General Discussion

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4.1 The influences of postnatal feeding on neurodevelopment

The central question of the first part of this thesis is whether formula feeding supplemented with long chain polyunsaturated fatty acids (LCPUFAs) has a positive effect on neurodevelopmental outcome of healthy term infants. Our double blind randomized trial indicated that there is a beneficial effect of LCPUFA supplementation on the quality of general movements at 3 months but not on neurodevelopmental outcome at 18 months of age. The importance of LCPUFAs in the functioning of the nervous system is widely acknowledged\(^1,2\). This does not necessarily imply that formula feeding should be supplemented with LCPUFAs. Double blind randomized trials can give the highest possible evidence of the effectiveness of LCPUFA-supplementation on neurodevelopment. We have used three different neurodevelopmental assessments to detect functional and qualitative differences in neurodevelopmental outcomes at 3 and 18 months.

4.1.1 Differences between formula groups

The quality of general movements was assessed at 3 months. Test results are classified into definitely abnormal, mildly abnormal, normal suboptimal and normal optimal general movements\(^3\). Definitely abnormal general movements are associated with a high risk for cerebral palsy and complex minor neurological dysfunction. The latter can be considered as borderline cerebral palsy\(^3\). None of the infants described in this thesis had definitely abnormal general movements, which confirms that we investigated a healthy term population of infants. However, twenty-five percent of all infants showed mildly abnormal general movements, which can be considered as a risk factor for the development of minor neurological dysfunctions, attention problems and behaviour problems at school age, as has been demonstrated in a mixed population of infants with a low and a high risk for developmental problems\(^3,4\). Unfortunately, until now no data is available regarding the significance of mildly abnormal general movements in a population with a low risk for developing neurodevelopmental disorders. Therefore, follow-up of the current cohort of infants is needed to confirm possible long-term consequences of mildly abnormal general movements on later development.

The Bayley Scales at 18 months were used to detect delays in development. The Bayley scales have been designed to detect clinical developmental delays but are not sensitive enough to detect subtle differences in neurodevelopment. Furthermore, some large studies have demonstrated that the concurrent validity and the predictive validity of the Bayley Scales are limited\(^5,6\). This can partly be explained by the large variance in the measurements caused by inherent biological variability of expression of neurodevelopmental milestones. At the age of 18 months, we also used the sensitive neurological examination according to Hempel to assess developmental milestones but apart from that also the quality of motor performance\(^7,8\). Although developmental milestones can be assessed more objectively and quickly than the assessment of the quality of motor behaviour, the examination according to Hempel has proven its usefulness in the detection of small differences in studies investigating the effects of polychlorinated biphenyls (PCBs)\(^9,10\). At present, there is no data available about the (predictive) validity of the examination according to Hempel.

The findings of our randomized trial are in line with similar trials done by others investigating the effects of LCPUFA supplementation. The review of similar LCPUFA-
trials described in the introduction of this thesis (chapter 1 §1.3.3) showed that the effects of LCPUFA supplementation tended to be subtle and transient\textsuperscript{11}. Several other studies have also found a beneficial effect of LCPUFA supplementation on neurological function around the age of 3 months. One study showed a beneficial effect on the Brunet-Lezine Developmental Quotient at 4 months and several other studies found a favourable effect on visual function at 3 to 4 months of age\textsuperscript{12-15}. Randomized controlled trials that used developmental outcomes beyond the age of 4 months indicated little or no beneficial effect of LCPUFA supplementation\textsuperscript{11,16}. It must be stressed that it is inherently difficult to detect differences in neurological condition because of large physiological variations of motor behaviour\textsuperscript{17}. Especially at the age of 6-24 months a ‘latency’ of expression of minor neurological dysfunction takes place which makes the detection of minor neurological signs complicated, because most minor neurological dysfunction manifest itself only when more established complex neural functions develop at school age\textsuperscript{11,18}. At present, no LCPUFA supplementation studies have been published using long-term follow up assessments beyond the age of 24 months. The possibility that beneficial effects of LCPUFA supplementation are expressed at later ages can not be excluded, because many ‘latent’ minor neurological signs are being expressed at school age, i.e. at an age at which complex neuronal circuitries become functionally active\textsuperscript{18}. In summary, postnatal supplementation of LCPUFAs for two months induces subtle beneficial effects on the neurological condition at 3 months but not at 18 months.

4.1.2 Differences between formula fed and breastfed infants

When breastfed infants are compared with infants receiving formula feeding with or without LCPUFAs, we found that breastfed infants have less mildly abnormal and more optimal general movements at 3 months. A subgroup analysis of the breastfed group revealed that breastfeeding for more than 6 weeks was associated with more optimal and less mildly abnormal general movements. These findings were adjusted for obstetrical, social-economical status and other environmental circumstances by means of the Home Observation for Measurement of the Environment score (HOME)\textsuperscript{19} to find that they remained statistically significant. Another alternative explanation for the observed beneficial effect of breastfeeding could be that infants who have a more favourable neurological condition are more likely to be breastfed for longer durations. Although the latter two explanations were not supported by a study of Lucas and Morley who found that the IQ of children who had been fed human milk by nasogastric tube was 8 points higher at 8 year compared to children who received formula by nasogastric tube\textsuperscript{20}. It is of importance to note that the children in this study were not randomized with respect to breast or formula grouping. To prove that breastfeeding induces beneficial effects, double blind randomized trials must be performed. However, randomized controlled trials on breastfeeding are not ethically justified. Recently, it became clear that maternal IQ explains a great deal of the observed beneficial effects of breastfeeding on cognitive development compared with formula fed infants\textsuperscript{21}. Some studies indicate that breastfeeding is associated not only with a better cognitive development but also with a somewhat better neurodevelopmental outcome\textsuperscript{10,22,23}. For instance, Lanting et al. found that infants who received ≥ 6 weeks breastfeeding had more fluent movements at the age of 42 months\textsuperscript{10}. At the age of 18 months we could no longer find a beneficial effect of breastfeeding which could be explained by the ‘latency’ of expression of minor neurological signs at this age as has been described earlier in this section\textsuperscript{17}. Our negative study results at 18 months are in line with a similar large randomized trial of Auestad et al. 2001 who also showed no effect of
breastfeeding on neurodevelopmental outcome at 18 months\textsuperscript{24}. To summarize, breastfeeding for more than 6 weeks was associated with a better neurological condition at 3 months but not at 18 months.

4.2 Prenatal fatty acid status and neurodevelopment

The second part of this thesis deals with the question whether prenatal LCPUFA status affects neurodevelopmental outcome. Prenatal fatty acid status was associated with neurodevelopmental outcome at birth and at the ages 3 and 18 months after term, an effect that was independent of type of postnatal feeding. To assess the relationship between intrauterine fatty acid status and neurodevelopmental outcome we used the measurement of the fatty acid composition of the walls of the umbilical vessels as a proxy for prenatal fatty acid status of infants\textsuperscript{25}. Note that information is not yet available about the relationship between the fatty acid composition of the umbilical vessels and the brain. However, Markrides et al.\textsuperscript{26} provided evidence that erythrocyte DHA status of infants aged less than 48 weeks correlated with the DHA composition of the brain. Because the umbilical vein LCPUFA status is likely to be related to the concurrent prenatal erythrocyte LCPUFA status, it is plausible that umbilical LCPUFA status is also a valid marker of the LCPUFA content of the brain\textsuperscript{25}. Indeed, animal studies have indicated that dietary alterations in LCPUFAs during the growth spurt induce differences in both peripheral tissue and cerebral cortex LCPUFA composition that in turn induces changes in neurodevelopmental outcome\textsuperscript{1,27}. The relationships between the peripheral LCPUFA status and the LCPUFA contents of the brain seem to vanish beyond the age of 2 years at which age the growth spurt ends\textsuperscript{1}. A study of Carver et al.\textsuperscript{28} indicated that the fatty acid composition of erythrocytes measured at autopsy is not a reliable predictor of the fatty acid composition of the cerebral cortex of children aged 2 to 18 years.

Dijck et al\textsuperscript{29} demonstrated a positive relationship between prenatal LCPUFA status and the neurological condition immediately after birth in the groups studied in the present thesis. At 3 months, we found that infants showing mildly abnormal general movements had a somewhat less favourable prenatal LCPUFA profile compared to the infants with normal quality of general movements. The observed differences in LCPUFA status between normal and mildly abnormal general movements were small but statistically significant.

At 18 months we found that prenatal DHA and AA status showed a positive relationship with neurological condition as has been measured with the neurological optimality score (NOS) of the Hempel assessment. However, the positive relationship between AA and neurological condition did not retain its significance after correction for confounders. Of importance is to note that there was no linear relationship between the DHA status in the umbilical vein and the NOS. Infants who had DHA status within the lowest quartile, showed a less favourable NOS than infants with a higher DHA status. Therefore, it seems that a minimal threshold amount of DHA supply from the mother is necessary for optimal neurological condition at 18 months.

Remarkably, supplementation of LCPUFAs or breastfeeding did not modify the observed associations between prenatal fatty acid status and neurodevelopment at birth and at 3 and 18 months in the multivariate analyses. This supports the notion that prenatal LCPUFA supply is more important than postnatal feeding. There were no significant interaction effects of prenatal LCPUFA status and type of postnatal feeding on neurological condition at 3 and 18 months. Therefore, apart from the evaluation of the effects of postnatal feeding...
on neurodevelopment, also the relationships between the prenatal LCPUFA status and neurological outcome can be studied in this thesis.

We found that the relationship between prenatal LCPUFA status and the quality of general movements at 3 months only was related with the LCPUFA status in the umbilical arteries, carrying the LCPUFAs away from the foetus to the placenta. The umbilical artery fatty acid composition is positively affected by placental supply and fetal synthesis and negatively influenced by fetal extraction of fatty acids. We did not find any differences in umbilical vein fatty acid composition between infants with normal GMs and mildly abnormal GMs. This might imply that an inadequate maternal fatty acid supply was not a determinant of the quality of GMs in this study population. Instead, our data suggest that the development of mildly abnormal GMs might be associated particularly with the impaired extraction or increased dilution of AA and EFA driven by fetal metabolism and not by maternal supply. It could be that dilution of EFA and LCPUFAs in the umbilical artery is the result of an increased maternal supply of glucose to the placenta that induces extra de novo fatty acid synthesis from glucose, especially saturated fatty acids and MUFAs in the fetus.

A more detailed inspection of the relationships between prenatal DHA and AA status and neurodevelopment revealed that a higher AA status was more related with the early neurological condition (at birth and at 3 months) than with the neurological condition at 18 months. It should be kept in mind that only preliminary conclusions can be drawn from these aforementioned results regarding the relationship between prenatal LCPUFA status and neurological condition. More measurements in time are needed to trace the maternal LCPUFA status during gestation to draw definite conclusions regarding the relationship between the prenatal LCPUFA status and neurodevelopmental outcome of the foetus or infant.

Double blind randomized trials in which maternal supplementation of LCPUFAs during pregnancy improve the LCPUFA status of the foetus can give also valuable insights about the effects of improved LCPUFA status on neurological development.

A secondary aim of the analysis was to study the potential negative effects of trans fatty acids on neurodevelopment. Deesi and other members of the LCP-project had already found a negative relationship between the trans fatty acid content and LCPUFA status in the umbilical vein in the population studied in this thesis. Indeed, trans fatty acids were negatively related with the neurological optimality score at 18 months. The relationship between trans fatty acids and neurodevelopmental outcome was even stronger than that between the LCPUFA status and outcome. Multivariate regression analyses confirmed the association which also revealed that the association was independent of LCPUFA status.

This indicates that the presumably negative effects of trans fatty acids on neurodevelopment at 18 months can not only be explained by the decrease of the LCPUFA contents in the umbilical vessel lipids. Overall, the results are to be interpreted with caution because we report associations which are not cause-effect relationships between prenatal fatty acid status and neurodevelopment. Therefore these findings should be confirmed by other future studies (e.g. by randomized controlled trials). To illustrate this point, trans fatty acids may be a marker for an unhealthy lifestyle. However, in our study low socioeconomic status was not related with trans fatty acid intake in our study which is also in line with a recent report of the Dutch Ministry of Health, Welfare, and Sports. Since endogenous trans fatty acids synthesis is not possible, the prenatal trans fatty acid content in the umbilical wall is a representation of the dietary intake of trans fatty acids by the mother. These findings suggest that avoidance of the intake of trans fatty acids could prevent a less favourable neurological condition at 18 months. High quality studies are
urgently needed to confirm the relationship between maternal *trans* fatty acid intake and less favourable neurological condition.

### 4.3 Perspectives on future research

The effects of LCPUFA supplementation on neurodevelopmental outcome may be detected when large randomized trials are being performed with relatively high concentrations of LCPUFAs, in particular DHA (≥ 0.30%) and with little attrition at follow up. Potentially, subtle effects are more likely to be found in qualitative differences in motor development than in motor milestone achievements. Long-term follow-up of our study cohort is currently performed at school age at which age we are able to detect potential beneficial effects of LCPUFAs on complex motor and cognitive tasks.
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