Fatty acids in context
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INTRODUCTION

This second Part will first introduce fatty acids, and explain why they may form an important factor in the pathophysiology of MDD and related psychiatric disorders. In addition, we describe studies on fatty acid alterations in MDD, including our DELTA study. This will outline possibilities for fatty acid metabolism to contribute to further understanding of MDD, and personalizing its treatment and prognosis.

Subsequently, in the three chapters of this part, we describe our studies in which we zoomed in on fatty acid alterations in MDD, by providing solutions for statistical methodological issues\(^1\) (chapter 1), investigating bimodal distributions\(^2\) (chapter 2), and nutrigenetic influences\(^3\) (chapter 3). We conclude with a brief discussion of this second Part.

What are fatty acids?

Fatty acids are organic molecules that consist of hydrocarbon (CHx) chains of varying length, containing no [saturated fatty acids (SFAs)], one [monounsaturated fatty acids (MUFAs)] or multiple double bonds [polyunsaturated fatty acids (PUFAs)] (Figure 1).\(^4\) The mono- and polyunsaturated fatty acids are subdivided based on carbon atom number from which the first double bond is positioned, counted from the methyl (i.e. non-acid) end of the fatty acid chain (e.g. in omega-3 fatty acids, the first double bond is located between the third and fourth carbon atom). The major PUFAs belong to the omega (ω or n) -3, -6 and -9 series. In addition, fatty acids from all three main classes can be characterized as “long-chain” if they have over 20 carbon atoms in their chain (Figure 2). Of note, fatty acids can be adjusted by desaturase (adding an extra double bond) or elongase (adding an extra hydrocarbon group) enzymes.\(^5\)

Figure 1. Fatty acids in the membrane phospholipid bilayer. Left and right panels schematically depict (A) Saturated fatty acid; (B) monounsaturated fatty acid; (C) polyunsaturated fatty acid.
Why fatty acids?

**Epidemiological evidence**

Ecological, cross-sectional and prospective data. Humans are incapable of de novo omega-3 and -6 polyunsaturated fatty acid synthesis, and therefore depend on dietary intake. This essential nature could underlie several lines of epidemiological evidence suggesting an inverse association between dietary omega-3 fatty acid intake and the prevalence of psychiatric disorders. An important hint of this inverse association was provided in 1998 by ecological evidence showing a lower prevalence of psychiatric disorders in countries where more (fatty) fish is being consumed, the main dietary source of omega-3 fatty acids. Later, more detailed epidemiological cross-sectional studies largely supported this ecological evidence. In addition, prospective studies generally also corroborated this relationship, providing further support for a possible causal relationship. For example, we recently showed that a higher baseline dietary pattern score, based on omega-3 fatty acid intake, protected against depression over a 9-year period in the InChianti study, a population based study of older persons living in the Chianti region in Tuscany, Italy.
Comorbidity patterns. Additional epidemiological evidence for a role for fatty acid abnormalities in psychiatric disorders is found in comorbidity patterns. The high comorbidity rate of psychiatric disorders with cardiovascular disease (CVD) may hint at a partly shared underlying pathophysiology. Because both are associated with similar alterations in fatty acid intake, this may suggest that fatty acids are transdiagnostically involved in their mutual pathophysiology. In this regard, psychiatric and cardiovascular disease have been suggested to represent two sides of the same coin. Furthermore, diseases caused by genetic disturbances in fatty acid metabolism, such as peroxisomal disorders including X-linked adrenoleukodystrophy, can cause psychiatric symptoms including psychosis, anxiety and depressive behavior. Of note, a study reported that 39% of X-linked adrenoleukodystrophy patients presented with a psychiatric diagnosis, sign or symptom, including mood symptoms, PTSD and schizophrenia.

Evolutionary perspective. Interestingly, evolutionary data also suggest an association between an increase in psychiatric disorder prevalence and a decrease in omega-3 fatty acid intake – together with a relative increase in omega-6 fatty acid intake. Over the past 100-150 years, the ratio of omega-6 to omega-3 fatty acids in our modern Western diets has shown a steep increase from ~1-2:1 to ~20-30:1. Although alternative explanations have also been provided, including cultural changes, this may have contributed to a suggested parallel rise in burden of disease due to psychiatric disorders. Similar observations in more recently modernizing countries could suggest that history repeats itself.

We recently aimed at testing the hypothesis of a mismatch between our modern diet and our evolution-based biological make-up, and showed a positive association between a dietary mismatch score and depression in a large Dutch multiethnic population study [Healthy Life in an Urban Setting (HELIUS)], that survived correction for confounders.

Biological evidence

Lipids constitute more than half of the dry weight of the brain. Approximately one third is accounted for by polyunsaturated fatty acids, forming main building blocks of (neuronal) cell membranes (Figure 3). Through their functional and structural characteristics, fatty acids influence brain physiology in multiple ways, e.g. by affecting neuronal membrane structure, inflammatory regulation, and oxidative stress vulnerability, as described below.

Neuronal membrane structure. Fatty acids form the hydrophobic tails of membrane phospholipids. Double bonds cause curvatures in (poly)unsaturated fatty acids. If phospholipids contain fatty acids with more double bonds, the curvatures decrease adhesive van der Waals forces between them, producing a more fluid membrane. The other way around, saturated fatty acids can be more tightly packed, resulting in a more rigid/stiff membrane. The fluidity of the membrane influences lipid-protein interactions of membrane-bound proteins as neurotransmitter receptors and transporters and ion-channels. Thereby, (neuronal) membrane fatty acid composition can influence membrane potential and synaptic communication, providing an important potential pathway through which fatty acids may be involved in neuronal connectivity and thereby in psychiatric disorder pathophysiology. As an example, the geometrical characteristics of fatty acids in the presynaptic membrane have been suggested to facilitate exocytosis of neurotransmitter-containing synaptic vesicles. Interesting in this regard, the precise
effects of membrane fatty acids on exocytosis are thought to differ depending on both number and placement of double bonds and chain length of the concerning fatty acids.

**Inflammatory regulation.** Through (non)enzymatic oxygenation, fatty acids form the precursors of lipid peroxidation products (oxylipins), inflammatory regulating molecules as eicosanoids, including prostaglandins and leukotrienes (Figure 3). Importantly, inflammatory mediators derived from omega-3 and omega-6 fatty acids are thought to have opposing effects: those from omega-3 fatty acids are considered anti-inflammatory, while those of omega-6 fatty acids are generally pro-inflammatory. Of note, omega-3 and omega-6 fatty acids compete for the same enzymes for metabolization, not only in fatty acid chain remodeling (e.g. elongation and desaturation), but also mobilization (e.g. phospholipase A2-mediated membrane release) as well as inflammatory mediator production (e.g. cyclooxygenase-mediated prostaglandin production). So a greater supply of omega-6 fatty acid to these enzymes implies less metabolization capacity for omega-3 fatty acids. This potentially aggravates their mutually antagonizing effects. Given that many psychiatric disorders are thought to be transdiagnostically characterized by increased inflammation, the increase in dietary omega-6/omega-3-ratio described above may have an important underlying role.

*Figure 3.* Simplified scheme depicting the different roles of fatty acids in the phospholipid cell membrane and associations with other pathophysiological aspects. Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ω, omega.
Oxidative stress vulnerability. Many psychiatric disorders manifest themselves together with a transdiagnostic increase in oxidative stress, defined as an imbalance between increased free radical formation on the one hand, and decreased anti-oxidant defense on the other.\textsuperscript{33,34} Fatty acids strongly differ in their susceptibility to free radical attack (peroxidation), based on the number of double bonds. With every double bond, a fatty acid becomes exponentially more susceptible.\textsuperscript{4} This way, the fatty acid content of e.g. neuronal membranes may determine neuronal vulnerability to oxidative stress. In short, the more polyunsaturated fatty acids a neuronal membrane contains, the more vulnerable it becomes to oxidative stress.\textsuperscript{4} Moreover, peroxidation of polyunsaturated fatty acids result in the production of lipid peroxidation product, i.e. oxylipins. This peroxidation process can substantially alter the biological effects of a fatty acid. For each fatty acid, multiple oxylipin peroxidation products can be produced, each with distinct effects on e.g. inflammation or neurotransmission. However, because these oxylipins cannot be easily measured due to their volatile nature, precise biological effects remain unknown for most oxylipins.

How can we measure fatty acids?
Fatty acid can be measured using gas chromatography in diverse media. Fatty acid alterations have been mostly studied in peripheral samples, e.g. red blood cell membranes, given the impossibility of in vivo brain tissue sampling. Nevertheless, (I) blood concentrations generally show adequate correlations with central nervous system measures, as shown in vivo in cerebrospinal fluid, as well as in postmortem and animal brain studies,\textsuperscript{33,35-42} (II) fatty acids both passively and actively cross the blood-brain-barrier,\textsuperscript{43,44} and (III) findings in post-mortem brains generally corroborate findings from peripheral samples.\textsuperscript{45-47} Plasma fatty acid concentrations are thought to reflect (recent) changes of dietary intake, while red blood cell (membrane), liver and adipose tissue fatty acid concentrations are less directly affected by diet. It becomes increasingly clear that fatty acid concentrations are also substantially regulated by endogenous fatty acid metabolism.\textsuperscript{48} So apart from dietary influences, fatty acid alterations could originate from changes in (non)enzymatic fatty acid remodeling and/or degradation, e.g. in response to oxidative stress.

Fatty acid alterations in depression
Several studies investigated fatty acid concentrations in MDD, most studies focused on omega-3 and omega-6 fatty acids. A meta-analysis on fatty acid alterations in depression showed lower omega-3 fatty acid concentrations compared to controls.\textsuperscript{49} The meta-analysis did not find altered omega-6 concentrations, but did not test omega-6 to omega-3 ratio. Studies assessing this ratio generally find an increased omega-6 to omega-3 ratio. A decreased omega-3 fatty acid concentrations and an increased omega-6 to omega-3 ratio in MDD would be in line with the above evidence suggesting an important (patho)physiological role for omega-3 and -6 fatty acids in the brain.

In our DELTA study on patients with recurrent MDD, we not only measured omega-3 and omega-6 fatty acid concentrations, but extended our measurements to the saturated and mono-unsaturated fatty acids.\textsuperscript{50} Using this more extensive approach, we observed alterations in various other fatty acid concentrations, that extended beyond the omega-3 and omega-6 PUFAs. In brief, we showed lower concentrations of long chained fatty acids and higher concentrations of short chained fatty acids among all fatty acid classes (saturated,
monounsaturated and polyunsaturated), that were state-independent. In addition, we also observed lower omega-3 fatty acid concentrations and an increased omega-6 to omega-3 ratio. These findings showed that fatty acid alterations in recurrent MDD represent a trait, and form a broad pattern that is not limited to the omega-3 and omega-6 PUFAs.

**Zooming in on fatty acid alterations in depression**

Following our finding that fatty acid alterations in MDD form a trait-like pattern that extends beyond the omega-3 and omega-6 classes (i.e. involves generally lower concentrations of long chained and higher concentrations of short chained fatty acids of the saturated, monounsaturated and polyunsaturated fatty acid classes), we aimed at investigating this pattern in more detail in order to nuance findings. To this end, we had to think of solutions for statistical methodological issues that are common in fatty acid research, which we describe in chapter 1 in this second part. In addition, following observations of bimodal distributions of fatty acid concentrations in schizophrenia, we also tested bimodal distributions of fatty acid patterns in MDD, which we describe in chapter 2. Finally, in chapter 3 we describe our investigations on whether the observed fatty acid alterations and distributions may be explained by a nutrigenetic factor: a polymorphism in the fatty acid binding protein 2 (FABP2) gene involved in fatty acid uptake from the gut, and its influence on cardiovascular risk factor waist circumference.