Status of fat-soluble vitamins in breast-fed preterm infants

Evaluation of a modified vitamin A supplementation regimen

Anne Karin Aurvåg

Department of Pediatrics, Akershus University Hospital

Master thesis

Department of Nutrition, Medical Faculty

UNIVERSITY OF OSLO

February 2007

Table of contents

4

Preface

This master of "Status of fat-soluble vitamins in breast-fed preterm infants – Evaluation of a modified vitamin A supplementation regimen" is part of the study "Nutrition, growth and development among very low birth weight infants" headed by Ph.D.-student Christine Henriksen at the Department of Nutrition at the University of Oslo. After being introduced to the results of the pilot study (1) about preterm infants and status of fat-soluble vitamins, I was inspired to make this subject my master degree. How to optimally nourish and supply very low birth weight infants with vitamins is an important and challenging question. These last three years since the inclusion of the first patient started, have provided me with valuable insight and overview and new knowledge in this field. This has resulted in a strong wish to continue the work in the clinical setting focusing on optimalization of nutrition to this very vulnerable group.

Thanks to:

First and foremost my supervisors, Prof. Dr. Med. Britt Nakstad and Prof. Dr. Med. Per Ole Iversen, who introduced me to the scientific arena, provided me with advice and response along the way and were a great help all the way.

Christine Henriksen, who with great enthusiasm have introduced me to this part of the clinical nutrition discipline. Thank you for always taking time to help!

Elin Bjørge Løken for helping with the establishment of the database with nutritional products in use for the preterm infant, and master student/clinical nutritionist Ane Cecilie Westerberg for cooperating in collecting data and looking through the journals.

Pernille, for an interesting discussion many years ago in the neonatal intensive care unit at Hedmark Central Hospital on energy needs in these small infants.

All patients and their parents that accepted to join the study, with the result that knowledge about this topic has been greatly increased.

And last but not least to my dear husband Svein Christian for looking after our children so that I finally could finish this work.

Fetsund, February 2007   Anne Karin Aurvåg

Summary

Objective: Preterm infants usually have low retinol status. We have therefore evaluated a new protocol designed to improve vitamin A status in very low birth weight infants (VLBW, birth weight ≤ 1,500 g). Since the new protocol also changed the supplementation of vitamins D, E and K, their plasma concentrations were also analyzed to characterize the infants' status of all fat-soluble vitamins.

Design: An open intervention trial was conducted where vitamin A was given in a human milk fortifier. The daily intake of vitamin A was increased by 8% compared to the previous regimen (reference), and given mixed with human milk instead of as a bolus. In this modified regimen, the intake of the other fat-soluble vitamins also changed due to different vitamin content in the supplementation used. Vitamin D intake was reduced by 10%; vitamin E intake was reduced by 6% in the modified protocol and vitamin K intake was increased by 132% compared to the reference regimen. Blood samples were collected at inclusion and at discharge from hospital. The plasma concentrations of the different vitamins were analyzed with high performance liquid chromatography. The daily intake of all the fat-soluble vitamins and their plasma concentrations were compared to the vitamin protocol normally used in Norwegian hospitals.

Results: Sixty VLBW infants were included and 53 completed the study. At discharge from hospital, the reference group had lower plasma retinol concentrations compared to the modified group (0.30 vs. 0.45 µM, P=0.005). Fewer infants in the modified group had plasma retinol levels below 0.35 µM (indicating reduced hepatic stores) compared to the infants in the reference group (44% vs. 69%, $P = 0.04$). At discharge the reference group had higher plasma 25(OH)vitamin D than the modified group (171 vs. 110 µM, P=0.003). The plasma concentrations of α-tocopherol were not significantly different between the two groups (31 vs. 39 µM) at discharge from hospital. The plasma phylloquinone concentration was non-significantly lower in the reference group compared to the modified group (1.0 vs. 2.1 ng/ml).

8

Conclusions: The modified regimen improved vitamin A status among VLBW infants at discharge compared to the reference protocol. More studies are needed to establish the optimal plasma retinal concentration for preterm infants and how this can be achieved. Whether this will translate into lower vitamin A-related morbidity and/or mortality in these children warrants further investigation. Vitamin D status was also improved in the modified group by giving a plasma concentration in the normal range compared to the high mean level in the reference group. Vitamin E status was similar in the two groups and vitamin K status was not significantly improved.

Abbrevations

AGA	adequate for gestational age
AAP	American Academy of Pediatrics
BPD	bronchopulmonary dysplasia
CLD	chronic lung disease
ELBW	extremely low birth weight
EN	enteral nutrition
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
GA	gestational age
IUGR	intrauterine growth retardation
IVH	intraventricular haemorrhage
LBW	low birth weight
NCPAP	nasal continuous positive airway pressure
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
PN	parenteral nutrition
RDS	respiratory distress syndrome
ROP	retinopathy of prematurity
SGA	small for gestational age
VLBW	very low birth weight
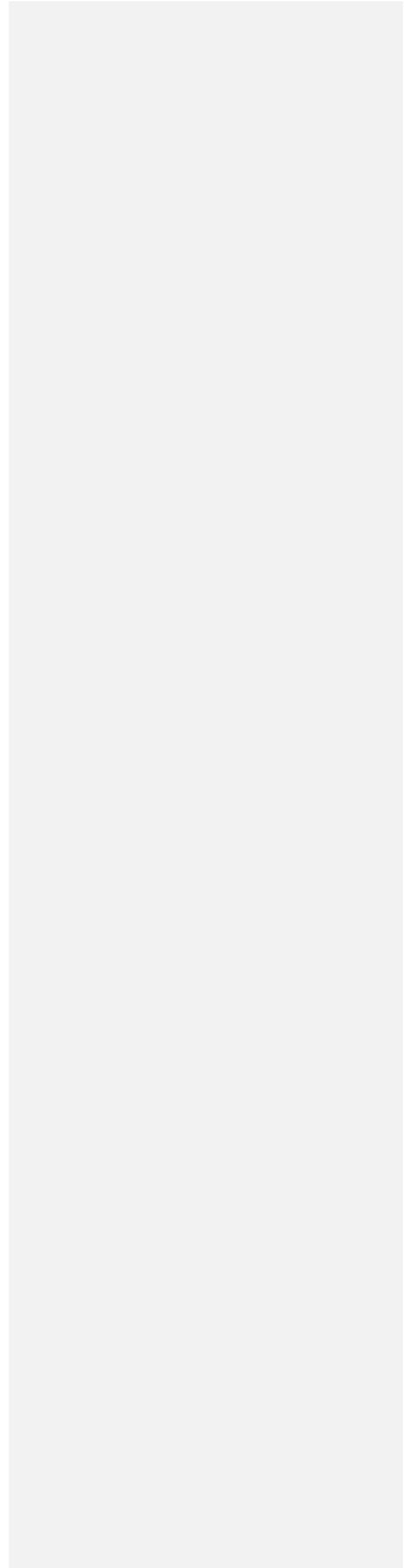
1. Introduction

1.1 Definition of preterm infants

Infants born prior to 36 weeks of completed gestation are called preterm infants (2). These infants can be categorized in groups based on birth weight. Low birth weight infants (LBW) have a birth weight less than 2500 g. Infants with birth weight below 1500 g are termed very low birth weight (VLBW), whereas those with birth weight less than 1000 g are termed extremely low birth weight (ELBW). Infants born smaller than expected for their gestational age (GA) are often denoted "small for gestational age" (SGA) and "intrauterine growth retarded" (IUGR). SGA infants are below the 10th or 3rd percentile for weight at birth, depending on source of definition (2). This is probably a heterogeneous group containing both the infants who are born small due to genetic disposition and the infants who are IUGR because of lack of nutrient supply according to their needs. The prevalence of IUGR is much higher among preterm infants than among term infants. Early nutritional intervention is important for later outcomes in these infants, as nutrition plays a key role in the development of multiple organ systems. The problem in managing this nutrient intervention is an immature or dysfunctional gastrointestinal tract and poor tolerance of parenteral nutrition (3;4).

1.2 Prevalence of preterm birth

Approximately 850 very low birth weight infants (<1500 g) are born in Norway every year. This accounts for approximately 1.5 % of the total number of births which amounts to nearly 60 000. In the last 30 years survival of premature children with birth weight below 1500 g has risen because of more knowledge about treatment during pregnancy and after birth.

1.3 Survival and clinical conditions

The incidence and severity of all complications of prematurity are roughly inversely related to gestation and birth weight. Adequate nutrition during the first days after birth is probably of vital importance for optimal growth and further development. Early nutritional deficits are linked to impairments of intellectual performance, reduced work capacity and elevated risk of coronary heart disease and metabolic syndrome later in life (5).

Although apnoea is associated with immaturity of the respiratory control system, it may also be the presenting sign of other diseases or pathophysiological conditions that affect preterm infants. Apnoea is defined as the cessation of pulmonary airflow for a specific time interval, usually longer than 10 to 20 seconds. Bradycardia often accompanies prolonged apnoea. Apnoea is divided into two groups, central and obstructive apnoea. Central apnoea refers to a complete cessation of airflow and respiratory efforts with no chest wall movements, causing a lack of activity in the respiratory control centre. In obstructive apnoea, no airflow is exhibited, but the chest wall movements continue. A combination of these two events, mixed apnoeas, is the most frequent type seen in preterm infants. The incidence of apnoea increases as gestational age decreases (6;7).

Immaturity of the respiratory system with surfactant deficiency results in respiratory distress syndrome (RDS). This disorder is also called hyaline membrane disease or surfactant deficiency syndrome and is treated with oxygen, N-CPAP (nasal continuous positive airway pressure), surfactant administration and mechanical ventilation (8).

Chronic lung disease (CLD) was earlier named bronchopulmonary dysplasia (BPD). This is usually defined as the need for supplemental oxygen at 36 weeks postmenstrual age. It results from a combination of lung immaturity, oxygen toxicity, inflammatory and free radical mediated lung injury. Babies with CLD may require

supplemental oxygen for months and are at increased risk of respiratory infections
(7;8).

Periventricular or intraventricular haemorrhage (IVH) is due to bleeding from the
immature rich capillary bed of the germinal matrix lining (7) the central ventricles.
Risk factors include asphyxia and changes in cerebral blood flow due to hypertension
or rapid intravenous fluid infusion. It is graded by severity from grade I to grade IV.
While lower grades of IVH have a good prognosis, grades III and IV are often
associated with hydrocephalus and neurological abnormalities, such as cerebral palsy,
and higher mortality (7;8).

Severe neurodevelopmental impairments like cerebral palsy, mental retardation,
blindness and deafness occur in 10-15 % of VLBW infants. More subtle delays in
language, attention deficits and social and behavioural difficulties are common.
Eating disorders and growth impairments are also more often seen in this group (7;8).

Retinopathy of prematurity (ROP) results from a disruption of the normal process of
vascularisation of the retina, which in effect leads to new vessel formation and
fibrous scarring. Although ROP can result from excessive oxygen exposure most
cases occur in ELBW infants with multiple other problems. Severity is classified on
the basis of location and extent of ROP from grade 1 to grade 4. Most mild ROP
regresses spontaneously, but eye examinations are required to detect progressive
ROP, which requires therapy to reduce the chances of blindness (9).

Hypoglycaemia is common in the preterm infant due to decreased glycogen stores
and increased glucose requirements. Hyperglycaemia can also occur in VLBW
infants, due to high glucose infusion rates, reduced insulin secretion and impaired
insulin sensitivity (8).

From 25 weeks GA the gastrointestinal tract is structurally ready and can absorb
nutrients, but due to immature gut motility and delayed gastric emptying feeding
problems are frequent. Thus, feed intolerance and gastro-oesophageal reflux are
common (10). There is evidence that rapid advancement of enteral feeds is associated

with increased risk of necrotising enterocolitis (NEC) (11;12). This is due to an inflammatory process in the bowel wall that may lead to bowel necrosis. Alterations in gut blood flow, hypotension, hypoxia, infection and the way of feeding have all been implicated, but their exact contribution remains unclear (8). A systematic review concluded that early introduction of enteral feeding shortens the time to full feeds as well as the length of hospitalisation without an increase in the rate of NEC (13). Breast milk has been demonstrated to have a protective effect against NEC (14).

1.4 Nutrition

1.4.1 Growth

At birth the preterm infant is in a phase of extreme growth. Normally there is a period of about a week before the infant has reached its initial birth weight. Among ELBW infants, this initial weight loss can be approximately 15-20 % of the birth weight (15). Weight gain is only possible after this first period (16) and when the energy intake exceeds energy expenditure. The initial weight loss seen in preterm infants is by some experts thought to be a catabolic state, caused by low nutrient supply in addition to changes in fluid balance (15;17). Ziegler et al and others believe that the growth retardation often seen in preterm infants is due to malnutrition (17).

Postnatal growth restriction is common among preterm infants and more infants may be defined as SGA at the time of discharge from hospital than at time of birth (18). This indicates that the nutrient requirements have not been adequately met during hospital stay.

Studies indicate that the growth restriction starts at hospital, lasts through all childhood and may persist until age 4 to 7 years before these infants regain their lost prenatal growth (19). A Norwegian study showed that preterm infants with birth

weight less than 2 kg, by the time they were 5 and 11 years had lower weight and were shorter compared to children with normal birth weight (20).

Determining nutritional requirements in preterm infants requires an agreed-upon reference standard. The most commonly applied and accepted standard is that of intrauterine growth (16). However, this standard is rarely achieved in clinical practice, either in terms of growth rate or body composition (21). Nevertheless, intrauterine growth remains an ideal goal.

1.4.2 Nutritional requirements

At birth preterm infants have limited stores of many nutrients as accumulation from the mother to the fetus (accretion) occurs predominantly in the last trimester (2;7). They are poorly equipped to withstand inadequate nutrition as the endogenous reserves of an infant with a birth weight of 1000 g are theoretically only sufficient for four days if unfed (22). From continuous intravenous nutrient supply via the umbilical cord, the infant must adjust to exogenous supply of nutrients from the gastrointestinal tract and endogenous production of energy yielding nutrients in between meals (7).

Because of the small volume of the stomach and low tolerance of enteral feeds, it is generally accepted that the VLBW infant needs parenteral nutritional supplementation while enteral feeds are gradually increased (2).

Immature metabolic responses and limited stores of nutrients, in addition to other complications, make nutrition to this group important and complicated (23).

1.4.3 Macronutrients

In an article, Ziegler described the body composition of a reference fetus using literature reports of whole body chemical analysis of fetuses born prematurely (24). This report is still the accepted representation of human fetal nutrient accretion. The rate of fetal nutrient accretion and weight gain change throughout gestation. Fetal

energy accretion is 24 kcal/kg/day between 24 and 28 weeks, and increases to 28 kcal/kg/day through the rest of the gestation. An energy balance of 25-30 kcal/kg/day represents an achievable goal for premature infants. This accumulation is specific to a rate of protein and fat accretion. Protein accretion is 2 g/kg/day in the beginning of the third trimester and declines slightly. Fat accretion increases throughout the third trimester. Early gestation is therefore characterized by accumulation of lean tissue, while more fat and less lean tissue is accreted in late gestation. Because fat tissue is energy dense compared to lean tissue, the rate of weight gain is reduced from 18 g/kg/day at 24-28 weeks to 16 g/kg/day at 32-36 weeks.

To achieve this optimal accretion of nutrients and weight gain, it is essential to ensure adequate intake of macronutrients. The energy need of the preterm infant is approximately 105-130 kcal/kg/day and the protein need is approximately 3.5-4.0 g/kg/day (16). The daily protein accretion at around 2 g/kg/day in the third trimester is probably achieved if the protein intakes are accompanied by at least 110 kcal/kg/day enterally or 80 non-protein kcal/kg parenterally (21). Early administration of protein are associated with higher weight gain (25).

As fat is energy dense, it is the preferred source of energy, but there is a risk of malabsorption among VLBW infants, due to low pancreatic lipase, low bile salt pools and possible reduced activity of lingual lipase. Human milk that is not pasteurised is better tolerated than heat-treated human milk because it contains some bile salt stimulated lipase (26). Medium chain triglycerides are often used as an energy source for the preterm infant because of theoretically improved fat absorption. An advantage with respect to fat or nitrogen balance, when using this energy source has not, however, been demonstrated (27;28). The risk of developing essential fatty acid deficiency is great in the VLBW and ELBW infants. It is thus necessary to provide enough linoleic and linolenic acid to cover the need for essential fatty acids (29).

1.4.4 Parenteral nutrition

Most of the VLBW and ELBW preterm infants will receive parenteral nutrition for at least the first days after birth. The administration of parenteral nutrients is usually administered together with minimal enteral feeding of human milk (30). Given parenterally, approximately 120 kcal/kg/day and 3.5 g protein/kg/day are needed to ensure intrauterine growth and positive nitrogen balance (31). Early parenteral administration of protein as amino acids is associated with better growth parameters at 36 weeks of gestation and fewer infants with suboptimal head circumference at 18 months (25). Hyperglycaemia, hyperlipidemia and metabolic acidosis are metabolic complications seen in sick preterm infants when administering parenteral nutrition (32), and this is probably the reason why many are reluctant to increase parenteral infusions to reach optimal levels of nutrient accretion.

1.4.5 Enteral nutrition

Minimal enteral feeding is started as early as possible after birth. If available, the mothers own milk is used; alternatively donor milk (human milk collected from other lactating women) is used. Neither of these milk-alternatives is pasteurised in NICUs in Norway. The advantages of early and minimal enteral feeding have been shown (2). The use of human milk has advantages such as improved feed tolerance (33), reduced risk of NEC (14), reduced risk of sepsis (14;34) and long-term neurodevelopmental advantages (35-37). Specific bioactive factors, such as Ig A, lactoferrin, lysozyme, oligosaccharides, nucleotides, growth factors, enzymes and cellular components are all represented in human milk and will have a positive effect in the gut of the preterm infant. Some of the factors can enhance the development of the gut flora and some help in the maturation of the gut (7).

Normally human milk is supplemented with protein, minerals and vitamins when the preterm infant has achieved an intake of a certain volume of milk per kg. The different NICUs in Norway differ in which volume of milk is considered to be the appropriate milk volume for starting supplementation. The time and volume

appropriate to start supplementation also depends on the toleration of the enriched and hyper osmotic nutrition. Many of the VLBW infants receive enteral feed of approximately 18% of their weight, and this volume of 180 ml/kg is often considered full milk volume. Some NICUs do not enrich the human milk with proteins etc. before full milk volume is achieved, but in our department a supplementation is often started if considered possible at 150 ml/kg. Increasing the volume instead of supplementing the milk is a possibility to give the preterm infant more energy and nutrients. This may be possible in the LBW infant, but the VLBW and ELBW infants need supplemented milk. On the marked, there are different commercially produced human milk fortifiers, which mainly supply extra protein and minerals to the milk. The energy content of the milk will also be higher with this supplementation.

1.5 Fat-soluble vitamins

Vitamin A, D, E and K are fat-soluble vitamins. The premature infant need, as do all fetuses and term infants eventually, to receive supplementation of these vitamins in order to achieve normal development. Metabolites of the fat-soluble vitamins fulfil a multitude of functions essential for life.

In the last trimester of the pregnancy, an accumulation in the fetus of fat-soluble vitamins occurs. This explains the fact shown in the literature that premature infants at birth have lower plasma levels and lower reserves of fat-soluble vitamins than full-born infants. Plasma levels of vitamin K are low both in term-born and VLBW infants. There is little knowledge about the status of fat-soluble vitamins in the prematurely born infant fed human milk, but one recent study showed great differences in plasma levels between VLBW and term-born infants (1). Human milk contains only small amounts of fat-soluble vitamins. It is therefore standard procedure to supply these vitamins to the preterm infant.

1.5.1 Vitamin A

Vitamin A is an important micronutrient affecting infant health. Retinol, retinaldehyde and retinoic acid are compounds referred by the term vitamin A. Retinol may be obtained directly from foods of animal origin, be formed in the body from metabolism of β-carotene or supplied from a supplement as retinyl esters. Absorption of retinyl esters involves hydrolysis and complexation with bile acids in the gut lumen before uptake by enterocytes (21). The availability of a specific carrier protein, cellular retinol binding protein type 2 (RBP), may be limited in the preterm infant and it is essential for the metabolism of vitamin A within these cells and transfer into the lymphatic system (38). After absorption, retinol is bound to RBP in the liver and transported in plasma as the retinol-RBP complex. Circulating retinol is delivered to target tissues via a specific membrane receptor and is oxidised within the cell to its active metabolite, retinoic acid (39). The precise mechanisms by which retinoic acid affects intracellular activity are complex and incompletely defined (40;41).

Metabolites of vitamin A are involved in the regulation of growth and differentiation of various types of cells, including skin and epithelial cells of the respiratory tract (42). Moreover, vitamin A is required for the formation of the photosensitive visual pigment in the retina, and for normal reproductive and immune functions (42). Consequently, deficiency of vitamin A may predispose preterm infants to chronic lung disease and xerophtalmia and increase the risk for infections (43).

In developed countries, most infants are provided with sufficient vitamin A supplies. Term infants receive adequate levels of vitamin A in utero, and human milk contains sufficient amounts of vitamin A to maintain normal growth and development during their first 6 months (44). In contrast, preterm infants have low plasma concentrations of retinol and retinol binding protein at birth, reflecting low hepatic stores (44). Thus, preterm infants need supplementation (45), but controversy exists regarding the optimal dose and way of administration.

The plasma concentration of retinol is a commonly used biochemical marker of vitamin A status. Plasma retinol concentrations less than 0.70 µM indicate biochemical vitamin A deficiency (44), whereas levels below 0.35 µM reflect reduced hepatic stores and clinical signs of deficiency in infants older than six months (46). Low plasma retinol concentration is associated with an increased risk for developing chronic lung disease, with an odds ratio of 2.04 for each 0.25 µM reduction in plasma retinol (47). The plasma reference level according to the laboratory that analyzed the blood samples in this study, Vitas AS, is above 0.7 µM for both children and adults.

The purpose of nutrition therapy in preterm infants is to approximate intrauterine accretion ratio (16). Human milk is the preferred nutritional source in enteral feeding in preterm infants (16), but the content of vitamin A in human milk is too low to meet the high requirement among these infants. The vitamin A concentration of human milk varies according to several factors, but preterm milk has higher vitamin A content compared to term milk after the first week of lactation. At approximately 35 days postpartum age, the vitamin A content of preterm milk ranges from 83 to 100 µg per 100 ml according to Tsang et al (21). This differs somewhat from the vitamin A concentration in human milk samples from this current study, 54 µg per 100 ml. Vitamin A is usually given as a parenteral or enteral supplement, but the dosages for both parenteral and enteral vitamin A supplements vary markedly between paediatric institutions (2;16). The recommendations for vitamin A intake for preterm infants range from 30 to 1000 µg/day (16;21;48). It was reported that VLBW infants, given the reference Norwegian supplementation of vitamin A (750 µg/day), had a plasma retinol concentrations at discharge from hospital of 0.30 µM, indicating vitamin A deficiency (1). In contrast, the plasma concentration of retinol among term infants was 0.72 µM (1). Possible explanations for the observed low plasma retinol concentrations among the VLBW infants include the dose of vitamin A supplement, impaired intestinal absorption or subsequent transport and/or metabolism of vitamin A.

The results of a randomized trial and a systematic Cochrane review show that intramuscular vitamin A injection may reduce chronic lung disease and death in VLBW infants (49;50). This may suggest that an insufficient absorption of vitamin A is a likely explanation for the low plasma retinol levels found in VLBW infants. However, this route of administration is not implemented in most institutions, probably because the reduced morbidity and mortality obtained with repeated intramuscular injections must be weighed against the acceptability and tolerance of such treatment in VLBW infants (51). Hence there is a need to improve enteral vitamin A supplementation.

1.5.2 Vitamin D

There are different vitamin D metabolites (52). Exposure of the skin to ultraviolet light catalyses the synthesis of vitamin D_3 (cholecalciferol) from 7-dehydrocholesterol. The other major form of vitamin, D_2 (ergocalsiferol), is the product of the ultraviolet light-induced conversion of ergosterol in plants. Dietary vitamin D is readily absorbed from the small intestine and transported in chylomicrons to the liver where conversion to 25-hydroxy vitamin D (25(OH)vitamin D) takes place. 25(OH)vitamin D is further hydroxylated in the kidney to yield 1,25-dihydroxy vitamin D (1,25(OH)$_2$vitamin D) and 24,25-dihydroxy vitamin D (24,25(OH)$_2$vitamin D). 1,25(OH)$_2$vitamin D is the hormonal circulating and biologically active metabolite of vitamin D. In addition to ensuring adequate absorption of calcium, 1,25(OH)$_2$vitamin D contributes to plasma calcium regulation by increasing bone resorption synergistically with parathyroid hormone and stimulating the reabsorption of calcium by the kidney. Vitamin D is thus essential for the proper formation of the skeleton and for mineral homeostasis. Hypocalcemia and increased parathyroid hormone secretion induce synthesis of 1,25(OH)$_2$vitamin D after birth in both term-born and preterm neonates. Nevertheless, serum concentrations of 25(OH)vitamin D are a rate-limiting factor in the synthesis of 1,25(OH)$_2$vitamin D (53). A recent publication demonstrats a possible protective effect of vitamin D against development of multiple sclerosis (54). The vitamin D

requirement of humans can be met if their skin is exposed to a sufficient amount of sunlight or artificial ultraviolet light radiation. Obviously, such light exposure is not possible in the VLBW infants, and vitamin D must be supplemented.

Measurement of plasma concentration of 25(OH)vitamin D is the commonly used biochemical marker of vitamin D status. Among infants, plasma 25(OH)vitamin D concentration ranges from approximately 62 to 75 nM (55), whereas the reference range for children according to the analysing laboratory, Vitas AS, is between 25 to 130 nM and for adults the reference ranges from 50 to 150 nM.

Both in term and preterm newborn, 25(OH)vitamin D concentrations in cord blood are lower than 25(OH)vitamin D concentrations found in maternal blood. Newborn blood concentrations are correlated with blood concentrations in the mother (56). Maternal vitamin D deficiency will reduce the transplacental transfer to the fetus of 25(OH)vitamin D, resulting in lower stores in the infants at birth (57). Preterm infants are born with plasma concentrations of 25(OH)vitamin D that are 20-30% lower than their mother's plasma 25(OH)vitamin D (57). The vitamin D requirements of preterm infants are influenced by the body stores at birth, which in turn are related to the length of gestation and maternal stores. It is shown that in infants born after 28 wk of gestation, activation of vitamin D is operative as early as 24 h after birth. Thus, vitamin D supplementation just after birth improves vitamin D nutritional status as evidenced by rising plasma 25(OH)vitamin D concentrations (53).

Controversies exist regarding the ideal supplementation of vitamin D to the preterm infant. The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (48) recommends a supplementation of 25 μg/day, while the American Academy of Pediatrics (AAP) (16) finds no evidence to supply the preterm infant with more than 10 μg/kg/day. Koo et al (58) measured plasma 25(OH)vitamin D concentrations in preterm infants receiving 5, 10 or 20 μg/day. They showed that plasma 25(OH)vitamin D remained normal for 6 months while infants received less than 10 μg /day. Further, Porcelli et al (59) substantiates the adequacy of vitamin D intakes in the range of 5 to10 μg /day for preterm infants.

Hypercalcemia due to vitamin D intoxication always accompanies 25(OH)vitamin D concentrations above 220 nM (60). A study from Japan (61) showed no hypercalcemia in VLBW infants on high intakes of supplemented vitamin D from formula (median intake 25 μg/day, 9-68 μg/day) with a plasma 25(OH)vitamin D concentration of 175 nM. The risk for hypercalcemia because of raised plasma 25(OH)vitamin D concentrations is probably a reason for the study Backstrøm et al preformed on preterm infants (62). They showed that a dose of vitamin D of 5 μg/kg up to a maximum of 10 μg/day maintained normal vitamin D status and as good a bone mineral accretion as the recommended higher dose in Europe of 25 μg/day.

1.5.3 Vitamin E

The term vitamin E refers to eight naturally occurring compounds with characteristic biological activities. Although the biological activities of the different vitamin E compounds vary, they all show antioxidant capability with the ability to protect cellular membranes from oxidative destruction initiated at the molecular level by lipid peroxidation (63). The most important group, the tocopherols, is characterized by a ring system and a long, saturated side chain. There are four members of this group: the α-, β-, γ- and δ-tocopherols, which differ only in the number and position of methyl groups in the ring. The second group, the tocotrienols, differs from the tocopherols by having an unsaturated side chain. The most active form of vitamin E, α-tocopherol, is also the most widely distributed in nature (64). α-Tocopherol may be synthesized and is then called all-rac-α-tocopherol as the natural form is called RRR-α-tocopherol. The supplement of vitamin E to the preterm infant comes as all-rac-α-tocopherol bound to acetate (65).

Although the biologic roles of vitamin E are numerous, they are poorly understood. To efficiently act as a radical scavenger α-tocopherol must be located in membrane sites well exposed to reactive oxygen species (66). While its hydrophobic character favours its integration into the bi-layer cell membrane, the conjugated isoprene side-chain gives it an antioxidant property (67). It is also suggested that during vitamin E

deficiency states, the degeneration of the central nervous system that precedes the fall of plasma vitamin E concentration, may be linked to impaired neuronal protection against lipid peroxidation (67).

The primary signs of vitamin E deficiency are reproductive failure, muscular dystrophy and neurological abnormalities. Among preterm infants vitamin E deficiency-induced haemolytic anaemia has been reported. This syndrome has been associated with the use of formulas that contain high levels of PUFAs with inadequate vitamin E while providing supplemental iron, which functions as an oxidant.

The requirement for vitamin E increases when the intake of polyunsaturated fatty acids (PUFA) increases. Tsang et al (21) recommend an intake of at least 0.68 mg vitamin E/g linoleic acid in preterm infants. Absorption of α-tocopherol is variable, ranging from 20 to 80 % in various studies. Normal bile secretion and normal pancreatic function are essential for tocopherol absorption. Because of preterm infants' low body stores of tocopherol, their reduced intestinal absorption and the relatively greater growth rates associated with prematurity, it is difficult for these infants to achieve and maintain normal vitamin E status. Since the preterm infants are born with low stores of tocopherol in addition to decreased blood concentrations, early provision is necessary to correct the depleted state and prevent adverse consequences attributable to insufficient antioxidants. In studies of enteral nutrition, it has been shown that a daily dose of 10-25 mg of α-tocopherol-acetate given to 0.6-1.5 kg neonates may be required to produce and maintain normal vitamin E status (68). Oral supplementation of 17 mg of vitamin E per day may be required by preterm infants up to 3 months of age (21). Compared to other fat-soluble vitamins, vitamin E is relatively non-toxic when taken orally. The recommend intake of vitamin E for preterm infants vary from 5 to 17 mg/day (16;21). Treating VLBW infants with high dose vitamin E therapy has been proposed for preventing or limiting retinopathy of prematurity (ROP), intracranial haemorrhage, haemolytic anaemia and chronic lung disease (CLD) (69;70). A Cochrane report (71) concludes that this

therapy reduced the risk of intracranial haemorrhage and severe retinopathy, but increased the risk of sepsis. The conclusion is that evidence does not support the routine use of vitamin E supplementation by intravenous route at high doses, or aiming at serum levels greater than 81 μM. Doses of vitamin E exceeding 3.5 mg/kg/day by the parenteral route or 25 mg/kg/day by the enteral route are considered pharmacological and should thus be regarded as experimental (45).

Plasma concentration of α-tocopherol is commonly used to evaluate vitamin E status and a concentration of at least 12 μM indicates adequate nutritional status according to Farrell et al (72). The reference range for the plasma concentrations from Vitas AS is 16 to 36 μM for both adults and children.

1.5.4 Vitamin K

Vitamin K exists in two forms: 1. Vitamin K_1 or phylloquinone which is the plant form, and 2. Vitamin K_2, a series of compounds with unsaturated side chains, synthesized by bacteria and referred to as menaquinones. Animal tissue contains both phylloquinone and menaquinones (73).

Compounds with vitamin K activity are essential for the formation of prothrombin and for at least five other proteins involved in the regulation of blood clotting. Although vitamin K is also required for the biosynthesis of some other proteins found in the plasma, bone and kidney, defective coagulation of the blood is the only major sign of vitamin K deficiency (73).

Vitamin K_1 has been reported to be present in low to undetectable concentrations in cord blood. There is no correlation between maternal and cord blood levels, and only very small amounts of vitamin K cross the placenta from mother to fetus (74).

Under normal conditions, vitamin K is moderately well absorbed from the jejunum and ileum. Similar to all fat-soluble vitamins, vitamin K is absorbed from the intestine into the lymphatic system, requiring the presence of both bile salts and pancreatic secretions. Most of the bacteria comprising the normal intestinal flora of

human milk fed infants do not produce menaquinones, including Bifidobacterium, Lactobacillus and Clostridium species. Bacteria that produce menaquinones include Bacteroides fragilis and Escherichia coli, which are more common in formula fed infants (74).

The newborn infant has low plasma prothrombin levels. Because human milk contains low levels of vitamin K (2 μg/l) and the intestinal flora are limited, exclusively breastfed infants who do not receive vitamin K prophylaxis at birth are at risk of developing fatal intracranial haemorrhage secondary to vitamin K deficiency.

Vitamin K is routinely administered in large quantities at the time of birth. This routine prevents vitamin K deficiency bleeding (VKDB). This condition is most commonly seen in exclusively breastfed infants. VKDB comprises early (0-24 h), classic (1-7 d) and late (2-12 wk) syndromes according to the time of presentation (75). An intramuscular injection of vitamin K is routinely provided after birth, but in some places oral solutions are administered (76). Such prophylaxis is still uncommon in many parts of the world today and even its use in Europe varies widely from country to country as well as within countries (77). In preterm infants who weigh more than 1 kg at birth, the standard dose of 1 mg of phylloquinone is appropriate and among infants less than 1 kg, a dose of 0.3 mg/kg of phylloquinone is recommended (16). Exclusively breast-fed preterm infants probably receive inadequate dietary vitamin K and may require further supplementation to protect against late-onset VKDB (78).

Formulas for preterm infants provide sufficient vitamin K to meet daily needs thereafter. Lactating mothers easily achieve the recommended dietary allowance for vitamin K and the breast milk concentration is readily increased by increasing maternal vitamin K intake (77). The use of human milk fortifiers that contain supplemental vitamins will provide the additional vitamin K needed to meet the recommended intake. For infants fed human milk, vitamin supplements of A, D and E are readily available as oral solutions. None of these contain vitamin K. If preterm

infants are discharged on standard term infant formulas, they may not receive the recommended amounts of this vitamin.

Recommended intake of vitamin K for preterm infants varies from 2-3 μg/kg/day (48) to 7-9 μg/kg/day (16;21), but an intake as high as 100 μg/kg/day (79) has also been suggested. In addition, the recommendation involves prophylaxis at birth ranging from 0.1 mg to 1 mg intramuscularly or intravenously (80).

The assessment of vitamin K status is dependent on the concentrations of different vitamin K dependent clotting factors. In the neonate these are 25-75% of normal adult concentrations and there is little difference at the time of birth between 30- and 40-weeks-gestational age infants (81). Injections of vitamin K do not significantly alter the measurements of these clotting factors. Thus, the difference in coagulation between adults and newborns cannot totally be ascribed to vitamin K deficiency (82) and the coagulation differences may be limited by the availability of precursor proteins rather than the availability of vitamin K. The reference range for plasma concentration of phylloquinone from Vitas AS is 0.1 to 2.2 ng/ml.
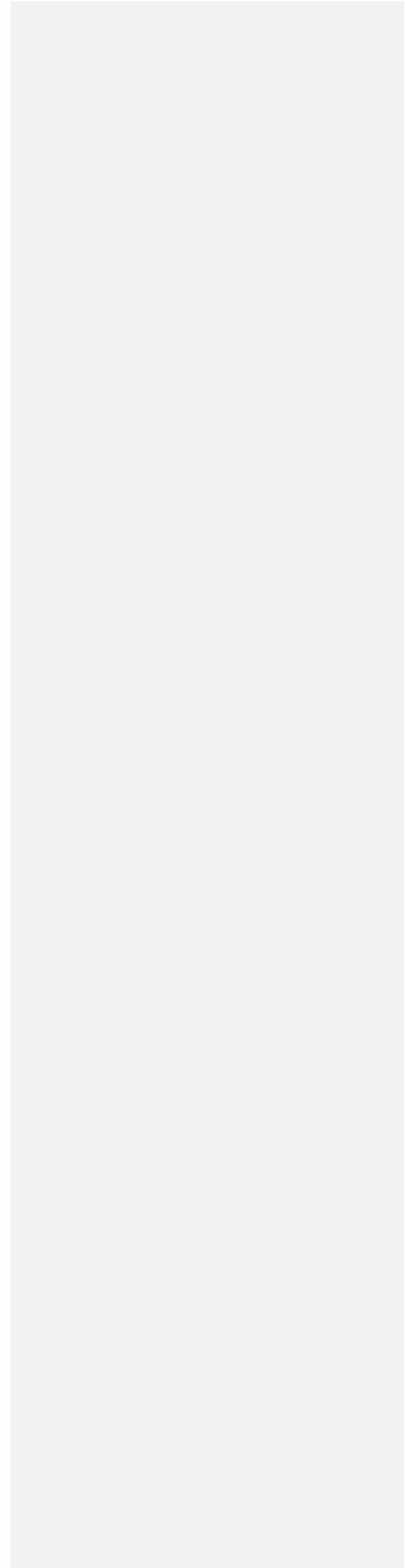
2. Aims

The aim of the present study was to compare a modified vitamin A regimen to the reference protocol normally used in NICUs in Norway. In the modified protocol, the dose of vitamin A was increased by 8% compared to the supplementation of vitamin A to the infants on the reference regimen. The supplementation was given according to the infants' actual weight and supplied continuously dispersed in the milk. The intervention aimed at increasing the plasma retinol concentration at discharge from hospital with the new supplementation regimen compared to the reference regimen.

HYPOTHESIS

Our main hypothesis was:

1. VLBW infants in the modified protocol will have higher plasma retinol concentration at discharge from hospital, compared to infants in the reference group.

The modified protocol introduced changes in the intake of the other fat-soluble vitamins D, E and K because of a different relative composition of vitamins in the modified supplement. We therefore hypothized:

2. VLBW infants in the modified protocol will have lower plasma concentrations of 25(OH)vitamin D compared to infants in the reference group.

3. VLBW infants in the modified protocol will have higher plasma concentrations of phylloquinone compared to infants in the reference group.

4. VLBW infants in the modified protocol will have plasma concentrations of α-tocopherol similar to the infants in the reference group.

3. Methods

3.1 Study subjects

All VLBW infants born between December 2003 and December 2005 at Akershus University Hospital were eligible for enrolment in an open intervention trial. Exclusion criteria were major congenital abnormalities, cerebral haemorrhage (grade 3 or 4) as determined by ultrasound examination, death and parental disapproval. During the inclusion period, 68 VLBW infants were born and 8 were excluded due to: refusal from the parents (n = 3), not meeting eligibility criteria including death and diseases (n = 4), or moving to another hospital (n = 1). The included VLBW infants (n=60) were assigned either to the reference or the modified vitamin A regimen.

Twenty-six infants in the reference group were included from December 2003 to September 2004, whereas 34 infants were enrolled in the modified group from October 2004 through November 2005. The two groups were comparable with regard to season variations, which may affect vitamin D status.

Written, informed consent was obtained from the parents and the Regional Ethics Committee approved the study. The current study is part of an ongoing larger multicenter study on supplementation of essential fatty acids and cognitive function.

3.2 Vitamin A supplementations

Infants (n = 26) assigned to the standard treatment received enteral and parenteral nutrition, consistent with the routine regimen of our Neonatal Intensive Care Unit (NICU). This reference oral supplement included human milk fortified with proteins and minerals (Presemp; Semper AB, Stockholm, Sweden) and multivitamins (Multibionta; Trophen Merck, Darmstadt, Germany), providing 750 µg retinol

equivalents per day regardless of infant weight. The supplement was given as a bolus once daily.

In the modified group (n = 34), the supplementation included a human milk fortifier with vitamins (Enfamil Human Milk Fortifier - Enfamil HMF; Mead Johnson Nutritionals, Evansville, Indiana, USA). The fortifier was dosed according to the infants' milk requirement, based on actual daily weight; thus, the dose of vitamin A increased as the infant gained weight (**Table 1**). The median duration of the intervention was 8 weeks.

Table 1. Daily intake of supplemented vitamin A by the reference and modified protocols.

Infant body weight (examples)	Reference protocol Presemp + Multibionta	Modified protocol Enfamil HMF
1,000 g		513 µg
1,500 g	750 µg	770 µg
2,000 g		1,026 µg

The vitamin A intake values are based on a daily intake of 180 ml human milk/kg.
HMF = human milk fortifier.

3.3 Dietary intake

Dietary intake of all VLBW infants during the neonatal period was calculated based on parenteral nutrition, human milk, formulas and oral supplements. The nutrient intake was calculated by a computer program (KBS; Department of Nutrition, University of Oslo, Norway) based on the Norwegian Food Composition Table (83). Data on commercially available products for preterm infants were obtained from the manufacturers and added to the database. Data on nutrient content in preterm human

milk (72 kcal, 3.5 g fat and 1.5 g protein per 100 ml) and donor milk were obtained from a report of Saarela et al (84). Data on vitamin A concentration in human milk from the food composition table were confirmed by high performance liquid chromatography (HPLC) analyses of 31 random milk samples from mothers in this study. Calculations were based on a mean concentration of vitamin A of 54 μg per 100 ml human milk. When the infants achieved a daily enteral milk intake of approximately 150 ml/kg, the milk used for feeding the infants in the reference group was fortified with Presemp. These infants also received oral vitamin supplement (Multibionta) containing 750 μg of vitamin A (retinyl palmitate), 12.5 μg of vitamin D and 2 mg of vitamin E (α-tocopherol). They also received one additional daily dose of 15 mg vitamin E (Vitamin E-NAF, 50 mg/ml; Ås Produksjonslab AS, Norway) from birth till 32 weeks of gestational age. Infants in the modified group also received fortified human milk when the daily enteral intake reached approximately 150 ml/kg. The milk was fortified with proteins, vitamins and minerals from a multinutrient fortifier (Enfamil HMF). They also received one additional daily dose of 10 mg vitamin E from birth to 32 weeks of gestational age to match the vitamin E supplementation in the reference group. Ten of the infants in the reference group and 12 infants in the modified group received parenteral vitamins (Vitalipid Infant; Fresenius Kabi, Bad Homburg, Germany), up to 4 ml/kg for the first few days of life (median 1 day, interquartile range 0-7 days). The infants received additional 0.5 mg vitamin K_1 (Kanavit Tropfen®; Medphano, Berlin, Germany) every third day orally according to the protocol of the department.

At discharge, 69% of the VLBW infants in the reference group and 64% of the VLBW infants in the modified group were breastfed, whereas the remaining infants were changed from donor human milk to term formula during the last days before discharge. When breastfeeding directly from the mother contributed with more than 50% of the total milk volume, the infants started with a multivitamin supplement (Nycopluss Multi; Nycomed Pharma AS, Oslo, Norway).

3.4 Blood sampling and analyses of vitamins

Venous blood samples (1 ml) from the VLBW infants were collected in EDTA containers at approximately one week of age and at discharge from the hospital. The blood samples were centrifuged, and plasma was stored at –80° C until further analyses. The plasma concentration of retinol, α-tocopherol, 25(OH)vitamin D and phylloquinone were measured by HPLC on a Hewlett Packard 1100 liquid chromatograph (Agilent Technologies; Palo Alta, CA, USA) with very high sensitivity. For detection of retinol, the method is linear at 0.1-10 µM and the lower limit of detection is 10 nM. The intra-assay coefficient of variation is 4.9-5.8 %, using known standards. For 25(OH)vitamin D, the method is linear at 5-400 nM and the lower limit of detection is 1-4 nM. CV is 5.2-5.8 %. For α- tocopherol, the method is linear at 1-200 µM and the lower limit of detection is 10 nM. CV is 4.6-4.8 %. For phylloquinone, the method is linear at 0.05-4 ng/ml, and the lower limit of detection was 0.01 ng/ml CV is 7.8-10 %.

3.5 Statistics

Normally distributed data are presented as mean and standard deviation (SD). Non-normally distributed data are presented as medians with interquartile range or minimum-maximum values. Categorical data are presented as percentages or actual numbers. Differences between groups were tested by the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. The change in plasma retinol during the study was tested by ANOVA (general linear model, repeated measurements). Statistical significance was defined as a *P* value < 0.05. Power estimation was performed with plasma retinol as the primary end point. Initially we aimed at increasing the plasma retinol concentration from 0.30 to 0.70 µM. To achieve this, the amount of supplementation could impose bloating and discomfort among the VLBW infants and even an increase to 0.50 µM is likely to be

Comment [p1]: Putt in ref. 8 her

important. Based on an earlier study (1), we estimated that we would need 25 infants in each group to detect a difference of 0.2 μM retinol, using a SD of 0.28 μM, 80% power and significance at 0.05.

4. Results

4.1 Patients characteristics

Three of the patients in the reference group and 4 in the modified group did not complete the study because of death or parent refusal, leaving 23 patients in the reference group and 30 patients in the modified group. Maternal and infant characteristics are presented in **Table 2**. The birth weight was 1,147 g vs. 1,155 g and the gestational age was 29.6 vs. 30.1 weeks in the reference group and modified group, respectively. No significant differences in patient characteristics were detected between the two groups at birth or enrolment.

Table 2. Maternal and infant characteristics.

	Reference protocol (n=26)	Modified protocol (n=34)
Birth weight (g)	1,147	1,155
Birth length (cm)	38.0	37.8
Gestational age (weeks)	29.6	30.1
SGA at birth (%)	38.5	36.4
Ratio male: female	1:1	1.4:1
Maternal age (years)	30.0	30.5
Maternal smoking (%)	22	22

Data are presented as median (interquartile range) or percentages. There were no significant differences between groups. SGA = small for gestational age (weight < 10 percentile for gestational age).

4.2 General feeding data

Feeding was started in the two groups on similar days after birth (first or second), and supply of 150 ml/kg/day was reached at similar ages (**Table 3**). The numbers of infants fed human milk versus mixed fed infants (human milk and formula) were similar in the two groups ($P>0.05$). There was no significant difference between the two groups regarding the number of infants receiving parenteral nutrition (PN). The two groups were also similar regarding the number of days the infants received PN during hospital stay. There was no significant difference for intake of energy (119 vs.123 kcal/kg/day) or protein (3.2 vs. 3.4 g/kg/day). There was no significant difference in the median duration of the intervention in the two groups (62 vs. 57 days) (**Table 4**). Moreover, there were no apparent differences in the incidence of feeding intolerance such as diarrhoea, abdominal distension or vomiting between the two groups (n = 2 in each group).

Table 3. Feeding data.

	Reference protocol (n = 26)	Modified protocol (n = 34)
Enteral feed started:		
-first day	73 %	79 %
-second day	27 %	21 %
Duration of PN (days)	5 (2-7)	3.5 (2-7)
Age when reached 150 ml/kg/day of enteral feeding (days)	6 (5-7)	6 (5-7)
Type of feeding:		
-human milk	61 %	60 %
-mixed (human milk + formula)	39 %	40 %

Data are presented as percent or median (interquartile range). There were no significant differences between groups. PN = parenteral nutrition.

4.3 Clinical events

There was no significant difference between the two groups in median weight gain or weight at discharge from the hospital (**Table 4**). Furthermore, there was no significant difference in the incidence of the following events (**Table 4**): mechanical ventilation (35 vs. 41%), days of nasal continuous positive airway pressure (N-CPAP; 6 vs. 8 days), median age at discharge (62 vs. 57 days) and days on antibiotics (1 vs. 3 days). One infant in each group received postnatal steroid treatment of dexametasone 0.5 mg/day in three days. Two infants with extremely low birth weight (birth weight 705 g and 830 g) in the modified group, died. Their deaths were not related to feeding protocols: one infant had major congenital malformations, not detected at time of inclusion, and the other died of multiorgan failure.

Table 4. Clinical events.

	Reference protocol (n=26)	Modified protocol (n=34)
Mechanical ventilation (%)	34.5	41.2
Duration of mechanical ventilation (days)	0 (0-2)	0 (0-7)
Duration of N-CPAP (days)	6 (4-23)	8 (0-38)
Age at discharge (days)	62 (52-78)	57 (49-90)
Antibiotic treatment (days)	1 (0-3)	3 (0-6)
Weight-gain (g/day)	26.6 (23.0-29.7)	25.6 (22.7-27.7)
Weight at discharge (g)	2,809 (2,380-3,354)	2,745 (2,439-2,990)
Mortality (number of infants)	0	2

Data are presented as median (interquartile range) or percentages. There are no significant differences between groups. N-CPAP = Nasal Continuous Positive Airway Pressure.

4.4 Intake of the different fat-soluble vitamins

The most important sources of fat-soluble vitamins were Vitalipid Infant if parenteral nutrition was given, and Multibionta or Enfamil HMF for enteral supplementation. The recommendations for the intake of the different fat-soluble vitamins are sometimes given per kg and sometimes given per day. Thus, to be able to compare the recommendations to the intake found in this study, the vitamin intakes per day are given in **Table 5** while the vitamin intakes per kg are given in **Tables 6**, **7**, **8** and **9**.

Table 5. Total intake of the different fat-soluble vitamins per day.

Mean Median (interquartile range)	Reference protocol	Modified protocol	P=value	Recommendations
Vitamin A **(µg)**	772	837	$P<0.01$	210-450 µg/kg/day (21)
	896	912		200-1000 µg/day (48)
	670-938	652-1126		30-80 µg/kg (16)
Vitamin D **(µg)**	10.6	9.5	$P<0.01$	3.75-10 µg/kg/day (21)
	12.6	10.8		25 µg/day (48)
	11.5-12.6	7.7-12.6		10 µg/kg/day (16)
Vitamin E **(mg)**	14.9	14.0	$P=0.59$	6-12 mg/kg/day (21)
	17.8	16.0		up to 17 mg/day
	17.3-18.0	8.7-19.5		5-15 mg/day (16)
Vitamin K **(µg)**	12.71	29.44	$P<0.01$	7-9 µg/kg/day (21)
	0.62	12.91		2-3 µg/kg/day (48)
	0.47-0.77	6.09-15.72		7-9 µg/kg/day (16)

Data are presented as mean, median and interquartile range.

4.4.1 Intake of vitamin A

Vitamin A intake related to gestational age is presented in **Figure 1** for a more visual approach in addition to **Table 5** and **Table 6**. During the total study period, infants in the modified group consumed slightly more vitamin A than infants in the reference group (585 vs. 546µg/kg/day; $P =0.66$). Infants in the modified group received significantly more vitamin A than infants in the reference group during 3 out of the 4 periods. The difference in vitamin A intake was greatest in the last period (gestational age > 37 weeks): 578 vs. 316 µg/kg/day ($P = 0.02$). This was due to increased intake of fortified human milk while the infants were gaining weight.
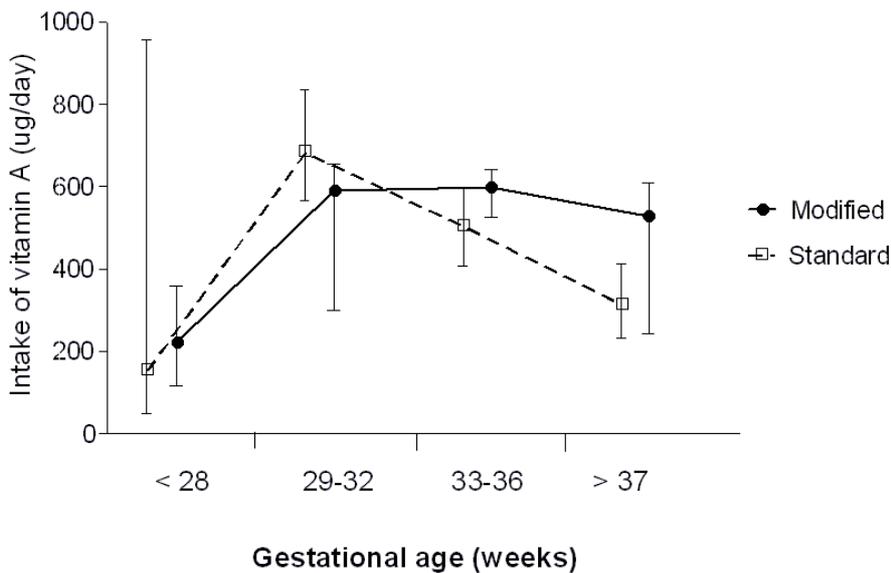

Figure 1. Intake of vitamin A at different gestational ages.
Data are presented as medians and interquartile range. There was a difference between the two groups during the observation period (ANOVA, $P < 0.001$).

Table 6. Intake of vitamin A.

Vitamin A (µg/kg) Mean Median (interquartile range)	Reference protocol n=26	Modified protocol n=34	*P*-value
Total intake µg/kg/day	537	524	*P*=0.66
	546	585	
	321-691	414-645	
Period 1 GA: < 28 weeks	415	440	*P*=0.06
	154	213	
	48-957	105-354	
Period 2 GA: 29-32 weeks	653	497	*P*<0.01
	685	590	
	567-837	294-659	
Period 3 GA: 33-36 weeks	501	565	*P*<0.01
	506	600	
	407-598	527-645	
Period 4 GA: > 37 weeks	361	469	*P*<0.01
	315	528	
	232-413	235-611	
Recommendations	210-450 µg/kg/day or 450-840 µg/kg/day with CLD (21) 200-1000 µg/day (48) 30-80 µg/kg/day (16)		

Data are presented as mean, median and interquartile range. GA= gestational age.

4.4.2 Intake of vitamin D

During the total study period, infants in the reference group had a vitamin D intake higher than infants in the modified group (7.4 vs. 6.4 µg/kg/day; $P<0.01$). The difference in vitamin D intake per kg infant between the two groups was greatest in period 2 (GA=29-32 weeks), which is the time right after birth for many of the infants. We suspect this, as in vitamin A intake, to be due to the fact that vitamin supplementation was given according to the infants daily weight as opposed to the fixed, standard dose given to the reference group. The intake of the vitamin supplementations in the reference group given as a single fixed dose per day was declining per kg body weight throughout the study because of weight gain in the infant, while the vitamin supplementation intake in the modified group was kept constant per kg infant during the study period.

Table 7. Intake of vitamin D.

Vitamin D (µg/kg) Mean Median (interquartile range)	Reference protocol n=26	Modified protocol n=34	*P*-value
Total intake **µg/kg/day**	7.5 7.4 4.6-9.9	6.0 6.4 4.9-7.2	*P*<0.01
Period 1 GA: < 28 weeks	6.0 1.7 0.03-14.8	6.0 2.7 0.1-4.5	*P*=0.09
Period 2 GA: 29-32 weeks	9.3 9.7 7.6-12.0	5.7 6.5 3.3-7.3	*P*<0.01
Period 3 GA: 33-36 weeks	6.8 6.7 5.3-8.2	6.4 6.6 5.9-7.1	*P*=0.05
Period 4 GA: > 37 weeks	5.1 4.3 2.9-5.4	5.4 5.9 2.9-6.8	*P*=0.06
Recommendations	3.75-10 µg/kg/day (21) 25 µg/day (48) 10 µg/kg/day (16)		

Data are presented as mean, median and interquartile range. GA= gestational age.

4.4.3 Intake of vitamin E

During the total study period the reference group and the modified group consumed the same amount of vitamin E, median values 10.4 vs. 10.3 mg/kg/day ($P<0.01$). The supplementation regimen was designed to provide equal doses of vitamin E in the modified and reference group. As Enfamil HMF contains vitamin E, the additional supplementation in the modified group was 10 mg as compared to the reference group who received 15 mg since Multibionta contains no vitamin E.

Table 8. Intake of vitamin E.

Vitamin E (mg/kg) Mean Median (interquartile range)	Reference protocol n=26	Modified protocol n=34	*P*-value
Total intake **mg/kg/day**	10.5 10.4 7.1-13.8	9.5 10.3 5.1-13.0	*P*<0.01
Period 1 GA: < 28 weeks	9.6 4.3 0.2-20.6	11.1 5.3 3.0-19.6	*P*=0.08
Period 2 GA: 29-32 weeks	13.1 13.6 10.8-16.7	9.6 11.3 5.2-14.3	*P*<0.01
Period 3 GA: 33-36 weeks	9.4 9.5 7.8-11.6	9.5 10.7 5.4-12.7	*P*=0.01
Period 4 GA: > 37 weeks	6.4 6.1 2.8-7.5	6.9 6.9 4.3-10.4	*P*=0.14
Recommendations	6-12 mg/kg/day, up to max 17 mg/kg (21) No recommendations from ESPGHAN (48) 5-15 mg/day (16)		

Data are presented as mean, median and interquartile range. GA= gestational age.

4.4.4 Intake of vitamin K

In addition to the vitamin K intramuscular injection of 0.5 mg at birth, the ELBW infants received 0.5 mg vitamin K orally every 3rd day if they were given antibiotics or if there was any sign of liver affection. The VLBW infants in the same situation received 1 mg vitamin K orally every 3rd day. This routine in our and other neonatal intensive care units (NICU) explains the extremely high mean intake values in period 1 (GA<28 weeks) in both groups (45.04 vs. 170.03 µg/kg/day; $P<0.01$). The intake of vitamin K in the reference group during the total study period was much lower than international recommendations, while the intake in the modified group was satisfactory due to supplementation of vitamin K from the milk fortifier, (0.38 vs. 7.71 µg/kg; $P<0.01$).

Table 9. Intake of vitamin K.

Vitamin K (µg/kg) Mean Median (interquartile range)	Reference protocol n=26	Modified protocol n=34	*P*-value
Total intake **µg/kg/day**	12.61	29.36	*P*<0.01
	0.38	7.71	
	0.34-0.42	4.95-8.62	
Period 1 GA: < 28 weeks	45.04	170.03	*P*<0.01
	0.45	48.39	
	0.24-27.89	0.41-89.07	
Period 2 GA: 29-32 weeks	20.46	32.86	*P*<0.01
	0.39	7.76	
	0.35-0.43	0.42-8.75	
Period 3 GA: 33-36 weeks	2.69	6.87	*P*<0.01
	0.37	7.77	
	0.35-0.41	6.67-8.39	
Period 4 GA: > 37 weeks	1.75	5.84	*P*<0.01
	0.39	6.79	
	0.29-0.49	0.32-7.96	
Recommendations	7-9 µg/kg/day (21)		
	2-3 µg/kg and in addition 0.5-1 mg IM injection (48)		
	7-9 µg /kg and in addition 0.3-1 mg IM injection (16)		

Data are presented as mean, median and interquartile range. GA= gestational age.

4.5 Plasma concentrations of the fat-soluble vitamins at inclusion and discharge

4.5.1 Plasma concentration of retinol

At inclusion (6 days of age), both groups had low plasma retinol concentrations, and there was no significant difference between the two groups (0.54 vs. 0.43 µM). The plasma concentration of retinol in the reference group decreased during the study period, but remained constant in the modified group (**Figure 2**). At discharge from hospital the reference group had lower plasma retinol concentration as compared to the modified group (0.30 vs. 0.45 µM, P=0.005). The difference between the reference and modified group at discharge (61 days of age) was higher in infants that had received parenteral vitamins (0.30 vs. 0.59 µM, reference and modified group, respectively, P=0.006) compared to those only given enteral vitamin. At discharge, more infants in the reference group had plasma retinol concentrations less than 0.35 µM as compared to the modified group (69 vs. 44%, $P = 0.04$) and many infants in both groups had plasma retinol concentrations below 0.70 µM: 88 vs. 73% in the reference and modified group, respectively.
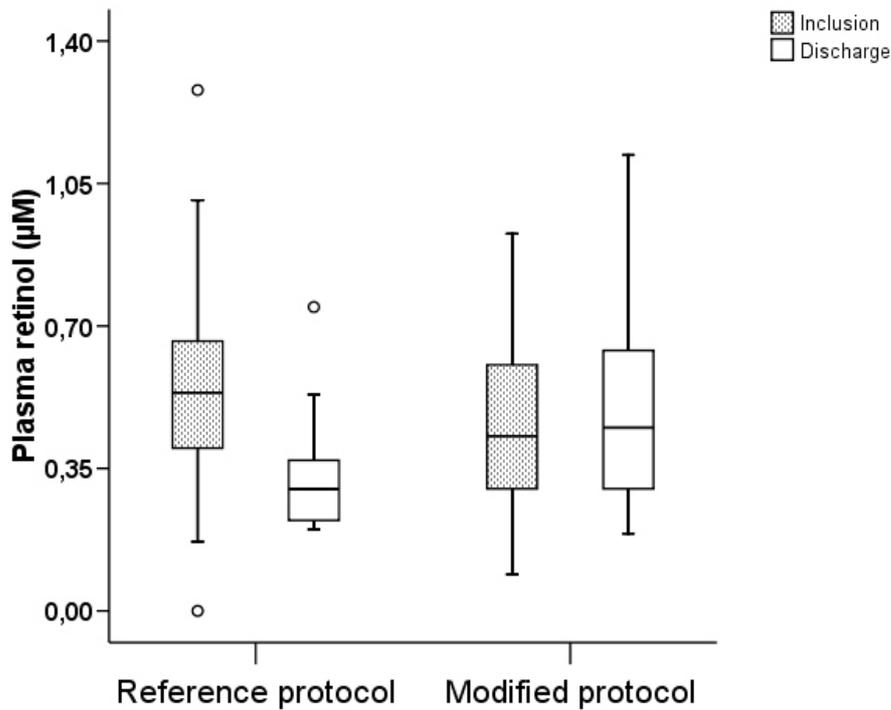
Figure 2. Plasma retinol concentrations at inclusion (6 days of age) and discharge (61 days of age).

The data are shown as box plots (minimum, 25th percentile, median, 75th percentile and maximum values), while outliers are shown as individual open circles. The plasma retinol concentration decreased on reference regimen during the study period, but remained constant in the modified group (ANOVA, P=0.012).

4.5.2 Plasma concentration of 25(OH)vitamin D

At inclusion (6 days of age), both groups had plasma concentrations in the normal range (**Figure 3**) and there was no significant difference between the two groups (81 vs. 70 nM, reference and modified group, respectively). Both groups had a significant rise in plasma 25(OH)vitamin D concentrations from inclusion to discharge. In the reference group, plasma 25(OH)vitamin D at discharge was higher (171 nM) than the

reference level of 150 nM for term born infants at six months age. At discharge, infants in the reference group had higher plasma concentrations of 25(OH)vitamin D compared to corresponding values in the modified group (171 vs. 110 nM, P=0.005). Lower plasma 25(OH)vitamin D concentrations than internationally recommended ($\leq$ 50 nM) were infrequent (one in each group).
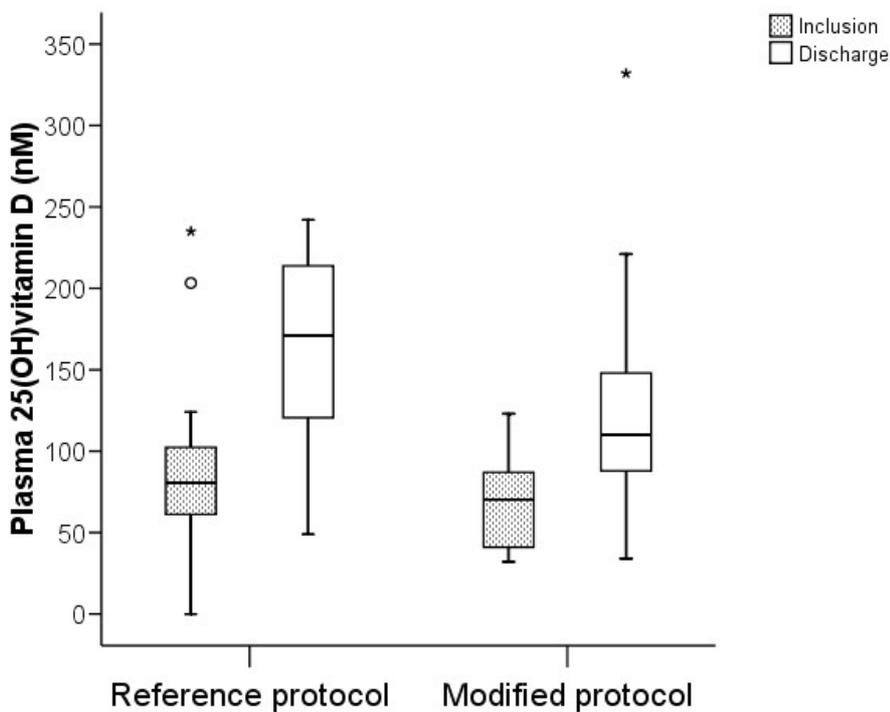

Figure 3. Plasma concentrations of 25(OH)vitamin D at inclusion and at discharge from hospital in the two groups.

The data are shown as box plots (minimum, 25th percentile, median, 75th percentile and maximum values), while outliers are shown as individual open circles.

4.5.3 Plasma concentration of α-tocopherol

At inclusion, the reference group had not significantly higher plasma α-tocopherol compared to the modified group (37 vs. 35 µM). At discharge from hospital there were no significant differences in plasma α-tocopherol concentration (31 vs. 39 µM) between the two groups. For the modified group, the median plasma concentration at discharge is slightly above the normal range (16-36 µM).
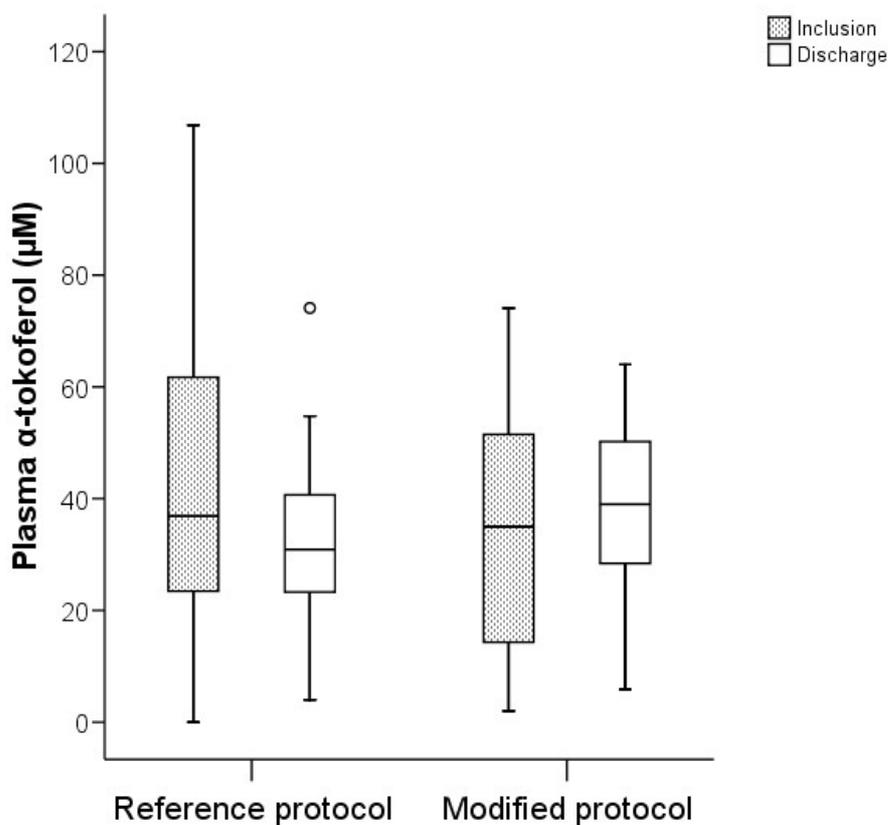

Figure 4. Plasma concentration of α-tocopherol at inclusion and at discharge from hospital in the two groups.

The data are shown as box plots (minimum, 25th percentile, median, 75th percentile and maximum values), while outliers are shown as individual open circles.

4.5.4 Plasma concentration of phylloquinone

Plasma levels of phylloquinone differed between the groups of infants (**Figure 5**). At inclusion (6 days of age), plasma phylloquinone concentration in both groups was high, but the plasma concentration in the modified group was significantly higher then the concentration in the reference group (49.50 vs. 8.87 ng/ml, P=0.03). These are plasma concentrations much higher than the reference range (0.13-1.19 µM) given by the AAP (16), and may reflect that the immediately-after-birth dose is far too high. There was no significant difference in plasma phylloquinone (1.01 vs. 2.10 ng/ml) between the two groups at discharge from hospital.
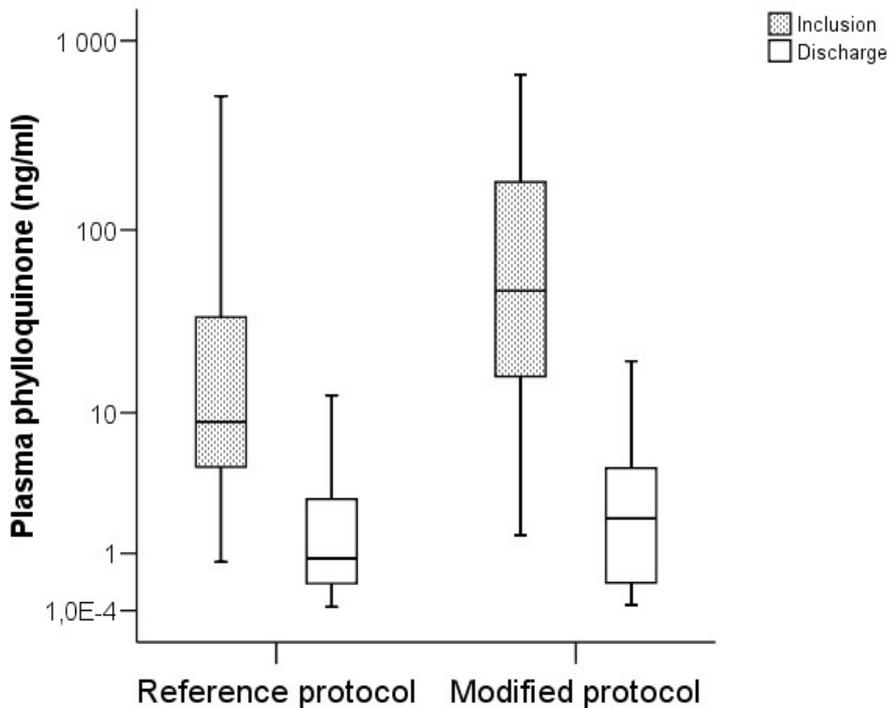

Figure 5. Plasma concentrations of phylloquinone at inclusion and at discharge from hospital.

The data are shown as box plots (minimum, 25th percentile, median, 75th percentile and maximum values), while outliers are shown as individual open circles.

5. Discussion

5.1 Vitamin A and plasma retinol

In this study of preterm infants weighing below 1500 g, two different vitamin A supplementation protocols were compared. In the modified protocol, infants received a higher daily dose of vitamin A, administered in a human milk fortifier, whereas infants in the reference group received vitamin A from a multivitamin supplement that was given as a bolus once a day. Our main finding was that infants in the modified group obtained higher plasma retinol levels at discharge from hospital as compared to the reference group. Importantly, more of the infants in the modified group reached plasma levels above 0.35 µM retinol, suggesting less vitamin A deficiency in this group. Many had plasma retinol concentrations below 0.70 µM, a level considered to be the lower limit for term infants and children (46). However, the optimal plasma retinol concentration for VLBW infants is not known and more research is needed to establish the optimal retinol concentration among this vulnerable group.

The plasma retinol concentration may have varied during the hospital stay and extremely high or low levels, and the possible duration of these may not have been detected. The pharmacokinetics of vitamin A are likely to vary between infants, and it is not clear whether a nadir or steady state plasma retinol value is the most important factor in terms of vitamin A status. Furthermore, measuring plasma concentrations of retinol may not be sufficient to assess vitamin A status properly in preterm infants (44). Plasma retinol may reflect the availability of its carrier named retinol-binding protein (RBP), which is typically low in the preterm infant, whereas un-bound retinol is likely to be the most significant parameter for vitamin A status. Moreover, in preterm infants the level of RBP is correlated to the retinol concentration in plasma (67). The concentration of RBP increases in parallel with plasma retinol, without reaching levels that are considered normal in the pediatric population (67).

Vitamin A is an important nutrient in early infancy during rapid growth. The beneficial effects of vitamin A are due to its ability to promote growth and differentiation of epithelial tissues, including skin, immune system, intestine and lungs. Retinoic acid is a derivative of vitamin A, and acts as a ligand for nuclear receptors acting as transcription factors for genes involved in growth and differentiation of several types of cells (85). Deficiency of vitamin A is a predisposing factor for chronic lung disease, respiratory distress, retinopathy of prematurity and infections often seen in the VLBW infants (44). Furthermore, vitamin A is necessary for the health of the anterior eye and is an essential constituent of visual pigment. Microscopic conjunctive changes consistent with vitamin A deficiency have been noted in preterm babies, and low plasma vitamin A concentrations have been associated with the development of retinopathy of prematurity (44).

Our present study confirms that preterm infants have low plasma concentrations of retinol at birth, and that in spite of supplementation, it is difficult to markedly increase retinol levels (47;86-88).This may be related to low intake, impaired absorption, higher requirements, inadequate transport and/or metabolism of vitamin A.

Vitamin A may be supplied intramuscularly, parenterally or orally. Intramuscular vitamin A injections may reduce chronic lung disease and death in ELBW infants (49). Porcelli et al (89) found retinol concentrations > 0.70 μM four weeks after birth in ELBW infants receiving 318 μg/kg/day of vitamin A, mainly as a part of parenteral lipids, compared with infants receiving 234 μg/kg/day. Enteral supplementations contain vitamin A as retinyl esters, which have to be hydrolysed in the intestine prior to absorption. Wardle et al (90) reported that oral supplementation (1,500 μg/day) of vitamin A failed to give plasma retinol significantly higher than the control group at 28 days of life, neither did it alter the incidence of chronic lung disease. Kennedy et al (91) also concluded that large enteral doses of vitamin A led to lower plasma retinol concentrations compared to intramuscular injections. These data

suggest that absorption of retinyl ester may be a limiting factor in neonatal bioavailability of retinol.

On the other hand, Delvin et al (67) reported that oral supplement containing 909 µg vitamin A gave adequate plasma retinol levels (on human milk to 0.84 µM and on formula to 1.01 µM) 30 days after birth. These infants had a higher birth weight (mean birth weight on human milk=1,746 g and on formula=1,795 g) than infants in our study.

Landman et al (88) suggested that oral supplementation of 1,500 µg retinyl esters/day provided plasma concentrations similar to intramuscular injections (600 µg every second day), including only a small number of selected VLBW infants.

Very high oral doses of retinyl esters efficiently reduce mortality in infants. In a randomised, double blind, placebo controlled trail with 11,619 newborn infants in South India, 7,200 µg of retinyl esters were given orally twice, on day 1 and day 2 after delivery. This intervention promoted a 37 % decrease in mortality among infants with birth weight less than 2,000 g and beneficial differences in adverse events compared to placebo (92). We are not aware of studies that have tested such high doses in VLBW infants.

Several studies show an increase in plasma retinol levels after intramuscular injections of vitamin A (50;87;93), and a high plasma retinol concentration protects ELBW infants against chronic lung disease and death (49). Intramuscular injection has not been implemented as routine treatment for preterm infants; the reasons reported from neonatal intensive care units are that the benefit of the treatment is regarded as small and not proven (51).

Oral supplementation of 1,200 µg/kg/day of retinyl esters has been recommended for VLBW infants from start of full enteral feeding until discharge from the neonatal unit (43). In our present study, only 600 µg/kg/day was given using a commercially available human milk fortifier intended for preterm infants. Even though the plasma retinol levels at discharge were improved by the modified regimen, optimal retinol

levels might not have been reached. The amount of human milk fortifier cannot be increased beyond recommended doses, because it contains proteins and minerals. Future trials should therefore include increased amounts of retinyl esters in the human milk fortifiers.

Another way of enhancing absorption may be to give unesterified retinol or a mixture of retinyl esters and retinoic acid (an active retinol metabolite). The advantage of giving free retinol is that the intestinal esterase would not be required before absorption. The mixture of retinyl esters and retinoic acid has been shown to increase the amount of retinyl esters in rat lung tissue compared to giving retinyl palmitate only (94). More studies are needed before this can be considered as a treatment option in humans.

In summary, our modified protocol that was acceptable in means of dose and administration and well tolerated by the infants, improved vitamin A status at discharge and reduced the number of infants with low plasma retinol concentrations (below 0.35 μM) in VLBW infants, as compared to a reference protocol. More studies are needed to establish the optimal plasma retinol concentration for preterm infants and how this can be achieved.

5.2 Vitamin D and plasma 25(OH)vitamin D

Vitamin D intake was different in the two groups. The group on the reference vitamin regime had a median vitamin D intake of 7.4 μg/kg/day. This is a significantly higher intake than in the modified group, which had a median vitamin D intake of 6.4 μg/kg/day (**Table 7).** Since the recommendations for vitamin D intake is given as μg/day and not μg/kg/day, the relevant median vitamin D intake in the two groups were 12.6 μg/day vs. 10.8 μg/day (reference vs. modified group, respectively) (**Table 5**). In both groups this was lower than the recommendations in Europe (25 μg/day, according to ESPGHAN) (48), but matching the recommendations in America (10

µg/day, according to AAP) (16). Nevertheless, we observed a significant rise in the plasma 25(OH)vitamin D concentration during the hospital stay in both groups.

In the reference group, plasma 25(OH)vitamin D concentration was higher than the reference range, whereas the modified group had plasma 25(OH)vitamin D concentration in the upper part of the normal range. This may indicate that the recommendations for vitamin D supplementation need not be as high as 25 µg/day for human milk fed VLBW infants. Adequate plasma levels may probably be reached with a smaller daily dose, even lower than 10 µg/day (corresponding to 400 IE) since results given in this thesis show that even 12.6 µg/day seems to be too high.

Plasma concentrations of 25(OH)vitamin D in preterm infants are 20-30% lower than the plasma levels of their mothers (57). Both in term-born and preterm newborns 25(OH)vitamin D concentrations in cord blood are lower than those in maternal blood, and are correlated with concentrations in the mother (56). The vitamin D requirements of preterm infants are influenced by the body stores at birth, which in turn are related to the length of gestation and maternal stores. Preterm infants born after 28 weeks of gestation, appear to have the ability to absorb and hydroxylate vitamin D to produce 25(OH)vitamin D and the active metabolite $1,25(OH)_2$vitamin D from the first day of life (95-97). Vitamin D supplementation immediately after birth improves vitamin D nutritional status as evidenced by rising plasma 25(OH)vitamin D concentrations. Concentrations of $1,25(OH)_2$vitamin D in preterm infants up to 3 months of age are more than 2-3 times higher than those seen in older children. During this period, there is no significant correlation between vitamin D metabolite concentrations and calcium and phosphorus intake. At 30 d of age, there is also no influence of diet or metabolite indexes (53).

Adequate supplementation of vitamin D is, together with calcium, phosphorus and magnesium, essential for the formation of structural matrix of bone as well as function of soft tissues (98). Rickets is an example of extreme vitamin D deficiency. A state of deficiency occurs months before rickets is obvious on physical examination.

Vitamin D deficiency has been implicated in the etiology of the osteopenia of prematurity (99), but it is apparent that the main cause for this condition is a deficiency of calcium and phosphorus (100). Since absorption of calcium and phosphorus in preterm infants appears to be independent of vitamin D, it is unlikely that higher intakes of vitamin D would be beneficial (101).

Early neonatal hypocalcemia affects 75% of preterm infants during the first days of life, principally those born with very low birth weights (<1500 g) (102). Long time effects of vitamin D supplementation in infancy have been shown to be associated with bone mineral content and increased bone mineral density in pre-pubertal girls (103).

The effects of excessive vitamin D intake include hypercalcemia and hypercalciuria, leading to deposition of calcium in soft tissues and irreversible renal and cardiovascular damage. Serum 25(OH)vitamin D concentration higher than 220 nM is associated with hypercalcemia in adults (60).

The recommended daily dose of vitamin D for preterm infants remains controversial and ranges between 5 (58) and 25 μg/day. The recommendations from AAP are to supply preterm infants with 10 μg/day (16). This is in contrast to the recommendations from ESPGHAN where preterm infants on human milk should be supplied with a vitamin D intake of 25 μg/day (48).

Koo et al (58) measured plasma 25(OH)vitamin D concentrations in preterm infants receiving 5, 10 or 20 μg/day. They showed that plasma 25(OH)vitamin D remained normal for 6 months while infants received less than 10 μg/day. This is the key study to support the standard practice in North America and Australia, which is supplementation of a single daily dose of vitamin D of 10 μg/day. Backstrøm et al (62) reported that a daily vitamin D intake of 5 μg/day up to a maximum of 10 μg/day is sufficient for premature infants to maintain adequate vitamin D status. A recent study (59) substantiates the adequacy of vitamin D intakes in the range of 5-10 μg/day.

The rationale for the higher intake of vitamin D for preterm infants recommended by ESPGHAN (48) with 25 µg/day, is the risk of sub-clinical vitamin D deficiency in women in Europe (and thus their newborn infants) owing to the lack of fortification of commercial cow milk with vitamin D. Maternal vitamin D deficiency will reduce the transplacental transfer to the fetus of 25(OH)vitamin D, resulting in lower stores in the infants at birth (57). However, in a clinical trial comparing 50 vs. 10 µg/day given to preterm infants fed human milk or standard term formula, no difference in metabolic bone disease as assessed radiologically was observed (104). Vitamin D status measured as the plasma concentration of 25(OH)vitamin D was within the normal range for both infant groups and at the upper limit of the normal range for those infants who received 50 µg/d. In our study, plasma 25(OH)vitamin D concentration in the reference group supplemented with 12.5 µg/day indicates that supplementation in this range is too high and results in plasma concentration above the reference range.

5.3 Vitamin E and plasma α-tocopherol

In this study, both groups had similar intakes of vitamin E (10.4 vs. 10.3 mg/kg/day, reference and modified group, respectively). Since some of the recommendations for vitamin E intake are given irrespective of the infants' weight (mg/day instead of mg/kg/day), the actual median vitamin E intake in the two groups were 17.8 mg/day vs. 16.0 mg/day. Supplementation of this amount is definitely higher than both the recommended intake of Tsang et al (21) of 4 to 8 mg/day and of Fewtrell & Lucas (10) of 5 mg/day. The dose of vitamin E given in this study gives plasma α-tocopherol concentration at discharge from hospital within or just above the reference ranges (16-36 µM). The recommended daily dose of vitamin E varies between 6 and 12 mg/kg/day and in both groups the daily supplementation doses were within the upper level of this reference intake range.

The biology of vitamin E, α-tocopherol being the most abundant and active form, is complex. Vitamin E is an antioxidant with the ability to protect cellular and subcellular membranes from oxidative destruction initiated at the molecular level by lipid peroxidation (63). In order to effectively act as a radical scavenger, α-tocopherol must be located at membrane sites well exposed to reactive oxygen species (66). Vitamin E may be considered as the primary defence against potentially harmful oxidants and signs of deficiency are reproductive failure, muscular dystrophy and neurological abnormalities.

Total body content of α-tocopherol in the human fetus increases from about 1 mg at 5 months gestation to approximately 20 mg at term (105). Because the preterm infant is born with low stores of α-tocopherol in addition to a decreased blood concentration, early provision of vitamin E is necessary to correct the depleted state and prevent adverse consequences attributable to insufficient antioxidants (16).

Vitamin E requirements in preterm infants are not clearly defined possibly because the assessment of vitamin status is difficult to obtain. A concentration of at least 11.6 µM (0.5 mg/dl) is thought to indicate adequate nutritional status in adults (72). Most would agree that vitamin E concentration in tissue is the most appropriate parameter to measure in order to assess vitamin E status, although in preterm infants, only blood concentrations are usually available. In adults, tocopherol to total lipid ratio is considered to be a more appropriate test. Because of the marked influence of plasma lipids on circulating α-tocopherol concentrations, tocopherol data have been expressed as a function of the actual lipid concentration in many studies (72;106). These investigations have demonstrated that children do have significantly lower levels of plasma vitamin E than adults and a tocopherol-to-total lipid ratio of 0.6-0.8 mg/g may indicate adequate nutritional status. This ratio might be important to measure in the VLBW infant, in whom changes in lipid levels occur, ranging from very low levels at birth to high during intravenous feedings of fatty acids. The requirement for vitamin E increases when PUFA intake increases. In extreme

situations, the need for α-tocopherol is suggested to vary from as little as 5 mg to more than 20 mg/day (66).

Absorption of α-tocopherol is relatively inefficient, ranging from 20 to 80 % in various studies. Normal bile secretion and normal pancreatic function are essential. Premature infants present problems somewhat different from those of term-born infants of normal birth weight. Vitamin E deficiency is common among preterm infants receiving intensive care (21;107). Because of their low body stores of tocopherol, their reduced intestinal absorption and the relatively greater growth rates associated with prematurity, it is more difficult to achieve and maintain normal vitamin E status for these infants. Thus, oral supplementation of 17 mg/day of vitamin E may be required by premature infants up to 3 months of age (2) and compared to other fat-soluble vitamins, vitamin E is relatively non-toxic when taken orally.

The Committee on Fetus and Newborn of the American Academy of Pediatrics (108) has endorsed 23 to 46 μM (1 to 2 mg/dl) as the normal range of serum tocopherol levels. Johnsen et al (109) has proposed classifying of serum tocopherol levels as physiologic in the range 23 to 81μM as appropriate for prophylaxis and treatment of early retinopathy of prematurity, and the pharmacologic range from 93 to 116 μM as appropriate for treatment of severe retinopathy of prematurity. A Cochrane review (71) has shown that vitamin E supplementation resulting in serum levels above 81 μM, significantly reduces the risk for severe retinopathy among VLBW infants, but increases the risk of sepsis and of necrotizing enterocolitis (NEC) among infants treated for more than one week. Some institutions give pharmacological doses to infants that have developed the first stages of retinopathy of prematurity with successful results, including no more frequent episodes of sepsis or NEC (109).

Vitamin E deficiency-induced haemolytic anaemia has been reported among preterm infants (48). This syndrome has been associated with the use of formulas that contain high levels of PUFAs with inadequate vitamin E while providing supplemental iron, which functions as an oxidant.

In studies of enteral nutrition, it has been shown that administration of 10-25 mg/day of water-miscible α-tocopherol-acetate given to neonates weighing 0.6-1.5 kg, may be required to produce and maintain normal vitamin E status (68;110). Some preterm infants receiving this amount of supplemented vitamin E, may not maintain a plasma α-tocopherol concentration above 12 μM, especially if they receive iron-fortified formula (111).

5.4 Vitamin K and plasma phylloquinone

Plasma phylloquinone concentrations in both groups were very high at inclusion compared to earlier studies in healthy newborns (74) and in preterm infants (1;112;113). Similar to all fat-soluble vitamins, absorption is enhanced by dietary fat and is absorbed from the intestine into the lymphatic system, depending on the normal flow of both bile salts and pancreatic secretions. The low vitamin K plasma concentration in neonates is determined by a small transplacental passage from the mother to the infant (114) and compared to adults they have virtually no hepatic stores of menaquinones (vitamin K_2). This vitamin is synthesized by bacteria of the gut. The lactobacillus intestinal microflora that is typical in breastfed infants does not synthesize menaquinones, and thus their vitamin K status is low. A new window, addressing physiology and safety, seems to have been opened by oral solutions, including mixed micellar or low-dose preparations of vitamin K (76).

We demonstrated that during the hospital stay, the plasma phylloquinone concentration was significantly reduced in both groups, but the levels were still within the normal range (48). At discharge from hospital, infants in the modified group had higher phylloquinone concentration compared to the standard group, probably reflecting the higher intake in this group throughout the study (7.71 vs. 0.38 μg/kg/day), i.e. plasma concentration seems to be correlated to the amount of vitamin supplemented.

The requirements and need at birth for such high doses of vitamin K as those given routinely in Norway, are not well documented. An intramuscular injection of vitamin K is routinely provided at birth to prevent bleeding in the newborn. Bleeding, however, is almost exclusively seen in breastfed infants. This may be explained by low plasma prothrombin levels in the newborn infant. Adding to this is that human milk contains low levels of vitamin K (2 μg/l) and the intestinal flora has not been established. Therefore, exclusively breastfed infants who do not receive vitamin K prophylaxis at birth may be at high risk of developing fatal intracranial haemorrhage. Vitamin K activity is essential for synthesis of prothrombin as well as at least five other proteins involved in the regulation of blood clotting. Vitamin K is also required for the biosynthesis of other proteins in plasma, bone and kidney.

Several authors have suggested deleterious side effects from high vitamin K levels, such as childhood leukaemia and hepatoblastoma (115). Such findings have not, however, been confirmed by randomised studies. At present, it is not known whether high plasma vitamin K concentration in the neonatal period increases disease risks. However, this uncertainty should enhance attention of vitamin K supplementation in VLBW infants.

In preterm infants who weigh more than 1 kg at birth, the standard dose of 1 mg of phylloquinone is used. Among infants less than 1 kg, a dose of 0.3 mg/kg of phylloquinone is recommended (16). 0.4 mg/kg of vitamin K intravenously to neonates, in whom oral or intramuscular administration is not feasible, seems to be rational (116).

Prophylaxis is still uncommon in many parts of the world today. Even its use in Europe varies widely from country to country as well as within countries (77). Formulas for preterm infants provide sufficient vitamin K to meet daily needs thereafter. Human milk has low vitamin K content. Lactating mothers easily achieve the recommended dietary allowance for vitamin K and the breast milk concentration is readily increased by increasing maternal vitamin K intake (77). Human milk fortifiers that contain supplemental vitamins provide additional vitamin K needed to

meet the recommended intake of 7 to 9 µg/kg/day (48). For infants fed human milk, vitamin supplements of A, D and E are readily available as oral solutions, however, none of these contains vitamin K. Premature infants may therefore be discharged using formulas with insufficient vitamin substitution. Therefore, exclusively breast-fed preterm infants probably receive inadequate dietary vitamin K and may require further supplementation to protect against late-onset VKDB (78).

The recommended intake of vitamin K for preterm infants is not evidence-based, ranging from 2-3 µg/kg/day (48) to 100 µg/kg/day (79), with current prophylaxis at birth ranging from 0.1 mg to 1 mg IM or intravenously.

Kumar et al (112) demonstrated that parenteral administration of large amounts of vitamin K in preterm infants at 2 weeks of age, resulted in high plasma vitamin K levels that were similar to those in term formula-fed infants. Therefore, also referring to the paper by Henriksen et al (1), we may argue that the parenteral vitamin K dose routinely given to preterm infants in Norway should be reduced. Lately, high doses of a water-soluble vitamin K (menadione) preparation has been shown to be associated with red cell haemolysis and hyperbilirubinemia, leading to kernicterus in the preterm infants (117). Therefore, current vitamin K supplementation of preterm infants, particularly at 2 weeks, provides excessive amounts of vitamin K and this has a potential for unforeseen side effects.

Human milk generally contains less than 1 ug/dl. Supplementing mothers with 2.5 mg vitamin K a day orally for two weeks increased the content of vitamin K in the milk to 6.42 µg/dl (118). Without this maternal supplementation, it would not be possible to supply these infants with even the minimal recommended Vitamin K intake for VLBW infants of 2 µg/kg/day.

5.5 Methodological considerations

The major strengths of this study are that it is conducted in a population of preterm infants fed fortified human milk and that we have detailed information on the intake

of vitamin A and plasma retinol measurements at start and end of the study. We further intended to obtain higher and acceptable plasma retinol concentrations by modifying the protocol for orally given vitamins and not by intravenous or intramuscular injections. There are, however, some limitations. Enrolled preterm infants were assigned in a cohort fashion to either the standard or modified nutrition regimen group. The cohort design was chosen for practical reasons, acceptable in the NICU. We recognize that the cohort design was not as desirable as a randomised prospective trial; however, the two groups were very similar regarding all aspects known to influence vitamin A status, and thus they were comparable. Corticosteroids are known to raise plasma retinol concentration. In this study only one infant in each group received corticosteroids for a short period of three days, so the plasma retinol concentration at discharge in this regard, probably reflects true vitamin A status. Another limitation of this study is that only two blood samples were collected, one at inclusion (median 6 days of age) and the second at discharge (median 61 days of age).

6. Conclusion

The modified regimen improved the plasma concentration of vitamin A among VLBW infants at discharge compared to the reference protocol. The majority of infants in the reference group had plasma retinol concentrations below 0.35 μM compared to the modified group. This indicates lower hepatic stores in the reference group compared to the modified group and a more optimal vitamin A status in the latter. However, most infants in both groups had plasma retinol levels below 0.70 μM. More studies are needed to establish the optimal plasma concentration for preterm infants and how this can be achieved.

The status of vitamin D measured as plasma 25(OH)vitamin D concentration, improved in VLBW infants in the modified group compared to infants in the reference group. Infants in the modified group received smaller doses and obtained reference plasma levels as compared to infants on the reference protocol. Vitamin K status, measured as plasma phylloquinone concentration, did not significantly improve in infants in the modified protocol compared to those in the reference regimen. Vitamin E status, measured as plasma α-tocopherol concentration, was not significantly different in the two groups.

7. References

(1) Henriksen C, Helland IB, Ronnestad A, Gronn M, Iversen PO, Drevon CA. Fat-soluble vitamins in breast-fed preterm and term infants. Eur J Clin Nutr 2006 Feb 1;60(6):756-62.

(2) King C. Preterm and low birthweight nutrition. In: Shaw V, Lawson M, editors. Clinical paediatric dietetics. 2nd ed. Oxford: Blackwell Science; 2001. p. 53-66.

(3) Georgieff MK, Hoffman JS, Pereira GR, Bernbaum J, Hoffman-Williamson M. Effect of neonatal caloric deprivation on head growth and 1-year developmental status in preterm infants. J Pediatr 1985 Oct;107(4):581-7.

(4) Powls A, Botting N, Cooke RW, Pilling D, Marlow N. Growth impairment in very low birthweight children at 12 years: correlation with perinatal and outcome variables. Arch Dis Child Fetal Neonatal Ed 1996 Nov;75(3):F152-F157.

(5) Dewey K, Lutter C. Guiding principles for complementary feeding of the breastfed child. Washington DC, USA: WHO Division of Health Promotion and Protection, Food and Nutrition Program; 2006.

(6) Clarence W, Gowen Jr. Fetal and neonatal medicine. In: Kliegman RM, Marcdante KJ, Jenson HB, Behrman RE, editors. Nelson Essentials of Pediatrics. 5th ed. Philadelphia: Saunders; 2006. p. 271-336.

(7) Finne PH. Propedeutisk pediatri: vekst, utvikling, ernæring. Oslo: Universitetsforl.; 2001.

(8) Harding J. Neonatal problems: low birth weight, prematurity and jaundice in infancy. In: Roberton DM, Robinson MJ, editors. Practical paediatrics.New York: Churchill Livingstone; 2003. p. 317-27.

(9) de Vries LS, Rennie JM. Neurological problems in the newborn. In: Rennie JM, editor. Textbook of Neonatology. Elsevier Churchill Livingstone; 2005. p. 1148-69.

(10) Fewtrell M, Lucas A. Infant feeding. In: Rennie JM, editor. Roberton's Textbook of Neonatology.Philadelphia: Elsevier Churchill Livingstone; 2006. p. 281-324.

(11) Uauy RD, Fanaroff AA, Korones SB, Phillips EA, Phillips JB, Wright LL. Necrotizing enterocolitis in very low birth weight infants: biodemographic and clinical correlates. National Institute of Child Health and Human Development Neonatal Research Network. J Pediatr 1991 Oct;119(4):630-8.

(12) McKeown RE, Marsh TD, Amarnath U, Garrison CZ, Addy CL, Thompson SJ, Austin JL. Role of delayed feeding and of feeding increments in necrotizing enterocolitis. J Pediatr 1992 Nov;121(5 Pt 1):764-70.

(13) Tyson JE, Kennedy KA. Minimal enteral nutrition for promoting feeding tolerance and preventing morbidity in parenterally fed infants. Cochrane Database of Syst Rev 2005;(3):CD000504.

(14) Schanler RJ, Shulman RJ, Lau C, Smith EO, Heitkemper MM. Feeding strategies for premature infants: randomized trial of gastrointestinal priming and tube-feeding method. Pediatrics 1999 Feb;103(2):434-9.

(15) Carroll J, Slobodzian R, Steward DK. Extremely low birthweight infants: issues related to growth. MCN Am J Matern Child Nurs 2005 Sep;30(5):312-8.

(16) American Academy of Pediatrics. Nutritional needs of the preterm infant. In: Kleinman RE, editor. Pediatric nutrition handbook. 5th ed. Washington, D.C.: American Academy of Pediatrics; 2004. p. 23-54.

(17) Ziegler EE, Thureen PJ, Carlson SJ. Aggressive nutrition of the very low birthweight infant. Clin Perinatol 2002 Jun;29(2):225-44.

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

(19) Niklasson A, Engstrom E, Hard AL, Wikland KA, Hellstrom A. Growth in very preterm children: a longitudinal study. Pediatr Res 2003 Dec;54(6):899-905.

(20) Elgen I, Johansson KA, Markestad T, Sommerfelt K. A non-handicapped cohort of low-birthweight children: growth and general health status at 11 years of age. Acta Paediatr 2005 Sep;94(9):1203-7.

(21) Tsang RC. Nutritional needs of the preterm infant : scientific basis and practical guidelines. 1st ed. New York: Caduceus Medical Publishers; 1993.

(22) Heird WC, Driscoll JM, Jr., Schullinger JN, Grebin B, Winters RW. Intravenous alimentation in pediatric patients. J Pediatr 1972 Mar;80(3):351-72.

(23) Fewtrell M, Lucas A. Enteral feeding of the preterm infant. Current Paediatrics 2002 Apr;12(2):98-103.

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

(25) 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 2006 Mar;148(3):300-5.

(26) De CM, Vetrano G. [Absorption of proteins, carbohydrates and fats in the preterm neonate]. Pediatr Med Chir 1986;8:437-42.

(27) Hamosh M, Mehta NR, Fink CS, Coleman J, Hamosh P. Fat Absorption in Premature Infants: Medium-Chain Triglycerides and Long-Chain Triglycerides Are Absorbed From Formula at Similar Rates. J Pediatr Gastroenterol Nutr 1991 Aug;13(2):143-9.

(28) Sulkers EJ, Goudoever JB, Leunisse C, Wattimena JLD, Sauer PJJ. Comparison of Two Preterm Formulas With or Without Addition of Medium-Chain Triglycerides (MCTs). I: Effects on Nitrogen and Fat Balance and Body Composition Changes. J Pediatr Gastroenterol Nutr 1992 Jul;15(1):34-41.

(29) 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 1987 Nov;111(5):767-73.

(30) Berseth CL. Neonatal small intestinal motility: motor responses to feeding in term and preterm infants. J Pediatr 1990 Nov;117(5):777-82.

(31) Yu VY. Extrauterine growth restriction in preterm infants: importance of optimizing nutrition in neonatal intensive care units. Croat Med J 2005;46(5):737-43.

(32) Cairns P. Parenteral nutrition. In: Rennie JM, editor. Roberton's Textbook of Neonatology.Philadelphia: Elsevier Churchill Livingstone; 2006. p. 325-54.

(33) Lucas A. AIDS and human milk bank closures. Lancet 1987 May 9;1(8541):1092-3.

(34) Hylander MA, Strobino DM, Dhanireddy R. Human milk feedings and infection among very low birth weight infants. Pediatrics 1998;102(3):E38.

(35) Horwood LJ, Darlow BA, Mogridge N. Breast milk feeding and cognitive ability at 7-8 years. Arch Dis Child 2001 Jan;84(1):F23-F27.

(36) Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. Lancet 1992 Feb 1;339(8788):261-4.

(37) Schanler RJ. The use of human milk for premature infants. Pediatr Clin North Am 2001 Feb;48(1):207-19.

(38) Ong DE, Page DL. Cellular retinol-binding protein (type two) is abundant in human small intestine. J Lipid Res 1987 Jun;28(6):739-45.

(39) Harrison EH, Hussain MM. Mechanisms involved in the intestinal digestion and absorption of dietary vitamin A. J Nutr 2001 May;131(5):1405-8.

(40) Biesalski HK, Nohr D. Importance of vitamin-A for lung function and development. Mol Aspects Med 2003 Dec;24(6):431-40.

(41) Perrotta S, Nobili B, Rossi F, Di PD, Cucciolla V, Borriello A, Oliva A, Della RF. Vitamin A and infancy. Biochemical, functional, and clinical aspects. Vitam Horm 2003;66:457-591.

(42) Ross AC. Vitamin A and retinoids. In: Shils ME, editor. Modern nutrition in health and disease. 9th ed. Baltimore: Williams & Wilkins; 1999. p. 305-28.

(43) Shenai JP. Vitamin A supplementation in very low birth weight neonates: rationale and evidence. Pediatrics 1999 Dec;104(6):1369-74.

(44) Mactier H, Weaver LT. Vitamin A and preterm infants: what we know, what we don't know, and what we need to know. Arch Dis Child Fetal Neonatal Ed 2005 Mar;90(2):F103-F108.

(45) Greer FR. Fat-soluble vitamin supplements for enterally fed preterm infants. Neonatal Netw 2001 Aug;20(5):7-11.

(46) de Pee S, Dary O. Biochemical indicators of vitamin A deficiency: serum retinol and serum retinol binding protein. J Nutr 2002 Sep;132(9 Suppl):2895S-901S.

(47) Inder TE, Graham PJ, Winterbourn CC, Austin NC, Darlow BA. Plasma vitamin A levels in the very low birthweight infant - relationship to respiratory outcome. Early Hum Dev 1998 Sep;52(2):155-68.

(48) Nutrition and feeding of preterm infants. Committee on Nutrition of the Preterm Infant, European Society of Paediatric Gastroenterology and Nutrition. Acta Paediatr Scand Suppl 1987;336:1-14.

(49) Darlow BA, Graham PJ. Vitamin A supplementation for preventing morbidity and mortality in very low birthweight infants. Cochrane Database Syst Rev 2002;(4):CD000501.

(50) Tyson JE, Wright LL, Oh W, Kennedy KA, Mele L, Ehrenkranz RA, et al. Vitamin A supplementation for extremely-low-birth-weight infants. National Institute of Child Health and Human Development Neonatal Research Network. N Engl J Med 1999 Jun 24;340(25):1962-8.

(51) Ambalavanan N, Kennedy K, Tyson J, Carlo WA. Survey of vitamin A supplementation for extremely-low-birth-weight infants: Is clinical practice consistent with the evidence? J Pediatr 2004 Sep;145(3):304-7.

(52) DeLuca HF. The vitamin D story: a collaborative effort of basic science and clinical medicine. FASEB J 1988;2(3):224-36.

(53) Salle BL, Delvin EE, Lapillonne A, Bishop NJ, Glorieux FH. Perinatal metabolism of vitamin D. Am J Clin Nutr 2000 May;71(5 Suppl):1317S-24S.

(54) Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 2006 Dec 20;296(23):2832-8.

(55) Atkinson SA, Reinhardt TA, Hollis BW. Vitamin D activity in maternal plasma and milk in relation to gestational stage at delivery. Nutr Res 1987;7(10):1005-11.

(56) Glorieux FH, Salle BL, Delvin EE, David L. Vitamin D metabolism in preterm infants: serum calcitriol values during the first five days of life. J Pediatr 1981 Oct;99(4):640-3.

(57) Delvin EE, Glorieux FH, Salle BL, David L, Varenne JP. Control of vitamin D metabolism in preterm infants: feto-maternal relationships. Arch Dis Child 1982 Oct;57(10):754-7.

(58) Koo WW, Krug-Wispe S, Neylan M, Succop P, Oestreich AE, Tsang RC. Effect of three levels of vitamin D intake in preterm infants receiving high mineral-containing milk. J Pediatr Gastroenterol Nutr 1995 Aug;21(2):182-9.

(59) Porcelli P, Schanler R, Greer F, Chan G, Gross S, Mehta N, et al. Growth in human milk-Fed very low birth weight infants receiving a new human milk fortifier. Ann Nutr Metab 2000;44(1):2-10.

(60) Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr 1999 May;69(5):842-56.

(61) Nako Y, Tomomasa T, Morikawa A. Risk of hypervitaminosis D from prolonged feeding of high vitamin D premature infant formula. Pediatr Int 2004;46(4):439-43.

(62) Backstrom MC, Maki R, Kuusela AL, Sievanen H, Koivisto AM, Ikonen RS, Kouri T, Maki M. Randomised controlled trial of vitamin D supplementation on bone density and biochemical indices in preterm infants. Arch Dis Child Fetal Neonatal Ed 1999 May;80(3):F161-F166.

(63) Burton GW, Traber MG. Vitamin E: antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr 1990;10:357-82.

(64)   Traber MG. Vitamin E. In: Shils ME, editor. Modern nutrition in health and disease. 9th ed. Baltimore: Williams & Wilkins; 1999. p. 347-62.

(65)   Dillard CJ, Gavino VC, Tappel AL. Relative antioxidant effectiveness of alpha-tocopherol and gamma-tocopherol in iron-loaded rats. J Nutr 1983 Nov;113(11):2266-73.

(66)   Zipursky A. Vitamin E deficiency anemia in newborn infants. Clin Perinatol 1984 Jun;11(2):393-402.

(67)   Delvin EE, Salle BL, Claris O, Putet G, Hascoet JM, Desnoulez L, Messai S, Levy E. Oral Vitamin A, E and D Supplementation of Pre-Term Newborns either Breast-Fed or Formula-Fed: a 3-Month Longitudinal Study. J Pediatr Gastroenterol Nutr 2005 Jan;40(1):43-7.

(68)   Ronnholm KA, Dostalova L, Siimes MA. Vitamin E supplementation in very-low-birth-weight infants: long-term follow-up at two different levels of vitamin E supplementation. Am J Clin Nutr 1989 Jan;49(1):121-6.

(69)   Hittner HM, Godio LB, Rudolph AJ, Adams JM, Garcia-Prats JA, Friedman Z, Kautz JA, Monaco WA. Retrolental fibroplasia: efficacy of vitamin E in a double-blind clinical study of preterm infants. N Engl J Med 1981 Dec 3;305(23):1365-71.

(70)   Ehrenkranz RA, Bonta BW, Ablow RC, Warshaw JB. Amelioration of bronchopulmonary dysplasia after vitamin E administration. A preliminary report. N Engl J Med 1978 Sep 14;299(11):564-9.

(71)   Brion L, Bell E, Raghuveer T. Vitamin E supplementation for prevention of morbidity and mortality in preterm infants. Cochrane Database Syst Rev 2003;3:CD003665.

(72)   Farrell PM, Levine SL, Murphy MD, Adams AJ. Plasma tocopherol levels and tocopherol-lipid relationships in a normal population of children as compared to healthy adults. Am J Clin Nutr 1978 Oct;31(10):1720-6.

(73)   Olson RE. Vitamin K. In: Shils ME, editor. Modern nutrition in health and disease. 9th ed. Baltimore: Williams & Wilkins; 1999. p. 363-80.

(74)   Greer FR, Mummah-Schendel LL, Marshall S, Suttie JW. Vitamin K1 (phylloquinone) and vitamin K2 (menaquinone) status in newborns during the first week of life. Pediatrics 1988 Jan;81(1):137-40.

(75)   Hathaway WE. New insights on vitamin K. Hematol Oncol Clin North Am 1987 Sep;1(3):367-79.

(76)   Tandoi F, Mosca F, Agosti M. Vitamin K prophylaxis: leaving the old route for the new one? Acta Paediatr Suppl 2005 Oct;94(449):125-8.

(77) Greer FR. Vitamin K status of lactating mothers and their infants. Acta Paediatr Suppl 1999 Aug;88(430):95-103.

(78) Clarke P, Mitchell SJ, Sundaram S, Sharma V, Wynn R, Shearer MJ. Vitamin K status of preterm infants with a prolonged prothrombin time. Acta Paediatr 2005 Dec;94(12):1822-4.

(79) Greene HL, Hambidge KM, Schanler R, Tsang RC. Guidelines for the use of vitamins, trace elements, calcium, magnesium, and phosphorus in infants and children receiving total parenteral nutrition: report of the Subcommittee on Pediatric Parenteral Nutrient Requirements from the Committee on Clinical Practice Issues of the American Society for Clinical Nutrition. Am J Clin Nutr 1988 Nov;48(5):1324-42.

(80) Cornelissen M, von KR, Loughnan P, Schubiger G. Prevention of vitamin K deficiency bleeding: efficacy of different multiple oral dose schedules of vitamin K. Eur J Pediatr 1997 Feb;156(2):126-30.

(81) Andrew M, Paes B, Milner R, Johnston M, Mitchell L, Tollefsen DM, Castle V, Powers P. Development of the human coagulation system in the healthy premature infant. Blood 1988 Nov;72(5):1651-7.

(82) von KR, Gobel U. Oral vitamin K prophylaxis and late haemorrhagic disease of the newborn. Lancet 1994 Feb 5;343(8893):352.

(83) Food Composition Table. Oslo: Gyldendal undervisning; 2001.

(84) Saarela T, Kokkonen J, Koivisto M. Macronutrient and energy contents of human milk fractions during the first six months of lactation. Acta Paediatr 2005 Sep;94(9):1176-81.

(85) Austenaa LM, Carlsen H, Ertesvag A, Alexander G, Blomhoff HK, Blomhoff R. Vitamin A status significantly alters nuclear factor-kappaB activity assessed by in vivo imaging. FASEB J 2004;18(11):1255-7.

(86) Shenai JP, Chytil F, Jhaveri A, Stahlman MT. Plasma vitamin A and retinol-binding protein in premature and term neonates. J Pediatr 1981 Aug;99(2):302-5.

(87) Pearson E, Bose C, Snidow T, Ransom L, Young T, Bose G, Stiles A. Trial of vitamin A supplementation in very low birth weight infants at risk for bronchopulmonary dysplasia. J Pediatr 1992 Sep;121(3):420-7.

(88) Landman J, Sive A, Heese HD, Van der EC, Sacks R. Comparison of enteral and intramuscular vitamin A supplementation in preterm infants. Early Hum Dev 1992 Sep;30(2):163-70.

(89) Porcelli PJ, Greene H, Adcock E. A modified vitamin regimen for vitamin B2, A, and E administration in very-low-birth-weight infants. J Pediatr Gastroenterol Nutr 2004 Apr;38(4):392-400.

(90) Wardle SP, Hughes A, Chen S, Shaw NJ. Randomised controlled trial of oral vitamin A supplementation in preterm infants to prevent chronic lung disease. Arch Dis Child Fetal Neonatal Ed 2001 Jan;84(1):F9-F13.

(91) Kennedy KA, Stoll BJ, Ehrenkranz RA, Oh W, Wright LL, Stevenson DK, et al. Vitamin A to prevent bronchopulmonary dysplasia in very-low-birth-weight infants: has the dose been too low? The NICHD Neonatal Research Network. Early Hum Dev 1997 Jul 24;49(1):19-31.

(92) Rahmathullah L, Tielsch JM, Thulasiraj RD, Katz J, Coles C, Devi S, et al. Impact of supplementing newborn infants with vitamin A on early infant mortality: community based randomised trial in southern India. BMJ 2003 Aug 2;327(7409):254.

(93) Ambalavanan N, Wu TJ, Tyson JE, Kennedy KA, Roane C, Carlo WA. A comparison of three vitamin A dosing regimens in extremely-low-birth-weight infants. J Pediatr 2003 Jun;142(6):656-61.

(94) Ross AC, Ambalavanan N, Zolfaghari R, Li NQ. Vitamin A combined with retinoic acid increases retinol uptake and lung retinyl ester formation in a synergistic manner in neonatal rats. J Lipid Res 2006 May 9;47(8):1844-51.

(95) Markestad T, Aksnes L, Finne PH, Aarskog D. Plasma concentrations of vitamin D metabolites in premature infants. Pediatr Res 1984 Mar;18(3):269-72.

(96) Salle BL, Glorieux FH, Delvin EE, David LS, Meunier G. Vitamin D metabolism in preterm infants. Serial serum calcitriol values during the first four days of life. Acta Paediatr Scand 1983 Mar;72(2):203-6.

(97) Salle BL, Senterre J, Glorieux FH, Delvin EE, Putet G. Vitamin D metabolism in preterm infants. Biol Neonate 1987;52 Suppl 1:119-30.

(98) Widdowson EM, Southgate DA, Hey E. Fetal growth and body composition. In: Lindblad BS, editor. Perinatal nutrition.San Diego: Academic Press; 1988. p. 3-14.

(99) Lapillonne AA, Glorieux FH, Salle BL, Braillon PM, Chambon M, Rigo J, Putet G, Senterre J. Mineral balance and whole body bone mineral content in very low-birth-weight infants. Acta Paediatr Suppl 1994 Dec;405:117-22.

(100) Greer FR. Osteopenia of prematurity. Annu Rev Nutr 1994;14:169-85.

(101) Bronner F, Salle BL, Putet G, Rigo J, Senterre J. Net calcium absorption in premature infants: results of 103 metabolic balance studies. Am J Clin Nutr 1992 Dec;56(6):1037-44.

(102) Rosli A, Fanconi A. Neonatal hypocalcaemia. "Early type" in low birth weight newborns. Helv Paediatr Acta 1973 Nov;28(5):443-57.

(103) Zamora SA, Rizzoli R, Belli DC, Slosman DO, Bonjour JP. Vitamin D supplementation during infancy is associated with higher bone mineral mass in prepubertal girls. J Clin Endocrinol Metab 1999 Dec;84(12):4541-4.

(104) Evans JR, Allen AC, Stinson DA, Hamilton DC, St John BB, Vincer MJ, et al. Effect of high-dose vitamin D supplementation on radiographically detectable bone disease of very low birth weight infants. J Pediatr 1989 Nov;115(5 Pt 1):779-86.

(105) Dju MU, Filer LJJ. Vitamin E (tocopherol) in human fetuses and placentae. Etudes Neonatales 1952 Sep;1(3):49-62.

(106) Sokol RJ, Heubi JE, Iannaccone ST, Bove KE, Balistreri WF. Vitamin E deficiency with normal serum vitamin E concentrations in children with chronic cholestasis. N Engl J Med 1984 May 10;310(19):1209-12.

(107) Phillips B, Franck LS, Greene HL. Vitamin E levels in premature infants during and after intravenous multivitamin supplementation. Pediatrics 1987 Nov;80(5):680-3.

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

(109) Johnson L, Quinn GE, Abbasi S, Gerdes J, Bowen FW, Bhutani V. Severe retinopathy of prematurity in infants with birth weights less than 1250 grams: incidence and outcome of treatment with pharmacologic serum levels of vitamin E in addition to cryotherapy from 1985 to 1991. J Pediatr 1995 Oct;127(4):632-9.

(110) Gross SJ, Gabriel E. Vitamin E status in preterm infants fed human milk or infant formula. J Pediatr 1985 Apr;106(4):635-9.

(111) Huijbers WA, Schrijver J, Speek AJ, Deelstra BA, Okken A. Persistent low plasma vitamin E levels in premature infants surviving respiratory distress syndrome. Eur J Pediatr 1986 Aug;145(3):170-1.

(112) Kumar D, Greer FR, Super DM, Suttie JW, Moore JJ. Vitamin K status of premature infants: implications for current recommendations. Pediatrics 2001 Nov;108(5):1117-22.

(113) Costakos DT, Greer FR, Love LA, Dahlen LR, Suttie JW. Vitamin K prophylaxis for premature infants: 1 mg versus 0.5 mg. Am J Perinatol 2003 Nov;20(8):485-90.

(114) Greer FR, Marshall SP, Foley AL, Suttie JW. Improving the vitamin K status of breastfeeding infants with maternal vitamin K supplements. Pediatrics 1997 Jan;99(1):88-92.

(115) Roman E, Fear NT, Ansell P, Bull D, Draper G, McKinney P, et al. Vitamin K and childhood cancer: analysis of individual patient data from six case-control studies. Br J Cancer 2002 Jan 7;86(1):63-9.

(116) Raith W, Fauler G, Pichler G, Muntean W. Plasma concentrations after intravenous administration of phylloquinone (vitamin K(1)) in preterm and sick neonates. Thromb Res 2000 Sep 1;99(5):467-72.

(117) Allison AC. Danger of vitamin K to newborn. The Lancet 1955 Mar 26;265(6865):669.

(118) Benke PJ. The isotretinoin teratogen syndrome. JAMA 1984 Jun 22;251(24):3267-9.

8. Appendixes

Appendix 1: The Regional Ethics Committee Approval

Appendix 2: Information letter for parents with informed consent scheme

Appendix 3: Mother and child background information scheme

Appendix 4: Codes for KBS

Appendix 5: Example of text file in KBS

Appendix 6: Guidelines for enteral vitamin supplementation for the NICU

Appendix 7: Guidelines for nutritional intervention for the NICU

REGIONAL KOMITE FOR MEDISINSK FORSKNINGSETIKK

Helseregion Sør

Førsteamanuensis dr.med.
Per Ole Iversen
Institutt for ernæringsforskning
Universitetet i Oslo
Pb. 1046

Deres ref.: 20. februar 2002   **Vår ref.:** S-02023   **Dato:** 20.03.02

"Ernæring, vekst og utvikling blant svært premature barn".
Prosjektleder: Førsteamanuensis dr.med. Per Ole Iversen, Institutt for ernæringsforskning.
Universitetet i Oslo

Revidert pasientinformasjon

Vi takker for brev av 20. februar 2002 vedlagt revidert pasientinformasjon.

Komiteen finner at det er tatt hensyn til marknadene i komiteens brev av 06.02.02, og tilrår at
prosjektet gjennomføres.

Vi ønsker lykke til med prosjektet.

Vi beklager at svaret grunnet stor saksmengde, er blitt forsinket.

Med vennlig hilsen

Sigurd Nitter-Hauge (sign)
professor dr.med.
leder

Ola P. Hole
avdelingsleder
sekretær

Appendix 2

ERNÆRING, VEKST OG UTVIKLING BLANT PREMATURE BARN

Vi holder på med en studie av ernæring, vekst og utvikling blant 200 premature (for tidlig fødte) barn med fødselsvekt under 1500 gram. Alle barn som blir født eller innlagt ved Rikshospitalet, Sykehuset i Buskerud, Sykehuset i Vestfold eller Akershus Universitetssykehus i perioden 15.11.2003 til 15.11.2005 blir invitert til å delta i studien. Deltagelse er frivillig. Studien er vurdert og anbefalt av regional etisk komite.

Bakgrunn:

Små, premature barn har et annerledes og høyere næringsbehov enn barn som er født til termin. Deres eksakte behov er fortsatt ukjent. Det er vanlig at barna får intravenøs ernæring (dvs. direkte i blodbanen) den første tiden. Mengden morsmelk trappes gradvis opp, i den takt som barnet tåler. Morsmelken gis via en sonde (et lite rør til magesekken) til barnet er i stand til å suge selv.

Det er enighet om at morsmelk er den beste ernæring for premature barn, men morsmelk alene dekker likevel ikke næringsbehovet for de aller minste. Derfor er det vanlig å tilsette proteiner og mineraler til morsmelken. Det er også vanlig å gi tilskudd av vitaminer, jern og folsyre

Dersom det er behov for ekstra energi gis dette vanligvis i form av karbohydrater eller en blanding av karbohydrater/fett. Vi vet ikke nok om hvilken type fettsyrer som er best for premature barn på lang sikt. Formålet med denne studien er å sammenligne to ulike fettsyretilsetninger mens barnet ligger på sykehuset. Begge tilskuddene inneholder fettsyrer som er viktige for barnets vekst og utvikling, og som finnes naturlig i morsmelk.

Hva innebærer dette for dere:

- Moren vil bli spurt om sitt kosthold, bruk av kosttilskudd og røyking i svangerskapet
- Etter fødselen vil det bli tatt en blodprøve fra navlestrengen
- Barnet vil i tillegg til den gjeldende behandling få *en av to typer* vegetabilsk oljeblanding tilsatt i melken
- Journalopplysninger om barnets inntak, vekst og sykdommer blir benyttet
- Det vil bli tatt en prøve av morsmelken (ca 25 ml)
- Det vil bli tatt blodprøve av barnet (ca 1 ml) to ganger under sykehusoppholdet
- Dere vil få tilbud om en tverrfaglig undersøkelse av barnets vekst og utvikling, inkludert synsfunksjon ved 6 og 20 måneders alder

Blodprøvene fra barnet og navlestrengsblodet vil bli analysert for fettsyremønster, fettløselige vitaminer og antioksidanter. Morsmelken vil bli analysert for fettsyremønster for å beregne barnas inntak av fettsyrer.

Hva får dere igjen for å delta:

- Alle barna vil få et ekstra energitilskudd, som kan være gunstig for vekst og utvikling hos barna.
- Dere bidrar til at vi i fremtiden vil kunne gi bedre råd om ernæring til premature barn.

Behandling av data

Dere kan trekke dere fra studien når som helst uten å måtte begrunne dette nærmere.
Alle data vil bli behandlet i samsvar med Datatilsynets regler. Blodprøvene vil ikke blir brukt
til annet enn det som er beskrevet over. Alle persondata og eventuelt overflødig biologisk
materiale vil bli ødelagt etter at studien er avsluttet.

Din kontaktperson er:
Christine Henriksen, Institutt for ernæringsforskning
Telefon 22 85 15 26
E-post: christine.henriksen@basalmed.uio.no

Prosjektledere er:
Professor Christian A. Drevon og professor Per Ole Iversen,
Institutt for ernæringsforskning

SAMTYKKE

Undertegnede har fått skriftlig og muntlig informasjon om studien "Ernæring, vekst og
utvikling blant premature barn" og samtykker i deltagelse.

Morens navn: _____

Adresse:_____

Telefon, hjem:_____

Telefon, mobil:_____

Oslo / -2005 _____

Appendix 3

Fylles ut for alle deltagere i ernæringsstudien

Nummer: _____

A. Sjekkliste

	Utført
Underskrift samtykkeskjema	
Fyllt ut opplysninger om mor og barn (se baksiden)	
Levert spørreskjema om matvaner til mor *NB! Noter nummeret*	
Mottatt spørreskjema om matvaner	
Levert flaske til mormsmelksprøve	
Mottatt morsmelksprøve (4 uker)	
Gitt beskjed til behandlende sykepleier/lege og rekvirert blodprøve før oppstart av oljeblandingen (ved enteralt inntak 50-100ml melk/kg)	
Lagt bivirkningsskjema i journalen	
Gitt beskjed til melkekjøkken om nummer på flasken og oppstartsdato	
Ved evn overflytting til annet sykehus: • Gitt beskjed til lege/sykepleier • Sendt flasken med oljeblandingen	
Bedt behandlende lege om å rekvirert blodprøve før utskriving	
Kopiert kurve (inkl. bivirkningsskjemaet) og epikrise	

B. Opplysninger om mor:

Spørsmål	Svar
Hva er ditt fødselsår?	
Hva er din høyeste fullførte utdanning?	1. Ungdomsskole (9 år eller mindre) 2. Fagbrev e.l. (10-11 år) 3. Videregående skole (12 år) 4. Høyskole/universitet: (over 12 år)
Hvor ofte røkte du sigaretter under svangerskapet?	1. Aldri: 2. 1-9 ganger: 3. Flere ganger i måneden: 4. 1 gang i uken 5. 2-6 ganger i uken 6. Daglig _____ (antall)

B. Opplysninger fra barnets journal

Barnets navn:_____

Barnets fødselsdato:_____

Kjønn:_____

Navlestrengsblod ble tatt (ja/nei):_____

Hvis ikke, angi årsak:_____

Fødselesvekt:_____

Gestasjonsalder:_____

Appendix 4

KODER for ernæringsprodukter brukt til premature barn:

- 5173 Nan Ha 1, pulver
- 5174 Nan Ha 1, drf
- 5180 Nan 1, pulver
- 5181 Nan 1, drf
- 8078 Semper Energi
- 9705 Biovit
- 9743 Multibionta
- 129001 Glukose 10 %
- 129002 Glukose 5 %
- 129003 Pedamix
- 129004 Vaminolac
- 129005 Intralipid
- 129006 Vitalipid*Soluvit (Rikshopitalets løsning)
- 129007 Vitalipid blanding (A-hus)
- 129008 Morsmelk
- 129009 Bankmelk
- 129011 Prenan, pulver
- 129012 Prenan HY, pulver
- 129013 1/1 presemp + morsmelk
- 129014 ½ presemp + morsmelk
- 129015 1/1 presemp + bankmelk
- 129016 ½ presemp + bankmelk
- 129017 Presemp per pose
- 129018 Super Soluble Duocal
- 129020 Duocal MCT, pulver
- 129022 Nycoplus Multi, flyt
- 129023 Neo-fer, flyt
- 129024 Folsyre, flyt
- 129025 Vit E, flyt (kodes som mg, 15 mg = 0,3 ml)
- 129026 Vit K, flyt (kodes som mg: 1 ml = 20 mg, 0,5 mg = 0,025 ml)
- 129027 Nutramigen, pulver
- 129028 Neocate, pulver
- 129029 Profylac, pulver
- 129030 Colett omega 3, flyt
- 129031 Neocate, drf
- 129032 Profylac, drf
- 129033 Prenan HY, drf
- 129034 Prenan, drf
- 129035 1/1 Enfamil + morsmelk
- 129036 ½ Enfamil + morsmelk
- 129037 1/1 Enfamil + bankmelk
- 129038 ½ Enfamil + bankmelk
- 129039 Glukose 20%
- 129040 Glukose 15 %
- 129041 Enfamil HMF per pose
- 129042 MCT-olje
- 129043 Nutramigen, drf
- 129044 Soluvit løst i intralipid (A-Hus blanding)
- 129045 per 4 poser Enfamil HMF
- 129046 Olivenolje (brukes på A-hus)
- 129047 Soyaolje Mills (brukes telemark)

Appendix 5

Eksempel fra tekstfilen der barnas næringsinntak ble registrert

P 65001 1 xx.xx.xx 4 1460 1 (P = personlinje: randomiseringsnr 65, dag 001, fødselsdato xx.xx.xx, sykehusnr, fødselsvekt)

K 1 (Daglinje: dag 1)

M 129009 59 (Måltidslinje: barnet fikk 129009 som er bankemelk og mengden var 59 ml)

P 65002 1 xx.xx.xx 4 1460 1

K 2

M 129009 135 9743 0.5 129024 0.5 129025 15

P 65003 1 xx.xx.xx 4 1460 1

K 3

M 129009 152 9743 0.5 129024 0.5 129025 15

P 65004 1 xx.xx.xx 4 1460 1

K 4

M 129009 157 9743 0.5 129024 0.5 129025 15 129049 0.79

P 65005 1 xx.xx.xx 4 1460 1

K 5

M 129008 168 9743 0.5 129024 0.5 129025 15 129049 0.84

P 65006 1 xx.xx.xx 4 1460 1

K 6

M 129008 199 9743 0.5 129024 0.5 129025 15 129049 0.99

P 65007 1 xx.xx.xx 4 1460 1

K 7

M 129008 232 9743 0.5 129024 0.5 129025 15 129049 1.16

P 65008 1 xx.xx.xx 4 1460 1

K 8

M 129008 232 9743 0.5 129024 0.5 129025 15 129049 1.16

P 65009 1 xx.xx.xx 4 1460 1

K 9

M 129008 266 9743 0.5 129024 0.5 129025 15 129049 1.33

P 65010 1 xx.xx.xx 4 1460 1

K 10

M 129008 280 9743 0.5 129024 0.5 129025 15 129049 1.4

P 65011 1 xx.xx.xx 4 1460 1

K 11

M 129036 280 9743 0.5 129024 0.5 129025 15 129049 1.4

P 65012 1 xx.xx.xx 4 1460 1

K 12

M 129035 305 9743 0.5 129024 0.5 129025 15 129049 1.53

P 65013 1 xx.xx.xx 4 1460 1

K 13

M 129035 320 9743 0.5 129024 0.5 129025 15 129049 1.6

P 65014 1 xx.xx.xx 4 1460 1

K 14

M 129035 332 129024 0.25 129025 10 129049 1.66

P 65015 1 xx.xx.xx 4 1460 1

K 15

M 129035 336 129024 0.25 129025 10 129049 1.68

P 65016 1 xx.xx.xx 4 1460 1

K 16

M 129035 335 129024 0.25 129025 10 129049 1.68

P 65017 1 xx.xx.xx 4 1460 1

Appendix 6

Ernæringsprosjekt:

Resulterer i følgende næringstilskudd -

Vitamintilskudd per os:	
Vitamin E (50 mg/ml)	Start tilskudd samtidig med oppstart av Enfamil HMF, i redusert dose : 0.2 ml/dag. Barn som ikke får PN, kan starte med red. tilskudd av vitamin E som tidligere på dag 1.
Folsyre (0.2 mg/ml)	Start med tilskudd samtidig med oppstart av Enfamil HMF, i redusert dose : 0.25 ml/dag
Parenteralt vitamintilskudd:	
Vitalipid Infant – blandes i Intralipid	
Soluvit – løses i 10 ml Intralipid, og 1 ml/kg gis	

Se eget skjema for beregning av vitamininntak

Appendix 7

Ernæringsprosjekt:

Opptrapping av parenteral og enteral ernæring

n = dag nr når morsmelk 120 ml/kg/døgn eller mer

Dag nr / iv ml/kg/døgn	1	2	3	→ n	n + 1	n+2
Intralipid	2,5	5	10	10-15	5	0
Vitalipid infant	1	2	4	4	2	0
Soluvit	1	1	1	1	1	
Glukose/Pedamix iv	Rest av døgnvolum				60	nedtrapping

Dag nr / per os (po)	1	2	3	→ n	n + 1	n+2
Vitamin E (50 mg/ml)					0.2	0.2
Folsyre (0.2 mg/ml)					0.25	0.25
Jern (9 mg/ml)	Oppstart fra ønsket tidspunkt				0.75 2	
Morsmelk po	2-120 **				0	0
Morsmelk med HMF*					120	opptrapping
* antall poser/25 ml					1/2	1
** forslagsvis						

totalt	Dag nr	1	2	3	→ n	n + 1	n+2
Resulterer i følgende næringstilskudd							
Vitamin A	iv ug/kg	69	138	276	276	138	
	po ug/100 ml mm m/HMF					145	290
Til beregning							
Vitamin E	iv mg/kg	0.6	1.3	2.6	2.6	1.3	
	po mg/100 ml mm m/HMF					1.6	3.1
	po mg tilskudd (50 mg/ml)					10	10
Til beregning							
Folsyre	iv ug/kg	40	40	40	40	40	
	po ug/100 ml mm m/HMF					12.5	25
	po ug tilskudd (0.2 mg/ml)					50	50
Til beregning							
PN fett g/kg		0.6	1.2	2.4	3.4	1.2	

SGA-barn: må vurderes nøye, ingen oppstart av tilsetninger før på fullt melkeinntak