Hester Vlaardingerbroek

Content Matters!

QUANTITY AND QUALITY OF PARENTERAL NUTRITION FOR PRETERM INFANTS

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Content Matters!

QUANTITY AND QUALITY OF PARENTERAL NUTRITION FOR PRETERM INFANTS

Inhoud is belangrijk!

Hoeveelheid en kwaliteit van parenterale voeding
voor te vroeg geboren kinderen

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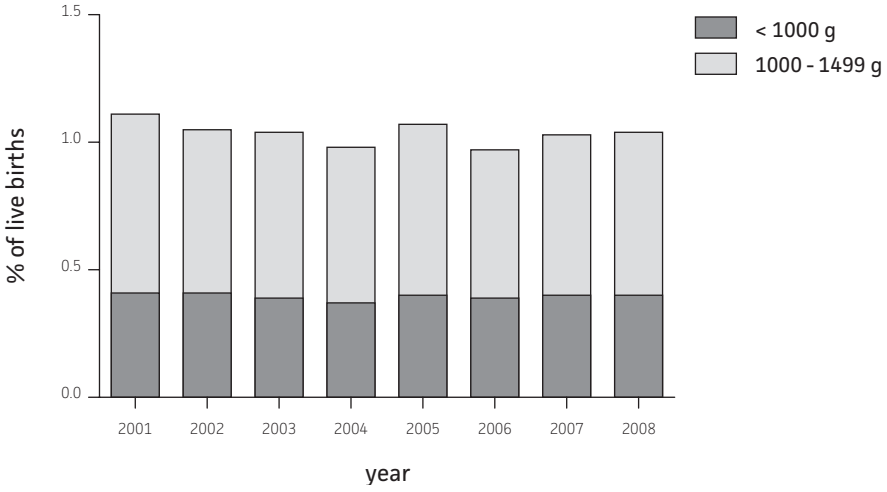
— Safety and efficacy of early and high-dose parenteral amino acid administration to preterm infants. Hester Vlaardingerbroek, Johannes B. van Goudoever, Chris H.P. van den Akker. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2009; 4: 021, 1.

CHAPTER 1
General introduction

Prematurity

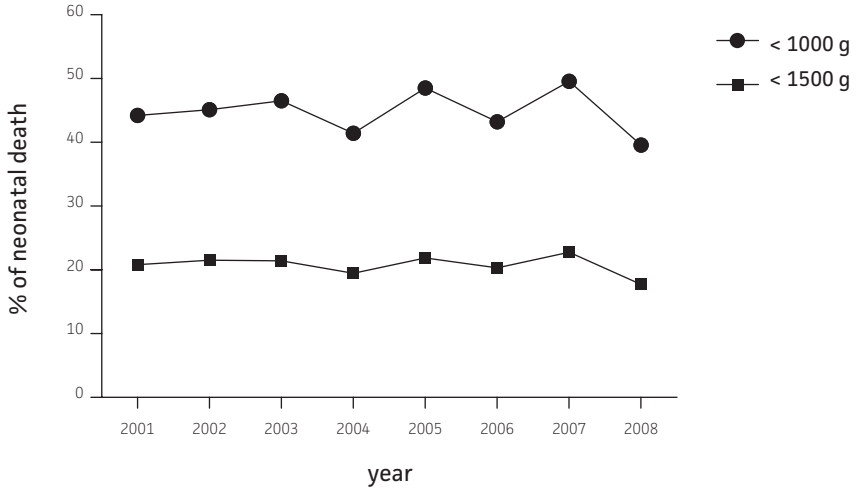
Preterm birth, defined as being born prior to 37 completed weeks of gestation, is the leading cause of perinatal morbidity and mortality in developed countries. In 2008 the incidence of preterm births was about 7 % of all live births in the Netherlands, while the incidence of very preterm births – that is < 32 weeks gestational age – was 1 %.[1] In many other developed countries, the incidence of preterm births is about 5-9 % of all live births, while it is 12-13 % in the USA.[2]
Infants can not only be classified based on gestational age, but also on birth weight: low birth weight (LBW; < 2500 g), very low birth weight (VLBW; < 1500 g), and extremely low birth weight infants (ELBW; < 1000 g).

FIGURE 1 Percent distribution of VLBW infants per live births in the Netherlands 2001-2008[1]



As shown in **FIGURE 1**, the incidence of ELBW and VLBW infants born alive in the Netherlands is stable over time. Compared to the era before the major innovations in neonatology, such as artificial ventilation and antenatal steroids, survival rates of very preterm infants raised substantially.[3] However, during the last decade the overall survival rate of infants born ELBW or VLBW did not change in the Netherlands (**FIGURE 2**). This is in agreement with survival rates in other developed countries.[4-5] Generally, the rate of survival to discharge home is approximately 85 % of VLBW infants.[5] The incidence of most morbidities such as late-onset sepsis, necrotizing enterocolitis, bronchopulmonary dysplasia, and intraventricular hemorrhage associated with prematurity has remained relatively stable as well.[5-6] Approximately 55 % of VLBW infants survive without complications, and approximately 30 % of VLBW infants survive with complications such

FIGURE 2 Percentage of neonatal death (day 1-28) per weight category for live births in the Netherlands 2001-2008[1]



as bronchopulmanory dysplasia, severe intraventricular hemorrhage, and/or necrotizing enterocolitis.[5] Survival rates for extremely preterm infants (< 27 week gestational age) are more or less comparable: 47-57 % of extremely preterm infants survive without severe neonatal morbidities.[7-8] At 2 years of age 71 % of these infants were free from neurodevelopmental disability.[7] The likelihood of survival without serious morbidities increases with gestational age. In addition, Tyson et al[9] and Kugelman et al[10] showed that exposure to antenatal corticosteroids, female sex, singleton gestation, and higher birth weight are also associated with a decrease in the risk of death or survival with neurodevelopmental impairment. This risk reduction was similar to that associated with a one-week increase in gestational age.[9]

Setting the stage

Malnutrition during the critical stages of development of preterm born infants is associated with long-lasting negative effects on growth[11] and neurodevelopment;[12] at least until school age and possibly also into adulthood.[13] Postnatal growth retardation is mainly caused by insufficient administration of protein and calories not meeting the requirements for achieving a growth velocity similar to fetal rates.[14] Clinical problems that preterm infants experience, especially during the first several days of life, often prevent matching nutritional needs. Moreover, nutrition is often not given the highest priority. As a result, postnatal growth failure is one of the most commonly observed morbidities in VLBW infants;[15-17] in extremely preterm infants incidences of 75 % have been reported at 28 days of life.[17] Regrettably, in most centers this has not declined much during the last decades.[16, 18] When energy intake is not limited, proteins are pivotal in achieving adequate growth.[19-20]

Parenteral amino acid administration

Due to immaturity of the gastrointestinal tract preterm infants do not tolerate full enteral feeding and are mainly dependent on parenteral nutrition during the first days of life. When receiving only glucose after birth, the estimated protein loss amounts to 1 % of the endogenous body protein per day.[21-22] The resulting protein deficit may be difficult if not impossible to recoup, and hampers the infants' growth and developmental potential.

Routine use of parenteral nutrition in the neonatal intensive care unit (NICU) was implemented in the early 1970s. The first amino acid solutions were based on fibrin and casein hydrolysates. Administration of these solutions was associated with various metabolic disturbances, e.g. hyperammonemia.[23] Disappointingly, the first generation of synthetic crystalline amino acid solutions appeared to cause adverse reactions, such as acidosis, as well.[24] Consequently, preterm infants were not given amino acid solutions during the first postnatal days, under the assumption that they could not tolerate these solutions. Later studies have shown that the complications were likely the result of the manufacturing method and the composition of the amino acid solutions, rather than the amino acids themselves.[25] From then on, crystalline amino acid solutions have been modified successfully to reduce the risk of complications.[26] Nevertheless, fear of metabolic derangements is still firmly rooted in clinical practice.

Safety of amino acid administration is generally based on biochemical parameters such as pH, urea and ammonia concentrations, and concentrations of potentially (neuro)toxic amino acids. However, none of these parameters are specific for amino acid intolerance[22, 27] as they are also influenced by the general clinical status of the neonate.[28] To overcome this general lack of safety markers for nutritional trials, recently an international consensus work group was set-up to agree on a range of outcome measures relevant to nutrition trials in infants and children < 3 years of age.[29]

Approaches to determine amino acid requirements for preterm infants

Amino acids play crucial roles during (early) life as precursor for proteins (and thus growth) and neurotransmitters, as transport molecules, and in cell signaling. Each amino acid has a unique function. However, not much is known about the amino acid requirements for (preterm) infants.

Different approaches can be used in determining the adequate amino acid requirements for preterm infants. First, the intake of the fetus of a similar gestational age can be regarded as suitable. Information about fetal protein requirements and metabolism is limited and most information comes from animal studies, in particular fetal sheep. Under physiological conditions in pregnant ewes, the fetal amino acid uptake exceeds the amount required for protein synthesis. The excess amount of amino acids is oxidized and contributes considerably to fetal energy generation.[30-31] These quantitative balance studies required blood sampling from both the venous and arterial umbilical vessels and measuring flow rates. In humans, this can only be performed safely around birth. Around elective cesarean section human studies on fetal leucine, valine, phenylalanine, and tyrosine kinetics were performed.[32-34] Results demonstrate that the amino acid uptake exceeded the amount that would be necessary for net protein

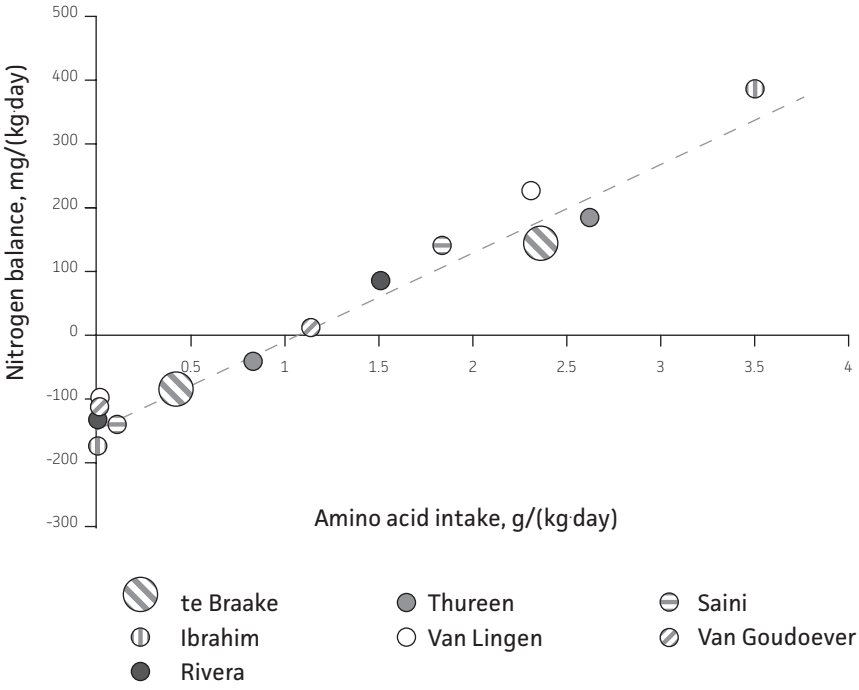
accretion, which indicates that also the human fetus oxidizes amino acids to generate energy.[32] A second method to determine the requirements is the factorial approach. The factorial approach combines the estimated growth rate of a fetus of a certain gestational age with the composition of newly formed tissue. Several drawback of the use of fetal metabolism as a reference standard for preterm infants can be pointed out. First, data on the composition of fetal tissue was derived a very long time ago (early 20th century onwards) from carcasses of deceased fetuses, with little or no knowledge on the condition of the mothers and their fetuses.[35] Secondly, the extra-uterine environment has very different physical and physiological properties than the intra-uterine environment. Thirdly, nutrients not used for tissue deposition, but used for energy generation, are thus not taken into account. Finally, at birth, most preterm infants are ill, requiring ventilatory support, antibiotic therapy and sometimes cardiotonic support. In these conditions, energy and protein need and metabolism will likely be different than in the physiological intra-uterine situation. A third approach is to base the requirements on the composition of human milk. However, the composition of human milk varies at different gestational ages, stages of lactation, and between lactating mothers.[36] VLBW infants fed their own mother's milk may become growth restricted if milk is not supplemented with so-called fortifiers, indicating that human milk composition is not adequately adapted to the nutritional need of the preterm infant below 32 weeks of gestation.[37] In conclusion, the methods of placental/fetal measurements, the factorial approach and (preterm) human milk analysis are all not adequate for determination of the nutritional requirements of preterm infants. Fortunately, more accurate methods are available to assess specific nutritional needs. From a biochemical standpoint, amino acid requirements are mainly determined by the rate of net protein synthesis, which depends on the availability of rate limiting amino acids[38] and on their oxidation rate. The utilization of the amino acid supply for protein synthesis depends for one part on sufficient concomitant energy intake. Generally, an energy supply of 30 to 40 kcal per g amino acids is recommended.[38] Methods to assess the adequacy of amino acid intake include anthropometry (weight and length gain), nitrogen balance, metabolic indices (e.g., amino acid concentration, albumin, pre-albumin, total protein concentrations, plasma urea concentration), whole-body nitrogen kinetics, specific amino acid kinetics and the indicator amino acid method.[38] For determination of the requirement of individual amino acids, the indicator amino acid method is an accurate method. For estimations of the total amino acid or protein requirement, the most widely used method is the nitrogen balance method. As summarized in the ESPGHAN guidelines, a minimum amino acid intake of 1.5 g/(kg·d) is necessary to prevent a negative nitrogen balance.[38] Higher intakes are needed to achieve physiological protein accretion and thus growth.

Timing and amount of amino acid administration

In early studies on parenteral amino acid administration to preterm infants, amino acid administration was initiated several days after birth.[39-40] During the last decades multiple studies have demonstrated that earlier parenteral amino acid administration at amounts of 1.0-2.3 g/(kg·d) can reverse a negative nitrogen or stable isotope

balance, which is indicative of protein accretion and thus growth, even at low caloric intake (**FIGURE 3**).[26, 40-44] This policy also increased plasma amino acid concentrations to reference values[41, 43] and has been associated with improved neurodevelopmental outcomes compared to infants who received no amino acids during the first postnatal days.[12] None of the studies with early amino acid administration up to 2.3 g/(kg·d) reported metabolic acidosis or hyper aminoacidemia.

FIGURE 3 Studies investigating the effects of different amounts of amino acid administration starting during the first two postnatal days in preterm neonates on nitrogen balance.
Legend identifies the primary author; size of the symbol indicates the number of infants. Data adapted from Embleton et al[203]



Recent studies focus on initiation of amino acid infusion at higher doses (≥ 2.4 g/(kg·d) within 24h after birth), together with infusion of glucose and sometimes lipids.[45-49] In addition to previous studies initiating amino acids at low doses, the studies using high doses show a reversal of a negative nitrogen balance into a positive balance, and thus anabolism (Figure 3).[47-49] In addition, plasma concentrations of all essential amino acids and of most non-essential amino acids increased and were more in concordance with reference ranges from healthy fetuses or breast-fed term infants.[48-49] Early higher-dose amino acid administration has beneficial effects on the synthesis of specific proteins as well. For example, upregulation of albumin synthesis with infusion of 2.4 g amino acids/(kg·d) from birth onwards has been demonstrated.[50] However, they were still lower than those measured in utero in fetuses between 28 and 35 weeks of gestation.[51]

Beside raising albumin synthesis rates, early higher-dose amino acid administration also raised the concentration and absolute synthesis rates of glutathione, the major intracellular anti-oxidant.[52] The commonly observed postnatal depletion of glutathione can thus partially be reversed with early amino administration. Potential negative side effects observed with early high-dose amino acid infusion – such as increased mean peak serum indirect bilirubin, lower base excess, lower concentrations of bicarbonate, and increased plasma urea nitrogen – were without clinical implications.[47-48] However, in the study of Blanco et al[45] high-dose amino acid infusion (up to 4 g/(kg·d) on day 3 of life) elevated urea and ammonia concentrations in the most immature infants. Overall, the studies with early high-dose amino acid administration show good efficacy during short-term follow-up without major side effects, although the study of Blanco et al warrants us to use high-dose amino acid administration (up to 4 g/(kg·d)) in the most immature infants. Short- and long-term effects are discussed in more detail in the 'benefits of early amino acid administration' section.

Current guidelines on amino acid intake in preterm infants, mainly based on small studies and expert opinion, are summarized in **TABLE 1**.[38, 53-54] According to these guidelines, amino acids should be started on the first postnatal day and preferably within hours after birth. Starting doses of 1.5 to 3 g/(kg·d) are recommended, with increments to a maximum dose of 4 g/(kg·d) in the next few days.[38, 53-54]

TABLE 1 Current recommendations for parenteral amino acid intake in preterm infants

Reference	Initiation of amino acids	Starting dose in g/(kg·d)	Target dose in g/(kg·d)
Simmer, 2007[54]	First postnatal day	2	-
Ehrenkranz, 2007[53]	Within hours after birth	3	4
ESPGHAN Committee on Nutrition, 2005[38]	First postnatal day	Minimum of 1.5	Maximum of 4

Despite these recommendations, administration of parenteral nutrition still varies widely between NICUs and countries and is often not in accordance with the recommendations.[55-60] Most NICUs start amino acid infusion in preterm infants between 0 and 36 h postnatally.[55-56] Starting doses vary widely: from as low as 0.5-1.0 g/(kg·d) up to 3.5 g/(kg·d). Some NICUs apply a stepwise procedure to reach the target dose of amino acids.[55, 57] However, the preference for a stepwise procedure is solely empirical, non-evidence based but based on fluid limitations, worries about intolerance, and fear of hyperglycemia in case of mixed glucose/amino acid solutions.

Quality of amino acid solutions

Amino acids can be divided into essential and non-essential amino acids, depending on whether they are solely derived from the diet (essential), or whether they can also be produced endogenously from other substrates in sufficient amounts (non-essential). Classically, isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine, tryptophan, and histidine are considered essential amino acids for adults. However, several metabolic processes are not fully developed in preterm and term

infants. Therefore, the following amino acids are conditionally essential for these infants: arginine, glutamine, glycine, proline, taurine, and tyrosine. Cysteine is also often defined as conditionally essential, but recent studies have demonstrated that this is not per se true for growing and healthy enterally fed infants.[61]

Every protein is composed of only 20 different amino acids and the order of amino acids is defined after translation from the DNA. When one of these amino acids is lacking in the (cyto)plasma, protein synthesis ceases and the remaining amino acids are oxidized. In addition, since protein synthesis and remodeling is a continuous process, an insufficient availability of certain (conditionally) essential amino acids may result in increased protein breakdown to provide sufficient amounts of this amino acid. The capacity of infants to regulate the above mentioned processes and thereby regulating amino acid concentrations is probably limited because of their immature kidney and liver functioning. Therefore, (temporary) overabundance of amino acids may occur, which may be detrimental to the infant.

Amino acids requirements differ for parenteral and enteral nutrition, due to bypassing splanchnic metabolism in case of parenteral nutrition. Therefore, not only the quantity, but also the quality (the composition) of the amino acid supply is crucial for achieving optimal growth and development. The overall composition of available parenteral amino acid solutions is probably not optimal, since individual requirements for parenterally-fed preterm infants are not known, except for tyrosine.[62] The lack of consensus and knowledge with regard to the 'optimal' amino acid pattern in parenteral solutions is clearly demonstrated by the diversity in the composition of current pediatric amino acid solutions (**TABLE 2**). Trophamine was originally formulated to match plasma amino acid concentrations of healthy, term, breast-fed infants. The composition of Primene is derived to mimic fetal and neonatal cord blood concentrations. Due to insolubility or instability in solution, these mixtures contain low amounts of or even lack the conditionally essential amino acids glutamine, tyrosine, and often cysteine, although the latter is regularly supplemented separately.[13]

To summarize, optimal growth and development requires focus on a perfectly balanced composition of (parenteral) nutrition for the preterm infant.

Benefits of early amino acid administration

For neonatologists, the ultimate goal of feeding preterm infants is to ameliorate the outcome of these infants to a level that is comparable to healthy term born infants. That is a postnatal growth rate that comes close to fetal growth rate with comparable tissue composition and a functional outcome similar to that of healthy term-born infants, as stated by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition and the American Academy of Pediatrics Committee on Nutrition.[13, 63-64] In daily practice, outcome is based on several criteria, such as postnatal growth in comparison to intrauterine growth charts[65] or to growth charts obtained from preterm infants,[66-68] incidence of specific neonatal diseases, duration of hospital stay and neurodevelopmental outcome. Studies on early amino acid administration have mainly investigated the effects in the direct postnatal phase; only a few studies have investigated medium- or long-term outcome parameters. Metabolic and endocrine long term outcome

TABLE 2 Amino acid concentrations of commercially available parenteral amino acid solutions, g/100 g amino acids

Product	Primene	Travasol	FreAmine III	TrophAmine	Aminoven	Vaminolact	Aminosyn	Aminosyn-PF	Novamine
% amino acids	10%	10%	10%	10%	10%	6.5%	10%	10%	10%
manufacturer	Baxter	Baxter	B. Braun	B. Braun	Fresenius Kabi	Fresenius Kabi	Hospira	Hospira	Hospira
ESSENTIAL									
Isoleucine	6.7	6.0	6.9	8.2	5.0	5.5	7.3	7.6	5.0
Leucine	9.9	7.3	9.1	14.0	7.4	10.8	9.5	11.9	6.9
Valine	7.6	5.8	6.6	7.8	6.2	5.5	8.1	6.6	6.7
Lysine	10.9	5.8	7.3	8.2	9.3	8.6	7.3	6.8	7.9
Methionine	2.4	4.0	5.3	3.4	4.3	2.0	4.0	1.8	5.0
Phenylalanine	4.2	5.6	5.6	4.8	5.1	4.2	4.7	4.3	6.9
Threonine	3.7	4.2	4.0	4.2	4.4	5.5	5.2	5.1	5.0
Tryptophan	2.0	1.8	1.5	2.0	2.0	2.2	1.6	1.8	1.7
Histidine	3.8	4.8	2.8	4.8	3.0	3.2	3.0	3.1	6.0
CONDITIONALLY ESSENTIAL									
Cysteine	1.9	0	0	0.1	0	1.5	0	0	0
Tyrosine	0.9	0.4	0	2.3*	0.4	0.8	0.9	0.6	0.3
Arginine	8.4	11.2	9.5	12.2	12.0	6.3	9.9	12.3	9.8
Glutamine	9.9	0	0	5.0	0	10.9	0	8.2	5.0
Glycine	4.0	10.3	14.0	3.6	11.0	3.2	12.9	3.9	6.9
Proline	3.0	6.8	11.2	6.8	11.2	8.6	8.7	8.1	6.0
Taurine	0.6	0	0	0.2	1.0	0.5	0	0.7	0
NON-ESSENTIAL									
Alanine	7.9	20.7	7.1	5.4	14.0	9.7	12.9	7.0	14.5
Aspartate	6.0	0	0	3.2	0	6.3	0	5.3	0
Serine	4.0	5.0	5.9	3.8	6.5	5.8	4.2	5.0	3.9

* Supplied as L-tyrosine (0.7 g/100 g amino acids) and N-acetyl-tyrosine (1.6 g/100 g amino acids).

parameters following intervention trials with parenteral nutrition have hardly been studied.

Recently reviewed observational studies and a few randomized clinical trials overwhelmingly support the short-term efficacy of early amino acid administration in reversing protein loss.[22,28] However, less is known about the longer-term outcomes of early amino acid administration. Most observational studies[69-71] and randomized clinical trials[69, 72-73] with high doses of parenterally administered amino acids or combined parenteral and enteral administration[14, 74] demonstrated improved growth at hospital discharge or 36 weeks postmenstrual age, although some did not.[46, 75] Beneficial long-term effects on neurodevelopment have been difficult to prove, since nutrition is only

one of the many variables determining neurodevelopmental outcome. Studies have shown that proteins are critical for the development of neurological functions and that malnutrition can alter neuronal density.[76-78] Disease itself may also negatively affect nutritional intake, as is seen in necrotizing enterocolitis (especially those with short-bowel syndrome).

Up till now, studies investigating the effect of high-dose parenteral amino acid administration to preterm infants do not exceed two years' follow-up. In infants with birth weights below 1000 g the effects of having received ≥ 3 g amino acids/(kg·d) or < 3 g amino acids/(kg·d) within the first 5 days of life were retrospectively investigated.[73] At 36 weeks' postmenstrual age, those who had received ≥ 3 g amino acids/(kg·d) during the early postnatal phase showed better weight, length and head circumference. However, at 18 months corrected age, effects on length and weight disappeared, whereas the effects on head circumference only remained in boys; there were no effects on neurodevelopment.[73] Only one follow-up study of a randomized trial in 62 infants reported potentially harmful effects of early high-dose amino acid administration.[45] This study showed that ELBW infants who received high-dose infusion of amino acids (up to 4.0 g amino acids/(kg·d) on day 3) had lower long-term anthropometric measurements and lower cognitive development at 18 months corrected gestational age.[79] However, at 2 years corrected age, the cognitive development was not significantly different between the groups anymore. Although only 63 % of surviving infants were studied at follow-up, and the study was clearly underpowered for neurodevelopmental or growth outcomes (n=32 infants), this study indicates that neonatologists should be cautious with early administration of high doses of amino acids (up to 4 g/(kg·d)), especially to the most immature (< 25 weeks GA) and ELBW infants. In contrast to this randomized study, a retrospective study in 148 ELBW infants showed that every extra gram of protein/(kg·d) or every extra 10 kcal/(kg·d) during the first week of life was associated with an increase of Mental Development Index scores of 8.2 and 4.6 points, respectively and a lower likelihood of length growth restrictions at 18 months.[12] In this study intake during the first week of life ranged from 0.5 to 2.8 g amino acids/(kg·d) and from 40 to 91 kcal/(kg·d). Nutritional intakes beyond the first week of life were not associated with neurocognitive outcome, indicating a very short window of opportunity to improve outcome.

Longer and more precise follow up is available for trials with enteral nutrient interventions. Well-known is the trial by Lucas et al[80] in which four weeks of an enriched formula instead of a standard formula supplemented to enterally fed preterm infants led to an improved neurodevelopmental outcome at 18 months corrected age. At 7.5 years of age, the cognitive development was still better in this group.[81] A subset of the original cohort was reevaluated at ages between 15 and 19 years. Those who received the enriched formula during the first month of life showed higher verbal IQ as well as larger caudate nucleus volumes on magnetic resonance imaging (the latter in boys only).[82-83] Recent randomized trials of a higher enteral (plus parenteral) protein plus energy intake during the first month[84] or year[85] of life show improved developmental scores at 3 months, but not at 9 months, corrected age[84] and increased occipitofrontal circumference and axonal diameters in the corticospinal tract.[85]

The relationship between postnatal growth and neurodevelopment has been illustrated in several studies. In a large observational study in 613 infants born < 30 weeks gestational age, gain in weight and body mass index to term age were associated with better neurodevelopmental outcomes at 18 months' corrected age.[86] In this study results were adjusted for various confounding factors such as neonatal morbidities, postnatal steroids and parental education. In another cohort study of 219 infants with a birth weight < 1250 g, the postnatal growth pattern during the first nine months of life, rather than weight status at birth, was significantly associated with neurological outcome at two years of age, also after adjusting for several morbidities.[87] In addition, a preterm infant's growth rate in the NICU (first few weeks of life) was positively correlated with neurodevelopment and growth outcome as well, as was demonstrated in a cohort of 500 ELBW infants.[88] Neurocognitive outcome at age five to eight was also associated with in-hospital weight gain and postdischarge head circumference growth in VLBW infants.[89-90]

In summary, most of the (retrospective) studies on long-term development indicate that the first few days of life provide a critical window and that nutrition should be part of immediate care in the preterm born infant.

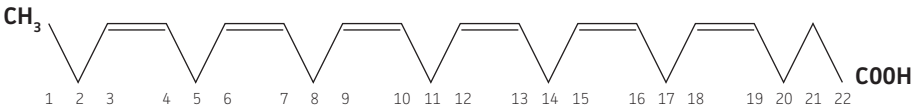
Parenteral lipid administration

As mentioned before, several studies have demonstrated that early parenteral amino acid administration (with starting doses of 1.0-3 g/(kg·d)) can reverse a negative nitrogen or stable isotope balance into a positive balance, which is indicative of protein accretion and thus growth, even at low caloric intake.[26, 40-44, 48] The relationship between protein accretion and energy supply appears to be curvilinear, with most of the beneficial effect of energy on protein gain at intakes of less than 50-60 kcal/(kg·d). Above this amount, the amount of administered amino acids itself will have a higher correlation with anabolism.[91] Optimal glucose and lipid intakes that maximize protein accretion and growth have not yet been determined.[44] Glucose is commonly initiated at a rate aimed to remain euglycemic; that is to minimize the endogenous hepatic glucose production rates but not to exceed the infant's utilization capacity. The initial requirement approximates 4 to 6 mg glucose/(kg·min) and provides 23 to 35 kcal/(kg·d).[92] This low caloric intake might thus not be enough to maximally stimulate protein synthesis. However, administration of higher dosages of glucose usually leads to hyperglycemia, which is associated with morbidity and mortality.[93] During the first few postnatal days, fluid intake in preterm infants is limited,[94] making parenteral lipid emulsions an attractive energy source because of their high energy density (8-9 kcal/g, more than twice than that of protein and glucose). Lipids are not only crucial for providing energy, but they also provide essential n-6 and n-3 fatty acids necessary for central nervous system development. In the absence of an exogenous lipid supply in combination with a very limited endogenous essential fatty acid (EFA) pool in preterm infants, EFA deficiency can develop as early as on the second day of life.[95-97] An inadequate exogenous supply of EFAs and/or their derivatized long-chain polyunsaturated fatty acids (LCPUFAs) during critical periods of rapid brain and retinal growth may lead to long-term impairment in neurodevelopment and visual function.[98]

Nomenclature and classification of fatty acids

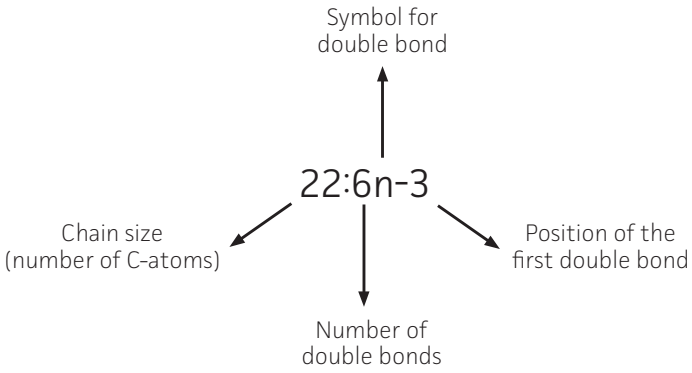
Most fatty acids consist of a straight chain of carbon atoms with a methylgroup (-CH_3) at one side and a carboxyl group (-COOH) at the opposite side (**FIGURE 4**).

FIGURE 4 Fatty acid structure and nomenclature, here docosahexaenoic acid (22:6n-3)



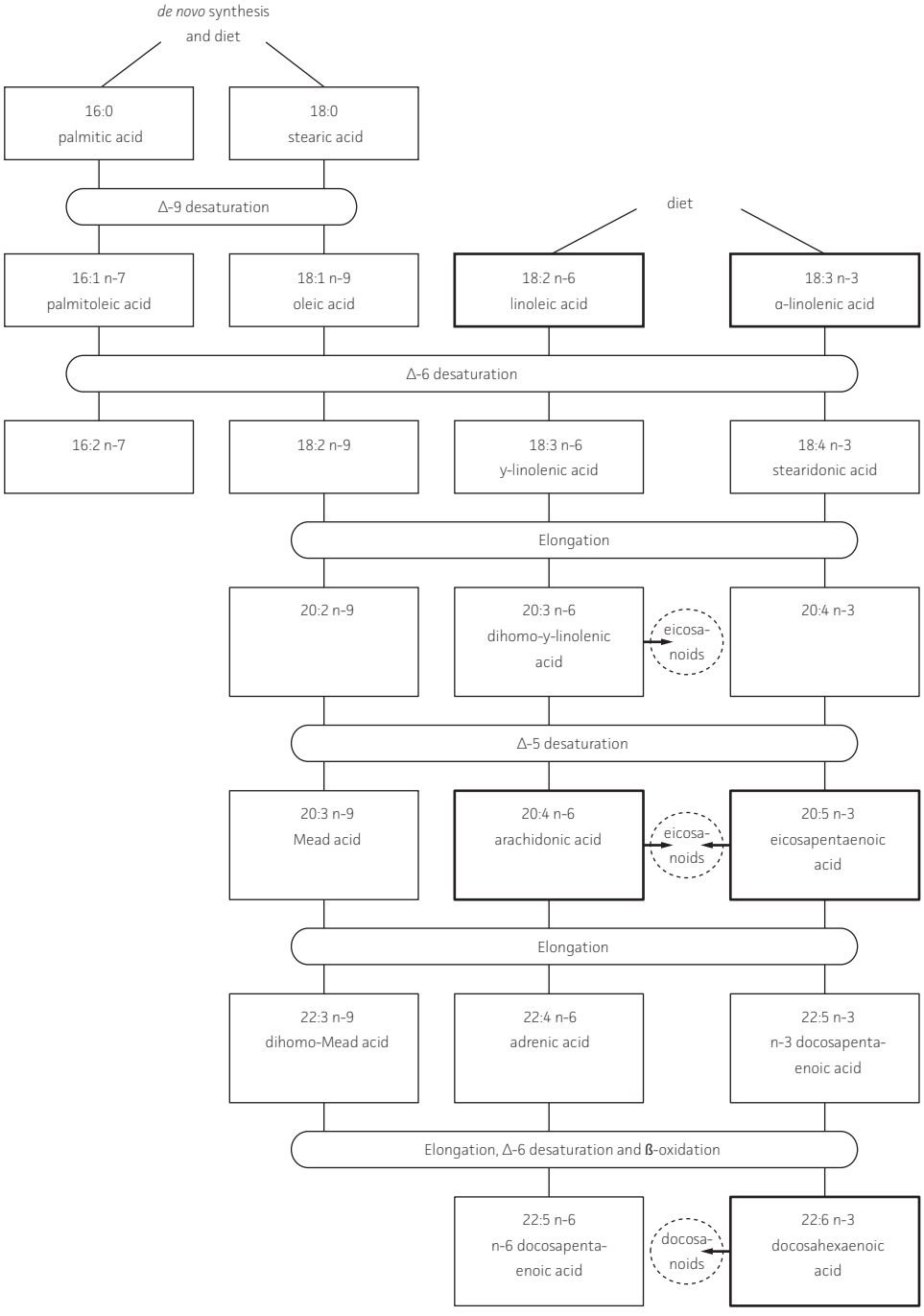
Fatty acids can be classified in various ways. Firstly, they can be classified based on the length of the carbon chain: short chain (≤ 6 carbon atoms), medium chain (8-10 carbon atoms), long chain (≥ 12 carbon atoms), or very long-chain (≥ 22 carbon atoms). Secondly, fatty acids can be classified according to the amount of double bonds in the carbon chain: saturated fatty acids (no double bonds), mono-unsaturated fatty acids (1 double bond), of polyunsaturated fatty acids (PUFA; ≥ 2 double bonds). The most commonly used nomenclature for fatty acids is based on the position of the first double bond counting from the methyl terminal (designated as n or ω) toward the carboxyl terminal. In this way, fatty acids can be divided into 'families', for examples n-3 family, n-6 family, and n-9 family, and are denoted as x:y(n-z). In this general formula, x is the number of carbon atoms, y is the number of double bonds, and z is the first double bond counted from the methyl terminus (**FIGURE 4 AND 5**).

FIGURE 5 General formula for fatty acids, here docosahexaenoic acid (22:6n-3)



Fatty acids can be desaturated (introduction of a double bond) and elongated (introduction of 2 additional carbon atoms) in a specific order (**FIGURE 6**). In contrast to plants, the mammalian body cannot synthesize the precursors of α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6), since it lacks the specific desaturation enzymes. Therefore, ALA and LA are considered essential fatty acids (EFAs). The n-3, n-6, and n-9 fatty acid families share the enzymes for desaturation and elongation of the parent fatty acids to their longer-chain derivatives. These enzymes have a preferred substrate order, with highest preference for the n-3 family and lowest for the n-9 family.[99]

FIGURE 6 The main pathways of fatty acid synthesis and interconversion



Thus, formation of end products depends on the concentration ratio between n-3, n-6, and n-9 fatty acids. This implies, for example, that an abundance of LA (18:2n-6) prevents the elongation and desaturation of oleic acid (18:1n-9) to Mead acid (20:3n-9), a fatty acid that is found in elevated concentrations during EFA deficiency. In addition, desaturation enzymes can be inhibited by high concentrations of their substrates and can be induced by low concentrations of their product.[99]

The LCPUFAs docosahexaenoic acid (DHA) and arachidonic acid (ARA) are considered the most important derivatives of ALA (n-3 family) and LA (n-6 family), respectively. High concentrations of ARA and especially of DHA are found in structural lipids of the cerebral cortex and in the outer segment of the retinal photoreceptors,[100-101] where they play important roles in neuronal function.[99, 102] During intrauterine development, more than 80 % of brain DHA accumulates between 26 and 40 weeks of gestation. As a consequence, all infants born preterm have low concentrations of brain DHA and are very vulnerable to suboptimal nutrition.[14] DHA also fulfills several other crucial roles in human physiology, including neuronal and cellular signal transmission,[103] neurogenesis,[104] immune function, and protection from oxidative stress, the latter two through the production of the docosanoids neuroprotectin D1 and Resolvin D.[103, 105] ARA, dihomo-α-linolenic acid (DGLA) (both n-6 LCPUFAs), and eicosapentaenoic acid (a n-3 LCPUFA) are precursors for eicosanoids. Eicosanoids can be subdivided into prostaglandins, prostacyclins, thromboxanes, and leukotrienes. The n-6 eicosanoids are considered mostly pro-inflammatory, whereas the n-3 eicosanoids are more anti-inflammatory.[106-107]

To a certain degree, infants are capable of de novo synthesis of the LCPUFAs ARA and DHA from the precursors LA and ALA, respectively.[108-112] However, the synthesis rates are insufficient to maintain adequate plasma and erythrocyte concentrations of these LCPUFAs, indicating that ARA and DHA might be conditionally essential fatty acids for preterm and term infants.[111, 113-114] Data from multiple studies in term infants have established that an exogenous supply of DHA of at least 0.2-0.3 % of total fatty acids enhances visual acuity and mental and psychomotor development.[111, 115-116] For preterm infants, an even larger supply might be necessary.

Tolerance and efficacy of early lipid administration

Among different NICUs, the time of initiation of parenteral lipid emulsions to preterm infants varies widely,[56-58] even though postponing lipid administration during this critical period of organ development may lead to insufficient energy supply for protein synthesis and a shortage of EFAs for normal brain development.

Newborn infants adapt very rapidly to parenteral lipid administration, with fat oxidation becoming the main source of energy within hours after starting lipid infusion.[117] In 2005, the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition stated that in newborn infants who cannot receive sufficient enteral feeding, administration of parenteral lipid emulsions should be started no later than on the third day of life but may be started on the first day of life.[64] However, in clinical practice the use of parenteral lipid emulsions in the preterm infant has been limited by concerns of impaired lipid tolerance, including increased albumin-

bound bilirubin displacement and impairment of oxygenation and development of bronchopulmonary dysplasia.[118] Clinically, tolerance of lipid administration is generally monitored by biochemical parameters. However, a specific indicator of lipid intolerance is lacking. Plasma triacylglycerol concentrations are a reflection of lipid clearance. In the case of hypertriacylglycerolemia, lipid infusion can be lowered or temporarily discontinued.[64, 119] The free fatty acid: albumin ratio is an important marker for identifying infants at risk, especially infants ≤ 28 weeks gestational age, for displacement of bilirubin from albumin by free fatty acids.[120-121] However, significant displacement of bilirubin does not occur until the free fatty acid: albumin molar concentration ratios are greater than five, while infusion rates of up to 3.25 g/(kg·d) do not result in ratios over four.[122] Therefore, it is unlikely that lipid infusion at rates of 3-3.5 g/(kg·d) results in increased incidence of hyperbilirubinemia or kernicterus.
In 2005, a meta-analysis of five randomized controlled trials (RCTs) comparing introduction of lipids within the first five days of life versus a later introduction showed no disadvantage for early introduction of lipid emulsions to preterm infants.[123] The primary outcomes of growth, death, and bronchopulmonary dysplasia were not significantly different between the 'early' and 'not early' lipid groups. The secondary outcomes of other pulmonary morbidities, including duration of respiratory support, duration of supplemental oxygen, pneumothorax, pulmonary hemorrhage, and pulmonary interstitial emphysema, and of necrotizing enterocolitis, retinopathy of prematurity, patent ductus arteriosus, sepsis, intraventricular hemorrhage, and significant jaundice also did not show significant differences between both groups.[123] However, cohort studies suggest advantages on neurodevelopment and growth and a reduction of early morbidity with early introduction of lipids and/or a higher energy intake.[12, 124-125] Therefore, future studies should define whether early introduction of lipids offers benefits on short- and long-term in VLBW infants.

Different lipid emulsions: benefits and drawbacks

Since the 1960s, safe commercial parenteral lipid emulsions have been widely used (see **TABLE 3**). These emulsions were developed for use in adults but are also used in children and infants. Parenteral lipid emulsions are composed of triacylglycerols and phospholipids; the latter serve as emulsifiers. In vivo, the triacylglycerols are partly hydrolyzed, and free fatty acids are released. The rate of hydrolyzation varies according to the type of triacylglycerol (e.g., length of the fatty acid, degree of saturation). For example, medium-chain triacylglycerols (MCTs) are hydrolyzed more quickly than long-chain triacylglycerols (LCTs).[126]
Purely soybean oil-based emulsions were the first lipid emulsions available for parenteral use and are still the most often used parenteral lipid emulsion.[127] However, pure soybean oil has several clinical disadvantages. First, soybean oil is very rich in n-6 PUFAs.[127] An excess intake of n-6 PUFAs may result in increased synthesis of proinflammatory eicosanoids[107, 128-129] and an increase in oxidative stress in the critically ill preterm infant.[130-132] This increase occurs while the preterm infant is already very susceptible to inflammation and to oxidative stress due to the high formation of free radicals (e.g., following oxygen administration and infections) and immature antioxidant systems.[52, 133-135]

TABLE 3 Composition of available lipid emulsions*

	FIRST GENERATION			SECOND GENERATION				THIRD GENERATION		
Lipid emulsion	Intralipid	Lipoven**	Liposyn III	Lipofundin MCT-soybean	Structolipid	Lipoven-MCT	ClinOleic	Omegaven	Lipoplus***	SMOFlipid
Manufacturer	Fresenius Kabi	Fresenius Kabi	Hospira	B. Braun	Fresenius Kabi	Fresenius Kabi	Baxter	Fresenius Kabi	B. Braun	Fresenius Kabi
OIL SOURCE, %										
Soybean	100	100	100	50	64	50	20	-	40	30
Coconut (MCT)	-	-	-	50	36	50	-	-	50	30
Olive	-	-	-	-	-	-	80	-	-	25
Fish	-	-	-	-	-	-	-	100	10	15
COMPOSITION OF MAJOR FATTY ACIDS, WT %										
MCTs										
Caproic acid (6:0)	-	-	-	0.5	0.1	0.2	-	-	-	Trace
Caprylic acid (8:0)	-	-	-	29	26	30	-	-	30	17
Capric acid (10:0)	-	-	-	20	10	17	-	-	19	12
Lauric acid (12:0)	-	-	-	1	0.2	0.2	-	-	-	0.2
Long chain triacylglycerols										
Myristic acid (14:0)	0.2	-	Trace	-	-	Trace	0.2	5	0.5	1
Palmitic acid (16:0)	11	12	11	7	7	7	12	12	6	9
Palmitoleic acid (16:1n-7)	-	-	Trace	-	-	0.2	1.5	9	0.6	2
Stearic acid (18:0)	4	5	4	2	3	3	2	4	2	3
Oleic acid (18:1n-9)	24	24	23	11	14	13	62	15	8	29
n-6 Long chain triacylglycerols										
Linoleic acid (18:2n-6)	53	53	53	29	35	27	19	4	24	19
Arachidonic acid (20:4n-6)	-	-	-	0.2	-	-	0.5	2	-	0.5
n-3 Long chain triacylglycerols										
α-linolenic acid (18:3n-3)	8	8	8	4	5	4	2	2	3	2
Eicosapentaenoic acid (20:5n-3)	-	-	-	-	-	-	-	19	3	3
Docosahexaenoic acid (22:6n-3)	-	-	-	-	-	-	0.5	12	2	2
OTHER COMPOUND										
α-Tocopherol (μmol/L)	87	132	NP	395	16	NP	75	505	455	500

* Data were provided by the manufacturers and adapted from Wanten et al.[202] MCT, medium-chain triacylglycerol; NP, not provided.

** Lipoven is also known as Lipovenoes.

*** Lipoplus is also known as Lipidem.

Second, soybean oil emulsions contain high concentrations of phytosterols, which are associated with the impairment of biliary secretion.[136] Phytosterols may act as the 'hepatotoxic' or 'cholestatic' component of soybean oil-based emulsions or may contribute to this phenomenon.[137] These disadvantages led to the development of lipid emulsions with altered compositions to enhance the beneficial effects of certain lipid sources and to minimize the risk of complications. It has been shown that with each reduction in the relative amount of soybean oil, there is a corresponding fall in the concentration of pro-inflammatory n-6 PUFAs[98] and phytosterols.

In second-generation lipid emulsions soybean oil was combined with coconut oil, providing MCTs, or with olive oil, thereby reducing the n-6 PUFA content (Table 3). Administration of these emulsions did not markedly modify fatty acid patterns in cell membranes compared to pre-infusion, as pure soybean oil emulsions do.[138] As said before, MCTs have faster plasma clearance (hydrolyzation) rates than the LCTs in soybean oil. Besides, MCTs do not accumulate in the liver, do not promote the synthesis of pro-inflammatory eicosanoids, nor serve as precursors for oxygen free-radical productions (peroxidation).[139] However, pure MCT emulsions are not tolerated and, even more importantly, will result in an EFA deficiency, making it obligatory to add another lipid type as well. Studies comparing the administration of MCT-soybean oil mixtures to pure soybean oil emulsions given to preterm infants demonstrate that MCT-soybean oil mixtures are well-tolerated in preterm infants.[140-144] However, these mixtures do not seem to spare EFAs and LCPUFAs from oxidation[140, 145] and result in lower LCPUFA concentrations compared to pure soybean oil emulsions during short-term infusion protocols.[140, 142-143] In addition, stable isotope techniques show that MCT-soybean oil mixtures result in a relatively lower protein accretion compared to pure soybean oil emulsions.[141] Thus, besides a reduced n-6 PUFA and phytosterol intake, partial replacement of soybean oil with MCTs does not seem to offer additional clinical benefits.

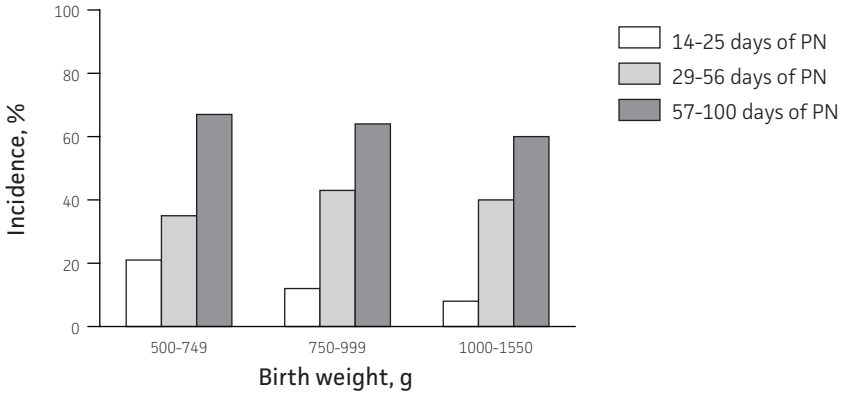
Olive oil is rich in monounsaturated fatty acids and naturally contains the antioxidant vitamin E (α-tocopherol), resulting in a potentially improved vitamin E status[146] and, therefore, improved oxidative stress defense. Studies comparing conventional soybean oil emulsions with olive oil-soybean oil emulsions in preterm infants show that olive oil-soybean oil emulsions seem safe and well-tolerated.[146-152] The administration of an olive oil-soybean oil emulsion results in increased plasma oleic acid concentrations (reflecting the higher oleic acid content of olive oil) and, despite enzyme competition, does not seem to enhance LA and ALA conversion to their long-chain metabolites,[146-147, 151] although one study[146] found higher concentrations of the PUFA intermediates 18:3n-6 and 20:3n-6. Whether the latter resulted in a higher production of pro-inflammatory eicosanoids was not analyzed. In addition, the higher vitamin E content of olive oil did not result in decreased peroxidation.[145-147, 150-151] A stable isotope study in preterm infants showed that olive oil-soybean oil emulsions have a different effect on glucose homeostasis compared to pure soybean oil emulsions.[153] The pure soybean oil emulsion resulted in dysregulated glucose production rates with subsequently higher glucose concentrations due to increased gluconeogenesis while maintaining the rate of glycogenolysis. Because both emulsions are similar with regard to components that

influence glucose metabolism (concentrations of free fatty acids and glycerol), this suggests a direct effect of the fatty acid composition of the emulsion.[153] This study could thus have important implications for clinical care, especially in infants with persistent hyperglycemia or hypoglycemia during lipid infusion.
Third-generation lipid emulsions are characterized by the addition of fish oil and are designed to reach a specific fatty acid pattern. Fish oil is rich in n-3 fatty acids (especially the n-3 LCPUFAs DHA and EPA), decreases the n-6:n-3 ratio and may maintain a better fatty acid balance in membrane phospholipids. The n-3 fatty acids in fish oil may reduce inflammatory and thrombotic responses while protecting tissue micro perfusion and immunity.[129] In addition, fish oil may play a role in the treatment and prevention of parenteral nutrition-associated liver disease/cholestasis.[154-155] Third-generation lipid emulsions are represented by a pure fish oil lipid emulsion, which should be used in combination with a soybean oil emulsion to prevent n-6 fatty acid deficiency; a ready-mixed emulsion of MCT, soybean oil, and fish oil; and a ready-mixed emulsion of soybean oil, MCTs, olive oil and fish oil. Since the introduction of the latter mixture, several studies in adults[156-160] and in children[161] have compared this emulsion with more conventional lipid emulsions. Besides preliminary data,[162-164] results of the administration of fish oil-containing emulsions to preterm infants suggest a slightly better liver tolerability, reduction in oxidative stress, a reduction in severe retinopathy, and improved plasma LCPUFA profile RCTs.[165-169] Hitherto, longer-term safety and efficacy of the LCT-MCT-olive oil-fish oil or pure fish oil emulsions in infants and children have not yet been studied.

Parenteral nutrition associated liver disease

The association between parenteral nutrition and the development of cholestasis in newborn infants emerged soon after the implementation of parenteral nutrition in the NICU. In 1971 Peden et al[170] described the first case of hepatic cholestasis and cirrhosis in a preterm infant receiving total parenteral nutrition. Cholestatic liver disease is one of the most common metabolic problems associated with (long-term) parenteral nutrition in preterm infants.
Parenteral nutrition associated liver disease (PNALD) is defined as cholestasis occurring with the administration of parenteral nutrition, if other specific causes of liver injury have been excluded, namely obstructive causes (e.g., extrahepatic biliary atresia), primary liver disease, and metabolic liver disease (e.g., hereditary tyrosinemia). The incidence of PNALD in infants who receive parenteral nutrition for at least 2 months might be as high as 50 % (**FIGURE 7**), and can lead to end-stage liver disease with the need for transplantation in infants who receive parenteral nutrition for more than 3 months.[171] Risk factors associated with PNALD are immature liver function, lack of enteral feeding, sepsis or infection, and potentially toxic substances or deficiencies in total parenteral nutrition.[171-172] Despite these risk factors, the precise etiology of PNALD in preterm infants remains unclear. In preterm infants, PNALD presents clinically with jaundice and increased plasma concentrations of direct bilirubin. In addition, serum bile acids and liver transaminases might be elevated.[171] Histological features of PNALD are intracellular and intracanalicular cholestasis, steatosis, and periportal inflammation, and

FIGURE 7 Incidence of parenteral nutrition (PN) associated liver disease in very low birth weight infants in relation to the duration of parenteral nutrition. Data adapted from Christensen et al[204]



eventually fibrosis. The only known effective treatment for PNALD is transition to full enteral feeding and discontinuation of parenteral nutrition. As long as the parenteral nutrition is continued, the liver disease is progressive and the mortality rate can be as high as 90 % within a year of diagnosis if infants cannot be weaned off parenteral nutrition or fail to receive a liver transplant.[173]

Lipids and PNALD

Accumulating evidence suggests that lipid emulsions may be an important contributor to the pathogenesis of PNALD. Studies in infants show that increased lipid loads trigger increased serum bilirubin concentrations.[174] Other studies suggest that phytosterols present in soybean oil are associated with the onset of cholestasis,[175] and that these compounds directly reduce the expression of bile acid transporter in cultured hepatocytes.[175-176] Several small, non-randomized trials suggest that switching from soybean oil-based to fish oil-based emulsions may be effective in treatment of PNALD. The beneficial effects of the substitution of soybean oil with fish oil emulsions are likely related to an improved bile flow (possibly due to lower phytosterols content), decreased steatosis, and a shift in the eicosanoid profile towards a less inflammatory state.[177-178] Reports of the beneficial effects of fish oil-based emulsions in infants and children with PNALD are limited to cohort studies, case reports and case series (n=216).[137, 154-155, 179-192] The use of fish oil decreased concentrations of bilirubin and liver enzymes and resulted in reversal of cholestasis in most of these infants and children with PNALD. However, the effects might be confounded by the fact that along with the replacement of soybean oil with fish oil-based lipids, the total daily amount of lipids was also reduced (to 1 g/(kg·d)). Reduction of the lipid load itself has been shown to reverse cholestasis as well.[174, 193] So far, there has been only one report on the adverse effects of fish oil. An infant with PNALD treated with fish oil monotherapy developed burr cell hemolytic anemia, which resolved after discontinuation of the fish oil therapy.[194] The mechanism responsible for

the development of burr cells is unknown but might be related to changes in the lipid composition of the red cell membrane. Another concern with long-term use of pure fish oil emulsions is the development of EFA deficiency. Before fish oil-based emulsions can be widely implemented in the clinical care of preterm infants either from birth onwards or as a treatment in patients with PNALD, well-designed RCTs are imperative to evaluate the safety and efficacy.

Thesis outline and aims

Postnatal nutrition has a large impact on short- and long-term outcome of preterm infants, as illustrated by the association between nutrient supply in the first week of life and later cognitive development in VLBW infants. However, optimal parenteral intakes of amino acids and lipids are not yet established. Not only the quantity, but also the quality and timing of parenteral nutrition might be important for well-being during the neonatal period and beyond. Therefore, the aims of this thesis are as follows:

— To investigate the safety and efficacy of the administration of lipid emulsions from birth onwards to VLBW infants compared to administration from the second day of life onwards.
— To investigate the safety and efficacy of increasing the amino acid intake from 2.4 to 3.6 g/(kg·d) in combination with lipid administration in VLBW infants.
— To investigate if a multi-component lipid emulsion – based on soybean oil, MCTs, olive oil, and fish oil – is superior compared to a pure soybean oil-based emulsion with respect to short- and longer-term outcome in VLBW infants.

In **CHAPTER 2** a systematic review of the literature and meta-analysis was performed to determine the most suitable timing of initiation of lipids and the most suitable lipid composition for parenteral nutrition in VLBW infants.

PART II describes a large randomized controlled trial in VLBW infants randomized to receiving 2.4 g amino acids/(kg·d) without lipids during the first two days of life, to early lipid administration plus 2.4 g amino acids/(kg·d) or to early lipid administration plus 3.6 g amino acids/(kg·d) from birth onwards.
In **CHAPTER 3** the safety and efficacy – in terms of nitrogen balance, urea metabolism and growth – of early lipid administration with or without a higher amount of amino acids is described. **CHAPTER 4** describes the effects on protein synthesis, breakdown, and oxidation by using leucine, α-ketoisocaproic acid, phenylalanine, and tyrosine stable isotopes. In **CHAPTER 5** the effects on albumin metabolism in these infants is evaluated. In **CHAPTER 6** the effects of early lipid introduction and higher amounts of amino acids on anti-oxidant defense mechanisms are analyzed, based on the synthesis rates of the major intracellular non-enzymatic antioxidant glutathione and on parameters of oxidative stress.

PART III compares pure soybean oil-based lipid emulsions with a multicomponent lipid emulsion containing soybean oil, MCTs, olive oil, and fish oil and with a pure fish oil-based emulsion.

CHAPTER 7 AND 8 describe a randomized controlled trial in VLBW infants randomized to a pure soybean oil-based emulsion or to a multicomponent emulsion in a blinded study design. In these chapters the short-term safety and efficacy of the pure soybean oil emulsion and the multicomponent emulsion are compared.

CHAPTER 9 describes a randomized controlled trial in preterm pigs. Preterm pigs have physiological similarities with human preterm infants, and these pigs are, therefore, suitable for studying the effects of parenteral and enteral nutrition.[195-200] The piglet has a similar development of the intestine, similar pathways of lipid and lipoprotein metabolism, similar perinatal timing of brain growth spurt and brain myelinisation, and similarity in essential nutrient requirements and natural milk fatty acid composition.[200] Due to the higher growth speed and maturation, the piglet can be viewed as an accelerated model of postnatal growth and development,[196] making it possible to study nutritional effects in a relatively shorter period of time than in infants. In addition, the piglet model has been shown to be a model for investigation of parenteral nutrition related hepatobiliary dysfunction.[197, 199, 201] In this trial preterm piglets were randomized to enteral nutrition or to total parenteral nutrition with either a pure soybean oil-based emulsion, a pure fish oil-based emulsion, or the multicomponent emulsion. We investigated if fish oil could prevent the development of PNALD and we unravelled some of the potential mechanisms of the development of PNALD.

PART IV summarizes the results of these studies, puts them into perspective and speculates on future studies (**CHAPTER 10 AND 11**).

REFERENCES

1 Stichting Perinatale Registratie Nederland. 10 jaar Perinatale Registratie Nederland, de grote lijnen. Zutphen: Drukkerij Tesink; 2011.
2 Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. Jan 5 2008;371(9606):75-84.
3 Philip AG. The evolution of neonatology. Pediatr Res. Oct 2005;58(4):799-815.
4 Tommiska V, Heinonen K, Lehtonen L, et al. No improvement in outcome of nationwide extremely low birth weight infant populations between 1996-1997 and 1999-2000. Pediatrics. Jan 2007;119(1):29-36.
5 Fanaroff AA, Stoll BJ, Wright LL, et al. Trends in neonatal morbidity and mortality for very low birthweight infants. American journal of obstetrics and gynecology. Feb 2007;196(2):147 e1-8.
6 Lemons JA, Bauer CR, Oh W, et al. Very low birth weight outcomes of the National Institute of Child health and human development neonatal research network, January 1995 through December 1996. NICHD Neonatal Research Network. Pediatrics. Jan 2001;107(1):E1.
7 de Waal CG, Weisglas-Kuperus N, van Goudoever JB, Walther FJ. Mortality, Neonatal Morbidity and Two Year Follow-Up of Extremely Preterm Infants Born in the Netherlands in 2007. PLoS ONE. 2012;7(7):e41302.
8 Express Group. Incidence of and risk factors for neonatal morbidity after active perinatal care: extremely preterm infants study in Sweden (EXPRESS). Acta Paediatr. Jul 2010;99(7):978-92.
9 Tyson JE, Parikh NA, Langer J, et al. Intensive care for extreme prematurity--moving beyond gestational age. The New England journal of medicine. Apr 17 2008;358(16):1672-81.
10 Kugelman A, Bader D, Lerner-Geva L, et al. Poor Outcomes at Discharge Among Extremely Premature Infants: A National Population-Based Study. Arch Pediatr Adolesc Med. Feb 6 2012.
11 Berry MA, Abrahamowicz M, Usher RH. Factors associated with growth of extremely premature infants during initial hospitalization. Pediatrics. Oct 1997;100(4):640-6.
12 Stephens BE, Walden RV, Gargus RA, et al. First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics. May 2009;123(5):1337-43.
13 Hay WW, Jr., Lucas A, Heird WC, et al. Workshop summary: nutrition of the extremely low birth weight infant. Pediatrics. Dec 1999;104(6):1360-8.
14 Dinerstein A, Nieto RM, Solana CL, Perez GP, Otheguy LE, Larguia AM. Early and aggressive nutritional strategy (parenteral and enteral) decreases postnatal growth failure in very low birth weight infants. J Perinatol. Jul 2006;26(7):436-42.
15 Thureen P, Heird WC. Protein and energy requirements of the preterm/low birthweight (LBW) infant. Pediatr Res. May 2005;57(5 Pt 2):95R-8R.
16 Hulst JM, van Goudoever JB, Zimmermann LJ, et al. The effect of cumulative energy and protein deficiency on anthropometric parameters in a pediatric ICU population. Clin Nutr. Dec 2004;23(6):1381-9.
17 Martin CR, Brown YF, Ehrenkranz RA, et al. Nutritional practices and growth velocity in the first month of life in extremely premature infants. Pediatrics. Aug 2009;124(2):649-57.
18 Ehrenkranz RA. Growth outcomes of very low-birth weight infants in the newborn intensive care unit. Clin Perinatol. Jun 2000;27(2):325-45.

19 van Goudoever JB, Sulkers EJ, Lafeber HN, Sauer PJ. Short-term growth and substrate use in very-low-birth-weight infants fed formulas with different energy contents. Am J Clin Nutr. Mar 2000;71(3):816-21.

20 Kashyap S, Schulze KF, Ramakrishnan R, Dell RB, Heird WC. Evaluation of a mathematical model for predicting the relationship between protein and energy intakes of low-birth-weight infants and the rate and composition of weight gain. Pediatr Res. Jun 1994;35(6):704-12.

21 Dusick AM, Poindexter BB, Ehrenkranz RA, Lemons JA. Growth failure in the preterm infant: can we catch up? Seminars in perinatology. Aug 2003;27(4):302-10.

22 Denne SC, Poindexter BB. Evidence supporting early nutritional support with parenteral amino acid infusion. Seminars in perinatology. Apr 2007;31(2):56-60.

23 Johnson JD, Albritton WL, Sunshine P. Hyperammonemia accompanying parenteral nutrition in newborn infants. J Pediatr. Jul 1972;81(1):154-61.

24 Heird WC, Dell RB, Driscoll JM, Jr., Grebin B, Winters RW. Metabolic acidosis resulting from intravenous alimentation mixtures containing synthetic amino acids. The New England journal of medicine. Nov 9 1972;287(19):943-8.

25 Hay WW, Jr. Strategies for Feeding the Preterm Infant. Neonatology. Oct 2 2008;94(4):245-54.

26 Saini J, MacMahon P, Morgan JB, Kovar IZ. Early parenteral feeding of amino acids. Arch Dis Child. Oct 1989;64(10 Spec No):1362-6.

27 Thureen PJ, Hay WW, Jr. Early aggressive nutrition in preterm infants. Semin Neonatol. Oct 2001;6(5):403-15.

28 Kashyap S. Is the early and aggressive administration of protein to very low birth weight infants safe and efficacious? Curr Opin Pediatr. Apr 2008;20(2):132-6.

29 Koletzko B, Szajewska H, Ashwell M, et al. Documentation of functional and clinical effects of infant nutrition: setting the scene for COMMENT. Annals of nutrition & metabolism. 2012;60(4):222-32.

30 Lemons JA, Adcock EW, 3rd, Jones MD, Jr., Naughton MA, Meschia G, Battaglia FC. Umbilical uptake of amino acids in the unstressed fetal lamb. The Journal of clinical investigation. Dec 1976;58(6):1428-34.

31 van Veen LC, Teng C, Hay WW, Jr., Meschia G, Battaglia FC. Leucine disposal and oxidation rates in the fetal lamb. Metabolism: clinical and experimental. Jan 1987;36(1):48-53.

32 van den Akker CH, Schierbeek H, Minderman G, et al. Amino acid metabolism in the human fetus at term: leucine, valine, and methionine kinetics. Pediatr Res. Dec 2011;70(6):566-71.

33 van den Akker CH, Schierbeek H, Dorst KY, et al. Human fetal amino acid metabolism at term gestation. The American journal of clinical nutrition. Jan 2009;89(1):153-60.

34 Chien PF, Smith K, Watt PW, Scrimgeour CM, Taylor DJ, Rennie MJ. Protein turnover in the human fetus studied at term using stable isotope tracer amino acids. Am J Physiol. Jul 1993;265 (1 Pt 1):E31-5.

35 Ziegler EE, O'Donnell AM, Nelson SE, Fomon SJ. Body composition of the reference fetus. Growth. Dec 1976;40(4):329-41.

36 Molto-Puigmarti C, Castellote AI, Carbonell-Estrany X, Lopez-Sabater MC. Differences in fat content and fatty acid proportions among colostrum, transitional, and mature milk from women delivering very preterm, preterm, and term infants. Clin Nutr. Feb 2011;30(1):116-23.

37 Kuschel CA, Harding JE. Multicomponent fortified human milk for promoting growth in preterm infants. Cochrane Database Syst Rev. 2004(1):CD000343.

[38] Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R. 3. Amino acids. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR). J Pediatr Gastroenterol Nutr. Nov 2005;41 Suppl 2:S12-8.

[39] Yu VY, James B, Hendry P, MacMahon RA. Total parenteral nutrition in very low birthweight infants: a controlled trial. Arch Dis Child. Sep 1979;54(9):653-61.

[40] van Lingen RA, van Goudoever JB, Luijendijk IH, Wattimena JL, Sauer PJ. Effects of early amino acid administration during total parenteral nutrition on protein metabolism in pre-term infants. Clin Sci (Lond). Feb 1992;82(2):199-203.

[41] Anderson TL, Muttart CR, Bieber MA, Nicholson JF, Heird WC. A controlled trial of glucose versus glucose and amino acids in premature infants. J Pediatr. Jun 1979;94(6):947-51.

[42] Rivera A, Jr., Bell EF, Bier DM. Effect of intravenous amino acids on protein metabolism of preterm infants during the first three days of life. Pediatr Res. Feb 1993;33(2):106-11.

[43] Van Goudoever JB, Colen T, Wattimena JL, Huijmans JG, Carnielli VP, Sauer PJ. Immediate commencement of amino acid supplementation in preterm infants: effect on serum amino acid concentrations and protein kinetics on the first day of life. J Pediatr. Sep 1995;127(3):458-65.

[44] Thureen PJ, Anderson AH, Baron KA, Melara DL, Hay WW, Jr., Fennessey PV. Protein balance in the first week of life in ventilated neonates receiving parenteral nutrition. Am J Clin Nutr. Nov 1998;68(5):1128-35.

[45] Blanco CL, Falck A, Green BK, Cornell JE, Gong AK. Metabolic responses to early and high protein supplementation in a randomized trial evaluating the prevention of hyperkalemia in extremely low birth weight infants. J Pediatr. Oct 2008;153(4):535-40.

[46] Clark RH, Chace DH, Spitzer AR. Effects of two different doses of amino acid supplementation on growth and blood amino acid levels in premature neonates admitted to the neonatal intensive care unit: a randomized, controlled trial. Pediatrics. Dec 2007;120(6):1286-96.

[47] Ibrahim HM, Jeroudi MA, Baier RJ, Dhanireddy R, Krouskop RW. Aggressive early total parental nutrition in low-birth-weight infants. J Perinatol. Aug 2004;24(8):482-6.

[48] te Braake FW, van den Akker CH, Wattimena DJ, Huijmans JG, van Goudoever JB. Amino acid administration to premature infants directly after birth. J Pediatr. Oct 2005;147(4):457-61.

[49] Thureen PJ, Melara D, Fennessey PV, Hay WW, Jr. Effect of low versus high intravenous amino acid intake on very low birth weight infants in the early neonatal period. Pediatr Res. Jan 2003;53(1):24-32.

[50] van den Akker CH, Te Braake FW, Schierbeek H, et al. Albumin synthesis in premature neonates is stimulated by parenterally administered amino acids during the first days of life. Am J Clin Nutr. Oct 2007;86(4):1003-8.

[51] van den Akker CH, Schierbeek H, Rietveld T, et al. Human fetal albumin synthesis rates during different periods of gestation. Am J Clin Nutr. Oct 2008;88(4):997-1003.

[52] Te Braake FW, Schierbeek H, de Groof K, et al. Glutathione synthesis rates after amino acid administration directly after birth in preterm infants. Am J Clin Nutr. Aug 2008;88(2):333-9.

[53] Ehrenkranz RA. Early, aggressive nutritional management for very low birth weight infants: what is the evidence? Seminars in perinatology. Apr 2007;31(2):48-55.

[54] Simmer K. Aggressive nutrition for preterm infants--benefits and risks. Early Hum Dev. Oct 2007;83(10):631-4.

[55] Collins CT, Chua MC, Rajadurai VS, et al. Higher protein and energy intake is associated with increased weight gain in pre-term infants. Journal of paediatrics and child health. Mar 2010;46(3):96-102.

[56] Hans DM, Pylipow M, Long JD, Thureen PJ, Georgieff MK. Nutritional practices in the neonatal intensive care unit: analysis of a 2006 neonatal nutrition survey. Pediatrics. Jan 2009;123(1):51-7.

[57] Lapillonne A, Fellous L, Mokthari M, Kermorvant-Duchemin E. Parenteral nutrition objectives for very low birth weight infants: results of a national survey. Journal of pediatric gastroenterology and nutrition. May 2009;48(5):618-26.

[58] Grover A, Khashu M, Mukherjee A, Kairamkonda V. Iatrogenic malnutrition in neonatal intensive care units: urgent need to modify practice. JPEN J Parenter Enteral Nutr. Mar-Apr 2008;32(2):140-4.

[59] Hopewell J, Miletin J. Parenteral nutrition in very low birth weight infants in the United Kingdom and Ireland. Ir Med J. Feb 2012;105(2):42-5.

[60] Kirk EL. Audit to determine whether current parenteral nutrition regimens for pre-term infants on the neonatal unit are in accordance with international guidelines. Archives of disease in childhood. 2009;94(7):e2.

[61] Riedijk MA, Voortman G, van Beek RH, Baartmans MG, Wafelman LS, van Goudoever JB. Cyst(e)ine requirements in enterally fed very low birth weight preterm infants. Pediatrics. Mar 2008;121(3):e561-7.

[62] Roberts SA, Ball RO, Moore AM, Filler RM, Pencharz PB. The effect of graded intake of glycyl-L-tyrosine on phenylalanine and tyrosine metabolism in parenterally fed neonates with an estimation of tyrosine requirement. Pediatr Res. Jan 2001;49(1):111-9.

[63] American Academy of Pediatrics Committee on Nutrition: Nutritional needs of low-birth-weight infants. Pediatrics. May 1985;75(5):976-86.

[64] Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R. 1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR). J Pediatr Gastroenterol Nutr. Nov 2005;41 Suppl 2:S1-87.

[65] Fenton TR. A new growth chart for preterm babies: Babson and Benda's chart updated with recent data and a new format. BMC pediatrics. Dec 16 2003;3:13.

[66] Christensen RD, Henry E, Kiehn TI, Street JL. Pattern of daily weights among low birth weight neonates in the neonatal intensive care unit: data from a multihospital health-care system. J Perinatol. Jan 1 2006;26(1):37-43.

[67] Ehrenkranz RA, Younes N, Lemons JA, et al. Longitudinal growth of hospitalized very low birth weight infants. Pediatrics. Aug 1999;104(2 Pt 1):280-9.

[68] Pauls J, Bauer K, Versmold H. Postnatal body weight curves for infants below 1000 g birth weight receiving early enteral and parenteral nutrition. Eur J Pediatr. May 1998;157(5):416-21.

[69] Kotsopoulos K, Benadiba-Torch A, Cuddy A, Shah PS. Safety and efficacy of early amino acids in preterm <28 weeks gestation: prospective observational comparison. J Perinatol. Dec 2006;26(12):749-54.

[70] Vogt RA, Gargus RA, Tucker R, McKinley L, Vohr BR. Impact of early postnatal nutrition on growth in extremely low birth weight infants born small for gestational age. Paper presented at: PAS2004; PAS2004:2525.

[71] Geary CA, Fonseca RA, Caskey MA, Malloy MH. Improved growth and decreased morbidities in <1000 g neonates after early management changes. J Perinatol. May 2008;28(5):347-53.
[72] Kashyap S, Abildskov K, Holleran SF, Ramakrishnan R, Towers HM, Sahni R. Effects of early aggressive nutrition in infants with birth weight (BW) <1250g: a randomized controlled trial. Paper presented at: PAS2007; E-PAS2007: 5912.2
[73] Poindexter BB, Langer JC, Dusick AM, Ehrenkranz RA. Early provision of parenteral amino acids in extremely low birth weight infants: relation to growth and neurodevelopmental outcome. J Pediatr. Mar 2006;148(3):300-5.
[74] Wilson DC, Cairns P, Halliday HL, Reid M, McClure G, Dodge JA. Randomised controlled trial of an aggressive nutritional regimen in sick very low birthweight infants. Arch Dis Child Fetal Neonatal Ed. Jul 1997;77(1):F4-11.
[75] Blanco CL, Falck A, Green BK, Cornell JE, Gong AK. Metabolic Responses to Early and High Protein Supplementation in a Randomized Trial Evaluating the Prevention of Hyperkalemia in Extremely Low Birth Weight Infants. J Pediatr. Jun 25 2008; 153(4):535-40.
[76] Soto-Moyano R, Fernandez V, Sanhueza M, et al. Effects of mild protein prenatal malnutrition and subsequent postnatal nutritional rehabilitation on noradrenaline release and neuronal density in the rat occipital cortex. Brain Res Dev Brain Res. Aug 5 1999;116(1):51-8.
[77] Winick M, Rosso P. The effect of severe early malnutrition on cellular growth of human brain. Pediatr Res. Mar 1969;3(2):181-4.
[78] Morgane PJ, Mokler DJ, Galler JR. Effects of prenatal protein malnutrition on the hippocampal formation. Neurosci Biobehav Rev. Jun 2002;26(4):471-83.
[79] Blanco CL, Gong AK, Schoolfield J, et al. Impact of Early and High Amino Acid Supplementation on ELBW Infants at 2 Years. J Pediatr Gastroenterol Nutr. May 2012;54(5):601-7.
[80] Lucas A, Morley R, Cole TJ, et al. Early diet in preterm babies and developmental status at 18 months. Lancet. Jun 23 1990;335(8704):1477-81.
[81] Lucas A, Morley R, Cole TJ. Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ. Nov 28 1998;317(7171):1481-7.
[82] Isaacs EB, Gadian DG, Sabatini S, et al. The effect of early human diet on caudate volumes and IQ. Pediatric research. Mar 2008;63(3):308-14.
[83] Isaacs EB, Morley R, Lucas A. Early diet and general cognitive outcome at adolescence in children born at or below 30 weeks gestation. J Pediatr. Aug 2009;155(2):229-34.
[84] Tan M, Abernethy L, Cooke R. Improving head growth in preterm infants--a randomised controlled trial II: MRI and developmental outcomes in the first year. Arch Dis Child Fetal Neonatal Ed. Sep 2008;93(5):F342-6.
[85] Dabydeen L, Thomas JE, Aston TJ, Hartley H, Sinha SK, Eyre JA. High-energy and -protein diet increases brain and corticospinal tract growth in term and preterm infants after perinatal brain injury. Pediatrics. Jan 2008;121(1):148-56.
[86] Belfort MB, Rifas-Shiman SL, Sullivan T, et al. Infant growth before and after term: effects on neurodevelopment in preterm infants. Pediatrics. Oct 2011;128(4):e899-906.
[87] Latal-Hajnal B, von Siebenthal K, Kovari H, Bucher HU, Largo RH. Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. J Pediatr. Aug 2003;143(2):163-70.

88 Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics. Apr 2006;117(4):1253-61.

89 Franz AR, Pohlandt F, Bode H, et al. Intrauterine, early neonatal, and postdischarge growth and neurodevelopmental outcome at 5.4 years in extremely preterm infants after intensive neonatal nutritional support. Pediatrics. Jan 2009;123(1):e101-9.

90 Kan E, Roberts G, Anderson PJ, Doyle LW. The association of growth impairment with neurodevelopmental outcome at eight years of age in very preterm children. Early Hum Dev. Jun 2008;84(6):409-16.

91 Thureen PJ, Hay WW, Jr. Intravenous nutrition and postnatal growth of the micropremie. Clin Perinatol. Mar 2000;27(1):197-219.

92 Chawla D, Thukral A, Agarwal R, Deorari AK, Paul VK. Parenteral nutrition. Indian journal of pediatrics. Apr 2008;75(4):377-83.

93 Kao LS, Morris BH, Lally KP, Stewart CD, Huseby V, Kennedy KA. Hyperglycemia and morbidity and mortality in extremely low birth weight infants. J Perinatol. Dec 2006;26(12):730-6.

94 Bell E, Acarregui M. Restricted versus liberal water intake for preventing morbidity and mortality in preterm infants. Cochrane Database Syst Rev. 2008(1):CD000503.

95 Hay WW, Jr. Intravenous nutrition of the very preterm neonate. Acta Paediatr Suppl. Oct 2005;94(449):47-56.

96 Farrell PM, Gutcher GR, Palta M, DeMets D. Essential fatty acid deficiency in premature infants. Am J Clin Nutr. Aug 1988;48(2):220-9.

97 Friedman Z, Frolich JC. Essential fatty acids and the major urinary metabolites of the E prostaglandins in thriving neonates and in infants receiving parenteral fat emulsions. Pediatr Res. Aug 1979;13(8):932-6.

98 Driscoll DF, Bistrian BR, Demmelmair H, Koletzko B. Pharmaceutical and clinical aspects of parenteral lipid emulsions in neonatology. Clin Nutr. Jun 25 2008;27(4):497-503.

99 Innis SM. Essential fatty acids in growth and development. Prog Lipid Res. 1991;30(1):39-103.

100 Connor WE, Neuringer M, Reisbick S. Essential fatty acids: the importance of n-3 fatty acids in the retina and brain. Nutrition reviews. Apr 1992;50(4 (Pt 2)):21-9.

101 Neuringer M, Anderson GJ, Connor WE. The essentiality of n-3 fatty acids for the development and function of the retina and brain. Annu Rev Nutr. 1988;8:517-41.

102 Youdim KA, Martin A, Joseph JA. Essential fatty acids and the brain: possible health implications. Int J Dev Neurosci. Jul-Aug 2000;18(4-5):383-99.

103 Innis SM. Dietary (n-3) fatty acids and brain development. The Journal of nutrition. Apr 2007;137(4):855-9.

104 Novak EM, Dyer RA, Innis SM. High dietary omega-6 fatty acids contribute to reduced docosahexaenoic acid in the developing brain and inhibit secondary neurite growth. Brain Res. Oct 27 2008;1237:136-45.

105 Waitzberg DL, Torrinhas RS. Fish oil lipid emulsions and immune response: what clinicians need to know. Nutr Clin Pract. Aug-Sep 2009;24(4):487-99.

106 Calder PC. Rationale for using new lipid emulsions in parenteral nutrition and a review of the trials performed in adults. Proc Nutr Soc. May 11 2009:1-9.

107 Wanten GJ. Parenteral lipids in nutritional support and immune modulation. Clin Nutr suppl. 2009;4:13-7.

[108] Carnielli VP, Wattimena DJ, Luijendijk IH, Boerlage A, Degenhart HJ, Sauer PJ. The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and linolenic acids. Pediatr Res. Jul 1996;40(1):169-74.

[109] Carlson SE, Carver JD, House SG. High fat diets varying in ratios of polyunsaturated to saturated fatty acid and linoleic to linolenic acid: a comparison of rat neural and red cell membrane phospholipids. The Journal of nutrition. May 1986;116(5):718-25.

[110] O'Connor DL, Hall R, Adamkin D, et al. Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective, randomized controlled trial. Pediatrics. Aug 2001;108(2):359-71.

[111] Koletzko B, Lien E, Agostoni C, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. Journal of perinatal medicine. 2008;36(1):5-14.

[112] Haggarty P. Effect of placental function on fatty acid requirements during pregnancy. Eur J Clin Nutr. Dec 2004;58(12):1559-70.

[113] Uauy R, Mena P, Wegher B, Nieto S, Salem N, Jr. Long chain polyunsaturated fatty acid formation in neonates: effect of gestational age and intrauterine growth. Pediatr Res. Jan 2000;47(1):127-35.

[114] Szitanyi P, Koletzko B, Mydlilova A, Demmelmair H. Metabolism of 13C-labeled linoleic acid in newborn infants during the first week of life. Pediatr Res. May 1999;45(5 Pt 1):669-73.

[115] European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies. Scientific Opinion. DHA and ARA and visual development. Scientific substantiation of a health claim related to docosahexaenoic acid (DHA) and arachidonic acid (ARA) and visual development persuant to Article 14 of Regulation (EC) No 1924/20061. The EFSA journal. 2009;941:1-14.

[116] Makrides M, Gibson RA, McPhee AJ, et al. Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. Jama. Jan 14 2009;301(2): 175-82.

[117] Pierro A, Carnielli V, Filler RM, Smith J, Heim T. Metabolism of intravenous fat emulsion in the surgical newborn. J Pediatr Surg. Jan 1989;24(1):95-101; Discussion -2.

[118] Cooke RW. Factors associated with chronic lung disease in preterm infants. Arch Dis Child. Jul 1991;66(7 Spec No):776-9.

[119] American Society for Parental end Enteral Nutrition. Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. JPEN J Parenter Enteral Nutr. Jan-Feb 2002;26(1 Suppl):106SA-7SA.

[120] Amin SB. Effect of free fatty acids on bilirubin-albumin binding affinity and unbound bilirubin in premature infants. JPEN J Parenter Enteral Nutr. Jul-Aug 2010;34(4):414-20.

[121] Spear ML, Stahl GE, Paul MH, Egler JM, Pereira GR, Polin RA. The effect of 15-hour fat infusions of varying dosage on bilirubin binding to albumin. JPEN J Parenter Enteral Nutr. Mar-Apr 1985;9(2):144-7.

[122] Hammerman C, Aramburo MJ. Decreased lipid intake reduces morbidity in sick premature neonates. J Pediatr. Dec 1988;113(6):1083-8.

[123] Simmer K, Rao SC. Early introduction of lipids to parenterally-fed preterm infants. Cochrane Database Syst Rev. 2005(2):CD005256.

[124] Ehrenkranz RA, Das A, Wrage LA, et al. Early nutrition mediates the influence of severity of illness on extremely LBW infants. Pediatr Res. Jun 2011;69(6):522-9.

[125] Eleni dit Trolli S, Kermorvant-Duchemin E, Huon C, Bremond-Gignac D, Lapillonne A. Early lipid supply and neurological development at one year in very low birth weight (VLBW) preterm infants. Early Hum Dev. Mar 2012;88 Suppl 1:S25-9.

[126] Adolph M. Lipid emulsions in parenteral nutrition. Annals of nutrition & metabolism. 1999;43(1):1-13.

[127] Waitzberg D. Evolution of parenteral lipid emulsions. Clin Nutr Suppl. 2005;1(3):5-7.

[128] Calder PC. The 2008 ESPEN Sir David Cuthbertson lecture: Fatty acids and inflammation - From the membrane to the nucleus and from the laboratory bench to the clinic. Clin Nutr. Feb 2010;29(1):5-12.

[129] Grimm H, Mertes N, Goeters C, et al. Improved fatty acid and leukotriene pattern with a novel lipid emulsion in surgical patients. European journal of nutrition. Feb 2006;45(1):55-60.

[130] Alexander-North LS, North JA, Kiminyo KP, Buettner GR, Spector AA. Polyunsaturated fatty acids increase lipid radical formation induced by oxidant stress in endothelial cells. J Lipid Res. Oct 1994;35(10):1773-85.

[131] Krohn K, Koletzko B. Parenteral lipid emulsions in paediatrics. Curr Opin Clin Nutr Metab Care. May 2006;9(3):319-23.

[132] Pitkanen OM, Hallman M, Andersson SM. Correlation of free oxygen radical-induced lipid peroxidation with outcome in very low birth weight infants. J Pediatr. May 1990;116(5):760-4.

[133] Gitto E, Reiter RJ, Karbownik M, et al. Causes of oxidative stress in the pre- and perinatal period. Biol Neonate. 2002;81(3):146-57.

[134] Perrone S, Salvi G, Bellieni CV, Buonocore G. Oxidative stress and nutrition in the preterm newborn. J Pediatr Gastroenterol Nutr. Dec 2007;45 Suppl 3:S178-82.

[135] Saugstad OD. Oxidative stress in the newborn--a 30-year perspective. Biol Neonate. 2005;88(3):228-36.

[136] Clayton PT, Whitfield P, Lyer K. The role of phytosterols in the pathogenesis of liver complications of pediatric parenteral nutrition. Nutrition. Jan 1998;14(1):158-64.

[137] Puder M, Valim C, Meisel JA, et al. Parenteral fish oil improves outcomes in patients with parenteral nutrition-associated liver injury. Ann Surg. Sep 2009;250(3):395-402.

[138] Carpentier YA, Portois L, Hacquebard M. Intravascular metabolism of lipid emulsions containing ω3 fatty acids. Clin Nutr Suppl. 2007;2:3-5.

[139] Chan S, McCowen KC, Bistrian B. Medium-chain triglyceride and n-3 polyunsaturated fatty acid-containing emulsions in intravenous nutrition. Curr Opin Clin Nutr Metab Care. Mar 1998;1(2):163-9.

[140] Lehner F, Demmelmair H, Roschinger W, et al. Metabolic effects of intravenous LCT or MCT/LCT lipid emulsions in preterm infants. J Lipid Res. Feb 2006;47(2):404-11.

[141] Liet JM, Piloquet H, Marchini JS, et al. Leucine metabolism in preterm infants receiving parenteral nutrition with medium-chain compared with long-chain triacylglycerol emulsions. Am J Clin Nutr. Mar 1999;69(3):539-43.

[142] Rubin M, Naor N, Sirota L, et al. Are bilirubin and plasma lipid profiles of premature infants dependent on the lipid emulsion infused? J Pediatr Gastroenterol Nutr. Jul 1995;21(1):25-30.

[143] Rubin M, Moser A, Naor N, Merlob P, Pakula R, Sirota L. Effect of three intravenously administered fat emulsions containing different concentrations of fatty acids on the plasma fatty acid composition of premature infants. J Pediatr. Oct 1994;125(4):596-602.

144 Lima LA, Murphy JF, Stansbie D, Rowlandson P, Gray OP. Neonatal parenteral nutrition with a fat emulsion containing medium chain triglycerides. Acta Paediatr Scand. May 1988;77(3):332-9.

145 Roggero P, Mosca F, Gianni ML, et al. F2-isoprostanes and total radical-trapping antioxidant potential in preterm infants receiving parenteral lipid emulsions. Nutrition. May 2010;26(5): 551-5.

146 Gobel Y, Koletzko B, Bohles HJ, et al. Parenteral fat emulsions based on olive and soybean oils: a randomized clinical trial in preterm infants. J Pediatr Gastroenterol Nutr. Aug 2003;37(2):161-7.

147 Deshpande GC, Simmer K, Mori T, Croft K. Parenteral lipid emulsions based on olive oil compared with soybean oil in preterm (<28 weeks' gestation) neonates: a randomised controlled trial. J Pediatr Gastroenterol Nutr. Nov 2009;49(5):619-25.

148 Gawecka A, Kornacka MK, Luckiewicz B, Rudzinska I. [Tolerance of two lipid emulsions used in parenterally-fed premature infants - a comparative study]. Medycyna wieku rozwojowego. Jul-Sep 2008;12(3):782-8.

149 Gawecka A, Michalkiewicz J, Kornacka MK, Luckiewicz B, Kubiszewska I. Immunologic properties differ in preterm infants fed olive oil vs soy-based lipid emulsions during parenteral nutrition. JPEN J Parenter Enteral Nutr. Jul-Aug 2008;32(4):448-53.

150 Pitkanen OM, Luukkainen P, Andersson S. Attenuated lipid peroxidation in preterm infants during subsequent doses of intravenous lipids. Biol Neonate. 2004;85(3):184-7.

151 Webb AN, Hardy P, Peterkin M, et al. Tolerability and safety of olive oil-based lipid emulsion in critically ill neonates: a blinded randomized trial. Nutrition. Nov-Dec 2008;24(11-12):1057-64.

152 Demirel G, Oguz SS, Celik IH, Erdeve O, Uras N, Dilmen U. The metabolic effects of two different lipid emulsions used in parenterally fed premature infants - A randomized comparative study. Early Hum Dev. Jan 12 2012;88(7):499-501.

153 van Kempen AA, van der Crabben SN, Ackermans MT, Endert E, Kok JH, Sauerwein HP. Stimulation of gluconeogenesis by intravenous lipids in preterm infants: response depends on fatty acid profile. Am J Physiol Endocrinol Metab. Apr 2006;290(4):E723-30.

154 Ekema G, Falchetti D, Boroni G, et al. Reversal of severe parenteral nutrition-associated liver disease in an infant with short bowel syndrome using parenteral fish oil (Omega-3 fatty acids). J Pediatr Surg. Jun 2008;43(6):1191-5.

155 Gura KM, Duggan CP, Collier SB, et al. Reversal of parenteral nutrition-associated liver disease in two infants with short bowel syndrome using parenteral fish oil: implications for future management. Pediatrics. Jul 2006;118(1):e197-201.

156 Antebi H, Mansoor O, Ferrier C, et al. Liver function and plasma antioxidant status in intensive care unit patients requiring total parenteral nutrition: comparison of 2 fat emulsions. JPEN J Parenter Enteral Nutr. May-Jun 2004;28(3):142-8.

157 Genton L, Karsegard VL, Dupertuis YM, et al. Tolerance to a lipid emulsion containing a mixture of soybean, olive, coconut and fish oils compared with a standard fat emulsion containing only soybean oil. Clin Nutr. 2004;23(4):793.

158 Grimm H. A balanced lipid emulsion - A new concept in parenteral nutrition. Clin Nutr Suppl. 2005;1(3):25-30.

159 Mertes N, Grimm H, Furst P, Stehle P. Safety and efficacy of a new parenteral lipid emulsion (SMOFlipid) in surgical patients: a randomized, double-blind, multicenter study. Annals of nutrition & metabolism. 2006;50(3):253-9.

[160] Schlotzer E, Kanning U. Elimination and tolerance of a new parenteral lipid emulsion (SMOF)--a double-blind cross-over study in healthy male volunteers. Annals of nutrition & metabolism. 2004;48(4):263-8.

[161] Goulet OJ, Corriol O, Alcindor L, et al. A randomized, double-blind study of SMOF 20% vs. Intralipid 20% in infants and children on long-term parenteral nutrition. e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism. 2006;1(2):191.

[162] Rayyan M, Allegaert K, Devlieger H. Parenteral nutrition with a lipid emulsion containing a mixture of soybean oil, olive oil, medium chain triglycerides and fish oil - a randomized, double-blind study in premature infants. Pediatr Crit Care Med. 2007;8((Suppl)):A318.

[163] Tomsits E, Pataki M, Tolgyesi A, et al. The use of n-3 containing lipid emulsion in the parenteral nutrition of premature babies requiring mechanical ventilation. Clin Nutr Suppl. 2007;2(2):148-9.

[164] Tomsits E, Pataki M, Tolgyesi A, et al. Evaluation of the safety and tolerability of SMOFlipid 20% compared to Intralipid 20% in parenteral nutrition of premature babies. Pediatr Crit Care Med. 2007;8 ((Suppl)):A247.

[165] Tomsits E, Pataki M, Tolgyesi A, Fekete G, Rischak K, Szollar L. Safety and Efficacy of a Lipid Emulsion Containing a Mixture of Soybean Oil, Medium-chain Triglycerides, Olive Oil, and Fish Oil: A Randomised, Double-blind Clinical Trial in Premature Infants Requiring Parenteral Nutrition. J Pediatr Gastroenterol Nutr. Jun 3 2010;51(4):514-21.

[166] Skouroliakou M, Konstantinou D, Koutri K, et al. A double-blind, randomized clinical trial of the effect of omega-3 fatty acids on the oxidative stress of preterm neonates fed through parenteral nutrition. Eur J Clin Nutr. Sep 2010;64(9):940-7.

[167] Pawlik D, Lauterbach R, Turyk E. Fish-Oil Fat Emulsion Supplementation May Reduce the Risk of Severe Retinopathy in VLBW Infants. Pediatrics. Jan 3 2011;127(2):223-8.

[168] D'Ascenzo R, D'Egidio S, Angelini L, et al. Parenteral Nutrition of Preterm Infants with a Lipid Emulsion Containing 10% Fish Oil: Effect on Plasma Lipids and Long-Chain Polyunsaturated Fatty Acids. J Pediatr. Feb 28 2011;159:33-8.

[169] Rayyan M, Devlieger H, Jochum F, Allegaert K. Short-term use of parenteral nutrition with a lipid emulsion containing a mixture of soybean oil, olive oil, medium-chain triglycerides, and fish oil: a randomized double-blind study in preterm infants. JPEN J Parenter Enteral Nutr. Jan 2012;36 (1 Suppl):81S-94S.

[170] Peden VH, Witzleben CL, Skelton MA. Total parenteral nutrition. J Pediatr. Jan 1971;78(1):180-1.

[171] Carter BA, Shulman RJ. Mechanisms of disease: update on the molecular etiology and fundamentals of parenteral nutrition associated cholestasis. Nature clinical practice. May 2007;4(5):277-87.

[172] Klein CJ, Ravenis M, Kusenda C, Scavo L. Parenteral nutrition-associated conjugated hyperbilirubinemia in hospitalized infants. Journal of the American Dietetic Association. Nov 2010;110(11):1684-95.

[173] Wales PW, de Silva N, Kim JH, Lecce L, Sandhu A, Moore AM. Neonatal short bowel syndrome: a cohort study. J Pediatr Surg. May 2005;40(5):755-62.

[174] Colomb V, Jobert-Giraud A, Lacaille F, Goulet O, Fournet JC, Ricour C. Role of lipid emulsions in cholestasis associated with long-term parenteral nutrition in children. JPEN J Parenter Enteral Nutr. Nov-Dec 2000;24(6):345-50.

[175] Clayton PT, Bowron A, Mills KA, Massoud A, Casteels M, Milla PJ. Phytosterolemia in children with parenteral nutrition-associated cholestatic liver disease. Gastroenterology. Dec 1993;105(6):1806-13.

[176] Carter BA, Taylor OA, Prendergast DR, et al. Stigmasterol, a soy lipid-derived phytosterol, is an antagonist of the bile acid nuclear receptor FXR. Pediatr Res. Sep 2007;62(3):301-6.

[177] Diamond IR, Sterescu A, Pencharz PB, Wales PW. The rationale for the use of parenteral omega-3 lipids in children with short bowel syndrome and liver disease. Pediatric surgery international. Jul 2008;24(7):773-8.

[178] de Meijer VE, Gura KM, Le HD, Meisel JA, Puder M. Fish Oil-Based Lipid Emulsions Prevent and Reverse Parenteral Nutrition-Associated Liver Disease: The Boston Experience. JPEN J Parenter Enteral Nutr. Jul 1 2009;33:541-7.

[179] Cheung HM, Lam HS, Tam YH, Lee KH, Ng PC. Rescue treatment of infants with intestinal failure and parenteral nutrition-associated cholestasis (PNAC) using a parenteral fish-oil-based lipid. Clin Nutr. Apr 2009;28(2):209-12.

[180] Chung PH, Wong KK, Wong RM, Tsoi NS, Chan KL, Tam PK. Clinical experience in managing pediatric patients with ultra-short bowel syndrome using omega-3 fatty acid. Eur J Pediatr Surg. Mar 2010;20(2):139-42.

[181] de Meijer VE, Le HD, Meisel JA, Gura KM, Puder M. Parenteral fish oil as monotherapy prevents essential fatty acid deficiency in parenteral nutrition-dependent patients. J Pediatr Gastroenterol Nutr. Feb 2010;50(2):212-8.

[182] Diamond IR, Sterescu A, Pencharz PB, Kim JH, Wales PW. Changing the paradigm: omegaven for the treatment of liver failure in pediatric short bowel syndrome. J Pediatr Gastroenterol Nutr. Feb 2009;48(2):209-15.

[183] Gura K, Strijbosch R, Arnold S, McPherson C, Puder M. The role of an intravenous fat emulsion composed of fish oil in a parenteral nutrition-dependent patient with hypertriglyceridemia. Nutr Clin Pract. Dec 2007;22(6):664-72.

[184] Gura KM, Lee S, Valim C, et al. Safety and efficacy of a fish-oil-based fat emulsion in the treatment of parenteral nutrition-associated liver disease. Pediatrics. Mar 2008;121(3):e678-86.

[185] Lee SI, Valim C, Johnston P, et al. Impact of fish oil-based lipid emulsion on serum triglyceride, bilirubin, and albumin levels in children with parenteral nutrition-associated liver disease. Pediatr Res. Dec 2009;66(6):698-703.

[186] Rollins MD, Scaife ER, Jackson WD, Meyers RL, Mulroy CW, Book LS. Elimination of soybean lipid emulsion in parenteral nutrition and supplementation with enteral fish oil improve cholestasis in infants with short bowel syndrome. Nutr Clin Pract. Apr 2010;25(2):199-204.

[187] Sigalet D, Boctor D, Robertson M, et al. Improved Outcomes in Paediatric Intestinal Failure with Aggressive Prevention of Liver Disease. Eur J Pediatr Surg. Oct 28 2009;19(6):348-53.

[188] Soden JS, Lovell MA, Brown K, Partrick DA, Sokol RJ. Failure of resolution of portal fibrosis during omega-3 fatty acid lipid emulsion therapy in two patients with irreversible intestinal failure. J Pediatr. Feb 2010;156(2):327-31.

[189] Strijbosch RA, van den Hoonaard TL, Olieman JF, Escher JC, Alwayn IP, Meijers-Ijsselstijn H. [Fish oil in prolonged parenteral nutrition in children--omega-3-fatty acids have a beneficial effect on the liver]. Nederlands tijdschrift voor geneeskunde. 2010;154:A2003.

[190] Le HD, de Meijer VE, Robinson EM, et al. Parenteral fish-oil-based lipid emulsion improves fatty acid profiles and lipids in parenteral nutrition-dependent children. Am J Clin Nutr. Jul 20 2011;94(3):749-58.

[191] Reniers D, Rajakumar I, Ratko S, Atkison P. Use of parenteral fish oil to reverse cholestasis induced by parenteral nutrition in infants with intestinal failure: single-centre case series. Can J Hosp Pharm. Jan 2012;65(1):27-30.

[192] Sant'Anna AM, Altamimi E, Clause RF, et al. Implementation of a multidisciplinary team approach and fish oil emulsion administration in the management of infants with short bowel syndrome and parenteral nutrition-associated liver disease. Can J Gastroenterol. May 2012;26(5):277-80.

[193] Cober MP, Teitelbaum DH. Prevention of parenteral nutrition-associated liver disease: lipid minimization. Current opinion in organ transplantation. Jun 2010;15(3):330-3.

[194] Mallah HS, Brown MR, Rossi TM, Block RC. Parenteral fish oil-associated burr cell anemia. J Pediatr. Feb 2010;156(2):324-6.e1.

[195] Sangild PT, Petersen YM, Schmidt M, et al. Preterm birth affects the intestinal response to parenteral and enteral nutrition in newborn pigs. The Journal of nutrition. Sep 2002;132(9):2673-81.

[196] Puiman P, Stoll B. Animal models to study neonatal nutrition in humans. Curr Opin Clin Nutr Metab Care. Sep 2008;11(5):601-6.

[197] Truskett PG, Shi EC, Rose M, Sharp PA, Ham JM. Model of TPN-associated hepatobiliary dysfunction in the young pig. Br J Surg. Jul 1987;74(7):639-42.

[198] Mei J, Xu R. The piglet as a model for studying parenteral nutrition. In: Xu R, Cranwell PD, eds. The neonatal pig: gastrointestinal physiology and nutrition. Nottingham: Nottingham University Press; 2003:309-35.

[199] Hyde MJ, Amusquivar E, Laws J, et al. Effects of Lipid-Supplemented Total Parenteral Nutrition on Fatty Liver Disease in a Premature Neonatal Piglet Model. Neonatology. 2008;93(2):77-86.

[200] Innis SM. The colostrum-deprived piglet as a model for study of infant lipid nutrition. The Journal of nutrition. Feb 1993;123(2 Suppl):386-90.

[201] Duerksen DR, Van Aerde JE, Chan G, Thomson AB, Jewell LJ, Clandinin MT. Total parenteral nutrition impairs bile flow and alters bile composition in newborn piglet. Digestive diseases and sciences. Sep 1996;41(9):1864-70.

[202] Wanten GJ, Calder PC. Immune modulation by parenteral lipid emulsions. Am J Clin Nutr. May 2007;85(5):1171-84.

[203] Embleton ND. Optimal protein and energy intakes in preterm infants. Early Hum Dev. Dec 2007;83(12):831-7.

[204] Christensen RD, Henry E, Wiedmeier SE, Burnett J, Lambert DK. Identifying patients, on the first day of life, at high-risk of developing parenteral nutrition-associated liver disease. J Perinatol. May 2007;27(5):284-90.

CHAPTER 2

Parenteral lipid administration to very low birth weight infants – early introduction of lipids and use of new lipid emulsions: a systematic review and meta-analysis

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ABSTRACT

BACKGROUND

The use of parenteral lipid emulsions in preterm infants has been limited by concerns regarding impaired lipid tolerance. As a result, the time of initiation of parenteral lipid infusion to very low birth weight (VLBW) infants varies widely among different neonatal intensive care units. However, lipids provide energy for protein synthesis and supply essential fatty acids that are necessary for central nervous system development.

OBJECTIVE

The objective was to summarize the effects of initiation of lipids within the first two days of life and the effects of different lipid compositions on growth and morbidities in VLBW infants.

METHODS

A systematic review and meta-analysis of publications identified in a search of PubMed, EMBASE, and Cochrane databases was undertaken. Randomized controlled studies were eligible if information on growth was available.

RESULTS

The search yielded 14 studies. No differences were observed in growth or morbidity with early lipid initiation. We found a weak favorable association of non-purely soybean oil-based emulsions with the incidence of sepsis (RR 0.75; 95% CI 0.56 to 1.00).

CONCLUSIONS

The initiation of lipids within the first two days of life in VLBW infants appears to be safe and well tolerated; however, beneficial effects on growth could not be shown for this treatment nor for the type of lipid emulsion. Emulsions that are not purely soybean oil-based might be associated with a lower incidence of sepsis. Large-scale randomized controlled trials in preterm infants are warranted to determine whether early initiation of lipids and lipid emulsions that are not purely soybean oil-based result in improved long-term outcomes.

CHAPTER 2

Meta-analysis of IV lipid supply to VLBW infants

Introduction

Postnatal growth failure is one of the most commonly observed morbidities in very low birth weight (VLBW) infants.[1-3] Proteins are the driving force for growth, and protein synthesis is an energy-demanding process. Therefore, sufficient energy should be administered to optimize this process. Lipids are an attractive energy source because of their high energy density and their supply of essential n-6 and n-3 fatty acids necessary for central nervous system development. An inadequate exogenous supply of essential fatty acids (EFAs) and/or their derivatized long-chain polyunsaturated fatty acids (LCPUFAs) during the critical periods of rapid brain and retinal growth may lead to long-term impairment of neurodevelopment and visual function.[4] However, the use of parenteral lipid emulsions in preterm infants has been limited by concerns regarding impaired lipid tolerance, including increased albumin-bound bilirubin displacement, impairment of oxygenation, and bronchopulmonary dysplasia (BPD). Among different neonatal intensive care units (NICUs), the time of initiation of parenteral lipid emulsions to preterm infants varies widely,[5-7] even though postponing lipid administration during this critical period of organ development may lead to insufficient energy supply for protein synthesis and a shortage of EFAs for normal brain development. A Cochrane meta-analysis compared initiation of lipids in preterm infants in five studies before and after five days of life. The primary outcomes of growth, death, and BPD were not different between 'early' (< five days) and 'not early' (> five days) initiation of lipids.[8] In 2005, the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition stated that in newborn infants who cannot receive sufficient enteral feeding, administration of parenteral lipid emulsions should be started no later than on the third day of life but may be started on the first day of life.[9] Therefore, the first objective of this systematic review and meta-analysis was to identify the most suitable timing for early introduction of parenteral lipids, and our determination was that initiation is best within the first two days of life. The ESPGHAN guidelines and available studies that were published after the Cochrane meta-analysis support our hypothesis.

Since the 1960s, safe commercial parenteral lipid emulsions have been widely used. Purely soybean oil-based emulsions were the first lipid emulsions available for parenteral use and are still the most often used parenteral lipid source.[10] However, in several newer emulsions, soybean oil is combined with other lipid sources, such as coconut oil (providing medium-chain triacylglycerols, MCTs), olive oil, and/or fish oil. Each type of lipid has different characteristics and potential benefits or disadvantages. Several of these recently developed mixed lipid emulsions have been shown in small studies to improve tolerance and short- and long-term outcomes, such as a lower incidence of

parenteral nutrition-associated liver disease (PNALD).[11-12] Nevertheless, it is not clear which lipid composition is most beneficial for a preterm infant. Therefore, the second objective of the present systematic review and meta-analysis was to identify the most suitable lipid composition for parenteral nutrition in VLBW infants.

Methods

The requirements of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement was followed.[13]

Search strategy for identification of studies

A PubMed (http://www.ncbi.nlm.nih.gov/pubmed), EMBASE (http://www.embase.com), and Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library, www.thecochranelibrary.com, Issue 8, 2011) search up to 23 February 2012 was conducted with the use of the following key terms (words in the title or abstract of the manuscript): 'lipid', 'fat', 'fatty acid', 'oil', 'parenteral', 'intravenous', 'infusion', the lipid type-related terms 'soy', 'soybean', 'medium chain triacylglycerol', 'olive', 'fish', 'n-6', 'n-3', and 'emulsion', and the population-related terms 'very low birth weight', 'preterm', and 'neonate'. The searches were limited to human studies. No language restriction was applied in the search. The complete PubMed, EMBASE, and Cochrane searches are shown elsewhere (see Appendix). We (HV and MABV) performed a manual search of reference lists of all relevant studies on this topic. The citations with abstracts were uploaded into a reference database (EndNote X3, Thomson Reuters) and checked for duplicates.

Data collection

HV and MABV independently selected the studies, and discrepancies were resolved by consensus. Studies were included if they met all of the following criteria: parallel-group randomized controlled trial (RCT) study design, preterm infants weighing < 1500 g admitted to a NICU who needed parenteral nutrition and who received any type of parenteral lipid emulsion within the first days of life, and growth included as an outcome measure. No restriction on the dose of lipid infusion was applied. Cohort studies, case series, case reports and trials studying only infants with congenital abnormalities were excluded.

Data extraction and management

Both reviewers (HV and MABV) read the selected articles. HV extracted, assessed, and coded all data for each study by using a form that was designed specifically for this review. Any SEM was replaced by the corresponding SD. For each study, HV entered final data into RevMan (RevMan version 5.1. 2011; Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration). MABV checked the extraction process and entered data. At each stage, any disagreement was resolved by discussion.

The extracted study data consisted of the following:

1 general study information, including title, first author, journal, and year of publication;
2 study design and characteristics of study participants (including number, gestational age (wk), birth weight (g), and specific inclusion and exclusion criteria per study);
3 type of intervention and control treatment (including duration, start of lipid administration, type of lipid, starting and final dose of administered lipids, and cointerventions in addition to lipids); and
4 outcome measures.

Outcome measures were divided into the primary outcome rate of weight gain and 16 secondary outcomes as follows:

1 death;
2 incidence of BPD defined as oxygen therapy or any form of respiratory support at 36 weeks postmenstrual age;
3 duration of respiratory support (days);
4 supplemental oxygen (days);
5 incidence of necrotizing enterocolitis (NEC) stage ≥ 2 on Bell's staging system;[14]
6 retinopathy of prematurity (ROP), defined as any stage of ROP during the weeks after birth observed by direct or indirect ophthalmoscope, as defined by International Classification of Retinopathy of Prematurity classification;[15]
7 significant patent ductus arteriosus (PDA) diagnosed clinically or by echocardiograph as needing treatment, either conservatively by fluid restriction, diuretics, indomethacin/ibuprofen, or by surgery;
8 sepsis, defined as a positive blood culture;
9 intraventricular hemorrhage (IVH), all grades, and severe IVH (grades 3 and 4) of Papile classification;[16]
10 significant jaundice, necessitating phototherapy or exchange transfusion;
11 PNALD defined as conjugated bilirubin > 2 mg/dL or 34.2 μmol/L, with or without increased liver enzymes in the absence of other causes;[17]
12 EFA defined by triene/tetraene ratio > 0.05;[18]
13 hypertriacylglycerolemia (serum triacylglycerol concentrations > 200 mg/dL or > 2.3 mmol/L);[19]
14 hypoglycemia (blood glucose concentration < 45 mg/dL or < 2.5 mmol/L) or hyperglycemia (blood glucose concentration > 150 mg/dL or > 8.3 mmol/L);[20]
15 signs of lipid peroxidation (F2-isoprostanes concentration); and
16 long-term neurodevelopmental outcome at ~ two years corrected age, including mental retardation (Bayley Scales of Infant Development Mental Developmental Index < 70), cerebral palsy, legal blindness (< 20/200 visual acuity), and hearing deficit (aided or < 60 dB on audiometric testing). Neurodevelopmental impairment was defined as having one of the aforementioned deficits.

ASSESSMENT OF STUDY QUALITY

The level of evidence of each article was established following the Oxford Centre for Evidence-Based Medicine Level of Evidence scale.[21] The quality of the RCTs was assessed by two authors (HV and MABV) by using the Jadad criteria[22] (0 to 5-point rating scale, with 5 as the maximum score).

Data analysis

Analyses were performed by using Review Manager software (RevMan version 5.1, 2011; Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration). Two-sided P values ≤ 0.05 were considered to be significant.

MEASURES OF TREATMENT EFFECT

The first comparison of this study was early (≤ 2 days) compared with late (> 2 days) initiation of lipids in preterm infants. A second comparison was performed to compare parenteral purely soybean oil-based emulsions with any other type of lipid emulsion. For both comparisons, the primary and 16 secondary outcomes were compared. To analyze treatment effect and calculate a pooled mean of outcomes reported in ≥ 2 studies, the Mantel-Haenszel method was used for categorical outcomes, and the inverse variance method was used for continuous outcomes. The data from each study were summarized in forest plots and summary estimates with 95% confidence intervals (CI) were calculated. For outcomes measured on a continuous scale, the weighted mean difference with a 95% CI was used. In assessing the treatment effects for categorical outcomes, the risk ratio (RR) with a 95% CI was used.

ASSESSMENT OF HETEROGENEITY

For all outcome measures, we assessed statistical heterogeneity by calculating the Q-statistic ($P < 0.05$ considered as heterogeneous) and the I^2 statistic ($I^2 > 50\%$ was considered heterogeneous) to assess in what amount the data from the included studies were heterogeneous.[23]

ASSESSMENT OF REPORTING BIASES

To detect publication bias, a funnel plot was constructed. However, there were an insufficient number of studies to permit proper evaluation of publication bias and to evaluate potential asymmetry of the funnel plot by Begg and Egger tests.

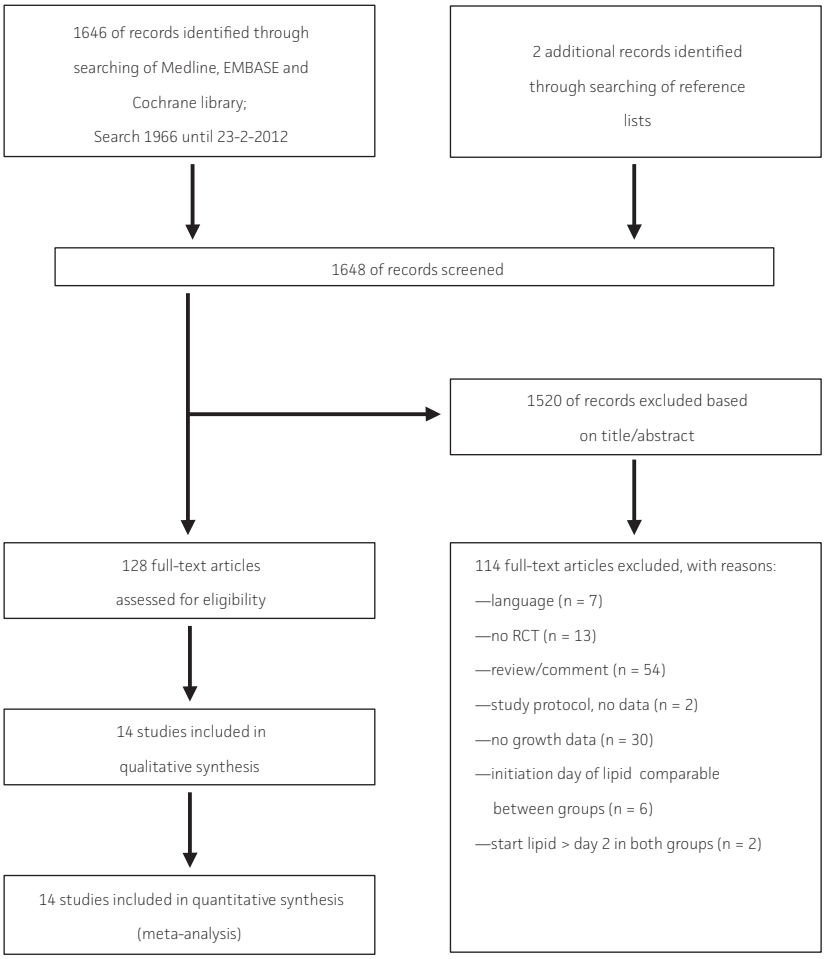
SENSITIVITY ANALYSES

In cases of low study quality, great variability in study protocols (e.g., lipid dose, duration of lipid administration), or other arbitrary findings, a sensitivity analysis was performed by removing this particular study and examining whether the results would significantly change.

Results

Fourteen of 128 potential studies on the effects of parenteral lipid emulsions on clinical outcomes met our predefined inclusion criteria (**FIGURE 1**). Four studies compared early with late introduction of lipid emulsions, nine studies compared different lipid emulsions, and one study compared both. Reasons for exclusion are outlined in Figure 1.

FIGURE 1 Overview of the selection process throughout the study. PubMed, www.ncbi.nlm.gov/pubmed; EMBASE, www.embase.com; Cochrane, www.cochrane.org. RCT, randomized controlled trial.



Early compared with late introduction of lipids

Characteristics of the included studies assessing the effect of early introduction of lipids are shown in **TABLE 1**. In three studies, infants included weighed < 1500 g; in the other studies, inclusion was on the basis of gestational age, rather than birth weight,[24] or was not specified.[25] Lipid emulsions were initiated between 12 hours and 2 days after birth in the intervention groups and between day 5 and day 8 in the control groups.

TABLE 1 Characteristics of studies assessing the effect of early introduction of lipids*

First author, year reference	Study location	Study period	Design	Population	n	Intervention and duration	Quality**
Brownlee, 1993[24]	UK	1990-1991	RCT	Preterm infants, GA 24-36 weeks	129	Start TPN with lipid (soybean oil emulsion 20%) and amino acids < 36h vs on day 6	1
Gilbertson, 1991[26]	UK	No data	RCT	Preterm infants, birth weight <1500 g	29	Start lipid (soybean oil emulsion 20%) on day 1 vs day 8	1
Gunn, 1978[25]	Canada	1974-1975	RCT	Preterm infants	40	Start lipid (soybean oil emulsion 10%) and amino acids on day 2 vs after day 7	1
Sosenko, 1993[27]	USA	1990-1991	RCT	Preterm infants, birth weight 600-1000 g	133	Start lipid (soybean oil emulsion 20%) < 12h postnatally vs after day 7	3
Wilson, 1997[28]	Northern Ireland	1990-1992	RCT	Preterm infants, birth weight <1500 g	125	Start lipid (MCT - soybean oil emulsion 10% vs soybean oil emulsion 10%) on day 2 vs day 5, start amino acids <12h vs on day 3, early minimal enteral feeding	3

* GA, gestational age; MCT, medium-chain triacylglycerol; RCT, randomized controlled trial; TPN, total parenteral nutrition.

** Quality was assessed by using the Jadad et al[22] criteria (0-5 point rating scale, with 5 as the maximum score).

In the studies of Brownlee et al,[24] Gilbertson et al,[26] and Gunn et al,[25] the randomization method was not described or was inappropriate. None of the studies were blinded, and follow-up was described in all studies. Baseline characteristics and outcome measures of the studies are shown in **TABLE 2**. In these five studies, a total of 456 infants were included: 233 in the intervention groups and 223 in the control groups. Sosenko et al[27] performed separate analyses for infants with birth weights of 600-800 g and 801-1000 g; therefore, we considered the results of each weight category as a separate study.

TABLE 2 Baseline characteristics and outcome measures of patients in studies comparing early with late introduction of lipids*

First author, year[reference]	n	Male	Gestational age	Birth weight	Rate of weight gain	Days to regain birth	Rate of head circumference growth	Death first 28 days	Death before discharge	BPD	Duration of respiratory support	Duration of supplemental O_2	NEC	ROP	PDA	Sepsis
		n (%)	weeks	g	g/(kg·d)	days	cm/wk	n (%)	n (%)	n (%)	days	days	n (%)	n (%)	n (%)	n (%)
Intervention																
Brownlee, 1993[24]	63	ND	29 (23–33)**	1144 (539–1748)**	19 ± 8***	ND	ND	ND	11 (17)	20 (32)	ND	20 (2–75)**	ND	ND	NS	ND
Gilbertson, 1991[26]	16	ND	28.6 ± 0.5	1150 ± 240	ND	11.4 ± 7.7	0.5 ± 0.4	1 (6)	1 (6)	2 (13)	9.9 ± 14.7	19.6 ± 25.0	1 (6)	0	4 (25)	2 (13)
Gunn, 1978[25]	20	9 (45)	32.2 ± 3.4	1700 ± 554	ND	12.8 ± 9.0	ND	ND	3 (15)	ND	5 (2–16)**	11 (3–31)**	ND	ND	ND	ND
Sosenko, 1993[27] 600-800 g	42	17 (40)	ND	709****	ND	NS	ND	18 (43)	20 (48)	20 (50)	40****	32****	3 (7)	31 (73)	22 (52)	34 (82)
Sosenko, 1993[27] 801-1000 g	28	18 (64)	ND	915****	ND	ND	ND	3 (11)	3 (11)	11 (38)	37****	18****	2 (7)	20 (71)	21 (75)	19 (67)
Wilson, 1997[28]	64	34 (53)	27 ± 2	925 ± 221	ND	9 (6–11)**	ND	ND	15 (23)	14 (22)	ND	26 (3–48)*****	4 (6)	ND	ND	32 (50)
Control																
Brownlee, 1993[24]	66	ND	29 (24–36)**	1147 (415–1647)**	21 ± 9	ND	ND	ND	14 (21)	20 (30)	ND	21 (2–127)**	ND	ND	ND	ND
Gilbertson, 1991[26]	13	ND	28.8 ± 2.1	1090 ± 324	ND	10.1 ± 4.8	0.5 ± 0.4	2 (15)	2 (15)	3 (23)	15.8 ± 16.3	25.3 ± 23.2	1 (8)	1 (8)	6 (46)	5 (38)
Gunn, 1978[25]	20	8 (40)	32.3 ± 3.5	1868 ± 781	ND	13.8 ± 4.1	ND	ND	6 (30)	ND	5 (1–12)**	9 (3–23)**	ND	ND	ND	ND
Sosenko, 1993[27] 600-800 g	37	18 (49)	ND	708****	ND	NS	ND	7 (19)	9 (24)	25 (68)	46****	40****	5 (14)	29 (79)	26 (70)	33 (89)
Sosenko, 1993[27] 801-1000 g	26	17 (65)	ND	888****	ND	ND	ND	5 (19)	7 (27)	7 (27)	25****	17****	3 (11)	15 (58)	20 (77)	16 (63)
Wilson, 1997[28]	61	32 (52)	27 ± 2	933 ± 242	ND	12 (9–17)**	ND	ND	15 (25)	14 (23)	ND	19 (3–51)*****	4 (7)	ND	ND	40 (66)

* BPD, bronchopulmonary dysplasia; EFA, essential fatty acid; HyperTG, hypertriacylgycerolemia; IVH, intraventricular hemorrhage; ND: no data; NEC, necrotizing enterocolitis; NS: in study described as not different between treatment groups, no data presented; PDA, patent ductus arteriosus; PNALD, parenteral nutrition associated liver disease; ROP, retinopathy of prematurity.

First author, year[reference]	IVH all grades	IVH ≥ 3	Jaundice	PNALD	EFA deficiency	HyperTG	Hypo-glycemia	Hyper-glycemia	F2-isoprostane concentration	Neuro-development
	n (%)		n (%)			n (%)	n (%)	n (%)		
Intervention										
Brownlee, 1993[24]	NS	ND	NS	ND	ND	ND	ND	ND	ND	ND
Gilbertson, 1991[26]	5 (31)	ND	7 (44)	ND	ND	3 (19)	7 (44)	6 (38)	ND	ND
Gunn, 1978[25]	ND	ND	17 (85)	ND	ND	ND	ND	ND	ND	ND
Sosenko, 1993[27] 600-800 g	19 (45)	NS	ND	ND	ND	ND	ND	ND	ND	ND
Sosenko, 1993[27] 801-1000 g	10 (37)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Wilson, 1997[28]	ND	ND	ND	ND	ND	22 (34)	ND	18 (28)	ND	ND
Control										
Brownlee, 1993[24]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gilbertson, 1991[26]	7 (54)	ND	5 (38)	ND	ND	1(8)	5 (38)	8 (62)	ND	ND
Gunn, 1978[25]	ND	ND	18 (90)	ND	ND	ND	ND	ND	ND	ND
Sosenko, 1993[27] 600-800 g	21 (57)	NS	ND	ND	ND	ND	ND	ND	ND	ND
Sosenko, 1993[27] 801-1000 g	12 (47)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Wilson, 1997[28]	ND	ND	ND	ND	ND	18 (30)	ND	24 (39)	ND	ND

** Median; range in parentheses.

*** Mean ± SD (all such values).

**** SD, range, or IQR was not presented and could not be calculated from available data.

***** Median; IQR in parentheses.

OUTCOME MEASURES

Three of five studies only reported that growth was no different between treatment groups, rather than specifying exact growth rates. Brownlee et al[24] reported mean weight gain during hospital admission (not significantly different between groups) and Gilbertson et al[26] presented growth only during the first week of life instead of during total hospital admission (more weight loss in the intervention group; days to regain birth weight did not differ between groups). Because of a lack of consistency in this outcome measure, a meta-analysis of weight gain during hospital admission was not possible. However, the individual study results suggested that earlier initiation of lipid emulsions does not have an effect on growth rates during the total hospital stay. This finding was further supported by the equal number of days to regain birth weight in both treatment groups (on the basis of two studies; mean difference: 0.20; 95% CI: -3.11 to 3.51; P=0.91; n= 60) (**FIGURE 2A**).

Death during the first 28 days was reported in two studies and involved 162 infants. Sosenko et al[27] reported that mortality of infants weighing 600-800 g was higher in the early-lipid group, whereas it was no different in the total population (600-1000 g) or in the Gilbertson et al study.[26] A meta-analysis of these studies did not show a significant effect of early introduction of lipids on mortality during the first 28 days. The heterogeneity of these studies was shown by an I^2 of 56%. Sensitivity analysis with removal of the 600-800 g subgroup and combined analyses of the 600-1000 g in Sosenko et al[27] did not change the results of the meta-analysis. All five studies reported death during the total hospital stay. Individual studies did not find significant differences in overall mortality, except in the 600-800 g birth weight group in the Sosenko et al study.[27] Meta-analysis did not show significant effects of early lipid administration on mortality during hospital stay.

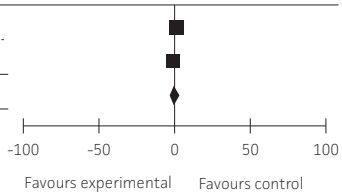
The incidence of BPD (or chronic lung disease, CLD) was reported in four studies. Brownlee et al[24] reported the diagnosis of CLD on the basis of 28 days of oxygen dependence. In Gilbertson et al,[26] Sosenko et al,[27] and Wilson et al,[28] BPD/CLD was based on history plus radiology appearance in infants who required supplemental oxygen after 28 days of life. Individual study results and meta-analyses of these three studies did not show an effect of early lipid introduction on the incidence of BPD/CLD. Only Gilbertson et al[26] reported data on duration of respiratory support and supplemental oxygen in a manner that could be used for meta-analysis. No individual study found significant differences in these outcomes.

The incidence of NEC and sepsis was reported in four studies. Gilbertson et al[26] defined septicemia as a positive blood culture or a clinical picture with hematologic evidence indicating infection. Sosenko et al[27] defined sepsis as a positive blood culture result associated with compatible clinical signs. Wilson et al[28] did not specify the diagnosis of sepsis. No significant effect of early lipid introduction was found in individual studies or in our meta-analysis. The incidence of ROP, PDA, and IVH (all grades) was reported in three studies. Again, no effect of early lipid introduction was detected in individual studies or in the combined meta-analysis. None of the studies presented data on the incidence of IVH ≥ grade 3, PNALD, or EFA deficiency. Two studies reported the incidence of significant jaundice necessitating phototherapy in a manner that could be

FIGURE 2 A-L Meta-analysis of the effects of initiation of lipids within the first two days compared with after day two of life (random effects). IV, inverse variance; M-H, Mantel-Haenszel.

A - DAYS TO REGAIN BIRTH WEIGHT

	Experimental			Control			Mean Difference	Mean Difference
Study or subgroup	Mean	SD	n	Mean	SD	n	Weight	IV, Random, 95% CI
Gilbertson, 1991[26]	11,4	7.7	16	10.1	4.8	13	52.1%	1.30 [-3.29, 5.89]
Gunn, 1978[25]	12.8	9	17	13.8	4.1	14	47.9%	-1.00 [-5.79, 3.79]
Total (95% CI)			**33**			**27**	**100%**	**0.20 [-3.11, 3.51]**

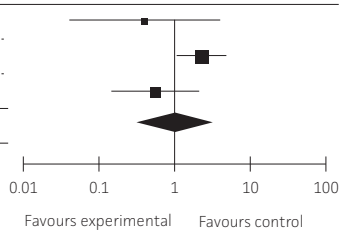


Heterogeneity: $Chi^2 = 0.46$, df = 1 (P = 0.50); $I^2 = 0\%$

Test for overall effect: Z = 0.12 (P = 0.91)

B - DEATH FIRST 28 DAYS

	Experimental		Control			Risk Ratio M-H,	Risk Ratio
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI	M-H, Random, 95% CI
Gilbertson, 1991[26]	1	16	2	13	18.1%	0.41 [0.04, 4.00]	
Sosenko, 1993, 600-800 g[27]	18	42	7	37	48.2%	2.27 [1.07, 4.81]	
Sosenko, 1993, 801-1000 g[27]	3	28	5	26	33,7%	0.56 [0.15, 2.10]	
Total (95% CI)		**86**		**76**	**100%**	**1.03 [0.32, 3.30]**	
Total events	22		14				

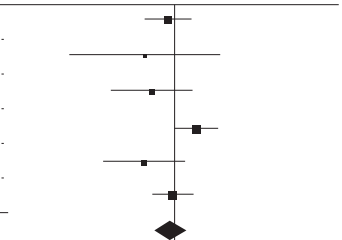


Heterogeneity: $Tau^2 = 0.58$; $Chi^2 = 4.55$, df = 2 (P = 0.10); $I^2 = 56\%$

Test for overall effect: Z = 0.06 (P = 0.95)

C - DEATH BEFORE DISCHARGE

	Experimental		Control			Risk Ratio M-H,	Risk Ratio
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI	M-H, Random, 95% CI
Brownlee, 1993[24]	11	63	14	66	22.8%	0.82 [0.40, 1.67]	
Gilbertson, 1991[26]	1	16	2	13	4.1%	0.41 [0.04, 4.00]	
Gunn, 1978[25]	3	20	6	20	11.4%	0.50 [0.14, 1.73]	
Sosenko, 1993, 600-800 g[27]	20	42	9	37	24.7%	1.96 [1.02, 3.75]	
Sosenko, 1993, 801-1000 g[27]	3	28	7	26	11.3%	0.40 [0.11, 1.38]	
Wilson, 1997[28]	15	64	15	61	25.7%	0.95 [0.51, 1.78]	
Total (95% CI)		**233**		**233**	**100%**	**0.90 [0.55, 1.45]**	
Total events	53		53				



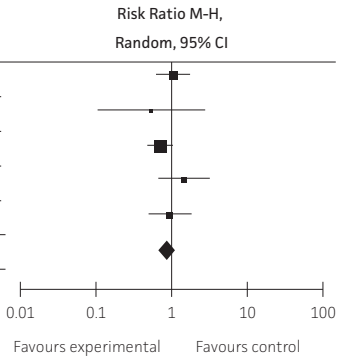
Heterogeneity: $Tau^2 = 0.14$; $Chi^2 = 8.34$, df = 5 (P = 0.14); $I^2 = 40\%$

Test for overall effect: Z = 0.45 (P = 0.65)

FIGURE 2 Continued

D - BRONCHOPULMONARY DYSPLASIA

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI
Brownlee, 1993[24]	20	63	20	66	25.5%	1.05 [0.63, 1.75]
Gilbertson, 1991[26]	2	16	3	13	2.5%	0.54 [0.11, 2.77]
Sosenko, 1993, 600-800 g[27]	20	42	25	37	45.0%	0.70 [0.48, 1.04]
Sosenko, 1993, 801-1000 g[27]	11	28	7	26	11.0%	1.46 [0.67, 3.19]
Wilson, 1997[28]	14	64	14	61	15.9%	0.95 [0.50, 1.83]
Total (95% CI)		**213**		**203**	**100%**	**0.88 [0.68, 1.14]**
Total events	67		69			

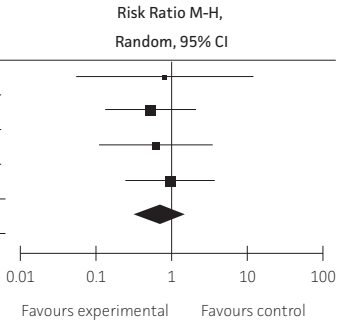


Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 3.80$, df = 4 (P = 0.43); $I^2 = 0\%$

Test for overall effect: Z = 0.96 (P = 0.34)

E - NECROTIZING ENTEROCOLITIS

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI
Gilbertson, 1991[26]	1	16	1	13	8.9%	0.81 [0.06, 11.77]
Sosenko, 1993, 600-800 g[27]	3	42	5	37	34.2%	0.53 [0.14, 2.06]
Sosenko, 1993, 801-1000 g[27]	2	28	3	26	21.7%	0.62 [0.11, 3.41]
Wilson, 1997[28]	4	64	4	61	35.2%	0.95 [0.25, 3.64]
Total (95% CI)		**150**		**137**	**100%**	**0.70 [0.32, 1.55]**
Total events	10		13			

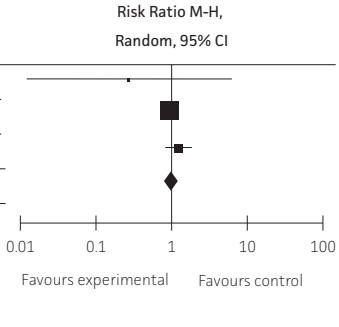


Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.40$, df = 3 (P = 0.94); $I^2 = 0\%$

Test for overall effect: Z = 0.88 (P = 0.38)

F - RETINOPATHY OF PREMATURITY

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI
Gilbertson, 1991[26]	0	16	1	13	0.5%	0.27 [0.01, 6.23]
Sosenko, 1993, 600-800 g[27]	31	42	29	37	72.4%	0.94 [0.74, 1.21]
Sosenko, 1993, 801-1000 g[27]	20	28	15	26	27.1%	1.24 [0.83, 1.85]
Total (95% CI)		**86**		**76**	**100%**	**1.01 [0.82, 1.24]**
Total events	51		45			

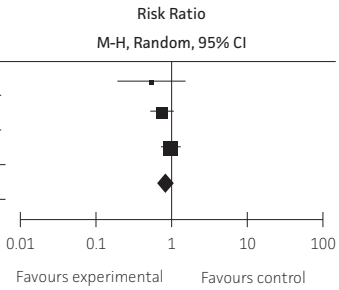


Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 1.96$, df = 2 (P = 0.38); $I^2 = 0\%$

Test for overall effect: Z = 0.08 (P = 0.94)

G - PATENT DUCTUS ARTERIOSUS

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	**Events**	**Total**	**Events**	**Total**	**Weight**	**Random, 95% CI**
Gilbertson, 1991[26]	4	16	6	13	5.7%	0.54 [0.19, 1.52]
Sosenko, 1993, 600-800 g[27]	22	42	26	37	40.7%	0.75 [0.52, 1.06]
Sosenko, 1993, 801-1000 g[27]	21	28	20	26	53.6%	0.97 [0.72, 1.32]
Total (95% CI)		**86**		**76**	**100%**	**0.85 [0.66, 1.09]**
Total events	47		52			

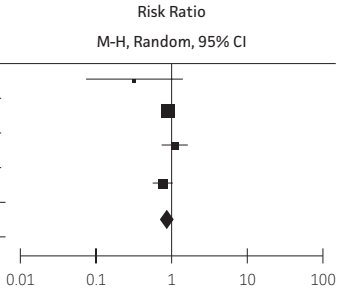


Heterogeneity: Tau² = 0.01; Chi² = 2.28, df = 2 (P = 0.32); I² = 12%

Test for overall effect: Z = 1.32 (P = 0.19)

H - SEPSIS

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	**Events**	**Total**	**Events**	**Total**	**Weight**	**Random, 95% CI**
Gilbertson, 1991[26]	2	16	5	13	1.9%	0.33 [0.07, 1.41]
Sosenko, 1993, 600-800 g[27]	34	42	33	37	48.9%	0.91 [0.75, 1.09]
Sosenko, 1993, 801-1000 g[27]	19	28	16	26	20.2%	1.10 [0.74, 1.64]
Wilson, 1997[28]	32	64	40	61	29.1%	0.76 [0.56, 1.03]
Total (95% CI)		**150**		**137**	**100%**	0.88 [0.72, 1.08]
Total events	87		94			

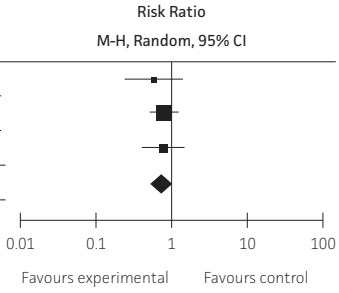


Heterogeneity: Tau² = 0.01; Chi² = 4.32, df = 3 (P = 0.23); I² = 31%

Test for overall effect: Z = 1.22 (P = 0.22)

I - INTRAVENTRICULAR HEMORRHAGE, ALL GRADES

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	**Events**	**Total**	**Events**	**Total**	**Weight**	**Random, 95% CI**
Gilbertson, 1991[26]	5	16	7	13	14.3%	0.58 [0.24, 1.40]
Sosenko, 1993, 600-800 g[27]	19	42	21	37	59.0%	0.80 [0.52, 1.23]
Sosenko, 1993, 801-1000 g[27]	10	28	12	26	26.7%	0.77 [0.40, 1.48]
Total (95% CI)		**86**		**76**	**100%**	**0.76 [0.54, 1.06]**
Total events	34		40			



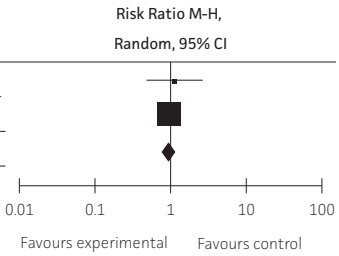
Heterogeneity: Tau² = 0.00; Chi² = 0.41, df = 2 (P = 0.82); I² = 0%

Test for overall effect: Z = 1.64 (P = 0.10)

FIGURE 2 Continued

J - SIGNIFICANT JAUNDICE

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI
Gilbertson, 1991[26]	7	16	5	13	6.6%	1.14 [0.47, 2.75]
Gunn, 1978[25]	17	20	18	20	93.4%	0.94 [0.75, 1.19]
Total (95% CI)		**36**		**33**	**100%**	**0.96 [0.76, 1.20]**
Total events	24		23			

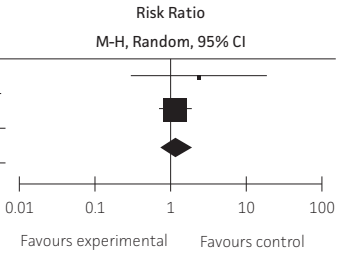


Heterogeneity: Tau² = 0.00; Chi² = 0.25, df = 1 (P = 0.62); I² = 0%

Test for overall effect: Z = 0.39 (P = 0.70)

K - HYPERTRIACYLGLYCEROLEMIA

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI
Gilbertson, 1991[26]	3	16	1	13	5.5%	2.44 [0.29, 20.75]
Wilson, 1997[28]	22	64	18	61	94.5%	1.16 [0.70, 1.95]
Total (95% CI)		**80**		**74**	**100%**	**1.21 [0.74, 2.00]**
Total events	25		19			

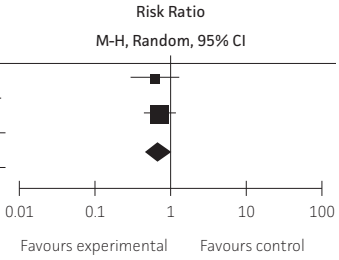


Heterogeneity: Tau² = 0.00; Chi² = 0.44, df = 1 (P = 0.51); I² = 0%

Test for overall effect: Z = 0.76 (P = 0.45)

L - HYPERGLYCEMIA

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI
Gilbertson, 1991[26]	6	16	8	13	30.0%	0.61 [0.28, 1.31]
Wilson, 1997[28]	18	64	24	61	70.0%	0.71 [0.43, 1.18]
Total (95% CI)		**80**		**74**	**100%**	**0.68 [0.45, 1.04]**
Total events	24		32			



Heterogeneity: Tau² = 0.00; Chi² = 0.12, df = 1 (P = 0.73); I² = 0%

Test for overall effect: Z = 1.80 (P = 0.07)

used for meta-analysis and meta-analysis did not show an effect of early introduction of lipids on significant jaundice. Hypertriacylglycerolemia, defined as triacylglycerol concentrations > 200 mg/dL (2.3 mmol/L), was not specified in the included studies. However, when the lower threshold of 1.5 mmol/L defined by Gilbertson et al[26] was used, two studies could be included in the meta-analysis that did not show manipulation by early lipid introduction. The incidence of hypoglycemia was reported only in the study of Gilbertson et al[26] and was not significantly different between treatment groups. The incidence of hyperglycemia was reported by Gilbertson et al[26] and Wilson et al.[28] Gilbertson defined hyperglycemia as glucose concentrations > 8.0 mmol/L (144 mg/dL), and Wilson defined it as glucose concentrations > 11.0 mmol/L with glucosuria. A meta-analysis of these two studies did not show an effect of early lipid introduction. None of the included studies presented data on F2-isoprostane concentrations or neurodevelopment. The results of the performed meta-analyses are shown in **FIGURE 2, B-L**. Sensitivity analysis by removing studies with a Jadad score < 3[24-26] and studies in which the lipid intervention was part of a package of more aggressive parenteral and/or enteral nutrition[24-25, 28] did not significantly change the results.

Comparison of lipid emulsions

Characteristics of the ten studies that compared different lipid emulsions are shown in **TABLE 3**. The inclusion of preterm infants was on the basis of gestational age in three studies,[29-31] of birth weight in two studies,[28, 32] of both,[33-36] or not specified[37] in others. Lipid emulsions with MCT-soybean, olive-soybean, soybean-MCT-fish, or soybean-MCT-olive-fish were compared with pure soybean oil emulsions or with MCT-soybean oil emulsions. In one study, the treatment group received an MCT-soybean oil emulsion combined with earlier amino acid administration and early minimal feeding.[28] In the studies of Lehner et al,[34] Rubin et al,[30] and Tomsits et al,[31] the method of randomization was not appropriately described. The studies by Despande et al,[29], Lehner et al,[34] Rayyan et al,[33] Rubin et al,[30] Skouroliakou et al,[35] and Tomsits et al[31] were described as double-blinded. However, the method of double blinding was appropriately described only in the studies of Despande et al,[29] Rayyan et al,[33] and Skouroliakou et al.[35] Follow-up was described in all studies. In **TABLE 4**, baseline characteristics and outcome measures of the studies are shown. A total of 499 infants were included: 249 in the intervention groups and 250 in the control groups.

OUTCOME MEASURES

None of the studies presented the rate of weight gain during the total period of hospital stay. Lima et al[37] and Tomsits et al[31] presented the rate of weight gain during the first two weeks, which was no different between groups. Rayyan et al[33] presented the rate of weight gain during the study period (days 7-14), which was no different between groups. Three other studies[29-30, 32] mentioned only that rate of weight gain during hospital admission was not different between treatment groups, rather than specifying growth rates. Four studies presented only weight at day 8,[34] day 14,[36] or the final weight,[28, 35] instead of growth rate. Meta-analysis of the studies by Lima et al[37] (MCT-soybean oil), Rayyan et al[33] (soybean-MCT-olive-fish), and Tomsits et al[31] (soybean-

TABLE 3 Characteristics of studies comparing different lipid emulsions*

First author, year[reference]	Study location	Study period	Design	Population	n	Intervention	Quality**
D'Ascenzo, 2011[32]	Italy	2007–2008	RCT	Preterm infants, birth weight 500-1249 g	48	50% MCT - 40% soybean - 10% fish vs. 50% MCT - 50% soybean	3
Demirel, 2012[36]	Turkey	2010	RCT	Preterm infants, birth weight < 1500 g, GA < 32 weeks	40	80% Olive - 20% soybean vs. 100% soybean	2
Deshpande, 2009[29]	Australia	2006–2007	RCT	Preterm infants, GA 23 to < 28 weeks	45	80% Olive - 20% soybean vs. 100% soybean	5
Lehner, 2006[34]	Hungary	No data	RCT	Preterm infants, GA 25-37 weeks, birth weight < 3000 g	12	20% MCT - 80% soybean vs. 100% soybean	1
Lima, 1988[37]	UK	No data	RCT	Preterm + term infants, combined analysis	51	50% MCT - 50% soybean vs. 100% soybean	3
Rayyan, 2012[33]	Belgium	2004–2006	RCT	Preterm infants, GA < 34 weeks, birth weight 500-2000 g	53	30% Soybean - 30% MCT - 25% olive - 15% fish vs. 100% soybean	5
Rubin, 1995[30]	Israel	No data	RCT	Preterm infants, GA < 35 weeks	33	50% MCT - 50% soybean vs. 100% soybean	1
Skouroliakou, 2010[35]	Greece	2008–2009	RCT	Preterm infants, GA < 32 weeks, birth weight < 1500 g	32	30% Soybean - 30% MCT - 25% olive - 15% fish vs. 100% soybean	5
Tomsits, 2010[31]	Hungary	2004–2006	RCT	Preterm infants, GA < 34 weeks	60	30% Soybean - 30% MCT - 25% olive - 15% fish vs. 100% soybean	1
Wilson, 1997[28]	Northern Ireland	1990–1992	RCT	Preterm infants, birth weight < 1500 g	125	50% MCT - 50% soybean (10% solution) on day 2 (and start amino acids at < 12 h, early minimal enteral feeding) vs. 100% soybean (10% solution) on day 5 (and start amino acids on day 3)	3

* GA, gestational age; MCT, medium-chain triacylglycerol; RCT, randomized controlled trial.

** Quality was assessed by using the Jadad et al[22] criteria (0-5 point rating scale, with 5 as the maximum score).

MCT-olive-fish) did not show a significant effect of lipid emulsion on growth rate during the first two weeks of life (weighted mean difference: 0.07; 95% CI: -2.46 to2.59; P=0.96; n=158) (**FIGURE 3A**).

Of the secondary outcomes, meta-analyses could be performed for the following variables: death before discharge, duration of respiratory support, and the incidence of sepsis, hypertriacylglycerolemia, and hyperglycemia. Hypertriacylglycerolemia was defined as a triacylglycerol concentration > 1.9-3.4 mmol/L[28, 33, 36] or not specified.[34] Hyperglycemia was defined as glucose concentration > 8 mmol/L,[37] > 11 mmol/L,[35] or > 11 mmol/L plus glucosuria.[28] Lipid sources that were not purely soybean oil-based caused a 25% reduction in sepsis episodes (RR: 0.75; 95% CI: 0.56 to 1.00; P=0.05; 3 studies; n=197). Other outcomes were not affected by the type of lipid emulsion. The results of the performed meta-analyses are shown in **FIGURE 3, B-F**.

Sensitivity analysis performed by removing studies with a Jadad score < 3,[30-31, 34, 36] studies in which the lipid intervention was part of a package of more aggressive parenteral and/or enteral nutrition,[28] or studies in which the lipid intervention was after the first few days of life[33] did not change the results significantly, except for the outcome of sepsis. When the studies of Demirel et al[36] or Wilson et al[28] were removed from this analysis, the type of lipid emulsion did not make a significant difference in the incidence of sepsis.

Discussion

In this systematic review, the benefits and adverse effects of lipid introduction within the first two days of life and the effects of the type of lipid were reported. The meta-analysis of lipid introduction within the first two days of life was based on five studies in 456 preterm infants. The results of this meta-analysis suggest that the initiation of lipids within the first two days of life is well-tolerated, does not offer significant benefits on growth, and does not cause a higher incidence of adverse events. For the primary outcome, growth during hospital admission, the reporting of results was too inconsistent to perform a meta-analysis. However, results of individual studies suggest no effect from the initiation of lipids before or after day two of life on growth during hospital admission. Time required to regain birth weight was also not significantly different between treatment groups. The difference of two days when lipids are introduced is probably too small an interval to result in persistent differences in growth. This finding was supported by a previous meta-analysis of five RCTs performed by Simmer and Rao,[8] which reported that a five day difference in the initiation of lipids did not result in growth benefits in the early -lipid group. In addition to the studies by Brownlee et al,[24] Gilbertson et al,[26] and Sosenko et al,[27] which were included in our meta-analysis, Simmer and Rao also included Alwaidh et al[38] and Hammerman et al.[39] We excluded these latter two studies[38-39] because lipids were initiated after day two in both treatment groups.

A meta-analysis of the effects on the secondary outcomes death and incidence of BPD, NEC, ROP, PDA, sepsis, IVH, significant jaundice, hypertriacylglycerolemia, or hyperglycemia did not show significant differences between initiation of lipids before or on day two compared with after day two. For the secondary outcomes of duration of

TABLE 4 Baseline characteristics and outcome measures of patients in studies comparing different lipid emulsions

First author, year[reference]	n	Male	Gestational age	Birth weight	Rate of weight gain	Days to regain birth	Rate of head circumference growth	Death first 28 days	Death before discharge	BPD	Duration of respiratory support	Duration of supplemental O_2	NEC	ROP	PDA	Sepsis
		n (%)	weeks	g	g/(kg·d)	days	cm/wk	n (%)	n (%)	n (%)	days	days	n (%)	n (%)	n (%)	n (%)
Intervention																
D'Ascenzo, 2011[32]	24	ND	29 ± 2**	1017 ± 203	NS	ND	NS	ND	ND	ND	ND	ND	ND	ND	ND	ND
Demirel, 2012[36]	20	9 (45)	30 ± 3	1300 ± 480	3.2***	ND	ND	ND	ND	NS	ND	ND	NS	0	ND	4 (20)
Deshpande, 2009[29]	24	14 (58)	26 ± 1	801 ± 211	NS	ND	NS	1 (4)	1 (4)	ND	ND	ND	ND	ND	ND	ND
Lehner, 2006[34]	6	6 (100)	31 ± 2	1573 ± 170	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lima, 1988[37]	26	ND	32 ± 1	1588 ± 750	-4.9 ± 12.7	ND	ND	ND	6 (23)	ND	ND	ND	ND	ND	ND	ND
Rayyan, 2012[33]	26	16 (59)	30 ± 2	1336 ± 409	13.0 ± 25.5	ND	ND	ND	1 (4)	ND	2***	ND	ND	ND	ND	ND
Rubin, 1995[30]	15	11 (73)	32 ± 2	1500 ± 400	NS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Skouroliakou, 2010[35]	14	ND	28 ± 3	1210 ± 170	NS	ND	NS	ND	ND	ND	12 ± 8	ND	ND	ND	ND	0
Tomsits, 2010[31]	30	ND	32 ± 2	1662 ± 418	5.5 ± 5.7	ND	ND	ND	ND	ND	5 ± 5	ND	ND	ND	ND	ND
Wilson, 1997[28]	64	34 (53)	27 ± 2	925 ± 221	ND	9 (6–11)****	ND	ND	15 (23)	14 (22)	ND	26 (3–48)****	4 (6)	ND	ND	32 (50)
Control																
D'Ascenzo, 2011[32]	24	ND	28 ± 1	1009 ± 211	NS	ND	NS	ND	ND	ND	ND	ND	ND	ND	ND	ND
Demirel, 2012[36]	20	12 (60)	29 ± 4	1253 ± 458	3.5***	ND	ND	ND	ND	NS	ND	ND	NS	0	ND	7 (35)
Deshpande, 2009[29]	21	10 (48)	26 ± 1	848 ± 184	NS	ND	NS	0	0	ND	ND	ND	ND	ND	ND	ND
Lehner, 2006[34]	6	3 (50)	33 ± 1	1782 ± 290	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lima, 1988[37]	25	ND	32 ± 1	1674 ± 600	-3.6 ± 15.5	ND	ND	ND	7 (28)	ND	ND	ND	ND	ND	ND	ND
Rayyan, 2012[33]	27	8 (31)	30 ± 2	1364 ±340	15.7 ± 11.1	ND	ND	ND	2 (7)	ND	3***	ND	ND	ND	ND	ND
Rubin, 1995[30]	18	11 (61)	31 ± 2	1400 ± 400	NS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Skouroliakou, 2010[35]	18	ND	30 ± 2	1140 ± 260	NS	ND	NS	ND	ND	ND	9 ± 6	ND	ND	ND	ND	0
Tomsits, 2010[31]	30	ND	32 ± 2	1677 ± 412	5.1 ± 5.1	ND	ND	ND	ND	ND	4 ± 5	ND	ND	ND	ND	ND
Wilson, 1997[28]	61	32 (52)	27 ± 2	933 ± 242	ND	12 (9–17)****	ND	ND	15 (25)	14 (23)	ND	19 (3–51)****	4 (7)	ND	ND	40 (66)

First author, year[reference]	IVH all grades	IVH ≥ 3	Jaundice	PNALD	EFA deficiency	HyperTG	Hypo-glycemia	Hyper-glycemia	F2-isoprostane concentration	Neuro-development
	n (%)		n (%)			n (%)	n (%)	n (%)		
Intervention										
D'Ascenzo, 2011[32]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Demirel, 2012[36]	ND	1 (5)	ND	ND	ND	0	ND	ND	ND	ND
Deshpande, 2009[29]	ND	ND	ND	ND	ND	ND	ND	ND	3238 ± 1173	ND
Lehner, 2006[34]	ND	ND	ND	ND	ND	0	ND	ND	ND	ND
Lima, 1988[37]	ND	ND	ND	ND	ND	ND	ND	6 (23)	ND	ND
Rayyan, 2012[33]	ND	ND	ND	ND	ND	0	ND	ND	ND	ND
Rubin, 1995[30]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Skouroliakou, 2010[35]	ND	ND	ND	ND	ND	ND	ND	3 (21)	ND	ND
Tomsits, 2010[31]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Wilson, 1997[28]	ND	ND	ND	ND	ND	22 (34)	ND	18 (28)	ND	ND
Control										
D'Ascenzo, 2011[32]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Demirel, 2012[36]	ND	2 (10)	ND	ND	ND	0	ND	ND	ND	ND
Deshpande, 2009[29]	ND	ND	ND	ND	ND	ND	ND	ND	3323 ± 1158	ND
Lehner, 2006[34]	ND	ND	ND	ND	ND	0	ND	ND	ND	ND
Lima, 1988[37]	ND	ND	ND	ND	ND	ND	ND	9 (36)	ND	ND
Rayyan, 2012[33]	ND	ND	ND	ND	ND	0	ND	ND	ND	ND
Rubin, 1995[30]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Skouroliakou, 2010[35]	ND	ND	ND	ND	ND	ND	ND	2 (11)	ND	ND
Tomsits, 2010[31]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Wilson, 1997[28]	ND	ND	ND	ND	ND	18 (30)	ND	24 (39)	ND	ND

* BPD, bronchopulmonary dysplasia; EFA, essential fatty acid; HyperTG, hypertriacylgycerolemia; IVH, intraventricular hemorrhage; ND: no data; NEC, necrotizing enterocolitis; NS: in study described as not different between treatment groups, no data presented; PDA, patent ductus arteriosus; PNALD, parenteral nutrition associated liver disease; ROP, retinopathy of prematurity.

** Mean ± SD (all such values).

*** SD or IQR was not presented and could not be calculated from available data.

**** Median; IQR in parentheses.

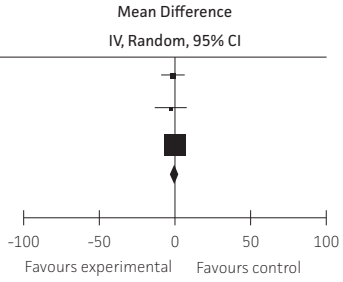
FIGURE 3 A-F Meta-analysis of the effects of lipid emulsions that are not purely soybean oil-based compared with purely soybean oil-based emulsions (random effects). IV, inverse variance; M-H, Mantel-Haenszel.

A - RATE OF WEIGHT GAIN, G/(KG·D)

	Experimental			Control			Mean Difference	Mean Difference
Study or subgroup	Mean	SD	n	Mean	SD	n	Weight	IV, Random, 95% CI
Lima, 1988[37]	-4.9	12.7	26	-3.6	15.5	25	10.4%	-1.30 [-9.09, 6.49]
Rayyan, 2012[33]	13	25.5	26	15.7	11.1	27	5.5%	-2.70 [-13.36, 7.96]
Tomsits, 2010[31]	5.5	5.7	30	5.1	5.1	30	84.1%	0.40 [-2.34, 3.14]
Total (95% CI)			**82**			**82**	**100%**	**0.05 [-2.46, 2.56]**

Heterogeneity: Tau² = 0.00; Chi² = 0.43, df = 2 (P = 0.81); I² = 0%

Test for overall effect: Z = 0.04 (P = 0.97)

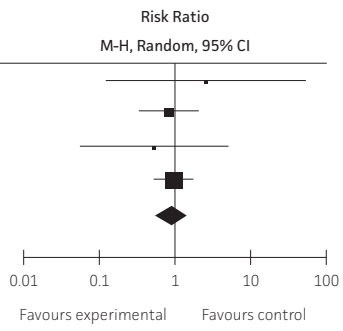


B - DEATH BEFORE DISCHARGE

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI
Deshpande, 2009[29]	1	24	0	21	2.5%	2.64 [0.11, 61.54]
Lima, 1988[37]	6	26	7	25	28.3%	0.82 [0.32, 2.11]
Rayyan, 2012[33]	1	26	2	27	4.6%	0.52 [0.05, 5.39]
Wilson, 1997[28]	15	64	15	61	64.6%	0.95 [0.51, 1.78]
Total (95% CI)		**140**		**134**	**100%**	**0.91 [0.55, 1.51]**
Total events	23		24			

Heterogeneity: Tau² = 0.00; Chi² = 0.72, df = 3 (P = 0.87); I² = 0%

Test for overall effect: Z = 0.36 (P = 0.72)

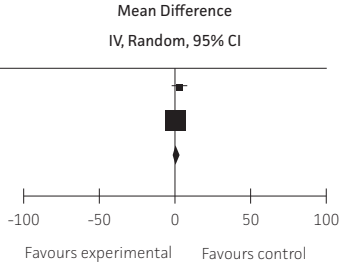


C - DURATION OF RESPIRATORY SUPPORT, DAYS

	Experimental			Control			Mean Difference	Mean Difference
Study or subgroup	Mean	SD	n	Mean	SD	n	Weight	IV, Random, 95% CI
Skouroliakou, 2010[35]	12.2	8.2	14	9.1	6.2	18	19.3%	3.09 [-2.06, 8.24]
Tomsits, 2010[31]	4.6	5.4	30	4.1	4.5	30	80.7%	0.50 [-2.02, 3.02]
Total (95% CI)			**44**			**48**	**100%**	**1.00 [-1.26, 3.26]**

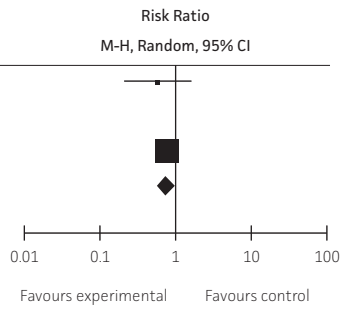
Heterogeneity: Tau² = 0.00; Chi² = 0.78, df = 1 (P = 0.38); I² = 0%

Test for overall effect: Z = 0.87 (P = 0.39)



D - SEPSIS

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI
Demirel, 2012[36]	4	20	7	20	7.6%	0.57 [0.20, 1.65]
Skouroliakou, 2010[35]	0	14	0	18		Not estimable
Wilson, 1997[28]	32	64	40	61	92.4%	0.76 [0.56, 1.03]
Total (95% CI)		**98**		**99**	**100%**	**0.75 [0.56, 1.00]**
Total events	36		47			

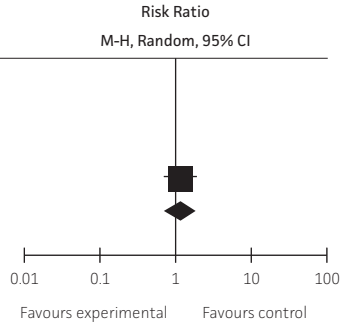


Heterogeneity: Tau² = 0.00; Chi² = 0.27, df = 1 (P = 0.60); I² = 0%

Test for overall effect: Z = 1.96 (P = 0.05)

E - HYPERTRIACYLGLYCEROLEMIA

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI
Demirel, 2012[36]	0	20	0	20		Not estimable
Lehner, 2006[34]	0	6	0	6		Not estimable
Rayyan, 2012[33]	0	26	0	27		Not estimable
Wilson, 1997[28]	22	64	18	61	100%	1.16 [0.70, 1.95]
Total (95% CI)		**116**		**114**	**100%**	**1.16 [0.70, 1.95]**
Total events	22		18			

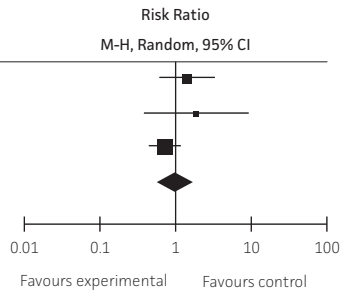


Heterogeneity: Not applicable

Test for overall effect: Z = 0.58 (P = 0.56)

F - HYPERGLYCEMIA

	Experimental		Control			Risk Ratio M-H,
Study or subgroup	Events	Total	Events	Total	Weight	Random, 95% CI
Lima, 1988[37]	6	26	9	25	30.4%	1.44 [0.60, 3.46]
Skouroliakou, 2010[35]	3	14	2	18	10.8%	1.93 [0.37, 10.01]
Wilson, 1997[28]	18	64	24	61	58.8%	0.71 [0.43, 1.18]
Total (95% CI)		**104**		**104**	**100%**	**0.98 [0.56, 1.74]**
Total events	30		32			



Heterogeneity: Tau² = 0.08; Chi² = 2.76, df = 2 (P = 0.25); I² = 28%

Test for overall effect: Z = 0.05 (P = 0.96)

respiratory support and supplemental oxygen, the incidence of PNALD, EFA deficiency, hypoglycemia, signs of lipid peroxidation, and long-term neurodevelopmental outcome, no meta-analysis could be performed because of to insufficient data.

One of the included studies in the meta-analysis (i.e., Sosenko et al[27]) reported higher mortality in the subgroup of infants with a birth weight of 600-800 g. However, this study has been criticized on methodological grounds because the subgroup analysis was post-hoc, antenatal corticosteroid use was significantly lower in the early-lipid group, and initial illness severity was not recorded.[28] The results of our meta-analysis are in agreement with the meta-analysis of Simmer et al (five-days difference in the initiation of lipids).[8] The absence of an effect on BPD and mortality was also in agreement with the meta-analyses of Fox et al and Wilson et al, both of which were published in abstract form only.[40-41]

A limitation of all included studies is that the amino acid intake was rather low or even absent during the first few days compared with current recommendations,[42] resulting in general undernutrition of the studied preterm infants. Several studies suggest that the initiation of lipids in combination with an adequate amount of amino acids may make a difference in growth. However, these studies were excluded from this systematic review for reasons explained below. The study by Ibrahim et al[43] was excluded because no growth outcomes were reported. However, the researchers reported a higher nitrogen balance, as a proxy of lean tissue growth, without increased incidence of metabolic or respiratory complications with introduction of high doses of amino acids (3.5 g/(kg·d)) plus lipids (3.0 g/(kg·d)) within the first two hours of life compared with 48 hours after birth. The RCTs by Drenckpohl et al[44] and Tan et al[45] were excluded because lipids were initiated on or before day two in both treatment groups. Drenckpohl et al[44] showed that introduction of 2.0 g/(kg·d) compared with 0.5 g/(kg·d) of pure soybean oil-based lipid infusion in combination with 3 g amino acids/(kg·d) on day one to VLBW infants (n=48 and n=52, respectively) improved energy intake, decreased weight loss, allowed an earlier regain of birth weight, and decreased incidence rates of NEC (although the incidence in the control group was higher than their annual average) and ROP, whereas the incidence of other common morbidities, such as BPD, was unchanged. Hypertriacylglycerolemia was observed more frequently in the higher-lipid group (15% compared with 4% of infants), which was as expected. In the study by Tan et al,[45] hyperalimentation (20% more dextrose, protein and fat starting at day 1, n=68) compared with standard nutrition (n=74) resulted in a reduction of postnatal growth failure without adverse clinical effects. RCTs on the effect of lipids on long-term development are lacking. However, cohort studies suggest developmental advantages with early introduction of lipids and/or a higher energy intake.[46-48] In the cohort study of Eleni dit Trolli et al[47] and Stephens et al,[48] a higher energy (and lipid) intake in the first weeks was associated with a higher developmental outcome at 1 year corrected age and higher Mental Development Index scores and lower likelihood of length growth restrictions at 18 months' corrected age, respectively. In addition, Ehrenkranz et al[46] showed that the total daily energy intake during the first week of life in extremely low birth weight infants was associated with decreased early morbidity. Therefore, the lack of differences in growth and morbidities with early introduction of lipids as shown by

this meta-analysis, should not exclude the use of lipids within the first few days of life in VLBW infants.

In addition to the effect of earlier initiation of lipids, the effects of the type of lipid emulsion on growth and adverse outcomes on 499 preterm infants from ten studies were reported in the present systematic review and meta-analysis. No statistically significant differences were observed for the primary outcome (growth during hospital admission) or during the first weeks of life on the basis of the type of lipid emulsion used. The type of lipid also did not affect the secondary outcomes of death, incidence of BPD, duration of respiratory support and supplemental oxygen, incidence of NEC, hypertriacylglycerolemia, and hyperglycemia. The included studies did not report data on the following outcomes: incidence of ROP, PDA, IVH, jaundice, PNALD, EFA deficiency, or hypoglycemia; signs of lipid peroxidation; or long-term neurodevelopmental outcome. This review shows that lipid emulsions that are not purely soybean oil-based (e.g., MCT-soybean, olive-soybean, and soybean-MCT-olive-fish emulsions; **TABLE 5**) are weakly associated with fewer episodes of sepsis than pure soybean oil emulsions (RR: 0.75; 95% CI: 0.56 to 1.00; based on 2 studies). This finding might be explained by the lower amount of n-6 fatty acids, because an excess intake of n-6 PUFAs may result in increased synthesis of proinflammatory eicosanoids.[49-51] In addition, the n-3 fatty acids in fish oil may reduce inflammatory responses while protecting immunity.[51] However, more adequately powered RCTs are necessary to confirm the effect of type of lipid emulsion on the prevention and possible treatment of sepsis episodes.

A general limitation of this systematic review is that studies were excluded when growth data were not presented. We used growth as our primary outcome because the ultimate goal of nutritional intervention is to support normal growth and development (i.e., comparable to term-born infants) without increasing the incidence of adverse events. A second limitation is that outcome measures were defined inconsistently in the studies, which made it necessary to adjust our predefined criteria of certain outcomes, such as hypertriacylglycerolemia and hyperglycemia. However, because both individual studies and our meta-analysis found no differences between treatment groups for these outcomes, we believe that the adjustments did not change the results. Another limitation is that we could not perform separate meta-analyses for the different lipid emulsions because insufficient data were available per outcome and per type of emulsion.

TABLE 5 Composition of available lipid emulsions*

	FIRST GENERATION			SECOND GENERATION				THIRD GENERATION		
Lipid emulsion	Intralipid	Lipoven**	Liposyn III	Lipofundin MCT-soybean	Structolipid	Lipoven-MCT	ClinOleic	Omegaven	Lipoplus***	SMOFlipid
Manufacturer	Fresenius Kabi	Fresenius Kabi	Hospira	B. Braun	Fresenius Kabi	Fresenius Kabi	Baxter	Fresenius Kabi	B. Braun	Fresenius Kabi
OIL SOURCE, %										
Soybean	100	100	100	50	64	50	20	-	40	30
Coconut (MCT)	-	-	-	50	36	50	-	-	50	30
Olive	-	-	-	-	-	-	80	-	-	25
Fish	-	-	-	-	-	-	-	100	10	15
COMPOSITION OF MAJOR FATTY ACIDS, WT %										
MCTs										
Caproic acid (6:0)	-	-	-	0.5	0.1	0.2	-	-	-	Trace
Caprylic acid (8:0)	-	-	-	29	26	30	-	-	30	17
Capric acid (10:0)	-	-	-	20	10	17	-	-	19	12
Lauric acid (12:0)	-	-	-	1	0.2	0.2	-	-	-	0.2
Long chain triacylglycerols										
Myristic acid (14:0)	0.2	-	Trace	-	-	Trace	0.2	5	0.5	1
Palmitic acid (16:0)	11	12	11	7	7	7	12	12	6	9
Palmitoleic acid (16:1n-7)	-	-	Trace	-	-	0.2	1.5	9	0.6	2
Stearic acid (18:0)	4	5	4	2	3	3	2	4	2	3
Oleic acid (18:1n-9)	24	24	23	11	14	13	62	15	8	29
n-6 Long chain triacylglycerols										
Linoleic acid (18:2n-6)	53	53	53	29	35	27	19	4	24	19
Arachidonic acid (20:4n-6)	-	-	-	0.2	-	-	0.5	2	-	0.5
n-3 Long chain triacylglycerols										
α-linolenic acid (18:3n-3)	8	8	8	4	5	4	2	2	3	2
Eicosapentaenoic acid (20:5n-3)	-	-	-	-	-	-	-	19	3	3
Docosahexaenoic acid (22:6n-3)	-	-	-	-	-	-	0.5	12	2	2
OTHER COMPOUND										
α-Tocopherol (μmol/L)	87	132	NP	395	16	NP	75	505	455	500

* Data were provided by the manufacturers and adapted from Wanten et al.[202] MCT, medium-chain triacylglycerol; NP, not provided.

** Lipoven is also known as Lipovenoes.

*** Lipoplus is also known as Lipidem.

In summary, available data show that lipid administration within the first two days to preterm infants seems safe and well tolerated; however, beneficial effects on growth were not observed. Despite the lack of growth benefits, the use of lipids within the first few days of life in VLBW infants should not be withheld. Further well-designed and adequately powered studies are necessary to determine the effects of early lipid administration with or without a higher amino acid intake on neurodevelopmental outcome. The use of lipid emulsions that are not purely soybean oil-based in preterm infants may result in a lower incidence of sepsis, whereas other adverse events were not affected by type of lipid. The type of lipid emulsion did not make a difference in the growth of preterm infants during hospital stays. Future large-scale RCTs in preterm infants are thus warranted to show whether these lipid emulsions result in improved long-term outcomes.

REFERENCES

1 Thureen P, Heird WC. Protein and energy requirements of the preterm/low birthweight (LBW) infant. Pediatr Res. May 2005;57(5 Pt 2):95R-8R.

2 Hulst JM, van Goudoever JB, Zimmermann LJ, et al. The effect of cumulative energy and protein deficiency on anthropometric parameters in a pediatric ICU population. Clin Nutr. Dec 2004;23(6):1381-9.

3 Martin CR, Brown YF, Ehrenkranz RA, et al. Nutritional practices and growth velocity in the first month of life in extremely premature infants. Pediatrics. Aug 2009;124(2):649-57.

4 Driscoll DF, Bistrian BR, Demmelmair H, Koletzko B. Pharmaceutical and clinical aspects of parenteral lipid emulsions in neonatology. Clin Nutr. Jun 25 2008;27(4):497-503.

5 Grover A, Khashu M, Mukherjee A, Kairamkonda V. Iatrogenic malnutrition in neonatal intensive care units: urgent need to modify practice. JPEN J Parenter Enteral Nutr. Mar-Apr 2008;32(2):140-4.

6 Hans DM, Pylipow M, Long JD, Thureen PJ, Georgieff MK. Nutritional practices in the neonatal intensive care unit: analysis of a 2006 neonatal nutrition survey. Pediatrics. Jan 2009;123(1):51-7.

7 Lapillonne A, Fellous L, Mokthari M, Kermorvant-Duchemin E. Parenteral nutrition objectives for very low birth weight infants: results of a national survey. J Pediatr Gastroenterol Nutr. May 2009;48(5):618-26.

8 Simmer K, Rao SC. Early introduction of lipids to parenterally-fed preterm infants. Cochrane Database Syst Rev. 2005(2):CD005256.

9 Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R. 1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR). J Pediatr Gastroenterol Nutr. Nov 2005;41 Suppl 2:S1-87.

10 Waitzberg D. Evolution of parenteral lipid emulsions. Clin Nutr Suppl. 2005;1(3):5-7.

11 Lilja HE, Finkel Y, Paulsson M, Lucas S. Prevention and reversal of intestinal failure-associated liver disease in premature infants with short bowel syndrome using intravenous fish oil in combination with omega-6/9 lipid emulsions. J Pediatr Surg. Jul 2011;46(7):1361-7.

12 Le HD, de Meijer VE, Robinson EM, et al. Parenteral fish-oil-based lipid emulsion improves fatty acid profiles and lipids in parenteral nutrition-dependent children. Am J Clin Nutr. Jul 20 2011;94(3):749-58.

13 Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. Jul 21 2009;6(7):e1000097.

14 Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg. Jan 1978;187(1):1-7.

15 International Committee for the Classification of Retinopathy of Prematurity. The International Classification of Retinopathy of Prematurity revisited. Arch Ophthalmol. Jul 2005;123(7):991-9.

16 Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr. Apr 1978;92(4):529-34.

17 Robinson DT, Ehrenkranz RA. Parenteral nutrition-associated cholestasis in small for gestational age infants. J Pediatr. Jan 2008;152(1):59-62.

18 Cober MP, Teitelbaum DH. Prevention of parenteral nutrition-associated liver disease: lipid minimization. Current opinion in organ transplantation. Jun 2010;15(3):330-3.
19 Putet G. Lipid metabolism of the micropremie. Clin Perinatol. Mar 2000;27(1):57-69, v-vi.
20 Sinclair JC, Bottino M, Cowett RM. Interventions for prevention of neonatal hyperglycemia in very low birth weight infants. Cochrane Database Syst Rev. 2009(3):CD007615.
21 OCEBM Levels of Evidence Working Group. The Oxford 2011 Levels of Evidence. 2011; http://www.cebm.net/index.aspx?o=5653.
22 Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials. Feb 1996;17(1):1-12.
23 Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of interventions, Version 5.1.0 [updated March 2011] 2011.
24 Brownlee KG, Kelly EJ, Ng PC, Kendall-Smith SC, Dear PR. Early or late parenteral nutrition for the sick preterm infant? Arch Dis Child. Sep 1993;69(3 Spec No):281-3.
25 Gunn T, Reaman G, Outerbridge EW, Colle E. Peripheral total parenteral nutrition for premature infants with the respiratory distress syndrome: a controlled study. J Pediatr. Apr 1978;92(4):608-13.
26 Gilbertson N, Kovar IZ, Cox DJ, Crowe L, Palmer NT. Introduction of intravenous lipid administration on the first day of life in the very low birth weight neonate. J Pediatr. Oct 1991;119(4):615-23.
27 Sosenko IR, Rodriguez-Pierce M, Bancalari E. Effect of early initiation of intravenous lipid administration on the incidence and severity of chronic lung disease in premature infants. J Pediatr. Dec 1993;123(6):975-82.
28 Wilson DC, Cairns P, Halliday HL, Reid M, McClure G, Dodge JA. Randomised controlled trial of an aggressive nutritional regimen in sick very low birthweight infants. Arch Dis Child Fetal Neonatal Ed. Jul 1997;77(1):F4-11.
29 Deshpande GC, Simmer K, Mori T, Croft K. Parenteral lipid emulsions based on olive oil compared with soybean oil in preterm (<28 weeks' gestation) neonates: a randomised controlled trial. J Pediatr Gastroenterol Nutr. Nov 2009;49(5):619-25.
30 Rubin M, Naor N, Sirota L, et al. Are bilirubin and plasma lipid profiles of premature infants dependent on the lipid emulsion infused? J Pediatr Gastroenterol Nutr. Jul 1995;21(1):25-30.
31 Tomsits E, Pataki M, Tolgyesi A, Fekete G, Rischak K, Szollar L. Safety and Efficacy of a Lipid Emulsion Containing a Mixture of Soybean Oil, Medium-chain Triglycerides, Olive Oil, and Fish Oil: A Randomised, Double-blind Clinical Trial in Premature Infants Requiring Parenteral Nutrition. J Pediatr Gastroenterol Nutr. Jun 3 2010;51(4):514-21.
32 D'Ascenzo R, D'Egidio S, Angelini L, et al. Parenteral Nutrition of Preterm Infants with a Lipid Emulsion Containing 10% Fish Oil: Effect on Plasma Lipids and Long-Chain Polyunsaturated Fatty Acids. J Pediatr. Feb 28 2011;159:33-8.
33 Rayyan M, Devlieger H, Jochum F, Allegaert K. Short-term use of parenteral nutrition with a lipid emulsion containing a mixture of soybean oil, olive oil, medium-chain triglycerides, and fish oil: a randomized double-blind study in preterm infants. JPEN J Parenter Enteral Nutr. Jan 2012;36(1 Suppl):81S-94S.
34 Lehner F, Demmelmair H, Roschinger W, et al. Metabolic effects of intravenous LCT or MCT/LCT lipid emulsions in preterm infants. J Lipid Res. Feb 2006;47(2):404-11.
35 Skouroliakou M, Konstantinou D, Koutri K, et al. A double-blind, randomized clinical trial of the effect of omega-3 fatty acids on the oxidative stress of preterm neonates fed through parenteral nutrition. Eur J Clin Nutr. Sep 2010;64(9):940-7.

[36] Demirel G, Oguz SS, Celik IH, Erdeve O, Uras N, Dilmen U. The metabolic effects of two different lipid emulsions used in parenterally fed premature infants - A randomized comparative study. Early Hum Dev. Jan 12 2012;88(7):499-501.

[37] Lima LA, Murphy JF, Stansbie D, Rowlandson P, Gray OP. Neonatal parenteral nutrition with a fat emulsion containing medium chain triglycerides. Acta Paediatr Scand. May 1988;77(3):332-9.

[38] Alwaidh MH, Bowden L, Shaw B, Ryan SW. Randomised trial of effect of delayed intravenous lipid administration on chronic lung disease in preterm neonates. J Pediatr Gastroenterol Nutr. Apr 1996;22(3):303-6.

[39] Hammerman C, Aramburo MJ. Decreased lipid intake reduces morbidity in sick premature neonates. J Pediatr. Dec 1988;113(6):1083-8.

[40] Fox GF, Wilson DC, Ohlsson A. Effect of early vs. late introduction of intravenous lipid to preterm infants on death and chronic lung disease (CLD) – Results of meta-analysis +1250. Pediatr Res. 1998;43(4, suppl 2):S214.

[41] Wilson DC, Fox GF, Ohlsson A. Meta-analysis of effects of early or late introduction of intravenous lipid to preterm infants on mortality and chronic lung disease. J Pediatr Gastroenterol Nutr. 1998;26(5):599 (abstract).

[42] van den Akker CH, Vlaardingerbroek H, van Goudoever JB. Nutritional support for extremely low-birth weight infants: abandoning catabolism in the neonatal intensive care unit. Curr Opin Clin Nutr Metab Care. Mar 6 2010;13:327-35.

[43] Ibrahim HM, Jeroudi MA, Baier RJ, Dhanireddy R, Krouskop RW. Aggressive early total parental nutrition in low-birth-weight infants. J Perinatol. Aug 2004;24(8):482-6.

[44] Drenckpohl D, McConnell C, Gaffney S, Niehaus M, Macwan KS. Randomized trial of very low birth weight infants receiving higher rates of infusion of intravenous fat emulsions during the first week of life. Pediatrics. Oct 2008;122(4):743-51.

[45] Tan MJ, Cooke RW. Improving head growth in very preterm infants--a randomised controlled trial I: neonatal outcomes. Arch Dis Child Fetal Neonatal Ed. Sep 2008;93(5):F337-41.

[46] Ehrenkranz RA, Das A, Wrage LA, et al. Early nutrition mediates the influence of severity of illness on extremely LBW infants. Pediatr Res. Jun 2011;69(6):522-9.

[47] Eleni dit Trolli S, Kermorvant-Duchemin E, Huon C, Bremond-Gignac D, Lapillonne A. Early lipid supply and neurological development at one year in very low birth weight (VLBW) preterm infants. Early Hum Dev. Mar 2012;88 Suppl 1:S25-9.

[48] Stephens BE, Walden RV, Gargus RA, et al. First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics. May 2009;123(5):1337-43.

[49] Calder PC. The 2008 ESPEN Sir David Cuthbertson lecture: Fatty acids and inflammation - From the membrane to the nucleus and from the laboratory bench to the clinic. Clin Nutr. Feb 2010;29(1):5-12.

[50] Wanten GJ. Parenteral lipids in nutritional support and immune modulation. Clin Nutr suppl. 2009;4:13-7.

[51] Grimm H, Mertes N, Goeters C, et al. Improved fatty acid and leukotriene pattern with a novel lipid emulsion in surgical patients. European journal of nutrition. Feb 2006;45(1):55-60.

[52] Wanten GJ, Calder PC. Immune modulation by parenteral lipid emulsions. Am J Clin Nutr. May 2007;85(5):1171-84.

CHAPTER 2

Appendix

Search strategy

Pubmed search strategy (result 1043 hits):

(lipids[mesh:noexp] OR fatty acids[mesh] OR lipid[tw] OR lipids[tw] OR fat[tw] OR fatty acid*[tw] OR oil*[tw] OR soy*[tw] OR triglyc*[tw] OR triacylgl*[tw] OR olive*[tw] OR fish*[tw] OR omega-6[tw] OR omega-3[tw] OR n-6[tw] OR n-3[tw])
AND
(emulsion*[tw] OR parenter*[tw] OR intraven*[tw] OR infus*[tw])
AND
(low birth weight*[tw] OR premature*[tw] OR preterm*[tw] OR pre-term*[tw])
NOT (animals[mesh] NOT humans[mesh])

EMBASE search strategy (result 1428 hits):

(lipids/de OR 'fatty acid'/exp OR (lipid OR lipids OR fat OR (fatty NEXT/1 acid*) OR oil* OR soy* OR triglyc* OR triacylgl* OR olive* OR fish* OR omega-6 OR omega-3 OR n-3 OR n-6):ti,ab,de)
AND
(emulsion* OR parenter* OR intraven* OR infus*):de,ab,ti
AND
(('low birth' NEXT/1 weight*) OR premature* OR preterm* OR (pre NEXT/1 term*)):de,ab,ti NOT ([animals]/lim NOT [humans]/lim)

Cochrane search strategy (result 7 hits):

(lipid OR fat OR fatty acid OR oil OR soy OR soybean OR (medium AND chain AND triglyceride OR triacylglycerol) OR olive OR fish OR n-6 OR omega-6 OR n-3 OR omega-3)
AND
(emulsion OR parenteral OR intravenous OR infusion)
AND
(Very low birth weight) OR premature OR preterm OR neonate)

Excluded studies

Exclusion criteria	n	Excluded studies (first author, year, reference)
Language	7	Bai, 2005;[1] Ceccarelli, 1995;[2] Gawecka, 2008;[3] Paust, 1986;[4] Sluncheva, 2010;[5] Tamaro, 1989;[6] Wolf, 1968[7]
No randomized controlled trial	13	Eleni dit Trolli, 2012;[8] Gnigler, 2011;[9] Hopewell, 2012;[10] Janeiro, 2010[11] Lapillonne, 2011;[12] Martin, 2009;[13] Martinez, 1987;[14] Reid, 1977;[15] Rochow, 2012;[16] Rubecz, 1979;[17] Vakrilova, 2011;[18] Wells, 1989;[19] Yunis, 1992;[20]
Review or comment	54	[No author listed], 1980;[21] Ben, 2008;[22] Bourgeois, 1974;[23] Bryan, 1976;[24] Camelo Jr, 2005;[25] Chan, 1998;[26] Chaudhari, 2006;[27] Chawla, 2008;[28] Clark, 2003;[29] Corpeleijn, 2011;[30] Deckelbaum, 2003;[31] Deshpande, 2011;[32] Driscoll, 2008;[33] Ehrenkranz, 2007;[34] Giovannini, 1995;[35] Hay Jr, 2005;[36] Hay, 2008;[37] Heine, 2002;[38] Heird, 1975;[39] Heird, 1995;[40] Heird, 1996;[41] Helbock, 1995;[42] Innis, 1992;[43] Kerner, 2006;[44] Koletzko, 1998;[45] Koletzko, 2002;[46] Krohn, 2006;[47] Lai, 2006;[48] Lima, 1989;[49] Lipsky, 1995;[50] Lucas, 1994;[51] Lucas, 1994;[52] Malan, 1976;[53] Mason, 2011;[54] Mirtallo, 2010;[55] Neu, 1992;[56] Pinchasik, 2001;[57] Pitkanen, 2004;[58] Putet, 2000;[59] Sala-Vila, 2007;[60] Schreier, 1979;[61] Schutzman, 2008;[62] Simmer, 2005;[63] Simmer, 2007;[64] Smithers, 2008;[65] Taylor, 2010;[66] Te Braake, 2007;[67] Ulrich, 1996;[68] Valentine, 2007;[69] Van Beek, 1995;[70] Velaphi, 2011;[71] Victorin, 1971;[72] Vlaardingerbroek, 2009;[73] Ziegler, 2009[74]
Study protocol, no study data	2	Kapoor, 2011;[75] Morgan, 2011[76]
No growth data presented	30	Bassiouny, 2009;[77] Brans, 1986;[78] Brans, 1987;[79] Brans, 1988;[80] Cooke, 1983;[81] Cooke, 1985;[82] Cooke, 1985;[83] Cooke, 1987;[84] Gawecka, 2008;[85] Gobel, 2003;[86] Gustafson, 1972;[87] Gustafson, 1974;[88] Gutcher, 1991;[89] Hammerman, 1988;[90] Haumont, 1989;[91] Haumont, 1992;[92] Ibrahim, 2004;[93] Kesiak, 2010;[94] Koksal, 2011;[95] Liet, 1999;[96] McClead, 1991;[97] Murdock, 1995;[98] Pereira, 1979;[99] Pitkanen, 2004;[100] Rhodes, 1991;[101] Roggero, 2010;[102] Rubin, 1991;[103] Rubin, 1994;[104] Shoji, 2011;[105] Spear, 1990[106]
Initiation day of lipids comparable between groups	6	Bulbul, 2011;[107] Cairns, 1996;[108] Drenckpohl, 2008;[109] Kerzner, 1983;[110] Tan, 2008;[111] Zlotkin, 1981[112]
Start lipids after day 2 in both groups	2	Alwaidh, 1996;[113] Vaidya, 1995[114]

REFERENCES

1 Bai XM, Liu ZJ, Li SJ, Xin P, Li G. Comparison of two parenteral nutrition methods in low birth weight premature infants. Chinese Journal of Contemporary Pediatrics. 2005;7(4):325-8.

2 Ceccarelli PL, Gentili A, Grillone G, et al. Use of 20% soy oil emulsion as lipidic nutrient in neonatal NPT. Rivista Italiana di Nutrizione Parenterale ed Enterale. 1995;13(3):205-10.

3 Gawecka A, Kornacka MK, Luckiewicz B, Rudzinska I. [Tolerance of two lipid emulsions used in parenterally-fed premature infants - a comparative study] Porownanie tolerancji dwoch emulsji tluszczowych na bazie oleju sojowego i oliwy z oliwek stosowanych w zywieniu pozajelitowym noworodkow urodzonych przedwczesnie. Med Wieku Rozwoj. Jul-Sep 2008;12(3):782-8.

4 Paust H, Park W, Brosicke H, Schroder H. [Fats in the parenteral nutrition of the child with special reference to the premature infant] Fett in der parenteralen Ernahrung des Kindes unter besonderer Berucksichtigung des Fruhgeborenen. Beitr Infusionther Klin Ernahr. 1986;13:178-90.

5 Sluncheva B. [Strategies for nutrition of the preterm infant with low and very low birth weight]. Akush Ginekol (Sofiia). 2010;49(2):33-9.

6 Tamaro G, Visconti P, Colonna F, et al. Monitoring of lipid tolerance in premature infants of very low weight during total parenteral nutrition with Intralipid. Giornale Italiano di Chimica Clinica. 1989;14(3):173-8.

7 Wolf H, Lohr H. [Fat infusion in the newborn during the first day of life] Fettinfusionen bei Fruhgeborenen am ersten Lebenstag. Monatsschr Kinderheilkd. Jun 1968;116(6):262-5.

8 Eleni dit Trolli S, Kermorvant-Duchemin E, Huon C, Bremond-Gignac D, Lapillonne A. Early lipid supply and neurological development at one year in very low birth weight (VLBW) preterm infants. Early Hum Dev. Mar 2012;88 Suppl 1:S25-9.

9 Gnigler M, Navarro-Psihas S, Schlenz B, Kiechl-Kohlendorfer U, Rüdiger M. Improved weight gain in VLBW infants after the introduction of a computer-based plan for individualized parenteral nutrition. Monatsschr Kinderheilkd. 2011;159:(Suppl) 343.

10 Hopewell J, Miletin J. Parenteral nutrition in very low birth weight infants in the United Kingdom and Ireland. Ir Med J. Feb 2012;105(2):42-5.

11 Janeiro P, Cunha M, Marques A, Moura M, Barroso R, Carreiro H. Caloric intake and weight gain in a neonatal intensive care unit. European Journal of Pediatrics. 2010;169(1):99-105.

12 Lapillonne A, Fellous L, Kermorvant-Duchemin E, French neonatal d. Use of parenteral lipid emulsions in French neonatal ICUs. Nutr Clin Pract. Dec 2011;26(6):672-80.

13 Martin CR, Brown YF, Ehrenkranz RA, et al. Nutritional practices and growth velocity in the first month of life in extremely premature infants. Pediatrics. Aug 2009;124(2):649-57.

14 Martinez M, Ballabriga A. Effects of parenteral nutrition with high doses of linoleate on the developing human liver and brain. Lipids. Mar 1987;22(3):133-8.

15 Reid WD. Metabolic response to intralipid infusions in the neonatal period. Adv Exp Med Biol. 1977;82:201-3.

16 Rochow N, Fusch G, Muhlinghaus A, et al. A nutritional program to improve outcome of very low birth weight infants. Clin Nutr. Feb 2012;31(1):124-31.

17 Rubecz I, Mestyan J, Varga P, Soltesz G. Metabolic and hormonal responses of low birthweight infants to intravenously infused calories not exceeding the maintenance energy expenditure. Arch Dis Child. Jul 1979;54(7):499-505.

18 Vakrilova L, Slancheva B, Emilova Z, Radulova P, Hitrova S, Petrova G. Early parenteral nutrition of very low birth weight infants: Practical application. Intensive Care Med. 2011;37(Suppl 2):S398.
19 Wells DH, Ferlauto JJ, Forbes DJ, et al. Lipid tolerance in the very low birth weight infant on intravenous and enteral feedings. JPEN J Parenter Enteral Nutr. Nov-Dec 1989;13(6):623-7.
20 Yunis KA, Oh W, Kalhan S, Cowett RM. Glucose kinetics following administration of an intravenous fat emulsion to low-birth-weight neonates. Am J Physiol. Nov 1992;263(5 Pt 1):E844-9.
21 Safety of intralipid. Lancet. Nov 8 1980;2(8202):1020-1.
22 Ben XM. Nutritional management of newborn infants: practical guidelines. World J Gastroenterol. Oct 28 2008;14(40):6133-9.
23 Bourgeois J, Genoud J. Parenteral feeding in premature and newborn infants. Pediatrie. 1974;29(6):655-61.
24 Bryan H, Shennan A, Griffin E, Angel A. Intralipid-its rational use in parenteral nutrition of the newborn. Pediatrics. Dec 1976;58(6):787-90.
25 Camelo Jr JS, Martinez FE. Nutritional dilemmas in extremely low birth weight infants and their effects on childhood, adolescence and adulthood. Jornal de Pediatria. 2005;81(1 SUPPL. 1):S33-S42.
26 Chan S, McCowen KC, Bistrian B. Medium-chain triglyceride and n-3 polyunsaturated fatty acid-containing emulsions in intravenous nutrition. Curr Opin Clin Nutr Metab Care. Mar 1998;1(2):163-9.
27 Chaudhari S, Kadam S. Total parenteral nutrition in neonates. Indian Pediatr. Nov 2006;43(11):953-64.
28 Chawla D, Thukral A, Agarwal R, Deorari AK, Paul VK. Parenteral nutrition. Indian J Pediatr. Apr 2008;75(4):377-83.
29 Clark RH, Wagner CL, Merritt RJ, et al. Nutrition in the neonatal intensive care unit: how do we reduce the incidence of extrauterine growth restriction? J Perinatol. Jun 2003;23(4):337-44.
30 Corpeleijn WE, Vermeulen MJ, Van Den Akker CH, Van Goudoever JB. Feeding very-low-birth-weight infants: Our aspirations versus the reality in practice. Annals of Nutrition and Metabolism. 2011;58(SUPPL. 1):20-9.
31 Deckelbaum RJ. Intravenous lipid emulsions in pediatrics: Time for a change? Journal of Pediatric Gastroenterology and Nutrition. 2003;37(2):112-4.
32 Deshpande G, Simmer K. Lipids for parenteral nutrition in neonates. Curr Opin Clin Nutr Metab Care. Mar 2011;14(2):145-50.
33 Driscoll DF, Bistrian BR, Demmelmair H, Koletzko B. Pharmaceutical and clinical aspects of parenteral lipid emulsions in neonatology. Clin Nutr. Aug 2008;27(4):497-503.
34 Ehrenkranz RA. Early, aggressive nutritional management for very low birth weight infants: what is the evidence? Semin Perinatol. Apr 2007;31(2):48-55.
35 Giovannini M, Riva E, Agostoni C. Fatty acids in pediatric nutrition. Pediatr Clin North Am. Aug 1995;42(4):861-77.
36 Hay Jr WW. Intravenous nutrition of the very preterm neonate. Acta Paediatrica, International Journal of Paediatrics. 2005;94(SUPP 449):47-56.
37 Hay WW, Jr. Strategies for feeding the preterm infant. Neonatology. 2008;94(4):245-54.
38 Heine RG, Bines JE. New approaches to parenteral nutrition in infants and children. J Paediatr Child Health. Oct 2002;38(5):433-7.

[39] Heird WC, Driscoll JM, Jr. Use of intravenously administered lipid in neonates. Pediatrics. Jul 1975;56(1):5-7.
[40] Heird WC. Amino acid and energy needs of pediatric patients receiving parenteral nutrition. Pediatric Clinics of North America. 1995;42(4):765-89.
[41] Heird WC, Gomez MR. Parenteral nutrition in low-birth-weight infants. Annu Rev Nutr. 1996;16:471-99.
[42] Helbock HJ, Ames BN. Use of intravenous lipids in neonates. J Pediatr. May 1995;126(5 Pt 1):747-8.
[43] Innis SM. n-3 fatty acid requirements of the newborn. Lipids. Nov 1992;27(11):879-85.
[44] Kerner JA, Jr., Poole RL. The use of IV fat in neonates. Nutr Clin Pract. Aug 2006;21(4):374-80.
[45] Koletzko B. Lipid supply and metabolism in infancy. Curr Opin Clin Nutr Metab Care. Mar 1998;1(2):171-7.
[46] Koletzko B. Parenteral lipid infusion in infancy: Physiological basis and clinical relevance. Clinical Nutrition. 2002;21(SUPPL. 2):53-65.
[47] Krohn K, Koletzko B. Parenteral lipid emulsions in paediatrics. Curr Opin Clin Nutr Metab Care. May 2006;9(3):319-23.
[48] Lai NM, Rajadurai SV, Tan KH. Increased energy intake for preterm infants with (or developing) bronchopulmonary dysplasia/ chronic lung disease. Cochrane Database Syst Rev. 2006;3:CD005093.
[49] Lima LA. Neonatal parenteral nutrition with medium-chain triglycerides: rationale for research. JPEN J Parenter Enteral Nutr. May-Jun 1989;13(3):312-7.
[50] Lipsky CL, Spear ML. Recent advances in parenteral nutrition. Clin Perinatol. Mar 1995;22(1):141-55.
[51] Lucas A. Early use of lipid infusions in very low birth weight infants. J Pediatr. Aug 1994;125(2):329-30.
[52] Lucas A, Sosenko IRS, Bancalari E. Early use of lipid infusions in very low birth weight infants [2]. Journal of Pediatrics. 1994;125(2):329-30.
[53] Malan AF, Higgs SC, de VHH. Intravenous lipid and growth in small premature infants. Pediatrics. Dec 1976;58(6):917-8.
[54] Mason DG, Puntis JWL, McCormick K, Smith N. Parenteral nutrition for neonates and children: A mixed bag. Archives of Disease in Childhood. 2011;96(3):209-10.
[55] Mirtallo JM, Dasta JF, Kleinschmidt KC, Varon J. State of the art review: Intravenous fat emulsions: Current applications, safety profile, and clinical implications. Ann Pharmacother. Apr 2010;44(4):688-700.
[56] Neu J. Nutritional support of the high-risk, very low birth weight, and premature infant. Current Opinion in Pediatrics. 1992;4(2):212-6.
[57] Pinchasik D. From TPN to breast feeding - Feeding the premature infant - 2000: Part I. Parenteral nutrition. American Journal of Perinatology. 2001;18(2):59-72.
[58] Pitkanen OM. Parenteral lipids and the preterm infant: between Scylla and Charybdis. Acta Paediatr. Aug 2004;93(8):1028-30.
[59] Putet G. Lipid metabolism of the micropremie. Clin Perinatol. Mar 2000;27(1):57-69, v-vi.
[60] Sala-Vila A, Barbosa VM, Calder PC. Olive oil in parenteral nutrition. Curr Opin Clin Nutr Metab Care. Mar 2007;10(2):165-74.

[61] Schreier K. Lipids in the parenteral nutrition of prematures. Acta Chirurgica Scandinavica. 1979;145(Suppl. 494):210-1.

[62] Schutzman DL, Porat R, Salvador A, Janeczko M. Neonatal nutrition: a brief review. World J Pediatr. Nov 2008;4(4):248-53.

[63] Simmer K, Rao SC. Early introduction of lipids to parenterally-fed preterm infants. Cochrane Database Syst Rev. 2005(2):CD005256.

[64] Simmer K. Aggressive nutrition for preterm infants--benefits and risks. Early Hum Dev. Oct 2007;83(10):631-4.

[65] Smithers LG, Gibson RA, McPhee A, Makrides M. Effect of long-chain polyunsaturated fatty acid supplementation of preterm infants on disease risk and neurodevelopment: a systematic review of randomized controlled trials. Am J Clin Nutr. Apr 2008;87(4):912-20.

[66] Taylor SN, Kiger J, Finch C, Bizal D. Fluid, electrolytes, and nutrition: minutes matter. Adv Neonatal Care. Oct 2010;10(5):248-55.

[67] te Braake FW, van den Akker CH, Riedijk MA, van Goudoever JB. Parenteral amino acid and energy administration to premature infants in early life. Semin Fetal Neonatal Med. Feb 2007;12(1):11-8.

[68] Ulrich H, Pastores SM, Katz DP, Kvetan V. Parenteral use of medium-chain triglycerides: a reappraisal. Nutrition. Apr 1996;12(4):231-8.

[69] Valentine CJ, Puthoff TD. Enhancing parenteral nutrition therapy for the neonate. Nutr Clin Pract. Apr 2007;22(2):183-93.

[70] van Beek RH, Carnielli VP, Sauer PJ. Nutrition in the neonate. Curr Opin Pediatr. Apr 1995;7(2):146-51.

[71] Velaphi S. Nutritional requirements and parenteral nutrition in preterm infants. S Afr J Clin Nutr. 2011;24:(Suppl 1) 27-31.

[72] Victorin L, Gustafson A, Kjellmer I, Olegard R. Intravenous administration of fat to premature infants. Acta Paediatr Scand. Jan 1971;60(1):102.

[73] Vlaardingerbroek H, van Goudoever JB, van den Akker CH. Initial nutritional management of the preterm infant. Early Hum Dev. Nov 2009;85(11):691-5.

[74] Ziegler EE, Carlson SJ. Early nutrition of very low birth weight infants. Journal of Maternal-Fetal and Neonatal Medicine. 2009;22(3):191-7.

[75] Kapoor V, Glover R, Malviya MN. Soy oil based versus alternative lipid emulsions for parenterally fed preterm infants (protocol). Cochrane Database Syst Rev. 2011.

[76] Morgan C, Herwitker S, Badhawi I, et al. SCAMP: standardised, concentrated, additional macronutrients, parenteral nutrition in very preterm infants: a phase IV randomised, controlled exploratory study of macronutrient intake, growth and other aspects of neonatal care. BMC Pediatr. 2011;11:53.

[77] Bassiouny MR, Almarsafawy H, Abdel-Hady H, Nasef N, Hammad TA, Aly H. A randomized controlled trial on parenteral nutrition, oxidative stress, and chronic lung diseases in preterm infants. J Pediatr Gastroenterol Nutr. Mar 2009;48(3):363-9.

[78] Brans YW, Dutton EB, Andrew DS, Menchaca EM, West DL. Fat emulsion tolerance in very low birth weight neonates: effect on diffusion of oxygen in the lungs and on blood pH. Pediatrics. Jul 1986;78(1):79-84.

[79] Brans YW, Ritter DA, Kenny JD, Andrew DS, Dutton EB, Carrillo DW. Influence of intravenous fat emulsion on serum bilirubin in very low birthweight neonates. Arch Dis Child. Feb 1987;62(2):156-60.

[80] Brans YW, Andrew DS, Carrillo DW, Dutton EP, Menchaca EM, Puleo-Scheppke BA. Tolerance of fat emulsions in very-low-birth-weight neonates. Am J Dis Child. Feb 1988;142(2):145-52.

[81] Cooke RJ, Burckhart GJ. Hypertriglyceridemia during the intravenous infusion of a safflower oil-based fat emulsion. J Pediatr. Dec 1983;103(6):959-61.

[82] Cooke RJ, Buis M, Zee P, Yeh YY. Safflower oil emulsion administration during parenteral nutrition in the preterm infant. 2. Effect on triglyceride and free fatty acid levels. J Pediatr Gastroenterol Nutr. Oct 1985;4(5):804-7.

[83] Cooke RJ, Zee P, Yeh YY. Safflower oil emulsion administration during parenteral nutrition in the preterm infant. 1. Effect on essential fatty acid status. J Pediatr Gastroenterol Nutr. Oct 1985;4(5):799-803.

[84] Cooke RJ, Yeh YY, Gibson D, Debo D, Bell GL. Soybean oil emulsion administration during parenteral nutrition in the preterm infant: effect on essential fatty acid, lipid, and glucose metabolism. J Pediatr. Nov 1987;111(5):767-73.

[85] Gawecka A, Michalkiewicz J, Kornacka MK, Luckiewicz B, Kubiszewska I. Immunologic properties differ in preterm infants fed olive oil vs soy-based lipid emulsions during parenteral nutrition. JPEN J Parenter Enteral Nutr. Jul-Aug 2008;32(4):448-53.

[86] Gobel Y, Koletzko B, Bohles HJ, et al. Parenteral fat emulsions based on olive and soybean oils: a randomized clinical trial in preterm infants. J Pediatr Gastroenterol Nutr. Aug 2003;37(2):161-7.

[87] Gustafson A, Kjellmer I, Olegard R, Victorin L. Nutrition in low-birth-weight infants. I. Intravenous injection of fat emulsion. Acta Paediatr Scand. Mar 1972;61(2):149-58.

[88] Gustafson A, Kjellmer I, Olegard R, Victorin LH. Nutrition in low-birth-weight infants. II. Repeated intravenous injections of fat emulsion. Acta Paediatr Scand. Mar 1974;63(2):177-82.

[89] Gutcher GR, Farrell PM. Intravenous infusion of lipid for the prevention of essential fatty acid deficiency in premature infants. Am J Clin Nutr. Dec 1991;54(6):1024-8.

[90] Hammerman C, Aramburo MJ. Decreased lipid intake reduces morbidity in sick premature neonates. J Pediatr. Dec 1988;113(6):1083-8.

[91] Haumont D, Deckelbaum RJ, Richelle M, et al. Plasma lipid and plasma lipoprotein concentrations in low birth weight infants given parenteral nutrition with twenty or ten percent lipid emulsion. J Pediatr. Nov 1989;115(5 Pt 1):787-93.

[92] Haumont D, Rossle C, Clercx A, et al. Modifications of surfactant phospholipid pattern in premature infants treated with curosurf: clinical and dietary correlations. Biol Neonate. 1992;61 Suppl 1:37-43.

[93] Ibrahim HM, Jeroudi MA, Baier RJ, Dhanireddy R, Krouskop RW. Aggressive early total parental nutrition in low-birth-weight infants. J Perinatol. Aug 2004;24(8):482-6.

[94] Kesiak M, Nowiczewski M, Talar T, Gulczynska E. Early use of intravenous lipids in two different doses in the group of very low birth weight newborns - RCT. Early Human Development. 2010;86:S86.

[95] Koksal N, Kavurt AV, Cetinkaya M, Ozarda Y, Ozkan H. Comparison of lipid emulsions on antioxidant capacity in preterm infants receiving parenteral nutrition. Pediatr Int. Aug 2011;53(4):562-6.

[96] Liet JM, Piloquet H, Marchini JS, et al. Leucine metabolism in preterm infants receiving parenteral nutrition with medium-chain compared with long-chain triacylglycerol emulsions. Am J Clin Nutr. Mar 1999;69(3):539-43.

[97] McClead RE, Jr., Lentz ME, Coniglio JG, Meng HC, Gozs S. The effect of three intravenous fat emulsions containing different concentrations of linoleic and alpha-linolenic acids on the plasma total fatty acid profile of neonates. J Pediatr Gastroenterol Nutr. Jan 1991;12(1):89-95.

[98] Murdock N, Crighton A, Nelson LM, Forsyth JS. Low birthweight infants and total parenteral nutrition immediately after birth. II. Randomised study of biochemical tolerance of intravenous glucose, amino acids, and lipid. Arch Dis Child Fetal Neonatal Ed. Jul 1995;73(1):F8-12.

[99] Pereira GR, Fox WW, Stanley CA. The effect of Intralipid on pulmonary function and triglyceride metabolism in premature infants. Acta Chirurgica Scandinavica. 1979;145(Suppl. 494):207-9.

[100] Pitkanen OM, Luukkainen P, Andersson S. Attenuated lipid peroxidation in preterm infants during subsequent doses of intravenous lipids. Biol Neonate. 2004;85(3):184-7.

[101] Rhodes PG, Reddy NS, Downing G, Carlson SE. Effects of different levels of intravenous alpha-linolenic acid and supplemental breast milk on red blood cell docosahexaenoic acid in very low birth-weight infants. J Pediatr Gastroenterol Nutr. Jul 1991;13(1):67-71.

[102] Roggero P, Mosca F, Gianni ML, et al. F2-isoprostanes and total radical-trapping antioxidant potential in preterm infants receiving parenteral lipid emulsions. Nutrition. May 2010;26(5):551-5.

[103] Rubin M, Harell D, Naor N, et al. Lipid infusion with different triglyceride cores (long-chain vs medium-chain/long-chain triglycerides): effect on plasma lipids and bilirubin binding in premature infants. JPEN J Parenter Enteral Nutr. Nov-Dec 1991;15(6):642-6.

[104] Rubin M, Moser A, Naor N, Merlob P, Pakula R, Sirota L. Effect of three intravenously administered fat emulsions containing different concentrations of fatty acids on the plasma fatty acid composition of premature infants. J Pediatr. Oct 1994;125(4):596-602.

[105] Shoji H, Hisata K, Suzuki M, et al. Effects of parenteral soybean oil lipid emulsion on the long-chain polyunsaturated fatty acid profile in very-low-birth-weight infants. Acta Paediatr. Jul 2011;100(7):972-6.

[106] Spear ML, Spear M, Cohen AR, Pereira GR. Effect of fat infusions on platelet concentration in premature infants. JPEN J Parenter Enteral Nutr. Mar-Apr 1990;14(2):165-8.

[107] Bulbul A, Okan F, Bulbul L, Nuhoglu A. Effect of low versus high early parenteral nutrition on plasma amino acid profiles in very low birth-weight infants. J Matern Fetal Neonatal Med. Jul 20 2011.

[108] Cairns PA, Wilson DC, Jenkins J, McMaster D, McClure BG. Tolerance of mixed lipid emulsion in neonates: effect of concentration. Arch Dis Child Fetal Neonatal Ed. Sep 1996;75(2):F113-6.

[109] Drenckpohl D, McConnell C, Gaffney S, Niehaus M, Macwan KS. Randomized trial of very low birth weight infants receiving higher rates of infusion of intravenous fat emulsions during the first week of life. Pediatrics. Oct 2008;122(4):743-51.

[110] Kerzner B, Sloan HR, Lubin AH, et al. The use of Intralipid 10% and 20% in very low birthweight premature infants. Acta Chir Scand Suppl. 1983;517:135-48.

[111] Tan MJ, Cooke RW. Improving head growth in very preterm infants--a randomised controlled trial I: neonatal outcomes. Arch Dis Child Fetal Neonatal Ed. Sep 2008;93(5):F337-41.

[112] Zlotkin SH, Bryan MH, Anderson GH. Intravenous nitrogen and energy intakes required to duplicate in utero nitrogen accretion in prematurely born human infants. J Pediatr. Jul 1981;99(1):115-20.

[113] Alwaidh MH, Bowden L, Shaw B, Ryan SW. Randomised trial of effect of delayed intravenous lipid administration on chronic lung disease in preterm neonates. J Pediatr Gastroenterol Nutr. Apr 1996;22(3):303-6.

[114] Vaidya UV, Bhave SA, Pandit AN. Parenteral nutrition (PN) in the management of very low birth weight (VLBW) babies--a randomized controlled trial. Indian Pediatr. Feb 1995;32(2):165-70.

PART 2 – QUANTITY OF PARENTERAL NUTRITION

CHAPTER 3

Safety and efficacy of early parenteral lipid and high-dose amino acid administration to very low birth weight infants: a randomized controlled trial

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SUBMITTED

ABSTRACT

OBJECTIVE

We aimed to assess the efficacy and safety of early parenteral lipid and high-dose amino acid administration from birth onwards in very low birth weight (VLBW, birth weight < 1500 g) infants.

STUDY DESIGN

Inborn VLBW infants were randomized to receive 2.4 g amino acids/(kg·d) (control group), or 2.4 g amino acids/(kg·d) plus 2-3 g lipids/(kg·d) (AA+lipid group), or 3.6 g amino acids/(kg·d) plus 2-3 g lipids/(kg·d) (high AA+lipid group) from birth onwards. The primary outcome was nitrogen balance. The secondary outcomes were biochemical parameters, urea rate of appearance, growth rates, and clinical outcome.

RESULTS

One hundred forty-four infants (birth weight 862 ± 218 g; gestational age 27.4 ± 2.2 weeks) were included. The nitrogen balance on day 2 was significantly higher in both intervention groups compared with the control group. High amino acid administration did not further improve nitrogen balance compared to standard amino acid dose plus lipids and was associated with high plasma urea concentrations and high rates of urea appearance. No differences in other biochemical parameters, growth, or clinical outcomes were observed.

CONCLUSIONS

In VLBW infants administration of parenteral amino acids combined with lipids from birth onwards improved conditions for anabolism and growth, as shown by improved nitrogen balance. Higher amino acid administration did not further improve the nitrogen balance, but lead to increased amino acid oxidation. Early lipid initiation and high-dose amino acid were well tolerated, as demonstrated by a lack of serious biochemical disturbances or adverse clinical outcome.

CHAPTER 4

Adaptive regulation of amino acid metabolism in very low birth weight infants

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SUBMITTED

ABSTRACT

BACKGROUND

An anabolic state can be achieved upon parenteral amino acid administration in the immediate postnatal phase, despite a low energy intake. Optimal dosing of both amino acid and energy intake has yet to be established.

OBJECTIVE AND METHODS

To quantify the efficacy of early initiation of parenteral lipids and higher amounts of amino acids on metabolism and protein accretion, 28 very low birth weight (VLBW) infants were randomized to receive 2.4 g amino acids/(kg·d) (control), 2.4 g amino acids/(kg·d) plus 2-3 g lipid/(kg·d) (AA+lipid), or 3.6 g amino acids/(kg·d) plus 2-3 g lipid/(kg·d) (high AA+lipid) from birth onwards. On postnatal day 2 we performed a stable isotope study with [1-^{13}C]phenylalanine, [ring-D_4]tyrosine, [U-$^{13}C_6$, ^{15}N]leucine, and [methyl-D_3]α-ketoisocaproic acid.

RESULTS

Adding lipids only did not influence leucine and phenylalanine metabolism, while adding both lipids and additional amino acids increased protein synthesis rates based on both phenylalanine and leucine data. This resulted in higher net protein balances. In addition, high amino acid intake significantly increased phenylalanine hydroxylation rates to tyrosine making more tyrosine available for protein synthesis.

CONCLUSIONS

Administration of 3.6 g amino acid/(kg·d) plus 2-3 g lipid/(kg·d) increased protein balances and phenylalanine hydroxylation rates in the acute phase following preterm birth in VLBW infants compared to an intake of 2.4 g amino acid/(kg·d) with or without lipids.

CHAPTER 5

Albumin synthesis is enhanced by early parenteral lipid and high-dose amino acid administration to very low birth weight infants

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SUBMITTED

ABSTRACT

BACKGROUND

Albumin is one of the most important plasma proteins and plays a key role in many physiological processes like preserving of colloid osmotic pressure, scavenging radicals, and binding and transporting bilirubin, hormones, and drugs. However, albumin concentrations are often low during the first days of life in preterm infants.
We hypothesized that early parenteral lipid and high-dose amino acid (AA) administration from birth onwards to very low birth weight (VLBW) infants increases hepatic albumin synthesis rates.

METHODS

Inborn VLBW infants were randomized to receive 2.4 g amino acids/(kg·d) (control group), 2.4 g amino acids/(kg·d) plus 2 g lipids/(kg·d) (AA+lipid group), or 3.6 g amino acids/(kg·d) plus 2 g lipids/(kg·d) (high AA+lipid group) from birth onwards. On postnatal day 2, infants received primed continuous infusion of [U-$^{13}C_6$,^{15}N]leucine. Mass spectrometry was used to determine the fractional and absolute albumin synthesis rates (FSR and ASR, respectively).

RESULTS

Twenty-eight infants (median gestational age 27 (IQR 25 – 28) weeks, birth weight 810 (IQR 679 – 998) g were studied in total. In the high AA+lipid group FSR and ASR almost doubled compared to the control group – median FSR increased from 7 %/d (control group) to 12 %/d, while the median ASR increased from 84 mg/(kg·d) to 160 mg/(kg·d).

CONCLUSION

Starting parenteral lipids and high-dose amino acids within 6 hours after birth increases albumin synthesis rates in VLBW infants.

CHAPTER 6

Increased amino acid and early lipid administration do not up-regulate glutathione synthesis, nor increase oxidative stress in very low birth weight infants

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ABSTRACT

BACKGROUND

Preterm neonates are subjected to increased oxidative stress, which is associated with bronchopulmonary dysplasia, retinopathy of prematurity and periventricular leukomalacia. Previously, we demonstrated that early amino acid administration in preterm infants increased glutathione (GSH) concentration, the main non-enzymatic intracellular antioxidant.

OBJECTIVE

The objective was to investigate whether additional amino acids and energy administration would further increase GSH synthesis and to investigate whether early lipid administration would increase oxidative stress.

DESIGN

Very low birth weight (VLBW) infants were randomized to receiving 2.4 or 3.6 g amino acids/(kg·d) with or without 2 g lipids/(kg·d) from birth onwards. On day 2, infants received a primed, continuous infusion of [U-^{13}C]glycine to determine intra-erythrocyte GSH synthesis rates. Oxidative stress markers were measured in urine. Data are presented as median (IQR).

RESULTS

Thirty-one VLBW infants with gestational age 27 ($25^{2}/_{7}$-$28^{2}/_{7}$) weeks and birth weight 820 (670-1000) g were included. Total GSH concentration, GSH synthesis, and oxidative stress markers did not change upon increasing the amino acid intake to 3.6 g/(kg·d) or by lipid administration from birth onwards. Total median values were: GSH concentration 1.8 (1.4 –2.0) mmol/L; fractional synthesis rate 41 (37 – 49) %/d; and absolute synthesis rate 6.5 (5.3 –8.5) mg/(kg·d).

CONCLUSION

Increasing amino acid intake to 3.6 g/(kg·d) with a concomitant increase in energy intake from birth onwards in VLBW infants did not increase GSH synthesis. Lipid infusion directly following birth did not increase oxidative stress.

PART 3 – QUALITY OF PARENTERAL NUTRITION

CHAPTER 7

Growth and fatty acid profiles in very low birth weight infants receiving a soybean, medium-chain triacylglycerol, olive, and fish oil-containing lipid emulsion from birth onwards, a randomized controlled trial

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SUBMITTED

ABSTRACT

BACKGROUND

Very low birth weight (VLBW) infants are dependent on parenteral nutrition following birth. A balanced composition of parenteral lipid emulsions might offer benefits on growth and neurodevelopment and might prevent liver injury often observed in long-term parenteral nutrition with pure soybean oil.

OBJECTIVE

Our aim was to evaluate the safety and efficacy of a multicomponent lipid emulsion containing 30 % soybean oil, 30 % medium-chain triacylglycerol, 25 % olive oil, and 15 % fish oil compared to a conventional pure soybean oil emulsion in VLBW infants.

DESIGN

We conducted a double-blind randomized controlled trial in inborn VLBW infants randomized to parenteral nutrition with the multicomponent emulsion (study group) or to the pure soybean oil emulsion (control group) from birth onwards at a dose of 2-3 g/(kg·d) until infants were on full enteral nutrition. Efficacy was based on plasma fatty acid profiles and growth rates. Safety was evaluated based on hematology and biochemistry parameters, on potentially harmful phytosterol concentrations, and on clinical neonatal outcome parameters.

RESULTS

Ninety-six infants were included. In the study group, plasma eicosapentaenoic acid and docosahexaenoic acid concentrations were higher than in the controls. Hematology, biochemistry, and neonatal outcome were not different between groups, while plasma concentrations of phytosterols were higher in the control group. Infusion of the multicomponent emulsion was associated with higher weight and head circumference *z*-scores during admission.

CONCLUSIONS

The multicomponent lipid emulsion was well tolerated and associated with improved growth and plasma fatty acid profiles in VLBW infants in comparison to the pure soybean oil emulsion.

CHAPTER 8

Multicomponent lipid emulsion containing a mixture of soybean oil, medium-chain triacylglycerol, olive oil and fish oil does not reduce oxidative stress in very low birth weight infants; a double-blind randomized controlled trial

Denise Rook, Hester Vlaardingerbroek, Yvonne Muizer, Kristien Dorst, Julia Kuligowski, Javier Escobar, Maximo Vento, Marijn J. Vermeulen, Johannes B. van Goudoever, Henk Schierbeek

ABSTRACT

BACKGROUND

Parenteral lipids are an integral part of nutrition of preterm infants, but have been associated with increased oxidative stress. In preterm infants, oxidative stress is associated with major neonatal morbidities like bronchopulmonary dysplasia and periventricular leukomalacia. Traditional lipid emulsions are mainly manufactured from soybean oil and are rich in n-6 polyunsaturated fatty acids (PUFAs), which are highly susceptible for lipid peroxidation resulting in increased oxidative stress.

OBJECTIVE

To compare the effect of administration of a multicomponent lipid emulsion containing soybean oil, medium-chain triacylglycerols, olive oil, and fish oil versus a traditional pure soybean oil emulsion on oxidative stress markers and glutathione (GSH) kinetics in very low birth weight (VLBW) infants in a double-blind randomized controlled trial.

METHODS

VLBW infants (birth weight < 1500 g) were randomized to receiving one of the lipid emulsions from birth onwards. All infants received glucose and amino acids (2.4-3.6 g/(kg·d)) from birth onwards. On day 2, infants received a primed, continuous infusion of [U-^{13}C]glycine, a precursor for glutathione synthesis, to determine intraerythrocyte glutathione synthesis rates. Oxidative stress markers were measured in urine. Data are presented as median (interquartile range).

RESULTS

Twenty-four infants with a gestational age of $26^{6}/_{7}$ ($25^{2}/_{7}$ – 28) weeks and birth weight of 783 (649 – 980) g were included. GSH concentration (1.8 (1.4 – 2.0) mmol/L), fractional synthesis rate (41 (38 – 52) %/d) and absolute synthesis rate (6.6 (5.5 – 9.3) mg/(kg·d)) were not different between the two lipid emulsion groups. Total isofurans were reduced upon administration of the multicomponent lipid emulsion while the other oxidative stress markers, including isoprostanes, were not different between the lipid emulsions.

CONCLUSION

The multicomponent lipid emulsion, containing a mixture of soybean oil, medium-chain triacylglycerols, olive oil, and fish oil does not result in reduced oxidative stress compared to a pure soybean oil emulsion.

CHAPTER 9

New generation lipid emulsions prevent phytosterolemia and cholestasis in chronic parenterally-fed preterm pigs

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* Both authors contributed equally to this work

SUBMITTED

ABSTRACT

BACKGROUND

During their first weeks of life, preterm infants are often dependent on total parenteral nutrition (TPN). However, prolonged TPN exposure is associated with the development of parenteral nutrition associated liver disease (PNALD). Fish oil-based lipid emulsions can reverse PNALD; yet it is unknown if they can also prevent PNALD.

METHOD

As a model for preterm infants, preterm pigs received TPN for 14 days with either 100 % soybean oil (IL), 100 % fish oil (OV), or a mixture of soybean, MCT, olive, and fish oil (SL). A reference group was fed milk formula enterally (EN). We also measured FXR function in cultured pig hepatocytes.

RESULTS

In TPN-fed pigs, serum direct bilirubin, GGT and plasma bile acids increased after the 14-day treatment, but were highest in IL pigs. Liver histopathology showed signs of cholangitis, steatosis, and neutrophil infiltrate mainly in IL pigs. In comparison with EN, all TPN pigs had suppressed hepatic expression of FXR, and its downstream target genes including BSEP and CYP7A1. In contrast, hepatic CYP7A1 protein abundance was increased in all TPN groups, but highest in the IL group. OSTα gene expression was the highest in the IL group and paralleled plasma bile acid concentrations. In cultured hepatocytes, bile acid-induced BSEP expression was inhibited by both phytosterol and IL treatment.

CONCLUSION

In preterm TPN-fed pigs, soybean oil emulsions differentially alter the expression of hepatic FXR and key downstream target genes resulting in cholestasis and this was prevented with both fish oil-based emulsions. Phytosterols and soybean oil emulsions negatively impact FXR-dependent gene expression in cultured hepatocytes and may explain the association between phytosterolemia and cholestasis.

PART 4 – DISCUSSION AND SUMMARY

CHAPTER 10
General discussion

PARTLY BASED ON:
Optimizing parenteral nitrogen intake for preterm infants.
H. Vlaardingerbroek, M.J. Vermeulen, C.H.P. van den Akker, J.B. van Goudoever.
Nutrients 2013, in press

CHAPTER 10
General discussion

As stated in **CHAPTER 1**, nutrition is an essential part of the acute care of preterm infants, especially in very low birth weight (VLBW) infants. During the first days of life VLBW infants are dependent on parenteral nutrition. The last decades, research has focused on determining the amount and composition of parenteral amino acid and lipid solutions that can be administered safely to improve outcome in these VLBW infants. Many questions remained to be answered. In this thesis we analyzed the effects of early initiation of lipids with and without a higher dose of parenteral amino acids on various biochemical and clinical outcomes. In addition, two different lipid emulsions were compared in both a clinical study and an animal model. The implications of this thesis are discussed below.

Early high-dose amino acid administration

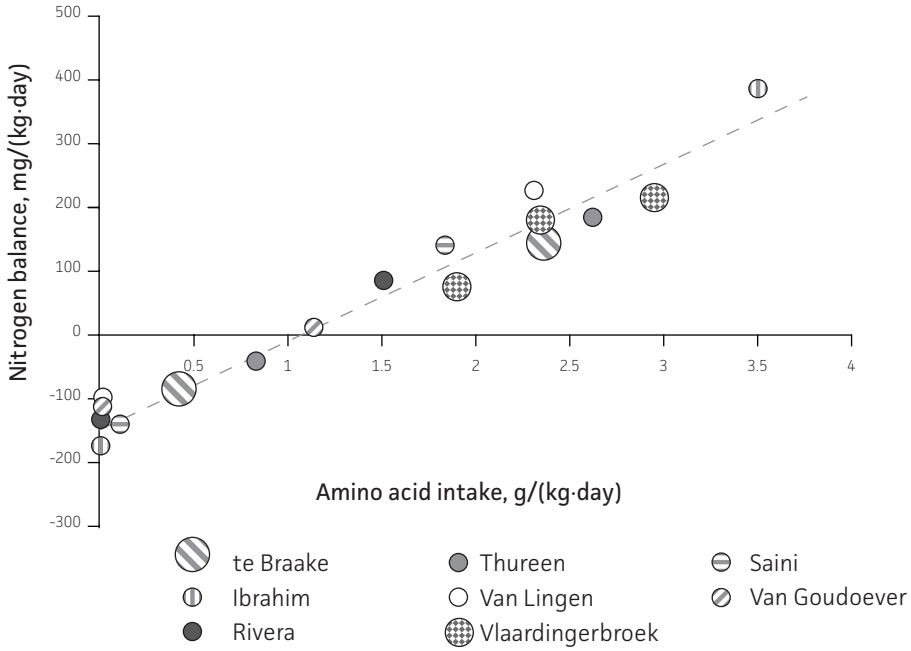
In utero, the fetal amino acid uptake exceeds the amount that is necessary for net protein accretion, which indicates that the human fetus oxidizes amino acids to generate energy.[1-3] After preterm birth survival, growth, and development are dependent on nutritional supply of amino acids, fat, and carbohydrates. A decade ago many VLBW infants received only glucose during the first few days of life, resulting in large protein deficits and weight loss. Administration of glucose and amino acids within a few hours after birth prevents infants from a catabolic state. Nowadays it has become clear that preterm infants, with their little nutritional reserves, need administration of total parenteral nutrition soon after birth.

Beneficial effects of amino acid administration in the acute postnatal phase

The effect of protein intake can be assessed in several ways. Gold standard is clinical outcome measures (including growth and functional outcome), requiring large study cohorts of randomized clinical trials and preferably long-term follow-up which is difficult to achieve. Methods measuring the acute effect of protein intake include the relatively simple method of nitrogen balance (the calculated difference between nitrogen intake and urinary losses), the more advanced methods of amino acid metabolism and protein accretion by means of stable isotopes, and measurements of plasma amino acid concentrations.

Several studies have demonstrated that administration of 1-2.5 g amino acids/(kg·d) starting within the first two days after birth can reverse a negative nitrogen balance into a positive balance, thus leading to anabolism.[4-9] More recent studies demonstrated that the nitrogen balance can be improved further by administration of amino acids up to 3.6 g/(kg·d) from birth onwards.[10-12] Results are summarized in **FIGURE 1**. In our trial, described in **CHAPTER 3**, infants were randomized to 2.4 g amino acids/(kg·d) without lipids during the first two days of life, or to early lipid administration plus 2.4 g amino acids/(kg·d) or plus 3.6 g amino acids/(kg·d) from birth onwards. Higher actual amino acid intake correlated positively with nitrogen balance. The intention to treat

FIGURE 1 Studies investigating the effects of different amounts of amino acid administration starting during any of the first two postnatal days in preterm neonates on nitrogen balance. Legend identifies the primary author; size of the symbol indicates the number of infants. Nitrogen intakes are the actual intakes in each study. Data adapted from Embleton et al.[141]

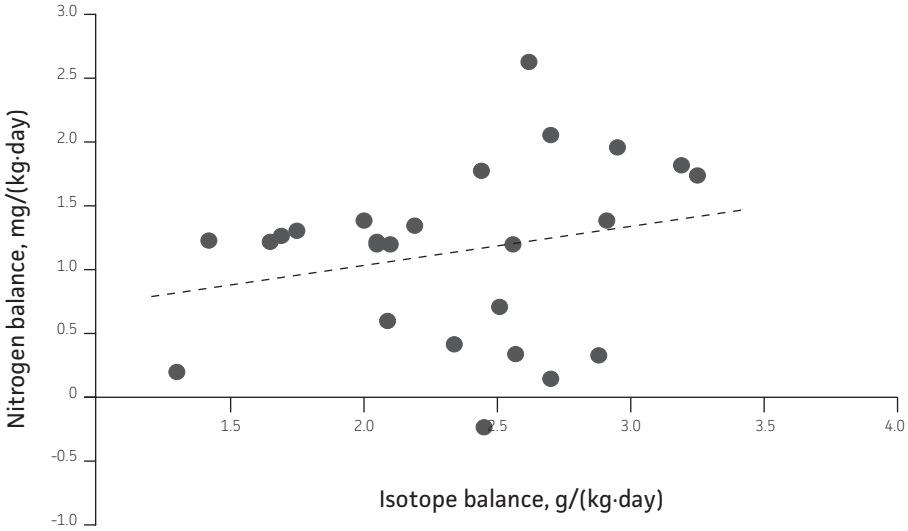


analysis (based on group randomization instead of actual intake) could not demonstrate such an effect, due to adjustments of the amino acid dosage in up to 81 % of the infants in the high amino acid group, according the local protocol stating that plasma urea concentrations should not exceed 10 mmol/L (28 mg/dL). However, the clinical relevance of plasma urea as a safety measure is debatable and will be discussed later in this chapter.

Stable isotope studies, including our trial described in **CHAPTER 4**, demonstrated that early amino acid administration in preterm infants especially increases the protein synthesis rate, rather than suppresses the proteolytic rate, resulting in the higher protein balances.[5, 7-9, 12] The discrepancy in results using these two methods can be explained in three ways. First, it could be coincidence, because the infants described in **CHAPTER 4** were only a subset from those studied in **CHAPTER 3**. Second, isotope studies are generally considered a more specific method to analyze the effects of amino acid administration on metabolism. Nitrogen balance techniques are based on measurement of end product concentrations, while stable isotope techniques track the metabolic processes in amino acid metabolism and can give information about several rates of intermediate metabolism. Third, in the isotope study, both enteral and parenteral nutritional intakes were kept stable during the 8 hour study period, while the intake was

reduced in the majority (81 %) of infants during the 24 hour-nitrogen balance study. This might explain the lack of correlation between the isotope and nitrogen balances (**FIGURE 2**). We conclude that the data based on isotope studies reflect the nutritional effects more purely than the nitrogen balance data, suggesting that higher-dose amino acid administration to VLBW infants results in a more anabolic state during the first postnatal phase. However, this does not indicate that nitrogen balances are useless. Nitrogen balances are non-invasive and can give an easy to obtain, general indication of anabolism, especially during stable nutritional intake. Therefore, we still recommend measurement of nitrogen balances as a basic measurement during nutritional intervention studies.

FIGURE 2 Association between protein balances based on the isotope study and on the nitrogen balance study (R^2 = 0.054).



Additional beneficial effects of high-dose amino acid administration in the acute phase are the higher plasma amino acid concentrations (**CHAPTER 3**). This is beneficial since the amino acid that is available in the lowest amount will determine the rate of protein synthesis: an insufficient amount of one of the amino acids can lead to increased oxidation rates of all other amino acids and can also result in increased protein breakdown to make this lacking amino acid available for the synthesis of other proteins. The plasma amino acid concentrations were more comparable to reference ranges in healthy breast-fed term neonates[13] and in second and third trimester human fetuses[14]. However, Blanco et al[15] found higher plasma amino acid concentrations in extremely low birth weight infants randomized to high-dose amino acid administration (up to 4.0 g/(kg·d)). In our study, plasma concentrations of valine, leucine, lysine and threonine were above or in the upper range of these references, suggesting that the amount of these amino acids in our currently used amino acid solution (Primene, Baxter) is too high. On the other hand, Primene probably contains insufficient amounts of asparagine,

cystine, glutamate, and methionine, as these concentrations were below the reference ranges. As stated before, the lowest available amino acid determines the rate of protein synthesis. Hence, not only a higher quantity of amino acid administration, but also improved quality of amino acid solutions is a promising mechanism to improve amino acid usage for protein synthesis. Since we do not know parenteral amino acid requirements, except for tyrosine,[16] efforts should be made to determine the optimal amino acid composition in parenteral amino acid solutions. As has been shown for enterally fed infants,[17-18] this can be done by indicator amino acid methods. A more optimal amino acid composition will improve protein synthesis, without a concomitant oxidation of amino acids that are in excess.

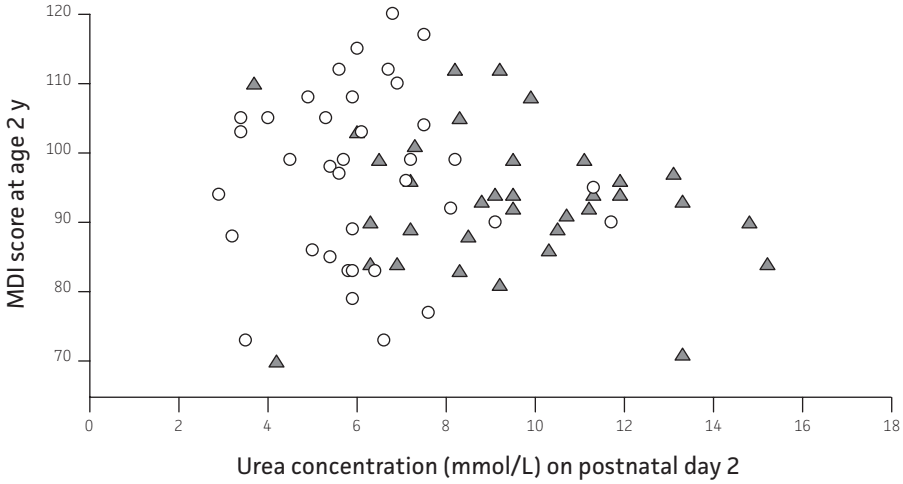
Another observed beneficial effect of high-dose amino acid administration in the acute phase is increased synthesis of proteins such as albumin.[19] (**CHAPTER 5**) In light of the many important functions of albumin, the higher albumin synthesis rates upon high-dose amino acid administration might have a major impact on outcome of these VLBW infants. That albumin concentrations were not increased is not surprising, since we only measured the influence of high amino acid supplementations for two days. This period is too short to detect differences in such a large pool. Furthermore, in a previous study we demonstrated increased glutathione absolute synthesis rates upon amino acid administration from birth onwards compared to glucose only.[20] However, high-dose amino acid plus lipid administration from birth onwards did not increase glutathione synthesis rates further (**CHAPTER 6**). Since markers of oxidative stress (**CHAPTER 6**) and the incidence of neonatal morbidities associated with increased oxidative stress, such as bronchopulmonary dysplasia, were also not different (**CHAPTER 3**), this might indicate that there is no need for upregulation of glutathione synthesis in these VLBW infants. In conclusion, high-dose amino acid plus lipid administration offers short-term beneficial effects on protein accretion and amino acid concentrations. The safety of high-dose amino acid administration will be discussed in more detail in the following paragraph.

Safety markers and potential drawbacks of amino acid administration

A well-known shortcoming in any of the above mentioned studies on early amino acid administration in preterm infants is that we do not know the proper safety markers of amino acid administration. The question is if overdosage (i.e., leading to toxic concentrations of amino acids or their metabolites) of amino acids is possible and how to measure potential amino acid overdosage or intolerance. Many nutritional trials set safety thresholds for upper limits of urea concentration. However, the clinical relevance of plasma urea as a safety measure is debatable. Various clinical trials in infants with high-dose amino acid infusion did not find increased plasma urea concentrations[8, 10, 12, 21-22] nor a correlation between plasma urea nitrogen and protein intake.[23] Other research groups, including ours, found higher plasma urea nitrogen concentrations in infants infused with higher amounts of amino acids.[11, 24-25] (**CHAPTER 3**) In the study of Blanco et al,[26] described before, mean peak urea concentrations were already very high (19.6 ± 6.8 mmol/L) and even ranged up to 36 mmol/L in some of the most immature infants (≤ 24 weeks gestational age). Ammonia concentrations were also elevated in these infants (~ 100 mmol/L), where normal values during early life in fasting preterm

infants are 70 ± 25 mmol/L.[27] A very wide range can be measured in cord blood.[28] Causal relationships between high urea concentrations and neonatal morbidity or mortality have not been described,[26-27, 29] (**CHAPTER 3**) and long-term consequences of high urea concentrations in preterm infants are not known,[30] although we could not demonstrate an association between plasma urea concentrations and mental developmental index scores in our previous study comparing administration of 2.4 g amino acids/(kg·d) from birth onwards with administration of glucose only during the first two days of life (**FIGURE 3**).[31] However, it is well known and recently reaffirmed that increased plasma urea concentrations are also a reflection of dehydration and immature kidney function.[32-33] Therefore, rather than a reflection of protein intolerance, urea concentrations are a reflection of hydration status, kidney function, successful ammonia detoxification following amino acid oxidation and thus part of normal physiology in preterm life. Hence, a range of urea concentrations that can be regarded as physiological and safe in VLBW infants during the first days of life should be established by adequately powered studies that relate urea concentrations during these first days of life with long-term outcome. This relation can be established most appropriately in studies that do not adjust the amount of amino acid intake based on urea concentrations. In the absence of such a relationship, the measurement of urea concentrations during the first days of life as a marker of amino acid intolerance should be questioned. Until this safe range of urea concentrations during the first days of life in VLBW infants is established we would recommend amino acid dose reduction when urea concentrations are > 12 mmol/L.[34]

FIGURE 3 Association between plasma urea concentrations and mental developmental index (MDI) scores at two years corrected age in infants with normal outcome. Data are from our previous study comparing administration of glucose and amino acid administration (2.4 g/(kg·d)) from birth onwards (grey triangles) with administration of glucose only during the first two days of life (open circles).[31]



Recently, more attention has been paid to electrolyte disturbances after early nutrient supplementation. Recent studies report on increased incidence of hypophosphatemia and hypokalemia upon early and high-dose administration of amino acids and lipids.[35-36] Despite higher nutritional intakes, in many NICUs electrolyte supplementations is often delayed to the second or third day of life.[35, 37-38] It has been suggested that high amino acid intakes and concomitant higher insulin concentrations and protein synthesis induce progressive depletion of potassium and phosphate[39] as for each gram of protein retained, also 0.3 mmol phosphate is required.[40] In case of limited phosphate supply, endogenous phosphate is mobilized, leading to hypophosphatemia and increased plasma calcium concentrations, which can be monitored by diminished urinary phosphate excretion and calciuria.[36, 39-41] In the study by Moltu et al[36] it was hypothesized that the lower phosphate concentrations led to impaired macrophage functioning leading to their observed high sepsis rate. Whether these effects can easily be prevented by early phosphate suppletion has not been studied. In our study, we did not measure phosphate concentrations. However, average phosphate intakes were 1.6 mmol/(kg·d), twice the amount compared to the study reporting hypophosphatemia.[36] Sodium, potassium, and calcium concentrations were not different between groups. The lack of an increase in calcium concentration may be considered an indirect proof of appropriate phosphate administration. Future studies should demonstrate if and how fluid and electrolyte management needs to be adjusted when providing enhanced nutritional regimens in VLBW infants.

Longer-term effects of amino acid administration

Studies on early amino acid administration have mainly focused on the effects in the acute postnatal phase; only a few studies have investigated the effects on growth and development in the more stable phase until discharge home or even the long-term effects into adulthood. Despite the fact that in most NICUs nutritional support provided to each preterm infant is part of daily care and nutrient intake has been shown to have the largest impact on growth differences among NICUs,[42] postnatal growth deficit is still one the most frequently observed morbidities in VLBW infants.[43-45] Postnatal growth is not only determined by nutritional intake and metabolic status, but also by the severity of coexisting morbidities and by the (epi)genetic potential.[46] The risk of postnatal growth failure increases with decreasing birth weight and gestational age, and is independently associated with male gender, the need for assisted ventilation on the first day, the need for respiratory support at 28 days, the need for postnatal steroids, and occurrence of necrotizing enterocolitis.[44]

The importance of nutrition in the early, and possibly critical, stages of life is well recognized[47] and the term 'programming' has been used to emphasize that early nutrition is not only crucial for meeting immediate nutritional needs, but also for its potentially long-lasting effects throughout life. Intrauterine and postnatal growth restriction in preterm infants has been associated with short stature[48-50] and adverse neurodevelopmental outcome.[51-53]

Long-term considerations about optimal growth add to the clinical problem that optimal growth velocities for preterm infants are not known. In several studies postnatal

growth is based on intrauterine growth charts[54] or growth charts obtained from preterm infants.[55-58] Intrauterine growth curves as a reference have several limitations. First, they are actually birth weight curves implying cross-sectional data, instead of longitudinal data. Second, 40-50 % of preterm delivery is associated with (some) intrauterine growth restriction giving an underestimation of the normal distribution of intrauterine weight.[59-60] Additionally, growth charts bridging size at birth with postnatal growth are scarce. In 2008, the first continuous growth chart for infants born from 24 weeks gestational age onwards up till 24 months of age was published.[58] However, many of the included infants were ill and hence have possibly not used their full growth potential. The question arises as well whether these infants have been fed in an optimal manner, as we continue to develop improved nutritional managements. These curves thus give insight how the individual preterm infant grows as compared to his/her peers of comparable gestational age, but do not give insight into how the infants should grow ideally. Despite these drawbacks on growth curves and reference growth, the American Academy of Pediatrics Committee on Nutrition and the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition[37, 61-62] state that neonatologists should strive for a postnatal growth rate that not only duplicates fetal growth rate but also mimics fetal body composition and functional outcome similar to that of healthy term-born infants. However, it has been shown that at the recommended intake of 110-120 kcal/(kg·d),[37] fat accretion is higher in preterm infants than in a reference fetus.[46, 63] As a consequence, at term age, preterm infants will have a higher relative fat content than full-term infants at birth and also fat is more distributed around the visceral organs.[64-65] In addition, many infants who are prenatally and/or postnatally growth restricted will overcome this in the later in-hospital and post-discharge phases, when increased amounts of lipids are consumed as part of fortified (post-discharge) formulas. This (post-discharge) catch-up growth has been shown to be beneficial for neurodevelopment[53] and bone development,[51, 53, 66] but can also lead to decreased insulin sensitivity visceral obesity,[64, 67-72] which has been associated with an adverse metabolic and cardiovascular adult health profile or the development of the so called 'early origins of adult disease'.[73-76] Hence, a dilemma exists: inadequate nutritional support increases the risk of neurodevelopmental impairment and bone disease, while better nutritional support increases the risk of an adverse adult health profile in later life. The most logic approach seems to avoid initial suboptimal growth rates, so that catch-up growth in the later stages may not be necessary. Whether nutritional intervention can accomplish this when the child is no longer dependent on parenteral nutrition and tube feeding, still needs to be studied in prospective cohorts. Factors like feeding tolerance, oral feeding skills and individual appetite (all probably influenced in the neonatal phase) may limit the effect of dietary interventions.

Most observational studies[77-80] and randomized clinical trials[21] with high doses of parenteral administered amino acids or combined parenteral and enteral administration[81-82] demonstrated improved growth at hospital discharge or 36 weeks postmenstrual age, whereas some[24-25] including our study (**CHAPTER 3**) did not. The lack of consistency in growth outcomes in early nutritional intervention studies is

probably related to the duration of intervention (e.g., only a few days or several weeks), the difference in total amino acid load between intervention groups, and differences in patient characteristics, such as gestational age and illness severity. Infants in our study were relatively small for gestational age (average birth weight *z*-score of -1.7 to -2.6) and being small for gestational age (birth weight *z*-score < -2) was associated with lower growth rates, but did not affect neonatal morbidities. However, although we did not observe improved growth upon nutritional intervention with high-dose amino acids, growth rates were fairly high in all groups, with an average in-hospital weight gain of 26 g/(kg·d). Future large size studies should demonstrate if early high-dose amino acid administration causally relates with longer-term improved growth outcomes, without increasing the risk of metabolic syndrome.

Beneficial long-term effects of nutrition on neurodevelopment have been difficult to prove because nutrition is only one of the many variables determining neurodevelopment. Disease itself might also negatively affect nutritional intake. Second, on average, the time on total parenteral nutrition is usually limited to periods of less than a week. Furthermore, we should decide which outcome measures are most indicative for improved function and subsequently are beneficial for infant (and adult) health.[83] For example, studies have shown that proteins are critical to the development of neurological functions and that malnutrition can alter neuronal density.[84-86] But does cerebral magnetic resonance imaging at term or 12 months corrected age predict neurodevelopmental outcome sufficiently? Or should we perform functional test of psychomotor development, such as Bayley scales at 24 months corrected age, Kaufman ABC at the age of 7 years, or IQ tests once infants are adolescents? To date, studies investigating the effect of high-dose parenteral amino acid administration to preterm infants have not exceeded two years' follow-up. Most of these studies on long-term development[80, 87-88] indicate that the first few days of life might provide a critical window and that nutrition should be part of immediate care in the preterm born infant.

Early lipid administration

While protein synthesis is the main determinant of growth, the energy generated by glucose and lipid oxidation finances the cost of this energy demanding process. The optimal glucose and lipid intakes and their proportions ensuring the energy supply to maximize protein accretion and growth have not yet been determined.[9] The ESPGHAN Committee of Nutrition recommends the use of parenteral lipid emulsions within the first few days of life in preterm infants.[37] Despite these recommendations, initiation of parenteral lipid emulsions is postponed beyond the first few days in many NICUs,[89-91] due to concerns regarding impaired lipid tolerance, impairment of oxygenation, and increased oxidative stress,[92-94] which is associated with major neonatal morbidities like bronchopulmonary dysplasia[95] and periventricular leukomalacia.[96] However, meta-analyses have not shown any association between early lipid initiation and an increased risk for these common neonatal morbidities.[97-98] In our study, early lipid administration also did not increase markers of oxidative stress nor the incidence of oxidative stress-related neonatal morbidities (**CHAPTER 3 AND 6**), although mortality rates appeared

higher upon early lipid administration, but this difference did not reach statistical significance and seemed a matter of coincidence because of lower than average mortality rates in the control group.
Plasma clearance of infused lipid emulsions can be monitored by the assessment of plasma triacylglycerol concentrations, although it is unclear at what concentrations adverse effects may occur.[99] Preterm infants might be at a higher risk for hypertriacylglycerolemia than term infants due to their relatively limited muscle and fat mass and, therefore, decreased hydrolytic capacity of the enzyme lipoprotein lipase.[37, 100] The ESPGHAN committee suggests to check plasma triacylglycerol concentrations with each increase of 1.0 g parenteral lipids/(kg·d) and weekly after the maximal dose is achieved. They recommend to reduce the dosage of parenteral lipid emulsions if the serum triacylglycerol concentration exceeds 250 mg/dL (2.85 mmol/L),[37] while the American equivalent organization, the American Society for Parenteral and Enteral Nutrition (ASPEN), recommends to discontinue parenteral lipid administration if plasma triacylglycerol concentrations exceeds 200 mg/dL (2.26 mmol/L).[101] Both these recommendations and the common practice of frequent monitoring of triacylglycerol are based on poor scientific base, and there are no scientifically based guidelines on critical values and subsequent alterations in infusion rate.[102] In our study as described in **CHAPTER 3**, early lipid administration did not result in a higher incidence of hypertriacylglycerolemia (triacylglycerol concentrations > 3 mmol/L). Moreover, the occurrence of hypertriacylglycerolemia was not associated with a higher prevalence of neonatal morbidities such as necrotizing enterocolitis, sepsis, bronchopulmonary dysplasia, retinopathy of prematurity, and intraventricular hemorrhage.
Future studies, preferably studies that do not adjust lipid dosage based on triacylglycerol concentrations, should demonstrate which triacylglycerol concentrations in VLBW infants can be regarded as safe, both at short-term and at long-term. Until that time, we recommend measurement of triacylglycerol concentration within approximately 1-2 days after initiation or adjustment of lipid infusion, and to lower the dosage if plasma concentrations are above 3.0 mmol/L (265 mg/dL). Considering the good tolerance and many (theoretical) advantages of early lipid administration on protein synthesis and anabolism, on amino acid tolerance (**CHAPTER 3-6**), and on the prevention of an essential fatty acid deficiency, we recommend initiation of lipids within hours after birth. Long-term follow-up studies should demonstrate whether the use of lipid emulsions from birth onwards will have long-lasting (positive) effects on growth and neurodevelopment.

Search for the optimal composition of the lipid emulsion

Pure soybean oil-based emulsions, available since the 1960s, are the most often used lipid emulsions worldwide.[103] However, as said before, these emulsions have been linked to increased pulmonary vascular resistance, impaired pulmonary gas exchange, hyperbilirubinemia, parenteral nutrition associated liver disease (PNALD), enhanced oxidative stress, and adverse immunologic effects such as increased rates of infection and sepsis.[104-106] Additionally, the high linoleic acid (LA) and α-linolenic acid (ALA) content of pure soybean oil emulsions can induce low blood concentrations

of their bioactive long-chain polyunsaturated fatty acid metabolites, especially of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[104-105] EPA is important for immune function,[107-108] while DHA plays a crucial role in neurodevelopment.[109-110] Data from multiple studies in term infants have established that an exogenous supply of DHA of at least 0.2-0.3 % of total fatty acid intake enhances visual acuity and mental and psychomotor development.[111-113] For preterm infants, an even larger supply might be necessary, because they miss the physiologic supply of preformed DHA after early termination of materno-fetal transfer of these fatty acids[114] and because of insufficient endogenous synthesis rates of DHA.[111, 115-116] In newer lipid emulsion soybean oil is partly or completely replaced with medium-chain triacylglycerols, olive oil, and/or fish oil. In our randomized controlled trial in VLBW infants, as described in **CHAPTER 7**, we compared biochemical and clinical outcomes of administration of 2-3g/(kg·d) of a pure soybean oil emulsion or a multicomponent emulsion containing soybean oil, medium chain triacylglycerols, olive oil, and fish oil from birth onwards. While pure soybean oil emulsions are devoid of DHA, the multicomponent emulsion contained 2 % of total fatty acids as DHA. This prevented a decrease in the concentrations of DHA and EPA and these effects remained on day 14, while the majority of infants were already on full enteral feeding (containing less preformed DHA and EPA) by that time. Weight gain during the first weeks of life, bronchopulmonary dysplasia, hypertriacylglycerolemia, hyperglycemia, or death before discharge were not significantly different between lipid emulsions. In our meta-analysis (**CHAPTER 2**),[97] mixed lipid emulsions were associated with a 25 % reduction in sepsis episodes compared to pure soybean oil emulsions (RR 0.75; 95 % CI 0.56 – 1.00; P = 0.05). When we add our trial data (**CHAPTER 7**), we find a further reduction in sepsis episodes (RR 0.72; 95 % CI 0.56 – 0.94; P = 0.02). The number needed to treat with an emulsion that is not purely soybean oil-based to prevent one episode of late onset sepsis amounts 9 (95 % CI 4 – 111), which should be regarded as clinically highly relevant. Sepsis is of course associated with adverse growth.[56] In our trial we observed an additional beneficial effect of the multicomponent emulsion. Weight gain and head circumference gain during hospital admission were significantly improved compared to the infants receiving the soybean oil emulsion (**CHAPTER 7**). In our study, sepsis was not associated with reduced growth rates. If confirmed in another study, we speculate that the improved growth was associated with the anti-inflammatory characteristics of the multicomponent emulsion.

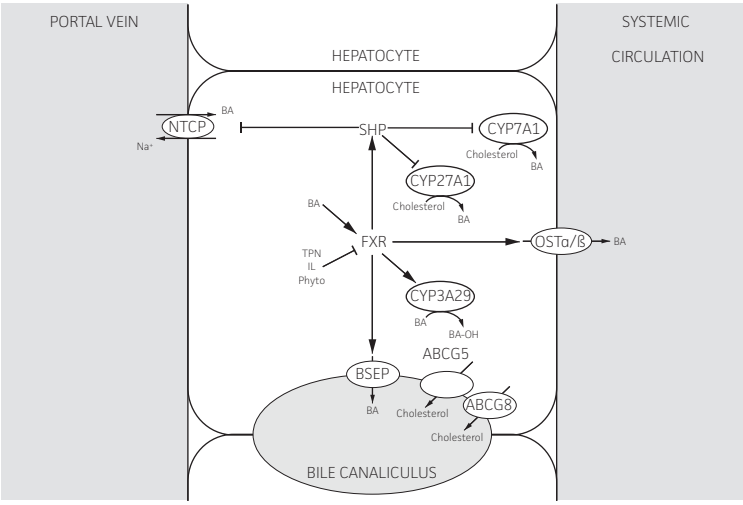
Previous literature comparing the effect of multicomponent lipid emulsions and pure soybean oil emulsions on oxidative stress is conflicting.[117-121] In these studies, parenteral lipids were supplied either in low doses from birth onwards or lipids were not started directly from birth onwards. In our study, lipids were started directly from birth onwards with a starting dose of 2 g/(kg·d) and 3 g/(kg·d) on the following days. As described in **CHAPTER 8**, we demonstrated that the multicomponent lipid emulsion did not result in reduced oxidative stress compared to pure soybean oil emulsion, although isofurans were reduced. Glutathione concentration and synthesis rates were also not altered following administration of these different lipid emulsions from birth onward in VLBW infants. In addition, the incidences of common neonatal morbidities associated with increased oxidative stress were also not different between groups.

Lipids and parenteral nutrition associated liver disease

As stated before, during their first days of life preterm infants are dependent on parenteral nutrition. However, parenteral nutrition is associated with the development of parenteral nutrition associated liver disease (PNALD), which ultimately can lead to fatal liver failure. While the etiology of PNALD is still unknown, risk factors associated with PNALD are multifactorial and include immature hepatic function, lack of enteral feeding resulting in less cholekinetic triggers, infection or sepsis, toxin exposure, or nutrient deficiencies.[122] In parenterally fed infants and children with PNALD, fish oil emulsions in low doses reduced serum triacylglycerol and direct bilirubin concentrations, improved liver function, and in some of these children fish oil administration even reversed PNALD.[123-134] However, it is unknown if fish oil-containing lipid emulsions can also prevent the development of PNALD. Therefore, we performed a study in preterm chronically TPN-fed pigs, as a model for preterm infants, to study if fish oil containing emulsions can prevent the development of PNALD, and to unravel supposed mechanisms of the beneficial effect of fish oils (**CHAPTER 9**). Fourteen days of continuous infusion of TPN produced dramatic hepatic findings. Although liver growth was maintained in all pigs, the livers of pigs fed pure soybean oil showed histopathologic microvascular steatosis and neutrophil infiltration suggesting active liver injury. This contrasted to the relative benign findings noted in the enterally fed pigs and in the pigs receiving either a pure fish oil-based emulsion or the multicomponenent emulsion (SMOFlipid, 15 % fish oil). We believe that phytosterols, which are found in soybean oil emulsions but are absent in pure fish oil emulsions, are one of the major reasons why pure soybean oil emulsions are associated with PNALD and why pure fish oil emulsions can reverse or prevent it. The mechanism is believed to be due to phytosterol's negative effect on Farnesoid X receptor (FXR), a key bile acid sensor involved in bile acid homeostasis (**FIGURE 4**). In all TPN-fed pigs mRNA expression of FXR target genes involved in bile acid synthesis (CYP7A1), bile acid import into the hepatocyte (NTCP), and bile acid export to the bile ducts (BSEP) were reduced. In contrast to reduced mRNA expression of CYP7A1, we demonstrated that protein expression of CYP7A1 was significantly upregulated. Thereby, bile acid synthesis via the classic pathway was significantly upregulated in the pure soybean oil-fed pigs compared to enterally fed pigs and to the pigs receiving either the pure fish oil emulsion or the multicomponent emulsion. These effects almost certainly contributed to bile acid accumulation as was observed in plasma and livers of pure soybean oil-fed pigs. Bile acid build-up from increased bile acid production and poor canalicular export as seen in these pigs most likely caused the compensatory upregulation of OSTα expression which is an integral alternative bile acid exporter, shunting bile acid into the bloodstream. Gene expression of CYP3A29, which is involved in bile acid hydroxylation, was highest in the IL group suggesting some degree of hepato-protection via bile acid metabolism. In a hepatocyte experiment we demonstrated that the phytosterols in TPN itself negatively impact FXR expression directly. Hence, phytosterols likely serve as disruptive ligands that perturb physiologic FXR functions which in turns dysregulate its downstream targets leading to increased bile acid synthesis and poor canalicular export. This then leads to a compensatory upregulation of alternative bile acid exporters to shunt the bile acids

directly into the systemic circulation (Figure 4). It thus seems that it is not the fish oil, but the reduction in soybean oil that prevented or reversed PNALD.
As stated before, many studies have shown reversal of PNALD when infants were switched from a soybean oil-based emulsion to a pure fish oil-based emulsions. However, much debate has arisen questioning whether or not the lower lipid dose (1 g/(kg·d)) in previous studies with pure fish oil emulsion is the primary preventative factor against PNALD rather than the composition of the lipid emulsions.[135-137] In our porcine and human study however, both groups received the same amount of lipids. Our study therefore strongly supports the idea that it is the composition, and not the lipid load that causes PNALD.

FIGURE 4 Main principles of nuclear receptor-dependent regulation of hepatobiliary bile acid (BA) transporters and enzymes as studied in this thesis. The central regulator of bile metabolism is bile acid activated farnesoid X receptor (FXR). FSR is a direct positive regulator for canalicular bile acid export via the bile-salt export pump (BSEP) and alternative bile acid export into the systemic circulation via the organic solute transporter (OSTα/β). In addition, FXR is a direct positive regulator for bile acid hydroxylation via cytochrome P450 3A29 (CYP3A29). Via the common transcriptional inhibitor small heterodimer partner (SHP), FSR indirectly inhibits basolateral Na^+/ taurocholate cotransporter (NTCP) bile acid uptake as well as bile acid synthesis via cholesterol 7-hydroxylase (CYP7A1, classic pathway) and sterol 27-hydroxylase (CYP27A1, alternative pathway). Cholesterol is either converted into bile acids or transported by ABCG5/G8 into the bile. FXR expression can be downregulated by total parenteral nutrition (TPN), especially by phytosterols (phyto) in soybean oil emulsions (IL).



Alternative ways to explain our findings include the fact pure soybean oil emulsions contain a much lower α-tocopherol concentration than fish oil emulsions: the α-tocopherol content of the pure soybean oil emulsion is 87 μmol/L, while the pure fish oil emulsion and the multicomponent emulsion contain 505 and 500 μmol/L

α-tocopherol. Alpha-tocopherol is added to fish oil emulsions to counteract the possible peroxidation of the higher number of double bonds in the LCPUFAs, while no α-tocopherol is added to pure soybean oil emulsions by the manufacturers. Increased serum α-tocopherol concentrations might be associated with the preservation of liver function, beneficial effects on immune function and clinical outcome[123, 138]. Therefore, differences in the amount of α-tocopherol provided by these lipid emulsions might also explain some of the harmful effects of soybean oil emulsions on the development of PNALD. Studies with equal α-tocopherol doses in different solutions or different doses in similar solutions are thus needed to elucidate this further.

How can we relate the porcine data with the findings in our preterm infant study? In our porcine study, bilirubin concentrations were increased after 14, but not after 7 days of parenteral nutrition with pure soybean oil compared with fish oil-containing emulsions. On day 14, the livers already showed progressive signs of PNALD. In our study in preterm infants, direct bilirubin concentrations on day 7 were not different between lipid groups, nor were peak indirect bilirubin concentrations during admission at the neonatal intensive care unit. The porcine model is an accelerated model of the situation in human infants. Therefore, despite the many beneficial effects of fish oil in the porcine model, the duration of total parenteral nutrition in most preterm infants was probably too short and the incidence of PNALD too low to detect biochemical and clinical benefits of the fish oil-containing emulsion in the studied VLBW infants as a whole group. However, for infants who turn out to be dependent on long-term TPN after, for example, necrotizing enterocolitis or anatomical bowel disruption, starting from birth onwards with relatively lower doses of soybean oil-containing emulsions, might be clinically very relevant. Unfortunately, due to low power with small number of infants on long-term TPN, we could not demonstrate this.

Clinical implications and future perspective

Before continuing with the clinical implications and future perspective, we have to emphasize on a general limitation of the clinical study described in this thesis. We used a complex randomized 5-armed model to study the effects of early lipid and high-dose amino acid administration (quantity) and to compare two different lipid emulsions (quality). We choose this model to guarantee sufficient infants per group and per outcome measure. Type of lipid did not affect the primary outcomes of the quantity study and amino acid dosage did not affect the primary outcomes of lipid quality. Therefore, we ignored the type of lipid emulsion in the analyses on the quantity of parenteral nutrition; in the analyses on quality of the lipid emulsion we ignored the amount of amino acid administration. Since infants were randomized and equally distributed to the five nutritional regimens and to the quantity and quality study, we believe that this complex mixed model did not bias our main results. However, we cannot exclude that this model might have affected minor secondary outcomes, such as biochemistry data.

Quantity of parenteral nutrition

Studies so far provide strong evidence for the beneficial effects of parenteral administration of early lipid administration and > 3 g amino acids/(kg·d) from birth onwards in VLBW infants on protein balances, growth, and neurodevelopment. However, since urea concentrations were lowest in the infants receiving early lipids and a standard amount of amino acids, we hypothesize that the protein/energy ratio is more determining for the usage of amino acid for protein synthesis, and thus growth, than the absolute amount of amino acids administered. This suggests that we should supply more energy (lipid) when administering amino acids at a high dose. Future studies should demonstrate if VLBW infants tolerate and benefit from lipid dosages of > 3 g/(kg·d) from birth onwards. Additionally, improvements in the composition of amino acid solutions will probably also (partially) improve amino acid usage for protein synthesis. In this thesis we investigated the effects of early lipid and high-dose amino acid administration in VLBW infants. Generally, more mature infants tolerate enteral feeding faster, and are therefore less dependent on parenteral nutrition. Nevertheless, since early nutrition gives a head start for later development, we would recommend administration of parenteral lipid and (high-dose) amino acid immediately after birth in all preterm neonates that do not tolerate full enteral feeding. Withholding adequate amounts of nutrition, even for a short period of time, might deprive infants of their full developmental potential. Considering the risks of excess weight for length gain, especially between term and 3 months corrected age, future studies should demonstrate the optimal parenteral and enteral intake beyond the acute postnatal phase to guarantee a balanced growth pattern during hospital admission and post-discharge. It might be prudent to restrict the use of energy-fortified (post-discharge) formulae in infants showing catch-up growth.[139]

When administering a hyperalimentation regimen, more attention should also be given to optimal fluid management and route of administration. Initial fluid restriction, beneficial for lung development and ductus arteriosus closure, implies using more concentrated TPN products and thus the requirement of rapidly implanted deeply inserted vascular catheters, also for more mature patients. Also the hyperalimentation requires more attention on electrolyte balances to avoid refeeding syndrome.

Quality of parenteral nutrition

The quality of available parenteral amino acid solutions is difficult to judge, since the exact requirements for individual amino acids in parenterally fed preterm infants are not known,[140] except for tyrosine.[16] Future studies using indicator amino acid techniques should demonstrate the optimal composition of amino acid solutions for preterm infants to achieve optimal growth and development. Based on the observed plasma amino acid concentrations that were below or above reference ranges, we speculate that a lower amount of valine, leucine, lysine, and threonine, and a higher amount of cysteine and glutamine is more optimal for this purpose.

What can we conclude about the quality of lipid emulsions? Considering the many drawbacks of pure soybean oil-based emulsions and the benefits of the multicomponent emulsion on maintenance of liver integrity, on a potentially

lower sepsis incidence and improved growth, and on neurodevelopment, we would recommend administration of the multicomponent emulsion instead of pure soybean oil-based emulsions in VLBW infants from birth onwards. We would not recommend the use of the pure fish oil-based emulsion. The use of pure fish oil-based emulsions might cause deficiencies of n-6 PUFAs, due to its high content of n-3 LCPUFAs. Second, pure fish oil emulsions are only available as 10 % solution. To supply the same amount of calories, double the volume has to be administered, while most VLBW infants are fluid restricted in the acute postnatal phase. Future studies in preterm infants, and especially in infants at risk for development of PNALD, e.g., infants on long-term parenteral nutrition due to short-bowel syndrome or microvillus inclusion disease, should demonstrate if the multicomponent emulsion prevents the development of PNALD and improves neurodevelopment. Our trial was underpowered for this purpose, since only 3 infants received long-term parenteral nutrition and developed cholestasis.

To conclude, we learned that high-dose amino acid plus lipid administration improves conditions for anabolism and growth, but also increases amino acid oxidation and successful urea synthesis. In addition, fish oil-containing lipid emulsions are associated with a lower incidence of late-onset sepsis and improved growth and may prevent the development of parenteral nutrition associated liver disease, probably due to its lower phytosterol content.

REFERENCES

1 Chien PF, Smith K, Watt PW, Scrimgeour CM, Taylor DJ, Rennie MJ. Protein turnover in the human fetus studied at term using stable isotope tracer amino acids. Am. J. Physiol. Jul 1993;265(1 Pt 1):E31-5.

2 Van den Akker CH, Schierbeek H, Dorst KY, et al. Human fetal amino acid metabolism at term gestation. Am. J. Clin. Nutr. Jan 2009;89(1):153-60.

3 van den Akker CH, Schierbeek H, Minderman G, et al. Amino acid metabolism in the human fetus at term: leucine, valine, and methionine kinetics. Pediatr Res. Dec 2011;70(6):566-71.

4 Anderson TL, Muttart CR, Bieber MA, Nicholson JF, Heird WC. A controlled trial of glucose versus glucose and amino acids in premature infants. J. Pediatr. Jun 1979;94(6):947-51.

5 Rivera A, Jr., Bell EF, Bier DM. Effect of intravenous amino acids on protein metabolism of preterm infants during the first three days of life. Pediatr. Res. Feb 1993;33(2):106-11.

6 Saini J, MacMahon P, Morgan JB, Kovar IZ. Early parenteral feeding of amino acids. Arch. Dis. Child. Oct 1989;64(10 Spec No):1362-6.

7 Van Goudoever JB, Colen T, Wattimena JL, Huijmans JG, Carnielli VP, Sauer PJ. Immediate commencement of amino acid supplementation in preterm infants: effect on serum amino acid concentrations and protein kinetics on the first day of life. J. Pediatr. Sep 1995;127(3):458-65.

8 Van Lingen RA, Van Goudoever JB, Luijendijk IH, Wattimena JL, Sauer PJ. Effects of early amino acid administration during total parenteral nutrition on protein metabolism in pre-term infants. Clin Sci (Lond). Feb 1992;82(2):199-203.

9 Thureen PJ, Anderson AH, Baron KA, Melara DL, Hay WW, Jr., Fennessey PV. Protein balance in the first week of life in ventilated neonates receiving parenteral nutrition. Am J Clin Nutr. Nov 1998;68(5):1128-35.

10 Ibrahim HM, Jeroudi MA, Baier RJ, Dhanireddy R, Krouskop RW. Aggressive early total parental nutrition in low-birth-weight infants. J. Perinatol. Aug 2004;24(8):482-6.

11 Te Braake FW, Van den Akker CH, Wattimena DJ, Huijmans JG, Van Goudoever JB. Amino acid administration to premature infants directly after birth. J. Pediatr. Oct 2005;147(4):457-61.

12 Thureen PJ, Melara D, Fennessey PV, Hay WW, Jr. Effect of low versus high intravenous amino acid intake on very low birth weight infants in the early neonatal period. Pediatr. Res. Jan 2003;53(1):24-32.

13 Scott PH, Sandham S, Balmer SE, Wharton BA. Diet-related reference values for plasma amino acids in newborns measured by reversed-phase HPLC. Clin. Chem. Nov 1990;36(11):1922-7.

14 Cetin I, Corbetta C, Sereni LP, et al. Umbilical amino acid concentrations in normal and growth-retarded fetuses sampled in utero by cordocentesis. Am. J. Obstet. Gynecol. Jan 1990;162(1):253-61.

15 Blanco CL, Gong AK, Green BK, Falck A, Schoolfield J, Liechty EA. Early Changes in Plasma Amino Acid Concentrations during Aggressive Nutritional Therapy in Extremely Low Birth Weight Infants. J Pediatr. Dec 1 2011;158:543-8.e1.

16 Roberts SA, Ball RO, Moore AM, Filler RM, Pencharz PB. The effect of graded intake of glycyl-L-tyrosine on phenylalanine and tyrosine metabolism in parenterally fed neonates with an estimation of tyrosine requirement. Pediatr Res. Jan 2001;49(1):111-9.

17 Huang L, Hogewind-Schoonenboom JE, de Groof F, et al. Lysine requirement of the enterally fed term infant in the first month of life. Am J Clin Nutr. Dec 2011;94(6):1496-503.

18 Huang L, Hogewind-Schoonenboom JE, van Dongen MJ, et al. Methionine requirement of the enterally fed term infant in the first month of life in the presence of cysteine. Am J Clin Nutr. May 2012;95(5):1048-54.

19 Van den Akker CH, Te Braake FW, Schierbeek H, et al. Albumin synthesis in premature neonates is stimulated by parenterally administered amino acids during the first days of life. Am. J. Clin. Nutr. Oct 2007;86(4):1003-8.

20 Te Braake FW, Schierbeek H, De Groof K, et al. Glutathione synthesis rates after amino acid administration directly after birth in preterm infants. Am. J. Clin. Nutr. Aug 2008;88(2):333-9.

21 Kashyap S, Abildskov K, Holleran SF, Ramakrishnan R, Towers HM, Sahni R. Effects of early aggressive nutrition in infants with birth weight (BW) <1250g: a randomized controlled trial. Paper presented at: PAS2007; E-PAS2007:5912.2.

22 Bulbul A, Okan F, Bulbul L, Nuhoglu A. Effect of low versus high early parenteral nutrition on plasma amino acid profiles in very low birth-weight infants. J Matern Fetal Neonatal Med. Jun 2012;25(6):770-6.

23 Ridout E, Melara D, Rottinghaus S, Thureen PJ. Blood urea nitrogen concentration as a marker of amino-acid intolerance in neonates with birthweight less than 1250 g. J. Perinatol. Feb 2005;25(2):130-3.

24 Blanco CL, Falck A, Green BK, Cornell JE, Gong AK. Metabolic Responses to Early and High Protein Supplementation in a Randomized Trial Evaluating the Prevention of Hyperkalemia in Extremely Low Birth Weight Infants. J Pediatr. Jun 25 2008; 153(4):535-40.

25 Clark RH, Chace DH, Spitzer AR. Effects of two different doses of amino acid supplementation on growth and blood amino acid levels in premature neonates admitted to the neonatal intensive care unit: a randomized, controlled trial. Pediatrics. Dec 2007;120(6):1286-96.

26 Blanco CL, Falck A, Green BK, Cornell JE, Gong AK. Metabolic responses to early and high protein supplementation in a randomized trial evaluating the prevention of hyperkalemia in extremely low birth weight infants. J. Pediatr. Oct 2008;153(4):535-40.

27 Usmani SS, Cavaliere T, Casatelli J, Harper RG. Plasma ammonia levels in very low birth weight preterm infants. J. Pediatr. Nov 1993;123(5):797-800.

28 DeSanto JT, Nagomi W, Liechty EA, Lemons JA. Blood ammonia concentration in cord blood during pregnancy. Early Hum. Dev. Apr 1993;33(1):1-8.

29 Rosenthal P. Assessing liver function and hyperbilirubinemia in the newborn. National Academy of Clinical Biochemistry. Clinical chemistry. Jan 1997;43(1):228-34.

30 van den Akker CH, Vlaardingerbroek H, van Goudoever JB. Nutritional support for extremely low-birth weight infants: abandoning catabolism in the neonatal intensive care unit. Curr Opin Clin Nutr Metab Care. Mar 6 2010;13:327-35.

31 Van den Akker CH, Te Braake FW, Weisglas-Kuperus N, Van Goudoever JB. Gender specific effects of early postnatal amino acid administration on long term outcome. Submitted.

32 Senterre T, Rigo J. Blood urea nitrogen during the first 2 weeks of life in VLBW infants receiving high protein intakes. Pediatr Res. 2011;70(5):767.

33 De Curtis M, Rigo J. Nutrition and kidney in preterm infant. J Matern Fetal Neonatal Med. Apr 2012;25 Suppl 1:55-9.

34 Radmacher PG, Lewis SL, Adamkin DH. Early amino acids and the metabolic response of ELBW infants (< or = 1000 g) in three time periods. J Perinatol. Jun 2009;29(6):433-7.

35 Bonsante F, Iacobelli S, Chantegret C, Martin D, Gouyon JB. The effect of parenteral nitrogen and energy intake on electrolyte balance in the preterm infant. Eur J Clin Nutr. Oct 2011;65(10):1088-93.

36 Moltu SJ, Strommen K, Blakstad EW, et al. Enhanced feeding in very-low-birth-weight infants may cause electrolyte disturbances and septicemia - A randomized, controlled trial. Clin Nutr. Sep 21 2012.

37 Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R. 1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR). J. Pediatr. Gastroenterol. Nutr. Nov 2005;41 Suppl 2:S1-87.

38 Elstgeest LE, Martens SE, Lopriore E, Walther FJ, te Pas AB. Does parenteral nutrition influence electrolyte- and fluid balance in preterm infants in the first days after birth? PLoS ONE. 2010;5(2):e9033.

39 Jamin A, D'Inca R, Le Floc'h N, et al. Fatal effects of a neonatal high-protein diet in low-birth-weight piglets used as a model of intrauterine growth restriction. Neonatology. Jun 2010;97(4):321-8.

40 Rigo J, Pieltain C, Viellevoye R, Bagnoli F. Calcium and phosphorus homeostasis: pathophysiology. In: Buonocore G, Bracci R, Weindling M, eds. Neonatology. A practical approach to neonatal management. Milan: Springer; 2012:333-53.

41 Boehm G, Kirchner B. [Calcium and phosphorus homeostasis of hypotrophic newborn infants fed breast milk]. Padiatr Padol. 1988;23(4):285-92.

42 Olsen IE, Richardson DK, Schmid CH, Ausman LM, Dwyer JT. Intersite differences in weight growth velocity of extremely premature infants. Pediatrics. Dec 2002;110(6):1125-32.

43 Lemons JA, Bauer CR, Oh W, et al. Very low birth weight outcomes of the National Institute of Child health and human development neonatal research network, January 1995 through December 1996. NICHD Neonatal Research Network. Pediatrics. Jan 2001;107(1):E1.

44 Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. Pediatrics. May 2003;111(5 Pt 1):986-90.

45 De Curtis M, Rigo J. Extrauterine growth restriction in very-low-birthweight infants. Acta Paediatr. Dec 2004;93(12):1563-8.

46 Sauer PJ. Can extrauterine growth approximate intrauterine growth? Should it? Am. J. Clin. Nutr. Feb 2007;85(2):608S-13S.

47 Senterre T, Rigo J. Reduction in postnatal cumulative nutritional deficit and improvement of growth in extremely preterm infants. Acta Paediatr. Feb 2012;101(2):e64-70.

48 Odberg MD, Sommerfelt K, Markestad T, Elgen IB. Growth and somatic health until adulthood of low birthweight children. Arch Dis Child Fetal Neonatal Ed. May 2010;95(3):F201-5.

49 Hack M, Schluchter M, Cartar L, Rahman M, Cuttler L, Borawski E. Growth of very low birth weight infants to age 20 years. Pediatrics. Jul 2003;112(1 Pt 1):e30-8.

50 Farooqi A, Hagglof B, Sedin G, Gothefors L, Serenius F. Growth in 10- to 12-year-old children born at 23 to 25 weeks' gestation in the 1990s: a Swedish national prospective follow-up study. Pediatrics. Nov 2006;118(5):e1452-65.

[51] Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics. Apr 2006;117(4):1253-61.

[52] Franz AR, Pohlandt F, Bode H, et al. Intrauterine, early neonatal, and postdischarge growth and neurodevelopmental outcome at 5.4 years in extremely preterm infants after intensive neonatal nutritional support. Pediatrics. Jan 2009;123(1):e101-9.

[53] Latal-Hajnal B, von Siebenthal K, Kovari H, Bucher HU, Largo RH. Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. J. Pediatr. Aug 2003;143(2):163-70.

[54] Fenton TR. A new growth chart for preterm babies: Babson and Benda's chart updated with recent data and a new format. BMC Pediatr. Dec 16 2003;3:13.

[55] Christensen RD, Henry E, Kiehn TI, Street JL. Pattern of daily weights among low birth weight neonates in the neonatal intensive care unit: data from a multihospital health-care system. J. Perinatol. Jan 1 2006;26(1):37-43.

[56] Ehrenkranz RA, Younes N, Lemons JA, et al. Longitudinal growth of hospitalized very low birth weight infants. Pediatrics. Aug 1999;104(2 Pt 1):280-9.

[57] Pauls J, Bauer K, Versmold H. Postnatal body weight curves for infants below 1000 g birth weight receiving early enteral and parenteral nutrition. Eur. J. Pediatr. May 1998;157(5):416-21.

[58] Niklasson A, Albertsson-Wikland K. Continuous growth reference from 24th week of gestation to 24 months by gender. BMC pediatrics. 2008;8:8.

[59] Fedrick J, Adelstein P. Factors associated with low birth weight of infants delivered at term. Br J Obstet Gynaecol. Jan 1978;85(1):1-7.

[60] Greisen G. Estimation of fetal weight by ultrasound. Hormone research. 1992;38(5-6):208-10.

[61] American Academy of Pediatrics Committee on Nutrition: Nutritional needs of low-birth-weight infants. Pediatrics. May 1985;75(5):976-86.

[62] Hay WW, Jr., Lucas A, Heird WC, et al. Workshop summary: nutrition of the extremely low birth weight infant. Pediatrics. Dec 1999;104(6):1360-8.

[63] van Goudoever JB, Sulkers EJ, Lafeber HN, Sauer PJ. Short-term growth and substrate use in very-low-birth-weight infants fed formulas with different energy contents. Am J Clin Nutr. Mar 2000;71(3):816-21.

[64] Uthaya S, Thomas EL, Hamilton G, Dore CJ, Bell J, Modi N. Altered adiposity after extremely preterm birth. Pediatr. Res. Feb 2005;57(2):211-5.

[65] Escribano J, Luque V, Ferre N, et al. Effect of protein intake and weight gain velocity on body fat mass at 6 months of age: the EU Childhood Obesity Programme. Int J Obes (Lond). Apr 2012;36(4):548-53.

[66] Lapillonne A, Salle BL, Glorieux FH, Claris O. Bone mineralization and growth are enhanced in preterm infants fed an isocaloric, nutrient-enriched preterm formula through term. Am J Clin Nutr. Dec 2004;80(6):1595-603.

[67] Roggero P, Gianni ML, Amato O, et al. Postnatal growth failure in preterm infants: recovery of growth and body composition after term. Early Hum. Dev. Aug 2008;84(8):555-9.

[68] Cooke RJ, Griffin I. Altered body composition in preterm infants at hospital discharge. Acta Paediatr. Aug 2009;98(8):1269-73.

[69] Kashyap S, Ohira-Kist K, Abildskov K, et al. Effects of quality of energy intake on growth and metabolic response of enterally fed low-birth-weight infants. Pediatr. Res. Sep 2001;50(3):390-7.

[70] Finken MJ, Keijzer-Veen MG, Dekker FW, et al. Preterm birth and later insulin resistance: effects of birth weight and postnatal growth in a population based longitudinal study from birth into adult life. Diabetologia. Mar 2006;49(3):478-85.

[71] Rotteveel J, van Weissenbruch MM, Twisk JW, Delemarre-Van de Waal HA. Infant and childhood growth patterns, insulin sensitivity, and blood pressure in prematurely born young adults. Pediatrics. Aug 2008;122(2):313-21.

[72] Rotteveel J, van Weissenbruch MM, Twisk JW, Delemarre-Van de Waal HA. Insulin sensitivity in prematurely born adults: relation to preterm growth restraint. Horm Res Paediatr. 2011;75(4):252-7.

[73] Barker DJ. The fetal and infant origins of adult disease. BMJ. Nov 17 1990;301(6761):1111.

[74] Lucas A, Morley R, Cole TJ. Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ. Nov 28 1998;317(7171):1481-7.

[75] Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet. Mar 29 2003;361(9363):1089-97.

[76] Kerkhof GF, Willemsen RH, Leunissen RW, Breukhoven PE, Hokken-Koelega AC. Health Profile of Young Adults Born Preterm: Negative Effects of Rapid Weight Gain in Early Life. J Clin Endocrinol Metab. Sep 19 2012;97:4498-506.

[77] Kotsopoulos K, Benadiba-Torch A, Cuddy A, Shah PS. Safety and efficacy of early amino acids in preterm <28 weeks gestation: prospective observational comparison. J. Perinatol. Dec 2006;26(12):749-54.

[78] Vogt RA, Gargus RA, Tucker R, McKinley L, Vohr BR. Impact of early postnatal nutrition on growth in extremely low birth weight infants born small for gestational age. Paper presented at: PAS2004:2525.

[79] Geary CA, Fonseca RA, Caskey MA, Malloy MH. Improved growth and decreased morbidities in <1000 g neonates after early management changes. J Perinatol. May 2008;28(5):347-53.

[80] Poindexter BB, Langer JC, Dusick AM, Ehrenkranz RA. Early provision of parenteral amino acids in extremely low birth weight infants: relation to growth and neurodevelopmental outcome. J. Pediatr. Mar 2006;148(3):300-5.

[81] Dinerstein A, Nieto RM, Solana CL, Perez GP, Otheguy LE, Larguia AM. Early and aggressive nutritional strategy (parenteral and enteral) decreases postnatal growth failure in very low birth weight infants. J. Perinatol. Jul 2006;26(7):436-42.

[82] Wilson DC, Cairns P, Halliday HL, Reid M, McClure G, Dodge JA. Randomised controlled trial of an aggressive nutritional regimen in sick very low birthweight infants. Arch. Dis. Child. Fetal Neonatal Ed. Jul 1997;77(1):F4-11.

[83] Koletzko B, Szajewska H, Ashwell M, et al. Documentation of functional and clinical effects of infant nutrition: setting the scene for COMMENT. Annals of nutrition & metabolism. 2012;60(4):222-32.

[84] Morgane PJ, Mokler DJ, Galler JR. Effects of prenatal protein malnutrition on the hippocampal formation. Neurosci. Biobehav. Rev. Jun 2002;26(4):471-83.

[85] Soto-Moyano R, Fernandez V, Sanhueza M, et al. Effects of mild protein prenatal malnutrition and subsequent postnatal nutritional rehabilitation on noradrenaline release and neuronal density in the rat occipital cortex. Brain Res. Dev. Brain Res. Aug 5 1999;116(1):51-8.

[86] Winick M, Rosso P. The effect of severe early malnutrition on cellular growth of human brain. Pediatr. Res. Mar 1969;3(2):181-4.

[87] Blanco CL, Cornell JE, Ramamurthy RS, Gong AK. Two Year Follow-Up Study From: The Effect of Early and Higher Protein Supplementation on Prevention of Hyperkalemia in Extremely Low Birth Weight (ELBW) Infants. Paper presented at: PAS2008:5630.7.

[88] Stephens BE, Walden RV, Gargus RA, et al. First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics. May 2009;123(5):1337-43.

[89] Grover A, Khashu M, Mukherjee A, Kairamkonda V. Iatrogenic malnutrition in neonatal intensive care units: urgent need to modify practice. JPEN. J. Parenter. Enteral Nutr. Mar-Apr 2008;32(2):140-4.

[90] Hans DM, Pylipow M, Long JD, Thureen PJ, Georgieff MK. Nutritional practices in the neonatal intensive care unit: analysis of a 2006 neonatal nutrition survey. Pediatrics. Jan 2009;123(1):51-7.

[91] Lapillonne A, Fellous L, Mokthari M, Kermorvant-Duchemin E. Parenteral nutrition objectives for very low birth weight infants: results of a national survey. J. Pediatr. Gastroenterol. Nutr. May 2009;48(5):618-26.

[92] Helbock HJ, Motchnik PA, Ames BN. Toxic hydroperoxides in intravenous lipid emulsions used in preterm infants. Pediatrics. Jan 1993;91(1):83-7.

[93] Laborie S, Lavoie JC, Chessex P. Increased urinary peroxides in newborn infants receiving parenteral nutrition exposed to light. J Pediatr. May 2000;136(5):628-32.

[94] Alexander-North LS, North JA, Kiminyo KP, Buettner GR, Spector AA. Polyunsaturated fatty acids increase lipid radical formation induced by oxidant stress in endothelial cells. J Lipid Res. Oct 1994;35(10):1773-85.

[95] Cooke RW. Factors associated with chronic lung disease in preterm infants. Arch Dis Child. Jul 1991;66(7 Spec No):776-9.

[96] Saugstad OD. The oxygen radical disease in neonatology. Indian journal of pediatrics. Sep-Oct 1989;56(5):585-93.

[97] Vlaardingerbroek H, Veldhorst MA, Spronk S, van den Akker CH, van Goudoever JB. Parenteral lipid administration to very-low-birth-weight infants--early introduction of lipids and use of new lipid emulsions: a systematic review and meta-analysis. Am J Clin Nutr. Aug 2012;96(2):255-68.

[98] Simmer K, Rao SC. Early introduction of lipids to parenterally-fed preterm infants. Cochrane Database Syst Rev. 2005(2):CD005256.

[99] Shulman RJ, Phillips S. Parenteral nutrition in infants and children. J Pediatr Gastroenterol Nutr. May 2003;36(5):587-607.

[100] Valentine CJ, Puthoff TD. Enhancing parenteral nutrition therapy for the neonate. Nutr Clin Pract. Apr 2007;22(2):183-93.

[101] American Society for Parental and Enteral Nutrition. Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. JPEN J Parenter Enteral Nutr. Jan-Feb 2002;26(1 Suppl):1SA-138SA.

[102] Neu J. Is it time to stop starving premature infants? J Perinatol. Jun 2009;29(6):399-400.

[103] Waitzberg D. Evolution of parenteral lipid emulsions. Clin Nutr Suppl. 2005;1(3):5-7.

[104] Calder PC, Jensen GL, Koletzko BV, Singer P, Wanten GJ. Lipid emulsions in parenteral nutrition of intensive care patients: current thinking and future directions. Intensive Care Med. Jan 14 2010;36(5):735-49.

[105] Krohn K, Koletzko B. Parenteral lipid emulsions in paediatrics. Curr Opin Clin Nutr Metab Care. May 2006;9(3):319-23.

[106] Koletzko B, Goulet O. Fish oil containing intravenous lipid emulsions in parenteral nutrition-associated cholestatic liver disease. Curr Opin Clin Nutr Metab Care. May 2010;13(3):321-6.

[107] Wanten GJ. Parenteral lipids in nutritional support and immune modulation. Clin Nutr suppl. 2009;4:13-7.

[108] Calder PC. Rationale for using new lipid emulsions in parenteral nutrition and a review of the trials performed in adults. Proc Nutr Soc. May 11 2009:1-9.

[109] Innis SM. Essential fatty acids in growth and development. Prog Lipid Res. 1991;30(1):39-103.

[110] Youdim KA, Martin A, Joseph JA. Essential fatty acids and the brain: possible health implications. Int J Dev Neurosci. Jul-Aug 2000;18(4-5):383-99.

[111] Koletzko B, Lien E, Agostoni C, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. Journal of perinatal medicine. 2008;36(1):5-14.

[112] European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies. Scientific Opinion. DHA and ARA and visual development. Scientific substantiation of a health claim related to docosahexaenoic acid (DHA) and arachidonic acid (ARA) and visual development persuant to Article 14 of Regulation (EC) No 1924/20061. The EFSA journal. 2009;941:1-14.

[113] Makrides M, Gibson RA, McPhee AJ, et al. Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. Jama. Jan 14 2009;301(2):175-82.

[114] Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. Early Hum Dev. Jun 1980;4(2):121-9.

[115] Uauy R, Mena P, Wegher B, Nieto S, Salem N, Jr. Long chain polyunsaturated fatty acid formation in neonates: effect of gestational age and intrauterine growth. Pediatr Res. Jan 2000;47(1):127-35.

[116] Szitanyi P, Koletzko B, Mydlilova A, Demmelmair H. Metabolism of 13C-labeled linoleic acid in newborn infants during the first week of life. Pediatr Res. May 1999;45(5 Pt 1):669-73.

[117] Skouroliakou M, Konstantinou D, Koutri K, et al. A double-blind, randomized clinical trial of the effect of omega-3 fatty acids on the oxidative stress of preterm neonates fed through parenteral nutrition. Eur J Clin Nutr. Sep 2010;64(9):940-7.

[118] Koksal N, Kavurt AV, Cetinkaya M, Ozarda Y, Ozkan H. Comparison of lipid emulsions on antioxidant capacity in preterm infants receiving parenteral nutrition. Pediatr Int. Aug 2011;53(4):562-6.

[119] Roggero P, Mosca F, Gianni ML, et al. F2-isoprostanes and total radical-trapping antioxidant potential in preterm infants receiving parenteral lipid emulsions. Nutrition. 2010;26(5):551-5.

[120] Gobel Y, Koletzko B, Bohles HJ, et al. Parenteral fat emulsions based on olive and soybean oils: a randomized clinical trial in preterm infants. Journal of pediatric gastroenterology and nutrition. Aug 2003;37(2):161-7.

[121] Webb AN, Hardy P, Peterkin M, et al. Tolerability and safety of olive oil-based lipid emulsion in critically ill neonates: a blinded randomized trial. Nutrition. Nov-Dec 2008;24(11-12):1057-64.
[122] Carter BA, Shulman RJ. Mechanisms of disease: update on the molecular etiology and fundamentals of parenteral nutrition associated cholestasis. Nature clinical practice. May 2007;4(5):277-87.
[123] Antebi H, Mansoor O, Ferrier C, et al. Liver function and plasma antioxidant status in intensive care unit patients requiring total parenteral nutrition: comparison of 2 fat emulsions. JPEN J Parenter Enteral Nutr. May-Jun 2004;28(3):142-8.
[124] de Meijer VE, Gura KM, Le HD, Meisel JA, Puder M. Fish Oil-Based Lipid Emulsions Prevent and Reverse Parenteral Nutrition-Associated Liver Disease: The Boston Experience. JPEN J Parenter Enteral Nutr. Jul 1 2009;33:541-7.
[125] Diamond IR, Sterescu A, Pencharz PB, Wales PW. The rationale for the use of parenteral omega-3 lipids in children with short bowel syndrome and liver disease. Pediatric surgery international. Jul 2008;24(7):773-8.
[126] Gura K, Strijbosch R, Arnold S, McPherson C, Puder M. The role of an intravenous fat emulsion composed of fish oil in a parenteral nutrition-dependent patient with hypertriglyceridemia. Nutr Clin Pract. Dec 2007;22(6):664-72.
[127] Gura KM, Duggan CP, Collier SB, et al. Reversal of parenteral nutrition-associated liver disease in two infants with short bowel syndrome using parenteral fish oil: implications for future management. Pediatrics. Jul 2006;118(1):e197-201.
[128] Gura KM, Lee S, Valim C, et al. Safety and efficacy of a fish-oil-based fat emulsion in the treatment of parenteral nutrition-associated liver disease. Pediatrics. Mar 2008;121(3):e678-86.
[129] Puder M, Valim C, Meisel JA, et al. Parenteral fish oil improves outcomes in patients with parenteral nutrition-associated liver injury. Ann Surg. Sep 2009;250(3):395-402.
[130] Premkumar MH, Carter BA, Hawthorne KM, King K, Abrams SA. High Rates of Resolution of Cholestasis in Parenteral Nutrition-Associated Liver Disease with Fish Oil-Based Lipid Emulsion Monotherapy. J Pediatr. Nov 16 2012.
[131] Muhammed R, Bremner R, Protheroe S, Johnson T, Holden C, Murphy MS. Resolution of parenteral nutrition-associated jaundice on changing from a soybean oil emulsion to a complex mixed-lipid emulsion. J Pediatr Gastroenterol Nutr. Jun 2012;54(6):797-802.
[132] Angsten G, Finkel Y, Lucas S, Kassa AM, Paulsson M, Engstrand Lilja H. Improved Outcome in Neonatal Short Bowel Syndrome Using Parenteral Fish Oil in Combination With omega-6/9 Lipid Emulsions. JPEN J Parenter Enteral Nutr. 2012;36(5): 587-95.
[133] Le HD, de Meijer VE, Robinson EM, et al. Parenteral fish-oil-based lipid emulsion improves fatty acid profiles and lipids in parenteral nutrition-dependent children. Am J Clin Nutr. Jul 20 2011;94(3):749-58.
[134] Le HD, de Meijer VE, Zurakowski D, Meisel JA, Gura KM, Puder M. Parenteral fish oil as monotherapy improves lipid profiles in children with parenteral nutrition-associated liver disease. JPEN J Parenter Enteral Nutr. Sep-Oct 2010;34(5):477-84.
[135] Cober MP, Teitelbaum DH. Prevention of parenteral nutrition-associated liver disease: lipid minimization. Current opinion in organ transplantation. Jun 2010;15(3):330-3.
[136] Colomb V, Jobert-Giraud A, Lacaille F, Goulet O, Fournet JC, Ricour C. Role of lipid emulsions in cholestasis associated with long-term parenteral nutrition in children. JPEN J Parenter Enteral Nutr. Nov-Dec 2000;24(6):345-50.

[137] Nehra D, Fallon EM, Carlson SJ, et al. Provision of a Soy-Based Intravenous Lipid Emulsion at 1 g/kg/d Does Not Prevent Cholestasis in Neonates. JPEN J Parenter Enteral Nutr. Jul 5 2012.

[138] Grimm H, Mertes N, Goeters C, et al. Improved fatty acid and leukotriene pattern with a novel lipid emulsion in surgical patients. European journal of nutrition. Feb 2006;45(1):55-60.

[139] Amesz EM, Schaafsma A, Cranendonk A, Lafeber HN. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. J Pediatr Gastroenterol Nutr. Feb 2010;50(2):200-7.

[140] Vlaardingerbroek H, van den Akker CH, de Groof F, et al. Amino acids for the neonate: search for the ideal dietary composition. Neoreviews. 2011;12:506-16.

[141] Embleton ND. Optimal protein and energy intakes in preterm infants. Early Hum. Dev. Dec 2007;83(12):831-7.

CHAPTER 11

Summary
Samenvatting

CHAPTER 11
Summary

PART 1 – INTRODUCTION

Functional outcome of preterm infants is highly related to the nutrient supply provided during the first few weeks of life. Especially amino acids and proteins seem to play a pivotal role in growth and neurodevelopment. However, optimal parenteral intakes of amino acids and lipids are not yet established. As a consequence, almost all very low birth weight (VLBW; birth weight < 1500 g) infants develop a protein and energy deficit during the first weeks of life and are growth-impaired upon hospital discharge. In addition, not only the quantity, but also the quality and timing of parenteral nutrition might be important for well-being during the neonatal period and beyond.
In the general introduction we describe the general background and aims of the studies presented in this thesis. We give a introduction to the role of parenteral amino acids and lipids and their potential benefits and drawbacks. Furthermore the potential relation between the composition of lipid emulsions and the development of parenteral nutrition associated liver disease (PNALD) is described.

Chapter 2

In this chapter we present a systematic review of the literature until February 2012 and meta-analysis that was performed to determine the most suitable timing of initiation of lipids and the most suitable composition of parenteral lipid emulsions for VLBW infants. Based on the available literature, initiation of lipids within the first two days of life in VLBW infants seems safe and well tolerated; however, beneficial effects on growth could not be demonstrated for this treatment nor for the type of lipid emulsion. We demonstrated that lipid emulsions that are not purely soybean oil-based are associated with a lower incidence of sepsis.

PART 2 – QUANTITY OF PARENTERAL NUTRITION

This part describes a large randomized controlled trial in VLBW infants randomized to receiving 2.4 g amino acids/(kg·d) without lipids during the first two days of life, to early lipid administration plus 2.4 g amino acids/(kg·d), or to early lipid administration plus 3.6 g amino acids/(kg·d) from birth onwards.

Chapter 3

In this chapter we assessed the efficacy and safety of early parenteral lipid and high-dose amino acid administration from birth onwards in 144 VLBW infants. Most VLBW infants tolerated early lipid administration starting at birth, with no increased incidence of adverse events. Furthermore, early lipid administration improved nitrogen balances, thus creating conditions for anabolism and growth. Higher amino acid administration combined with early lipid administration did not further improve the nitrogen balance, but did lead to increased amino acid oxidation. However, when expressed as actual

amino acid intake, a higher amino acid intake correlated positively with nitrogen balance. Plasma amino acid concentrations of most amino acids were higher in the high-dose amino acid group and were more in concordance to reference ranges. No differences in other biochemical parameters, growth, or clinical outcomes were observed. We concluded that early lipid initiation and high-dose amino acids were well tolerated, as demonstrated by a lack of serious biochemical disturbances or adverse clinical outcome and resulted in improved conditions for anabolism and growth.

Chapter 4

To quantify the efficacy of early initiation of parenteral lipids and higher amounts of amino acids on metabolism and protein accretion we performed a stable isotope study in a subset of infants described in the previous chapter. On the second day of life, 28 infants received a primed, continuous infusion of [1-^{13}C]phenylalanine, [ring-D_4] tyrosine, [U-$^{13}C_6$,^{15}N]leucine, and [methyl-D_3]α-ketoisocaproic acid. Plasma enrichments were analyzed by mass spectrometry techniques. We demonstrated that early lipid administration did not change phenylalanine and leucine metabolism, while early lipids plus high-dose amino acid administration increased protein synthesis rates based on both phenylalanine and leucine data. This resulted in higher net protein balances. In addition, high-dose amino acid intake significantly increased phenylalanine hydroxylation rates to tyrosine making more tyrosine available for protein synthesis. Hence, early lipid plus higher-dose amino acid administration to VLBW infants resulted in a more anabolic state during the acute postnatal phase.

Chapter 5

In plasma of the infants studied in Chapter 4, albumin was purified to analyze albumin synthesis rates. Albumin is one of the most important plasma proteins and plays a key role in many physiological processes like preserving of colloid osmotic pressure, scavenging radicals, and binding and transporting bilirubin, hormones, and drugs. However, albumin concentrations are often low during the first days of life in preterm infants. Early parental lipid and high-dose amino acid administration from birth onwards to VLBW infants increased hepatic albumin synthesis rates compared to administration of standard amounts of amino acids and no lipids during the first days of life.

Chapter 6

Preterm neonates are exposed to a high amount of oxidants and have a lower antioxidative capacity. Therefore they are subjected to increased 'oxidative stress', which is associated with bronchopulmonary dysplasia and periventricular leucomalacia. We hypothesized that early lipid and high-dose amino acid administration would result in an upregulation of the antioxidant defense mechanism by increased synthesis of glutathione, the main non-enzymatic intracellular antioxidant, without increasing oxidative stress due to the lipid administration. A subset of infants described in Chapter 3 received a primed, continuous infusion of [U-^{13}C]glycine, a precursor for glutathione synthesis, on the second day of life. Intra-erythrocyte glutathione synthesis rates were calculated and urinary oxidative stress markers were analyzed. Early lipid administration

and high-dose amino acid administration did not increase glutathione concentration, glutathione synthesis, or oxidative stress markers. Therefore, it seems safe to initiate lipids and high-dose amino acids from birth onwards in VLBW infants, although this does not improve the defense mechanisms against oxidative stress.

PART 3 – QUALITY OF PARENTERAL NUTRITION

Since the 1960s, commercial parenteral lipid emulsions have been widely used. Purely soybean oil-based emulsions were the first lipid emulsions available for parenteral use and are still the most often used parenteral lipid emulsion. However, in several newer emulsions, soybean oil is combined with other lipid sources, such as coconut oil (providing medium-chain triacylglycerols, MCTs), olive oil and/or fish oil. Each type of lipid has different characteristics and potential benefits and disadvantages. Several of these recently developed multicomponent lipid emulsions have been shown to improve tolerance and short- and long-term outcomes, such as a lower incidence of PNALD. Nevertheless, it is not clear which lipid composition is most beneficial for a preterm infant. In this part pure soybean oil-based lipid emulsions are compared with a multicomponent lipid emulsion containing soybean oil, MCTs, olive oil, and fish oil and with a pure fish oil-based lipid emulsion.

Chapter 7

The efficacy and safety of the multicomponent emulsion compared to a pure soybean-oil based emulsion was assessed in a double blinded randomized controlled trial in 96 VLBW infants. Lipid emulsions were administered from birth onwards at a dose of 2-3 g/(kg·d) until infants were on full enteral nutrition. We demonstrated that the multicomponent lipid emulsion improved plasma fatty acid profiles of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), fatty acids crucial for neurodevelopment and immune function. In addition, administration of the multicomponent emulsion was associated with improved growth until hospital discharge. The multicomponent emulsion was well tolerated since hematology, biochemistry, and neonatal outcome were not different between groups, while plasma concentrations of phytosterols were lower in the multicomponent emulsion group, which might reduce the risk on the development of PNALD.

Chapter 8

Fish oil containing lipid emulsion contain more n-3 long-chain polyunsaturated fatty acids than plant-based lipid emulsions and contain more added α-tocopherol, which both likely decrease oxidative stress. To compare the effects of administration of the multicomponent lipid emulsion and the pure soybean oil emulsion on oxidative stress markers and glutathione synthesis rates we performed a stable isotope study in a subset of infants described in the previous chapter. On the second day of life, 24 infants received a primed, continuous infusion of [U-^{13}C]glycine, a precursor for glutathione synthesis, to determine intra-erythrocyte glutathione synthesis rates. Oxidative stress markers were measured in urine. Glutathione concentrations, synthesis rates, and oxidative stress markers were not different between the two lipid emulsions.

Hence, we could not demonstrate reduced oxidative stress in the infants receiving the multicomponent emulsion.

Chapter 9

Prolonged administration of total parenteral nutrition is associated with the development of PNALD. Fish oil-based lipid emulsion can reverse PNALD; however, it is unknown if they can also prevent PNALD in preterm infants. As a model for preterm infants, we randomly assigned preterm pigs to total parenteral nutrition with either a pure soybean oil-based emulsion, a pure fish oil-based emulsion, or the multicomponent emulsion to test if lipid emulsions with varying amounts of fish oil can prevent PNALD. A reference group was enterally fed with milk formula. Fourteen days of continuous infusion of total parenteral nutrition produced dramatic hepatic findings. Although liver growth was maintained in all pigs, the livers of pure soybean oil-fed pigs showed histopathologic microvascular steatosis and neutrophil infiltration suggesting active liver injury. This contrasted to the relative benign findings noted in the enterally fed pigs and in the pigs receiving either the pure fish oil-based or the multicomponent emulsions. The results also suggested that phytosterols, which are found in soybean oil emulsions but are devoid in pure fish oil emulsions, are one of the major reasons why pure soybean oil-based emulsions are associated with PNALD and why pure fish oil-based emulsions can reverse or prevent it. The mechanism is believed to be due to phytosterol's negative effect on Farnesoid X receptor (FXR) a key bile acid sensor involved in bile acid homeostasis.

PART 4 – DISCUSSION AND SUMMARY

Chapter 10 provides a general discussion of our findings in relation to the current literature. Furthermore we give directions for future research. The main conclusions obtained from the studies described in this thesis are the following.

Lipid administration from birth onwards to VLBW infants:

— improves conditions for anabolism and growth, as shown by improved nitrogen balances
— is well tolerated and does not increase oxidative stress.

High-dose amino acid plus lipid administration from birth onwards to VLBW infants:

— improves conditions for anabolism and growth, as shown by improved protein balances
— increases amino acid oxidation and thereby increases urea synthesis
— seems safe as demonstrated by a lack of serious biochemical disturbances or adverse clinical outcome.

In comparison to pure soybean oil emulsions, fish oil-containing lipid emulsions:

— are associated with a lower incidence of infections and improve growth
— may prevent the development of parenteral nutrition associated liver disease
— are well tolerated, but do not reduce oxidative stress

Samenvatting

DEEL 1 – INTRODUCTIE

Wanneer een foetus nog in de baarmoeder zit, krijgt het een constante aanvoer van aminozuren en energie van de moeder via de placenta en navelstreng. De aminozuren zijn bouwstoffen voor eiwitten en daardoor voor de aanleg van organen en spieren. De foetus gebruikt de aminozuren voor groei, maar ook als brandstof. Vanaf de tweede helft van de zwangerschap krijgt de foetus ook vetten via de navelstreng, die eveneens gebruikt worden als brandstof. Daarnaast leveren vetten belangrijke bouwstoffen voor de aanleg van het centrale zenuwstelsel. Na de geboorte zijn te vroeg geboren kinderen afhankelijk van de voeding die hen wordt toegediend. Gedurende de eerste levensdagen verdragen te vroeg geboren kinderen met een zeer laag geboortegewicht (VLBW kinderen, geboortegewicht < 1500 gram) nog vrijwel geen melkvoeding en zijn ze afhankelijk van infuusvoeding. Infuusvoeding bestaat uit suiker (glucose), aminozuren en vetten. De lichamelijke en geestelijke ontwikkeling van te vroeg geboren kinderen heeft een grote relatie met voeding en groei in de eerste levensweken. De optimale hoeveelheid aminozuren en vetten (energie) in de infuusvoeding is echter nog niet bekend. Aminozuren en glucose worden in vrijwel alle neonatologie afdelingen direct vanaf de geboorte toegediend, terwijl vetten vaak pas na een paar dagen worden gegeven. Het blijkt dat niet alleen de hoeveelheid infuusvoeding, maar ook de samenstelling en het moment van toedienen belangrijk is voor een goede lichamelijke en geestelijke ontwikkeling van VLBW kinderen. Momenteel hebben vrijwel alle VLBW kinderen na de eerste levensweek een eiwit- en energietekort en zijn ze bij ontslag naar huis (rond de uitgerekende datum) minder goed gegroeid dan dat ze in de baarmoeder gedaan zouden hebben.
Onderzoek naar infuusvoeding is daarom van groot belang om VLBW kinderen een goede start te geven en te zorgen dat hun groei en ontwikkeling niet onder doet voor die van op tijd geboren kinderen. Met het onderzoek beschreven in dit proefschrift hebben we geprobeerd hier een bijdrage aan te leveren.

In de inleiding beschrijven we de algemene achtergrond en doelen van de studies die in dit proefschrift beschreven worden. We beschrijven de rol van aminozuren en vetten in infuusvoeding en de mogelijke voor- en nadelen hiervan. Bovendien beschrijven we de mogelijke relatie tussen de samenstelling van vetemulsies en de ontwikkeling van leverziekten die met infuusvoeding in verband worden gebracht (parenterale voeding geassocieerde leverziekten, PNALD). PNALD wordt gekarakteriseerd door verstopping en vernietiging van de galwegen, wat kan resulteren in vervetting van de lever waardoor deze uiteindelijk niet meer kan functioneren.

Hoofdstuk 2

In hoofdstuk 2 presenteren we een systematische samenvatting van diverse studies die tot februari 2012 verschenen zijn om het beste moment van starten van vetten en de samenstelling van deze vetten in infuusvoeding voor VLBW kinderen te bepalen. De uitkomsten van deze studies hebben we door middel van een meta-analyse met elkaar vergeleken. Gebaseerd op deze studies lijkt het veilig om binnen de eerste twee levensdagen met vetten te starten. We konden echter geen voordelen van eerder starten van vetten of van het type vetten aantonen op de groei van deze kinderen. In dit hoofdstuk demonstreerden we ook dat vetemulsies die niet geheel van sojabonenolie gemaakt zijn, geassocieerd zijn met minder infecties.

DEEL 2 – HOEVEELHEID INFUUSVOEDING

In dit deel beschrijven we een grote studie waarin VLBW kinderen per loting verdeeld zijn over drie verschillende groepen: 2.4 g aminozuren/(kg·dag) zonder vetten gedurende de eerste twee levensdagen (standaard voedingsbeleid van de afdeling), 2.4 g aminozuren/(kg·dag) plus vetten vanaf de geboorte, of 3.6 g aminozuren/(kg·dag) plus vetten vanaf de geboorte.

Hoofdstuk 3

In dit hoofdstuk bepaalden we de effectiviteit en veiligheid van het toedienen van vetten en een hogere dosering aminozuren vanaf de geboorte in 144 VLBW kinderen. De meeste VLBW kinderen verdroegen de vetten vanaf de geboorte goed. Bovendien verhoogde vroege vettoediening de stikstofbalans, een maat voor eiwitgroei, en zorgde daarmee voor betere omstandigheden om te kunnen groeien. Vroege vettoediening plus hogere aminozuurtoediening verhoogde de stikstofbalans niet verder, maar verhoogde wel de verbranding van aminozuren, mogelijk om meer energie te maken. Plasma aminozuurconcentraties waren hoger in de groep die een hogere dosing aminozuren kreeg en deze concentraties waren meer in overeenstemming met referentiewaarden. We vonden geen verschillen in andere biochemische uitkomsten van veiligheid, in de incidentie van ziekten die geassocieerd worden met vroeggeboorte, of in groei. We concludeerden dat het toedienen van vetten vanaf de geboorte en hogere dosering aminozuren aan VLBW kinderen veilig is en zorgt voor betere omstandigheden om te groeien.

Hoofdstuk 4

Om de effectiviteit van vroege vettoediening en hogere dosering aminozuren op eiwitstofwisseling en eiwitgroei te kwantificeren hebben we een stabiele isotopen studie verricht in een deel van de kinderen die in het vorige hoofdstuk beschreven werden. Op de tweede levensdag kregen deze kindenen een infuus met daarin een aantal gelabelde aminozuren en producten hiervan: [1-^{13}C]phenylalanine, [ring-D_4] tyrosine, [U-$^{13}C_6$,^{15}N]leucine, en [methyl-D_3]α-ketoisocaproic acid. Na 6, 7 en 8 uur werd bloed afgenomen uit een infuus en werd de hoeveelheid van dit gelabelde aminozuur (verrijking) in plasma bepaald. We toonden aan dat vroege vettoediening de stofwisseling van phenylalanine en leucine (twee aminozuren) niet veranderde, terwijl

vroege vettoediening plus een hoge dosering aminozuren de aanmaaksnelheid van eiwitten verhoogde, waardoor deze kinderen een hogere eiwitbalans hadden en dus meer groei vertoonden. Bovendien verhoogde een hoge dosis aminozuren de omzetting van phenylalanine naar tyrosine, zodat er meer tyrosine beschikbaar was voor het maken van eiwitten.

Hoofdstuk 5

Uit het plasma van dezelfde kinderen als beschreven in hoofdstuk 4 werd albumine gezuiverd om de aanmaaksnelheid van albumine te kunnen meten. Albumine is een van de belangrijkste eiwitten in plasma en speelt een sleutelrol in veel fysiologische processen, zoals het handhaven van de colloid osmotische druk, wegvangen van vrije radicalen en het binden en transporteren van bilirubine, hormonen en medicijnen. Ondanks deze belangrijke functies, is de albumine concentratie in VLBW kinderen vaak laag tijdens de eerste levensdagen. We toonden aan dat vroege vettoediening plus hoge dosering aminozuren de albumine aanmaaksnelheid verhoogde.

Hoofdstuk 6

Te vroeg geboren kinderen hebben meer blootstelling aan oxidanten en beschikken over minder beschermende antioxidanten. Daardoor hebben zij verhoogde "oxidatieve stress", wat geassocieerd is met bronchopulmonaire dysplasie (chronische longziekte bij te vroeg geboren kinderen) en periventriculaire leucomalacie (hersenschade). We voorspelden dat vroege vettoediening en hoge dosering aminozuren het antioxidant verdedigingsmechanisme tegen deze schade zou verhogen door een hogere aanmaaksnelheid van glutathion, de belangrijkste niet-enzymatische intracellulaire antioxidant, zonder dat vroege vettoediening de oxidatieve stress zou verhogen. Op de tweede levensdag verrichtten we een stabiele isotopenstudie in een deel van de kinderen beschreven in hoofdstuk 3. Deze kinderen kregen een infuus met gelabeld [U-^{13}C]glycine, de precursor voor glutathion synthese. De glutathion aanmaaksnelheid in rode bloedcellen en oxidatieve stress markers in urine werden gemeten. Vroege vettoediening en hoge dosering aminozuren verhoogde de glutathion concentratie, glutathion aanmaaksnelheid of oxidatieve stress markers niet. Op basis hiervan lijkt het veilig om vetten en hoge dosering aminozuren vanaf de geboorte toe te dienen aan VLBW kinderen. Het helpt echter niet om de verdedigingsmechanismen tegen oxidatieve stress te versterken.

DEEL 3 – SAMENSTELLING VAN INFUUSVOEDING

Vanaf de jaren '60 worden vetemulsies voor infuusvoeding over de hele wereld gebruikt. De eerste emulsies die beschikbaar waren en nog steeds het meeste gebruikt worden zijn emulsies die volledig gebaseerd zijn op sojabonenolie. In verschillende nieuwere vetemulsies wordt sojabonenolie deels vervangen door andere oliesoorten, zoals kokosolie (levert middellangeketenvetzuren, MCTs), olijfolie en/of visolie. Elk type olie heeft verschillende eigenschappen en mogelijke voor- en nadelen. Studies in voornamelijk volwassenen en kinderen hebben aangetoond dat deze gecombineerde vetemulsies de tolerantie en lange-termijn uitkomsten kunnen verbeteren. Het is echter

nog niet duidelijk welke samenstelling van vetemulsies het meeste voordelen biedt voor te vroeg geboren kinderen.
In dit deel wordt een pure sojabonenolie emulsie vergeleken met een samengestelde emulsie, bestaand uit sojabonenolie, MCTs, olijfolie en visolie, en met een pure visolie emulsie.

Hoofdstuk 7

De effectiviteit en veiligheid van de samengestelde emulsie in vergelijking tot de pure sojabonenolie emulsie werd onderzocht in een dubbel geblindeerde studie waarin 96 VLBW kinderen per loting een van deze twee emulsies toegediend kregen. De vetemulsies werden vanaf de geboorte toegediend in een dosering van 2-3 gram/(kg·dag) totdat de kinderen geen infuusvoeding meer nodig hadden en enkel gevoed konden worden met melk. We toonden aan dat de samengestelde emulsie het vetzuurprofiel van docosahexaeenzuur (DHA) en eicosapentaeenzuur (EPA), vetzuren die erg belangrijks zijn voor de ontwikkeling van het zenuwstelsel en voor de immuunfunctie, in plasma verbeterde. Ook was toediening van de samengestelde emulsie geassocieerd met een betere groei gedurende de ziekenhuisopname. De samengestelde emulsie werd goed verdragen, aangezien we geen verschillen vonden in hematologische en biochemische bloeduitslagen en in de incidentie van veel voorkomende ziekten. Bovendien waren de plasma concentraties van phytosterolen lager in de groep die de samengestelde emulsie kreeg, wat mogelijk het risico op het ontstaan van PNALD verlaagd.

Hoofdstuk 8

Visolie bevat van nature meer n-3 langeketenvetzuren dan plantaardige oliën, terwijl er daarnaast meer vitamine E aan wordt toegevoegd. Beiden dragen bij aan een mogelijke vermindering van oxidatieve stress. Om de verschillen op oxidatieve stress en de aanmaaksnelheid van glutathion van de samengestelde emulsie en de pure sojabonenolie emulsie te vergelijken, verrichtten we een stabiele isotopenstudie in een deel van de kinderen die in het voorgaande hoofdstuk beschreven zijn. Deze studie was hetzelfde van opzet als de studie beschreven in hoofdstuk 6. De glutathion concentraties, glutathion aanmaaksnelheid en oxidatieve stress markers waren niet verschillend tussen de twee groepen. We konden dus geen verminderde oxidatieve stress aantonen in de VLBW kinderen die de samengestelde emulsie toegediend kregen.

Hoofdstuk 9

Langdurige toediening van infuusvoeding is geassocieerd met het ontwikkelen van PNALD. Visolie emulsies kunnen PNALD genezen; echter het is niet bekend of ze het ontstaan van PNALD ook kunnen voorkomen bij te vroeg geboren kinderen. Om te testen of emulsies met visolie het ontstaan van PNALD kunnen voorkomen hebben we een studie uitgevoerd in te vroeg geboren biggen als een model voor te vroeg geboren kinderen. De biggen werden per loting verdeeld over infuusvoeding met de pure sojabonenolie emulsie, met de samengestelde emulsie, of met een pure visolie emulsie. Als referentiegroep werd een groep biggen met melk gevoed. Na 14 dagen

infuusvoeding vonden we dramatische effecten in de lever. Hoewel de levergroei bij alle biggen hetzelfde was, vertoonden de levers van de biggen in de pure sojabonenolie groep histopathologisch tekenen van microvasculaire steatose (leververvetting) en neutrofiele infiltratie (ontstekingsreactie), wat bij actieve leverschade past. Dit was in tegenstelling tot de relatieve gunstige effecten die we in de melk-gevoede biggen en de biggen met de visolie bevattende emulsies zagen. We hebben sterke aanwijzingen dat phytosterolen, plantaardige sterolen die in sojabonenolie gevonden worden maar niet in visolie, één van de belangrijkste veroorzakers zijn van PNALD. Dat zou verklaren waarom PNALD vooral gezien wordt bij sojabonenolie en waarom pure visolie emulsies dit juist zou kunnen genezen of voorkomen. We denken dat dit komt door het negatieve effect van phytosterolen op Farnesoid X receptor (FXR), dat een sleutelrol speelt als galzuur sensor in de galzuurhomeostase.

DEEL 4 – DISCUSSIE EN SAMENVATTING

In hoofdstuk 10 wordt een algemene discussie gegeven van onze bevindingen in relatie tot de huidige literatuur. Bovendien worden er aanbevelingen gedaan voor toekomstig onderzoek.

De belangrijkste conclusies van de studies die beschreven worden in dit proefschrift zijn de volgende.
Toediening van vetten vanaf de geboorte aan VLBW kinderen:
— verbetert de omstandigheden voor anabolisme en groei, zoals aangetoond door middel van verhoogde stikstofbalansen
— wordt goed verdragen en verhoogt oxidatieve stress niet.

Toediening van vetten plus hoge dosering aminozuren vanaf de geboorte aan VLBW kinderen:
— verbetert de omstandigheden voor anabolisme en groei, zoals aangetoond door middel stabiele isotopenstudies naar aminozuurstofwisseling
— verhoogt aminozuuroxidatie en verhoogt daardoor ureumsynthese
— lijkt veilig, aangezien we geen ernstige biochemische verstoringen of nadelige klinische uitkomsten vonden.

Visolie bevattende emulsies, in vergelijking tot pure sojabonenolie emulsies:
— zijn geassocieerd met een lagere incidentie van infectie en verbeteren groei
— kunnen de ontwikkeling van parenterale voeding geassocieerde leverziekte voorkomen
— worden goed verdragen, maar verlagen oxidatieve stress niet.

PART 5 – APPENDICES

List of publications and awards
Authors' affiliations
Curriculum vitae
Dankwoord
PhD portfolio
Abbreviations

List of publications and awards

Journal papers

- Vlaardingerbroek H, Vermeulen MJ, Rook D, Van den Akker CHP, Dorst K, Wattimena DJ, Vermes A, Schierbeek H, Van Goudoever JB. Safety and efficacy of early parenteral lipid and high-dose amino acid administration to very low birth weight infants: a randomized controlled trial. Submitted.
- Vlaardingerbroek H, Roelants JA, Rook D, Dorst K, Schierbeek H, Vermes A, Vermeulen MJ, Van Goudoever JB, Van den Akker CHP. Adaptive regulation of amino acid metabolism in very low birth weight infants. Submitted.
- Vlaardingerbroek H, Schierbeek H, Rook D, Vermeulen MJ, Dorst K, Vermes A, Van Goudoever JB, Van den Akker CHP. Albumin synthesis is enhanced by early parenteral lipid and high dose amino acid administration to very low birth weight infants. Submitted.
- Rook D, Vlaardingerbroek H, Muizer Y, Dorst K, Kuligowski J, Escobar J, Vento M, van Goudoever JB, Schierbeek H. Increased amino acid and early lipid administration do not up-regulate glutathione synthesis, nor increase oxidative stress in very low birth weight infants.
- Vlaardingerbroek H, Vermeulen MJ, Carnielli V, Vaz FM, Van den Akker CHP, Van Goudoever JB. Growth and fatty acid profiles in very low birth weight infants receiving a soybean, medium-chain triacylglycerol, olive, and fish oil-containing lipid emulsion from birth onwards. Submitted.
- Rook D, Vlaardingerbroek H, Muizer Y, Dorst K, Kuligowski J, Escobar J, Vento M, Vermeulen MJ, van Goudoever JB, Schierbeek H. A multicomponent lipid emulsion containing a mixture of soybean oil, medium-chain triglycerides, olive oil and fish oil does not reduce oxidative stress in very low birth weight infants; a double-blind randomized controlled trial.
- Vlaardingerbroek H, Ng K, Stoll B, Benight N, Kluijtmans LAJ, Kulik W, SquiresEJ, Finegold MJ, Van Goudoever JB, Burrin DB. New generation lipid emulsions prevent phytosterolemia and cholestasis in chronic parenterally-fed preterm pigs. Submitted.
- Vlaardingerbroek H, Vermeulen MJ, Van den Akker CHP, Van Goudoever JB. Optimizing parenteral nitrogen intake for preterm infants. Accepted in Nutrients.
- Rook D, Schierbeek H, Vento M, Vlaardingerbroek H, van der Eijk AC, Longini M, Buonocore G, van Goudoever JB, Vermeulen MJ. No differences in clinical outcome or oxidative stress between an initial fraction of inspired oxygen of 30% and 65% during resuscitation of preterm infants after birth: a double-blind, randomized controlled trial. Submitted.
- Vlaardingerbroek H, Van den Akker CHP, Van der Schoor SRD, Van Goudoever JB. Amino acid homeostasis in the preterm infant. Nestle Nutr Workshop Ser. 2013; 74 (In press).

- Vlaardingerbroek H, Veldhorst MA, Spronk S, van den Akker CH, van Goudoever JB. Parenteral lipid administration to very-low-birth-weight infants-early introduction of lipids and use of new lipid emulsions: a systematic review andmeta-analysis. Am J Clin Nutr 2012;96(2):255-68.
- Vlaardingerbroek H, van den Akker CH, de Groof F, Hogewind-Schoonenboom JE, Huang L, Riedijk M, van der Schoor S, Huang Y, Van Goudoever JB. Amino acids for the neonate: search for the ideal dietary composition. Neoreviews 2011;12:506-16.
- van den Akker CH, Vlaardingerbroek H, van Goudoever JB. Nutritional support for extremely low-birth weight infants: abandoning catabolism in the neonatal intensive care unit. Curr Opin Clin Nutr Metab Care 2010;13:327-35.
- Vlaardingerbroek H, van Goudoever JB, van den Akker CH. Initial nutritional management of the preterm infant. Early Hum Dev 2009;85(11):691-5.
- Vlaardingerbroek H, Van Goudoever JB, Van den Akker CH. Safety and efficacy of early and high-dose parenteral amino acid administration to preterm infants. CAB Reviews 2009;4: 032, 1.
- Vlaardingerbroek H, van der Flier M, Borgstein JA, Lequin MH, van der Sluis IM. Fatal Aspergillus rhinosinusitis during induction chemotherapy in a child with acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2009;31(5):367-9.
- Vlaardingerbroek H, Hornstra G, de Koning TJ, Smeitink JA, Bakker HD, de Klerk HB, Rubio-Gozalbo ME. Essential polyunsaturated fatty acids in plasma and erythrocytes of children with inborn errors of amino acid metabolism. Mol Genet Metab 2006;88(2):159-65.
- Vlaardingerbroek H, Hornstra G. Essential fatty acids in erythrocyte phospholipids during pregnancy and at delivery in mothers and their neonates: comparison with plasma phospholipids. Prostaglandins, leukotrienes, and essential fatty acids 2004;71(6):363-74.

Book chapters

- Vlaardingerbroek H, Van Goudoever JB. Parenteral nutrition - Amino acids. In: Patole SK, ed. Nutrition for the preterm neonate, 2013 In Press.

Awards

- Young Investigator Award nominee, European Society for Pediatric Research, Annual meeting of the European Academy for Pediatric Societies, October 2012, Istanbul, Turkey.
- Society for Pediatric Research Student Research Award, Annual meeting of the Pediatric Academic Societies, May 2011, Denver (CO), USA.
- Young Investigator Exchange Program, International Pediatric Research Foundation, 2010
- Stipend of the Ter Meulen Fonds, Royal Netherlands Academy of Arts and Sciences, 2010
- Research Fellowship grant, SSWO, Rotterdam, The Netherlands, 2010.
- Travel grant for the annual conference of the Society for the Study of Inborn Errors of Metabolism, September 2002, Dublin, Ireland

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Curriculum Vitae

Hester Vlaardingerbroek was born on August 25th 1979 in Rijsbergen, The Netherlands. She passed her secondary school exam (gymnasium) at the Mencia de Mendoza Lyceum in Breda in 1997. In the same year she started studying Biological Health Sciences at Maastricht University. During her study she was involved in a longitudinal study on the fatty acid status in pregnant women and their offspring. She graduated in 2003 after completing a study on the fatty acid status of children with a disorder of amino acid metabolism (Prof.dr. G. Hornstra and dr. M.E. Rubio-Gozalbo).

In 2000 Hester started her medical training at the Maastricht University. During her training she was an intern at the neonatal intensive care unit of the Hospital el Milagro, Argentina. After obtaining her medical degree in 2006, she worked a few months at the pediatric and obstetric wards of the Alotau General Hospital, Papua New Guinea. After her return to The Netherlands, she was a resident at the Medium Care department of the Sophia Children's Hospital in Rotterdam. In 2007 she started her research fellowship at the department of Neonatology of the Erasmus MC – Sophia Children's Hospital in Rotterdam, The Netherlands (supervised by Prof.dr. J.B. van Goudoever, dr. M.J. Vermeulen, and dr. C.H.P. van den Akker), on the studies presented in this thesis. From May to November 2010 she performed part of her research fellowship in the Children's Nutrition Research Center of Baylor College of Medicine, Houston (TX), USA, under supervision of Prof.dr. D.B. Burrin and dr. B. Stoll. As of August 2012 she continued working as a resident at the Medium Care department of the Sophia Children's Hospital in Rotterdam. In January 2013 she started her training in Pediatrics (AIOS) at the AMC – Emma Children's Hospital in Amsterdam, The Netherlands (supervisors: Prof.dr. A.P. Bos and dr. D.K. Bosman). Hester lives in Rotterdam together with Ward Mouwen and their daughter Yentl.

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De leden van de kleine promotiecommissie: Prof.dr. Reiss, Prof.dr. Tibboel, en Prof.dr. Lafeber, dank voor het beoordelen van mijn proefschrift.
Prof.dr. Hokken-Koelega, Prof.dr. Van der Ploeg, Prof.dr. Sauer, bedankt voor het plaatsnemen in de grote commissie.

Dear Prof.dr. Burrin, dear Doug, when we met at Maaike Riedijk's thesis defense I was still unaware that I would be part of your lab one year later! Thank you for welcoming me in your research group and arranging my stay at Jane's place. I appreciated your efforts to make my stay as smooth and meaningful as possible.

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Dear Jane, you were my home and family in Houston. Thank you for your great hospitality, our nice talks, the many nights out, the Sunday ballets, the theater in the park, our road trip, and many more. We have a lot in common, except our age….

Mijn paranimfen: Nanda en Pauline. Lieve Nanda, eerst was je die onbekende AGNIKO die af en toe diensten deed op de neo, daarna collega onderzoeker en nu mijn paranimf! De vele Doppio's, onze supergeslaagde neo-retraite in Thorn, het laatste gezamelijke congres in Vancouver, de leuke verjaardagen, ik hoop dat er nog vele mooie momenten samen mogen volgen! Fijn dat je vandaag naast me wilt staan. Lieve Pauline, studiemaatje uit Maastricht. Ik noemde het al bij je bruiloft, we zijn elkaars spiegel. Gezondheidswetenschappen, geneeskunde, promoveren, werken in het Erasmus, mama worden, ik jouw ceremoniemeester, jij mijn paranimf. Ik hoop dat er nog vele gezellige borrels, heerlijke etentjes, gezellige middagen (wie weet straks wel in de speeltuin) zullen volgen. Fijn dat je mijn paranimf wilt zijn.

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wat ben je toch een heerlijke vrolijke meid. Na een lange dag is er niets zo fijn als thuiskomen bij die grote lach van jou!

PhD Portfolio

Summary of PhD training and teaching activities

DEPARTMENT

Pediatrics, division of Neonatology, Erasmus MC – Sophia Childen's Hospital

PHD PERIOD

October 2007 – July 2012

PROMOTOR

Prof.dr. J.B. van Goudoever

CO-PROMOTORS

Dr. C.H.P. van den Akker, Dr. M.J. Vermeulen

1. PhD training	Year	Workload (ECTS)
GENERAL COURSES		
Good medical Practice, Erasmus MC	2008	1.0
Classical Methods for Data-analysis (CC02), Erasmus MC	2009	5.7
Animal Science, Baylor College of Medicine, Houston, USA	2010	0.8
Biomedical English Writing and Communication, Erasmus MC	2011	4.0
SPECIFIC COURSES		
Biomedic Research Techniques, Erasmus MC	2008	0.1
Methodologie van patiëntgebonden onderzoek en voorbereiding van subsidieaanvragen, Erasmus MC	2010	0.2
Nutrition Summer School of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, Ameland, The Netherlands	2011	2.0
NATIONAL AND INTERNATIONAL CONFERENCES		
Annual meeting of the Pediatric Academic Societies, Hawaii, USA	2008	1.0
Annual meeting of the Benelux Association for Stable Isotope Scientists, Arnhem, The Netherlands	2008	0.4
Annual meeting of the European Society for Pediatric Research, Nice, France	2008	1.0
Annual meeting of the European Society for Pediatric Research, Hamburg, Germany	2009	1.0
Annual meeting of the Society for Pediatric Research, Vancouver, Canada	2010	1.0
Annual meeting of the Society for Pediatric Research, Denver, USA	2011	1.0
Annual meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, Sorrento, Italy	2011	1.0
Annual meeting of the European Academy of Paediatric Societies, Istanbul, Turkey	2012	1.0

SEMINARS AND WORKSHOPS

Annual PhD day, Erasmus MC	2008, 2009	0.2
Annual Pediatric Research day, Erasmus MC	2008-2010	0.3
Research meetings Moeder en Kind Centrum, Erasmus MC	2008-2011	0.2
Young Investigator meeting, Nederlandse Vereniging van Kindergeneeskunde, Veldhoven, The Netherlands	2009	0.1
Current issues in nutritional support for critically ill neonates and children, Erasmus MC	2010	0.1
Fellowdagen neonatologie	2011, 2012	1.0

Oral Presentations

Dutch Neonatology meeting, VUMC, Amsterdam	2008	1.0
Annual meeting of the European Society for Pediatric Research, Nice, France (poster symposium)	2008	1.0
Flamisch-Dutch Neonatology Meeting, Antwerpen, Belgium	2009	1.0
Annual meeting of the European Society for Pediatric Research, Hamburg, Germany	2009	1.0
Annual meeting of the Society for Pediatric Research, Vancouver, Canada	2010	1.0
Fellowdagen neonatologie, Groningen, The Netherlands	2011	1.0
Annual meeting of the Pediatric Academic Societies, Denver, USA (poster symposium)	2011	1.0
Annual meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, Sorrento, Italy	2011	1.0
Fellowdagen neonatologie, Utrecht, The Netherlands	2012	1.0
Annual meeting of the European Academy of Paediatric Societies, Istanbul, Turkey (oral and poster symposium)	2012	2.0

Poster Presentations

Annual meeting of the Pediatric Academic Societies, Hawaii, USA	2008	1.0
Annual meeting of the European Society for Pediatric Research, Nice, France	2008	1.0
Annual meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, Sorrento, Italy	2011	1.0
Annual meeting of the European Academy of Paediatric Societies, Istanbul, Turkey	2012	1.0

2. Teaching	Year	Workload (ECTS)
LECTURING		
Training nurses, Erasmus MC	2008-2012	0.5
Training residents and neonatologists, Erasmus MC	2008-2012	0.5
Darmfalen team Sophia Children's hospital	2008	0.1
Pharmacy, Erasmus MC	2008, 2012	0.2
Pediatricians, regional hospitals of Erasmus MC	2008-2011	0.2
SUPERVISING MASTER'S THESES		
Jacoline de Groot, medical student Erasmus MC	2009	0.5
Jorine Roelants, medical student Erasmus MC	2010	2.0
OTHER		
Peer review of articles for international scientific journals	2009-2012	0.5

Abbreviations

3-Cl-Tyr	3-chloro-tyrosine
3-N-Tyr	3-nitro-tyrosine
8OhdG/2dG	8-hydroxy-2'-deoxyguanosine/2-deoxyguanosine ratio
AA	Amino acid
ALT	Alanine aminotransferase
ARA	Arachidonic acid
ASR	Absolute synthesis rate
AST	Aspartate aminotransferase
BPD	Bronchopulmonary dysplasia
BSEP	Bile salt export pump
CDCA	Chenodeoxycholic acid
CRIB	Clinical risk index for babies
CYP27A1	Sterol 27-hydroxylase
CYP3A29	Cytochrome P450 3A29
CYP7A1	Cholesterol 7-hydroxylase
DHA	Docosahexaenoic acid
DMSO	Dimethyl sulfoxide
EFA	Essential fatty acid
ECF	Ethyl chloroformate
EN	Enteral
EPA	Eicosapentaenoic acid
FiO_2	Fraction of inspired oxygen
FSR	Fractional synthesis rate
FXR	Farnesoid X receptor
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GC/C/IRMS	Gas chromatograph combustion isotope ratio mass spectrometer
GC/MS	Gas chromatography-mass spectrometry
GGT	Gamma glutamyl transferase
GMP	Good manufacturing practice
GSH	Glutathione
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IgG-HRP	Horseradish Peroxidase-Conjugated Immunoglobulin G
IL	Intralipid
IRDS	Infant respiratory distress syndrome
IVH	Intraventricular hemorrhage
KH	Krebs-Henseleit
KIC	α-ketoisocaproic acid
LC/IRMS	Liquid chromatography isotope ratio mass spectrometry
LCPUFA	Long chain polyunsaturated fatty acid
Leu	Leucine
MCT	Medium chain triacylglycerol
MPE	Mole percent excess

NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
NOD	Non-oxidative disposal
NOLD	Non-oxidative leucine disposal
NTCP	Na^+/taurocholate cotransporter
OBCA	Obeticholic acid
OSTα/ß	Organic solute transporters alpha and beta
o-Tyr/Phe	o-Tyrosine/phenylalanine ratio
OV	Omegaven
PBS	Phosphate-buffered saline
PDA	Patent ductus arteriosus
Phe	Phenylalanine
PN	Parenteral nutrition
PNALD	Parenteral nutrition-associated liver disease
PUFA	Polyunsaturated fatty acid
PVL	Periventricular leukomalacia
Q	Flux
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RBC	Red blood cell
RCT	Randomized controlled trial
ROP	Retinopathy of prematurity
ROS	Reactive oxygen species
RP	Rate of amino acid release from protein
S	Rate of utilization of amino acid for protein synthesis
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SGA	Small for gestational age
SHP	Small heterodimer partner
SL	SMOFlipid
TAP	Total antioxidant potential
TBS	Tris-buffered saline
TG	Triacylglycerol
TPN	Total parenteral nutrition
TTR	Tracer-tracee-ratios
UDCA	Ursodeoxycholic acid
UPLC/MS/MS	Ultra-performance liquid chromatography coupled to tandem mass spectrometry
VLBW	Very low birth weight