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List of abbreviations

CE cholesterol ester(s)
LCPUFA long chain polyunsaturated fatty acid(s) (>C20)
MDI mental development index
PDI psychomotor development index
PLT platelet(s)
RBC erythrocyte(s)
RN ribonucleotide(s)
SDS standard deviation score
SGA small for gestational age
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Chapter 1 describes the state of the art of long chain polyunsaturated fatty acids (chain length >C20; LCPUFA) in infant nutrition. LCPUFA are important components of cell membranes, notably in the brain and retina, and precursors of eicosanoids. Before birth, LCPUFA accumulation in the fetus results from placental transfer of maternal LCPUFA. After birth, breast milk supplies the infant with all the LCPUFA of the ω6 and ω3 series it needs. The infant who receives formula must synthesize LCPUFAω3 and LCPUFAω6 from linoleic (18:2ω6) and α-linolenic (18:3ω3) acids, respectively, or must receive these LCPUFA with the formula feeding. The inability of humans and other mammals to synthesize 18:2ω6 and 18:3ω3 is the reason for their essentiality. They are the precursors of two independent families of LCPUFA: the linoleic acid series (or ω6 series) and the α-linolenic acid series (or ω3 series). The two quantitatively major LCPUFA are docosahexaenoic (22:6ω3) and arachidonic (20:4ω6) acids. The latter is widespread throughout the body. Docosahexaenoic acid has a highly specific distribution and is found at high levels in the brain and retina, particularly in synaptic membranes and the membranes of photoreceptors. In humans desaturation and elongation of ω3 and ω6 fatty acids occurs in the endoplasmatic reticulum. Elongation also takes place in the mitochondria. The synthesis of 20:4ω6 progresses by alternating steps of elongation and desaturation involving the Δ5- and Δ6-desaturases. The synthesis of 22:6ω3 from 18:3ω3 requires not only these steps, but also subsequent chain elongation, Δ6-desaturation and chain shortening by peroxisomal β-oxidation. The latter three reactions were previously considered to be catalyzed by a single Δ5-desaturase. The present idea is that notably Δ6-desaturation and possibly peroxisomal β-oxidation are the rate limiting steps.

Since preterms may have relatively immature enzyme systems for the synthesis of LCPUFA, the small reserve at birth might be insufficient to fulfill their high requirements. Currently there is growing evidence, that the circulating levels of 20:4ω6 and 22:6ω3, as observed in breast-fed infants, cannot be reached in formula-fed infants by only manipulating the contents of 18:2ω6 and 18:3ω3 in their formula. It seems that 20:4ω6 is only conditionally essential for (pre)term infants if the Δ6-desaturation step is inhibited for a long period by an excess of the competing 20:5ω3 in the diet. For 22:6ω3 the situation is very different. Both preterm and term infants who received formulae without 22:6ω3 not only showed low RBC 22:6ω3 contents but also a RBC 22:6ω3 status related response on visual acuity and electroretinogram responses. The latter has been particularly found in studies in preterms. In formula-fed infants contents of 22:6ω3 in brain tissue are also lower in comparison with breast-fed infants. Therefore, there is general agreement that 22:6ω3 is conditionally essential in preterm infants. Chapter 1 also deals with
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LCPUFA metabolism during pregnancy and in the brain, and reviews recent studies on the fatty acid composition of human milk and on the LCPUFA status of infants who receive formula either without LCPUFA, with ribonucleotides (RN) or with LCPUFA.

Chapter 2 gives background information on the studies presented in the thesis. The studies were performed in low birthweight (<2500 g) infants, both preterms and small for gestational age (SGA) term infants. We conducted 2 randomized supplementation studies in formula-fed infants. In the first study (described in Chapter 3), we supplemented ribonucleotides (RN), substances naturally occurring in human milk. In the second study (described in Chapter 4), we made use of fish oil as a source of LCPUFAω3 and evening primrose oil as a source of 18ω6. The supplements in both studies were given up to 6 weeks of age. The aims of the supplementation studies were to investigate whether the additions of preterm formula would raise LCPUFA status in cholesterol esters (CE), erythrocytes (RBC), and in the second study also platelets (PLT), to levels encountered in breast-fed babies (reference group). In addition, the infants of both supplementation studies were studied for growth up to 6 weeks of age (third study: described in Chapter 5) and subsequently, for development at 19 months (fourth study: described in Chapter 6). The aims of the third study were to explore for correlation between growth parameters of weight, length and occipito-frontal circumference (and from this calculated brainweight) on the one side and 22ω6, 20ω4 intakes during the period days 10-42 and RBC and CE 22ω6 and 20ω4 contents on days 10 and 42 on the other. The aims of the fourth study (as described in Chapter 6) were to identify which factors of nutrition, fatty acid status, growth, and perinatal and of the mother may effect the outcome of mental and psychomotor development at 19 months.

In Chapter 3 we described the effects on LCPUFA status of supplementation of RN to preterm formula. It has been suggested that dietary RN augment LCPUFA synthesis in neonates by induction of hepatic and/or intestinal desaturases. Human milk RN belong to the, so called, non-protein nitrogen fraction. Formulas that are based on cow's milk have low RN concentrations. We investigated whether RN supplementation of a regular formula for premature infants (PRE; PRE+RN) raised RBC and plasma CE LCPUFA of low birthweight babies to those encountered in breast-fed counterparts. PRE and PRE+RN contain 18ω3 but no appreciable LCPUFAω3. From days 11 to 42, 31 babies received PRE and 37 PRE+RN. Eleven breast-fed babies served as reference group. RBC and CE fatty acids were determined on days 11, 21 and 42. The courses of various RBC ω3-fatty acid ratios may especially reflect desaturase-chain elongation activities during feeding with PRE and PRE+RN. Differences of the ratios RBC 20.5ω3/18.3ω3, 22.5ω3/18.3ω3 and

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22:6ω3/18:3ω3 during the course of the study between the PRE and PRE+RN fed infants could not be distinguished. This applied also for the courses of the RBC ω6-fatty acid ratios 20:3ω6/18:2ω6, 20:4ω6/18:2ω6, 22:4ω6/18:2ω6 and 22:5ω6/18:2ω6. Above mentioned ratios of ω3 and ω6 fatty acids were lower on day 42 than those of breast-fed babies. On day 42, both 22:6ω3 and 22:5ω3 were, however, higher in RBC of breast-fed babies, compared with those of PRE and PRE+RN fed babies. In addition, there were no differences in RBC and CE fatty acid courses of PRE and PRE+RN fed infants. On day 42, formula-fed babies had lower RBC and CE LCPUFAω3, notably 22:6ω3, and LCPUFAω6, notably 20:4ω6, compared with breast-fed babies. Subdivision into gestational age and bodyweight matched subgroups gave similar results. We concluded that, RN do not result in higher RBC and CE LCPUFA in a group of formula-fed, predominantly preterm, low birthweight infants. This study confirmed the importance of formula 22:6ω3 supplementation because of immature perinatal Δ⁶desaturation capacity.

Chapter 4 addresses the question whether addition of evening primrose as a source of 18:3ω6 and fish oils as a source of LCPUFAω3 to formula raises LCPUFA in plasma CE, RBC and PLT to levels encountered in breast-fed babies. Low birthweight infants (gestational ages 35.7±2.8 weeks) received LCP1 formula (n=16; 0.31 % 18:3ω6, 0.17% 20:5ω3 and 0.20 % 22:6ω3), LCP2 formula (n=13; 0.32 % 18:3ω6, 0.34% 20:5ω3 and 0.43% 22:6ω3) or human milk (n=16) from birth to day 42. Fatty acids were measured on days 10±2, 20±3 and 42±3. LCP1 and LCP2 dose-dependently raised 20:5ω3 and 22:6ω3 (in CE, RBC and PLT). Their levels on day 42 exceeded those of breast-fed babies, except for RBC 22:6ω3. RBC 22:5ω3 (LCP1 and LCP2) and PLT 22:5ω3 (LCP2) were comparable with those of breast-fed infants. CE 18:3ω6 exceeded, and 20:3ω6 (in CE, RBC and PLT) was comparable with, that of breast-fed babies. Levels of 20:4ω6 (CE, RBC, PLT), 22:4ω6 (RBC, PLT) and 22:5ω6 (RBC, PLT) were below those of breast-fed infants. Considering all infants, there were positive relations between dietary percentages 20:5ω3 and 22:6ω3 on the one hand and percentages 20:5ω3 and 22:6ω3 (in CE, RBC and PLT) on the other. Dietary 20:5ω3 and LCPUFAω3 were inversely related to 20:4ω6 and LCPUFAω6 (in CE, RBC and PLT) respectively. Infants fed with LCP1 and LCP2 showed, however, no differences in parameters of LCPUFAω6 status on day 42. We concluded that 18:3ω6 does not augment 20:4ω6 to those of breast-fed infants. It may, however, improve 20:3ω6 status. Fish oil dose-dependently raised 20:5ω3 and 22:6ω3, but decreased 20:4ω6 and other LCPUFAω6. There is, however, a limit to this decline. Platelets seem a useful compartment for the study of LCPUFA status in newborns.

In Chapter 5 we report the relationships between the contents of 20:4ω6 and
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22:6ω3 in RBC and plasma CE on the one hand, and anthropometric measures and growth rates on the other, in the period post-natal days 10-42. One hundred forty-three low birthweight infants received preterm formula without LCPUFA (n=81), with LCPUFA (n=29) or mother’s own milk (n=33). RBC 20:4ω6 contents on day 10 were correlated (p <0.05) with standard deviation score (SDS) weight in all formula-fed infants, and with SDS weight and SDS length in breast-fed infants. Brainweight was related to RBC 22:6ω3 and CE 22:6ω3 contents on both days 10 and 42 in formula-fed infants. Correlations between brainweight and 22:6ω3 status on day 42 were similar in subclasses of term SGA, preterm AGA and preterm SGA infants, who received formula. In AGA preterm babies gain of brainweight and occipito-frontal circumference showed relations with RBC and CE 22:6ω3 contents on day 42. Linear regression models were constructed for brainweight and SDS occipito-frontal circumference on day 42. These brain growth parameters were partially (21-34%) explained by 22:6ω3 status on day 42 and protein intake from days 10 to 42. We concluded that parameters of post-natal 20:4ω6 status are more related to intrauterine growth than to post-natal growth. Parameters of post-natal brain growth are related to RBC 22:6ω3 and CE 22:6ω3 contents, and to dietary protein intake. These results point to the importance of dietary 22:6ω3 for braingrowth during the first post-natal weeks. Because RBC and CE 22:6ω3 levels on day 42 are higher in infants who received formula with 18:3ω6 and LCPUFAω3, we suggest that during the first 6 weeks of life brain growth can be influenced by addition of LCPUFAω3 to formula.

Chapter 6 describes the outcome of development at 19 months in relation to early nutrition, fatty acid status and a number of perinatal, maternal, and anthropometric data in low birthweight infants receiving preterm formula without LCPUFA (n=75), preterm formula with 18:3ω6 and LCPUFAω3 (evening primrose oil, and fish oil at two doses; n=28), or their mother’s own milk (n=27), from birth to day 42. The fatty acid composition of plasma CE, RBC and PLT were determined on days 10, 20 and 42. Bayley's mental development (MDI) and psychomotor development (PDI) indices were assessed at 19 months. The diet groups showed no significant differences in neurodevelopment, but infants who received formula with the highest LCPUFAω3 percentage tended to have higher PDI than those who received formula with lower LCPUFAω3 percentage. Correlation studies showed that PDI related to parameters of LCPUFAω6 (notably 20:3ω6 and 20:4ω6) status on day 10 in breast-fed infants, and that both PDI and MDI related to parameters of LCPUFAω3 and LCPUFAω6 status in the period days 10-42 in formula-fed infants. The most consistent and strongest correlations were between neurodevelopment and parameters of LCPUFAω3 on day 42, in infants who received formula with 18:3ω6 and LCPUFAω3. In plasma CE, RBC and PL the strength of the correlations between PDI and 22:6ω3 contents (day
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42) were as follows: PLT > RBC > CE. In addition, in the infants who received formula with LCPUFA<o>3 and 18:3ω6 the strongest influence of dietary LCPUFA<o>3 was found in the relationship between 22:6ω3 intake in the period days 0-20 with PDI. MDI in these infants was for 20% explained by RBC 22:5ω3 day 42, whereas PDI was for 51% explained by PLT 22:6ω3 day 42. We concluded that a relevant part of development of low birthweight infants at 19 months can be explained by LCPUFA status at age 10-42 days. Our data gave evidence that neurodevelopment of formula-fed low birthweight infants benefits from augmentation of their LCPUFAω3 status by the addition of fish oil.

Chapter 7 contains the general discussion. It opens with a summary of our findings on the importance of LCPUFAω3 status in relation to function. Next we discuss the most optimal LCPUFAω3 composition for preterm formula, based on the functional and biochemical outcomes of these studies. Furthermore, the importance of the LCPUFAω3 supplementation and the LCPUFA status in the formula-fed infants are discussed against the background of human milk as a reference. Development was consistently related to the status of all LCPUFAω3 (20:5ω3, 22:5ω3 and 22:6ω3) and brain growth to 22:6ω3 status. Psychomotor development at 19 months seems to be dose-dependently related to both dietary 22:6ω3 intake and 22:6ω3 status. Our findings of an association of both dietary 22:6ω3 and 22:6ω3 status during the first 6 post-natal weeks with psychomotor development are in line with other reports on visual development in very preterm and term infants. Like in our study with infants who received formula with 0.43% 22:6ω3, the infants in the other reports showed at study end higher RBC 22:6ω3 contents than the human milk reference group. The one and only relationship between RBC 22:5ω3 status and MDI in the infants who received formula with LCPUFAω3 and 18:3ω6 raises the question whether 22:5ω3 is of importance for cognitive development and 22:6ω3 not. The formula-fed low birthweight infant supplemented with fish oil is provided with all LCPUFAω3 related to both cognitive and motor developments. Our studies provide no arguments for the essentiality of 20:4ω6 in post-natal nutrition. However, supplementation of preterm formula with 18:3ω6 is indicated, since it augments 20:3ω6 status and because it is unknown whether the decrease of 20:4ω6 status due to LCPUFAω3 supplementation may be enhanced by the absence of 18:3ω6 in the formula. The statistical relationship between 22:4ω6 and psychomotor development appeared manifest only in the infants who received formula with LCPUFA. Except for 22:4ω6, the status of all the LCPUFA that related to brain growth, and psychomotor and mental development was similar or even higher in the infants who received the formula supplemented with the highest LCPUFAω3 dose (0.80%), compared with breast-fed infants. Possibly, the most important advantage of human milk from the LCPUFA point of view is its LCPUFA
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balance. This may be important for the infant to reach balance between the LCPUFA precursors of the eicosanoids. We found contents of 22:6ω3 in mature milk of mothers of low birthweight infants that may cause intakes of 11 ± 6 mg/kg/day. These intakes are lower than a current recommendation for formula-fed preterm infants (35-75 mg/kg/day). Mothers of LBW infants should be encouraged to eat fish, or preferably to consume purified fish oil. The greatest challenge in future LCPUFA research might be to elucidate the involvement of LCPUFA in the intrauterine conditions which finally result in the birth of SGA low birthweight infants.