This Part of the thesis is based on (or adapted from) the following publications:

Focus on fatty acids in the neurometabolic pathophysiology of psychiatric disorders. Mocking RJ, Assies J, Ruhé HG, Schene AH. J Inherit Metab Dis. Under Review.


The aim of this thesis was to focus on fatty acids and their relation with other (patho)physiological aspects in Major Depressive Disorder (MDD), in order to improve disease understanding. By conducting several studies on the role of fatty acids in the pathophysiology, recurrence and treatment of MDD, we looked for new ways beyond the monoaminergic theory to (I) improve the understanding of interactions among pathophysiological aspects, (II) reduce MDD heterogeneity by definition of subgroups with more comparable diagnostic characteristics, and (III) delineate profiles associated with prognosis and treatment outcome. This to provide anchor points to personalize treatment in the future, in order to increase treatment efficacy, reduce recurrence rates, and minimize cardiovascular comorbidity. Ultimately, we aimed to open new opportunities to reduce the burden of disease by MDD and related psychiatric disorders.

In this final chapter we will first summarize the results of the described studies, and subsequently discuss the implications and recommendations of our findings for future research and clinical practice.

**SUMMARY OF MAIN FINDINGS**

In Part I, we explained why we studied MDD, by describing its large burden of disease and identifying opportunities to reduce it. In Part II, which includes three chapters, we first introduced fatty acids as a possible factor in the pathophysiology of MDD that may contribute to a reduction in its worldwide burden.

The relation between fatty acids and MDD has been put forward based on epidemiological and biological evidence suggesting a role for fatty acids in MDD and related psychiatric disorders. We first reviewed this evidence in the introduction of Part II, including our own findings (not integrally included in this thesis). These findings showed links between diets with low omega-3 fatty acid intake and (I) more cross-sectional psychopathology in the HELIUS study\(^1\) and (II) more prospective depression in the InChianti study\(^2\) (see Table 1 in Part I of this thesis for an overview of studies). These epidemiological findings can be explained by biological evidence showing that omega-3 fatty acids constitute 5-10% of the dry weight of the brain (with several important structural and functional characteristics), while we humans cannot synthesize them and therefore rely on dietary intake that is often inadequate.

In the remainder of the introduction of Part II we reviewed research on fatty acid alterations in MDD, including our own research comparing fatty acid concentrations in patients with recurrent MDD to matched controls in the DELTA study.\(^3\) Apart from lower omega-3 and relatively higher omega-6 fatty acid concentrations that were also reported previously, we additionally showed a state-independent pattern of fatty acid alterations that consisted of higher concentrations of short chain and lower concentrations of long chain fatty acids from the saturated, monounsaturated, and polyunsaturated series.

After this introduction, Part II continues with a focus on methodological issues in chapter 1. In order to effectively study the above described pattern of alterations in a large number of different fatty acids, we proposed and applied methodological statistical solutions including
the use of indices to achieve data-reduction. Using these indices, we showed that fatty acid alterations in recurrent MDD can be summarized as lower overall fatty acid unsaturation and shorter chain length. Interestingly, chapter 2 showed that these fatty acid characteristics were bimodally distributed, i.e. one subgroup of patients showed substantially lower fatty acid unsaturation and shorter chain length than the other. This suggests two biologically distinct categories of MDD patients. In order to find the origin for this subdivision, in chapter 3 we investigated a nutrigenetic polymorphism in the fatty acid binding protein 2 gene that is responsible for gut uptake of long-chain fatty acids. While this polymorphism could not explain the bimodal distribution, it was associated to the cardiovascular disease risk factor waist circumference through an effect on fatty acid concentrations.

In Part III, we introduced fatty acids’ pathophysiological context in MDD. Because of the structural and functional roles of fatty acids in the brain described above, fatty acid metabolism can be expected to have several cross-links with other (patho)physiological aspects. Two relevant examples of these aspects are neuroendocrinological stress and emotional processing. Preclinical data suggests relations of these two aspects with fatty acid metabolism. In Part III we first described our studies on neuroendocrinological stress and emotional processing on their own in MDD.

We studied neuroendocrinological stress using the end products of the hypothalamic-pituitary-adrenal (HPA)-axis: cortisol and DHEAS. In the DELTA study on recurrent MDD in chapter 4, we showed a trait of higher diurnal salivary cortisol concentrations compared to controls, that did not change during new episodes or stress, but could be lowered by preventive cognitive therapy. Likewise, in chapter 5, we observed a trait consisting of a steeper diurnal decline of DHEAS and a diurnally flattened cortisol/DHEAS ratio. Interestingly, cortisol/DHEAS ratio was associated with cognitive therapy success: a higher cortisol/DHEAS ratio led to a worse clinical outcome. In the DELPHI study on acutely depressed unmedicated MDD patients we observed no study-entry HPA-axis alterations compared to matched controls. However, in this study we did find an association between the HPA-axis and clinical outcome of treatment with the antidepressant paroxetine: patients that remitted after treatment showed a decrease in HPA-axis setpoint with increasing HPA-axis flexibility.

Reviewing related neuroendocrinological studies by others showed a similar picture of in general higher cortisol in MDD, but also with several inconsistencies remaining with increasing evidence for an inverted U-shape of the association of HPA-axis activity with MDD and its clinical course. Given these inconsistencies, it is not surprising that interventions targeted at the HPA-axis have not yet shown sufficiently consistent effects for clinical implementation. Instead of improving clinical care by targeting the HPA-axis with interventions, we stated that using HPA-axis parameters to predict treatment response or prognosis seems a more feasible approach in the short term.

Regarding emotional processing, MDD patients seem to exhibit negative biases in dealing with emotional information. Using magnetic resonance imaging (MRI), small (~1%) decreases in the sizes of brain structures relevant for emotional processing have been found. However, changes in brain activity associated with disturbances in emotional processing may be more pronounced in MDD. Indeed, in the DIADE study covered in chapter 6, we
showed that MDD patients differed in brain activity during emotion regulation from healthy controls but also from patients with bipolar disorder. Moreover, these differences depended on symptom state (depressed versus remitted). Likewise, in the DELTA-neuroimaging study, we showed that in patients with remitted recurrent MDD, the daily variability in negative emotions was associated with functional brain network organization.\textsuperscript{11}

Reviewing related emotional processing studies by other and us showed that negative biases are a core aspect in MDD pathophysiology. Clinical translational efforts are being made using emotional processing test batteries.\textsuperscript{12} Studies of brain structure and activity alterations associated with disturbed emotional processing apply increasingly advanced network perspectives.\textsuperscript{13} Nevertheless, limited transdiagnostic specificity and statistical concerns may hamper effective clinical neuroimaging applications thus far.

\textbf{Part IV} covers the hypothesized associations between fatty acid metabolism and the pathophysiological aspects introduced above in Part III: neuroendocrinological stress and emotional processing.

Regarding the relation between fatty acid metabolism and neuroendocrinological stress as expressed in HPA-axis activity, we reviewed the preclinical literature suggesting a “vicious” circle of bidirectional effects of cortisol on fatty acid metabolism. Long-chain omega-3 fatty acids may reduce HPA-axis (re)activity, and vice versa, cortisol influences fatty acid synthesis, mobilization and degradation cumulating in lower long-chain omega-3 fatty acid concentrations. In line with this, in \textbf{chapters 7} and \textbf{8} we observed associations between cortisol and fatty acid parameters in two independent samples of MDD patients from our DELTA and DELPHI studies: higher cortisol was associated with lower fatty acid unsaturation, e.g. resulting in lower omega-3 fatty acid concentrations. In addition, this relation between cortisol and fatty acid parameters was associated with treatment response. Moreover, in \textbf{chapter 9} we described a randomized placebo-controlled clinical trial in MDD patients with comorbid diabetes, and showed that supplementation of the omega-3 fatty acid EPA may reduce HPA-axis activity, further supporting a causal relationship.\textsuperscript{16}

Regarding the relation between fatty acid metabolism and emotional processing, we reviewed biological evidence showing that fatty acids influence structure and function of brain areas involved in emotional processing. Moreover, initial clinical studies observed associations between fatty acids and emotional processing, including a reduced bias towards fearful facial stimuli after placebo-controlled omega-3 fatty acid supplementation. Likewise, in MDD patients of the DELPHI study described in \textbf{chapter 10},\textsuperscript{17} we observed associations between the omega-6 fatty acid arachidonic acid and reactivity to emotional faces of a central emotional processing brain area: the amygdala. In detail, MDD patients showed a more negative association between arachidonic acid and left amygdala reactivity than controls.

Next to the above relations between fatty acids, neuroendocrinological stress and emotional processing, fatty acids have been hypothesized to be related to several other pathophysiological aspects. We finished Part IV by reviewing evidence on some of these suggested relations, including our own work on the relation between fatty acids and folate/one-carbon metabolism showing that both MDD and posttraumatic stress disorder (PTSD)
are associated with disturbances in folate/one-carbon metabolism including increased homocysteine and lower folate, that were associated with omega-3 fatty acids EPA and DHA. Importantly, disturbances in folate/one-carbon metabolism including high homocysteine and low folate are considered risk factors for the development of cardiovascular disease.

Finally, following the evidence presented in the preceding Parts for a role of fatty acids in depression, Part V details the use of fatty acid supplementation as a clinical application in a meta-analysis in MDD (chapter 11) and another in peripartum depression (chapter 12). For MDD, we observed a medium sized overall beneficial effect, that was larger for studies supplementing a higher EPA dose and when added to an antidepressant. In peripartum depression we observed important subgroup differences: there was a large effect of omega-3 fatty acid supplementation in postpartum depression, compared to no or negligible effects antepartum, nor for depressive symptoms not meeting criteria for depression. By presenting a cautionary note in chapter 13 on the risk of fatty acid supplementation, particularly during pregnancy, we aimed at continuing the academic debate on omega-3 fatty acid supplementation in MDD. To further investigate the potential risks of fatty acid supplementation, we presented a third meta-analysis on the effects of fatty acid supplementation on oxidative stress in chapter 14. While we observed no overall effect, potentially important subgroup differences emerged: in subjects with CVD-risk omega-3 fatty acids decreased oxidative stress (measured using various parameters), while higher EPA dose in healthy participants had the potentially harmful effect of increasing oxidative stress. These findings stress the importance of personalized nutritional or nutraceutical therapy, in order to find the optimal balance between beneficial and harmful effects for each individual. Moreover, these findings point to potentially harmful effects of nutraceuticals sold over the counter. Nevertheless, taken together, we conclude Part V with the suggestions that omega-3 fatty acid supplementation has (I) a similar overall effect size as antidepressants with more effect for higher EPA dose in an add-on design, (II) a relatively safe and tolerable known side-effect profile, and (III) relatively low costs. Large, well-executed, long-term clinical trials may help clinicians and guideline-makers to decide whether to implement omega-3 fatty acid supplementation for MDD in routine clinical practice.

In brief, Part I identifies MDD as a major health concern; Part II introduces fatty acids as a potential means to alleviate MDD’s disease burden, given epidemiological and biological evidence suggesting their importance for brain structure and functioning. Chapters 1-3 focus on fatty acid alterations in MDD, showing a state-independent bimodally distributed pattern consisting of shorter and less unsaturated fatty acids, that could not be explained by a nutrigenetic polymorphism in the fatty acid binding protein 2 gene. To better understand this fatty acid pattern, we studied its pathophysiological context by linking it to other aspects relevant in MDD: neuroendocrinological stress and emotional processing. We first studied these other aspects on their own in Part III. We showed state-independent increases in neuroendocrinological stress in MDD, expressed as a higher cortisol/DHEAS ratio (chapters 4 and 5), and altered activity of brain areas involved in emotional processing (chapter 6). In Part IV, we showed evidence for bidirectional associations between the observed increased neuroendocrinological stress and the fatty acid pattern of lower unsaturation and shorter chain length (chapters 7-9). Moreover, fatty acids were associated with emotional processing brain activity (chapter 10). These results led us to investigate clinical application of fatty
acids in Part V where we showed that the long chained and unsaturated omega-3 fatty acid EPA is a promising intervention for MDD (chapter 11) and postnatal depression (chapter 12). Nevertheless, given the vulnerability of unsaturated fatty acid for oxidation, clinicians should balance positive effects with potential biological negative effects on the long term (chapters 13 and 14).

Thereby, this thesis provides evidence for a role of fatty acid alterations in the pathophysiology of MDD. In addition, fatty acid alterations proved to be associated with alterations in other pathophysiological aspects, including the neuroendocrinological stress and emotional processing. Clinically, meta-analyses showed that EPA supplementation may be an effective intervention in (postpartum) depression, particularly in the form of add-on treatment with EPA. Moreover, our findings of associations between alterations in fatty acid concentrations and clinical course suggest inroads to personalize treatment and prognosis in the future.

Below, we will aim at further integrating findings and discuss the implications for future research and clinical practice. We will look back on the research opportunities outlined in the introduction of this thesis (improve disease understanding, personalize treatment and prognosis, and augment therapy response), and see to what extent our research may have contributed.

INTEGRATING FINDINGS

Fatty acids in disease understanding

Focus on pathophysiological context

This thesis contributes to the increasing evidence for an interesting role of fatty acid metabolism in the pathogenesis of MDD. Based on their basic biological status of forming the essential components of all outer and subcellular cell membranes, fatty acid alterations seem to be involved in MDD’s etiology and pathophysiology. This importance is further substantiated by widespread associations with other pathophysiological aspects including neuroendocrinological stress (HPA-axis activity), inflammation and emotional processing brain network structure and function. Thereby, fatty acids may form part of a neurometabolic network. Observed trait alterations in this network may constitute vulnerability for (recurrent) MDD, e.g. leading to reduced resilience to homeostatic disturbances by various external (psychological) stressors.

In detail, neuroendocrinological stress leading to HPA-axis activation and increased cortisol concentrations may decrease fatty acid unsaturation and chain length, particularly in vulnerable subjects with a more negative association between cortisol and fatty acid alterations. Through bidirectional effects this may lead to a vicious circle further increasing cortisol and decreasing fatty acid unsaturation and chain length. This decrease consequently leads to a stiffening of the neuronal membrane, hampering effective neuronal communication. This may affect emotional processing, as a result of less prefrontal control over limbic responses, given that the prefrontal cortex is particularly rich in unsaturated and long-chained fatty acids. As such, this cumulative process may be part of the pathophysiology of MDD. How supplementing long-chained and polyunsaturated omega-3 fatty acids may partly restore the neurometabolic balance will be discussed below (see section 2.3).
However, as in many areas of biological psychiatric research, several inconsistencies remain. For example, although the outspoken fatty acid alterations (e.g. lower polyunsaturated omega-3 fatty acids, higher omega-6/omega-3 ratio) in the DELTA study in recurrent MDD patients have been largely corroborated in a meta-analysis, we did not see them in an independent sample of acutely unmedicated MDD patients from the DELPHI study. Of note, the same held true for HPA-axis alterations between DELTA and DELPHI samples. Interestingly, the difference from controls in the relationship between fatty acid metabolism and HPA-axis activity actually was consistent between the two studies. This suggests an altered, more negative, relationship between fatty acid metabolism and HPA-axis activity in MDD, with differences between samples depending on where they are located on this association line: high cortisol and low fatty acid unsaturation versus relatively low cortisol and slightly higher unsaturation. This could mean that the inconsistencies in findings for the individual pathophysiological aspects could be caused by a different location (i.e. being located at another point) on the underlying association line (Figure 1). Differences in clinical or other factors between these two studies that could explain the differential placement on this association line remain yet unknown. Nevertheless, incorporating the relatively consistent relationship between fatty acids and the HPA-axis in future studies could lead to clearer findings than when studying both individual pathophysiological aspects in isolation.

Figure 1. Possible differential placement of samples on the association line between HPA-axis activity and fatty acid alterations. Squares represent a fictional sample with high cortisol and low fatty acid unsaturation, starts represent another fictional sample with relatively low cortisol and high fatty acid unsaturation. The association line shows a negative relationship that is consistent between the two samples.
**Compose more homogeneous groups through biology**

A potential way to improve disease understanding would be to reduce diagnostic heterogeneity. As shown in this thesis, biological parameters including fatty acid concentrations and indices, HPA-axis activity, and their relation, provide ways to subgroup patients, both cross-sectionally (i.e. profiling) and longitudinally (i.e. staging).\[^{5,8-10,15,17,34-36}\]

Meaningful subdivisions could result in more homogeneous patient groups, which might lead to clearer findings in pathophysiology and treatment studies.

Regarding cross-sectional subdivisions, we observed bimodal distributions of fatty acid alterations in recurrent MDD patients.\[^{5}\] This mix of two unimodal distributions suggests two distinct biologically definable subgroups. Nevertheless, the causal factor and clinical relevance of this subgroup division remain yet unknown and are an important subject of further study, e.g. by linking bimodal group status to clinical parameters as follow-up course. From a longitudinal perspective, in recurrent MDD patients we observed both trait and state effects in HPA-axis activity and fatty acid, but also one-carbon metabolism.\[^{3,7,8,14,15,20,32}\]

Disentangling these state- and trait effects may provide insight in the stability and nature of biological alterations during different stages of the disease, thereby providing a way to reduce longitudinal heterogeneity, e.g. to use HPA-axis parameters to indicate in which patients additional preventive interventions are needed to prevent imminent recurrence. In addition, our finding that HPA-axis activity was partly state-independent may stimulate future research into underlying gene-environment interactions.\[^{7,8}\]

Regarding gene-environment interactions, we showed that nutrigenetic influences on fatty acid (**FABP2**-genotype) and one-carbon metabolism (**MTHFR**-genotype) could be used to subgroup MDD-patients.\[^{34,35}\] This subdivision seemed to hold clinical relevance, given observed associations with CVD risk and MDD clinical course, respectively.\[^{34,35,37,38}\]

For example, patients with the **FABP2** Ala54Thr-polymorphism showed higher concentrations of long chain fatty acids that mediated a lower waist circumference.\[^{34}\] This suggests that this nutrigenetic factor might contribute to future CVD-risk detection. Another example of a nutrigenetic factor that could be relevant in the prediction of clinical course in psychiatry comes from a study in schizophrenia, where subgroups based on nutrigenetic variation in folate absorption determined clinical effects of folate and vitamin B\(_{12}\) on negative symptoms.\[^{39}\]

**Transdiagnostic pathophysiological differences and similarities**

Interestingly, similar to other pathophysiological areas including neuroimaging findings (e.g. corticolimbic imbalance), alterations in fatty acid metabolism observed in various psychiatric disorders seem to be relatively comparable.\[^{21}\] This might suggest that observed alterations in fatty acid metabolism may be part of a general transdiagnostic vulnerability pattern for psychiatric diseases, instead of an MDD specific pathway.

While fatty acid metabolism have been most often studied in MDD, findings in other psychiatric disorders seem to be comparable. Meta-analyses on fatty acid alterations in schizophrenia also showed overall lower omega-3 fatty acids concentrations,\[^{40}\] although inconsistencies remain, including our finding of higher concentrations of omega-3 fatty acids recently published in Schizophrenia Bulletin.\[^{41}\] In addition, in the first study comparing fatty acid metabolism of PTSD patients with controls, we observed lower omega-3 DHA and alterations in one-carbon metabolism that were relatively similar to those in MDD.\[^{18,19}\]
Moreover, also other diseases as CVD, Alzheimer’s and Parkinson’s disease, as well as normal ageing have all been associated with similar fatty acid alterations (lipid peroxidation products included).21

These transdiagnostic similarities could suggest that the differences between disorders are too detailed to be detected by the studies that have been performed thus far. Within the observed general pattern, specific more subtle differences could differentiate between disorders. These more subtle alterations may also explain different clinical outcomes despite a partly shared underlying pathophysiology. Multivariate pattern analyses also integrating the relations between individual factors seem most suitable to delineate these transdiagnostic differences.

Alternatively, the transdiagnostically similar alterations could resemble a general vulnerability pattern for mental illness.21,42 Although alterations would in that case be poorly differentiating between disorders, this would hold promise for interventions: if an intervention would be able to target this general vulnerability pattern, the different associated negative outcomes might benefit simultaneously. If so, transdiagnostic similarities would limit the potential to profile patients into homogeneous groups within a diagnostic category to personalize treatment. Nevertheless, it may be that the alterations are still specific for certain profiles, but that these profiles extent beyond current diagnostic categories. Unsupervised pattern recognition using e.g. metabolomics could identify potential transdiagnostic alterations, that can subsequently be used to better profile patients clinically.

**Biologically personalizing psychiatry**

A scarcity of clinically applicable data on expected prognosis and treatment response results in the currently applied ‘trial and error’ approach in contemporary clinical psychiatry. If we would be able to better understand and predict response and prognosis, we could make more evidence based clinical decisions on what therapy is indicated and, maybe even more important, not indicated. Improved personal profiling and subsequent testing in RCTs comparing personalized versus the current ‘trial and error’ based treatment allocation could lead to larger effect sizes and thereby the translation, of amongst others the findings of this thesis, to clinical practice.

This thesis contains several examples of biological factors that improve our knowledge of course and outcome, including prospective recurrence and treatment response (and/or resistance) to antidepressant and cognitive therapy. In the DELPHI study, we showed that fatty acid alterations could prospectively predict antidepressant response.14,17 HPA-axis alterations in the DELTA-study predicted the recurrence-preventing effects of cognitive psychotherapy.7,8 Moreover, the direction of the associations between fatty acids and HPA-axis alterations was also associated with response.14,17 Furthermore, CVD-risk could be predicted using nutrigenetic variation in fatty acid alterations.34

These associations between alterations in fatty acid metabolism and clinically relevant outcomes have also been observed in other disorders. As an example, in a study in subjects with ultra-high risk to first-episode psychosis, omega-3 fatty acids and concentrations of the omega-9 monounsaturated fatty acid nervonic acid aided in predicting transition
into psychosis. In another study, DHA signaling was one of the top biological pathways overrepresented in validated biomarkers predicting suicidality in samples of women with bipolar disorder, depression, schizoaffective disorder or schizophrenia and men with bipolar disorder. Furthermore, omega-3 fatty acids and their omega-6 ratio could predict depression onset during interferon-alpha treatment.

This suggests that the pathophysiological processes that are reflected by these neurometabolic alterations not only play a role in disease vulnerability and/or onset, but also in the clinical consequences during follow-up (e.g. disease progression and/or treatment response). Effects of fatty acids on inflammation in the brain and periphery, neuronal and endothelial membrane stiffness, and oxidative stress vulnerability are likely mediators of these clinical consequences.

**Augment therapy response using fatty acids**

Although several effective treatment options are available, low response rates point to an urgent need for improved antidepressant treatment strategies. In this thesis, we described studies that tested whether fatty acids could be used to augment currently available therapeutic options.

Although our own RCT on omega-3 fatty acid supplementation to patients with depression and comorbid diabetes showed no clinical effect, by combining all available evidence in a meta-analysis we showed a pooled effects size that was comparable to that for antidepressants and numerically comparable to a preceding Cochrane meta-analysis. In addition, we showed that effects were larger in patients using antidepressants when higher doses of EPA were used. Moreover, we showed promising effects in postnatal depression. These findings suggest that omega-3 fatty acids could be used in postnatal depression and as an augmentation strategy in MDD to theoretically double the effect of antidepressants, which will be incorporated in the upcoming version of the guideline describing the standard of care for MDD in the Netherlands.

Interestingly, we observed a dose-response relationship for the antidepressant effects of EPA, while DHA showed no effect. This seems in contrast to the observation that DHA is the fatty acid that is most consistently found in lower concentrations in patients with psychiatric disorders including depression. This apparent contradiction questions the rationale that low DHA resembles a shortage/deficit that should be corrected by supplementation. In addition, while DHA is the most abundant fatty acid in the brain, EPA only represents ≤1% of total brain fatty acids. Furthermore, although EPA can be transformed into DHA, evidence suggests that EPA is rapidly and extensively β-oxidized (generating acetyl-CoA) after entry into the brain. Of note, this process results in little to no extra EPA available in the brain after supplementation and the β-oxidized products are not specific for EPA. All in all, this suggests that EPA's efficacy cannot be explained by a direct effect in the brain, and that EPA is not only a precursor of DHA but that DHA and EPA have distinct roles as outlined below. An explanation could be that the effects of supplemented EPA are mainly mediated by peripheral anti-inflammatory actions. EPA, given its multiple double bonds, is prone for enzymatic and non-enzymatic oxidation. This may be aggravated by the pro-oxidative environment found in psychiatric disorders.
oxidation products generally have anti-inflammatory properties. Given that inflammation is thought to transdiagnostically underlie many psychiatric disorders including depression, this could explain why supplemented EPA is more efficacious than DHA. Interestingly, a recent study corroborated this view, by showing that inflammation serves as a positive predictive biomarker for response to omega-3 fatty acid supplementation in depression. In detail, this proof-of-concept study in 155 depressed patients showed that combined inflammation biomarkers (IL-1ra, IL-6, hs-CRP, leptin, adiponectin) could predict response to EPA vs. DHA or placebo. In detail, only patients with high pre-treatment inflammation biomarker concentrations benefited significantly more from EPA supplementation (40% remission) compared to DHA or placebo (≤25% remission). This is corroborative with the idea that EPA reduces the increased inflammatory response in MDD (and other psychiatric disorders), which makes it a promising candidate (add-on) treatment in specific subgroups with elevated inflammation markers.

The observation that supplementing the low DHA concentrations seems to have less therapeutic effect requires an alternative interpretation than low DHA as a deficit. Although it seems plausible given the epidemiological, evolutionary and biological evidence presented earlier, a causal deficiency should result in (clear) improvement after DHA supplementation, which is not substantiated by (our) pooled meta-analytical results. Several explanations might clarify this apparent contradiction. First, due to its six double bonds, DHA is even more susceptible to oxidation than EPA. If not already oxidized \textit{ex vivo} in the supplementation capsules (i.e. becoming rancid), DHA could easily become oxidized after ingestion \textit{in vivo}, especially given the pro-oxidant state observed in psychiatric disorders. Supplementation capsules most often contain tocopherols as antioxidants, but these probably do not provide full protection against oxidation \textit{ex vivo} and especially not \textit{in vivo}. Resulting oxidative metabolites of DHA are not yet routinely measured and therefore not completely understood, but these may induce negative effects as well which potentially contribute to the observed negative findings. This \textit{in} and \textit{ex vivo} oxidation of supplemented DHA would hamper effective restoration of central nervous system DHA concentrations, and may even lead to adverse clinical outcomes, particularly during vulnerable periods as pregnancy.

As an alternative to the idea of low DHA as a shortage/deficit, low DHA could be interpreted as an adaptive process in reaction to increased oxidative stress (as seen in psychiatric disorders). As mentioned before, DHA is even more susceptible to oxidation than EPA. Following the interpretation of low DHA as an adaptive process, cells sense increased oxidative stress, and in response lower the DHA content of their membranes, in order to reduce their vulnerability for the oxidative stress present. This lower membrane vulnerability to oxidative stress (i.e. lower peroxidizability) comes at the “cost” of lower membrane fluidity, adversely affecting membrane functioning including membrane bound proteins as neurotransmitter-receptors. This way, low membrane DHA content and its neuropsychiatric consequences can be interpreted as a logical by-product of underlying increases in oxidative stress. e.g. from mitochondrial dysfunction. Following this line of thought, it can be expected that “correcting” this adaptive response by supplementing DHA has no, or even deleterious effects, since additional DHA will be oxidized and not incorporated in the membranes. If this hypothesis is correct, instead, it should better be tried to correct the underlying oxidative stress.
Unfortunately, effectively lowering oxidative stress levels is not that easy, particularly in the brain. Current antioxidant supplementation does not seem to be effective, coined as the antioxidant paradox. This can be explained because supplemented antioxidants (I) do not enter the brain; (II) distort endogenous antioxidant responses and physiological oxidative stress; and (III) not always act as an antioxidant in vivo. Alternatively, it may be more effective to prevent oxidative stress from arising in the first place. Although possibly less commercially exploitable due to the difficulty of producing a marketable product and implementing it, several interventions aimed at managing oxidative stress are under investigation. For example, oxidative stress could be lowered by lifestyle improvement including stopping smoking, increasing physical exercise, and limiting excess caloric intake. Indeed, research (in other areas of medicine than psychiatry) shows that if oxidative stress can be diminished, fatty acid alterations seem to (partially) recover. For example, bariatric surgery-induced weight loss reduced lipid peroxidation. Pioneering research efforts to systematically study these lifestyle interventions in psychiatry already show promising results.

Interestingly, reducing inflammation by EPA supplementation could also reduce oxidative stress, thereby allowing for reactive increases in DHA that could mediate the observed therapeutic effects. In addition, omega-3 fatty acids like EPA may have some direct free radical scavenging effects on their own and as such they may directly interfere with increased oxidative stress. However, in our third meta-analysis, we showed no significant overall effect of omega-3 fatty acid supplementation on oxidative stress. A potential explanation could be the subgroup differences: in participants with CVD-risk, supplementation decreased oxidative stress, while in healthy participants higher EPA-dose increased oxidative stress, suggesting a window of opportunity. All in all, findings point to a complex homeostatic balance involving oxidative stress, fatty acid metabolism and inflammation. Distorting this balance with supplementation of artificially high concentrations of omega-3 fatty acids may result in potentially harmful increases in oxidative stress in healthy participants, while on the other hand, patients with an already disbalanced homeostasis may in some cases benefit from ameliorating this existing disbalance by supplementing omega-3 fatty acids.

### RESEARCH IMPLICATIONS

Next to the directions for future research outlined in the individual parts of this thesis, here we provide some overall comments on future research designs that could help to further increase MDD treatment efficacy, reduce recurrence rates, and minimize cardiovascular comorbidity.

In order to further disentangle the role of fatty acids and their peroxidation products in psychiatric disorder pathophysiology and to optimally exploit their promise for clinical application, future studies would benefit from a combined pathophysiological and clinical design, in order to make causal inferences on a biological level from randomized controlled trials. A potential focus would be the interplay between oxidative stress, DHA-concentrations and EPA-supplementation in augmentation studies aiming at inflammatory modulation. Detailed measurement of peroxidation products using lipidomics in a systems biology
approach, preferably repeatedly at an interval of several weeks during a well-controlled long-term intervention will meaningfully quantify alterations in metabolic patterns, of which the dynamics could be studied on a shorter timescale to get a better insight in the underlying mutual connections.\textsuperscript{63}

Furthermore, personalizing interventions should receive special attention. Following the line of thought presented above, future trials should use (patterns of) inflammatory, oxidative stress and nutrigenetic markers to \textit{a priori} identify the subjects that benefit versus those that are potentially harmed by supplementation, in order to increase the effect size for clinical outcomes.\textsuperscript{6,72} Alternatively, fatty acids could be used as markers to identify subgroups of patients that will benefit from other interventions, e.g. antidepressants or even neurostimulation (given associations of fatty acids with brain network structure and function\textsuperscript{49,74}). We expect that pathophysiologically tailored treatment strategies will increase effect sizes in individual studies and later meta-analyses.

Future randomized controlled trials mutually combining add-on lifestyle interventions [e.g. diet (among others less omega-6), physical exercise] and investigation of (adjuvant) novel oxidative-stress-relieving treatments seem to hold promise. For example, effects on oxidative stress of polyphills containing e.g. N-acetylcysteine affecting the one-carbon cycle, but also psychotherapy (e.g. cognitive behavioral therapy),\textsuperscript{7,75-80} may be interesting topics of future investigation. In addition, it might be worthwhile to look for ways to prevent disrupted mitochondrial oxidative stress formation, i.e. mitochondrial therapy.\textsuperscript{42,62,81,82} Importantly, these studies should combine clinical outcomes with biochemical parameters, for example oxidative stress and (non-)enzymatic lipid peroxidation products, to understand underlying biological aspects.\textsuperscript{21} A resulting decrease in oxidative stress and increase in co-intake of essential nutrients as trace elements and amino acids may have the additional advantage that increased dietary intake of omega-3 fatty acids can likely be incorporated better into the neuronal membrane.\textsuperscript{68}

Adaptive trial designs,\textsuperscript{83,84} incorporating patient preferences in randomization and applying nationwide harmonized registration and intervention protocols (like in oncology) could lead to very valuable data while still being feasible. Effective sharing of curated data would provide datasets that are large enough to effectively apply machine learning on the individual level, enabling the creation of independent discovery and test samples.\textsuperscript{85-89} In an iterative approach, these study designs could directly influence clinical practice and vice versa.

A more experimental line of research concerns the quantum-characteristics of fatty acids, particularly DHA. DHA has been proposed to possess unique properties from a quantum mechanical perspective. Through its atomic configuration, DHA is thought to facilitate membrane tunneling of electrons. This membrane tunneling capabilities enable precise and rapid depolarization of membranes with a high DHA content. These quantum properties of DHA may explain its extreme conservation - i.e. very high concentrations relative to the rest of the body - in the neuronal synapse and retina, where precise and rapid membrane depolarization is essential for adequate cell functioning. Moreover, because of these unique quantum characteristics, DHA has been hypothesized to be a driving facilitator of brain development through evolution, as detailed elsewhere.\textsuperscript{26} The precise neuroscientific and clinical consequences of these fascinating ideas have yet to be investigated further.
Figure 2. Theoretical framework. Schematic representation of the theoretical framework of the present DELTA-neuroimaging study. The four selected levels of perspective (endocrinology/metabolism, brain circuits, affective neuropsychology and symptoms), their respective subdomains, and their connections have been depicted. The horizontal straight arrows show potential bidirectional relationships (for readability bidirectional relationships between eg, anhedonia and cognitive reactivity are not shown), the horizontal curved arrow shows membrane fluidity balance, colored arrows show potential connections, dashed arrows show inhibiting effects and vertical grey arrows show possible underlying pathways. Abbreviations: DELTA, Depression Evaluation Longitudinal Therapy Assessment; DHEAS, dehydroepiandrosterone-sulfate; GABA, γ-aminobutyric acid; HPA, hypothalamic-pituitary-adrenal; PFC, prefrontal cortex; vStr, ventral striatum; VTA, ventral tegmental area; TPN, task positive network; DMN, default mode network; dACC, dorsal anterior cingulate cortex; pgACC, pregenual anterior cingulate cortex; Amy, amygdala; ‘Hot’ neuro-Ψ, affective neuropsychology; Cogn. react., cognitive reactivity; Dysf. attit., dysfunctional attitudes.
Our ongoing research projects

The research in this thesis leads to several new scientific questions, for which additional research projects are currently ongoing. In the DELTA-Neuroimaging study, the included remitted unmedicated patients with recurrent MDD almost completed a 2.5-year follow-up. Patients that experienced a recurrence during follow-up were invited to repeat baseline measures, together with a matched patient that was not depressed at the time of reassessment. This provides the interesting opportunity to (I) investigate how alterations in biopsychosocial measures prospectively change within-person during a new episode, and (II) predict recurrence in a high-risk sample from baseline biopsychosocial measures. Through a collaboration with the University of California, San Diego, we applied advanced metabolomics/lipidomics techniques to gain further insights in metabolic pathways in MDD pathophysiology and their association with prospective recurrence, which are currently being analyzed. In addition, in collaboration with the Universities of Ghent and Oxford, an extensive neuropsychological and neuroimaging protocol [including several hot cognitive emotional processing tasks, structural and functional neuroimaging approaches, including measurements of white matter structure and glutamate and gamma-aminobutyric acid (GABA) concentrations using magnetic resonance spectroscopy] will enable us to further study the links between metabolic pathways and brain network function/alterations (Figure 2).

To further study transdiagnostic similarities and differences in a clinically representative sample, in the CANTAB/biobank study at the department of psychiatry of the AMC, all patients admitted to the psychiatry department are invited to participate. Among the obtained measures are cortisol in hair and fatty acids in red blood cells. Subjects will be extensively phenotyped clinically – also using dimensional DSM-free symptom questionnaires. Thereby, we hope to be able to study to what extent predictive effects for treatment response exist and translate to clinical practice. In addition, in a lifestyle program implemented in the mood disorders program of our department we investigate to what extent lifestyle changes moderate symptom and CVD-risk improvement.

Next, we hope to further study bimodal or even multimodal distributions of fatty acid alterations and their causes and consequences in the general population. Therefore, in the multiethnic Helius longitudinal population study, fatty acid profiles have been measured in a representative subsample of participants. This has been combined with extensive food-frequency questionnaires and a mental health phenotyping protocol that is relatively elaborate/extensive for such a large epidemiological study.

Finally, ongoing treatment studies include measurements of fatty acid profiles as a potential predictor and/or moderator of treatment response. These include (I) a study testing the effectiveness of ketamine to prevent suicide in suicidal subjects admitted to the hospital that includes fatty acid measurements as a possible treatment effect predictor/modifier, (II) a study testing whether activation of negative schemata shortly before ECT sessions in the course of ECT treatment may enhance treatment efficacy, and finally (III) a pilot study on the effect of deep brain stimulation in treatment resistant anorexia nervosa.
CLINICAL IMPLICATIONS

Results from this thesis, particularly from the meta-analyses in Part V, provide support for the clinical application of omega-3 fatty acid supplementation in selected patients with MDD. Especially supplementation products with a relatively high EPA content used as an augmentation strategy, seem to be able to double antidepressant effectiveness. Moreover, omega-3 fatty acids for postnatal depression also show clinical promise.

Nevertheless, clinicians should always critically evaluate available evidence and monitor treatment effects. While omega-3 fatty acids have a generally mild side effect profile, long-term more negative effects of fatty acid supplementation have not been studied systematically thus far. The above-provided idea that decreases in DHA do not necessarily represent shortages but may reflect an adaptive process, nuances implications for treatment. True deficits should be supplemented, whereas decreases as adaptive responses could potentially be hindered or even be made harmful by supplementation. Moreover, fatty acids in capsules may be prone to oxidation in and ex vivo, leading to production of biologically active, possibly harmful lipid peroxidation products. An example of unintended negative effects of supplements was blockage of the health-promoting effects of exercise and even an increase in long-term mortality. A more sophisticated/correct interpretation (adaptation versus deficit) of fatty acid alterations as outlined above could help to provide a clearer indication of where and when supplementing is warranted or should be avoided. While for MDD our meta-analysis provided evidence that EPA as an add-on strategy could double effect size, evidence for other indications (including treatment of subclinical depressive symptoms) is less convincing or even disappointing.

As a more effective alternative, lowering oxidative stress and thereby preventing fatty acid alterations through physical exercise and a healthy diet seem to be beneficial for prevention and treatment of psychiatric symptoms and CVD-risk. Therefore, these interventions should be more routinely implemented in (mental) healthcare. Some medical professionals seem reluctant to integrate these “non-medical” interventions as physical exercise and diet in their daily practice. This may explain why some of these interventions seem to have been expropriated by “integrative” practitioners. Consequently, these non-medical interventions – although effective according to standard scientific criteria – may receive the stigma “alternative”, which could hamper further implementation in healthcare. Overcoming these prejudices on the basis of robust scientific evidence seems pivotal to deliver optimal care. Societies as the International Society for Nutritional Psychiatry Research and the “Vereniging voor Arts en Voeding” could help in bridging these gaps between evidence and daily practice.
Finally, in spite of consensus recommendations and guidelines, appropriate surveillance of anthropometric and metabolic parameters has not yet been rigorously implemented in psychiatric care.\textsuperscript{106} Obstacles to implementation need to be overcome by making CVD-risk monitoring mandatory.\textsuperscript{107} The concept ‘metabolic syndrome’ encompasses a cluster of CVD-risk factors and may be a helpful tool for clinicians to assess CVD-risk. Although there is continuing debate regarding the metabolic syndrome criteria and concept, this clustering of risk factors is unequivocally linked to an increased risk for developing type 2 diabetes mellitus and CVD.\textsuperscript{108-110} Thereby, the concept metabolic syndrome could guide clinicians which/when psychiatric patients should receive treatment for their increased CVD-risk.\textsuperscript{111}

**LIMITATIONS AND STRENGTHS**

While several limitations and strengths of the performed studies have already been noted in the individual chapters of this thesis, some overall points can be identified.

**Limitations**

Crucial is of course whether fatty acid alterations represent epiphenomena of or risk factors for MDD, which still remains difficult to disentangle. Complicating in this regard is the daunting number of mutually interacting metabolic pathways that are under both environmental (e.g. diet) and genetic influence.\textsuperscript{21} Several arguments can be made for a causal role of fatty acid alterations. First, diseases with genetic defects in fatty acid metabolism (e.g. X-ALD) are associated with psychiatric symptoms including depression.\textsuperscript{74,112} Second, influencing fatty acid alterations seems to improve psychiatric symptoms, including depression.\textsuperscript{22,23,48} Third, associations are not only cross-sectional,\textsuperscript{3,33,55} but also prospective,\textsuperscript{2,21} and have a firm biological rationale given the structural and functional roles of fatty acids in the brain.\textsuperscript{21,27,113,114} A relationship is also supported from an evolutionary perspective, with a supposed mismatch between our genetic make-up which precludes synthesis of essential omega-3 fatty acids and decreasing dietary intake of omega-3 fatty acids.\textsuperscript{1}

Nevertheless, given the complexity and multitude of factors influencing fatty acid metabolism, (partly) epiphenomenal effects cannot be ruled out yet.\textsuperscript{21} Psychiatric disorders are often characterized by suboptimal lifestyles, which may have profound impact on fatty acid metabolism. For example, diet, smoking, and physical inactivity may all influence fatty acid concentrations and cause the observed alterations.\textsuperscript{21} Nevertheless, given the important roles of fatty acids in (patho)physiology, these resulting fatty acid alterations may partially mediate the known detrimental effects of a suboptimal lifestyle on mental health.\textsuperscript{21} Also medication, including major psychotropic drugs, are associated with metabolic changes leading to dyslipidaemia and CVD-risk. Nevertheless, in our studies we corrected for medication effects where applicable; in some studies we even exclusively included medication free subjects.\textsuperscript{13,15} An extra argument against the idea that the metabolic changes are merely secondary to psychotropic drug use is that the evidence for the bilateral association between metabolic alterations, CVD and psychiatric disorders predates psychotropic agents.\textsuperscript{21}
Furthermore, studies in this thesis were designed using DSM-IV diagnostic criteria, while the DSM-5 has already been introduced. Since the classification of depressive episodes (i.e. recurrences) has not substantially changed in DSM-5, this - together with our generally relatively specific in- and exclusion criteria - will not lead to difficulties in translating the results when DSM-5 will be used for clinical purposes. Nevertheless, concerns regarding the validity of DSM criteria remain an important issue. More specific results could be seen if fatty acids would be linked to data-driven subgroups or transdiagnostic pathophysiological dimensions instead of DSM-based disorders, e.g. using the Research domain criteria (RDoC). In addition, evidence is available that minor depression is responsible for an even larger cumulative burden of disease. Concepts including burn-out, which is not included in the DSM, would be interesting to additionally include from a more dimensional perspective.

Finally, the message of our meta-analyses that omega-3 fatty acid supplementation helps in the treatment of depression may have ironic negative effects on lifestyle. Evidence is available that the attributed positive health effects of supplements may “license” unhealthy behavior. A perceived invulnerability due to the conceived protective effects of supplements may lead to more risky and less healthy behavior. This may worsen the existing situation in which people know that certain foods are unhealthy, but cannot be motivated to change their eating habits. In addition, dietary supplements are responsible for an estimated 23000 emergency room presentations in the U.S. each year, e.g. due to choking or adverse reactions. Attention for these areas in future studies and psychoeducation may hopefully combat paradoxical negative by-effects. Nevertheless, these negative by-effects should also be outweighed against beneficial placebo effects that may be associated with supplement use and add up to the pharmacological beneficial effects seen in comparisons against placebo.

**Strengths**

Studies in the current thesis have several distinct strengths. First, studies were performed by experienced interdisciplinary (international and/or multicenter) research groups, combining expertise from all measured perspectives. This interdisciplinary approach, combining a wide range of biological (fatty acid metabolism, HPA-axis, brain networks, inflammation, nutrigenetics, one-carbon metabolism, oxidative stress), but also psychological and social aspects, made that cross-links between research areas could be investigated, crossing dualistic boundaries.

Second, for our research we effectively combined several assessment procedures, including neuroimaging, mass-spectroscopy, chromatography, genetics, immunoassays, e-health, and anthropomorphy. Third, in order to answer our research questions we applied diverse statistical methods including multiple imputation, multilevel mixed modeling, propensity scores and neuroimaging and meta-analysis statistics. Fourth, this thesis combines diverse research designs (patient-control, prospective cohort, epidemiological, randomized controlled trials, meta-analyses) to provide multiple approaches for the research questions under investigation. Fifth, by combining several studies and sharing data, we worked in a cost-effective way, which will become increasingly important because a growing regulatory pressure will make it more expensive and harder to obtain new data.
All in all, we aimed to bring nuance to the story on the role of fatty acids in depression and in a broader perspective psychiatry in general. By providing the interpretation of fatty acid alterations as an adaptive response, we oppose the widely marketed story of fatty acid supplementation as a panacea for all health problems. Nevertheless, we did not throw out the baby with the bathwater, by providing specific indications and possibilities where fatty acids can be used to improve diagnosis, prognosis and treatment of MDD and related psychiatric disorders.

EPILOGUE

Studies in this thesis have been developed and published in a scientific culture that was in transition. In response to scandals and decreasingly self-evident public trust in general, several initiatives are under way to further improve scientific practice.

Because studies in thesis were primarily funded by an unconditional grant from the Academic Medical Center, there was no external pressure on results or decisions to publish. Nevertheless, editorial and reviewers’ decisions that may have had some influence on the content of the published papers might have been partly (unconsciously) guided by external motives. Moreover, the unconditional grant made it possible to study research questions chosen out of scientific interest, which may not necessarily align with the interests of public stakeholders.

In addition, while study protocols were published a priori in dedicated repositories and all hypothesis were formulated a priori, not all of them have been published beforehand in detail to facilitate optimal control as is currently experimented with in some journals. Furthermore, while a large number of papers in this thesis have been published under open access conditions, not all papers are publicly available yet. Regarding other communication about our findings, in contacts with media we have aimed to bring across nuanced stories, which is not always easy in a mass media culture that is dominated by click bait headlines.

Applying the scientific perspective to investigate scientific practice itself could help to further optimize the scientific culture. Also the interaction between science and politics would be of interest. For example, the current potentially suboptimal practice of distributing research funding based on quantitative measures as number of publications and impact factors seems to be still quietly accepted. This approach seems to create several perverse incentives leading to questionable research practices. An interesting alternative approach can be thought of that uses data on actual obtained deliverables from research funded by earlier funding rounds. This data can be used to develop and constantly update an evidence-based model to predict what research will show the best cost-effectiveness. This model can be used to help decisions on how to distribute research funding, taking serendipity into account. The apparent reluctance of science to investigate itself in order to further improve, could be seen as relatively conservative for a discipline that is all about progressing knowledge limits.
CONCLUSION

Although – contrary to some popular belief – omega-3 polyunsaturated fatty acids are certainly no miracle molecules, increasing evidence confirms an important role for fatty acid alterations in the pathophysiology of MDD and psychiatric disorders in general. Associations with other pathophysiological aspects including biological stress (HPA-axis and oxidative stress), inflammation and structure and function of brain area’s involved in emotional processing, form a complex neurometabolic network that seems to increase vulnerability for MDD (recurrence) and affects treatment outcomes. Clinically, this network can be influenced by lifestyle modification. If this proves to be unfeasible, supplementation of the omega-3 fatty acid EPA may be an effective surrogate in the form of add-on treatment in specific subpopulations, possibly due to the anti-inflammatory effects of its oxidation products. Several lines of research are currently being explored further, including the use of fatty acids as biomarkers to predict antidepressant treatment efficacy using lipidomics. Ultimately, improved neurometabolic insights could hopefully contribute to a reduction of the large and still growing burden of disease from MDD and related psychiatric disorders.


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