Psychiatric Services Basel-land); Principal Investigator: Silke Bachmann; Investigators: Lars Wöckel, and Simone Heitzer (Clienia Littenheid); Principal Investigator: Bruno Rhiner; Investigators: Amir Yamini (Child and Adolescent Psychiatric Services Thurgau); Principal Investigator: Suzanne Erb; Investigators: Michael Schmid (Child and Adolescent Psychiatric Services St. Gallen); Principal Investigator: Ulrich Müller-Knapp; Investigator: Ioannis Christodoulakis (Klinik Sonnenhof). UH and Burkhardt Seifert (retired) are statistical consultants. Renate Drechsler is head of the neuropsychology department and Edna Grünblatt head of the department for translational molecular psychiatry (Department of Child and Adolescent Psychiatry, University Hospital of Zurich). Martin Hersberger is head of the division of Clinical Chemistry and Biochemistry at the University Children's Hospital Zürichand his PhD student Ivan Hartling of the division of Clinical Chemistry and Biochemistry who will analyse the bioactive lipids; Romuald Brunner (University of Heidelberg), Jürgen Drewe (University of Basel), and Julia Braun (Epidemiology, Biostatistics, and Prevention Institute, University of Zürich) are members of the Data Monitoring Committee. Jenny Peterson, Clinical Trials Pharmacy (Kantonsapotheke) Zürich, responsible for the packaging, handling, and quality of the study medication.

Acknowledgements

We would like to give our sincerest thank the following people for their valuable contribution to this case-control study: all the adolescents participating in this study; for their support in laboratory analyses: T Christ, A Krzystek, C Zeder; for their support in study management and data assessment: A Zacher, D Salinas, S Pleus, S Probst, Msc students PUK; and all the the study nurses for their contributions.

Author contributions

Conceptualization of current case-control analysis: EO, IHA, JB. Conceptualization and execution of Omega-3 pMDD study: IH, GB, KS, UH, SW, Omega-3 study team. Recruitment of controls (including data collection): EO, AZ, DS, SP, SP, OW. Data analysis: EO, IHA, JB. Writing of original draft: EO, IHA, JB. Writing (review & editing): EO, IHA, OW, SE, MA, NB, KS, SW, MS, MBZ, IH, GB, JB

7 Discussion and conclusions

This PhD project aimed to investigate the associations of n-3 Polyunsaturated fatty acids (PUFA), iodine, and iron with paediatric and perinatal depressive disorders. This thesis comprises two research studies: a data analysis within the Nutrition during pregnancy and early development (NuPED) study; and a case-control study in adolescents with and without Paediatric major depressive disorder (pMDD). The first study, the NuPED study, is a prospective cohort study following 242 South African women from birth until 12 months postpartum. It is the basis of **Manuscript 1**, where the aim was to investigate the associations of n-3 PUFA at <18 weeks gestation with perinatal depression at different time points throughout pregnancy until 12 months postpartum. The second study is a matched case-control study in 190 Swiss adolescents with and without diagnosed pMDD and is the basis for **Manuscript 2, 3, and 4**. **Manuscript 2** investigated associations of n-3 PUFA status and intake with pMDD. **Manuscript 3** investigated the relationship between iodine status, thyroid functions and pMDD. Finally, **Manuscript 4** investigated associations of iron status with pMDD.

This chapter will start by summarising this thesis' main findings and discuss them in the context of existing literature. Next, general methodological considerations will be discussed. Finally, this chapter will suggest future research topics within the field of nutritional psychiatry based on this thesis' findings.

7.1 Main findings

Evidence from the last years suggests a role of diet and nutrients in the aetiology and symptom management of Major depressive disorder (MDD) [44], a leading cause of disability worldwide [396]. The link between depression and modifiable nutritional risk factors is of particular interest in vulnerable groups such as adolescents and pregnant women, due to their increased nutrient requirements as well as increased risk for developing depression during their respective life stage. The following paragraphs will summarise first the findings of the NuPED cohort study in South African pregnant women, followed by a summary of the case-control study in Swiss adolescents with and without pMDD.

Perinatal depression can negatively affect the mother's and her offspring's health [64, 66]. Since n-3 PUFA have been suggested to play a role in the depression's aetiology [156], associations between n-3 PUFA status during early pregnancy and perinatal depression were assessed within the NuPED study, following 242 women throughout pregnancy and up to 12 months postpartum. The women's depression was assessed at $<$18, 22, and 36 weeks gestation and at 6 and 12 months postpartum using the Edinburgh Postnatal Depression Scale (EPDS). The Red blood cell (RBC) total Fatty acids (FA) composition at $<$18 weeks gestation was analysed. RBC Eicosapentaenoic acid (EPA) status was negatively, and the Arachidonic acid (AA)/EPA ratio was positively associated with EPDS scores at 12 months postpartum. Further, higher RBC Docosahexaenoic acid (DHA) and n-3 index were associated with lower odds for depression, while higher n-6/n-3 PUFA and AA/EPA ratios early in pregnancy were associated with higher odds for depression at 12 months postpartum. Overall, results suggest that women with a higher RBC n-3 PUFA status during early pregnancy may be at lower risk for depression at 12 months postpartum.

Depression most often emerges in adolescence [42]. There is evidence that adolescents have poorer eating habits with respect to diet quality compared to adults [47]. Thus, studying the associations between nutrients and depression could be particularly interesting in this age group. A matched case-control study in 95 adolescents with diagnosed pMDD and 95 healthy controls aged 13-17 was conducted to assess associations of n-3 PUFA, iodine, and iron status among Swiss adolescents with pMDD. The Children's Depression Rating Scale-Revised (CDRS-R) was used to assess depression and the Perceived stress scale (PSS) to assess the adolescent's perceived stress. RBC's total FA composition was analysed, and n-3 PUFA intake was assessed using a focused Food frequency questionnaire (FFQ). Iodine status was determined by measuring Urinary iodine concentration (UIC) and thyroid status by measuring Thyroid stimulating hormone (TSH) and Thyroxine (T4) in serum. Iron status (Serum ferritin (SF), Soluble transferrin receptor (sTfR)) and inflammation (C-reactive protein (CRP), α-1-acid-glycoprotein (AGP)) were assessed using a multiplex immunoassay. Further, gut inflammation was assessed by measuring Intestinal fatty acid binding protein (I-FABP) using a commercially available Enzyme-linked immunosorbent assay (ELISA). Finally, the history of Iron deficiency (ID) diagnosis and treatment was assessed with a self-reported questionnaire.

Overall, mean RBC EPA, DHA, and subsequently n-3 index were lower among cases than controls. Also, the n-6/n-3 PUFA ratio was higher in cases than controls. Higher RBC EPA and DHA were associated with lower odds for depression. EPA and DHA intake correlated with RBC EPA and DHA status, respectively. However, α-linoleic acid, 18:3n-3 (ALA), EPA and DHA intake did not differ significantly between cases and controls. These results suggest that a higher RBC n-3 PUFA status in Swiss adolescents may be associated with a lower risk for pMDD. Also, the lower n-3 PUFA status in adolescents with pMDD compared to healthy counterparts could not be

explained by differences in n-3 PUFA intake. Generally, 20% of adolescents had a very low n-3 index (n-3 index <4%), and 81% of adolescents had a low n-3 index (n-3 index <6%). Also, an n-3 index >6% was associated with lower odds for depression. The observed very low and low (<4% and <6%) n-3 index among the participating adolescents is alarming and highlights the need for public health measures to increase n-3 PUFA status among adolescents.

The median UIC did not differ between cases and controls, and indicated adequate iodine nutrition among the adolescents. However, significantly lower TSH and T4 concentrations were found in cases compared to the controls. The prevalence of hypothyroxinaemia (characterised by low T4 and normal TSH levels) was higher among the cases than among the controls (15% vs 4%). These results suggest iodine nutrition to be adequate and not being different between adolescents with and without pMDD. The observed increased prevalence of hypothyroxinaemia among cases compared to controls might therefore be unrelated to iodine status. Potential risk factors for hypothyroxinaemia could involve abnormal lipid profiles, poor iron status, and antidepressant treatment.

No difference in SF levels among cases compared to controls could be observed. However, there were significantly lower sTfR concentrations among cases than in controls and a trend for higher Body iron stores (BIS) among cases compared to controls. CRP, AGP, and I-FABP concentrations were significantly higher among cases compared to controls. Furthermore, a total of 44% cases reported ever being diagnosed with ID, compared to 26% of controls. Finally, 16% of cases took iron supplementation at study inclusion compared to 4% among the controls. Overall, no difference in SF between adolescents with and without pMDD could be observed. However, higher sTfR concentrations among controls and higher BIS concentrations among cases indicate a better iron status among adolescents with depression compared to controls. This might be partly explained by higher proportion of cases taking iron supplements at study inclusion compared to controls. Also, the cases had significantly higher systemic inflammatory markers than the controls. However, whether increased systemic inflammation among cases is related to higher rate of ID diagnosis and iron treatment among adolescents with pMDD remains unclear.

For this thesis, we expected to gain better insight into the associations between nutritional status and depression by focusing on adolescents and pregnant women as vulnerable groups to poor nutrient status. Indeed, the findings concerning n-3 PUFA status were similar among pregnant women and adolescents. Therefore, the role of n-3 PUFA status within depression might be similar in these two populations. According to our results, iodine nutrition seems unrelated to the Hypothalamic-pituitary-thyroid (HPT) axis alterations observed in depressed patients. However, possible risk factors for hypothyroxinaemia such deficiencies in other nutrients, abnormal lipid profiles and anti-depressant treatment require further research. Finally, for iron research

within depression, our results suggest controlling for iron treatment to avoid possible differences in SF status being masked by past or current iron treatment. Overall, the higher proportion of ID diagnosis and treatment among adolescents with pMDD indicate a possible bi-directional relationship between ID and pMDD.

7.1.1 Main findings in the context of theories on the aetiology of depressive disorders

The literature provides strong and diverse evidence for biochemical alterations being involved in the aetiology of depressive disorders. In section 2.1.3 different theories behind the aetiology of depressive disorders have been summarised within four fields of brain health: (1) neurochemical processes; (2) plasticity, structure and neurogenesis; (3) neuroendocrine processes; and (4) immune system and inflammation. In sections 2.2.3, 2.3.3, 2.4.3 n-3 PUFA, iodine, and iron have been discussed in the context of their role in the aetiology of depression. Thus, this section will discuss this PhD project's main findings in the context of the named fields of brain health.

Firstly, n-3 PUFA, iodine, and iron play important roles in brain development by being involved in processes such as myelination, neurotransmission, or synaptic plasticity. Alterations in brain structure and function have been associated with depression before [4]. However, for now, alterations in brain structure and function due to nutrient deficiency during critical periods of neuronal development have been shown to be irreversible even after restoring nutrient sufficient conditions [168, 281, 290]. Thus, the question arises to what extent treatment or symptom management of depression after critical periods of neuronal development can be influenced by the modifiable diet. Depression seems to be a multifactorial disease with many different risk factors such as socio-demographic causes, adverse childhood experiences, hereditary biological factors, and physical disease (section 2.1) [16–18]. Structural changes caused by nutrient deficiency in critical periods of neuronal development might remain irreversible after modifying the habitual diet or supplementing nutrients. Nevertheless, the overall risk for depression could be decreased by reducing the biological risk factors for depression through nutritional interventions. Therefore, a better understanding of the biological pathophysiology and the nutrients' role in depression could contribute to the prevention of depressive disorders and even an individually tailored treatment, especially for treatment-resistant depression.

The results from both studies conducted within this PhD project show n-3 PUFA being associated with paediatric and perinatal depression. Therefore these studies support the evidence of n-3 PUFA being involved in the aetiology of depression, especially in the field of (4) immune system and inflammation having a possible impact on the (1) neurochemical processes, and (2) plasticity, structure and neurogenesis. Further, we have seen associations of thyroid status with paediatric depression unrelated to iodine

status. Thus, we provide evidence for the HPT axis being involved in the aetiology of depression through (3) neuroendocrine processes. However, these alterations seem to be unrelated to iodine's nutritional status. Further, even though we might not have observed associations between iron status and depression, such an association could have been masked by the higher proportion of depressed adolescents being diagnosed with ID and receiving iron supplements compared to the controls. Finally, when investigating in the context of iron status, we found increased systemic and gut inflammation among depressed patients compared to the controls, supporting the evidence on (4) the immune system's and inflammation's role in the aetiology of depression. However, intervention studies will need to follow observational findings to conclude mechanistic pathways behind peripheral and central changes in the nutritional status being involved in depressive disorders. Suggestions for future research will be provided in section 7.2.1.

7.1.2 Potential relevance of main findings for public health and clinics

Based on the results of this PhD thesis, suggestions concerning the single nutrients can be formulated, which could be translated into public health measures addressing the prevention of depression among women throughout pregnancy and adolescents.

Overall, the n-3 index among South African pregnant women and Swiss adolescents was $<6\%$. In both study populations, the recommended n-3 index of $>6\%$ for healthy cognitive development [327] and a n-3 index $>8\%$ for low risk of Coronary heart disease (CHD) [140] was not met (see sec. 2.2.2). While an n-3 index $>6\%$ was associated with lower odds for depression among Swiss adolescents, no associations between an n-3 index of $>8\%$ and depression could be found. This finding might suggest that an n-3 index of $>8\%$ might not be more beneficial than an n-3 index of $>6\%$ in the context of depression prevention. Therefore, our results could contribute to a general recommendation for an n-3 index $>6\%$ in preventing depressive disorders and ensuring healthy cognitive development [327]. An adequate iodine status among Swiss adolescents could be observed. This thesis' case-control study was not designed to assess the iodine status of a representative Swiss population. Nevertheless, these results suggest that the Swiss government's monitoring and salt iodisation programs effectively ensure adequate iodine nutrition among Swiss adolescents, which should therefore be preserved. Finally, the iron status among Swiss adolescents with pMDD was found to be better compared to healthy counterparts, suggesting appropriate medicinal and nutritional monitoring of patients in psychiatric care. Thus, the increase of the n-3 index as a significant public health urge is left to be addressed in South African pregnant women and the general Swiss adolescent population.

7.1.3 Methodological considerations

Both studies conducted within this PhD project were observational, which therefore does not allow drawing causal conclusions on nutrients' relationship with depressive disorders. A significant strength of the NuPED study is its prospective design and the follow-up period of 12 months after giving birth, although the study had a high loss to follow-up in the postnatal periods. The results from the NuPED study were obtained by doing a data analysis. Since the PhD candidate was not involved in the recruitment and data assessment process, the focus of this section will be on the experiences from conducting the case-control study. Details on the strengths and limitations of the NuPED study can be found in section 3.4.

For the matched case-control study design, the specific matching and especially the selection of cases from a Randomized control trial (RCT) conducted in adolescents with clinically diagnosed pMDD is a significant strength. By combining an observational design with the selection of cases from a RCT, we could minimise the selection bias within the case group since RCT apply to a much narrower population than observational studies [397] by having very specific inclusion and exclusion criteria. Therefore, we could provide exceptional results in a very difficult to access vulnerable group of adolescents. The methodological strengths and limitations, e.g. biological sample acquisition and analysis, are described in more detail within the manuscripts in sections 4.4, 5.4, and6.4.

From September 2019 to April 2020, the recruitment process for the controls was exclusively done through schools, sports clubs, and leisure time activities. However, after the outbreak of the Covid-19 pandemic, recruitment via the mentioned means was no longer possible. Therefore, in May 2020, recruiting via social media was introduced with great success. While recruiting via "traditional" means was very slow, recruitment via social media outnumbered the participants recruited in the period before within one month.

The collaboration with depressed patients can be very challenging in terms of empathy for behaviours from the investigators to the individuals ("subjects"), but also in the amount of burden every individual ("subject") may carry in order to participate in a research study. Awareness of this fact influences the study, from designing and conducting it, up to evaluating the results (e.g. interpretation of loss to follow-ups and drop-outs along the study course).

In the case-case control study, the effort was made for the data assessment to be as comprehensive as possible and short as possible. Since subjects were either participating in their free time (after school, homework, and leisure time activities) or having severe depression, the study must be a minimum of an additional burden. Nevertheless, the controls' data assessment lasted between 3-4 hours, which was a very maximum according to the subject's exhaustion levels. Especially younger participants had more

difficulties staying concentrated until the end of the assessment. Such exhaustion and loss of concentration from the participants could influence the quality of assessed data, especially the assessed data on cognition (the data on cognition were not part of this thesis).

To summarise, the recruitment process using social media to inform possible subjects about the study was a big success. Further, future studies should make an effort not to exceed 4 hours of data assessment or even try to reduce the assessment time by prioritising assessment instruments. Keeping data assessment short could reduce the burden on the adolescents, but more importantly, it could ensure data quality, minimising bias caused by exhaustion of the participants.

7.2 Research in the context of depression

In this thesis, depression has been addressed as one disease with a multifactorial aetiology. In the past few years, singular opinions have emerged that this might not necessarily be true from a biological point of view. These opinions suggest that in medicinal research and the clinics, depression was referred to as a symptom of multiple and very different underlying disorders rather than a disease itself [398, 399]. Changing the perspective on how to perceive depression from being a disease to a symptom would change research strategies and, therefore, probably also treatment approaches. For instance, research would no longer look for common aberrant parameters within different depressive populations but instead focus on creating a decision tree that allows making individualised treatment approaches according to the individual's manifesting biological parameters. For instance, individuals showing increased peripheral and gut inflammation markers combined with a low n-3 PUFA intake could be administered anti-inflammatory drugs and a modification of their diet instead of traditional antidepressant treatment. This approach of seeing depression as a symptom rather than a disease could partly explain treatment-resistant depression since the applied medication might not effectively target the actual cause of the depression.

The following section will provide ideas and suggestions for future research regarding nutrients involved in depressive disorders. Some ideas and suggestions are based on the hypothesis that depression could be a symptom of multiple pathologies rather than a single multifactorial disease.

7.2.1 Suggestions for future research

Firstly, the hypothesis that depression might be a symptom rather than a disease could be addressed within the case-control study conducted as part of this thesis. Therefore, the different observed biological manifestations of n-3 PUFA, thyroid function, and iron status in the context of pMDD could be investigated in combination. So far, we have not investigated whether all of these manifestations are apparent within the same individuals simultaneously or present in different populations' sub-groups.

However, in the context of depression being a multifactorial disorder, possible interactions between the investigated nutrients could be examined. Such interactions have been shown, e.g. between n-3 PUFA and iron, when a combined deficiency of the nutrients compared to single deficiencies alone produced more significant disruptions in brain monoamine metabolism affecting memory deficits in male rats [306]. Also, interactions between iron and iodine have been shown in rats, where Iron deficiency anaemia (IDA) reduced the activity of Thyroperoxidase (TPO), a heme-containing enzyme which catalyses the first two steps of thyroid hormone synthesis (see fig. 2.14) [368]. Interactions between the HPT axis and lipid profiles could be observed in the context of risk factors for hypothyroxinaemia among women of childbearing age [210]. Furthermore, whether there is an interaction between the HPT and Hypothalamic-pituitary-adrenal (HPA) axis could be investigated. The HPA axis is involved in the "stress response" and shares similar brain regions in its regulation like the HPT axis. By investigating possible interactions between the HPT and HPA axis, the aetiology of the observed aberrant HPT axis parameters could be investigated. Finally, in the context of this case-control study, stool samples of adolescents were acquired.

Nutrients play a significant role in neuronal development as well as structural and functional alterations within the brain, all associated with depression. Therefore, it would be important to investigate whether children and adolescents whose mothers experienced a nutritional deficiency during pregnancy are at higher risk for depression or alterations in nutritional status associated with depression and to what extent these alterations might be reversible. However, when conducting observational studies, especially prospective ones, a significant challenge is the recruitment and follow-up of the subjects. In a prospective study investigating such a long-termed research question, the initial sample size would need to consider the general prevalence rate of pMDD and the drop-out rate, probably resulting in a considerable initial sample size. Especially for prospective studies, this is linked to high financial and professional expenses. On the other hand, by investigating this research question retrospectively within, e.g. this PhD project's case-control study, the results could be strongly biased by recall inaccuracies. A more feasible approach could involve accessing health data through health insurances since they usually are involved in the payment of treatment options such as, e.g. iron infusions.

Interventional studies will need to follow observational ones to establish the most efficient treatment and prevention strategies for depression. The efficacy of different treatment approaches could be compared within the different sub-groups of "treatment profiles" to investigate the hypothesis of depression being a symptom and not a multifactorial disease. A "treatment profile" could reflect the individual's biological alterations manifested in the context of depression. Consequently, different underlying pathophysiologies would require investigating biomarkers adapted for the corresponding treatment profile. The first implication of this approach would be that inclusion and exclusion criteria must also be applied to the biomarkers of interest. Then, one possible approach to monitoring "success" could be the combination of peripheral parameter measurements in combination with structural and functional brain imaging within the same individuals, which seem to play a significant role in depression (see sec. 2.1.3). By combining blood parameters with imaging, possible changes could be tracked in the periphery and within the Central nervous system (CNS). This approach of combing imaging with peripheral blood parameters could be applied to n-3 PUFA, thyroid hormones, and iron research.

7.3 Conclusions

This thesis found a lower n-3 PUFA status to be associated with higher odds for pMDD and perinatal depression. Furthermore, although unrelated to iodine status, the HPT axis was involved in the pathophysiology of paediatric depression. No associations between iron status and paediatric depression could be observed. However, the findings suggest that such an association between iron status and depression might be masked by more frequent iron supplementation in depressed adolescents. This thesis' findings provide a rationale to further investigate n-3 PUFA's and iron's role in depression, especially within the field of (4) immune system and inflammation having a possible impact on (1) neurochemical processes; and (2) plasticity, structure and neurogenesis. Finally, this thesis provides a rationale to investigate the relationship between the HPT and HPA axis possibly involved in depression through (3) neuroendocrine processes.

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