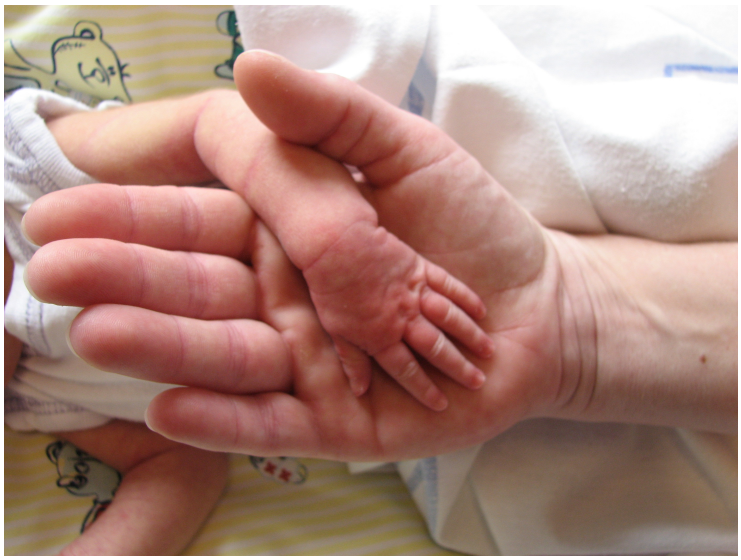
Ph.D. Thesis

Nutrition, Growth, and Allergic Diseases among Very Preterm Infants after Hospital Discharge



Gitte Zachariassen

Hans Christian Andersen Children's Hospital,
Odense University Hospital, Denmark
Faculty of Health Sciences
University of Southern Denmark

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SYDDANSK UNIVERSITET
UNIVERSITY OF SOUTHERN DENMARK

Preface

This Ph.D. thesis presents results obtained from a randomized controlled trial (RCT) on post discharge nutrition for very preterm infants and was initiated in collaboration with neonatologists from the Neonatal Units at four Paediatric Departments in Denmark. The trial went on from July 2004 – August 2008.

The trial has been carried out at the Departments of Paeditric at Holbaek Hospital, Kolding Hospital, Aarhus University Hospital at Skejby, and at Hans Christian Andersen Children's Hospital at Odense University Hospital in Odense, Denmark.
The present work was conducted during my employment as a research assistant at The Paediatric Research Unit at Hans Christian Andersen Children's Hospital, Odense University Hospital, Denmark, and the Institute of Clinical Research, Faculty of Health Sciences, University of Southern Denmark from March 2007 to May 2010.

This Ph.D. thesis is based on following papers.

1. Factors associated with successful establishment of breastfeeding in very preterm infants. Acta Paediatr. 2010 Jul;99(7):1000-4. Epub 2010 Feb 11.
2. Nutrient Enrichment of Mother's Milk and Growth of Very Preterm Infants after Hospital Discharge. Pediatrics. 2011 Apr;127(4):e995-e1003. Epub 2011 Mar 14.
3. Allergic diseases among very preterm infants according to nutrition after hospital discharge. Pediatric Allergy and Immunology. 2011 Feb 20. Epub ahead of print.

As supplemental material, data for two planned manuscripts are presented.

4. Protein-content in human milk from mothers of very preterm infants.
5. Feeding-pattern and -problems among very preterm infants according to nutrition - from hospital discharge until introduction of complementary feeding.

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Mead Johnson Nutritionals have donated the products used in the clinical trial.

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Abbreviations and definitions

AD	Atopic dermatitis defined as areas of scaly, erythematous, and itchy eczematous rash for at least 3 months
AGA	Appropriate for gestational age (-2 SDS > BW Z-score < 2 SDS)
Allergic/atopic symptoms	Bronchial asthma, recurrent wheezing (wheezy bronchitis), atopic dermatitis, allergic rhinoconjunctivitis, allergic urticaria, and/or food allergy
Allergic disease	Hypersensitivity reaction initiated by immunological mechanism(s), may be IgE-mediated or non-IgE-mediated
Atopic predisposition	At least one first-degree relative with documented atopic disease
BUN	Blood-urea nitrogen
BW	Birth weight in grams
Catch-up growth	Accelerated rates of growth following a period of growth failure in order to reach the growth reference of normal preterm or term infants
CA	Corrected age (weeks, months, or year after term)
CF	Complementary feeding
CI	Confidence interval
CMPA	Cow's milk protein allergy defined as proven or likely immunological mediated reaction to cow's milk protein diagnosed by controlled elimination / challenge procedures
CMP	Cow's milk protein
DEXA	Dual Energy X-ray Absortiometry
GA	Gestational age at birth (weeks and days)
HbA1C	Glycohemoglobin (haemoglobin A (1C))
HbF	Haemoglobin F (fetal haemoglobin)
HC	Head circumference (cm or mm)
HMA	Human milk analyzer
HMF	Human milk fortifier
IgE	Immunoglobulin E
ITT	Intention to treat
LBW	Low birth weight (weighing less than 2500g at birth)

MAP	Mean arterial blood pressure
Mix	Both breast- and bottle-feeding
MF	Mature or term formula
NEC	Necrotising enterocolitis
NICU	Neonatal intensive care unit
Non-SGA	BW Z-score > -2 SDS (in this study equivalent to AGA)
OR	Odds Ratio
PF	Preterm or premature formula
PMA	Postmenstrual age (GA + weeks and /or days since birth)
PP	Per protocol
RCT	Randomized controlled trial
REM	Random effect model
RW	Recurrent wheezing: at least two episodes of wheezing requirering bronchodilatator treatment and diagnosed by a physician
SD	Standard deviation
SGA	Small for gestational age (BW Z-score ≤ -2 SDS according to a reference)
S-IgE	Allergen Specific Immunoglobulin E
SPT	Skin prick test
Term	280 days PMA
UHM	Unfortified human milk
VPI	Very preterm infants (GA ≤ 32+0)
VLBW	Very low birth weight (weighing less than 1500g at birth)
Z-score	Standard deviation score (SDS). The difference between the actual growth and the expected reference growth divided with one standard deviation (ex.: (BW – reference BW)/1 SD)

1. Introduction

Despite advances in perinatal care, with improvements in lowering morbidity and mortality and advances in nutrition, growth failure remains a major problem for preterm infants who need neonatal intensive care (1;2). Once respiratory status is stabilized, nutrition becomes the most urgent challenge facing clinicians caring for high-risk newborns admitted to the neonatal intensive care unit (NICU) (3).
Growth and the accumulation of nutrient reserves are higher during the second and third trimester of pregnancy than at any other time during life, and it is essential to develop strategies to feed preterm infants in order to maintain a goal of normal in *utero* growth rates. Feeding less will continue to produce growth-restricted infants with limited growth- and developmental capacity particularly of the brain and its many essential functions. Feeding more may result in obesity that can have harmful consequences (4).

Agreement on recommendations about optimal nutrition and growth of very low birth weight (VLBW) preterm infants after hospital discharge has not yet been achieved. Close monitoring of growth after hospital discharge has been recommended, and if the infant is discharged with subnormal weight for age supplementation has been recommended.(5;6). A nutrient enriched formula can be used for non-breastfed very preterm infants, but the nutritional intervention with fortification of mother's milk for breastfed preterm infants after hospital discharge has not yet been proven optimal as a feeding strategy as regards growth and neurological development. The risk of developing allergic diseases due to exposure to allergens such as cow's milk protein is also unknown among very preterm infants.

Solely breastfeeding of very preterm infants at and beyond hospital discharge is a common practice in Denmark. Therefore, a randomized controlled trial (RCT) to investigate the effect of nutrient enrichment of mother's own milk while breastfeeding after hospital discharge, and at the same time characterizing very preterm infants and their mothers at hospital discharge, and evaluating the incidence of allergic diseases among preterm infants within the first year of life, was planned.

2. Background

2.1. Growth among preterm infants

Most VLBW infants are discharged before they reach term postmenstrual age (PMA) and at the time of discharge, many very preterm infants have deficits in accretion of energy, protein, minerals, and other nutrients. Nutrient deficit already in the first weeks of life can be directly related to postnatal growth retardation (2;7), and at hospital discharge VLBW preterm infants have often not achieved the median birth weight of the reference *fetus* at the same postmenstrual age (1;8), and nutritional support might still be relevant at and beyond hospital discharge.

Preterm infants must achieve catch-up growth in order to attain the growth-parameters of term infants of the same PMA. Some catch-up growth is observed in most VLBW infants, although the rate and time of catch-up differs between studies (9;10). Low birth weight infants as a group do catch-up, but many remain smaller compared to infants of normal birth weight (11;12). Infants born small for gestational age (SGA) and VLBW infants show slower rates of catch-up growth compared to appropriate for gestational age (AGA) VLBW infants (9;10;13;14).

Improvement of growth among VLBW preterm infants can be achieved by feeding a nutrient enriched formula during hospitalization and after hospital discharge (15;16). Human milk has though shown to have many benefits – especially on the IQ among infants born SGA and VLBW preterm infants (17;18). But human milk requires nutrient fortification to meet the protein and mineral needs of the rapidly growing preterm infant during hospitalization (3).

2.2. Nutritional needs for preterm infants

Nutritional needs are higher for preterm infants than for term infants because of less stores, altered absorption, and rapid growth rates. Extremely low birth weight infants have greater nutritional needs than VLBW infants, and enterally fed infants have greater nutritional needs than parentally fed infants (3). Preterm infants loose approximately 10-20% of their birth weight during the first 4-6 days of life with an expectation that the weight will be regained by 2-3 weeks of age. The weight loss results mainly from a concentration in the extracellular fluid compartment and is more pronounced in preterm than in term infants (19).
Nutrition of the very preterm infant therefore is an urgent challenge, and shortly after birth parental nutrition in combination with minimal enteral nutrition, also called hypocaloric priming

or trophic feeding, has to be started (20;21). Immediately after delivery, the mother of the preterm infant is encouraged to start pumping her breast milk. Preterm infants are routinely tube fed with expressed milk from the mother until they are developmentally and physiologically ready to begin the process of learning to suck, swallow, and breathe in a coordinated fashion (22).

While human milk offers many advantages, the quantity of protein and minerals is inadequate for the growing preterm infants. The protein-content in human milk decreases within the first months after birth for both preterm and term infants (23). Feeding solely human milk therefore may lead to insufficient intake of protein and energy, and the use of fortified human milk improves adequate growth and satisfies the specific nutritional requirements of preterm infants (24). Commercial human milk fortifiers (HMF) are available and increase the nutrient density of human milk. Nutrients of particular interest for the preterm infant include e.g. protein, calcium, and phosphorus. Fortified human milk and preterm formulas (PF), when fed adequate volume, will meet most nutritional requirements (3). Cow's milk based fortifiers have been used for years in many NICU's for nutrient enrichment / fortification of human milk e.g. Enfamil® human milk fortifier was introduced in 1984 and was reformulated with a higher protein-level in 2002 (25). In general, human milk fortification is continued throughout hospitalization and is easy to administer as long as the infant has a nasal-gastric tube. At the time when the infant is fully breastfed and ready for discharge, human milk fortification becomes a challenge.

2.3. Breastfeeding preterm infants

Breastfeeding-rates of very preterm infants at and beyond hospital discharge are reported to vary considerably in the literature. This could possibly be explained by the duration of maternity leave, mothers opportunities for spending time in the hospital with their infant(s) and different policies for supporting breastfeeding. An American retrospective study from 2008 with a cohort of 361 mother-infant pairs reported that 60% provided expressed milk feeding for their VLBW infants, but only 27% provided direct breastfeeding (26). In a Swedish study from 2007, 53% of preterm infants (GA < 37 weeks) were exclusively breastfed at discharge from the neonatal unit. The rate of exclusively breastfeeding was though lower among the most immature infants (born with GA < 32 weeks) (27). A Danish study from 2007 with 77 very preterm infants (GA ≤ 30 weeks) found 62% to be exclusively breastfed, 19% to be bottle-fed, 16% to be both breast- and bottle-fed, and 3% to be bottle-fed and supplemented with parental nutrition at hospital discharge

(28). A study from the Danish national birth cohort reported an overall prevalence of breastfeeding among term and preterm infants to be 88% at 1 month of age and 69% at 4 months of age, while the prevalence among low birth weight (LBW) infants at 4 months of age (chronological) was 56% (29).

Preterm infants are as already described routinely tube fed until they are developmentally and physiologically ready to begin the process of learning to suck, swallow, and breathe in a coordinated fashion which often occurs at 32-35 weeks PMA (22). One study found that preterm infants allowed early non-nutritive suckling at the breast were able to demonstrate nutritive suckling (≥ 5g milk-volume by test-weighing) as early as 30.6 weeks PMA (30). The same author found in another study that full breastfeeding was attained at a median age of 35 weeks (range 32 to 38 weeks) among 15 preterm infants (31). Kangaroo-care dyads have been found to breastfeed more exclusively and for a longer period compared to less or none skin-to-skin contact (32). Kangaroo-care with early skin-to-skin contact as early as possible is a routine in Danish neonatal units.

Feeding mother's milk and breastfeeding has many advantages and breastfeeding seems possible to establish among preterm infants, but in order to improve breastfeeding-establishing policies and to identify those who need extra attention, it is important to estimate the breastfeeding rate, and to characterize the mothers and the very preterm infants.

2.4. Health effects associated with nutrition of very preterm infants

In preterm infants, the beneficial effects of human milk generally relate to improvements in host defences, digestion, absorption of nutrients, gastrointestinal function, neuro-developmental outcomes, and maternal psychological well-being (33). Breastfeeding provides a broad multi-factorial anti-inflammatory defence for the infant (34). Even donor breast milk is associated with a lower risk of developing necrotising enterocolitis (NEC) compared to formula milk in preterm and low birth weight infants (35). Breastfed children also have shown significantly higher developmental scores in comparison with formula fed children. The benefit obtained from breastfeeding was most pronounced in children with low birth weight. Also, a significant benefit from breastfeeding on cognitive development was obtained for breastfeeding exposure for more than 8 weeks (18). Protective factors associated with breast milk probably even supersede the harm associated with smoking while breastfeeding (36).

Feeding solely human milk for very preterm infants during hospitalization will though lead to insufficient intake of protein and energy, and poor postnatal growth impairment – especially of the head, has been shown associated with increased levels of motor and cognitive impairments at 7 years of age (37). Rates of brain growth are highest in the last part of gestation and the first year of life, which is the critical period of catch-up growth among VLBW infants. In children born at term, IQ scores at 4 years were highest in children whose heads grew most during infancy (38). A randomized controlled study on high-energy and -protein diet for term and preterm infants with brain injuries showed improved head-growth at 1 year of age among infants fed more than the recommended intake of energy and protein, supporting the hypothesis that growth impairment due to lack of nutrients may decrease postnatal brain growth (39). Optimal nutrition for achieving catch-up growth during infancy among very preterm infants is therefore important in order to achieve head (brain) growth and decrease the risk of neurological impairment.

2.4.1. Development of allergic diseases among preterm infants

The expression of allergic diseases varies with age, and symptoms may disappear and be replaced by other symptoms. In infancy the main allergic symptoms are atopic dermatitis (AD), gastrointestinal symptoms, and recurrent wheezing (RW), whereas bronchial asthma and allergic rhino-conjunctivitis are the main problems later in childhood. Adverse reactions to food, mainly cow's milk protein (CMP), are most common in the first year of life, whereas allergy to inhalant allergens mostly occurs later. A variety of factors are known to influence the risk of allergic disease, such as atopic predisposition, exposure to allergens (e.g. cow's milk and egg), and environmental factors (40). It is also well known that the mode of early feeding influences the risk of food allergy and that breastfeeding compared with cow's milk formula feeding is associated with a lower frequency of AD, RW, and cow's milk protein allergy (CMPA) (40-42). The incidence of CMPA in infancy seems to be approximately 2 to 3 % in developed countries and the onset of diseases is in most cases closely related to the time of introduction to cow's milk products (43). A review on breastfeeding and allergic diseases concludes that there are beneficial effects of breastfeeding especially among infants with atopic predisposition. The same review concludes that exposure to small doses of cow's milk during the first days of life appears to increase the risk of CMPA, but does not affect the incidence of allergic diseases later on (44) Meanwhile, it remains to be established whether fortification of human milk with a cow's milk based fortifier for very preterm infants has any impact on the development of allergic diseases, such as cow's milk protein allergy.

2.4.2. Development of metabolic syndrome

Catch-up growth among preterm infants is often necessary in order to attain the growth-parameters of term infants of the same PMA, but catch-up growth has been suggested to increase the risk of developing "the metabolic syndrome" with obesity, cardiovascular disease, and insulin resistance in adulthood. An animal study have shown a reduced lifespan among male mice after in *utero* growth restriction and a postnatal period of accelerated growth with an obesity-inducing diet (45). A study among term infants found an association between increased risk of elevated systolic blood pressure at 7 years of age and catch-up growth with crossing weight-percentiles upward during early childhood, but infants born SGA were though not at risk (46). Preterm infants assigned to human milk (donor breast milk) have been found to have marked benefits up to 16 years later for all of the major components of the metabolic syndrome (blood pressure, leptin resistance, insulin resistance, and lipid profile) compared to formula fed infants. A positive dose-response association between the proportion of breast milk intake in the total feeding volume and later beneficial effect on blood pressure has been indicated as well (47-49).

There is definitely a dilemma with preterm infants that need sufficient nutrition with especially protein and fatty acids for promoting growth and brain-development on one side, and possible metabolic risks later in life by early induced catch-up growth (50;51). Based on the current knowledge, dietary restrictions resulting in poorer growth are not recommended for preterm infants and further investigations are of high priority (52).

In order to achieve the goal for optimal growth and development among preterm infants after hospital discharge – without any negative health effects, it is important to investigate whether nutrient enrichment of mother's milk while breastfeeding after hospital discharge is possible, and whether it influences growth, increases the risk of feeding problems, and the development of allergic diseases and metabolic syndrome in later life.

3. The aims of the thesis

This Ph.D. project and thesis is part of a study including later follow-up.

Primary aim:

1. For "healthy" very preterm infants, to compare the effect of human milk fortifier, added to mother's own milk while breastfeeding after hospital discharge, versus solely mother's milk given until 4 months corrected age (CA). Primary outcome was growth as measured by length, weight, and head circumference until 1 year CA.

Secondary aims:

2. To investigate breastfeeding rates among preterm infants at discharge, and to investigate the duration of breastfeeding, and the possibility of fortifying mother's own milk after hospital discharge while breastfeeding.
3. To investigate if there is any relationship between intake of fortifier added to mother's milk and blood-urea nitrogen, serum-phosphorus, and haemoglobin until 4 months CA.
4. To describe growth-pattern in general including catch-up growth among preterm infants (both SGA and non-SGA) fed different diets after hospital discharge and until 1 year CA.
5. To describe the occurrence of allergic diseases / symptoms in relation to nutrition in very preterm infants during the first year of life.
6. To investigate the relationship between type of nutrition and haemoglobin A1C (HbA1C) at 4 months CA.
7. To investigate the relationship between type of nutrition and blood pressure during the intervention-period until 4 months CA and at 1 year of age.
8. To describe the content of macronutrients in human milk until 4 months CA from mothers who delivered prematurely.
9. To investigate eating habits including frequency of meals and possible feeding problems (regurgitation and constipation) according to type of nutrition after hospital discharge.

Further outcomes beyond this thesis:
To investigate if there are any long term consequences of fortification of mother's milk after hospital discharge on growth (including catch-up), intelligence, and socio-psychological behaviour, and the risk of metabolic disease and allergic diseases at 6 years of age.

3.1. Hypotheses

1. It is possible to fortify mother's milk while breastfeeding after hospital discharge, without any disadvantages or risks of interfering with breastfeeding.

2. Growth is increased among "healthy" very preterm infants, who are fed human milk fortifier in combination with mother's milk while breastfeeding, compared to preterm infants who are fed solely mother's milk.

3. Growth of preterm infants born small for gestational age differs from preterm infants born appropriate for gestational age.

4. Preterm infants achieve catch-up growth during hospitalization and catch-up growth possibly continues within the first year(s) of life.

5. There is not an increased incidence of allergic diseases among very preterm infants supplemented with a human milk fortifier or fed a preterm formula, compared to exclusively breastfeeding after hospital discharge.

4. Ethics

The study was approved July 1.st 2004 by the Danish National Committee on Biomedical Research Ethics (J.nr. VF20030208), and handling of data and registrations were approved February 2006 by the Danish Data Protection Agency (J.nr. 2007-41-1349). Informed consent was obtained from parents of the very preterm infants participating in the intervention study after oral and written information.

There are no conflicts of interest. Mead Johnson Nutritionals donated the products used in the intervention study, but the company had no influence on the project, neither on the design nor on the products and methods used.

5. Materials and methods

5.1. Study-design and study-population in general

This Ph.D. thesis is based on a prospective, randomized population-based birth cohort study with consecutive recruitment of newborn infants with a GA ≤ 32+0 weeks and data-registration performed prospectively at four neonatal units in Denmark (Holbaek Hospital (HH), Kolding Hospital (KH), Hans Christian Andersen Children's Hospital at Odense University Hospital (OUH), and Aarhus University Hospital, Skejby (AUH)). The newborn infants were born and admitted to the neonatal units from July 2004 in Odense, December 2004 in Holbaek, March 2005 in Skejby, and May 2005 in Kolding, and until August 2008 for all units.

Feeding regimens were identical at the four neonatal units with early parenteral nutrition and early trophic feeding. Until PMA of at least 30 weeks, the infants were all fed expressed mother's milk and/or donor breast milk. Fortification with HMF was initiated day 10-14 after birth. Fortification of mother's own expressed milk was done until discharge, but with decreasing amounts during the last week(s) while the infant was improving to suck directly from the breast. If the mothers did not have enough milk of their own, the infants were supplemented with a preterm formula after 30 weeks PMA. The breastfed infants were discharged when sucking full amount direct from the breast and gaining weight. If the mothers decided not to breastfeed or breastfeeding stopped before discharge, the infants were bottle-fed, and they were discharged when bottle-fed without problems. SGA very preterm infants were fed like non-SGA infants, and not routinely given a larger volume or extra calories.
The counselling of the mother to breastfeed her infant(s) was done as a routine in the departments and according to the guidelines from The Danish National Board of Health (2003, 2005, 2006, and 2008) (53). Kangaroo-care with skin-to-skin contact was established as soon as possible also during ventilator-treatment. As soon as the very preterm infant was stable (respiratory and cardiovascular) skin-to-skin contact was established once a day (2-3 hours) when ventilator-treated and twice a day (2 x 2-3 hours) when treated with continuous positive airway pressure (CPAP). The mothers were all encouraged to start expressing milk as soon as possible after birth and later on to breastfeed if possible and if they wanted to. Nutritive suckling / breastfeeding was initiated on an individual basis at 34-36 weeks PMA where the infant sucked increasing amount of mother's milk directly from the breast. At the same time the amount of mother's milk in the feeding-tube and thereby the amount of fortifier decreased. Breastfeeding

was not possible in some mothers due to breast-surgery, chemotherapy, or other medication contraindicating breastfeeding. There were several available breast pumps at all departments.

Inclusion-criteria

GA ≤ 32+0 weeks

"Healthy" at time of randomization and not excluded due to diseases or circumstances possibly influencing eating ability and growth.

Exclusion-criteria

1. Death
2. Serious congenital or chromosomal anomalies
3. Surgery due to necrotising enterocolitis (NEC) or ductus arteriosus persistens (DAP)
4. Intraventricular haemorrhage (IVH) III-IV and/or periventricular leucomalacia (PVL)
5. Bronchopulmonary dysplasia (BPD)
6. Eating disability at 42 weeks PMA, including suspected adverse reactions to the intervention diet
7. Mothers with language problems (unable to communicate in Danish or English)
8. Severe social problems (mothers placed in institutions, alcohol, or drug abuse)
9. Families who moved out of the involved regions

Basic characteristics describing infants and mothers

At birth: birth weight (BW), GA, and single birth or multiple births were recorded for each infant. Based on patient records and questionnaires, information on mother's age, education, and smoking habits were obtained. Mother's social group was defined according to The Danish National Centre of Social Research based on education and occupation (54).

5.2. Part 1. Breastfeeding rate at hospital discharge

A population based observational part of the study describing breastfeeding rate and possible factors influencing breastfeeding until hospital discharge among very preterm infants and their mothers.

Data-registration was performed consecutively from birth until discharge for all not-excluded very preterm infants (with permission from the Danish Data Protection Agency).
Exclusion was due to diseases or circumstances that would influence the eating- and/or feeding-ability at discharge (see 5.1).

Basic characteristics describing infants and mothers

As described in 5.1.
In addition, PMA and weight at discharge were recorded for each infant

Outcome measures (secondary in the thesis)

At discharge, feeding practice (breastfeeding, bottle-feeding, or combined breast- and bottle-feeding) was recorded for each infant.

5.3. Part 2. Nutrient enrichment and growth after hospital discharge

A randomized controlled trial investigating the effect on growth when adding a human milk fortifier to mother's own milk while breastfeeding her very preterm infant(s) after hospital discharge.

Participants

Included were "healthy" very preterm infants whose parents accepted to participate in the intervention study by their signature after both written and oral information within the first two weeks from birth. Exclusion was due to diseases or circumstances that would influence the eating- and/or feeding-ability at discharge (as described in 5.1.) including verified cow's milk allergy diagnosed by controlled elimination/challenge procedures.

Sample size calculation

See statistics.

Intervention

Shortly before hospital discharge, the breastfed infants were randomized to either breastfeeding without supplementation (group A) or intervention with fortification (group B). Five packets of HMF (Enfamil® HM Fortifier, Mead Johnson Nutritional, Evansville IN, USA) (17.5 kcal, 1.375g protein / 5 packets) (composition in details in appendix 1) was added to a small amount (20-50 ml) of mother's expressed milk given in a bottle or with a small cup every day until 4 months CA if possible. Many of the mothers in the intervention group were fortifying a small amount of their own defrosted expressed milk after hospital discharge. Both breastfeeding groups were encouraged to breastfeed as long as possible. The study was not blinded due to the lack of a placebo-product without influence on breastfeeding, nutrition, and growth.

If the infant(s) were bottle-fed at discharge, the infant(s) were fed a preterm formula (PF) (group C) (Enfalac® Premature Formula, Mead Johnson Nutritionals, Njimegen, Netherlands) (68 kcal, 2g protein, 7.4g carbohydrate, and 3.5g fat / 100 ml) (composition in details in appendix 1) until 4 months CA.

The above products from Mead Johnson Nutritionals were chosen since they were already used and known at the four neonatal units involved in the trial.

Introduction to complementary food was not recommended until 4 months CA for any of the groups. At that time, they were recommended the same complementary feeding as term infants without any special restrictions.

Randomization

Sealed envelopes with randomization numbers made prior to the study-start for each of the four neonatal units involved were used for randomization. Envelopes contained even numbers (assigned to group A) or uneven numbers (assigned to group B). Multiple births were randomized together. Only doctors enrolled participants while both nurses and doctors assigned to the project made the randomization.

In case of change of nutrition after randomization, the infant continued in the study with parents' permission. If breastfeeding was not sufficient (group A and B) within the first month after discharge, the infant was supplemented with or changed to PF. If breastfeeding ceased between 1 and 2 months after discharge the infant was supplemented or changed to preterm or term formula based on an individual assessment made by the phycisian involved in the trial. If breastfeeding (group A and B) ceased later than 2 months after discharge the infant was supplemented or changed to term formula.

Basic characteristics of infants and parents

As described in 5.1.

In addition, length and head circumference (HC) were recorded at birth. Data on weight, length, and HC were obtained during hospitalization. Information on mother's previous breastfeeding experience was also obtained by interviews based on questionnaires at the time of randomization.

Primary outcome measures on growth

Infants were seen at the outpatient clinics at term, 2, 4, 6, and 12 months CA, where data on growth such as weight, length (crown-heel), and head-circumference (occipital-frontal) were obtained. During hospitalization, weight was measured on the same weighing machine each time and tape measures were used for length and head circumference. At the outpatient clinics the same weighing machines were used each time, tape measures were used for measuring HC, and infant measuring rods or stadiometers for measuring length (the infant lying until 1 year of age).

Secondary outcome measures

Feeding practice and breastfeeding duration

Data on duration of breastfeeding and feeding practice (breastfeeding with or without fortifier, feeding a preterm or term formula, or complementary feeding) during the intervention period were recorded.

Blood-samples

At randomization, discharge, term, and 4 months CA, blood was drawn to measure serum hemoglobin, serum phosphorus, and blood-urea nitrogen (BUN).

At 4 months CA, blood was also drawn to measure HbA1C. Haemoglobin F (HbF) was also measured.

Blood pressure

At time of randomization, at term, and at 2, 4, 6, and 12 months CA, the infant had their blood pressure measured. The mean blood pressure was recorded. Both doctors and nurses were measuring blood pressure at the departments and at the outpatient clinics.

Dropouts

Data from dropout infants were with the parents' permission used until the date of withdrawal.

5.4. Part 3. Allergic diseases during the first year of life

A population based part of the study describing allergic diseases in relation to nutrition in very preterm infants until 1 year of age.

Inclusion and exclusion criteria were as described in part 2, except that infants excluded from the RCT due to verified cow's milk allergy during the intervention period, or parents' decision not to participate in Part 2 due to severe family predisposition to allergic disease, were included in this part of the study, when the parents' accept was obtained.

Basic characteristics describing infants and parents

As described in 5.1.

In addition information on atopic predisposition (at least one first-degree relative with allergic disease), and previous breastfeeding experience were obtained by questionnaire based interviews at the time of randomization.

Outcome measures (secondary in the thesis)

At 4 and 12 months of age a standardized questionnaire based interview about allergic diseases/allergic symptoms such as urticaria, AD, gastrointestinal symptoms (colic, diarrhea, or vomiting without known infection), episodes of RW (diagnosed by a physician) and rhinitis/conjunctivitis, and treatment was performed by a paediatric nurse or doctor. CMPA was proven by controlled elimination/challenge test in a hospital setting. At 4 months CA a blood-sample was drawn for later analysis for specific IgE antibodies (egg-white, milk, peanut, dust mites (pteronyssinus and farinae), dog, cat, grass pollen, and latex) by ImmunoCAP 250 (Pharmacia detection limit 0.35 kIU/L). All blood-samples were analysed at Odense University Hospital. Data on duration of exclusively breastfeeding and introduction of formula and/or complementary food were recorded.

5.5. Part 4. Macronutrients in human milk

A population based part of the study describing macronutrients in human milk from mothers who delivered prematurely.

Inclusion and exclusion criteria were as described for part 2 (the RCT).

Outcome measures (secondary in the thesis)

Content of protein, fat, lactose and energy in human milk.

Milk samples during hospitalization

The mothers were expressing milk as soon as possible after birth (within hours) using breast pumps available at all departments.

The first milk sample for analysis of macronutrients was collected 2 weeks from birth, and then every second week until hospital discharge.

Milk sample collection: Each time the mother expressed milk during 24 hours (4-8 times) 2 ml human milk was stored in the same test-tube and frozen as soon as possible after 24 hours collection.

Milk samples after hospital discharge – at term (40 weeks PMA), 2, and 4 months CA

The mother emptied one breast by hand or with a breast pump once in 24 hours (no specific time during the day) and stored 10 ml of this in a test-tube. It was frozen as soon as possible.

Analysis of human milk samples

The milk-analyses were made using Human Milk Analyzer (HMA) from Miris AB, Sweden. The HMA measurement principle is based on mid-infrared transmission spectroscopy.

HMA was originally developed for the analysis of macronutrients in cow's milk, but has been modified and calibrated for human milk against reference methods for fat, protein, lactose, and total solids (55).

The HMA used for analyzing milk samples in this project was calibrated with reference-milk analysed with the methodologies of Rose-Gottlieb (fat), Kjeldahl (protein), dry-oven (solids / lactose) at a certified laboratory (Fødevarestyrelsen, Region Nord) for official controls of foods under the Danish Ministry of Food, Agriculture, and Fisheries in Aarhus, Denmark.

The energy content in the milk samples was calculated from the individual fat, protein, and lactose values using following equation:
Energy = (9.25 kcal/g x fat) + (4.40 kcal/g x protein) + (3.95 kcal/g x lactose)

All milk samples had been frozen and were analysed according to the guidelines from Miris A/B: defrosted in a refrigerator, heated in warm water until a temperature of 40° Celsius and homogenized before analysis.

5.6. Part 5. Feeding-pattern and -problems after hospital discharge

A part of the RCT investigating possible feeding-problems, when supplementing mothers own milk with a human milk fortifier, compared to exclusively breastfeeding and formula feeding after hospital discharge.

Included and excluded infants were the same as in Part 2 (the RCT).

Outcome measures (secondary in the thesis)

At time of randomization, at term, 2, and 4 months CA the mothers filled in a questionnaire on:

1. Nutrition: human milk with or without fortification, preterm formula, term formula, both human milk and formula, or complementary feeding during the last five days
2. Number of meals: how many meals (breast and/or bottle) each day during the last five days
3. Regurgitation-frequency: how many times each day during the last five days
4. Stool-frequency: how many stools each day during the last five days and use of anti-constipation medicine

If the infant received anti-constipation medicine they had to register if it was Movicol® (Junior) or Lactulose.

6. Data handling and statistics (part 1-5)

Data were analysed using STATA (Statacorp, College Station, TX, USA): version 9.2 in part 1, and version 11.0 in parts 2, 3, 4, and 5. In part 4, Microsoft Excel 2003 (Microsoft, Redmond, WA, USA) has also been used.

In general:
Age, gender, and primary outcome measures on growth (weight, length, and head circumference) were transferred to Z-scores or standard deviation scores (SDS) in order to be able to compare nutrition groups containing both genders in parts of the statistical analyses.

Part 1. Breastfeeding rate at hospital discharge

Z-score or standard deviation score (SDS) was calculated as the difference between the actual weight and the expected reference weight divided with 1 standard deviation (SD) (ex.: (BW – reference BW) / 1 SD). The growth reference used for calculating Z-scores for each gender was according to Marsal (56). Instead of comparing weight at certain PMA for each gender, the Z-scores were used for comparisons between groups. In this study, the very preterm infants were defined as SGA if weight Z-score at birth was below -2 SDS and large for gestational age if weight Z-score was above +2 SDS.

Group comparisons were conducted with t-test for continuous variables and chi2-test for categorical data or Wilcoxon rank-sum test when data were not parametrically distributed (BW and GA). Logistic regression was used to produce univariate odds ratio (OR) and 95% confidence intervals (CI). Multivariate logistic regression was used to determine which factors (weight Z-score at birth, PMA at discharge, multiple births, young mother, maternal social group, and smoking) were independently associated with breastfeeding at discharge. Excluded from the final model were factors that clinically were more a result rather than a cause of feeding practices such as weight and weight Z-score at discharge, factors strongly correlated to other factors in the model (SGA, BW, and maternal age), and insignificant factors not influencing the final model (gender, duration of hospitalization, and GA).

Part 2. Intervention with human milk fortifier after hospital discharge

Sample-size calculation was made ahead of the study-start based on growth in absolute terms with a standard deviation of 5 g/day, significance at 5%, the lowest weight-difference not to be missed at 2.5 g/day, and power at 90%. The sample-size calculation showed that at least 85 infants were needed in each group.

In order to compare nutrition groups Wilcoxon rank-sum test or t-test were used for continuous variables and chi2-test was used for categorical variables.
To evaluate factors influencing the duration of breastfeeding and introduction to complementary feeding a multiple logistic regression model with clinical relevant variables (nutrition group, mother's age, social group, smoking habit, previous breastfeeding experience, multiple births, gender, GA, and SGA) was used.

Age was calculated and shown as post-menstrual age (PMA) or corrected age (CA):
At birth = GA. At time of randomization = 34 weeks = 238 days. At time of discharge = 36 weeks = 252 days. At term = 40 weeks = 280 days. At 2 months CA = 341 days. At 4 months CA = 402 days. At 6 months CA = 463 days. At 1 year = 12 months CA = 645 days.

Growth converted to Z-scores

Z-scores were calculated for weight, length, and HC as described in chapter 6. Part1. The preterm infants were defined as SGA if weight Z-score was below -2 SDS at birth. In part 2, all Z-scores have been calculated according to Niklasson and Albertsson-Wikland (57). By linear interpolation weight, length, and HC was estimated at day 238 (randomization of the first infant), 252 (discharge of first infant), 280 (term) days PMA, and at 2, 4, 6, and 12 months CA, and then calculated as Z-scores according to the chosen reference. Mean Z-scores were used to calculate change in Z-score (delta Z-score) from day 238 PMA and until day 252 PMA, 280 PMA, 2, 4, 6, and 12 months CA. Multiple logistic regression was used to evaluate variables (gender, nutrition group, SGA/non-SGA, and multiple/single-birth) influencing delta Z-score.

Growth in absolute terms

Because data consists of repeated observations on the same subject taken over time and in order to exploit the full population sample, random effect models (REM) with intercept and slope random effects (58) have also been used from randomization time of the first infant (238 days PMA) until 12 months CA. According to clinical relevant variables possibly influencing growth

and likelihood-ratio-tests comparing random effect models, the following variables were included in the final REM: the age of the infants at the different times of measure, nutrition group, gender, multiple births, SGA, baseline-weight, -length, or -HC (weight, length, or HC at 238 days PMA), and a 4.grade polynomial on time interacting with gender and nutrition group. Weight was transformed by taking the square root. The residuals of the model showed normal distribution. Random intercept and random slope was added in order to account for the unobserved heterogeneity between individuals measured at multiple occasions.

Growth charts on weight, length, and HC from randomization and until 1 year of age were made. These growth charts are based on REM including the age at the different times of measurements, nutrition group, gender, and a 4.grade polynomial on time interacting with gender and nutrition group.

Intention-to-treat and per-protocol

All analyses on growth were calculated as both by intentions to treat (ITT) and treated per protocol (PP).

Outliers

Possible outliers from the dataset did not influence the results from regression-model-analysis on delta Z-scores or random effect model.

Blood-samples and blood pressure

A linear regression model was used for comparing the 3 nutrition groups possibly influencing hemoglobin, s-phosphorus, and BUN. Only infants who were still feed the assigned nutrition at the time of blood-sample were part of this analysis.

A multiple regression model was used to investigate the impact of GA, BW, the time of blood-sampling, and PMA on HbF and HbA1C.

Mean blood pressure was calculated at time of randomization, term, 2, 4, 6, and 12 months CA and analysed both by ITT and treated PP. A multivariate logistic regression analysis with variables possibly influencing blood pressure (nutrition group and gender) was performed.

Part 3. Allergic diseases according to nutrition until 1 year of age

The incidence is the percentage of infants with allergic symptoms until 1 year CA. The prevalence is the percentage of infants with allergic symptoms at 12 months CA. The prevalence is corrected for missing data at 12 months CA.

Group comparisons were conducted with univariat analysis: t-test for continuous variables and chi2-test for categorical data or Wilcoxon rank-sum test when data were nonparametric distributed (BW and GA). Logistic regression was used to calculate univariate odds ratio and 95% confidence intervals. Multivariate logistic regression was used to determine which clinical relevant factors (GA, BW, gender, atopic predisposition, nutrition group, time of introduction to complementary food, mother's age, social group, and parents smoking at home) possibly influenced the development of AD and/or RW before 12 months CA. The preterm infants were defined as SGA if weight Z-score was below -2 SDS at birth according to a reference (57). Analyses were performed by ITT and treated PP.

Part 4. Nutrient content in human milk from mothers who delivered prematurely

Comparison of protein-content in human milk was conducted with a t-test.

Part 5. Possible feeding problems according to nutrition after hospital discharge

Logistic regression was used to determine the type of nutrition (unfortified mother's / human milk (UHM), HMF, PF, mature formula (MF), both breast- and bottle-feeding (Mix), and complementary feeding (CF)) possibly influencing the number of meals each day, regurgitation, and/or use of anti-constipation medicine.

7. Results

A total number of 633 infants born with a GA ≤ 32+0 from July 2004 until August 2008 were recorded (GA 23+0 – 32+0 weeks and BW 428g – 2255g). Characteristics are shown inTable 1.

Table 1. Distribution and characteristics of infants at the four neonatal units and in total.

Very preterm infants (VPI)	Holbaek hospital	Kolding hospital	OUH HCA	AUH Skejby	Total
Initial registration	95	98	249	191	633
Transferred to (-) or received (+) from another hospital	(*)	+33	-15+2	-20	0
Participants part 1 on breastfeeding at discharge.					
Excluded	19	28	57	51	155
Study-cohort	76	103	179	120	478
Single birth (n) (%)	53 (70)	60 (58)	102 (57)	83 (69)	298 (62)
Breastfeeding at discharge (n) (%)	47 (62)	54 (52)	106 (59)	78 (65)	285 (60)
Bottle-feeding at discharge (n) (%)	28 (37)	43 (42)	67 (37)	29 (24)	167 (35)
Breast- & bottle-feeding at disch. (n) (%)	1 (1)	6 (6)	6 (3)	13 (11)	26 (5)
Participants part 2 on nutrient enrichment and growth (ITT).					
Excluded	19	28	59	51	157
Refused to participate	31	37	33	55	156
Study-cohort	45	66	144	65	320
Single birth (n) (%)	34 (76)	39 (59)	81 (56)	46 (71)	200 (63)
Randomized to no fortifier (gr. A) (n) (%)	12 (27)	21 (32)	40 (28)	29 (45)	102 (32)
Randomized to fortifier (gr. B) (n) (%)	14 (31)	21 (32)	49 (34)	21 (32)	105 (33)
Bottle-feeding (gr. C) (n) (%)	19 (42)	24 (36)	55 (38)	15 (23)	113 (35)
Blood-samples (n)	342	484	1389	965	3180
VPI who delivered blood-sample (n)	45	65	144	64	318
Participants part 3 on allergic diseases					
Excluded	19	26	57	51	153
Refused to participate	31	37	33	55	156
Initial Study-cohort (ITT)	45	68	146	65	324
Withdrawals	2	11	16	8	37
Lack of data before 1 year CA	1	1	1	1	4
Final Study-cohort	42	56	129	56	283
For Specific IgE-analyses	13	33	84	33	163
Participants part 4 on macronutrients in human milk					
Milk samples					736
Number of mothers					214
Participants part 5 on feeding problems					
Registrations on feedings	79	15	402	133	769
Number of VPI	34	60	136	56	286
Follow-up at 6 years (#)	43	55	127	52	277

(*) The youngest VPI according to GA were born at Department of Neonatology, Rigshospitalet in Copenhagen and transferred to Holbaek after birth. (#) VPI in the study by March 2010 - VPI that will be invited to the 6-year follow-up. Parents of these VPI received a letter in December 2009 – January 2010 with information on the project until December 2009, test-results if blood was drawn for IgE-analyses, and information about the follow-up.

7.1. Factors associated with successful establishment of breastfeeding

Of 633 eligible very preterm infants and their mothers, 155 infants were excluded (24% of initial cohort) as shown in Table 2.

The study-population consisted of 478 infants (GA 24+1 to 32+0 and BW 520g-2255g) distributed within the four neonatal units: Holbaek hospital: 76, Kolding hospital: 103, Hans Christian Andersen Children's Hospital at Odense University Hospital: 179, and Skejby hospital at Aarhus University Hospital: 120. There were 224 girls and 254 boys. A total of 180 infants (38%) were multiple births (166 twins, 11 triplets, and 3 quadruplets) and 113 infants (24%) were born SGA. No infants were large for gestational age making AGA and non-SGA identical groups. At discharge mean PMA was 37+2 (SD = 12 days), mean weight was 2634g (SD = 406g), 285 (60%) infants were exclusively breastfed, 167 (35%) were bottle-fed, and 26 (5%) were both breast- and bottle-fed.

Table 2. Excluded very preterm infants in part 1.

Cause of exclusion	**Number (% of total population)**
Death	34 (5)
Surgery due to Necrotising Enteroclitis (NEC) (n=30) or due to other gastrointestinal problems (n=2)	32 (5)
Mb cordis incl. Ductus Arteriosus Persistens (DAP)	12 (2)
Intraventricular Haemorrhage (IVH) (grade III or IV), Periventricular Leucomalacia (PVL), or Hydrocephalus	24 (4)
Malformations, incl Downs Syndrome (n=4)	9 (1)
Bronchopulmonary Dysplasia (BPD) (Oxygen dependent at discharge)	9 (1)
Language problems (Mothers who were not able to communicate in Danish or English)	12 (2)
Social problems (Mothers with cancer, drug-abuse, or placed at an institution)	9 (1)
Eating disability (1 still tube-fed at discharge)	4 (1)
Moved before discharge (6 out of the country, 2 to another region in DK, 2 born in other countries)	10 (2)
Total	155 (24)

Mean GA 27+6 (23+0 – 32+0). Mean BW 1062g (428g – 2121g). Twins 32%.

The following analyses are based on two feeding groups according to whether the infant was 1) exclusively breastfed, n=285 (60%), or 2) not exclusively breastfed, n=193 (40%) at discharge. Results are shown in Table 3.

For the final analysis, complete dataset were available on 409 very preterm infants and their mothers (86% of study-cohort (478) and 65% of initial cohort (633)). Information on mother's age was obtained among mothers of 474 infants. Information on smoking was obtained from mothers of 436 infants and 19% of 436 mothers smoked during pregnancy and lactation. Information on mother's social group was obtained from mothers of 423 infants and the mothers were divided into 5 different social groups: 1=high social group (12%), 2 (28%), 3 (6%), 4 (39%), and 5=low social group (15%).

In the multivariate logistic regression, a higher rate of breastfeeding at discharge was found among mothers of high social group (p=0.000) and mothers who did not smoke (p=0.003). There was a strong correlation between smoking and low social group with a mean social group 4.07 ± 1.02 and 3.00 ± 1.30 among mothers who smoke and mothers who did not smoke respectively (p=0.000). A higher rate of single birth infants tended to be breastfed at discharge though not significantly (p=0.09). A lower rate of young mothers (<25 years) were breastfeeding at discharge (p=0.007 univariate), though not significantly in the final model (p=0.28) but young age was also correlated with low social group (p=0.000).
Previous breastfeeding experience among mothers of 299 infants did not have any significant influence on breastfeeding.

Low weight Z-score at birth tended to be negatively correlated to breastfeeding at discharge (p=0.02 univariate), though not significant in the final model (p=0.09). Less SGA infants were exclusively breastfed at discharge (21.1% vs. 27.5% not exclusively breastfed), but not significantly (p=0.11 univariate). Weight Z-score at discharge seemed lower among exclusively breastfed infants though not significant (p=0.06 univariate). Change in weight Z-score from birth to discharge was 0.34 SDS (-1.36 to -1.02) among not exclusively breastfed and -0.10 SDS (-1.09 to -1.19) among exclusively breastfed infants (p=0.000 univariate).
The not exclusively breastfed group consisted of 167 solely formula fed and 26 formula and breastfed (combined) very preterm infants. Change in weight Z-score from birth to discharge was 0.41 (SD = 0.76) in the solely formula fed group compared to -0.15 (SD = 0.72) in the combined group and -0.10 (SD = 0.88) in the exclusively breastfed group.
Length and head circumference (HC) was not obtained on the exact day of discharge among all infants why it was not possible to calculate change in Z-score on length and HC from birth to discharge.

Including only single birth infants (n=253) in the final model the results did not change comparing exclusively breastfed with not exclusively breastfed infants. There was no significant differences except that maternal social group (p=0.02) and smoking (p=0.01) were still negatively correlated to breastfeeding at discharge. Change in weight Z-score among single birth was 0.28 SDS (formula fed) and -0.10 SDS (exclusively breastfed) (p=0.000 univariate).

Main results on factors associated with breastfeeding very preterm infants at hospital discharge

In 478 very preterm infants 60% were exclusively breastfed, 35% were exclusively bottle-fed, and 5% were both breast- and bottle-fed at discharge.

Mothers in a high social group (p=0.000) and “not smoking” (p=0.003) were significantly more often exclusively breastfeeding their preterm infant(s) at discharge.

Factors like low weight Z-score at birth, multiple births, and young mothers below 25 years were negatively correlated to exclusively breastfeeding at discharge.

Infant age at discharge and duration of hospitalization did not influence breastfeeding at discharge.

Table 3. Characteristics associated with nutrition at discharge among 478 very preterm infants in the study-cohort recruited from 4 neonatal units in Denmark from July 2004 – August 2008.

Characteristics	Exclusively bottle-fed with formula	Combined formula and breast-feeding	Exclusively breastfeeding at discharge?			**Univariate**	Final model (n=409) (86% of study-cohort, 65% of initial cohort)	
Preterm Infants	(n=167)[n]	(n=26)[n]	**No ([n]) (n=193)**	**Yes (n=285)**	p-value *	**Odds Ratio (95% CI)**		p-value
Gender (% male)	57.5	42.3	55.4	51.6	0.41	0.86 (0.59-1.24)	–	–
Birth weight (median) (g)	1270	1433	1285	1350	0.07	1.05 (1.00-1.11) #	–	–
GA at birth (median) (wk)	29.6	31.2	29.7	30.3	0.11	1.04 (0.94-1.15)	–	–
Weight Z-score at birth ±1SD (mean) (SDS)	-1.35 ± 1.30	-1.47 ± 0.88	-1.36 ± 1.25	-1.09 ± 1.24	0.02	1.19 (1.03-1.38)	1.17 (0.98-1.40)	0.09
SGA (weight Z-score < -2 SDS) (% in group)	27.5	26.9	27.5	21.1	0.11	0.70 (0.46-1.08)	–	–
Weight at discharge ±1SD (mean) (g)	2684 ± 403	2486 ± 351	2655 ± 401	2620 ± 409	0.35	–	–	–
PMA at discharge ±1SD (mean) (wk)	37.2 ± 1.7	37.5 ± 1.3	37.3 ± 1.7	37.4 ± 1.7	0.39	1.05 (0.94-1.17)	1.08 (0.94-1.23)	0.28
Weight Z-score at discharge ±1SD (mean) (SDS)	-0.93 ± 1.03	-1.61 ± 0.70	-1.02 ± 1.02	-1.19 ± 0.94	0.06	–	–	–
Change in weight Z-score, birth to discharge ±1SD (SDS)	0.41 ± 0.76	-0.15 ± 0.72	0.34 ± 0.77	-0.10 ± 0.88	0.000	0.53 (0.42-0.67)	–	–
Hospitalized ±1SD (mean) (days)	54.3 ± 17.0	47.9 ± 13.9	53.5 ± 17.0	53.6 ± 21.5	0.93	1.00 (0.99-1.01)	–	–
Multiple births (% multiple)	37.1	61.5	40.4	35.8	0.31	0.82 (0.56-1.20)	0.67 (0.43-1.06)	0.09
Mothers								
Maternal age ±1SD (mean) (y) (n=474)	29.8 ± 5.9	29.3 ± 3.8	29.7 ± 5.7	30.7 ± 4.5	0.06	1.04 (1.00-1.08)	–	–
Young mother (% <25y) (n=474)	19.8	0.0	17.1	8.8	0.007	0.47 (0.27-0.81)	0.70 (0.37-1.33)	0.28
Maternal social group ±1SD (mean) (1=high, 2, 3, 4, 5=low) (n=423)	3.7 ± 1.2	3.1 ± 1.4	3.6 ± 1.3	2.9 ± 1.3	0.000	0.65 (0.55-0.77)	0.71 (0.59-0.86)	0.000
Smoking (%) (n=436)	33.3	10.0	30.6	11.3	0.000	0.29 (0.17-0.48)	0.43 (0.25-0.76)	0.003

*Wilcoxon rank-sum test if median, t-test if continuous variables or chi2-test if categorical variables. # Odds Ratio per 100g.

7.2. Nutrient enrichment of human milk and growth of very preterm infants

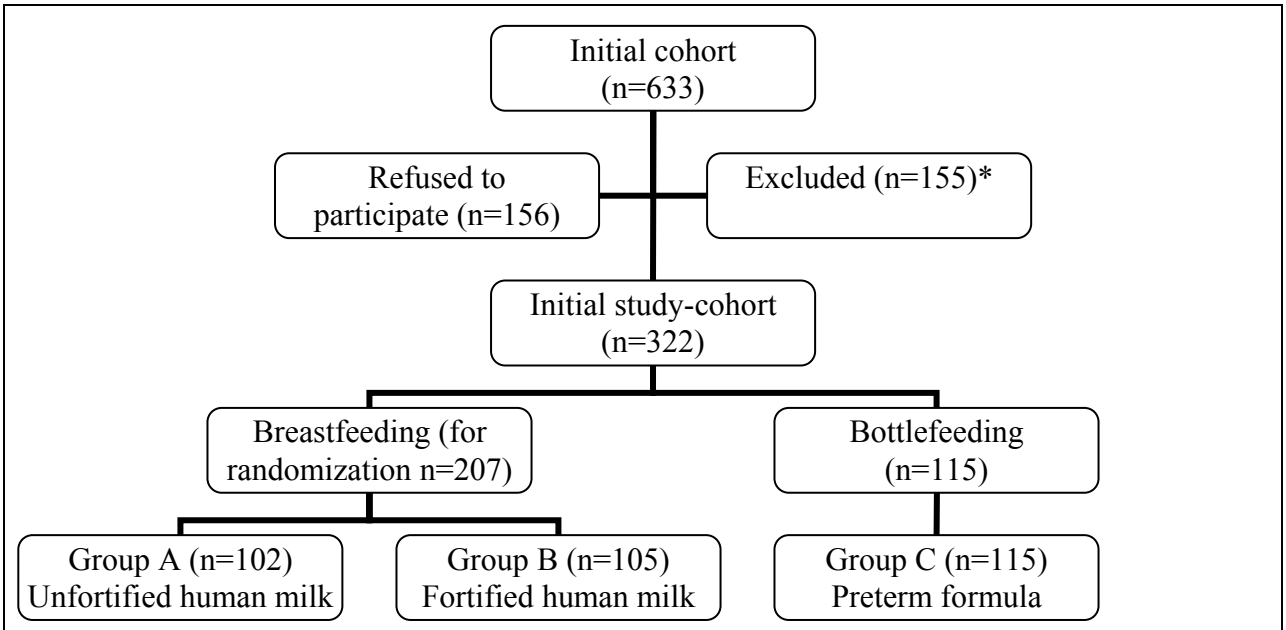
Before randomization the same number (n=155) of infants were excluded initially as in part 1. In addition, two very preterm infants were excluded after randomization due to eating disability and severe cerebral palsy in one case, and severe social problems, PVL and severe cerebral palsy in another case (Figure 1). The final study-cohort consisted of 320 (51%) very preterm infants, as parents of 156 (25%) refused to participate in the RCT and a total of 157 (25%) were excluded.

The excluded infants had a lower GA and BW whereas those with parents who refused to participate had a higher GA and BW compared to the study-cohort ($p \leq 0.002$). Compared with mothers who refused to participate, mothers in the study-cohort were older (30.8 years *vs* 29.3 years, $p = 0.003$) and more often breastfeeding (65% *vs* 50%, $p = 0.002$) (Table 4).

The total number of infants in the study-cohort (ITT analysis) was 320. The number that completed in their assigned nutrition groups was 283 (88%) at term, 211 (66%) at 2 months and 108 (34%) at 4 months (Figure 1). Due to parents choices many changes of nutrition between 2 and 4 months within all 3 nutrition groups were performed with 211 infants completing in their assigned nutrition groups until 2 months (for PP-analysis).
The gender distribution among these 211 infants is boys/girls: Group A 38/35, B 26/25, and C 54/33. ITT- and PP analyses were at 6 months based on 303 and 206 infants, and at 12 months based on 277 and 188 infants respectively.

Characteristics of the infants and their mothers in the nutrition groups are presented in Table 5. At discharge only one infant was fed both bottle and breast, but this infant was one of the infants excluded after randomization due to severe cerebral palsy and eating problems. Sixty-five percent (207/320) infants were exclusively breastfed (group A and B) and 35% (113/320) were bottle-fed with a preterm formula (group C) at discharge.

Figure 1. Participation flowchart during the intervention period.



	Group A	**Group B**	**Group C**	**Total number**
Number at randomization	**102**	**105**	**115**	**322**
Excluded	**0**	**0**	2*	
34 weeks PMA	**102**	**105**	**113**	**320 (ITT-group)**
Dropouts Changed nutrition	0 0	0 0	0 2	
36 weeks PMA	**102**	**105**	**111**	**318**
Dropouts Changed nutrition	3 9	0 14	0 9	
40 weeks PMA (Term)	**90**	**91**	**102**	**283 (88%)**
Dropouts Changed nutrition	2 15	0 40	3 12	
2 months CA	**73**	**51**	**87**	**211 (66%) (PP-group)**
Dropouts Changed nutrition	2 33	0 31	0 37	
4 months CA	**38**	**20**	**50**	**108 (34%)**

*Exclusion as described in Table 2 with the addition of two infants (1due to periventricular leucomalacia and 1 due to eating disability).

Table 4. Characteristics of initial cohort, excluded, refusals, and study-cohort.

Infants	Initial Cohort (n = 633)	Excluded (n = 157)	Refusal to participate (n = 156)	Study-Cohort (SC) (n = 320)	Refusals versus SC (p-value)
GA at birth (median) (min-max) (days)	208 (161-224)	195 (161-224)	214 (175-224)	208.5 (169-224)	0.002
BW (median) (min-max) (g)	1256 (428-2255)	1014 (428-2121)	1436 (520-2220)	1271 (535-2255)	0.001
Boys	330/631	78/155	77/156	175/320	ns
Weight Z-score at birth ±1SD (mean) (SDS)	-1.08 ± 1.22	-1.15 ± 1.40	-0.96 ± 1.23	-1.10 ± 1.11	ns
SGA at birth	136/624	39/148	29/156	68/320	ns
Multiple births	229/633	49/157	60/156	120/320	ns
PMA at discharge ±1SD (mean) (days)	–	–	262 ± 12	261 ± 12	ns
Weight Z-score at discharge ±1SD (mean) (SDS)	–	–	-1.29 ± 1.14	-1.11 ± 0.92	ns
Mothers					
Breastfeeding at discharge	–	–	78/156	207/320	0.002*
Mothers age ±1SD (mean) (years)	–	–	29.3 ± 5.1	30.8 ± 4.9	0.003*
Social group ±1SD (mean) (1=high and 5=low)	–	–	3.29 ± 1.31	3.13 ± 1.31	ns
Smoking	–	–	20/117	61/317	ns

*Also significant ($p<0.05$) in a multiple logistic regression model (GA, BW, weight Z-score at birth, multiple births, mother's age, smoking habit, social group, and breastfeeding at discharge).

Table 5. Characteristics of the nutrition groups.

Infants	A (n = 102)	B (n = 105)	A vs. B (p-value)	**A and B (n = 207)**	**C (n = 113)**	A and B vs. C (p-value)
GA at birth (median) (min-max) (days)	208.5 (169-224)	212 (171-224)	ns	**210 (169-224)**	**207 (176-224)**	ns
BW (median) (min-max) (g)	1260 (548-2255)	1320 (535-2100)	ns	**1287 (535-2255)**	**1233 (612-2140)**	ns
Weight Z-score at birth ±1SD (mean) (SDS)	-1.02 ± 1.16	-1.03 ± 1.05	ns	**-1.02 ± 1.10**	**-1.23 ± 1.13**	ns
SGA	20/102	21/105	ns	**41/207**	**27/113**	ns
Boys	58/102	52/105	ns	**110/207**	**65/113**	ns
SGA boys	11/20	10/21	ns	**21/41**	**13/27**	ns
Multiple births	27/102	42/105	0.04	**69/207**	**51/113**	0.04#
Baseline weight at day 238 PMA ±1SD (mean) (g)	**Girls** 1882±293 **Boys** 1964±287	**Girls** 1865±268 **Boys** 1988±268	ns ns	**Girls** 1872±278 **Boys** 1975±277	**Girls** 1895±358 **Boys** 1996±280	ns ns
Baseline Length at day 238 PMA ±1SD (mean) (cm)	**Girls** 43.9±2.5 **Boys** 44.2±2.5	**Girls** 43.8±2.1 **Boys** 44.9±2.0	ns ns	**Girls** 43.9±2.3 **Boys** 44.5±2.3	**Girls** 43.5±2.5 **Boys** 44.0±2.2	ns ns
Baseline HC at day 238 PMA ±1SD (mean) (cm)	**Girls** 30.8±1.4 **Boys** 30.9±1.3	**Girls** 30.4±1.1 **Boys** 31.2±1.2	ns ns	**Girls** 30.6±1.2 **Boys** 31.1±1.2	**Girls** 30.6±1.4 **Boys** 31.1±1.3	ns ns
PMA at discharge ±1SD (mean) (days)	264 ± 15	260 ± 10	0.04	**262 ± 13**	**259 ± 10**	ns
Weight Z-score at discharge ±1SD (mean) (SDS)	-1.22± 0.94	-1.22 ±0.88	ns	**-1.22 ± 0.91**	**-0.92 ± 0.92**	0.006#
Mothers						
Mother's age ±1SD (mean) (years)	30.9 ± 4.5	31.0 ± 4.3	ns	**31.0 ± 4.4**	**30.4 ± 5.8**	ns
Social group ±1SD (mean) (1=high and 5=low)	2.88 ± 1.27	2.89 ± 1.30	ns	**2.88 ± 1.28**	**3.59 ± 1.25**	0.000#
Smoking	13/99	10/105	ns	**23/204**	**38/113**	0.000#
Previous breast-feeding experience	35/93	33/95	ns	**68/188**	**43/109**	ns

Wilcoxon rank-sum test or t-test if continuous variables and chi2-test if categorical variables. #Also significant ($p<0.05$) in a multiple logistic regression model (variables: GA, BW, weight Z-score at birth, weight Z-score at discharge, multiple births, mother's age, smoking habit, social group, and previous breastfeeding experience).

Duration of breastfeeding

Duration of breastfeeding was not influenced by fortification. Mothers of multiple births stopped breastfeeding earlier than single birth infants (p=0.000), and mothers in the lowest social group (p=0.02) and younger mothers (p=0.03) also discontinued breastfeeding earlier. Mean duration of breastfeeding was 11.8 ± 7.7 weeks in group A and 10.6 ± 7.5 weeks after term in group B (no significant difference) (Figure 2). Mean age for introduction of complementary food was 16.4 ± 3.9 weeks (min 6.0 – max 27.4 weeks), 18.3 ± 4.4 weeks (min 8.4 – max 39.7 weeks), and 17.0 ± 3.4 weeks (min 7.1 – max 24.3 weeks) after term in group A, B, and C respectively. Older mothers (p=0.000) and group B compared to group A (p=0.002) introduced complementary food significantly later.

Overall growth (using Z-scores and growth in absolute terms)

All very preterm infants, no matter the nutrition group, showed a nadir in weight, length, and HC Z-score at 34 weeks PMA. Mean Z-scores however, did never drop below normal range (-2 SDS). All weight, length, and HC Z-scores increased irrespective of the nutrition group after 34 weeks PMA, but tended to decrease after 6 months CA to a mean weight Z-score: -0.69 (ITT) and -0.75 (PP), mean length Z-score: -0.05 (ITT) and -0.14 (PP), and mean HC Z-score: +0.10 (ITT) and +0.06 (PP) at 12 months CA (Figure 3, Figure 4 and appendix 2).

Boys compared to girls showed significant higher weight, length, and HC within all 3 nutrition groups from term (280 days PMA) until 1 year CA (645 days PMA) using random effect models (REM). Results are shown in tables in appendix 2 (ITT and PP), and growth charts based on ITT-analysis are shown in appendix 3. These growth-charts were made without using e.g. growth baseline-values.

Growth according to nutrition (using Z-scores and growth in absolute terms)

ITT: Infants in group C increased significantly more in length and weight Z-score compared to both breastfed groups (A and B) (Figure 3). Among boys, length Z-score increased significantly more in group C compared to both group A and B (2 and 4 months), while girls increased significantly more in length Z-score comparing group C with A (2 and 4 months), but not with B (Figure 5).

In addition, REM showed weight (2 – 6 months) and HC (term – 4 months) to be significantly higher among girls in group B compared to A (Table 6).

PP: Compared to A and B, group C increased significantly more in weight Z-score (term – 4 months) and length Z-score (2 and 4 months) (Figure 4). Girls in group C increased significantly more in weight Z-score compared to both A and B (term) and increased significantly more in length Z-score compared to A but not to B (2 - 6 months) (Figure 6).
In addition, REM showed length and HC to be significantly higher among girls in group B compared to A (2 – 4 months) (Table 6).

Catch-up growth (Z-scores)

In our study, non-SGA infants seemed to have achieved catch-up on HC at discharge, on weight at 2 months CA, and on length at 4 months CA., while SGA infants showed rapid catch-up growth on HC until term, on weight until 4 months, and length-growth even continued until 1 year CA (details in Figure 7, Figure 8 and in appendix 4).

Subgroup analyses (Z-scores)

Growth during hospitalization

Growth (weight, length, and HC Z-scores) during hospitalization among infants in the RCT. Weight Z-score increased from birth to discharge (day 252 PMA) in group C and decreased in A and B (comparable to results in part 1 with measurements on the exact day of discharge). Length Z-scores decreased while HC Z-score increased within all 3 nutrition-groups from birth to discharge (day 252 PMA). Results are shown in appendix 4.

Number of infants with Z-scores < -2 SDS at birth and during the first year of life

The number of infants with Z-scores < -2 SDS on weight, length, and HC increased from birth until 34 weeks PMA and then decreased mainly until term on weight, but after term and until 4 months on length. After term the number of infants with Z-scores < -2 SDS on both weight and length seemed to decrease more among infants supplemented with fortifier or fed a preterm formula – though not significant. Details are shown in appendix 4.

ITT subgroup analysis on infants with subnormal weight

SGA infants (n=68) compared to non-SGA increased significantly more in length Z-score during the entire study period with no significant difference comparing nutrition groups (Figure 7 and Figure 8). SGA boys increased significantly more in weight Z-score until 2 months compared to girls, while SGA girls increased significantly more in HC Z-score compared to boys until 6 months (both $p<0.05$).

Among 53 infants with subnormal weight at discharge, group C increased significantly more in delta weight Z-score compared to A and B until 2 months CA, but with no significant difference at 12 months CA.

Results on serum chemical determinations according to nutrition groups

Blood-samples from 265 infants at time of randomization, 177 at discharge, 60 at term, and 65 at 4 months CA (fed either A, B, or C at the time of blood-sample) showed significant higher levels of BUN in group C compared to both A and B at all 4 periods ($p<0.05$). Infants in group B had a significant higher level of BUN and s-phosphorus compared to group A at 4 months CA (Table 7). Results on HbA1C and HbF are shown in appendix 5.

Results on blood pressure

Procedures and equipment measuring blood pressure turned out to be different at the four departments. Results on blood pressure are shown in appendix 6.

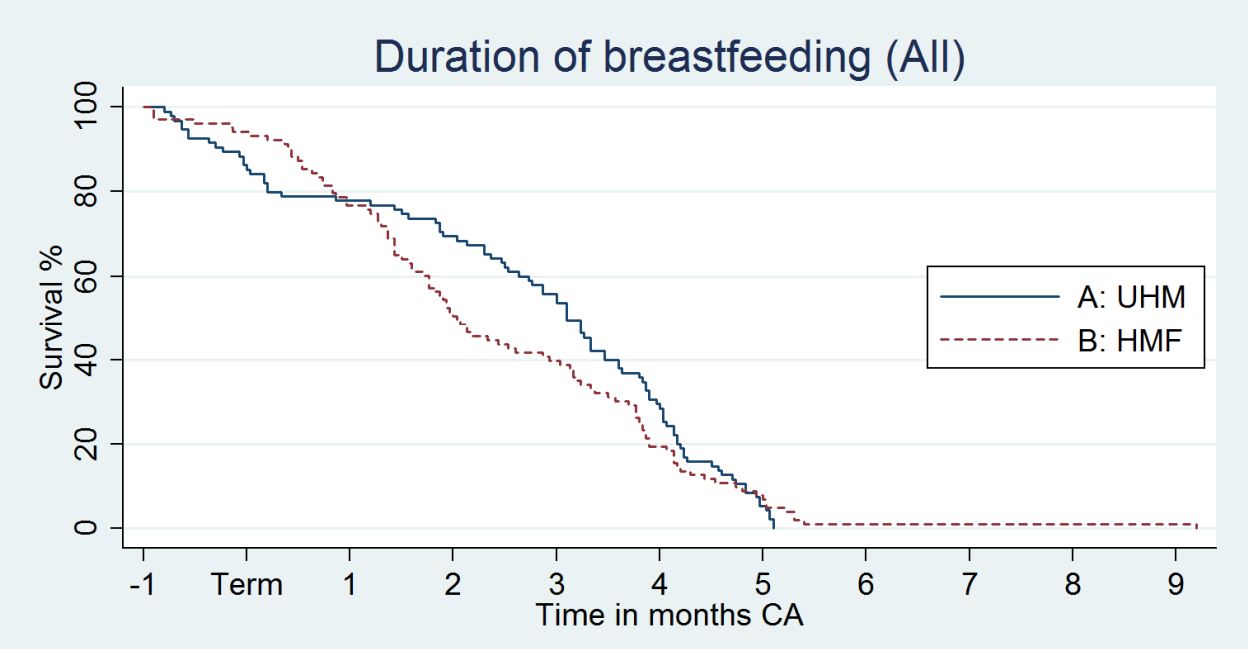
7.2.1. Main results on nutrient enrichment

At discharge 65% were breastfed (n=207) and 35% were bottle-fed with preterm formula (n=113). Comparing the breastfeeding-groups (A) mothers milk without (n=102) and (B) mothers milk with fortification (n=105), no significant difference in mean duration of breastfeeding after term (11.8 and 10.6 weeks respectively) was found.

Compared to group A and B, infants in C increased significantly more in weight Z-score until term and length Z-score until 6 months CA. No significant difference was found in weight, length, or head-circumference at 12 months CA between group A and B. At 12 months CA using REM, boys in group C were significantly longer and heavier compared to A and B, while girls in C were longer and heavier compared to A only. By PP analysis, girls in group B compared to A were longer and had larger HC at 2 and 4 months CA ($p<0.05$).

Higher protein-intakes were related to higher blood protein-levels.

Figure 2. Kaplan Meier plots illustrating duration of breastfeeding among very preterm infants (group A: Mothers milk / Unfortified Human Milk (UHM) and B: Mothers milk supplemented with Human Milk Fortifier (HMF)) for all breastfed very preterm infants and single birth.



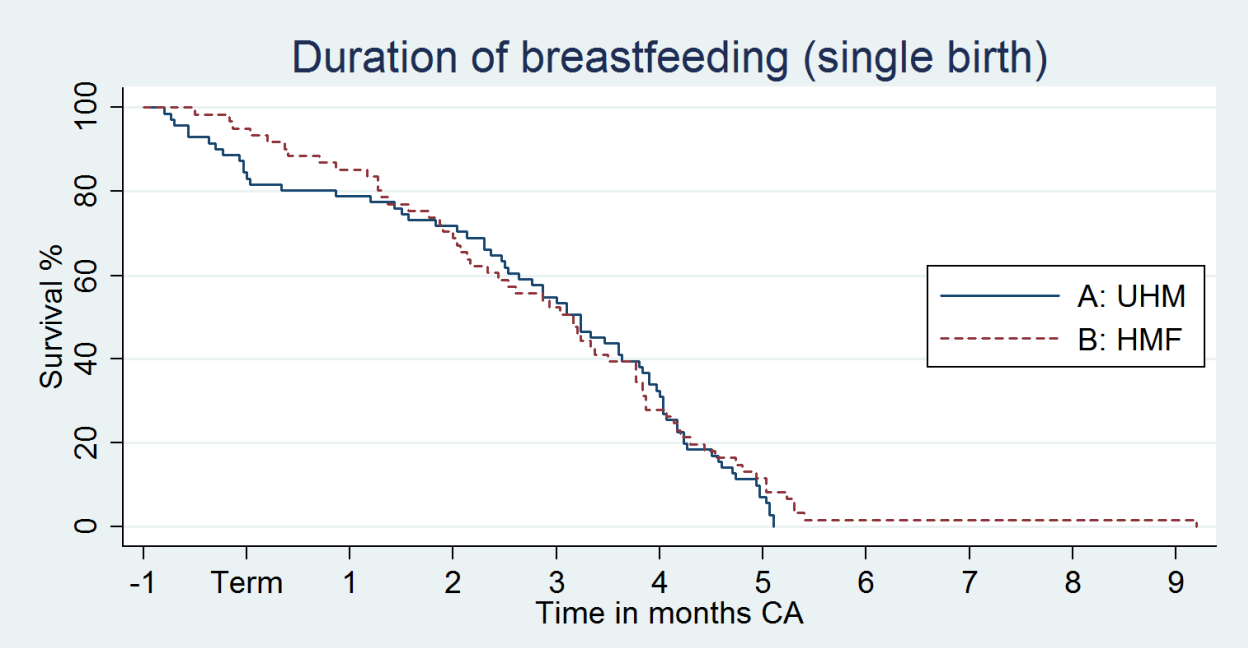


Figure 3. (ITT) Z-scores from birth to 12 months CA (both gender) according to nutrition-groups (mean Z-score ±1SD) and change in Z-score (delta Z-score from 34 weeks PMA). Significant difference in delta Z-score ($p<0.05$) comparing nutrition-groups shown as ● C > A and ▲C > B.

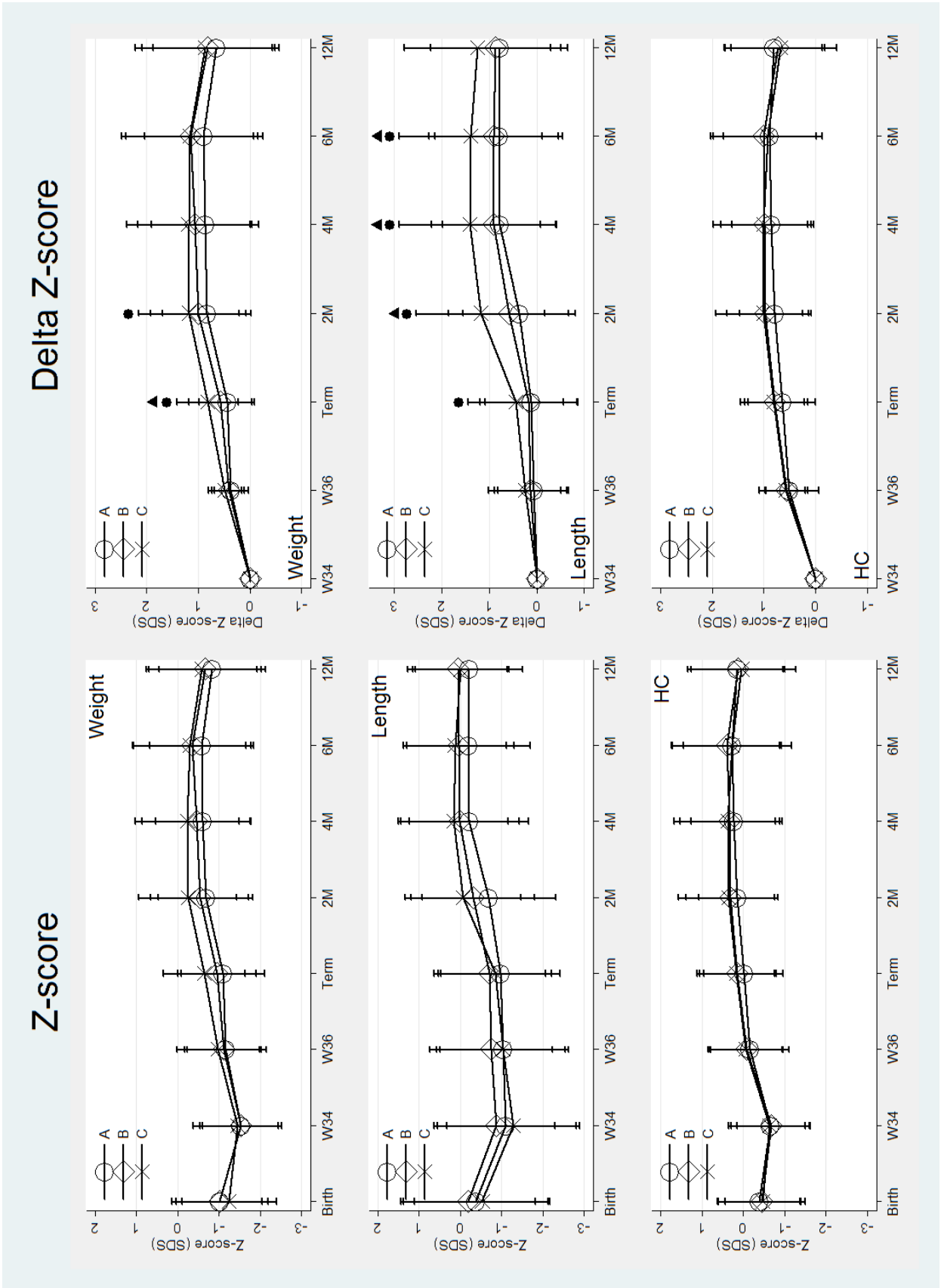


Figure 4. (PP) Z-scores from birth to 12 months CA (both gender) according to nutrition-groups (mean Z-score ±1SD) and change in Z-score (delta Z-score from 34 weeks PMA). Significant difference in delta Z-score ($p<0.05$) comparing nutrition-groups shown as ● C > A and ▲C > B.

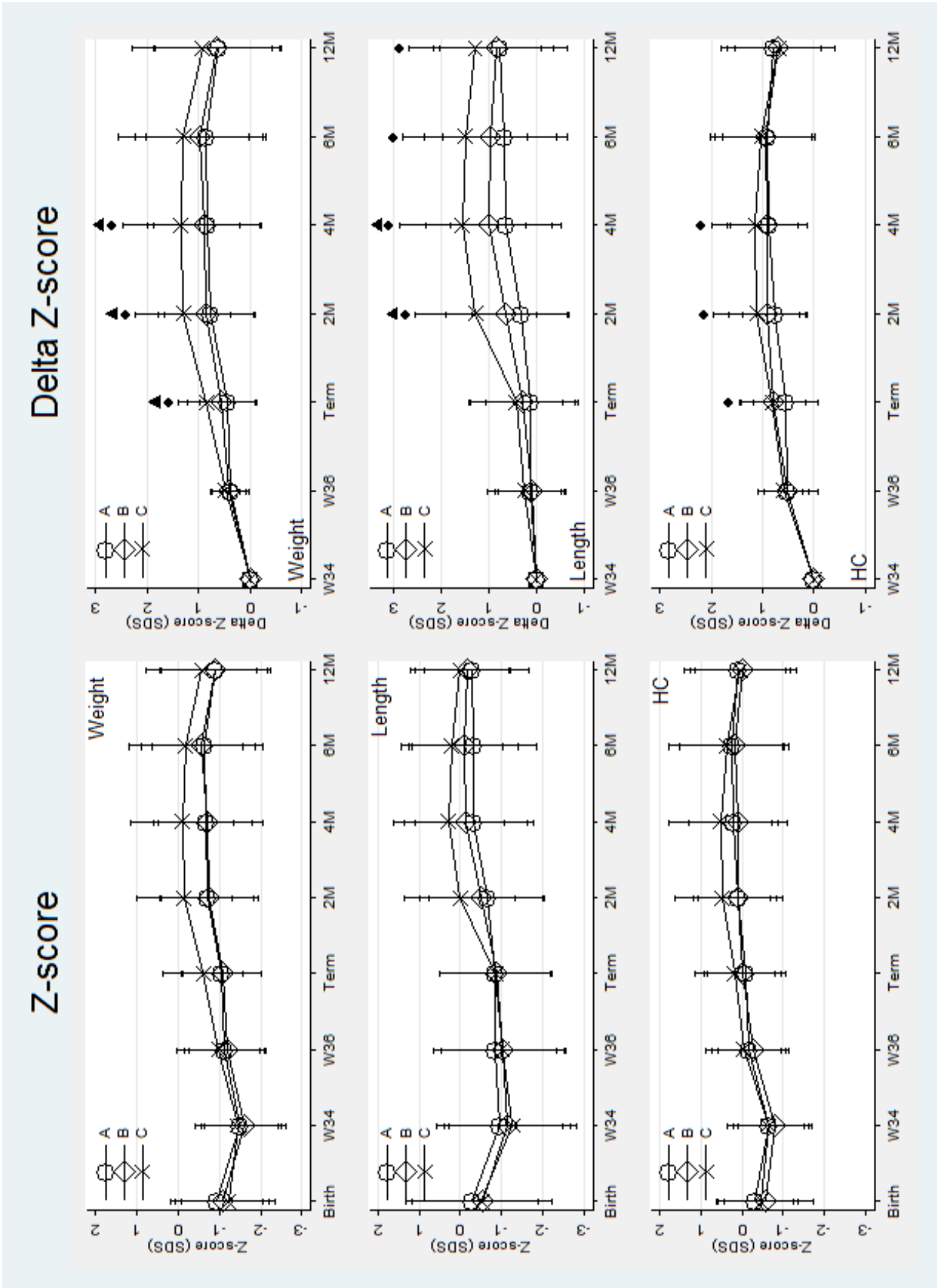


Figure 5. (ITT) Change in Z-score (delta Z-score from 34 weeks PMA) according to gender. Significant difference in delta Z-score ($p<0.05$) comparing nutrition-groups shown as ● C > A and ▲C > B.

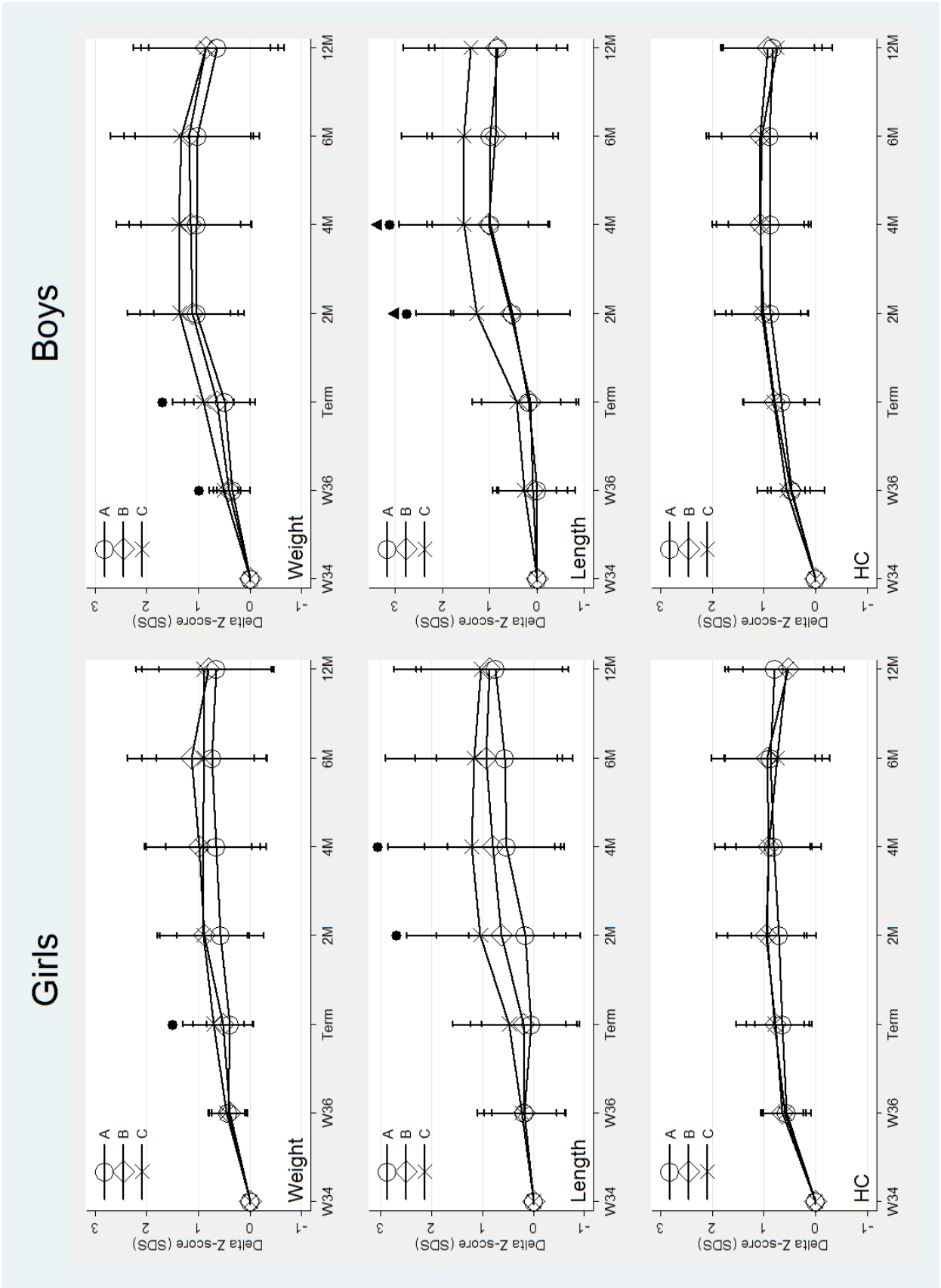


Figure 6. (PP) Change in Z-score (delta Z-score from 34 weeks PMA) according to gender. Significant difference in delta Z-score ($p<0.05$) comparing nutrition-groups shown as ● C > A and ▲C > B.

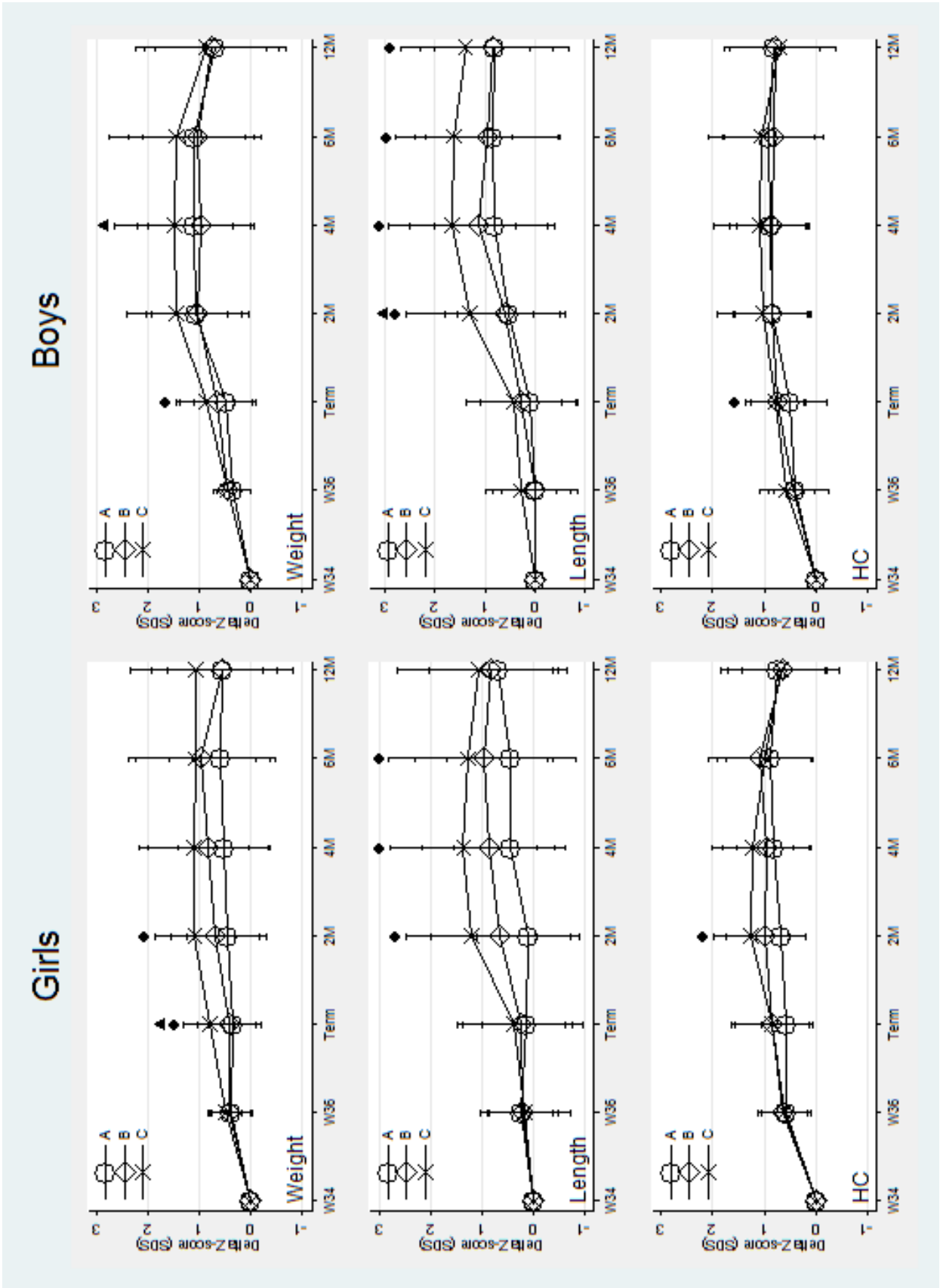


Figure 7. (ITT) Anthropometric data as Z-scores for AGA (=non-SGA) and SGA on weight, length, and HC (both gender).

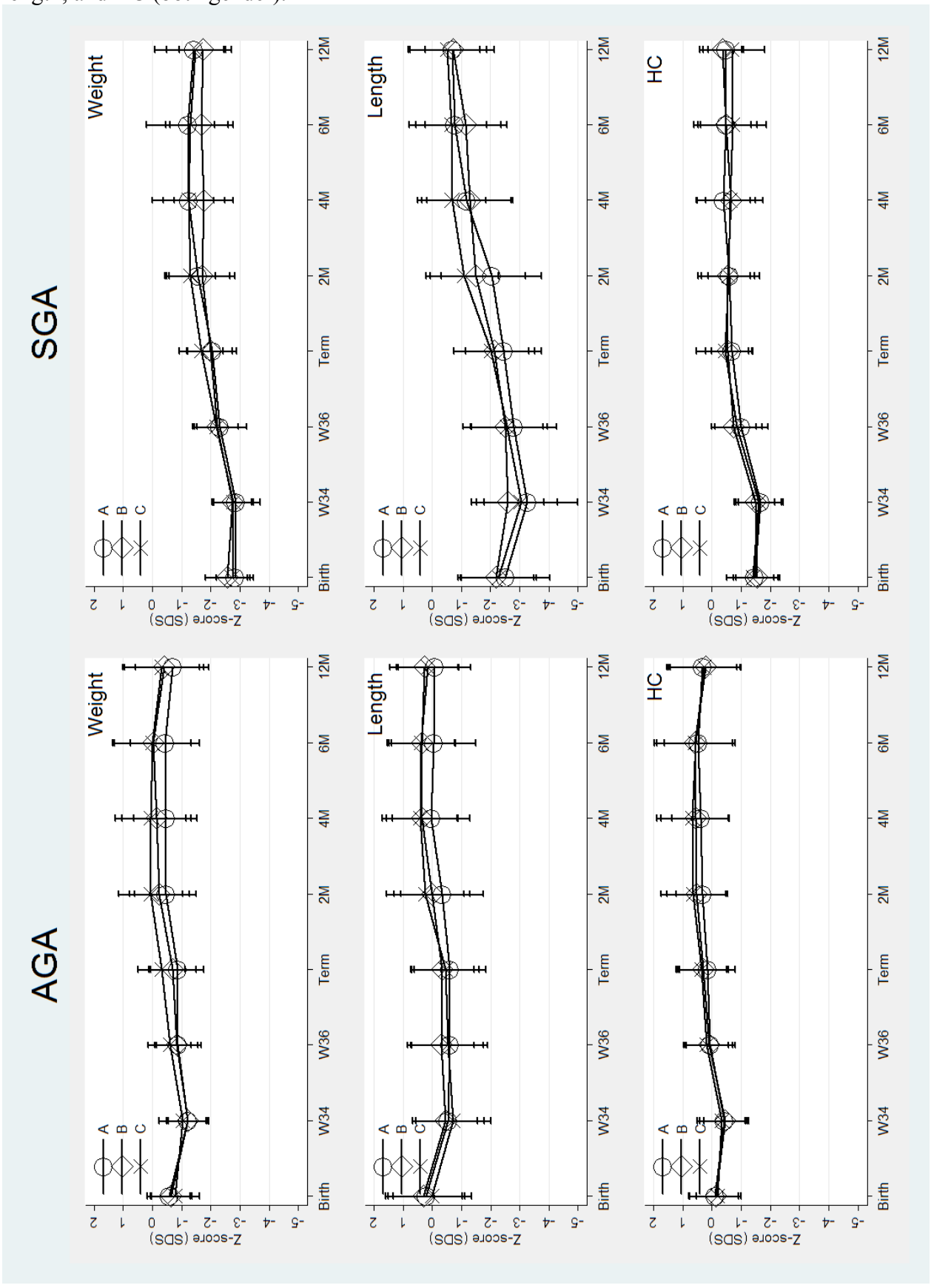


Figure 8. (PP) Anthropometric data as Z-scores for AGA (=non-SGA) and SGA on weight, length, and HC (both gender).

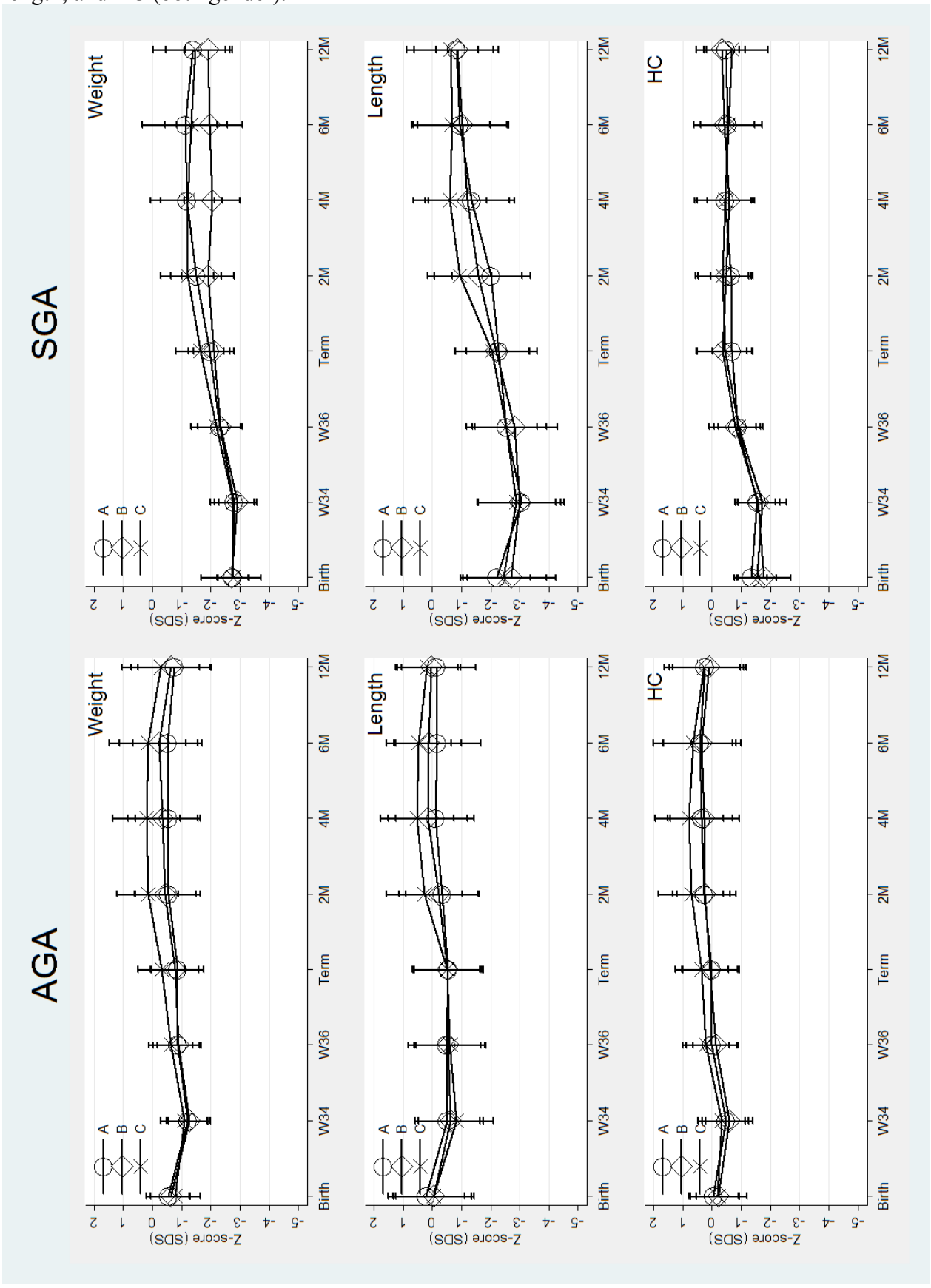


Table 6. Main results on growth according to nutrition and gender using REM with significant difference ($p<0.05$) (ITT and PP). See appendix 2 for details.

		By ITT (at n months CA)	Treated PP (at n months CA)
Weight	Girls	**B > A at 2, 4, and 6** C > A at 6 and 12	C > A at 4, 6, and 12 C > B at 12
	Boys	C > A at term, 2, 4, 6, and 12 C > B at 4, 6, and 12	C > A at 2, 4, and 6 C > B at 2, 4, 6, and 12
Length	Girls	C > A at 2, 4, 6, and 12	**B > A at 2 and 4 (p=0.059 at 6)** C > A at 2, 4, 6, and 12 C > B at 12
	Boys	C > A at term, 2, 4, 6, and 12 C > B at 2, 4, 6, and 12	C > A at (p=0.053 at term), 2, 4, 6, and 12 C > B at (p=0.053 at 2) 4, 6, and 12
HC	Girls	**B > A at term, 2, and 4** C > A at term, 2, and 4	**B > A at 2 and 4** C > A at term, 2, 4, and 6
	Boys	No significant difference	No significant difference

Table 7. Mean blood-urea nitrogen (BUN), s-phosphorus, and hemoglobin (whole blood). Type of nutrition (A, B, or C) on the day of blood-sample was known. Conversion factors from (59).

	Nutrition group	Week 34-35	Week 36-38	Week 39-40	4 months CA
BUN ±1SD (mean) (mmol/L) 1 mmol/L = 2.80 mg/dL	Numbers of VPI	265	177	60	65
	A	1.7 ± 0.6	1.5 ± 0.4	1.5 ± 0.8	2.2 ± 0.9
	B	1.8 ± 0.7	1.8 ± 0.7	1.9 ± 1.0	3.1 ± 1.2 *
	C	2.1 ± 0.9 #¤	2.5 ± 0.9 #¤	3.3 ± 1.3 #¤	4.2 ± 0.7 #¤
S-phosphorus ±1SD (mean) (mmol/L) 1 mmol/L = 3.10 mg/dL	Numbers of VPI	260	167	56	64
	A	2.09 ± 0.22	2.02 ± 0.23	1.87 ± 0.33	1.74 ± 0.44
	B	2.10 ± 0.27	2.09 ± 0.22	1.96 ± 0.27	1.96 ± 0.19 *
	C	2.16 ± 0.24	2.17 ± 0.22 ¤	2.17 ± 0.23 #¤	2.05 ± 0.11 ¤
Hemoglobin ±1SD (mean) (mmol/L) 1 mmol/L = 1.61 mg/dL	Numbers of VPI	207	161	60	61
	A	6.6 ± 1.2	6.4 ± 0.8	6.2 ± 0.7	7.3 ± 0.6
	B	6.8 ± 1.2	6.4 ± 0.8	6.1 ± 0.6	7.5 ± 0.6
	C	6.3 ± 0.9 &	6.1 ± 0.9	5.9 ± 0.6	7.2 ± 0.6

Significant difference ($p<0.05$) with higher levels of BUN comparing group C with A (¤) and B (#), group B with higher levels of BUN and s-phosphorus compared to A (*) at 4 months CA, and group C with lower levels of hemoglobin compared to B at time of randomization (&). Very preterm infants (VPI).

7.3. Allergic diseases during the first year of life

The study-cohort consisted of 324 (51%) very preterm infants, as parents of 156 (25%) refused to participate and 153 (24%) were excluded (Figure 9).

Parents of 3 infants chose not to participate in the RCT due to severe atopic predisposition (and were categorized as excluded in the RCT). None of them received human milk fortifier during their hospitalizations: A couple of twins (two boys) (GA 31+4) were exclusively breastfed until 6 months CA. No allergic symptoms were reported within the first year CA.
A girl (GA 32+0) was exclusively breastfed until 4 months CA. At 9 months CA the mother reported that the girl had an episode with rhinitis and conjunctivitis.

One boy (GA 29+3) with AD from 3 months CA was suspected of CMPA after hospital discharge (but was for other reasons excluded in the RCT). He received HMF added to mother's own milk or donor milk during hospitalization and was bottle-fed with preterm formula after hospital discharge. A skin prick test (SPT) to milk was positive (4 mm), while specific (Immunoglobulin E) IgE was <0.35 kIU/L to milk and egg respectively. Controlled elimination/challenge procedure with milk was negative and thus the suspicion of CMPA was not confirmed. There was no clinical suspicion on allergy to egg.

Characteristics of participating infants and parents are shown in Table 8.

Information on allergic symptoms was obtained in 90% (290/324) infants. Due to withdrawal of 7 infants before 12 months CA there were 283 infants remaining at 12 months CA (ITT-analysis). An overall incidence and prevalence (corrected for missing data from 4 VPI) of allergic symptoms at 12 months CA among those 283 infants is shown in Table 9.

Information on atopic predisposition was obtained from 78% (253/324) with 25% (63/253) predisposed to allergic diseases. Among breastfed infants (group A (21/86) + B (25/85)) 26% (46/171) were predisposed to allergic disease(s) compared to 21% (17/82) in the formula fed (group C) with no significant difference between groups.

The number of infants who completed in their assigned nutrition groups until 2 months CA was 205 (63%) with 199 (61%) remaining at 12 months CA (PP-group).

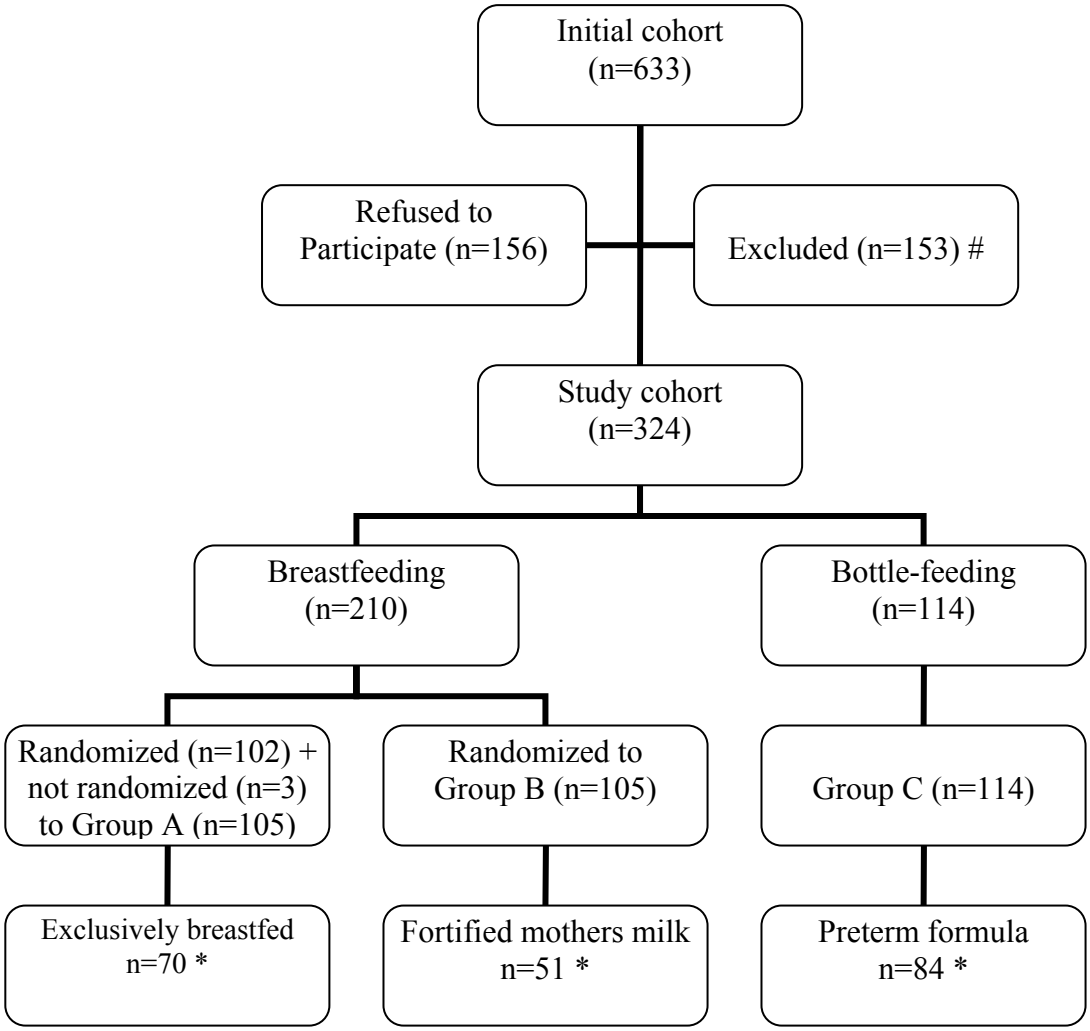
Comparing nutrition groups (A, B, and C, or just A and B) no difference was found regarding development of atopic dermatitis (AD) or recurrent wheezing (RW) during the first year of life, whereas atopic predisposition and gender were associated with an increased risk of developing AD and/or RW using both unvariat and multiple regression models. Infants with mothers in low social groups had a lower risk of developing AD in the univariat analysis (p=0.01), but not significant in the final model (ITT). The risk of developing AD increased with increasing age of the mother in the univariat analysis (p=0.03) (PP), but was not significant in the final model. Early introduction to complementary feeding was associated with an increased risk of developing RW in both univariat (p=0.02) and the final model (p=0.006) (PP). All results are shown in Table 10. There was no significant association between parental smoking and the development of RW or AD.

Specific IgE were analysed in blood-samples from 51% (163/320) infants at 4 months CA (Table 11). In two infants, specific IgE to cow's milk was detected: In a girl (GA 31+0), specific IgE (milk) was 0.38 kIU/L. She received HMF added to donor milk and mother's milk during hospitalization. She was exclusively breastfed at discharge and supplemented with a term formula and complementary food shortly before 4 months CA. She had two episodes with viral induced wheezing treated with bronchodilatator at 9 and 11 months CA while no other allergic symptoms were reported. In a boy (GA 29+4), specific IgE (milk) was 0.36 kIU/L. He also received HMF added to human milk during hospitalization. He was bottle-fed with preterm formula from hospital discharge until 4 months CA. He did not show any allergic symptoms until 1 year CA.

Main results on allergic diseases

The incidence during and prevalence at 12 months CA of RW was 39.2% and 32.7%, while AD was 18.0% and 12.1% respectively. Predisposition to allergic disease increased the risk of developing AD (p=0.04) (OR 2.6 (95% CI 1.0 – 6.4)) (PP) and the risk of developing RW (p=0.02) (OR 2.7 (95% CI 1.2 – 6.3)) (PP). Boys compared to girls had an increased risk of developing RW (p=0.003) (OR 3.1 (95% CI 1.5 – 6.5)) (PP). No difference was found between nutrition groups. None developed food allergy.

Figure 9. Participation flowchart. Treated PP (*) (completed in assigned nutrition-group until 2 months CA) **205**/211 and by ITT **283**/324 remained at 1 year of age.



(#) Exclusion: Death (n=34), serious congenital or chromosomal anomalies (n=9), surgery due to necrotizing enterocolitis (n=32), persistent ductus arteriosus (n=12), intraventricular haemorrhage III-IV and/or periventricular leucomalacia (n=25), bronchopulmonary dysplasia (n=9), eating disability at 42 weeks PMA (n=1), severe language problems (n=12), social problems (n=9), or moved (n=10).

Table 8. Characteristics of 324 preterm infants and their mothers.

Preterm infants	
GA at birth (median) (min-max) (weeks+days)	29+6 (24+1 – 32+0)
BW (median) (min-max) (g)	1283 (535 – 2255g)
SGA (weight Z-score < -2 SDS)	68/324
Boys	178/324
Multiple births	122/324
Predisposition to allergic disease Breastfed (A+B) vs. Formula fed (C)	63/253 46/171 (26%) vs. 17/82 (21%)
Introduction to complementary food ±1SD (mean) (weeks after term CA)	17.3 ± 4.0 weeks (after term)
Mothers	
Mother's age ±1SD (mean) (years)	30.7 ± 4.9
Social group ±1SD (mean) (1=high, 2, 3, 4, and 5=low)	3.14 ± 1.31
Parental smoking (one or two parents) smoking at home	116/319 (36%)
Maternal smoking Paternal smoking	61/319 (19%) 94/294 (32%)

Table 9. Incidence and prevalence of allergic symptoms within and at 12 months corrected age (CA) and mean age of onset of allergic symptoms among 283 very preterm infants.

Allergic symptoms	**Number of cases**	**Incidence until 12 months CA (%)**	**Median corrected age of onset of symptoms (min-max)**	**Prevalence (%) of symptoms at 12 months CA ($)**
Urticaria	7	2.5	7.2 (0.8 – 9.3)	0.0
Atopic dermatitis Total	51	18.0	6.1 (-2.6 – 11.9)	12.1
Treated with steroids	8	2.8	6.7 (0.2 – 11.9)	–
Treatment unknown	43	15.2	6.1 (-2.6 – 11.9)	–
Gastrointestinal symptoms	38	13.4	1.0 (-3.3 – 6.8)	1.4
Recurrent wheezing Total	111	39.2	7.1 (-0.4 – 12.0)	32.7
Treated with oral/inhaled bronchodilatators (*)	7 (oral) 23 (inh.)	10.6	8.0 (1.9 – 11.9)	–
Treated with inhaled glucocorticosteroids (#) (&)	31	11.0	6.1 (-0.4 – 11.1)	–
Treatment unknown	50	17.7	7.3 (-0.2 – 12.0)	–
Rhinitis ¤	27	9.5	2.1 (-2.7 – 11.8)	3.7
Conjunctivitis ¤	10	3.5	5.8 (-0.2 – 11.1)	1.8
One or more of the above symptoms	159	56.2	–	41.8

Treatment with: (*) beta-2-agonist oral or inhaled, (#) inhaled glucocorticosteroids, (&) 2 infants were also treated with leukotrienreceptor-antagonists. (¤) One child was treated with oral antihistamine (Clarityn®). ($) corrected for missing data from 4 infants at 12 months CA.

Table 10. Significant associations with development of atopic dermatitis and recurrent wheezing.

			Univariate (*)		Final model	
By ITT			p-value	**Odds Ratio (95% CI)**		p-value
Atopic dermatitis (AD)						
Group A, B, and C	+AD	-AD			(n=219)	
Atopic predisposition (n=223)	16/41	37/182	0.013	2.5 (1.2-5.2)	2.3 (1.1-5.0)	0.03
Maternal social group ±1SD (mean) (1=high, 2, 3, 4, 5=low)	2.69 ± 1.32 (n=51)	3.21 ± 1.30 (n=230)	0.011	0.7 (0.6-0.9)	–	ns
Group A and B					(n=153)	
Maternal social group ±1SD (mean) (1=high, 2, 3, 4, 5=low)	2.39 ± 1.15 (n=36)	2.99 ± 1.30 (n=156)	0.013	0.7 (0.5-0.9)	–	ns
Recurrent wheezing (RW)						
Group A, B, and C	+RW	-RW			(n=219)	
Boys (Both gender=283)	76/111	80/172	0.000	2.5 (1.5-4.1)	3.3 (1.8-6.1)	0.000
Group A and B					(n=153)	
Boys (Both gender=194)	50/73	51/121	0.000	3.0 (1.6-5.5)	4.1 (1.9-8.6)	0.000
Treated PP						
Atopic dermatitis (AD)						
Group A, B, and C	+AD	-AD			(total n=155)	
Atopic predisposition (n=159)	12/30	28/129	0.041	2.4 (1.0-5.6)	2.6 (1.0-6.4)	0.043
Group A and B					(total n=95)	
Mothers age ±1SD (mean) (year)	32.9 ± 4.6 (n=23)	30.6 ± 4.2 (n=95)	0.027	1.1 (1.0-1.3)	–	ns
Recurrent wheezing (RW)						
Group A, B, and C	+RW	-RW			(total n=155)	
Atopic predisposition (n=159)	19/55	21/104	0.049	2.1 (1.0-4.3)	2.7 (1.2-6.3)	0.016
Boys (Both gender=199)	49/72	61/127	0.007	2.3 (1.3-4.2)	3.1 (1.5-6.5)	0.003
Group A and B					(total n=95)	
Boys (Both gender=118)	26/39	34/79	0.017	2.6 (1.2-5.9)	3.6 (1.2-10.4)	0.018
Introduction to CF ± 1SD (mean time since term CA) (weeks)	16.0 ± 3.5 (n=39)	17.8 ± 3.7 (n=79)	0.020	0.98 (0.97-1.0)	0.97 (0.95-0.99)	0.006

(*) Wilcoxon rank-sum test if median, t-test if continuous variables or chi2-test if categorical variables. CF=complementary feeding. n=total number of very preterm infants in the model.

Table 11. Results on specific IgE-analyses to different allergens.

Specific IgE (n=161)	**Median (kIU/L) (min-max)**	**Number > 0.35 kIU/L**
Egg-white (n=161)	0.03 (0.02-0.14)	0
Milk (n=161)	0.03 (0.02-0.38)	2 *
Peanut (n=160)	0.00 (0.00-0.03)	0
Dust mite: Pteronyssinus n=157	0.01 (0.00-0.32)	0
Dust mite: Farinae n=153	0.00 (0.00-0.27)	0
Dog (n=149)	0.01 (0.00-0.16)	0
Cat (n=151)	0.00 (0.00-0.08)	0
Grass pollen (n=160)	0.00 (0.00-0.07)	0
Latex (n=159)	0.05 (0.03-0.14)	0

* a) 0.38 kIU/L and b) 0.36 kIU/L.

7.4. Macronutrients in human milk from mothers who delivered prematurely

A number of 214 mothers delivered 736 human milk samples (from 2 weeks after birth until 4 months CA).

Main results on macronutrients in human milk

Macronutrients in human milk showed decreasing content of protein, but stable contents of fat and lactose from birth to 4 months CA (Figure 10). Energy in human milk is shown in Figure 11.

Using t-test comparing protein-content in human milk at different times, there was a significant difference between the following weeks:
Four weeks: Human milk had lower content of protein compared to 2 weeks (p=0.000).
Six weeks: Human milk had lower content of protein compared to 4 weeks (p=0.024).
Eight weeks: Human milk had lower content of protein compared to 6 weeks (p=0.036).

Protein-contents in human milk samples from mothers of very preterm infants are shown in Figure 12, Figure 13, and Table 12.

Figure 10. Content of lactose, fat, and protein in human milk

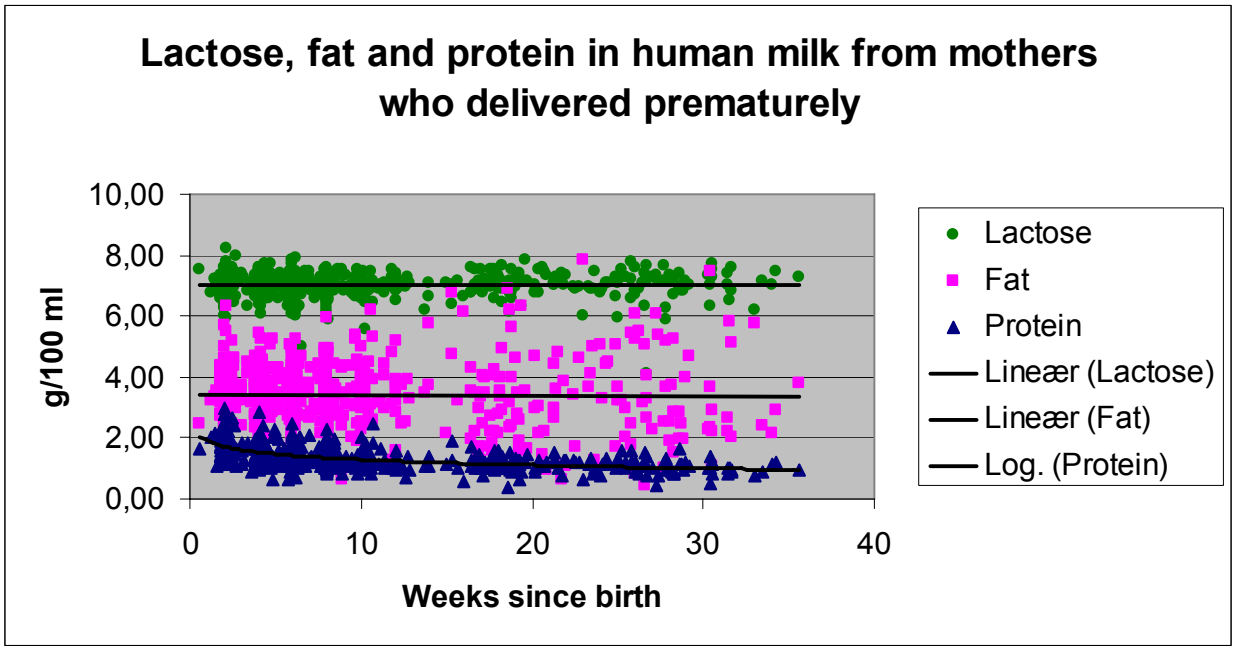


Figure 11. Energy in human milk

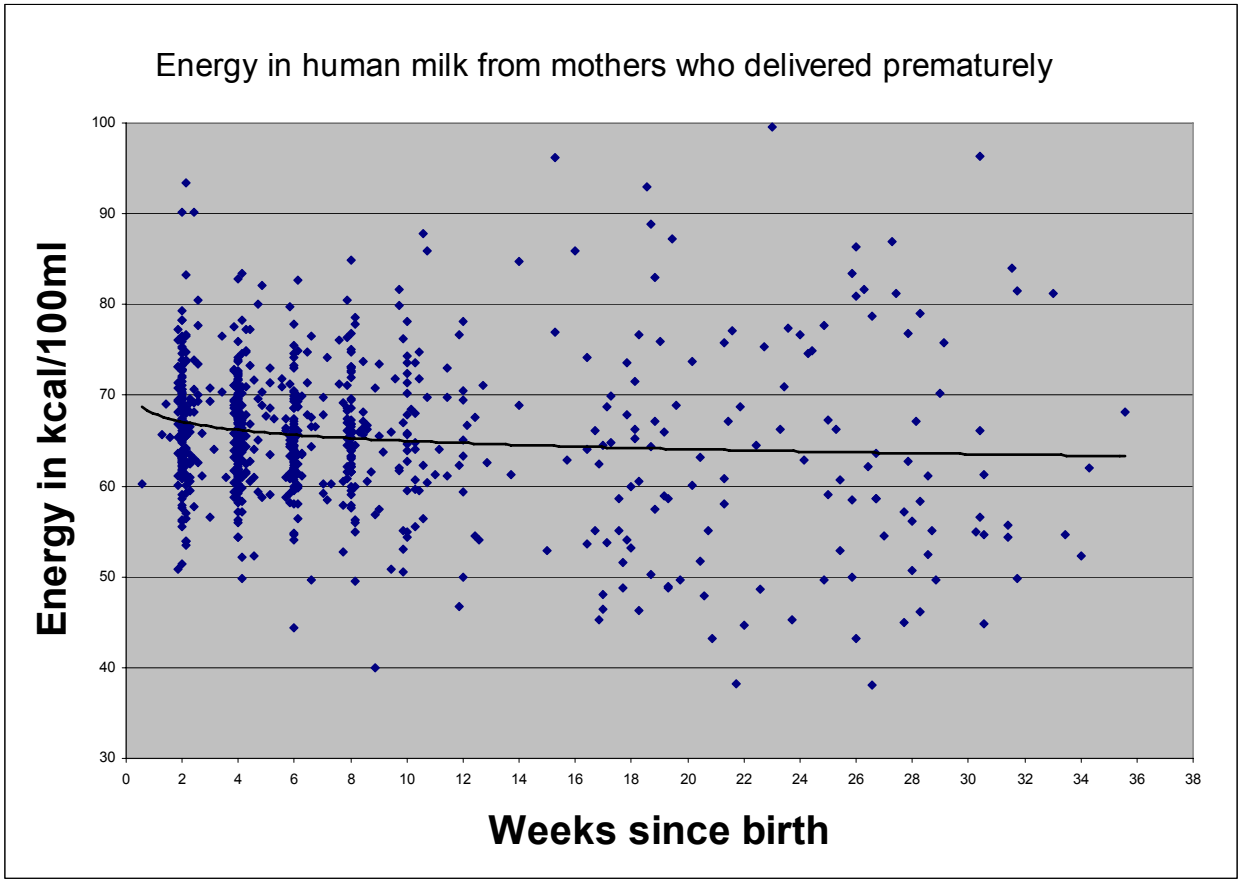


Figure 12. Protein-content in human milk from mothers of very preterm infants.

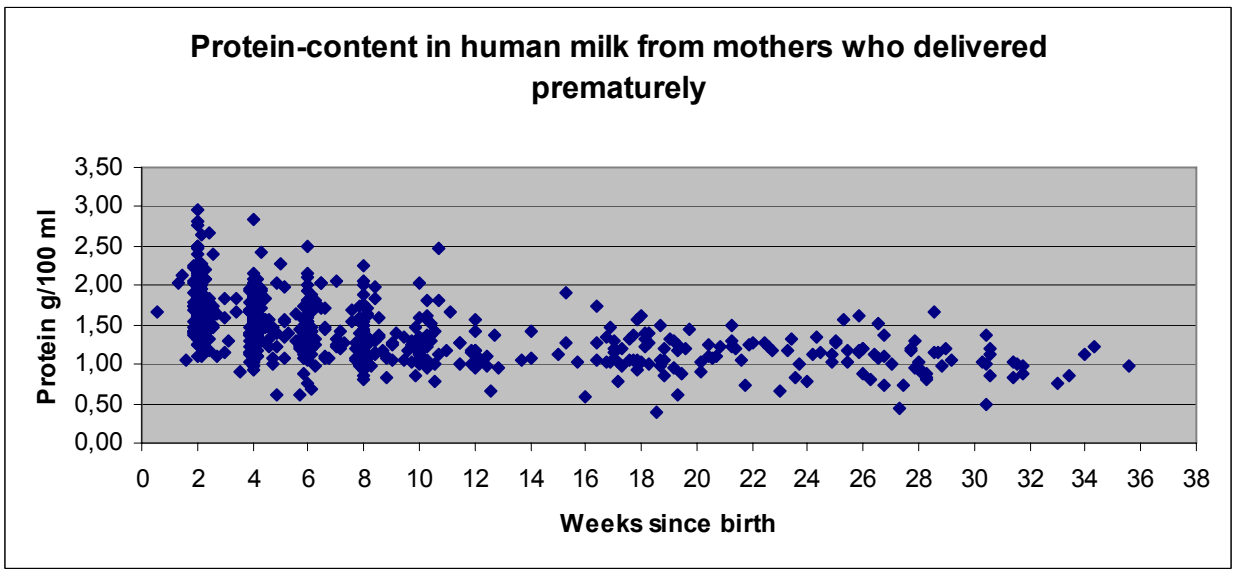


Figure 13. Mean protein-content in human milk from mothers who delivered prematurely

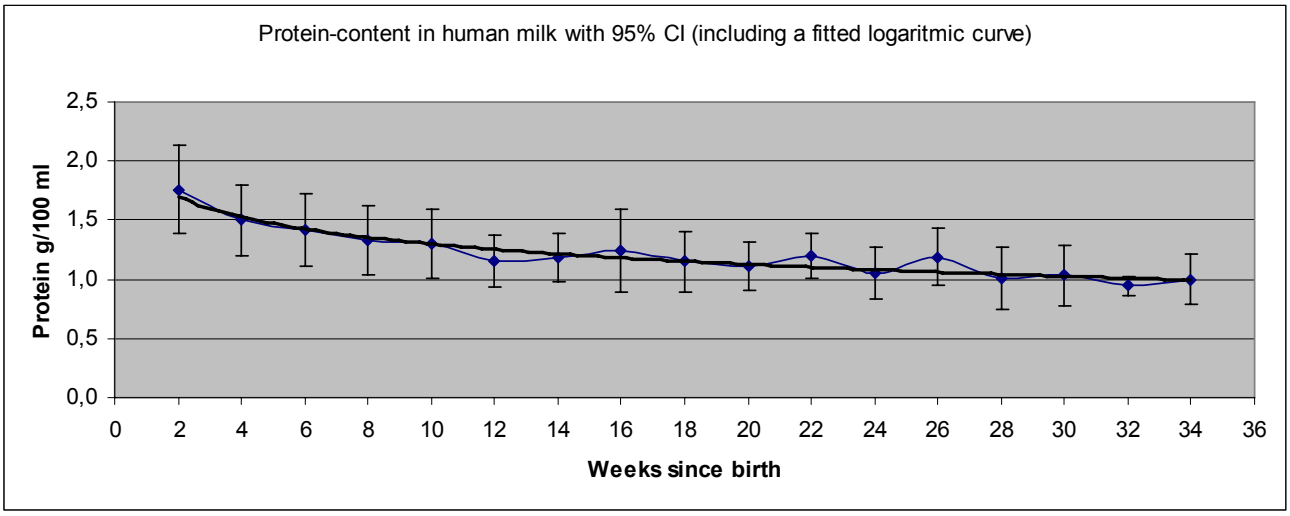


Table 12. Mean protein-content and SD in human milk samples

Weeks after birth	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	32
Number of milk Samples	155	175	110	90	48	21	3	12	29	16	11	11	18	17	9	5	4
Protein-conc. (mean) (g/100ml)	1.8	1.5	1.4	1.3	1.3	1.2	1.2	1.2	1.1	1.1	1.2	1.0	1.2	1.0	1.0	0.9	1.0
1 SD	0.4	0.3	0.3	0.3	0.3	0.2	0.2	0.4	0.3	0.2	0.2	0.2	0.2	0.3	0.3	0.1	0.2

7.5. Eating habits and feeding problems: Meals, regurgitation, and use of anti-constipation medicine

A total number of 769 questionnaires based on 286 (89% of study-cohort) infants were completed.

Main results on eating habits and possible feeding problems

Both breastfeeding groups (A and B) were fed more meals each day compared to formula fed groups (preterm and term formula). Results on number of meals each day are illustrated in Figure 14 and Table 13.

Many episodes of regurgitation were reported especially around 2 months CA with 74.0% among all very preterm infants. No significant difference was found on regurgitation comparing the breastfed groups. Results on regurgitation frequency each day is illustrated in Figure 15.

Infants fed formula (group C and mature formula (MF)) were more often treated with anti-constipation medicine compared with both breastfed groups (A and B). At term, group B received more anti-constipation medicine compared to group A. Results on use of anti-constipation medicine are illustrated in Figure 16 and Table 14.

Number of meals each day.

Questionnaires with information on "number of meals each day" (751/769=98%):

235 answered questions about meals each day in the period from week 34-37 PMA.

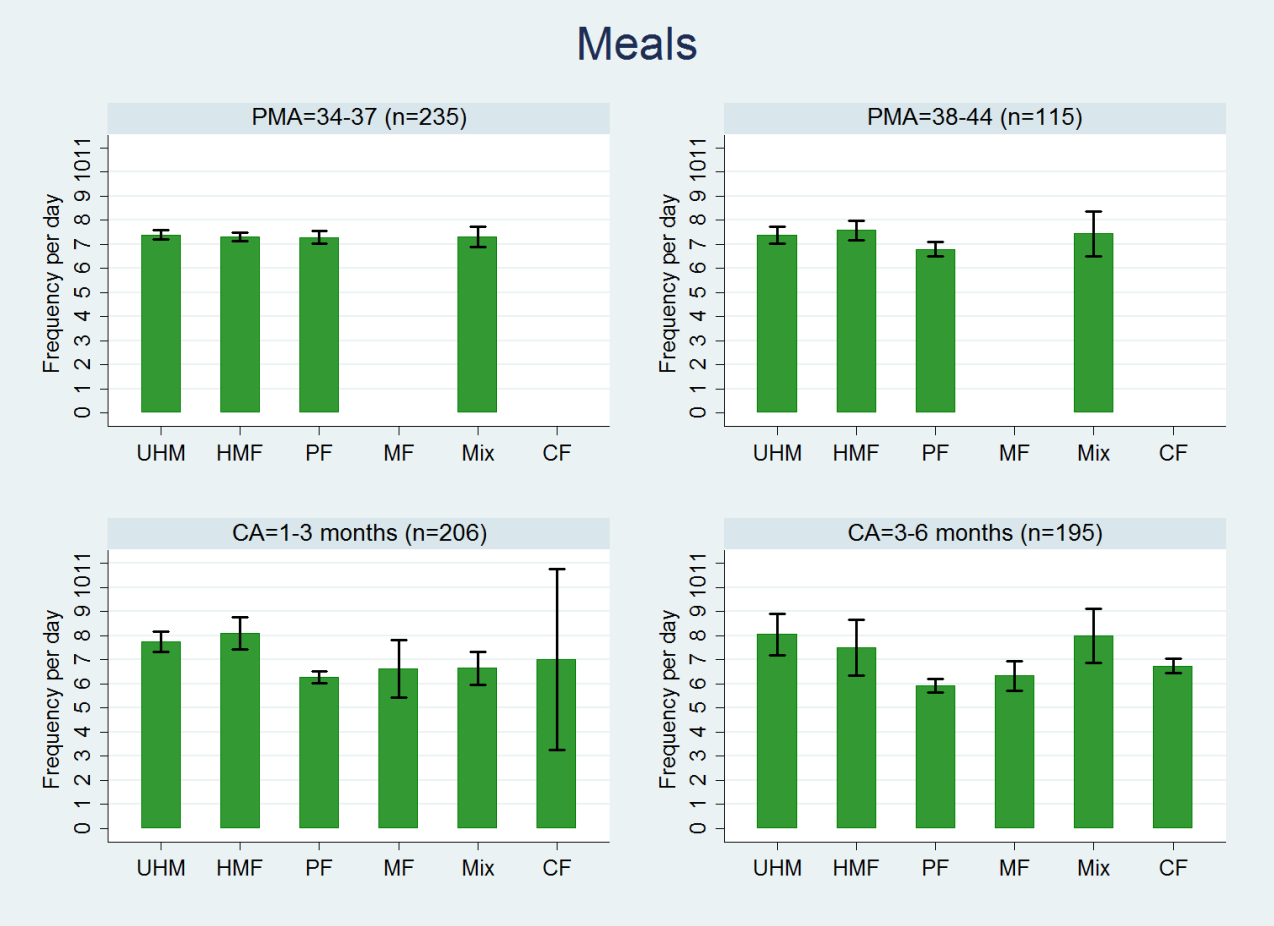
115 answered questions about meals each day in the period from week 38-44 PMA.

206 answered questions about meals each day in the period from 1-3 months CA.

195 answered questions about meals each day in the period from 3-6 months CA.

Both breastfeeding groups (A=UHM and B=HMF) were fed more meals each day compared to the three formula groups (C=PF, MF, and Mix) and complementary-fed (CF) group.

Figure 14. Mean number of meals each day with 95% CI.



UHM=unfortified human milk
HMF=human milk fortification added to mother's own milk
PF=preterm formula
MF=mature formula
Mix= both breast and bottle
CF=complementary feeding

Table 13. Significant difference in number of meals each day comparing nutrition groups.

	Comparing groups	Coefficient	p-value	95% CI
34-37 weeks PMA	–	–	Ns	–
38-44 weeks PMA	UHM vs. PF	0.58	0.032	0.05-1.10
	HMF vs. PF	0.78	0.001	0.31-1.25
1-3 months CA	UHM vs. PF	1.47	0.000	0.96-1.98
	UHM vs. MF	1.11	0.024	0.15-2.07
	UHM vs. Mix	1.10	0.000	0.37-1.82
	HMF vs. PF	1.83	0.000	1.21-2.45
	HMF vs. MF	1.47	0.005	0.44-2.49
	HMF vs. Mix	1.45	0.001	0.64-2.26
3-6 months CA	UHM vs. PF	2.12	0.000	1.42-2.82
	UHM vs. MF	1.72	0.001	0.74-2.70
	UHM vs. CF	1.32	0.000	0.64-2.00
	HMF vs. PF	1.57	0.002	0.58-2.55

Regurgitation among very preterm infants each day according to nurition.

Questionnaires with information on "regurgitation each day" (730/769=95%)

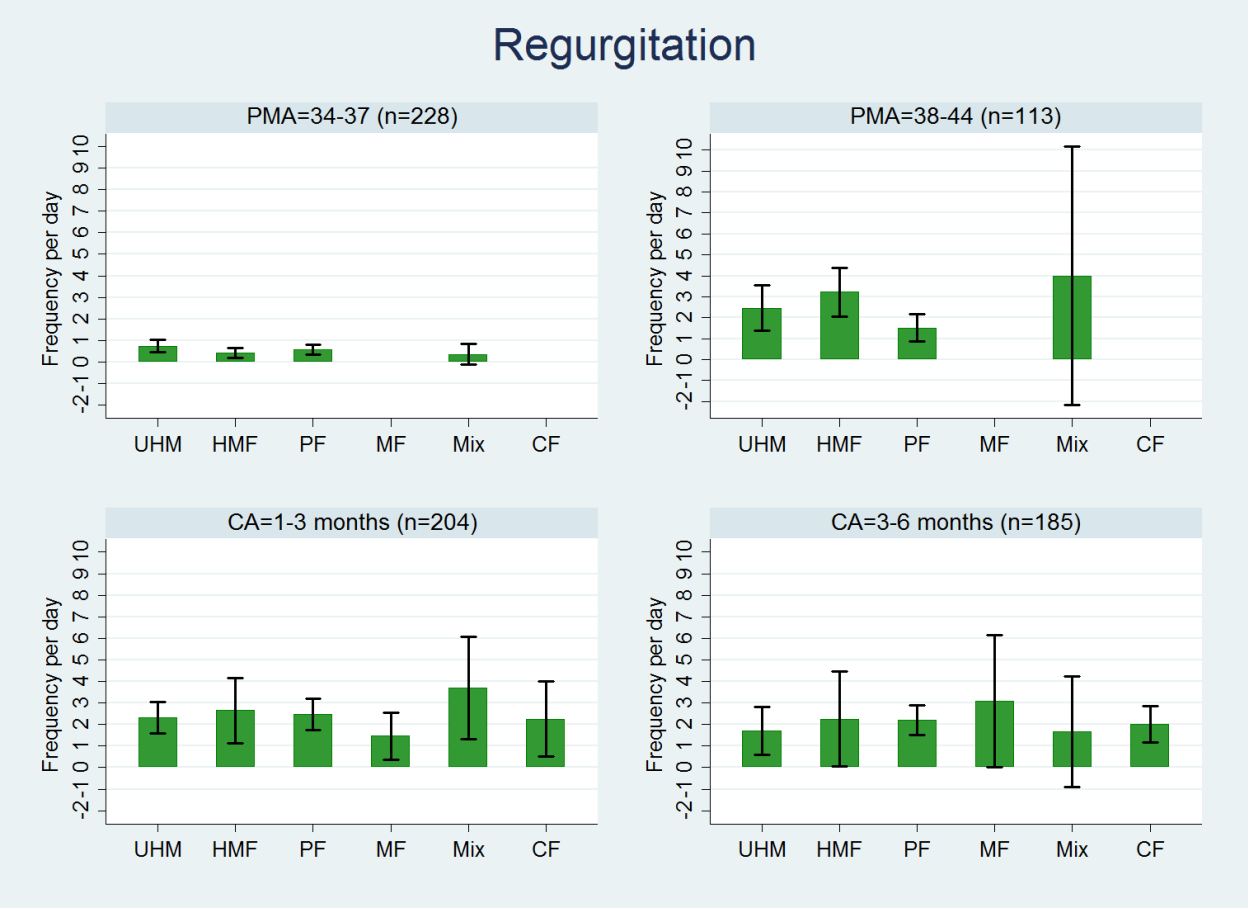
228 answered questions about regurgitation each day in the 1.st period from week 34-37 PMA.

113 answered questions about regurgitation each day in the 2.nd period from week 38-44 PMA.

204 answered questions about regurgitation each day in the 3.rd period from 1-3 months CA.

185 answered questions about regurgitation each day in the 4.th period from 3-6 months CA.

Figure 15. Frequency of regurgitation each day with 95% CI.



No significant difference on regurgitation between the groups in the first period.

No significant difference between the breastfeeding groups in the second period, but the infants in the fortified group (HMF) had more regurgitation compared to the infants fed PF (p=0.034) (1.72 95% CI 3.30 – 0.14) but not compared to infants fed unfortified human milk (UHM). No significant difference was found on regurgitation comparing groups in the third or fourth period.

Infants with reported regurgitation in the first period was 33.7%, in the second period was 65.5%, in the third period 74.0%, and in the fourth period was 62.2%.

Use of anti-constipation medicine.

Questionnaires with information on "use of anti-constipation medicine" (742/769=96%)

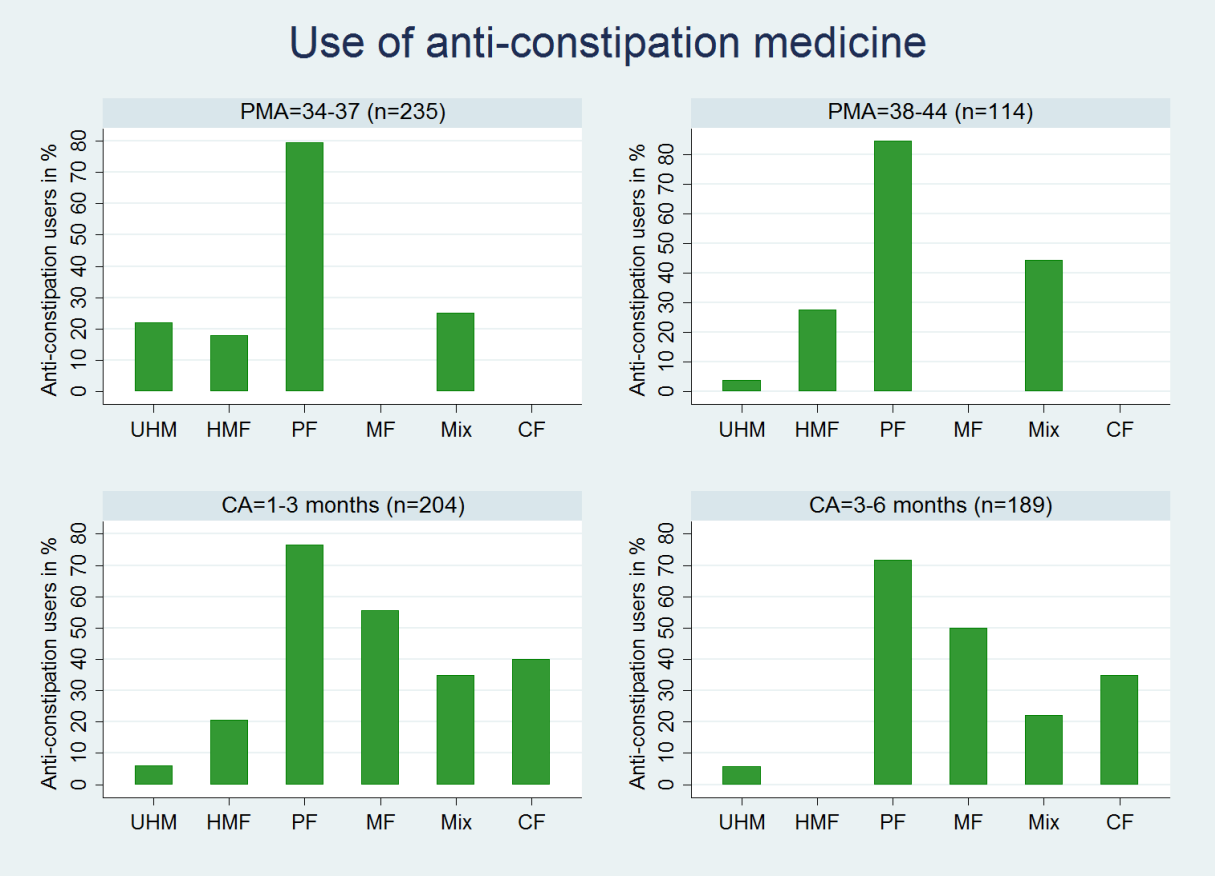
235 answered questions about medicine each day in the period from week 34-37 PMA.

114 answered questions about medicine each day in the period from week 38-44 PMA.

204 answered questions about medicine each day in the period from 1-3 months CA.

189 answered questions about medicine each day in the period from 3-6 months CA.

Figure 16. Percentage of very preterm infants using anti-constipation medicine.



38.7% of the infants received anti-constipation medicine in the period from 34-37 weeks.

43.0% of the infants received anti-constipation medicine in the period from 38-44 weeks.

45.1% of the infants received anti-constipation medicine in the period from 1-3 months CA.

42.9% of the infants received anti-constipation medicine in the period from 3-6 months CA.

Table 14. Significant difference in use of anti-constipation medicine comparing nutrition groups.

	Comparing groups	Coefficient	p-value	95% CI
34-37 weeks PMA	PF vs. UHM	0.57	0.000	0.44-0.71
	PF vs. HMF	0.62	0.000	0.48-0.75
38-44 weeks PMA	HMF vs. UHM	0.24	0.016	0.04-0.43
	PF vs. UHM	0.81	0.000	0.61-1.00
	Mix vs. UHM	0.41	0.007	0.11-0.70
	PF vs. HMF	0.57	0.000	0.40-0.74
1-3 months CA	PF vs. UHM	0.70	0.000	0.56-0.33
	MF vs. UHM	0.50	0.001	0.21-0.78
	Mix vs. UHM	0.29	0.005	0.09-0.49
	PF vs. HMF	0.56	0.000	0.39-0.72
	MF vs. HMF	0.35	0.025	0.04-0.65
3-6 months CA	PF vs. UHM	0.66	0.000	0.42-0.90
	MF vs. UHM	0.44	0.010	0.11-0.77
	CF vs. UHM	0.29	0.016	0.06-0.53
	PF vs. HMF	0.71	0.000	0.38-1.05
	MF vs. HMF	0.50	0.015	0.10-0.90
	CF vs. HMF	0.35	0.036	0.02-0.68

In general infants fed formula (C=PF and MF) were more often treated with anti-constipation medicine compared with both breastfed groups (A=UHM and B=HMF). At term, group B (HMF) received more anti-constipation medicine compared to group A (UHM).

8. General discussion

8.1. Study-design

The findings presented in this thesis originate from an intervention study on nutrition of very preterm infants after hospital discharge. The study includes a prospective and consecutive registration of very preterm infants from four neonatal units creating a population based birth cohort of very preterm infants followed until 1 year CA. Our study consisted of an observational part from birth to shortly before planned hospital discharge, followed by an interventional part. The intervention study was a randomized controlled trial with randomization shortly before hospital discharge in order to test if fortification of mother's own milk while breastfeeding was possible, and to test the effect of fortification of mother's own milk on growth among very preterm infants, compared to feeding solely mother's milk after hospital discharge. In our randomized trial, we compared a treatment group assigned to fortification of mother's milk with a control group not receiving fortification of mother's milk. A third nutrition group of infants fed a preterm formula was created without randomization. It is not possible to randomize to breastfeeding versus bottle-feeding. Therefore, based on the observational part of the study, we have characterized the breastfed compared to the bottle-fed infants and their mothers.

The primary aim of this study was to investigate the effect of human milk fortifier, added to mother's own milk while breastfeeding, on growth. The study was randomized but not blinded due to the lack of a placebo-product without influence on breastfeeding, nutrition, and growth. The most important advantage of a randomized trial is that selection bias will be eliminated by balancing both known and unknown factors influencing the outcome of the treatment.
Of the 633 eligible infants in our study, 157 were excluded due to death or diseases influencing eating ability and/or growth. The excluded group consisted of the youngest (GA) and smallest (BW) (including SGA) very preterm infants. Among parents of 156 infants who refused to participate in the intervention study, the mothers were younger and more often bottle-feeding their infants at discharge. Among 320 infants in the intervention study mothers of the bottle-fed infants more often had "multiple births", were more often smokers, and belonged to lower social groups compared to mothers of breastfed infants.

More girls and more multiple births were randomized to the fortification-group possibly influencing the outcome. Optimally randomization should have been done also according to e.g. gender and multiple versus single birth in order not to have groups with uneven distribution of

gender and multiple birth infants. Meanwhile, as the study was carried out at 4 centers, it was considered impossible to include these two parameters in the randomization process.

In our study, 25% were excluded and parents of further 25% refused to participate. Other studies dealing with preterm infants have shown similar problems with exclusion and refusals. In a Canadian study, 36% were excluded and 37% refused to participate (60). The parents often have experienced their infants critically ill and they can not manage also to participate in a study. In our study, dropouts / change of nutrition were as expected highest in the intervention-group (B) due to extra workload with expressing milk and fortification of mother's milk.
Breastfeeding should be provided as long as mutually desired by mother and infant / child according to ESPGHAN Commentary on Breastfeeding (61). In our study, the intervention was planned until 4 months CA, but in both breastfed groups (A and B) breastfeeding stopped between 2 and 4 months CA in most cases, making intervention with fortification further on to be difficult. The "oldest" infants in the study were born with a GA of 32+0 weeks and at the age of 2 months CA their mothers (if nursing / breastfeeding) have been expressing milk or breastfeeding for 4 months already. Therefore, it is a challenge to maintain an increasing amount of breast milk for the preterm infant several months after birth and especially when nursing multiple births. Due to some "changes of nutrition" during the intervention period both ITT and PP analyses were made. Overall, participation and compliance was high in our intervention study, and with the above considerations in mind, we believe that our results can be transferred to other populations of very preterm infants.
Care must be taken not to extrapolate the results of the RCT to patients excluded from the study. It would have been interesting to investigate the effect of fortification of human milk among the sick preterm infants who were excluded, but this was not the aim of the study.

8.2. Growth of preterm infants

It is a general problem to establish adequate growth in very preterm infants. It takes time to establish adequate dietary intakes in the immature infant, and infants often become malnourished during the initial hospitalization (2). A common goal for optimal nutrition of very preterm infants is important, and accurate and reproducible outcome measures and references for growth (weight, length, and head circumference) are important for consensus, in order to discuss the extent of nutritional supply during and after hospitalization.

8.2.1. Assessment of growth

Provision of energy and nutrients at levels to support optimal growth and development is the goal of nutritional support, but to demonstrate growth pattern among VLBW infants, monitoring of growth is important. Body weight comprises the total mass of infant's lean tissue, fat, and extracellular and intracellular fluid compartments. Weight gain or loss, therefore reflects changes in body-composition (19). Body-weight can be measured more accurately and reproducibly than linear growth even though change in linear growth is generally regarded as the best measure of assessing adequacy of dietary intake (19;62). It is difficult to measure total length of a sick preterm infant especially in the incubator. Therefore, measurement of knee-heel length has been advocated, but data suggests that these measurements are neither an accurate nor a more sensitive indicator of total linear growth in preterm infants (62). In our study, the infants had their crown-heel length measured with a tape measure during hospitalization, while an "infant measuring rod" / a stadiometer was used at and beyond hospital discharge.
Anthropometric data on weight, length, and HC has been obtained during hospitalization for all infants in the study. Weight was measured several times every week while length and HC was registered less often. Especially the registration of HC at birth and length at the day of discharge were missing. The main aim of the study and this thesis was to investigate nutrition and growth after hospital discharge for which reason not all anthropometric data during hospitalization have been presented.

8.2.2. Growth references

In order to discuss optimal growth and catch up growth, a reference describing growth is needed. Growth references for preterm infants from birth, through discharge, term, and during the first year of life are few, making it difficult to describe "optimal" growth of very preterm infants. Growth references can be "descriptive", depicting how children actually grow or "prescriptive", describing how children should optimally grow (63). Some preterm growth references (descriptive) are based on in-*utero*-measurements (ultrasound) (56) and some are based on measurements at birth (57). Growth references used for preterm infants from 40 weeks PMA can be based on mature or preterm infants, breastfed or formula fed infants, infants from same part of the world or pooled data from different parts of the world, like the WHO growth references (64). In our intervention study, we chose a descriptive reference based on measurements of weight, length, and head-circumference among Swedish infants from preterm and term birth to 24 months CA (57) as they probably reflect the growth of Danish preterm infants well. The

integration of a term-born growth curve until 24 months could explain why Z-scores in our nutrition groups tended to decrease among most infants from 6 to 12 months CA. Another explanation could be that low birth weight infants as a group has been shown at greater risk to remain smaller than normal birth weight peers throughout the years of growth until young adulthood, with the extremely low birth weight infants at greatest risk (63). A third explanation could be that the growth-pattern of both non-SGA and SGA preterm infants is different as compared to mature infants no matter the nutrition.

As different growth references vary in population and statistics, it is important to specify which growth reference has been used in a certain study. The new WHO growth references have been criticized because the references for both girls and boys are heavier as compared to the infant references used in the UK and by the US Centre for Disease Control and Prevention (65-67). On the contrary, we found the WHO growth references to be a little lighter especially at term compared to the Swedish population-based reference.

8.2.3. Catch-up growth and small for age

In the past, catch-up growth has been advised, especially for SGA infants because early enhanced nutritional intake in VLBW infants, leading to catch-up, has shown to be associated with better long-term neurodevelopmental outcome (63;68). However, because almost all preterm infants loose percentiles after birth, catch-up growth has been advised for basically all preterm infants (69).

To achieve the goal for catch-up growth, special formulas, taking the nutritional requirements of preterm infants into consideration, has been developed and evaluated in studies comparing preterm formula or post-discharge formula with a term formula and/or human milk (16;70-73). These studies have demonstrated that a significant proportion of LBW infants, regardless of how they were fed post-discharge, did catch-up, although not completely. The advantage of nutrient enrichment though seems to appear early (within 1-2 months post-term), suggesting that there is a finite period during which catch-up in response to higher nutrient intakes is most likely (8). In our study, the infants fed a preterm formula achieved catch-up at an earlier age compared to both breastfed groups. Non-SGA infants in our study seemed to have achieved catch-up on HC at discharge, on weight at 2 months CA, and on length at 4 months CA.

A study on growth among children born with a GA < 32 weeks found that SGA infants with rapid initial growth (during the first 3 months) already attained normal height for target height at 2 years of age while those with slow initial growth still showed persisting stunting at the age of 10 years (10). SGA infants in our study had greater catch-up growth compared to non-SGA

infants during the study-period, but with no significant difference comparing nutrition groups. All SGA infants showed rapid catch-up growth on HC until term, on weight until 4 months, and length-growth even continued until 1 year CA.

Both SGA and non-SGA infants in our study did achieve some catch-up growth during hospitalization but did not fully reach the chosen growth reference of the *fetus* / preterm infant with the same PMA before hospital discharge but seemed to be achieved before 1 year of age on all growth parameters. The group of infants with subnormal weight at discharge increased significantly more in weight when fed PF compared to both breastfed groups. The two sub-groups of infants with subnormal weight (at birth and/or at discharge) seemed to have a more rapid catch-up growth for a longer period compared to infants with "non-subnormal" weight, but the groups were however small, resulting in lower statistical power allowing no firm conclusion or recommendation.

8.3. Nutritional requirements and recommendations for preterm infants

Inadequate nutrition has also been proven to increase the risk of neurodevelopment impairment and bone-disease among preterm infants (74-76) as well as growth failure. Suboptimal nutrition or even malnutrition has been supposed to affect structural and functional development of the nervous system in the preterm brain, possibly affecting long term development of neurological functions (77). Preterm infants with "failure to thrive", especially SGA infants, had the lowest cognitive scores, significantly lower than both the non-SGA and SGA infants with normal postnatal growth (37;63;76).

In addition, inadequate nutrient intakes of calcium, phosphorus, and vitamin D, in combination with e.g. a prolonged period of parental nutrition, increase the risk of reduction in bone mineral content known as "*osteopenia* of prematurity"(78).

Substrate supply is therefore very important in very preterm infants in order to improve body and organ growth in general and brain growth in particular. Early initiation with adequate amount of calories and amino-acids in e.g. parental nutrition is recommended for the very preterm infant (79;80). Enteral feeding, particularly with breast milk, may be started within the first few days of life (3;81) and also timely increased.

Human milk has many advantages and is the preferred feeding for term infants (61). As a source of nutrients for preterm infants, human milk is however, not sufficient in the usual feeding volumes. Human milk therefore needs fortification with proteins and minerals in order to meet

the needs of the growing preterm infant (3;82) and may not meet the nutritional requirements of the growing preterm infant after hospital discharge either. The protein-content in human milk from mothers who delivered prematurely has been shown to decrease significantly within weeks and months after birth (23). Several weeks after birth, at the mean time of discharge ≈ 37 weeks PMA, the protein-content of mother's milk had decreased to a level equivalent to human milk 4-8 weeks after birth from mothers who delivered at term (23). We found the same significant decrease in protein-content in mother's milk, from 2-8 weeks after birth in milk samples from mothers in our study. At the time of discharge, usually 2-4 weeks before term, the protein-content in mother's milk was low and did not meet the needs of a growing preterm infant.

In order to meet the optimal nutritional requirements of the growing preterm infant, several studies have been published and the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) has recently (2010) published recommendations on enteral nutrient supply for preterm infants up to a weight of approximately 1800g with e.g. recommended protein-levels, calcium-, and phosphorus-levels (80).

Preterm formulas have been developed to meet the nutritional needs for preterm infants.
To meet the recommendations when feeding human milk, fortification can be done by different methods, either a "standard fortification" using the same standard amount of fortification based on the assumption of a standard composition of human milk, or an "individualized fortification". The latter can be done with "adjustable fortification" based on the infants metabolic response evaluated by BUN-levels, or a "targeted fortification" based on analysis of human milk (82-84). These recommendations on fortification of human milk are made for and are very useful for tube-fed infants during hospitalization, but have not yet been tried or verified as useful for breastfed very preterm infants when practicing breastfeeding directly from the breast after hospital discharge.

We found that formula fed infants increased more in growth compared to breastfed infants in weight Z-score from birth to discharge (in part 1 on the exact day of discharge, and in part 2 at day 252 = 36 weeks PMA. This can be explained by: 1. Breastfed infants losing weight during the period practicing breastfeeding, since they are changed to be fed on demand and not on schedule. 2. Formula fed infants received constantly all the nutrients they need because they did not have to practice breastfeeding. 3. The breastfed infants supplemented with HMF did not

receive enough fortification during hospitalization – and especially not during the breastfeeding establishing period.

A comparison of compositions of mother's milk and products for feeding preterm infants during hospitalization and after hospital discharge is shown in appendix 1. The "Premature Formula" used in our trial has almost the same composition as the two different "Discharge Formulas" for preterm infants with more energy, protein, calcium, and phosphorus compared to both human milk 8 weeks after preterm birth and a term formula. Adding only 5 packets of HMF to mother's milk does not provide as much energy and protein as the formulas. This probably explains some of the differences on growth in the RCT with increased growth in the formula group compared to both breastfed groups.

8.4. Breastfeeding preterm infants at discharge

In our prospective observational part of the study, 60% of 478 very preterm infants were exclusively breastfed at discharge and the corresponding number in the intervention study was 65% of 320 infants. All but one of the infants fed both mother's milk and formula at discharge refused to participate in the RCT. According to the literature, breastfeeding rates of preterm infants are reported to vary considerably. The definition of breastfeeding, also vary in the literature, with breastfeeding defined as "exclusively breastfeeding" or "breastfeeding including supplementation with a formula" (mixed feeding). A study from 2010 (85) describes variations in breastfeeding-rates in Europe with very low rates of exclusively breastfeeding and higher rates of mixed feeding. The highest breastfeeding rate was in the UK with 29% exclusively breastfeeding and 6% mixed feeding making 65% to be formula fed (85). In a study from 2002 with 119 mothers of single birth VLBW infants, it was found that 73% intended to breastfeed but only 34% (25% of total cohort) continued lactation beyond 40 weeks PMA. Mothers who continued breastfeeding were older, more often married, and had a higher level of education compared to those who discontinued lactation. Significant factors influencing lactation beyond 40 weeks PMA included start of milk expression before 6 hours post-delivery, expressing milk more than 5 times a day, and kangaroo care (86). An Italian study from 2008 found the highest probability of initiating and maintaining breastfeeding among infants with mothers aged between 27 and 34 years, with high educational levels, non-smokers, and with previous breastfeeding experience (87).

Very few studies describe the duration of breastfeeding among term and preterm infants. In our observational study and in the RCT, 60% and 65% of the infants were directly breastfed at discharge respectively. At the time of discharge, the infants had received human milk for an average of 8 weeks. In comparison, a Scandinavian study from 1996 found a breastfeeding rate among term infants 6 weeks *post partum* to be 77% (88).

Previous studies has shown a negative association of maternal smoking on breastfeeding initiation and duration (89-91). In our study, with 19% of the mothers of very preterm infants being smokers, we found a negative correlation between maternal smoking and breastfeeding. Mothers in low social groups, were also negatively correlated to breastfeeding at discharge. Smoking and mothers in low social groups were strongly correlated, but both variables were significant in the multivariate logistic regression model. The number of smoking mothers apparently has decreased during the past 2 decades in Denmark. A study from 1996 on term infants found 20% smoking mothers (88), and another study from 2006 found 26% smoking mothers during pregnancy (91), while a study from 1991 reported 38% mothers smoking in pregnancy and 42% smoking during lactation period (92). It has been shown that mothers perceived that a strong risk of harming the baby was posed by smoking while breastfeeding and that they received little encouragement to continue breastfeeding because of inability to stop smoking (93). This indicates a need for more consistency in promoting breastfeeding in spite of smoking *post partum*.

Studies have shown positive associations between breastfeeding and cognitive development also within the low birth weight group (17;18;94). Therefore, breastfeeding is also recommended for the low birth weight infants.

8.5. Nutrient enrichment of mother's milk for preterm infants after hospital discharge

To our knowledge, only one other group has studied fortification of breast milk for very preterm infants after discharge. A Canadian pilot-study followed growth until 1 year of age of human milk fed very preterm infants who were randomized to fortification of expressed mother's milk (half of their feedings) or not for 12 weeks after hospital discharge (60;95). The infants that received fortification remained longer and had a greater whole-body mineral content (both significant) until 12 months of age (95). The infants in the Canadian study were not direct

breastfed and were fed a higher amount of fortification (supplied roughly half the volume of human milk as nutrient enriched feedings (4 packets / 100 ml)) compared to the infants in our study. We chose to supplement with only 5 packets of fortifier each day (~1 packet / 100 ml) because of the risk of interfering with breastfeeding if using higher amounts. The duration of breastfeeding was not influenced by fortification of human milk in these amounts, showing that this fortification was practically possible, but we do not know if it would be so with higher amounts of fortification.

In our study, infants fed PF achieved weight and length catch-up growth earlier compared to infants fed mother's milk with or without fortification, probably due to feeding more protein as shown by elevated BUN-levels indicating elevated protein-metabolism. BUN-values has previously been shown as indicators of protein-metabolism (96) and even used as a method to fortify mother's milk for infants during hospitalization (adjustable fortification) (83). Agreement on optimal BUN-values for optimal growth among very preterm infants has not yet been achieved. Hall has recommended a BUN-value to be > 0.8 mmol/l (> 5 mg/dl) among preterm infants post-discharge, otherwise intervention is suggested (97). The PF fed infants in our study show BUN-values almost identical with PF-fed infants in a similar study on post-discharge nutrition comparing formulas for preterm infants (73). The difference between infants fed PF and breast milk could possibly be explained by feeding an increasing amount of formula / day (and thereby protein), while the breastfed infants received the same amount of fortifier and maybe even the same amount of human milk each day during the intervention period. The latter suggestion is based on studies on mature breastfed infants that has shown an increase in milk volume until 1 month CA, while after 1 month the mean day-to-day variability within subjects is fairly constant, but between subjects the volume differs between 600 and 900 ml (98;99). The exact volume intake within our three nutrition groups was not registered. Compared to infants fed unfortified mother's milk, those who completed fortification until 4 months CA had higher s-phosphorus- and BUN-values. Though this was not associated with increased weight gain, it may reflect a better growth potential.

Preterm girls seemed to benefit from fortification of mother's milk after hospital discharge compared to girls fed solely mother's milk. Fortification in the amount given in our study did not affect growth significantly at 1 year CA for both genders. Fortification though improved weight at 2-6 months CA and HC at term - 4 months CA (ITT) and length- and HC-growth at 2 - 4 months CA (PP) significantly among girls using REM. Studies based on body composition of

preterm infants have described boys to be programmed to grow faster and accrete more lean mass compared to girls. At 12 months CA boys were greater on lean mass, bone area, and bone mineral mass compared to girls (100). It has been suggested that preterm boys fed a high amount of protein seemed to benefit more from this diet (101). Looking at data on growth between 12 weeks and 18 months using standardized Z-scores, the same author (as the two previous mentioned studies) found that catch-up on length was apparent in preterm girls and not boys, suggesting that dietary intake during this period more adequately met the needs of girls than of boys (15). Our results may reflect that boys and girls are programmed differently according to growth and may have different nutritional needs, though a type 1 error should always be considered as a possibility. Further studies on growth according to gender differences are needed.

8.6. Metabolic syndrome – is there a risk?

Catch up growth may however, not be the only goal for feeding strategies of feeding preterm infants. Enhanced nutrition may increase the risk of metabolic and cardiovascular disease later in life (47;48;102). The definition of "the metabolic syndrome" and the metabolic abnormalities possibly affecting the development of this syndrome with increased risk of diabetes and cardiovascular disease is though not standardized in the literature (103;104). The mechanism(s) tying together anthropometric, physiological, and biochemical abnormalities are not completely understood and studies have to be carefully read and interpreted.

Based on epidemiological evidence and among predominantly term born populations, it has been hypothesized that the adoptions in the *fetus* due to e.g. undernourishment induce alterations in metabolism, hormonal output, and cardiac output, which result in central obesity, diabetes, and cardiovascular disease in middle age. Subjects with rapid catch-up growth are hypothesized at greatest risk for these consequences (50;105). However, as described, catch-up growth among preterm infants also have beneficial effects. Rapid growth of preterm infants during infancy, especially among those who failed to grow adequately *in-utero* or during the neonatal period, is regarded as a sign of good health and the resolution of chronic complications of prematurity. Especially catch-up of head size is associated with beneficial effects on cognitive development (62). This is however, a great dilemma in nutrition of preterm infants, with VLBW infant's demonstrating atypical low weight gain in the early years of life having a higher probability of less than optimal cognitive development over time, while those with excessive weight gain have a greater likelihood of later childhood and adult obesity, cardiovascular disease, and diabetes (63).

The protective effects of breastfeeding of mature infants regarding cardiovascular disease may be explained, at least partly, by the lower rates of weight gain, which may be related to differences in substrate intakes (106). Our study showed the same growth pattern with lower rates of weight- and length-gain (PP and ITT) among breastfed compared to PF fed infants. SGA infants though showed no significant difference on growth comparing nutrition groups while they showed more rapid catch-up on growth during the entire study-period compared to non-SGA infants.
It is still unknown if there is a risk for preterm infants of developing metabolic syndrome secondary to metabolic / nutritional events early in life (programming). This risk has been described small compared with the contribution from other risk factors, such as parental size, weight as an adolescent, and various lifestyle factors such as physical activity (107;108).
The balance between optimized growth and neurological outcomes without sacrificing metabolic and cardiovascular health among preterm infants still needs to be investigated. One study have examined preterm infants and reference infants at 35 and 36 weeks GA using DEXA, and found preterm infants to have a reduced fat-free mass and an increased global and central fat mass, that has been suggested to increase the risk of the metabolic syndrome (109). In a further follow-up, our infants will be tested on neurological development and growth including body-composition measured using Dual Energy X-ray Absortiometry (DEXA).

We also wanted to measure HbA1C at 4 months CA and blood pressure among very preterm infants during the study-period and to correlate these measures to later outcomes. Measuring HbA1C though turned out to be difficult due to unexpected constantly elevated levels of HbF at 4 months CA among preterm infants. Results are shown in appendix 5 (have been presented as a poster at The EAP-meeting in 2008). The method for measuring blood pressure without standardized equipment and procedures for VLBW infants turned out to be unreliable and evaluated to be invalid. The results are shown in appendix 6. Blood pressure will be measured in the follow-up study at 6 years of age with standardized equipment and procedures.

8.7. Allergic symptoms and feeding problems

Allergic symptoms among infants in their first year of life are often unspecific and only seldom represent a true allergic disease. The most common allergic symptoms are atopic dermatitis (AD) and recurrent wheezing (RW). When these conditions are caused by allergy, it will most often be a food allergy e.g. to milk or egg in this age group. We found an overall incidence of allergic symptoms among infants within 12 months CA to be 56.2% while the prevalence at 1 year CA was 41.8%. Predisposition to allergic disease and being a boy increased the risk of developing

AD and RW within the first 12 months CA. Information on predisposition was obtained by interviews at the time of randomization. Parents of 3 infants chose not to participate in the RCT due to severe atopic predisposition. These infants were all breastfed and because they were assigned to solely breastfeeding without randomization they can possibly bias the results on allergic disease. A larger number of infants participating without randomization could possibly lead to "reverse causality" with predisposed infants in one of the nutrition groups leading to an incorrect conclusion on nutrition and allergic diseases.
Nutrition with or without CMP after hospital discharge was not associated with the development of any allergic symptoms.

Studies describing allergic symptoms and studies on dietary prevention among preterm infants are very few. By the age of 18 months CA, Lucas et al found an overall incidence of one or more allergic symptoms among 777 preterm infants (BW <1850g) to be 44% (wheezing 22.5% and eczema / AD 19.4%). They found atopic predisposition, maternal smoking, vaginal delivery, and duration of ventilation to be associated with wheezing, and multiple births to be associated with atopic dermatitis (110). The same study-population was used for two randomized trials (A: banked donor milk *vs.* preterm formula and B: term *vs.* preterm formula) and it was found that CMP-based formula was not associated with an overall increased risk of developing allergic diseases, but in the subgroup with atopic predisposition, early exposure to CMP increased the risk of developing allergic diseases – especially AD and CMPA (111).

The allergic symptoms urticaria, rhinitis, and conjunctivitis are unspecific and difficult to interpret in this age group, also among preterm infants. In two children suspected of these symptoms no sensitization was found by SPT and specific IgE-analysis.

The main allergic symptoms reported in our study are RW and AD. Our results are based on standardized questionnaire based interviews performed by the doctors and/or nurses when the infants attended the outpatient clinics for follow-up examination at 4 and 12 months CA.

8.7.1. Recurrent wheezing

Chronic or recurrent respiratory morbidity has been described as common following preterm birth, particular if complicated by broncho-pulmonary dysplasia (BPD) (112;113). In our study, infants with BPD have been excluded from the RCT. A recent, but solely register- and population-based study on "administrative claims data", found that preterm infants (born ≤32

weeks) had a higher prevalence of persisting asthma later in childhood (11.7%) compared with term births (8%) (OR 1.51 (95% CI 1.40-1.63)) (114). A study from New Zealand based on parental reports at 12 months of age found an incidence of RW among very preterm infants (<33 weeks) to be 14.5% and among term infants to be 3%. They found significant risk factors to be parental history of asthma, maternal smoking, siblings at home, neonatal oxygen supplementation at 28, 36, and 40 weeks of gestation (115). Another study found risk factors for developing asthma to be chronic lung disease, neonatal mechanical ventilation, corticosteroids, and a higher childhood body mass index, while being a SGA girl and septic post birth to be protective factors among former preterm infants at 8 years of age (116). A Swedish birth cohort study (on preterm and term infants) found a cumulative incidence on recurrent wheezing (doctors diagnosed or any wheezing) to be 27% up to two years of age (117). The incidence and prevalence of RW up to one year of age among "healthy very preterm infants" were 39.2% and 32.7% respectively in our study, indicating that preterm birth might increase the risk of RW even though preterm infants with BPD were excluded from our study.

It is well recognized that in infants most cases of "acute expiratory wheeze" is associated with viral infections (118). In our study, we did not routinely register concurrent viral infections as a possible cause of wheezing episodes, but it was probably a major cause of RW, and preterm infants may have an increased risk of RW due to viral infections and prematurity in combination. Parental smoking is also known as a risk factor of developing RW in infancy and early childhood (119). In our study, parental smoking was though not significantly associated with an increased risk of developing RW. Later follow-up must reveal risk factors and the risk of bronchial asthma in this population.

8.7.2. Atopic dermatitis

A population based birth cohort study among term infants in Denmark found an incidence of AD to be 11% (120) and the cumulative 1-year prevalence to be 8.2% (121). An association between AD and being a boy and predisposed to *atopy* (maternal) was found (120). In a large Norwegian prospective cohort study, based on clinical examinations if parents reported allergic symptoms, was found a prevalence of AD at 1 year of life to be 13% among preterm infants (GA 28-35 weeks) and 10% among term infants. No significant difference between preterm and term infants (122). A much higher prevalence of AD at 12 months post-term of 35.8% was found in a British prospective study among preterm infants (GA ≤ 37+0), but in that study the diagnosis was based on questionnaires and home-visits by midwifes only (123). Our study is also a population based birth cohort of preterm infants, and the incidence and prevalence of AD at 1 year in our study

was 18% and 12.1% respectively, and seems to correlate very well with the results of the Norwegian study.

8.7.3. Feeding problems and gastrointestinal symptoms

In our study most of the gastrointestinal symptoms were reported early and mainly during the intervention period. Regurgitation of gastric content is common in preterm infants due to relatively large milk intake, horizontal posture, and immature lower sphincter function, so gastro-oesophageal reflux is a common symptom in very preterm infants and has been shown in some cases to trigger apnoea (124). Gastroesophageal reflux has been described as a clinical symptom responding to anti-reflux treatment (125). The infants in our study were not investigated by gastric pH-measurements and possible symptoms during hospitalization were not recorded, but at randomization, at term, and at 2 and 4 months CA registration of regurgitation was obtained. Supplementing with a cow's milk based protein fortifier to mother's own milk did not increase regurgitation compared to solely human milk fed infants in our study.

One study on primarily preterm infants and infant stools (the "Amsterdam" stool form scale) has described the amount of stool produced by formula fed infants to be significantly larger compared to breastfed infants (126). In our study, we did not look at stools but registered the use of anti-constipation medicine, and preterm formula fed infants did receive anti-constipation medicine significantly more often during the entire study-period compared to both breastfed groups, while infants supplemented with HMF were treated with anti-constipation medicine more often compared to exclusively breastfed infants during a period around term PMA. Overall, we could not find any significant feeding problems comparing fortification of mother's milk with solely mother's milk.

In our study, gastrointestinal symptoms were reported with an incidence of 13.4% with a prevalence of 1.4% at 1 year of age. Symptoms from the gastrointestinal tract are unspecific and are rarely due to allergic reactions. A Norwegian study (127) among preterm and term infants diagnosed 27 (9 preterm) of 555 with adverse reactions to cow's milk such as pain behavior, gastrointestinal symptoms (excessive vomiting or diarrhea), respiratory symptoms, and AD. Twenty six of these infants were diagnosed by elimination/challenge tests (all but one was exclusively breastfed). Non-IgE-mediated reactions were the most frequent and only one child with AD as the presenting symptom had a positive SPT as well as elevated IgE-level to cow's milk. At 1 year of age 13 were tolerant to cow's milk. No difference was found between preterm

and term infants. Adverse reactions to cow's milk (especially gastrointestinal symptoms) can be difficult to interpret and are possibly over diagnosed. In our study, 38 of 283 reported gastrointestinal symptoms, but none of these infants were suspected to have CMPA and did not have an elimination/challenge test preformed.

8.7.4. Sensitization and development of allergic disease

The development and phenotypic expression of allergic disease depends on a complex interaction between genetic and several environmental exposure to food and inhalant allergens, and non-specific adjuvant factors (e.g. tobacco smoke, air pollution, and infections) (40). Sensitization is an IgE response to a foreign antigen (allergen) as measured by *in vitro* IgE determination or SPT. At 4 months CA only 51% infants in our study had blood drawn for specific IgE-analysis, because many parents did not want their infant to experience a *veno*-puncture. A Norwegian study found one child with elevated IgE-levels to both egg and milk, and one child with only elevated IgE-levels to egg (127). In our study, one child was suspicious of CMPA due to AD and a positive SPT, but CMPA was not confirmed by elimination/challenge test, and further two infants (2/163) had positive IgE-reactions to cow's milk but none of them were suspected to have CMPA.

Breastfeeding possibly influences the occurrence of asthma and allergic disease through different mechanisms. The infant gut is immature, and may poorly eliminate multiple allergens or large quantities of allergens that can react with the immune system. Human milk contains substances that may influence the micro flora in the gut and possibly protect the intestinal mucosa. Cytokines / growth factors in human milk may also promote the maturation of gastrointestinal mucosa restricting the penetration of antigenic material and contribute to the anti-inflammatory effect of human milk (128). Especially preterm infants with an even more immature intestine have been suggested to have an increased risk of absorption of food allergens and development of food allergy (such as CMPA). In the literature, 3 cases of preterm infants, who presented signs suggestive of sensitization to CMP after supplementation of their mother's milk with a human milk fortifier, have been reported. The diagnoses were based on clinical response to elimination of the allergen from the diet only (129). In our study, 321 very preterm infants received a cow's milk based human milk fortifier during hospitalization, and only one infant was suspected to have CMPA after hospital discharge while he was fed a cow's milk based preterm formula but the diagnosis was not confirmed by elimination challenge test. Human milk, only supplemented with a small amount of HMF, might be protective to the immature gut. Another protective factor

might be that the very preterm infants in our study received solely human milk for at least 10 days before HMF was started. Multicomponent fortification of human milk for preterm infants is associated with improvements in growth, and support the use of fortification as a common practice in neonatal intensive care units (130;131). In our study, fortification was used also after hospital discharge and was not associated with an increased risk of developing allergic diseases among very preterm infants during the first year of life.

9. Conclusions and future perspectives

Although still controversial, the goal for nutrition of the preterm infant could be to supply nutrients to achieve the rate of growth and a body-composition, which would equal that of a normal *fetus* of the same postmenstrual age, and from term to equal the breastfed term infant of the same corrected age, and through the first year of life.

In very preterm infants, breastfeeding is recommended, but supplementation of mother's milk with protein is necessary in order to achieve optimal growth during hospitalization. After hospital discharge, optimal nutrition and supplementation is still unknown and for discussion.

We found that breastfeeding could be established in 60% of the "healthy" very preterm infants at discharge. However, mothers belonging to lower social groups and mothers who are smokers are less often breastfeeding their infants. An active nutrition- and feeding-policy during hospitalization is necessary in order to establish breastfeeding with a special attend on multiple births, infants with low weight for age, mothers of lower social groups, and smokers who want to breastfeed their very preterm infant(s) at and beyond hospital discharge.

Fortification of mother's milk, while exclusively breastfeeding after hospital discharge, was demonstrated to be possible and did not interfere with the duration of breastfeeding in our study. Preterm girls seemed to benefit temporarily from nutritional supply with fortification of mother's milk after hospital discharge compared to girls fed solely mother's milk. Fortification in the amount given in this study did not affect growth significantly at 1 year of age.

In our study, catch-up tended to be achieved early in HC (between discharge and term) and weight (between 2 and 4 months CA) but later in length (between 4 to 12 months CA) indicating a period after term for catch-up growth on weight and length. SGA infants showed greater catch-up growth compared to non-SGA infants during the study-period, but no significant difference on growth comparing nutrition groups. All SGA infants showed catch-up growth especially on weight until 4 months after term, and length growth even seemed to continue until 1 year CA.

Feeding problems such as regurgitation due to fortification of mother's milk were not increased compared to exclusively breastfed infants. Treatment with anti-constipation medicine was

primarily seen among preterm formula fed infants compared to both breastfeeding groups during the intervention period.

Cow's milk based fortification or preterm formula for preterm infants after hospital discharge did not increase the risk of developing allergic symptoms in this population. Allergy to cow's milk protein was not found among any of the infants that received a cow's milk based human milk fortifier during and after hospitalization. It may be of importance that HMF was not introduced until day 10-14, and that all the infants were fed only human milk until then. Predisposition to allergic disease and being a boy increased the risk of developing both atopic dermatitis and recurrent wheezing. Compared to other studies of preterm infants a high incidence and prevalence of recurrent wheezing were found, while atopic dermatitis was found to have an incidence and prevalence comparable to other studies.

Much is still unknown with regard to nutrition of preterm infants. Recent studies have shown hormones in human milk to be involved in energy balance regulation, possibly having a role in the regulation of growth and development in the neonatal period and in infancy. The long term consequence of these hormones (e.g. leptin, ghrelin, adiponectin, resistin, and obestatin) among both term and preterm infants especially on the development of the metabolic syndrome is unknown and need further investigation (132-134). Another growing research area is the regulation of the growth hormone (GH), insulin growth factor–1 (IGF1), and insulin which is different in *fetal* life compared to childhood and adulthood, and might be influenced and regulated differently in low birth weight infants with a possible association to long-term endocrine programming effects (135;136).

The content of protein in human milk is declining within weeks after birth, and the amount of protein will be inadequate in order to meet the nutritional needs of the growing preterm infant. Multi-component cow's milk based fortification of human milk for preterm infants is associated with improvements in growth, which supports the use of fortification as a common practice in neonatal intensive care units (130;131). A human-milk based fortifier has in one study been shown to reduce the risk of necrotizing enterocolitis among preterm infants (137), but similar studies and studies on long term health effects - such as reducing the possible risk factors like cow's milk protein allergy also needs to be evaluated.

Breastfeeding preterm infants instead of feeding a term formula should be promoted in all countries. After hospital discharge, a larger amount of fortifier added to mother's milk (fresh or defrosted) may be possible while breastfeeding and should be considered if supplementation with extra protein is needed. In countries where human milk fortifiers are not available, breastfeeding can be supplemented with a nutrient enriched formula, if available, in order to better meet the nutritional needs of the growing preterm infant, but studies on the effect of such a feeding strategy have still not been performed.

Close monitoring of growth during hospitalization and after hospital discharge is important in order to identify the infants with subnormal weight for age, infants that might be at risk of neurodevelopment impairment and bone-diseases. If catch-up growth on length and head circumference has been achieved before hospital discharge, supplementation to improve growth might not be necessary, though it is still unknown if supplementation after discharge improves neuro-developmental outcome. Both "healthy" and "sick" very preterm infants, who have not achieved catch-up on length and head circumference at discharge, should be supplemented with a fortifier added to mother's milk or a nutrient enriched (preterm or post discharge) formula after hospital discharge in order to improve growth and neuro-developmental outcome. A gradual return to normal for all growth variables while avoiding excessive weight gain should be the goal for nutrition of very preterm infants during and after hospitalization.

Later follow-up of the infants in our study (at 6 years of age) has been planned and will show whether fortification of human milk after hospital discharge improves neuro-developmental outcome or has other health effects such as early signs of metabolic syndrome and possible allergic diseases among the very preterm infants in our study.

10. Summaries

10.1. Summary in English

The aims of this Ph.D. thesis were: **1**. Primarily to investigate the effect, of adding human milk fortifier to mother's milk while breastfeeding very preterm infants after hospital discharge, on growth until 1 year corrected age (CA) **2**. Secondarily to describe breastfeeding rate and factors associated with breastfeeding among very preterm infants at hospital discharge. **3**. To describe possible feeding-problems during the intervention-period, and allergic diseases during the first year of life, among very preterm infants related to their nutrition after hospital discharge. **4**. To describe the content of macronutrients in human milk from mothers delivering very preterm.

This Ph.D. thesis is based on a prospective, randomized, and controlled interventional birth cohort study. A total of 633 very preterm infants with a gestational age (GA) ≤ 32+0 weeks were recruited consecutively from July 2004 until August 2008 of whom 157 were excluded due to diseases or circumstances influencing nutrition. Further 156 refused participation in the interventional part of the study, but data on breastfeeding, weight, and some epidemiological data until discharge were available. Results on breastfeeding rate at discharge were therefore based on data from 478 infants, and parents of 320 infants accepted participation in the intervention study. Of these 320 infants, 207 were exclusively breastfed and they were shortly before hospital discharge randomized to either breastfeeding without (group A) or with fortification (group B) until 4 months CA. Infants (n=113) who were bottle-fed at discharge (group C) were given a preterm formula (PF) until 4 months CA. Infants were examined at the outpatient clinics at term, and at 2, 4, 6, and 12 months CA, where parameters on growth, allergic diseases, possible feeding problems, blood-samples, and milk samples were obtained. Data on duration of exclusively breastfeeding and time of introduction to formula and/or complementary food were also recorded.

Among the 478 infants 60% (n=285) were exclusively breastfed, 35% (n=167) were exclusively bottle-fed, and 5% (n=26) were both breast- and bottle-fed at discharge. Compared to mothers in lower social groups and mothers who smoked, mothers in higher social groups and "non-smokers" were significantly (p=0.000 and p=0.003 respectively) more often breastfeeding their very preterm infants at discharge. Single birth infants tended more often to be breastfed (p=0.09). Infant age at discharge and duration of hospitalization did not influence breastfeeding at discharge. Increase in weight Z-score from birth to discharge was largest in the bottle-fed group

compared to the breastfed group (p=0.000), probably due to feeding practice the last week(s) of hospitalization.

In the intervention study, 207 exclusively breastfed very preterm infants were randomized to group A (n=102) and B (n=105) respectively. The duration of breastfeeding was not influenced by fortification of mother's milk after hospital discharge. There was no significant difference on growth comparing group A and B at 12 months CA. Both boys and girls in group C achieved catch-up in weight and length earlier as compared to group A and B. Per protocol (PP) analysis showed that girls, but not boys, were longer and had a larger head circumference but were not heavier in group B (n=51) compared to group A (n=73) at 2 and 4 months CA ($p<0.05$). Protein-concentration in mothers' milk declined significantly from 2 weeks (1.8 g/100 ml) to 6 weeks after birth (1.4 g/100 ml) and declined further to 1.2 g/100 ml 12 weeks after birth.

The incidence and the prevalence at 12 months CA of recurrent wheezing was 39.2% and 32.7% respectively, while atopic dermatitis was 18.0% and 12.1% respectively. Predisposition to allergic disease increased the risk of developing atopic dermatitis (p=0.04) (OR 2.6 (95% CI 1.0 – 6.4)), and the risk of developing recurrent wheezing (p=0.02) (OR 2.7 (95% CI 1.2 – 6.3)). Boys had an increased risk of developing recurrent wheezing (p=0.003) (OR 3.1 (95% CI 1.5 – 6.5)).

In conclusion breastfeeding can successfully be established in very preterm infants. Fortification of human milk after hospital discharge while breastfeeding was possible without influencing the duration of breastfeeding. Fortification in the amount given in this study did, however, not affect growth significantly at 1 year of age. An increased amount of protein was correlated with increased BUN-values indicating a better growth potential. Fortification of mother's milk or preterm formula was not associated with an increased risk of developing allergic diseases. Future follow-up of this cohort investigating e.g. growth, allergic diseases, and neuropsychological development is planned at 6 years of age. The definition of optimal growth and nutrition of preterm infants is though still a question for debate and further investigations are needed.

10.2. Summary in Danish / Dansk Resumé

Formålene med denne ph.d. afhandling var:
1. Primært at undersøge effekten af protein-berigning af modermælken samtidig med amning, på præmature børns vækst fra udskrivelsen og frem til 1 år korrigeret alder, uden at påvirke amme-varigheden. **2**. Sekundært at beskrive amme frekvensen og faktorer associeret med amning ved udskrivelsen blandt meget præmature nyfødte børn. **3**. At beskrive eventuelle spiseproblemer og allergiske sygdomme i første leveår blandt meget præmature børn i relation til deres ernæring efter udskrivelsen. **4**. At beskrive indholdet af makronæringsstoffer i modermælken hos mødre, som har født meget præmaturt.

Denne ph.d. afhandling er baseret på et prospektivt, randomiseret og kontrolleret fødsels- kohorte studie. Af 633 meget præmature børn med en gestationsalder ≤ 32+0 uger, som blev rekrutteret fortløbende fra juli 2004 og frem til august 2008, blev 157 ekskluderet pga. sygdomme og tilstande, som influerede på ernæringen. Derudover ønskede forældrene til 156 børn ikke at deltage i interventionsdelen af studiet, men data vedrørende amning, vægt og nogle epidemiologiske data indtil udskrivelsen var tilgængelige. Resultater vedrørende amme frekvensen ved udskrivelsen er således baseret på data fra 478 børn, hvoraf 320 deltog i interventionsstudiet. Af disse 320 børn blev 207 udelukkende ammet, og kort før udskrivelsen blev de randomiseret til enten amning uden (gruppe A) eller med protein-berigning (gruppe B) indtil 4 måneder korrigeret alder – hvis muligt. Børn som blev flaske-ernæret ved udskrivelsen (n=113) (gruppe C) fik en præmatur modermælkserstatning indtil 4 måneder korrigeret alder. Børnene blev set ambulant til terminen, 2, 4, 6 og 12 måneder, hvor vækst parametre, informationer vedrørende allergisk sygdom og eventuelle spiseproblemer, blodprøver og mælkeprøver blev indhentet. Data vedrørende amme varighed og tidspunktet for introduktion til modermælkserstatning og / eller overgangskost blev ligeledes registreret.

Blandt de 478 præmature børn blev 60 % (n=285) udelukkende ammet, 35 % (n=167) udelukkende flaskeernæret og 5 % (n=26) både ammet og flaskeernæret ved udskrivelsen. Mødre i høj socialgruppe (p=0.000) og "ikke rygere" (p=0.003) ammede oftere deres præmature børn ved udskrivelsen. Enkeltfødte havde en tendens til oftere at blive ammet (p=0.09). Barnets alder ved udskrivelsen og varigheden af indlæggelsen influerede ikke på amningen ved udskrivelsen. Øgningen i vægt Z-score fra fødsel til udskrivelse var størst blandt de flaskeernærede børn sammenlignet med de ammede børn (p=0.000), hvilket sandsynligvis skyldes ernærings-praksis de sidste par uger under indlæggelsen.

I interventionsstudiet blev 207 børn randomiseret til henholdsvis gruppe A (n=102) og B (n=105). Varigheden af amningen blev ikke influeret af berigningen af modermælken efter udskrivelsen. Ved 12 måneders alderen var der ingen signifikant forskel i væksten mellem gruppe A og B. Både drenge og piger i gruppe C opnåede ”catch-up” i vægt og længde tidligere end børnene i både gruppe A og B. Per protokol (PP) analyse viste at piger, men ikke drenge, var længere og havde større hovedomfang men ikke var tungere i gruppe B (n=51) sammenlignet med gruppe A (n=73) ved 2 og 4 måneder korrigeret alder ($p<0.05$). Protein koncentrationen i modermælken faldt signifikant fra 2 uger (1,8 g/100 ml) til 6 uger efter fødslen (1.4 g/100 ml) og faldt yderligere til 1.2 g/100 ml 12 uger efter fødslen.

Incidencen og prævalencen ved 12 måneders korrigeret alder vedrørende astmatisk vejrtrækning var henholdsvis 39,2 % og 32,7 %, mens atopisk dermatitis var henholdsvis 18,0 % og 12,1 %. Arvelig disposition for allergisk sygdom øgede risikoen for at udvikle atopisk dermatitis ($p=0.04$) (OR 2.6 (95 % CI 1.0 – 6.4)), og risikoen for at få astmatisk vejrtrækning ($p=0.02$) (OR 2.7 (95 % CI 1.2 – 6.3)). Drenge havde en øget risiko for at udvikle astmatisk vejrtrækning ($p=0.003$) (OR 3.1 (95 % CI 1.5 – 6.5)).

Det kan konkluderes, at amning kan etableres hos meget præmature nyfødte børn. Berigning af modermælken sammenlignet med ikke beriget modermælk efter udskrivelsen samtidig med amning, var muligt uden at influere på varigheden af amningen, men var i den givne mængde ikke associeret med øget vækst i 1 års alderen. Berigning af modermælken eller præmatur modermælkserstatning var ikke associeret med en øget risiko for at udvikle allergisk sygdom. Opfølgende undersøgelse af denne kohorte i 6 års alderen vil blandt andet undersøge vækst, den neuropsykologiske udvikling og risikoen for udvikling af allergiske sygdomme. Definitionen af optimal ernæring og vækst til præmature børn efter udskrivelsen er fortsat til diskussion og yderligere studier er nødvendige.

11. References

Reference List

(1) Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL, et al. Longitudinal growth of hospitalized very low birth weight infants. Pediatrics 1999 Aug;104(2 Pt 1):280-9.

(2) Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 2001 Feb;107(2):270-3.

(3) Groh-Wargo S, Sapsford A. Enteral nutrition support of the preterm infant in the neonatal intensive care unit. Nutr Clin Pract 2009 Jun;24(3):363-76.

(4) Hay WW, Jr. Strategies for feeding the preterm infant. Neonatology 2008;94(4):245-54.

(5) Aggett PJ, Agostoni C, Axelsson I, De CM, Goulet O, Hernell O, et al. Feeding preterm infants after hospital discharge: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 2006 May;42(5):596-603.

(6) Griffin IJ, Cooke RJ. Nutrition of preterm infants after hospital discharge. J Pediatr Gastroenterol Nutr 2007 Dec;45 Suppl 3:S195-S203.

(7) Schanler RJ. Post-discharge nutrition for the preterm infant. Acta Paediatr Suppl 2005 Oct;94(449):68-73.

(8) Heird WC. Determination of nutritional requirements in preterm infants, with special reference to 'catch-up' growth. Semin Neonatol 2001 Oct;6(5):365-75.

(9) Hack M, Weissman B, Borawski-Clark E. Catch-up growth during childhood among very low-birth-weight children. Arch Pediatr Adolesc Med 1996 Nov;150(11):1122-9.

(10) Knops NB, Sneeuw KC, Brand R, Hille ET, den Ouden AL, Wit JM, et al. Catch-up growth up to ten years of age in children born very preterm or with very low birth weight. BMC Pediatr 2005;5:26.

(11) Dodrill P, Cleghorn G, Donovan T, Davies P. Growth patterns in preterm infants born appropriate for gestational age. J Paediatr Child Health 2008 Jun;44(6):332-7.

(12) Chan GM, Armstrong C, Moyer-Mileur L, Hoff C. Growth and bone mineralization in children born prematurely. J Perinatol 2008 Sep;28(9):619-23.

(13) Gortner L, van HM, Thyen U, Gembruch U, Friedrich HJ, Landmann E. Outcome in preterm small for gestational age infants compared to appropriate for gestational age preterms at the age of 2 years: a prospective study. Eur J Obstet Gynecol Reprod Biol 2003 Sep 22;110 Suppl 1:S93-S97.

(14) Guo SS, Roche AF, Chumlea WC, Casey PH, Moore WM. Growth in weight, recumbent length, and head circumference for preterm low-birthweight infants during the first three years of life using gestation-adjusted ages. Early Hum Dev 1997 Feb 20;47(3):305-25.

(15) Cooke RJ, Embleton ND, Griffin IJ, Wells JC, McCormick KP. Feeding preterm infants after hospital discharge: growth and development at 18 months of age. Pediatr Res 2001 May;49(5):719-22.

(16) Lucas A, Fewtrell MS, Morley R, Singhal A, Abbott RA, Isaacs E, et al. Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. Pediatrics 2001 Sep;108(3):703-11.

(17) Slykerman RF, Thompson JM, Becroft DM, Robinson E, Pryor JE, Clark PM, et al. Breastfeeding and intelligence of preschool children. Acta Paediatr 2005 Jul;94(7):832-7.

(18) Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. Am J Clin Nutr 1999 Oct;70(4):525-35.

(19) Moyer-Mileur LJ. Anthropometric and laboratory assessment of very low birth weight infants: the most helpful measurements and why. Semin Perinatol 2007 Apr;31(2):96-103.

(20) Kuzma-O'Reilly B, Duenas ML, Greecher C, Kimberlin L, Mujsce D, Miller D, et al. Evaluation, development, and implementation of potentially better practices in neonatal intensive care nutrition. Pediatrics 2003 Apr;111(4 Pt 2):e461-e470.

(21) Tyson JE, Kennedy KA. Trophic feedings for parenterally fed infants. Cochrane Database Syst Rev 2005;(3):CD000504.

(22) Nye C. Transitioning premature infants from gavage to breast. Neonatal Netw 2008 Jan;27(1):7-13.

(23) Faerk J, Skafte L, Petersen S, Peitersen B, Michaelsen KF. Macronutrients in milk from mothers delivering preterm. Adv Exp Med Biol 2001;501:409-13.

(24) Reali A, Greco F, Fanaro S, Atzei A, Puddu M, Moi M, et al. Fortification of maternal milk for very low birth weight (VLBW) pre-term neonates. Early Hum Dev 2010 Apr 17.

(25) Mead Johnson Nutritionals. History Enfamil Human Milk Fortifier. www meadjohnson com 2010.

(26) Smith MM, Durkin M, Hinton VJ, Bellinger D, Kuhn L. Initiation of breastfeeding among mothers of very low birth weight infants. Pediatrics 2003 Jun;111(6 Pt 1):1337-42.

(27) Akerstrom S, Asplund I, Norman M. Successful breastfeeding after discharge of preterm and sick newborn infants. Acta Paediatr 2007 Oct;96(10):1450-4.

(28) Karin Kok og Helle S Vestergaard. Præmature børns ernæring og vækst fra udskrivelse til 1 år kronologisk alder. 2007.
Ref Type: Serial (Book,Monograph)

(29) Benn CS, Wohlfahrt J, Aaby P, Westergaard T, Benfeldt E, Michaelsen KF, et al. Breastfeeding and risk of atopic dermatitis, by parental history of allergy, during the first 18 months of life. Am J Epidemiol 2004 Aug 1;160(3):217-23.

(30) Nyqvist KH, Sjoden PO, Ewald U. The development of preterm infants' breastfeeding behavior. Early Hum Dev 1999 Jul;55(3):247-64.

(31) Nyqvist KH. Early attainment of breastfeeding competence in very preterm infants. Acta Paediatr 2008 Jun;97(6):776-81.

(32) Hake-Brooks SJ, Anderson GC. Kangaroo care and breastfeeding of mother-preterm infant dyads 0-18 months: a randomized, controlled trial. Neonatal Netw 2008 May;27(3):151-9.

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

(34) Hanson LA. Session 1: Feeding and infant development breast-feeding and immune function. Proc Nutr Soc 2007 Aug;66(3):384-96.

(35) Henderson G, Anthony MY, McGuire W. Formula milk versus maternal breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev 2007;(4):CD002972.

(36) Gartner LM, Morton J, Lawrence RA, Naylor AJ, O'Hare D, Schanler RJ, et al. Breastfeeding and the use of human milk. Pediatrics 2005 Feb;115(2):496-506.

(37) Cooke RW, Foulder-Hughes L. Growth impairment in the very preterm and cognitive and motor performance at 7 years. Arch Dis Child 2003 Jun;88(6):482-7.

(38) Gale CR, O'Callaghan FJ, Bredow M, Martyn CN. The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. Pediatrics 2006 Oct;118(4):1486-92.

(39) Dabydeen L, Thomas JE, Aston TJ, Hartley H, Sinha SK, Eyre JA. High-energy and -protein diet increases brain and corticospinal tract growth in term and preterm infants after perinatal brain injury. Pediatrics 2008 Jan;121(1):148-56.

(40) Halken S. Prevention of allergic disease in childhood: clinical and epidemiological aspects of primary and secondary allergy prevention. Pediatr Allergy Immunol 2004 Jun;15 Suppl 16:4-32.

(41) Muraro A, Dreborg S, Halken S, Host A, Niggemann B, Aalberse R, et al. Dietary prevention of allergic diseases in infants and small children. Part III: Critical review of published peer-reviewed observational and interventional studies and final recommendations. Pediatr Allergy Immunol 2004 Aug;15(4):291-307.

(42) Host A, Halken S. Primary prevention of food allergy in infants who are at risk. Curr Opin Allergy Clin Immunol 2005 Jun;5(3):255-9.

(43) Host A. Frequency of cow's milk allergy in childhood. Ann Allergy Asthma Immunol 2002 Dec;89(6 Suppl 1):33-7.

(44) van OJ, Kull I, Borres MP, Brandtzaeg P, Edberg U, Hanson LA, et al. Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966-2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. Allergy 2003 Sep;58(9):833-43.

(45) Ozanne SE, Hales CN. Lifespan: catch-up growth and obesity in male mice. Nature 2004 Jan 29;427(6973):411-2.

(46) Hemachandra AH, Howards PP, Furth SL, Klebanoff MA. Birth weight, postnatal growth, and risk for high blood pressure at 7 years of age: results from the Collaborative Perinatal Project. Pediatrics 2007 Jun;119(6):e1264-e1270.

(47) Singhal A. Early nutrition and long-term cardiovascular health. Nutr Rev 2006 May;64(5 Pt 2):S44-S49.

(48) Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. Lancet 2001 Feb 10;357(9254):413-9.

(49) Singhal A, Cole TJ, Fewtrell M, Lucas A. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. Lancet 2004 May 15;363(9421):1571-8.

(50) Cianfarani S, Germani D, Branca F. Low birthweight and adult insulin resistance: the "catch-up growth" hypothesis. Arch Dis Child Fetal Neonatal Ed 1999 Jul;81(1):F71-F73.

(51) O'Connor DL, Jacobs J, Hall R, Adamkin D, Auestad N, Castillo M, et al. Growth and development of premature infants fed predominantly human milk, predominantly premature infant formula, or a combination of human milk and premature formula. J Pediatr Gastroenterol Nutr 2003 Oct;37(4):437-46.

(52) Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet 2004 May 15;363(9421):1642-5.

(53) The Danish National Board of health. Sucesfull Breasfeeding 2003 and 2006, Recommandations for infant nutrition 2005, Breastfeeding 2008, Food for infants and small children 2009. Guidelines for healthcare professionals. Available at: www ssi dk Latest access-date 2010 Jan 1.

(54) The Danish National Centre For Social Research. Oversigt over de 5 socialgrupper. Available at: www sfi dk Latest access date 2010 Feb 2.

(55) Polberger S, Lonnerdal B. Simple and rapid macronutrient analysis of human milk for individualized fortification: basis for improved nutritional management of very-low-birth-weight infants? J Pediatr Gastroenterol Nutr 1993 Oct;17(3):283-90.

(56) Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996 Jul;85(7):843-8.

(57) Niklasson A, bertsson-Wikland K. Continuous growth reference from 24th week of gestation to 24 months by gender. BMC Pediatr 2008;8:8.

(58) Rabe-Hesketh S, Skrondal A. Multilevel and Longitudinal Modeling Using Stata. Second ed. Texas, USA: Stata Press; 2008.

(59) Behrman RE KRJH. Nelson Textbook of Pediatrics. 16.th. ed. W.B. Saunders Company; 2000.

(60) O'Connor DL, Khan S, Weishuhn K, Vaughan J, Jefferies A, Campbell DM, et al. Growth and nutrient intakes of human milk-fed preterm infants provided with extra energy and nutrients after hospital discharge. Pediatrics 2008 Apr;121(4):766-76.

(61) Agostoni C, Braegger C, Decsi T, Kolacek S, Koletzko B, Michaelsen KF, et al. Breast-feeding: A commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 2009 Jul;49(1):112-25.

(62) Thureen PJ and Hay WW. Neonatal Nutrition and Metabolism. Second ed. Cambridge University Press, NY, USA; 2006.

(63) Casey PH. Growth of low birth weight preterm children. Semin Perinatol 2008 Feb;32(1):20-7.

(64) World Health Organization, Geneva. WHO Child Growth Standards: Methods and development: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age. Available at: www who int/childgrowth/publications 2006.

(65) Binns C, Lee M. New growth standards. Lancet 2007 Nov 3;370(9598):1542.

(66) Binns C, Lee M. Will the new WHO growth references do more harm than good? Lancet 2006 Nov 25;368(9550):1868-9.

(67) CDC Growth Charts: United States. CDC growthcharts. Available at: www cdc growthcharts Latest access date November 2009 2009.

(68) Brandt I, Sticker EJ, Lentze MJ. Catch-up growth of head circumference of very low birth weight, small for gestational age preterm infants and mental development to adulthood. J Pediatr 2003 May;142(5):463-8.

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

(70) Chan GM. Growth and bone mineral status of discharged very low birth weight infants fed different formulas or human milk. J Pediatr 1993 Sep;123(3):439-43.

(71) Carver JD, Wu PY, Hall RT, Ziegler EE, Sosa R, Jacobs J, et al. Growth of preterm infants fed nutrient-enriched or term formula after hospital discharge. Pediatrics 2001 Apr;107(4):683-9.

(72) Wheeler RE, Hall RT. Feeding of premature infant formula after hospital discharge of infants weighing less than 1800 grams at birth. J Perinatol 1996 Mar;16(2 Pt 1):111-6.

(73) Cooke RJ, Griffin IJ, McCormick K, Wells JC, Smith JS, Robinson SJ, et al. Feeding preterm infants after hospital discharge: effect of dietary manipulation on nutrient intake and growth. Pediatr Res 1998 Mar;43(3):355-60.

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

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

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

(77) Ramenghi LA, Fumagalli M, Bassi L, Groppo M, De CA, Fanaro S, et al. Brain maturation of preterm newborn babies: new insights. J Pediatr Gastroenterol Nutr 2007 Dec;45 Suppl 3:S143-S146.

(78) Bozzetti V, Tagliabue P. Metabolic Bone Disease in preterm newborn: an update on nutritional issues. Ital J Pediatr 2009;35(1):20.

(79) Corpeleijn WE, van Goudoever JB. Early nutrient supply and the preterm infant. J Pediatr Gastroenterol Nutr 2010 Dec;51 Suppl 3:S141-S142.

(80) Agostoni C, Buonocore G, Carnielli VP, De CM, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr 2010 Jan;50(1):85-91.

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

(82) Arslanoglu S, Moro GE, Ziegler EE, The Wapm Working Group On Nutrition. Optimization of human milk fortification for preterm infants: new concepts and recommendations. J Perinat Med 2010 May;38(3):233-8.

(83) Arslanoglu S, Moro GE, Ziegler EE. Adjustable fortification of human milk fed to preterm infants: does it make a difference? J Perinatol 2006 Oct;26(10):614-21.

(84) Polberger S, Raiha NC, Juvonen P, Moro GE, Minoli I, Warm A. Individualized protein fortification of human milk for preterm infants: comparison of ultrafiltrated human milk protein and a bovine whey fortifier. J Pediatr Gastroenterol Nutr 1999 Sep;29(3):332-8.

(85) Bonet M, Blondel B, Agostino R, Combier E, Maier RF, Cuttini M, et al. Variations in breastfeeding rates for very preterm infants between regions and neonatal units in Europe: results from the MOSAIC cohort. Arch Dis Child Fetal Neonatal Ed 2010 Jun 10.

(86) Furman L, Minich N, Hack M. Correlates of lactation in mothers of very low birth weight infants. Pediatrics 2002 Apr;109(4):e57.

(87) Manganaro R, Marseglia L, Mami C, Paolata A, Gargano R, Mondello M, et al. Effects of hospital policies and practices on initiation and duration of breastfeeding. Child Care Health Dev 2008 Nov 17.

(88) Eriksen W. Breastfeeding, smoking and the presence of the child's father in the household. Acta Paediatr 1996 Nov;85(11):1272-7.

(89) Liu J, Rosenberg KD, Sandoval AP. Breastfeeding duration and perinatal cigarette smoking in a population-based cohort. Am J Public Health 2006 Feb;96(2):309-14.

(90) Donath SM, Amir LH. The relationship between maternal smoking and breastfeeding duration after adjustment for maternal infant feeding intention. Acta Paediatr 2004 Nov;93(11):1514-8.

(91) Giglia R, Binns CW, Alfonso H. Maternal cigarette smoking and breastfeeding duration. Acta Paediatr 2006 Nov;95(11):1370-4.

(92) Halken S, Host A, Husby S, Hansen LG, Osterballe O, Nyboe J. Recurrent wheezing in relation to environmental risk factors in infancy. A prospective study of 276 infants. Allergy 1991 Oct;46(7):507-14.

(93) Goldade K, Nichter M, Nichter M, Adrian S, Tesler L, Muramoto M. Breastfeeding and smoking among low-income women: results of a longitudinal qualitative study. Birth 2008 Sep;35(3):230-40.

(94) Agostoni C. Small-for-gestational-age infants need dietary quality more than quantity for their development: the role of human milk. Acta Paediatr 2005 Jul;94(7):827-9.

(95) Aimone A, Rovet J, Ward W, Jefferies A, Campbell DM, Asztalos E, et al. Growth and Body Composition of Human Milk-fed Premature Infants Provided With Extra Energy and Nutrients Early After Hospital Discharge: 1-year Follow-up. J Pediatr Gastroenterol Nutr 2009 Jul 21.

(96) Polberger SK, Axelsson IE, Raiha NC. Urinary and serum urea as indicators of protein metabolism in very low birthweight infants fed varying human milk protein intakes. Acta Paediatr Scand 1990 Aug;79(8-9):737-42.

(97) Hall RT. Nutritional follow-up of the breastfeeding premature infant after hospital discharge. Pediatr Clin North Am 2001 Apr;48(2):453-60.

(98) Neville MC, Keller R, Seacat J, Lutes V, Neifert M, Casey C, et al. Studies in human lactation: milk volumes in lactating women during the onset of lactation and full lactation. Am J Clin Nutr 1988 Dec;48(6):1375-86.

(99) Powers NG. Slow weight gain and low milk supply in the breastfeeding dyad. Clin Perinatol 1999 Jun;26(2):399-430.

(100) Cooke RJ, Rawlings DJ, McCormick K, Griffin IJ, Faulkner K, Wells JC, et al. Body composition of preterm infants during infancy. Arch Dis Child Fetal Neonatal Ed 1999 May;80(3):F188-F191.

(101) Cooke RJ, McCormick K, Griffin IJ, Embleton N, Faulkner K, Wells JC, et al. Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res 1999 Oct;46(4):461-4.

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

(103) Ford ES, Li C. Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? J Pediatr 2008 Feb;152(2):160-4.

(104) Lee S, Bacha F, Gungor N, Arslanian S. Comparison of different definitions of pediatric metabolic syndrome: relation to abdominal adiposity, insulin resistance, adiponectin, and inflammatory biomarkers. J Pediatr 2008 Feb;152(2):177-84.

(105) Forsen T, Eriksson JG, Tuomilehto J, Osmond C, Barker DJ. Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. BMJ 1999 Nov 27;319(7222):1403-7.

(106) Koletzko B. Long-term consequences of early feeding on later obesity risk. Nestle Nutr Workshop Ser Pediatr Program 2006;58:1-18.

(107) Greer FR. Post-discharge nutrition: what does the evidence support? Semin Perinatol 2007 Apr;31(2):89-95.

(108) Greer FR. Long-term adverse outcomes of low birth weight, increased somatic growth rates, and alterations of body composition in the premature infant: review of the evidence. J Pediatr Gastroenterol Nutr 2007 Dec;45 Suppl 3:S147-S151.

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

(110) Lucas A, Brooke OG, Cole TJ, Morley R, Bamford MF. Food and drug reactions, wheezing, and eczema in preterm infants. Arch Dis Child 1990 Apr;65(4):411-5.

(111) Lucas A, Brooke OG, Morley R, Cole TJ, Bamford MF. Early diet of preterm infants and development of allergic or atopic disease: randomised prospective study. BMJ 1990 Mar 31;300(6728):837-40.

(112) Greenough A. Long-term pulmonary outcome in the preterm infant. Neonatology 2008;93(4):324-7.

(113) Greenough A. Late respiratory outcomes after preterm birth. Early Hum Dev 2007 Dec;83(12):785-8.

(114) Dombkowski KJ, Leung SW, Gurney JG. Prematurity as a predictor of childhood asthma among low-income children. Ann Epidemiol 2008 Apr;18(4):290-7.

(115) Elder DE, Hagan R, Evans SF, Benninger HR, French NP. Recurrent wheezing in very preterm infants. Arch Dis Child Fetal Neonatal Ed 1996 May;74(3):F165-F171.

(116) Grischkan J, Storfer-Isser A, Rosen CL, Larkin EK, Kirchner HL, South A, et al. Variation in childhood asthma among former preterm infants. J Pediatr 2004 Mar;144(3):321-6.

(117) Lannero E, Wickman M, Pershagen G, Nordvall L. Maternal smoking during pregnancy increases the risk of recurrent wheezing during the first years of life (BAMSE). Respir Res 2006;7:3.

(118) Papadopoulos NG, Christodoulou I, Rohde G, Agache I, Almqvist C, Bruno A, et al. Viruses and bacteria in acute asthma exacerbations - A GA(2) LEN-DARE* systematic review. Allergy 2010 Nov 18.

(119) Carlsen KH, Carlsen KC. Respiratory effects of tobacco smoking on infants and young children. Paediatr Respir Rev 2008 Mar;9(1):11-9.

(120) Johnke H, Norberg LA, Vach W, Host A, Andersen KE. Patterns of sensitization in infants and its relation to atopic dermatitis. Pediatr Allergy Immunol 2006 Dec;17(8):591-600.

(121) Johnke H, Vach W, Norberg LA, Bindslev-Jensen C, Host A, Andersen KE. A comparison between criteria for diagnosing atopic eczema in infants. Br J Dermatol 2005 Aug;153(2):352-8.

(122) Kvenshagen B, Jacobsen M, Halvorsen R. Atopic dermatitis in premature and term children. Arch Dis Child 2009 Mar;94(3):202-5.

(123) Morgan J, Williams P, Norris F, Williams CM, Larkin M, Hampton S. Eczema and early solid feeding in preterm infants. Arch Dis Child 2004 Apr;89(4):309-14.

(124) Corvaglia L, Zama D, Gualdi S, Ferlini M, Aceti A, Faldella G. Gastro-oesophageal reflux increases the number of apnoeas in very preterm infants. Arch Dis Child Fetal Neonatal Ed 2009 May;94(3):F188-F192.

(125) Birch JL, Newell SJ. Gastrooesophageal reflux disease in preterm infants: current management and diagnostic dilemmas. Arch Dis Child Fetal Neonatal Ed 2009 Sep;94(5):F379-F383.

(126) Bekkali N, Hamers SL, Reitsma JB, Van Toledo L, Benninga MA. Infant Stool Form Scale: Development and Results. The Journal of PediatricsIn Press, Corrected Proof.

(127) Kvenshagen B, Halvorsen R, Jacobsen M. Adverse reactions to milk in infants. Acta Paediatr 2008 Feb;97(2):196-200.

(128) Oddy WH. The long-term effects of breastfeeding on asthma and atopic disease. Adv Exp Med Biol 2009;639:237-51.

(129) Vlieghe V, Roches AD, Payot A, Lachance C, Nuyt AM. Human milk fortifier in preterm babies: source of cow's milk protein sensitization? Allergy 2009 Nov;64(11):1690-1.

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

(131) Maggio L, Costa S, Gallini F. Human milk fortifiers in very low birth weight infants. Early Hum Dev 2009 Oct;85(10 Suppl):S59-S61.

(132) Savino F, Liguori SA, Lupica MM. Adipokines in breast milk and preterm infants. Early Hum Dev 2010 Feb 2.

(133) Savino F, Liguori SA, Fissore MF, Oggero R. Breast milk hormones and their protective effect on obesity. Int J Pediatr Endocrinol 2009;2009:327505.

(134) Savino F, Fissore MF, Liguori SA, Oggero R. Can hormones contained in mothers' milk account for the beneficial effect of breast-feeding on obesity in children? Clin Endocrinol (Oxf) 2009 Dec;71(6):757-65.

(135) Kajantie E. Insulin-like growth factor (IGF)-I, IGF binding protein (IGFBP)-3, phosphoisoforms of IGFBP-1 and postnatal growth in very-low-birth-weight infants. Horm Res 2003;60 Suppl 3:124-30.

(136) Dunger DB, Salgin B, Ong KK. Session 7: Early nutrition and later health early developmental pathways of obesity and diabetes risk. Proc Nutr Soc 2007 Aug;66(3):451-7.

(137) Sullivan S, Schanler RJ, Kim JH, Patel AL, Trawoger R, Kiechl-Kohlendorfer U, et al. An Exclusively Human Milk-Based Diet Is Associated with a Lower Rate of Necrotizing Enterocolitis than a Diet of Human Milk and Bovine Milk-Based Products. J Pediatr 2009 Dec 24.

12. Paper I.

Factors associated with successful establishment of breastfeeding in very preterm infants

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Authors:

G Zachariassen[1], J Faerk[2], C Grytter[3], BH Esberg[4], P Juvonen[1], S Halken[1]

1. Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark
2. Holbaek Hospital, Holbaek, Denmark
3. Kolding Hospital, Kolding, Denmark
4. Aarhus University Hospital, Skejby, Denmark

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REGULAR ARTICLE

Factors associated with successful establishment of breastfeeding in very preterm infants

G Zachariassen (Gitte.Zachariassen@ouh.regionsyddanmark.dk)[1], J Faerk[2], C Grytter[3], BH Esberg[4], P Juvonen[1], S Halken[1]

1.Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark
2.Holbaek Hospital, Holbaek, Denmark
3.Kolding Hospital, Kolding, Denmark
4.Aarhus University Hospital, Skejby, Denmark

Keywords
Breastfeeding, Smoking, Social class, Very preterm infants, Z-score

Correspondence
G Zachariassen, Pediatric Research Unit, Hans Christian Andersen Children's Hospital, Odense University Hospital, 5000 Odense C, Denmark.
Tel: +45 65412117 |
Fax: +45 66119162 |
Email: Gitte.Zachariassen@ouh.regionsyddanmark.dk

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Abstract

Aim: To describe feeding practices at hospital discharge in relation to characteristics of the very preterm infants (VPI) and their mothers.

Methods: *Design.* Prospective hospital-based registration of very preterm infants born with a gestational age ≤32 weeks in Denmark during 2004–2008. *Subjects.* Healthy mothers and VPI without diseases causing eating disabilities at discharge.

Results: A total of 478 VPI were registered. At discharge, 60% were exclusively breastfed, 35% were exclusively bottlefed, and 5% were both breast- and bottle-fed. Mothers of high social class ($p = 0.000$) and 'not smoking' ($p = 0.003$) were significantly more often breastfeeding their preterm infant(s) at discharge. Single births infants tended more often to be breastfed ($p = 0.09$). Infant age at discharge and duration of hospitalization did not influence breastfeeding at discharge. Increase in weight z-score from birth to discharge was largest in the bottlefeeding-group compared with the breastfeeding-group ($p = 0.000$) probably as a result of feeding practice the last week(s) of hospitalization.

Conclusion: Breastfeeding can successfully be established in very preterm infants. Mothers of low social classes, smokers, multiple birth and very preterm infants with low weight for age may need extra attention in breastfeeding establishing policies.

INTRODUCTION

In preterm infants the beneficial effects of human milk generally relate to improvements in host defences, digestion and absorption of nutrients, gastrointestinal function, neuro-developmental outcomes and maternal psychological well-being (1). Breastfeeding provides a broad multifactorial anti-inflammatory defence for the infant (2). Even donor breast milk is associated with a lower risk of developing necrotizing enterocolitis (NEC) compared with formula milk in preterm and low birth weight infants (3). Breastfed children also have shown significantly higher developmental scores in comparison with formula-fed children. The benefit obtained from breastfeeding was mostly pronounced in children with low birth weight. Also, a significant benefit from breastfeeding on cognitive development was obtained for breastfeeding exposure for more than 8 weeks (4). Preterm infants assigned to human milk (donor breast milk) have also been found to have marked benefits up to 16 years later for all of the major components of the metabolic syndrome (blood pressure, leptin resistance (suggestive of future obesity), insulin resistance, and lipid profile) compared with formula-fed infants, and a positive dose-response association between the volume of breast milk intake and later beneficial effect on lipid-profile as well (5–7). Protective factors associated with breast milk probably even supersede the harm associated with smoking while breastfeeding (8). Preterm infants are routinely tube fed until they are developmentally and physiologically ready to begin the process of learning to suck, swallow and breathe in a coordinated fashion which often occurs at 32-35 weeks gestational age (GA) (9) One study found that preterm infants allowed early non-nutritive sucking at the breast were able to demonstrate nutritive sucking (≥5 g milk-volume by test-weighing) as early as 30.6 weeks postmenstrual age (PMA) (10). The same author found in another study that full breastfeeding was attained at a median age of 35 weeks, between 32 and 38 weeks among 15 VPI (11). Kangaroo-care (KC) dyads have been found to breastfeed more exclusively and for a longer period (12).

In a Swedish study from 2007, 53% of preterm infants (GA <37 weeks) were exclusively breastfed at discharge from the neonatal unit. The rate of exclusively breastfeeding was lower for the most immature infants (born with GA<32

weeks) (13). The aim of this study was to estimate the breastfeeding rate, and to characterize the mothers and the VPI in order to identify those who need extra attention in breastfeeding establishing policies.

METHODS

Recruitment of VPI and data-registration was performed prospectively at four neonatal intensive care units (NICU) in Denmark [Holbaek Hospital (HH), Kolding Hospital (KH), Hans Christian Andersen Children's Hospital at Odense University Hospital (OUH) and Aarhus University Hospital in Skejby (AUH)]. The VPI were born and admitted to the NICU from July 2004 in Odense and from 2005 in Skejby, Kolding and Holbaek and until August 2008.

Feeding regimes were identical at the four paediatric departments with early parenteral nutrition and early trophic feeding. Until PMA of at least 30 weeks, the infants were all fed expressed mothers milk and/or donor breast milk. Fortification with Human Milk Fortifier (HMF) was initiated from day 10–14 from birth. Fortification of mother's own expressed milk was done until discharge, but with decreasing amounts during the last week(s) while the infant was improving sucking directly from the breast. The breastfed infants were discharged when sucking full amount direct from the breast and gaining weight. Mothers who did not have enough milk of their own were supplementing with a Preterm Formula after 30 weeks PMA. If the mothers decided not to breastfeed or breastfeeding stopped before discharge, infants were bottlefed and they were also discharged when sucking from the bottle without problems and gaining weight. Small for gestational age (SGA) VPI were fed like all other VPI, and they were not routinely given a larger volume or extra calories.

The counselling of the mother to breastfeed her infant(s) was done as a routine in the departments and according to the guidelines from The Danish National Board of Health (2003, 2005, 2006 and 2008) (14). Kangaroo-care with skin to skin contact (SSC) was established as soon as possible also during ventilator-treatment. As soon as the very preterm infant was stable (respiratory and cardiovascular), SSC was established once a day (2–3 h) when ventilator-treated and twice a day (2 × 2–3 h) when treated with continuous positive airway pressure (CPAP). The mothers were all encouraged to start expressing milk as soon as possible after birth and later on to breastfeed if possible and if they wanted to. Breastfeeding was not possible in some cases of breast-surgery, chemotherapy or other medication contraindicating breastfeeding. There were several available breast pumps at all departments.

At birth, birth weight (BW), GA, single birth, twin, triplets or quadruplets were recorded and at discharge, feeding practice, PMA and weight were recorded for each preterm infant. Based on patient records and questionnaires, information on mother's age, education and smoking habits were obtained. Mother's social class (SC) was defined according to The Danish National Centre of Social Research based on education and occupation (15).

Ethical consideration

The study was approved by the Danish National Committee on Biomedical Research Ethics (J.nr. VF20030208) and the registrations were approved at the Danish Data Protection Agency (J.nr.2007-41-1349).

Statistical analysis

Data were analysed using STATA (version 9.2, Stata Corporation, College Station, TX, USA).

Z-score or Standard deviation score (SDS) was calculated as the difference between the actual weight and the expected reference weight divided with 1 standard-deviation (SD) (ex.: (BW – ref. BW)/1 SD) according to a reference for each gender (16). Instead of comparing weight at certain PMA for each gender the z-scores were calculated for comparisons between groups. In this study the VPI were defined as SGA if weight z-score at birth was below –2 SDS.

Group comparisons were conducted with *t*-test for continuous variables and chi-squared test for categorical data or Wilcoxon rank-sum test when data were nonparametric distributed (BW and GA). Logistic regression was used to produce univariate odds ratio and 95% confidence intervals. Multivariate logistic regression was used to determine which factors (z-score at birth, PMA at discharge, multiple births, young mother, maternal social class and smoking) were independently associated with breastfeeding at discharge. Excluded from the final model are factors that from a clinical point of view are more a result of feeding practice rather than a cause (z-score and weight at discharge), factors strongly correlated to other factors in the model (SGA, BW and maternal age) and insignificant factors not influencing the final model (gender, duration of hospitalization, GA at birth).

RESULTS

A total number of 633 infants born with a GA ≤32 + 0 from July 2004 until August 2008 were recorded (GA 23 + 0 - 32 + 0 weeks and BW 428 g–2255 g). One hundred and fifty-five preterm infants were excluded (24% of initial cohort) because of death (5%), serious congenital or chromosomal anomalies (1%), surgery as a result of necrotizing enterocolitis (NEC) (5%) or ductus arteriosus persistens (DAP) (2%), intraventricular haemorrhage (IVH) III-IV and/or periventricular leucomalacia (PVL) (4%), bronchopulmonary dysplasia (BPD) (1%) or eating disability (1%). Mothers with language problems (unable to communicate in Danish or English) (2%), severe social problems (mothers placed in institutions, alcohol or drug abuse) (1%) or families who moved out of the involved regions (2%) were excluded.

A total number of 478 (GA 24 + 1 to 32 + 0 and BW 520 g–2255 g) VPI remained in the study population distributed within the four NICU's; HH; 76, KH; 103, OUH; 179 and AUH; 120. There were 224 girls and 254 boys. A total of 180 infants (38%) were multiple-birth (166 twins, 11 triplets and 3 quadruplets) and 113 infants (24%) were born SGA. At discharge mean PMA was 37 + 2 (SD ± 12 days)

and mean weight was 2634 g (SD ± 406 g), 285 (60%) infants were exclusively breastfed, 167 (35%) were bottlefed and 26 (5%) were both breast- and bottle-fed. The following analysis includes only two feeding-groups according to whether they were (1) exclusively breastfed, n = 285 (60%) or (2) not exclusively breastfed, n = 193 (40%) at discharge.

Results are illustrated in Table 1.

For the final analysis complete data-set were available on 409 VPI and their mothers [86% of study-cohort (478) and 65% of initial cohort (633)]. Information on mother's age was obtained among mothers of 474 VPI. Information on smoking was obtained from mothers of 436 VPI and 19% of 436 mothers smoked during pregnancy and lactation. Information on mother's social class was obtained from mothers of 423 VPI and the mothers were divided into 5 different social classes 1 = high SC (12%), 2 (28%), 3 (6%), 4 (39%) 5 = low SC (15%).

A higher rate of breastfeeding at discharge was found among mothers of high social class (p = 0.000) and mothers who did not smoke (p = 0.003). A higher rate of single birth VPI tended to be breastfed at discharge though not significant (p = 0.09). A lower rate of young mothers (<25 years) were breastfeeding at discharge (p = 0.007 univariate), though not significant in the final model (p = 0.28) probably because young age was correlated with low social class (p = 0.000). Low z-score at birth tended to be negatively correlated to breastfeeding at discharge (p = 0.02 univariate), though not significant in the final model (p = 0.09). The proportion of SGA-VPI was highest in the not exclusively breastfed group as compared with the exclusively breastfed group (27.5% vs 21.1%), but it was not significant (p = 0.11 univariate). Z-score at discharge seemed lower among exclusively breastfed VPI though not significant (p = 0.06 univariate). Change in z-score from birth to discharge was 0.34 SDS (−1.36 to −1.02) among not exclusively breastfed VPI and −0.10 SDS (−1.09 to −1.19) among exclusively breastfed VPI (p = 0.000 univariate). Previous breastfeeding experience among mothers of 299 VPI did not show any significant influence on breastfeeding.

Including only single birth VPI (n = 253) in the final model no significant differences were found except maternal SC (p = 0.02) and smoking (p = 0.01) that were still negatively correlated to breastfeeding at discharge. Change in z-score among singletons was 0.28 SDS and −0.10 SDS respectively (p = 0.000 univariate).

DISCUSSION

In our prospective observational study we found that 60% of 478 VPI to be exclusively breastfed at discharge. Blinded randomized trials comparing breastfeeding with formula feeding does not exist for obvious reasons. Therefore, an observational study is the best available study to characterize breastfed VPI at discharge.

According to the literature breastfeeding-rate of VPI are reported to vary considerably. This could possibly be explained by the duration of maternity leave, mother's

Table 1 Characteristics associated with breastfeeding at discharge among 478 VPI in the study-cohort recruited from 4 NICU in Denmark from July 2004–August 2008

	Exclusively breastfeeding at discharge			Univariate	Final model (n = 409) (86% of study-cohort, 65% of initial cohort)	
Characteristics	No (n = 193)	Yes (n = 285)	p-value*	Odds ratio (95% CI)		p-value
Preterm infants						
Gender (% male)	55.4	51.6	0.41	0.86 (0.59–1.24)		
Birth weight (g) (median)	1285	1350	0.07	1.05 (1.00–1.11)†		
GA at birth (week) (median)	29.7	30.3	0.11	1.04 (0.94–1.15)		
Z-score (SDS) at birth (mean)	−1.36 ± 1.25	−1.09 ± 1.24	0.02	1.19 (1.03–1.38)	1.17 (0.98–1.40)	0.09
SGA (weight z-score <−2) (% in group)	27.5	21.1	0.11	0.70 (0.46–1.08)		
Weight at discharge (g) (mean)	2655 ± 401	2620 ± 409	0.35			
PMA at discharge (week) (mean)	37.3 ± 1.7	37.4 ± 1.7	0.39	1.05 (0.94–1.17)	1.08 (0.94–1.23)	0.28
Z-score (SDS) at discharge (mean)	−1.02 ± 1.02	−1.19 ± 0.94	0.06			
Change in z-score (SDS) birth to discharge	0.34 ± 0.77	−0.10 ± 0.88	0.000	0.53 (0.42–0.67)		
Hospitalized (days) (mean)	53.5 ± 17.0	53.6 ± 21.5	0.93	1.00 (0.99–1.01)		
Multiple birth (% multiple)	40.4	35.8	0.31	0.82 (0.56–1.20)	0.67 (0.43–1.06)	0.09
Mothers						
Maternal age (years) (mean) (n = 474)	29.7 ± 5.7	30.7 ± 4.5	0.06	1.04 (1.00–1.08)		
Young mother (% <25 years) (n = 474)	17.1	8.8	0.007	0.47 (0.27–0.81)	0.70 (0.37–1.33)	0.28
Maternal social class (1 = high, 2, 3, 4, 5 = low) (mean) (n = 423)	3.6 ± 1.3	2.9 ± 1.3	0.000	0.65 (0.55–0.77)	0.71 (0.59–0.86)	0.000
Smoking (%) (n = 436)	30.6	11.3	0.000	0.29 (0.17–0.48)	0.43 (0.25–0.76)	0.003

*Wilcoxon rank-sum test if median, *t*-test if continuous variables or chi-squared test if categorical variables.
†Odds Ratio per 100 g.

opportunities for spending time in hospital with their infant(s) and different policies for supporting breastfeeding. An American retrospective study from 2008 with a cohort of 361 mother-infant pairs reported 60% provided expressed milk feeding for their very low birth weight (VLBW) infants, but only a total of 27% provided direct breastfeeding. They found direct breastfeeding positively associated with positive socio-demographic factors (17).

In a study from 2002 with 119 mothers of single birth VLBW infants found that 73% intended to breastfeed but only 34% (25% of total cohort) continued lactation beyond 40 weeks PMA. Mothers who continued breastfeeding were older, more often married and had more than a high school education compared with those who discontinued lactation. Significant factors influencing lactation beyond 40 weeks PMA included start of milk expression before 6 h post-delivery, expressing milk more than 5 times a day and kangaroo care (18). An Italian study from 2008 found the highest probability of initiating and maintaining breastfeeding among infants with mothers aged between 27 and 34 years, with high educational levels, non-smokers and with previous breastfeeding experience. This was also correlated to the infants who suckled in the first hour of life, had longer hospital stay, and where breastfed at discharge (19).

In our population 60% of the VPI were direct breastfed at discharge and thereby had received human milk for a mean duration of 1.8 months. Compared with the duration of breastfeeding among term infants, a Scandinavian study from 1996 found a breastfeeding-rate among term infants 6 weeks post partum to be 77% (20).

Previous studies have shown a negative association of maternal smoking on breastfeeding initiation and duration (21–23). In our study, we found 19% of the mothers of VPI were smokers and we also found a negative correlation between maternal smoking and breastfeeding in preterm infants. The number of smoking mothers has apparently decreased during the past 2 decades in Denmark. A study from 1996 on term infants found 20% smoking mothers (20), and another study from 2006 found 26% smoking mothers during pregnancy (23) while a study from 1991 reported 38% mothers smoking in pregnancy and 42% smoking during lactation period (24). It has been shown that mothers perceived that a strong risk of harming the baby was posed by smoking while breastfeeding and that they received little encouragement to continue breastfeeding because of inability to stop smoking (25). This indicates a need for more consistency in promoting breastfeeding in spite of smoking post partum.

Studies have shown positive associations between breastfeeding and cognitive development also within the low birth-weight group (4,26,27). In our study the number of SGA VPI did not differ significantly between groups, but a low z-score at birth seemed to be negatively correlated to breastfeeding at discharge. The change in z-score between groups was significant and is probably a result of the nutrition- and feeding-practices that includes being supplemented with formula if breastfeeding is not sufficient during the last week(s) of hospitalization. VPI learning to breastfeed are usually not gaining as much weight while practicing direct breastfeeding and fed on demand and not on schedule. Breastfeeding should be provided as long as mutually desired by mother and child according to ESPGHAN Commentary on Breast-feeding (28). ESPGHAN also stated in a Commentary on feeding preterm infants after hospital discharge; that preterm infants are often discharged from hospital care with body weights below the usual birth weight of healthy term infants. Close monitoring of growth during hospital stay and after discharge is recommended. Infants discharged with a subnormal weight for age are at increased risk of long-term growth failure, and they might need some kind of supplementation (29).

CONCLUSION

Breastfeeding is recommended and is possible to establish among VPI at discharge, however mothers of low social classes and smokers are less often breastfeeding. An active nutrition- and feeding-policy during hospitalization is necessary in order to establish breastfeeding with a special attend on multiple births, infants with low weight for age, and mothers of low social class and smokers who want to breastfeed their very preterm infant(s) at and beyond hospital discharge.

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References

1. Schanler RJ. The use of human milk for premature infants. *Pediatr Clin North Am* 2001; 48: 207–19.
2. Hanson LA. Session 1: Feeding and infant development breastfeeding and immune function. *Proc Nutr Soc* 2007; 66: 384–96.
3. Henderson G, Anthony MY, McGuire W. Formula milk versus maternal breast milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev* 2007; 4: CD002972.
4. Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. *Am J Clin Nutr* 1999; 70: 525–35.
5. Singhal A. Early nutrition and long-term cardiovascular health. *Nutr Rev* 2006; 64: S44–9.
6. Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. *Lancet* 2001; 357: 413–9.
7. Singhal A, Cole TJ, Fewtrell M, Lucas A. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. *Lancet* 2004; 363: 1571–8.
8. Gartner LM, Morton J, Lawrence RA, Naylor AJ, O'Hare D, Schanler RJ, et al. Breastfeeding and the use of human milk. *Pediatrics* 2005; 115: 496–506.
9. Nye C. Transitioning premature infants from gavage to breast. *Neonatal Netw* 2008; 27: 7–13.

10. Nyqvist KH, Sjoden PO, Ewald U. The development of preterm infants' breastfeeding behavior. *Early Hum Dev* 1999; 55: 247–64.
11. Nyqvist KH. Early attainment of breastfeeding competence in very preterm infants. *Acta Paediatr* 2008; 97: 776–81.
12. Hake-Brooks SJ, Anderson GC. Kangaroo care and breastfeeding of mother-preterm infant dyads 0–18 months: a randomized, controlled trial. *Neonatal Netw* 2008; 27: 151–9.
13. Akerstrom S, Asplund I, Norman M. Successful breastfeeding after discharge of preterm and sick newborn infants. *Acta Paediatr* 2007; 96: 1450–4.
14. The Danish National Board of Health. Successful breastfeeding 2003 and 2006, recommendations for infant nutrition 2005 and breastfeeding 2008. Guidelines for healthcare professionals. Copenhagen: The Danish National Board of Health, 2003, 2005, 2006 and 2008. [Generic].
15. The Danish National Centre For Social Research. Oversigt over de 5 socialgrupper. 2009. Available at: http://www.sfi.dk/ (accessed February 3, 2010).
16. Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr* 1996; 85: 843–8.
17. Smith MM, Durkin M, Hinton VJ, Bellinger D, Kuhn L. Initiation of breastfeeding among mothers of very low birth weight infants. *Pediatrics* 2003; 111: 1337–42.
18. Furman L, Minich N, Hack M. Correlates of lactation in mothers of very low birth weight infants. *Pediatrics* 2002; 109: e57.
19. Manganaro R, Marseglia L, Mamì C, Paolata A, Gargano R, Mondello M, et al. *Child Care Health Dev* 2009; 35: 106–11.
20. Eriksen W. Breastfeeding, smoking and the presence of the child's father in the household. *Acta Paediatr* 1996; 85: 1272–7.
21. Liu J, Rosenberg KD, Sandoval AP. Breastfeeding duration and perinatal cigarette smoking in a population-based cohort. *Am J Public Health* 2006; 96: 309–14.
22. Donath SM, Amir LH. The relationship between maternal smoking and breastfeeding duration after adjustment for maternal infant feeding intention. *Acta Paediatr* 2004; 93: 1514–8.
23. Giglia R, Binns CW, Alfonso H. Maternal cigarette smoking and breastfeeding duration. *Acta Paediatr* 2006; 95: 1370–4.
24. Halken S, Host A, Husby S, Hansen LG, Osterballe O, Nyboe J. Recurrent wheezing in relation to environmental risk factors in infancy. A prospective study of 276 infants. *Allergy* 1991; 46: 507–14.
25. Goldade K, Nichter M, Nichter M, Adrian S, Tesler L, Muramoto M. Breastfeeding and smoking among low-income women: results of a longitudinal qualitative study. *Birth* 2008; 35: 230–40.
26. Slykerman RF, Thompson JM, Becroft DM, Robinson E, Pryor JE, Clark PM, et al. Breastfeeding and intelligence of preschool children. *Acta Paediatr* 2005; 94: 832–7.
27. Agostoni C. Small-for-gestational-age infants need dietary quality more than quantity for their development: the role of human milk. *Acta Paediatr* 2005; 94: 827–9.
28. Agostoni C, Braegger C, Decsi T, Kolacek S, Koletzko B, Michaelsen KF, et al. Breast-feeding. A commentary by the ESPGHAN Committee on nutrition. *J Pediatr Gastroenterol Nutr* 2009; 49: 112–25.
29. Aggett PJ, Agostoni C, Axelsson I, De CM, Goulet O, Hernell O, et al. Feeding preterm infants after hospital discharge: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2006; 42: 596–603.

13. Paper II.

Nutrient enrichment of Human Milk and Growth of Very Preterm Infants after Hospital Discharge

Nutrient enrichment of Human Milk and Growth of Very Preterm Infants after Hospital Discharge

Authors:

Gitte Zachariassen[1], Jan Faerk[2], Carl Grytter[3], Birgitte H Esberg[4], Jacob Hjelmborg[5], Sven Mortensen[1], Henrik T Christesen[1], Susanne Halken[1].

1. Hans Christian Andersen Children's Hospital, Odense University Hospital
2. Paediatric Department, Holbaek Hospital.
3. Paediatric Department, Kolding Hospital.
4. Paediatric Department, Skejby, Aarhus University Hospital.
5. Institute of Biostatistics, Southern University of Denmark.

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Nutrient Enrichment of Mother's Milk and Growth of Very Preterm Infants After Hospital Discharge

AUTHORS: Gitte Zachariassen, MD,[a] Jan Faerk, MD, PhD,[b] Carl Grytter, MD,[c] Birgitte Hass Esberg, MD,[d] Jacob Hjelmborg, MSc, PhD,[e] Sven Mortensen, MD,[a] Henrik Thybo Christesen, MD, PhD,[a] and Susanne Halken, MD, DMSci[a]

[a]Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark; [b]Pediatric Department, Holbaek Hospital, Holbaek, Denmark; [c]Pediatric Department, Kolding Hospital, Kolding, Denmark; [d] Pediatric Department, Skejby, Aarhus University Hospital, Aarhus, Denmark; and [e] Institute of Biostatistics, Southern University of Denmark, Odense, Denmark

KEY WORDS
Fortification, breastfeeding, preterm infants, discharge, growth

ABBREVIATIONS
VLBW—very low birth weight
PMA—postmenstrual age
PF—preterm formula
SGA—small for gestational age
GA—gestational age
HMF—human milk fortifier
CA—corrected age
BW—birth weight
HC—head circumference
SUN—serum urea nitrogen
REM—random-effect model
ITT—intention to treat
PP—per protocol
AGA—appropriate for gestational age

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Address correspondence to Gitte Zachariassen, MD, Hans Christian Andersen Children's Hospital, Odense University Hospital, Sdr. Boulevard 29, DK-5000 Odense C.
E-mail: gitte.zachariassen@ouh.regionsyddanmark.dk

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WHAT'S KNOWN ON THIS SUBJECT: Very preterm infants are often discharged before they reach term, and at the time of discharge many have not achieved catch-up growth. Mother's milk is recommended but might not meet the needs of the growing preterm infant after discharge.

WHAT THIS STUDY ADDS: Fortification of mother's milk while breastfeeding her very preterm infant after hospital discharge is possible without influencing breastfeeding duration. The amount of fortification given in this study did not improve growth at 1 year of age compared with unfortified mother's milk.

abstract

OBJECTIVE: To determine if the addition of a multinutrient human milk fortifier to mother's milk while breastfeeding very preterm infants after hospital discharge is possible and whether it influences first-year growth.

METHODS: Of a cohort of 320 infants (gestational age: 24–32 weeks; birth weight: 535–2255 g), breastfed infants (65% [$n = 207$]) were randomly assigned shortly before hospital discharge to receive either unfortified ($n = 102$, group A) or fortified ($n = 105$, group B) mother's milk until 4 months' corrected age (CA). The remaining infants were bottle-fed with a preterm formula (group C). Follow-up was performed at term and at 2, 4, 6, and 12 months' CA.

RESULTS: Mean duration of breastfeeding after term was not significantly different between groups A and B (11.8 and 10.6 weeks, respectively). Weight, length, and head circumference were not significantly different between groups A and B at 12 months' CA. Compared with groups A and B, infants in group C had a higher increase in weight *z* score until term and in length *z* score until 6 months' CA. At 12 months' CA, boys in group C were significantly longer and heavier compared with those in groups A and B, whereas girls in group C were longer and heavier compared with those in group A only. A higher protein intake was related to a higher serum urea nitrogen level and growth.

CONCLUSIONS: Fortification of mother's milk after hospital discharge while breastfeeding very preterm infants was possible without influencing breastfeeding duration but did not significantly influence growth parameters at 1 year of age compared with unfortified mother's milk. *Pediatrics* 2011;127:e995–e1003

The majority of very low birth weight (VLBW) infants are discharged before they reach 40 weeks' postmenstrual age (PMA). At the time of discharge, many VLBW infants have deficits in accretion of energy, protein, minerals, and other nutrients. Nutrient deficit in the first weeks of life can be directly related to postnatal growth retardation,[1–3] and VLBW preterm infants have often not reached the median birth weight of the reference fetus at the same PMA at hospital discharge.[4] Some catch-up growth is observed in most VLBW infants, but the rate and time of catch-up differ between studies.[5,6] Although low birth weight infants as a group do catch up, many remain smaller than infants of normal birth weight.[7,8]

Improvement of growth among VLBW preterm infants can be achieved by feeding a nutrient-enriched preterm formula (PF).[9,10] However, human milk has been shown to have many benefits, especially on the IQ among infants born small for gestational age (SGA) and VLBW preterm infants.[11,12] Human milk does require nutrient fortification to meet the protein and mineral needs of the rapidly growing preterm infant during hospitalization.[13] After discharge, a nutrient-enriched formula can be used for low birth weight infants, but fortification of human milk for breastfed preterm infants with gestational age (GA) below 32 weeks has not yet been proven optimal as a feeding strategy to improve growth.

The aim of this study was to determine if addition of a multinutrient human milk fortifier (HMF) to a small sample of the mother's own milk while breastfeeding is possible and whether it improves growth of very preterm infants compared with unfortified mother's milk after hospital discharge.

METHODS

We performed a prospective, randomized, and controlled birth cohort study on infants with a GA of ≤32⅐ weeks who were recruited consecutively from 4 neonatal units in Denmark (Odense, Aarhus, Holbaek, and Kolding) from July 2004 to August 2008.

Exclusion criteria were severe diseases or circumstances influencing eating- and feeding-ability at discharge. Feeding regimen was identical at the 4 neonatal units as described previously.[14] Shortly before discharge, the breastfed infants were randomly assigned to receive either breastfeeding without intervention (group A) or intervention with fortification (group B). Five packets of HMF (Enfamil Human Milk Fortifier [Mead Johnson Nutritional, Evansville, IN]; 17.5 kcal and 1.375 g protein per 5 packets) were added to 20 to 50 mL of mother's fresh or defrosted expressed milk and given orally in a feeding bottle, or with a small cup, every day until 4 months' corrected age (CA). All mothers were encouraged to breastfeed as long as possible. The study could not be blinded because of the lack of a placebo product without influence on breastfeeding, nutrition, and growth.

Bottle-fed infants at discharge (group C) were given a PF (Enfalac Preterm Formula [Mead Johnson Nutritionals, Nijmegen, Netherlands]; 68 kcal, 2 g protein, 7.4 g carbohydrate, and 3.5 g fat per 100 mL) until 4 months' CA with a daily volume of approximately one-sixth of the infant's weight in grams. Randomization was performed by using sealed envelopes with randomization numbers made before study start for each department. Multiple births were randomly assigned together. Only physicians enrolled participants; both project nurses and physicians assigned to the project performed randomizations.

Infants were not excluded in case of change of nutrition after randomization. If breastfeeding was not sufficient (groups A and B) within the first month after discharge, the infants were supplemented with or changed to PF. If breastfeeding ceased between 1 and 2 months after discharge, the infants were supplemented or changed to preterm or mature formula on the basis of an individual assessment. If breastfeeding ceased later than 2 months after discharge, the infants were supplemented or changed to mature formula. Introduction to complementary food was not recommended until 4 months' CA for any of the groups.

Birth weight (BW), length, head circumference (HC), GA, and single- or multiple-birth information were recorded. Information on mother's age, education, occupation, smoking habits, and previous breastfeeding experience was obtained by interviews and questionnaires at the time of randomization. Mother's social group was defined according to the Danish National Centre of Social Research on the basis of education and occupation.[15]

Infants were seen at the outpatient clinics at term and at 2, 4, 6, and 12 months' CA. As primary outcome measures on growth, weight, length (crown to heel), and HC (occipital to frontal) were obtained. Data on breastfeeding and introduction of formula and/or complementary food were recorded. At randomization, discharge, term, and 4 months' CA, blood was drawn to measure serum hemoglobin, serum phosphorus, and serum urea nitrogen (SUN). Data obtained from dropouts were included until the date of withdrawal.

Ethics

The study was approved on July 1, 2004, by the Danish National Committee on Biomedical Research Ethics, and the handling of data and registra-

tions were approved February 2006 by the Danish Data Protection Agency. Informed consent was obtained from the parents after verbal and written information was provided.

Statistical Analysis

Sample-size calculation was made before study start with an SD of 5 g/day, significance at 5%, the lowest weight difference not to be missed at 2.5 g/day, and power at 90%.

The sample-size calculation showed that at least 85 infants were needed in each group.

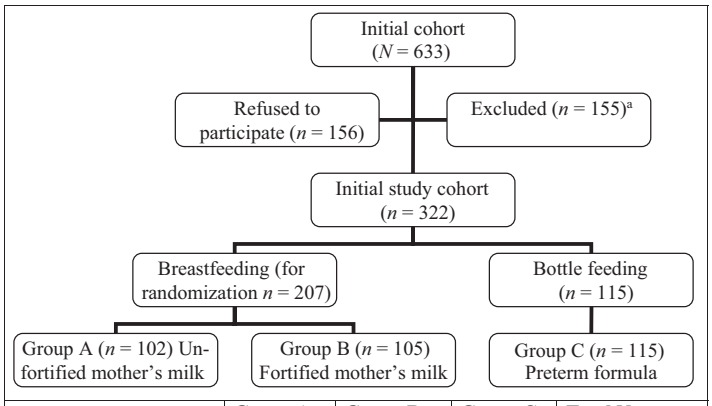
Data were analyzed by using Stata 11 (Stata Corp, College Station, TX). The Wilcoxon rank or Student's *t* test was used for continuous variables, the χ^2 test was used for categorical variables, and a multiple logistic regression model was used to compare nutrition groups. Factors that possibly influenced the duration of breastfeeding and time of introduction to complementary feeding were evaluated by a multiple logistic regression model with clinically relevant variables (nutrition group, mother's age, social group, smoking habit, previous breastfeeding experience, multiple births, gender, GA, and SGA).

To compare groups for primary outcomes on growth, *z* scores (SD scores) were calculated as the difference between the actual growth and the expected reference growth divided with 1 SD (eg, [BW − reference BW]/1 SD) for each gender. SGA was defined as a BW *z* score below −2 SD scores. All *z* scores were calculated by using Niklasson and Albertsson-Wikland[16] as a reference. By linear interpolation, weight, length, and HC were estimated at PMA day 238 (random assignment of first infants), day 252 (discharge of first infants), day 280 (term), and at 2, 4, 6, and 12 months' CA as a basis for *z* score calculation. Change in *z* score (Δ *z* score) was calculated from PMA day 238 until day 252, day 280, and 2, 4, 6, and 12 months' CA, respectively. Multiple logistic regression on the full sample was used to evaluate variables (gender, nutrition, SGA, and multiple births) that influenced Δ *z* score.

Because data consist of repeated observations on the same subject taken over time and to exploit the full sample, random-effect models (REMs) with intercept and slope random effects[17] from 238 days' PMA were also used. According to clinical relevant variables possibly influencing growth and likelihood-ratio-tests comparing REMs, the following variables were included in the final REM: the age of the infants at the time of measurements, gender, multiple births, SGA, baseline weight, length, and HC (weight, length, or HC at 238 days' PMA), nutrition, and fourth grade polynomial on time interacting with gender and nutrition. Weight was transformed by taking the square root. The residuals of the model showed normal distribution. Random intercept and random slope were added to account for the unobserved heterogeneity between children measured at multiple occasions.

Analyses on growth were calculated as both intention to treat (ITT) and per protocol (PP).

Multiple logistic regressions were used to evaluate variables (gender, nutrition, SGA, and subnormal weight at discharge) that might influence growth among the subgroups with subnormal weight.



	Group A	Group B	Group C	Total No.
No. at randomization	***n* = 102**	***n* = 105**	***n* = 115**	**322**
Excluded	**0**	**0**	2[a]	
34 wk PMA	**102**	**105**	**113**	**320 (ITT group)**
Dropouts	0	0	0	
Changed nutrition	0	0	2	
36 wk PMA	**102**	**105**	**111**	**318**
Dropouts	3	0	0	
Changed nutrition	9	14	9	
40 wk PMA (term)	**90**	**91**	**102**	**283 (88%)**
Dropouts	2	0	3	
Changed nutrition	15	40	12	
2 mo CA	**73**	**51**	**87**	**211 (66%) (PP group)**
Dropouts	2	0	0	
Changed nutrition	33	31	37	
4 mo CA	**38**	**20**	**50**	**108 (34%)**

FIGURE 1

Participation flow chart during the intervention period. [a] Exclusion because of death ($n = 34$), congenital or chromosomal anomalies ($n = 9$), surgery resulting from necrotizing enterocolitis ($n = 32$) or persistent ductus arteriosus ($n = 12$), intraventricular hemorrhage III to IV and/or periventricular leukomalacia ($n = 25$), bronchopulmonary dysplasia ($n = 9$), eating disability ($n = 5$), severe language problems ($n = 12$), social problems ($n = 9$), or moved out of involved regions ($n = 10$).

A linear regression model was used to compare the possible impact of nutrition on hemoglobin, serum phosphorus, and SUN.

RESULTS

The initial cohort consisted of 633 infants with a median GA of 29⅚ (range: 23⅞–32⅞) weeks and a median BW of 1256 g (range: 428–2255 g). Twenty-two percent were SGA, and 36% were multiple births. The final study cohort consisted of 320 infants with a median GA of 29⅚ (24⅛–32⅞) weeks, and a median birth weight of 1271 g (range: 535–2255 g); the parents of 156 (25%) infants refused to participate, and 157 (25%) were excluded (Fig 1).

The excluded infants had a lower GA and BW, whereas those with parents who refused to participate had a higher GA and BW compared with those in the study cohort ($P \leq .002$). Compared with mothers who refused to participate, mothers in the study cohort were older (30.8 vs 29.3 years; $P = .003$) and more often breastfeeding (65% vs 50%; $P = .002$). Characteristics of the study group infants and their mothers are listed in Table 1.

Duration of breastfeeding after term was not influenced by fortification (11.8 ± 7.7 [group A] vs 10.6 ± 7.5 weeks [group B] [mean ± 1 SD]; nonsignificant). Early discontinuation of breastfeeding was associated with multiple births ($P = .000$), low social group ($P = .02$), and low maternal age ($P = .03$). Introduction of complementary food occurred later in group B compared with group A (18.3 ± 4.4 vs 16.4 ± 3.9 weeks' CA; $P = .002$), and group C was in between them (17.0 ± 3.4 weeks). Late introduction of complementary food was associated with high maternal age ($P = .000$).

Of the 320 infants in the ITT study cohort, 283 (88%) remained in their assigned nutrition groups until term, 211 (66%) until 2 months, and 108 (34%) until 4 months' CA (Fig 1). Because of many changes of nutrition between 2 and 4 months in all 3 nutrition groups, PP analyses were performed among the 211 infants who remained in their assigned nutrition groups until 2 months' CA.

Boys showed significantly higher weight, length, and HC than girls within all 3 nutrition groups from term until 1 year of age (REM). Mean *z* score showed a nadir in weight, length, and HC at 34 weeks' PMA irrespective of nutrition group, although they were never below the normal range (Fig 2).

In the ITT analyses, infants in group C increased significantly more in length and weight in *z* scores compared with those in groups A and B. At 2 and 4 months, boys' length *z* score increased significantly more in group C compared with those in

TABLE 1 Characteristics of Nutrition Groups

	A ($N = 102$)	B ($N = 105$)	A vs B (P)	A and B ($N = 207$)	C ($N = 113$)	A and B vs C (P)
Infants						
GA at birth, median (min–max), d	208.5 (169–224)	212 (171–224)	NS	210 (169–224)	207 (176–224)	NS
BW, median (min–max), g	1260 (548–2255)	1320 (535–2100)	NS	1287 (535–2255)	1233 (612–2140)	NS
Weight *z* score at birth, mean ± SD scores	−1.02 ± 1.16	−1.03 ± 1.05	NS	−1.02 ± 1.10	−1.23 ± 1.13	NS
SGA, *n*/*N*	20/102	21/105	NS	41/207	27/113	NS
Boys, *n*/*N*	58/102	52/105	NS	110/207	65/113	NS
SGA boys, *n*/*N*	11/20	10/21	NS	21/41	13/27	NS
Multiple births, *n*/*N*	27/102	42/105	.04	69/207	51/113	.04[a]
Baseline weight at day 238 PMA, mean ± SD, g						
Girls	1882 ± 293	1865 ± 268	NS	1872 ± 278	1895 ± 358	NS
Boys	1964 ± 287	1988 ± 268	NS	1975 ± 277	1996 ± 280	NS
Baseline length at day 238 PMA, mean ± SD, cm						
Girls	43.9 ± 2.5	43.8 ± 2.1	NS	43.9 ± 2.3	43.5 ± 2.5	NS
Boys	44.2 ± 2.5	44.9 ± 2.0	NS	44.5 ± 2.3	44.0 ± 2.2	NS
Baseline HC at day 238 PMA, mean ± SD, cm						
Girls	30.8 ± 1.4	30.4 ± 1.1	NS	30.6 ± 1.2	30.6 ± 1.4	NS
Boys	30.9 ± 1.3	31.2 ± 1.2	NS	31.1 ± 1.2	31.1 ± 1.3	NS
PMA at discharge, mean ± SD, d	264 ± 15	260 ± 10	.04	262 ± 13	259 ± 10	NS
Weight *z* score at discharge, mean ±SD scores	−1.22 ± 0.94	−1.22 ± 0.88	NS	−1.22 ± 0.91	−0.92 ± 0.92	.006[a]
Mothers						
Mother's age, mean ± SD, y	30.9 ± 4.5	31.0 ± 4.3	NS	31.0 ± 4.4	30.4 ± 5.8	NS
Social group, mean ± SD (1 = high and 5 = low)	2.88 ± 1.27	2.89 ± 1.30	NS	2.88 ± 1.28	3.59 ± 1.25	.000[a]
Smoking, *n*/*N*	13/99	10/105	NS	23/204	38/113	.000[a]
Previous breastfeeding experience, *n*/*N*	35/93	33/95	NS	68/188	43/109	NS

Wilcoxon rank or Student's *t* test if continuous variables and χ^2 test if categorical variables. min indicates minimum; max, maximum; NS, not significant.

[a] Also significant ($P < .05$) in a multiple logistic regression model (variables: GA, BW, weight *z* score at birth, weight *z* score at discharge, multiple births, mother's age, smoking habit, social group, and previous breastfeeding experience).

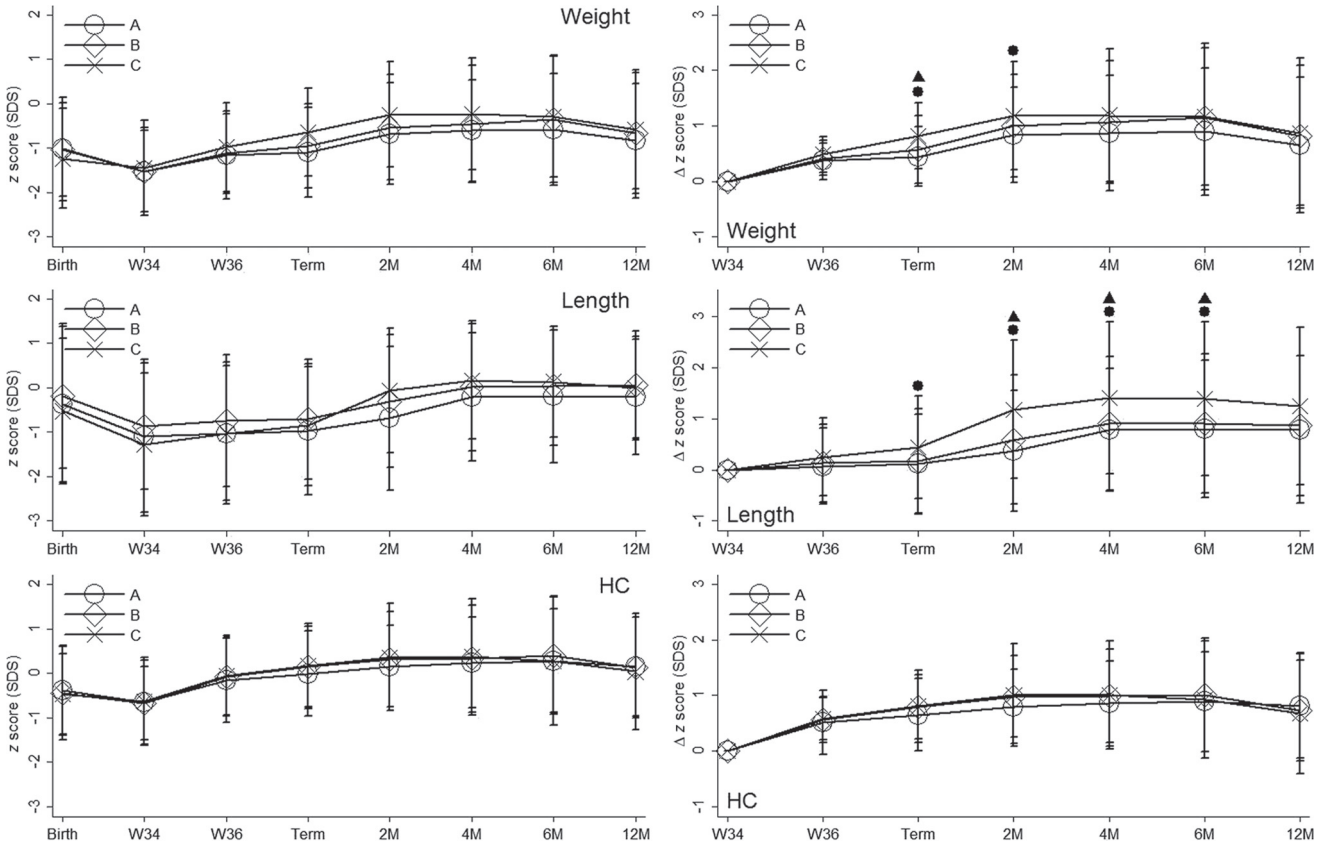


FIGURE 2

z scores from birth to 12 months of age (both genders) according to nutrition groups (mean *z* score ± 1 SD) (left column) and change in *z* score (Δ *z* score from 34 weeks' PMA) (right column) (ITT). Significant difference in Δ *z* score ($P < .05$) comparing nutrition-groups shown as: filled circles, C > A; filled triangles, C > B.

groups A and B, whereas girls in group C had a higher increase in length *z* score compared with those in group A only (Fig 3). In addition, weight at 2 to 6 months, and HC from term to 4 months, were significantly higher in girls of group B compared with those of group A (REM; Table 2).

In PP analyses, infants in group C increased significantly more in length and weight *z* score until 4 months' CA compared with those in groups A and B. Girls in group C increased significantly more in weight *z* score compared with those in groups A and B at term and increased significantly more in length *z* score compared with those in group A but not with those in group B (2–6 months). In addition, REM showed length and HC to be significantly higher among girls in group B compared with those in group A at 2 and 4 months. Results from term to 12 months' CA using REM are shown in Table 2.

In the ITT subgroup analyses on infants with subnormal weight, compared with appropriate for gestational age (AGA) infants, SGA infants ($n = 68$) increased significantly more in length *z* score during the entire study period with no difference when comparing nutrition groups (Fig 4). Among the 53 infants with subnormal weight at discharge, those in group C increased significantly more in weight *z* score compared with those in groups A and B until 2 months' CA but not at 12 months' CA.

Associations between nutrition group and selected biochemical parameters are shown in Table 3.

DISCUSSION

In preterm infants, close monitoring of growth has been recommended, and supplementation should be considered if the infant is discharged with subnormal weight for age.[18,19] The optimal method, amount, and duration of supplementation after hospital discharge are, however, unknown. We addressed these questions in an intervention study comparing the use of unfortified mother's milk with fortified milk. We found that fortification of breast milk while breastfeeding after discharge is possible; growth was improved among female infants, however, only during the intervention period and not at 12 months' CA. PF-fed infants increased more in growth compared with those fed mother's milk with or without fortification.

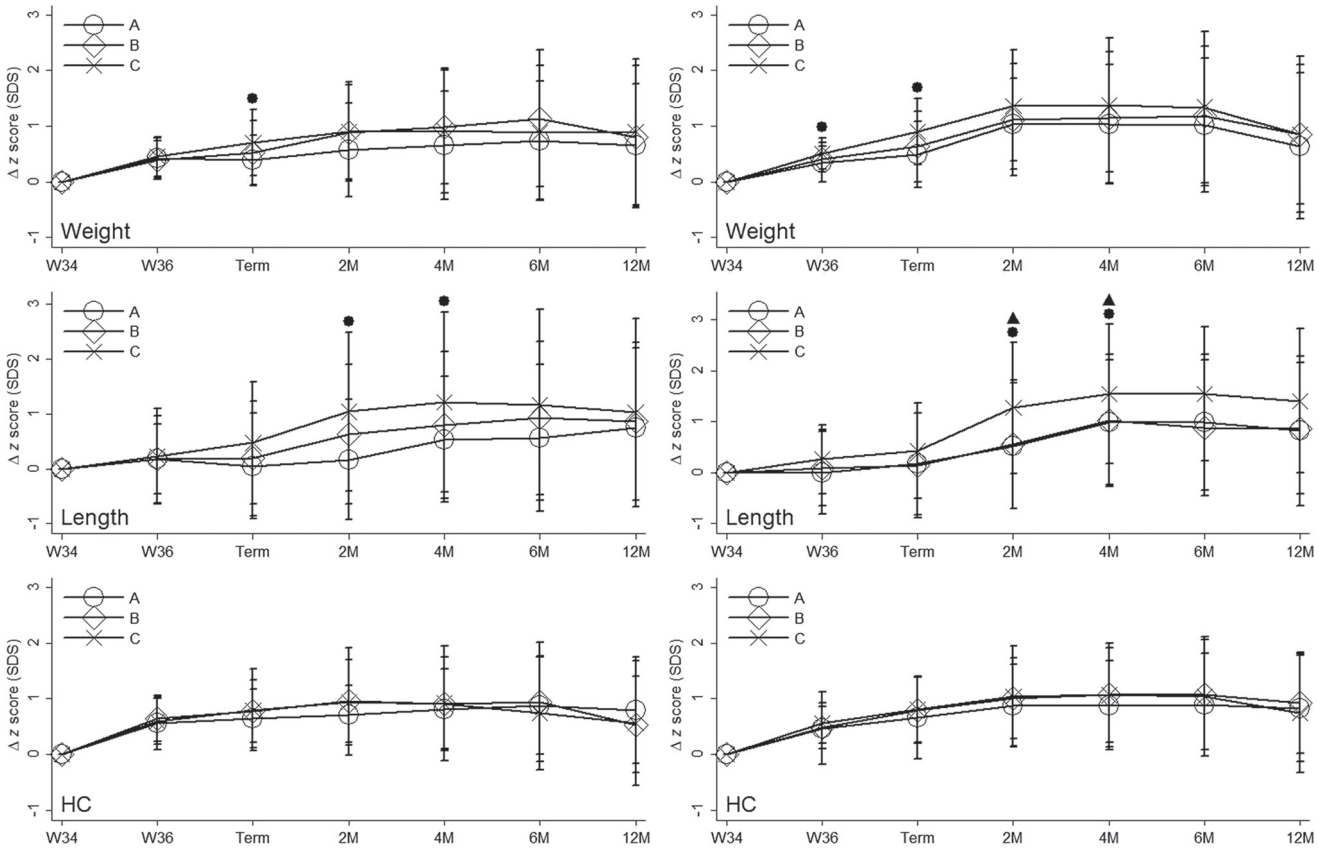


FIGURE 3

Change in *z* score (Δ *z* score from 34 weeks' PMA) (ITT) according to gender: left column, girls; right column, boys. Significant difference in Δ *z* score ($P < .05$) comparing nutrition groups shown as: filled circles, C > A; filled triangles, C > B.

Dropout, or change of nutrition after randomization, occurred mainly in the intervention group with a higher percentage of multiple births. Fortification of milk in multiple-birth-mothers seemed difficult, probably because of the need of more expressed milk and the feeding of more than 1 infant. The duration of breastfeeding was, however, not influenced by fortification of mother's milk, and the fortification group did not report more feeding problems (eg, regurgitation) compared with the exclusively breastfed group (data not shown).

Preterm infants must achieve catch-up growth to attain the growth parameters of term infants of the same PMA. To discuss optimal growth and catch-up growth, a reference describing normal growth is needed. The "optimal" growth of very preterm infants remains to be defined. Growth references for preterm infants from birth, through discharge, term, and during the first year of life are few. Some preterm growth references are based on intrauterine ultrasound measurements[20] and some are based on measurements at birth.[16] Growth references for preterm infants from 40 weeks' PMA can be based on mature or preterm infants, breastfed or formula-fed infants, or infants from the same part of the world or pooled data from different parts of the world (eg, World Health Organization growth references).[21] In our study, we chose a reference based on measurements of weight, length, and HC among Swedish preterm and term infants from birth to 24 months' CA[16] as they probably reflect the growth of Danish preterm infants well. The integration of a preterm and a term-born reference growth curve could explain why *z* scores in our study tended to decrease in most infants from 6 to 12 months' CA. Alternatively, the growth pattern of both very preterm AGA and SGA infants is different from term infants, irrespective of the nutrition.

Early enhanced nutritional intake in VLBW SGA infants leading to catch-up has been shown to improve HC growth and long-term neurodevelopmental outcome.[22] Because almost all preterm infants lose percentiles after birth, catch-up growth has been recommended for basically all preterm infants.[23] To achieve catch-up growth, special formulas aimed at meeting the nutritional requirements of preterm infants have been developed and evaluated in studies comparing preterm formula or postdischarge formula with a term formula and/or human

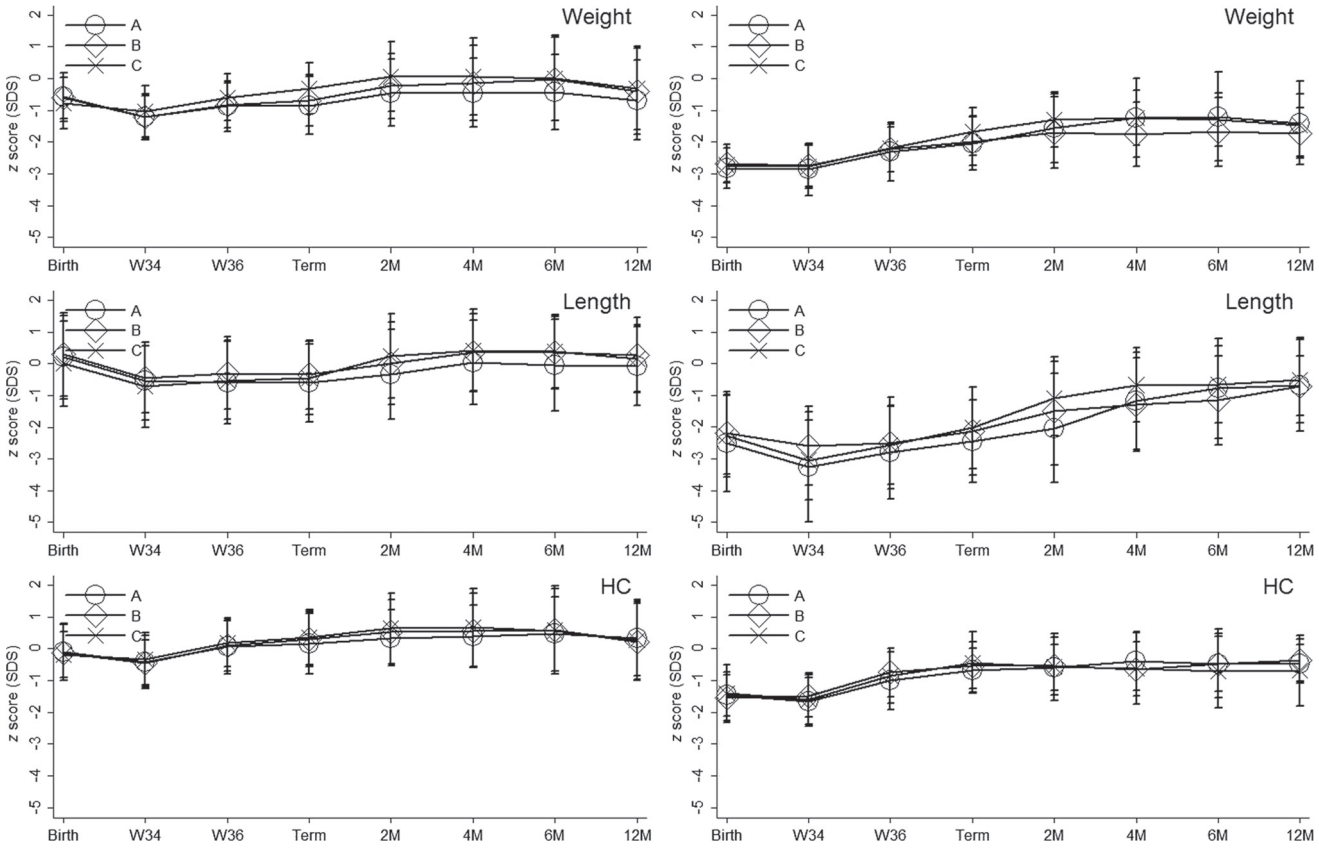


FIGURE 4
Anthropometric data as *z* scores for AGA (left column) and SGA (right column) on weight, length, and HC (both genders) (ITT).

TABLE 2 Main Results on Growth Using an REM

	ITT (at *n* mo CA)	PP (at *n* mo CA)
Weight		
Girls	B > A at 2, 4, and 6	C > A at 4, 6, and 12
	C > A at 6 and 12	C > B at 12
Boys	C > A at term, 2, 4, 6, and 12	C > A at 2, 4, and 6
	C > B at 4, 6, and 12	C > B at 2, 4, 6, and 12
Length		
Girls	C > A at 2, 4, 6, and 12	B > A at 2 and 4 ($P = .059$ at 6)
		C > A at 2, 4, 6, and 12
		C > B at 12
Boys	C > A at term, 2, 4, 6, and 12	C > A at ($P = .053$ at term), 2, 4, 6, and 12
	C > B at 2, 4, 6, and 12	C > B at ($P = .053$ at 2) 4, 6, and 12
HC		
Girls	B > A at term, 2, and 4	B > A at 2 and 4
	C > A at term, 2, and 4	C > A at term, 2, 4, and 6
Boys	No significant difference	No significant difference

Significant differences are defined as $P < .05$.

milk.[10,24–27] These studies show catch-up, although not completely, in a significant proportion of low birth weight infants, regardless of their nutrition after discharge. The advantage of nutrient enrichment seems to appear early (1–2 months after delivery), suggesting that there is a window of time during which catch-up in response to higher nutrient intake is most likely.[28]

In our study, PF-fed infants achieved weight and length catch-up earlier compared with those fed mother's milk irrespective of fortification, probably because of the higher load of protein and phosphorus as shown by elevated serum phosphorus and SUN levels. SUN values are indicators of protein metabolism[29] and can be used as a method for monitoring the need for fortification of mother's milk during hospitalization.[30] The optimal SUN value for optimal growth among preterm infants is, however, unknown. Hall has recommended a SUN value >0.8 mmol/L (>5 mg/dL) among preterm infants after discharge; otherwise, intervention is suggested.[31]

The PF-fed infants in our study had significantly higher SUN values compared with both breastfed groups; these values were similar to values of PF-fed infants in a study on postdischarge nutrition comparing formulas for preterm infants.[27] Compared with infants fed unfortified mother's milk, those who completed fortification until 4

TABLE 3 Mean SUN, Serum Phosphorus, and Hemoglobin (Whole Blood)

Nutrition Group	Weeks 34–35	Weeks 36–38	Weeks 39–40	4 mo CA
SUN, mean ± SD, mmol/L (1 mmol/L = 2.80 mg/dL)				
No. of infants	265	177	60	65
A	1.7 ± 0.6	1.5 ± 0.4	1.5 ± 0.8	2.2 ± 0.9
B	1.8 ± 0.7	1.8 ± 0.7	1.9 ± 1.0	3.1 ± 1.2[a]
C	2.1 ± 0.9[b,c]	2.5 ± 0.9[b,c]	3.3 ± 1.3[b,c]	4.2 ± 0.7[b,c]
Serum phosphorus, mean ± SD, mmol/L (1 mmol/L = 3.10 mg/dL)				
No. of infants	260	167	56	64
A	2.09 ± 0.22	2.02 ± 0.23	1.87 ± 0.33	1.74 ± 0.44
B	2.10 ± 0.27	2.09 ± 0.22	1.96 ± 0.27	1.96 ± 0.19[a]
C	2.16 ± 0.24	2.17 ± 0.22[b]	2.17 ± 0.23[b,c]	2.05 ± 0.11[b]
Hemoglobin, mean ± SD, mmol/L (1 mmol/L = 1.61 mg/dL)				
No. of infants	207	161	60	61
A	6.6 ± 1.2	6.4 ± 0.8	6.2 ± 0.7	7.3 ± 0.6
B	6.8 ± 1.2	6.4 ± 0.8	6.1 ± 0.6	7.5 ± 0.6
C	6.3 ± 0.9[d]	6.1 ± 0.9	5.9 ± 0.6	7.2 ± 0.6

Type of nutrition (A, B, or C) was registered on the day of blood sample. Conversion factors are from ref [41]. Significant difference ($P < .05$) with higher levels of SUN comparing group C with A[b] and B[c], group B with higher levels of SUN and serum phosphorus compared to A[a] at 4 months' CA, and group C with lower levels of hemoglobin compared to B at time of randomization.[d]

months' CA had higher serum phosphorus and SUN values. Although this finding was not associated with increased weight gain, it may reflect a better growth potential.

Inadequate nutrition of preterm infants may result not only in restricted catch-up growth but also in an increased risk of neurodevelopmental impairment and bone disease.[32–34] Conversely, enhanced nutrition may increase the risk of metabolic and cardiovascular disease in later life.[35–37] The protective effects of breastfeeding of mature infants on cardiovascular disease may, at least partly, be explained by the lower rates of early weight gain, possibly related to differences in substrate intakes.[38] Our study showed the same pattern with lower rates of weight and length gain among breastfed infants compared with PF-fed very preterm infants. SGA infants showed a more rapid catch-up growth compared with AGA infants during the study period but without significant differences in growth between nutrition groups. Although infants with subnormal weight at discharge increased significantly more in weight when fed PF compared with both breastfed groups, the small number of infants in these subgroups does not allow for general recommendations on feeding strategies on the basis of the present study.

To our knowledge, only one other group has studied the effect of fortification of breast milk in preterm infants after discharge. A Canadian pilot study observed growth until 1 year of age of human milk–fed preterm infants randomized to receive either fortification of expressed mother's milk (half of their feedings) or no fortification for 12 weeks after discharge.[39,40] Infants given fortification remained longer and had a higher whole body mineral content until 12 months of age.[40] The infants in the Canadian study were not directly breastfed and were fed a higher amount of fortification than the infants in our study. We chose to supplement with a lower dose of fortifier to lower the risk of interfering with breastfeeding. A larger amount of fortifier added to mother's milk (fresh or defrosted) may be possible while breastfeeding and should be considered until term or 2 months' CA to improve growth.

CONCLUSIONS

Fortification of mother's milk while breastfeeding very preterm infants after hospital discharge was possible and did not influence the duration of breastfeeding. However, fortification in the amount given in this study did not affect growth significantly at 1 year's CA. An increased amount of protein was associated with increased SUN values, indicating a better growth potential. The definition of optimal growth and nutrition of preterm infants is still a question of debate, and further investigations are needed.

ACKNOWLEDGMENTS

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REFERENCES

1. Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? *Pediatrics*. 2001;107(2):270–273
2. Schanler RJ. Post-discharge nutrition for the preterm infant. *Acta Paediatr Suppl*. 2005;94(449):68–73
3. Henriksen C, Westerberg AC, Rønnestad A, et al. Growth and nutrient intake among very-low-birth-weight infants fed fortified human milk during hospitalisation. *Br J Nutr*. 2009;102(8):1179–1186
4. Ehrenkranz RA, Younes N, Lemons JA, et al. Longitudinal growth of hospitalized very low birth weight infants. *Pediatrics*. 1999; 104(2 pt 1):280–289
5. Hack M, Weissman B, Borawski-Clark E. Catch-up growth during childhood among very low-birth-weight children. *Arch Pediatr Adolesc Med*. 1996;150(11):1122–1129
6. Knops NB, Sneeuw KC, Brand R, et al. Catch-up growth up to ten years of age in children born very preterm or with very low birth weight. *BMC Pediatr*. 2005;5:26
7. Dodrill P, Cleghorn G, Donovan T, Davies P. Growth patterns in preterm infants born

appropriate for gestational age. *J Paediatr Child Health*. 2008;44(6):332–337

8. Chan GM, Armstrong C, Moyer-Mileur L, Hoff C. Growth and bone mineralization in children born prematurely. *J Perinatol*. 2008; 28(9):619–623
9. Cooke RJ, Embleton ND, Griffin IJ, Wells JC, McCormick KP. Feeding preterm infants after hospital discharge: growth and development at 18 months of age. *Pediatr Res*. 2001; 49(5):719–722
10. Lucas A, Fewtrell MS, Morley R, et al. Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. *Pediatrics*. 2001;108(3): 703–711
11. Slykerman RF, Thompson JM, Becroft DM, et al. Breastfeeding and intelligence of preschool children. *Acta Paediatr*. 2005;94(7): 832–837
12. Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. *Am J Clin Nutr*. 1999;70(4): 525–535
13. Groh-Wargo S, Sapsford A. Enteral nutrition support of the preterm infant in the neonatal intensive care unit. *Nutr Clin Pract*. 2009; 24(3):363–376
14. Zachariassen G, Faerk J, Grytter C, Esberg B, Juvonen P, Halken S. Factors associated with successful establishment of breastfeeding in very preterm infants. *Acta Paediatr*. 2010;99(7):1000–1004
15. Danish National Centre For Social Research. Overview of the 5 social groups [In Danish]. Available at: www.sfi.dk/Default.aspx?ID=1179. Accessed February 2, 2010
16. Niklasson Albertsson-Wikland K. Continuous growth reference from 24th week of gestation to 24 months by gender. *BMC Pediatr*. 2008;8:8
17. Rabe-Hesketh S, Skrondal A. *Multilevel and Longitudinal Modeling Using Stata*. 2nd ed. College Station, TX: Stata Press; 2008
18. ESPGHAN Committee on Nutrition; Aggett PJ, Agostoni C, Axelsson I, et al. Feeding preterm infants after hospital discharge: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr*. 2006;42(5):596–603
19. Griffin IJ, Cooke RJ. Nutrition of preterm infants after hospital discharge. *J Pediatr Gastroenterol Nutr*. 2007;45(suppl 3): S195–S203
20. Márs al K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr*. 1996;85(7):843–848
21. World Health Organization. WHO child growth standards: methods and development—length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age. Available at: www.who.int/childgrowth/publications/technical_report_pub. Accessed December 1, 2010
22. Brandt I, Sticker EJ, Lentze MJ. Catch-up growth of head circumference of very low birth weight, small for gestational age preterm infants and mental development to adulthood. *J Pediatr*. 2003;142(5):463–468
23. Sauer PJ. Can extrauterine growth approximate intrauterine growth? Should it? *Am J Clin Nutr*. 2007;85(2):608S–613S
24. Chan GM. Growth and bone mineral status of discharged very low birth weight infants fed different formulas or human milk. *J Pediatr*. 1993;123(3):439–443
25. Carver JD, Wu PY, Hall RT, et al. Growth of preterm infants fed nutrient-enriched or term formula after hospital discharge. *Pediatrics*. 2001;107(4):683–689
26. Wheeler RE, Hall RT. Feeding of premature infant formula after hospital discharge of infants weighing less than 1800 grams at birth. *J Perinatol*. 1996;16(2 pt 1):111–116
27. Cooke RJ, Griffin IJ, McCormick K, Wells JC, Smith JS, Robinson SJ, Leighton M. Feeding preterm infants after hospital discharge: effect of dietary manipulation on nutrient intake and growth. *Pediatr Res*. 1998;43(3): 355–360
28. Heird WC. Determination of nutritional requirements in preterm infants, with special reference to "catch-up" growth. *Semin Neonatol*. 2001;6(5):365–375
29. Polberger SK, Axelsson IE, Räihä NC. Urinary and serum urea as indicators of protein metabolism in very low birthweight infants fed varying human milk protein intakes. *Acta Paediatr Scand*. 1990;79(8–9):737–742
30. Arslanoglu S, Moro GE, Ziegler EE. Adjustable fortification of human milk fed to preterm infants: does it make a difference? *J Perinatol*. 2006;26(10):614–621
31. Hall RT. Nutritional follow-up of the breastfeeding premature infant after hospital discharge. *Pediatr Clin North Am*. 2001;48(2): 453–460
32. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. *Pediatrics*. 2006;117(4):1253–1261
33. Lapillonne A, Salle BL, Glorieux FH, Claris O. Bone mineralization and growth are enhanced in preterm infants fed an isocaloric, nutrient-enriched preterm formula through term. *Am J Clin Nutr*. 2004;80(6): 1595–1603
34. Latal-Hajnal B, von Siebenthal K, Kovari H, Bucher HU, Largo RH. Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. *J Pediatr*. 2003;143(2):163–170
35. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet*. 2003;361(9363):1089–1097
36. Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. *Lancet*. 2001;357(9254):413–419
37. Singhal A. Early nutrition and long-term cardiovascular health. *Nutr Rev*. 2006;64(5 pt 2):S44–S49
38. Koletzko B. Long-term consequences of early feeding on later obesity risk. *Nestle Nutr Workshop Ser Pediatr Program*. 2006; 58:1–18
39. O'Connor DL, Khan S, Weishuhn K, et al. Growth and nutrient intakes of human milk-fed preterm infants provided with extra energy and nutrients after hospital discharge. *Pediatrics*. 2008;121(4):766–776
40. Aimone A, Rovet J, Ward W, et al. Growth and body composition of human milk-fed premature infants provided with extra energy and nutrients early after hospital discharge: 1-year follow-up. *J Pediatr Gastroenterol Nutr*. 2009;49(4):456–466
41. Behrman RE, Kliegman RM, Jenson HB. *Nelson Textbook of Pediatrics*. 16th ed. Philadelphia, PA: WB Saunders Company; 2000

14. Paper III.

Allergic diseases among very preterm infants according to nutrition after hospital discharge

Allergic diseases among very preterm infants according to nutrition after hospital discharge

Authors:

Gitte Zachariassen[1], Jan Faerk[2], Birgitte H Esberg[3], Fenger-Gron J[4], Sven Mortensen[1], Henrik T Christesen[1], Susanne Halken[1].

1. Hans Christian Andersen Children's Hospital, Odense University Hospital
2. Paediatric Department, Holbaek Hospital.
3. Paediatric Department, Skejby, Aarhus University Hospital.
4. Paediatric Department, Kolding Hospital.

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ORIGINAL ARTICLE

Allergic diseases among very preterm infants according to nutrition after hospital discharge

Gitte Zachariassen[1], Jan Faerk[2], Birgitte H. Esberg[3], Jesper Fenger-Gron[4], Sven Mortensen[1], Henrik T. Christesen[1] and Susanne Halken[1]

[1]Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense C, Denmark, [2]Pediatric Department, Holbaek Hospital, Holbaek, Denmark, [3]Pediatric Department, Skejby, Aarhus University Hospital, Aarhus N, Denmark, [4]Pediatric Department, Kolding Hospital, Kolding, Denmark

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Correspondence
Gitte Zachariassen, Hans Christian Andersen Children's Hospital, Odense University Hospital, DK - 5000 Odense C, Denmark
Tel.: +4565412117
Fax: +4566119162
E-mail: Gitte.Zachariassen@ouh.regionsyddanmark.dk

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To determine whether a cow's milk-based human milk fortifier (HMF) added to mother's milk while breastfeeding or a cow's milk-based preterm formula compared to exclusively mother's milk after hospital discharge, increases the incidence of developing allergic diseases among very preterm infants (VPI) during the first year of life.

Of a cohort of 324 VPI (gestational age 24–32 wk), the exclusively breastfed VPI were shortly before discharge randomized to breastfeeding without fortification or supplementing with a fortifier. Those not breastfed were fed a preterm formula. The intervention period was from discharge until 4 months corrected age (CA). Follow-up was performed at 4 and 12 months CA including specific IgE to a panel of allergens at 4 months CA.

The incidence during and prevalence at 12 months CA of recurrent wheezing (RW) was 39.2% and 32.7%, while atopic dermatitis (AD) was 18.0% and 12.1%, respectively. Predisposition to allergic disease increased the risk of developing AD ($p = 0.04$) [OR 2.6 (95% CI 1.0–6.4)] and the risk of developing RW ($p = 0.02$) [OR 2.7 (95% CI 1.2–6.3)]. Boys had an increased risk of developing RW ($p = 0.003$) [OR 3.1 (95% CI 1.5–6.5)]. No difference was found between nutrition groups. None developed food allergy.

Compared to exclusively breastfed, VPI supplemented with HMF or fed exclusively a preterm formula for 4 months did not have an increased risk of developing allergic diseases during the first year of life.

The expression of allergic diseases varies with age, and symptoms may disappear and be replaced by other symptoms. In infancy, the main atopic/allergic symptoms are atopic dermatitis (AD), gastrointestinal symptoms, and recurrent wheezing (RW), whereas bronchial asthma and allergic rhino-conjunctivitis are the main problems later in childhood. Adverse reactions to food, mainly cow's milk protein (CMP), are most common in the first year of life, whereas allergy to inhalant allergens mostly occurs later. A variety of factors are known to influence the risk of allergic disease, such as atopic predisposition, exposure to allergens (e.g., cow's milk and egg), and environmental factors (1). It is also well known that the mode of early feeding influences the risk of food allergy and that breastfeeding is associated with a lower frequency of AD, RW, and cow's milk protein allergy (CMPA) compared with cow's milk formula feeding (1–3).

Human milk is the best nutrition also for preterm infants, but it may lead to insufficient intake of protein and energy. The use of fortified human milk produces adequate growth and satisfies the specific nutritional requirements of preterm infants (4). Cow's milk-based fortifiers have been used for years in most neonatal intensive care units for nutrient enrichment/fortification of human milk, e.g. Enfamil® human milk fortifier (HMF) was introduced in 1984 and was

Abbreviations:
HMF,; human milk fortifier; PF,; preterm formula; VPI,; very preterm infants; GA,; gestational age in weeks; BW,; birth weight; SGA,; small for gestational age; PMA,; post-menstrual age; CA,; corrected age; AD,; atopic dermatitis; RW,; recurrent wheezing; CMPA,; cow's milk protein allergy; CMP,; cow's milk protein; SPT,; skin prick test; ITT,; intension to treat; PP,; per protocol.

reformulated with a higher protein level in 2002 (5). Preterm infants are usually discharged from hospital care early and with a body weight below the usual birth weight (BW) of a healthy term [40-wk post-menstrual age (PMA)] infant. Close monitoring of growth is recommended, and supplementation should be considered if the preterm infant is discharged with subnormal weight for age (6, 7). Meanwhile, the optimal nutrition to meet the needs of a growing preterm infant after hospital discharge, and at the same time to avoid possible negative health effects such as developing e.g., allergic diseases, still needs to be evaluated. As a part of a randomized controlled trial (RCT) on nutrient enrichment of mother's milk while breastfeeding after hospital discharge, we investigated whether there was an increased incidence of allergic disease among VPI supplemented with a cow's milk-based HMF or a preterm formula compared to exclusively breastfeeding after hospital discharge.

Patients and methods

In a prospective RCT on nutrition and growth after hospital discharge, VPI with a gestational age (GA) ≤ 32.0 wk were recruited consecutively from four neonatal intensive care units in Denmark (Holbaek, Kolding, Hans Christian Andersen Children's Hospital, Odense and Skejby, Aarhus). Exclusion criteria were diseases or circumstances influencing eating- and feeding ability at discharge. In case of exclusion from the RCT because of CMPA or parent's refusal of participation because of predisposition to allergic diseases, these VPI were kept in this study for follow-up with parent's acceptance.

Feeding regimens were identical at the four pediatric departments and are described in details in (8). Fortification with HMF (cow's milk based) was initiated from day 10–14 from birth for all VPI and fortification of mother's own expressed milk was performed until discharge, but with decreasing amounts during the last week(s) while the infant was improving sucking directly from the breast.

Shortly before hospital discharge, the breastfed VPI in the RCT were randomized to either breastfeeding without supplementation (group A) or intervention with fortification (five packets Enfamil® Human Milk Fortifier (HMF); Mead Johnson Nutritional, Evansville IN, USA) of mother's milk daily until 4 months corrected age (CA) (group B). VPI who were bottlefed were fed a preterm formula (Enfalac® Preterm Formula; Mead Johnson Nutritionals, Njimegen, Netherlands) (group C) until 4 months CA. For all groups, parents were instructed to refrain from introducing any complementary food until 4 months CA.

BW, GA, single birth, or multiple births were recorded. Based on interviews and questionnaires at the time of randomization, information on mother's age, education, and occupation, smoking habits, atopic predisposition (at least one-first-degree relative with reported allergic disease), and previous breastfeeding experience were obtained. Mother's social group was defined according to The Danish National Centre of Social Research based on education and occupation (9). Infants were examined at the outpatient clinics at term, 2, 4, 6, and 12 months CA. At 4 and 12 months of age, a standardized questionnaire-based interview about possible allergic symptoms such as; urticaria, AD (areas of scaly, erythematous, and itchy eczematous rash revealed by physical examination), gastrointestinal symptoms (colic, diarrhea, or vomiting without known infection), episodes of RW (at least two episodes of wheezing, most often associated with respiratory infections, and diagnosed by a physician), and rhinitis/conjunctivitis, and treatment was performed by a pediatric nurse or doctor. CMPA was proven by controlled elimination/challenge test in a hospital setting. At 4 months CA, a blood sample was drawn for later analysis for specific IgE antibodies (egg white, milk, peanut, dust mites (Dermatophagoides Pteronyssinus and Farinae), dog, cat, grass pollen, and latex) by ImmunoCAP 250 (Pharmacia, detection limit 0.35 kU/l). All blood samples were analyzed at Odense University Hospital. Data on duration of breastfeeding and introduction of formula and/or complementary food were recorded.

Ethics

The study was approved by the Danish National Committee on Biomedical Research Ethics (J.nr. VF20030208) and the registrations were approved by the Danish Data Protection Agency (J.nr.2007-41-1349). Informed consent was obtained from the parents after written and oral information.

Statistics

Data were analyzed using STATA (version 11, Statacorp, College Station, TX, USA).

The incidence is the percentage of new cases of VPI with allergic symptoms from birth to 12 months CA. The prevalence is the percentage of VPI having allergic symptoms at 12 months CA. The prevalence is corrected for missing data at 12 months CA.

Multivariate logistic regression was used to determine which clinical relevant factors (GA, BW, gender, atopic predisposition, nutrition, time of introduction to complementary food, mother's age, social group, parents smoking at home) possibly influenced the development of AD and/or RW before 12 months CA. The preterm infants were defined as SGA if the BW was below the expected weight by more than 2 SD according to a reference (10). Analyses were performed by intention to treat (ITT) and treated per protocol (PP).

Results

The study cohort consisted of 324 (51%) VPI, as parents of 156 (25%) refused to participate and 153 (24%) were excluded of an initial cohort of 633 VPI (Fig. 1). In the refusal group, VPI had a significant higher GA, BW, and younger mothers compared to the study cohort ($p < 0.05$), and 50% were breastfed at discharge while 65% were breastfed in the study cohort ($p = 0.002$). Characteristics of the 324 participating infants and their parents are shown in Table 1.

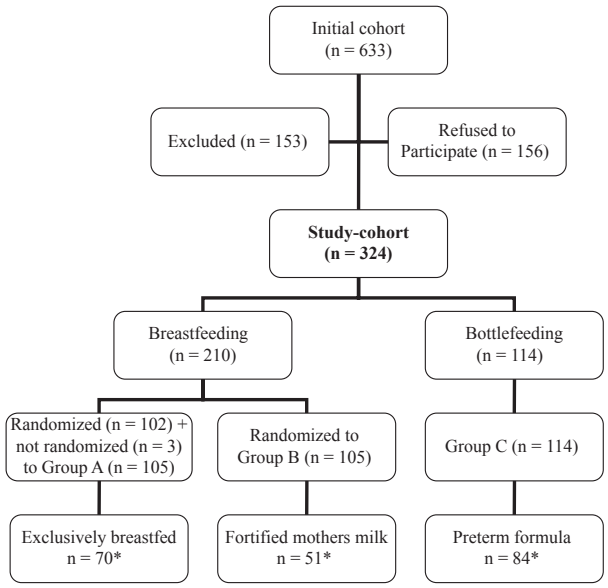


Figure 1 Participation flowchart. Treated per protocol (*) (n = 205). By ITT 283/324 remained in the study at 12 months corrected age.

Three breastfed VPI (a couple of twin-boys (GA 31.6) and a girl (GA 32.0)) were not randomized because of atopic predisposition and parent's request. None of them received HMF during their hospitalizations.

One boy (GA 29.4) with AD from 3 months CA was suspicious of CMPA after hospital discharge. He received HMF added to mother's own milk or donor milk during hospitalization and was bottlefed with preterm formula after hospital discharge. A skin prick test (SPT) was positive (4 mm), while specific IgE was <0.35 KIU/l to milk and egg, respectively. Controlled elimination/challenge procedure with milk was negative and thus the suspicion to CMPA was not confirmed. There was no clinical suspicion on allergy to egg.

Table 1 Characteristics of 324 preterm infants and their mothers

Preterm infants	
GA at birth (median) (min–max) (wk)	29 + 6 (24.1–32.0)
Birth weight (median) (min–max) (g)	1283 (535–2255 g)
SGA (weight *z*-score < 2 SD)	68/324
Boys	178/324
Multiple births	122/324
Predisposition to allergic disease	63/253
Introduction to complementary food ± SD (mean) (corrected age) (wk)	17.3 ± 4.0 wk
Mothers	
Mother's age ± SD (mean) (yr)	30.7 ± 4.9
Social group ± SD (mean) (1 = high, 2, 3, 4 and 5 = low)	3.14 ± 1.31
Very preterm infants with parents (one or two) smoking at home	116/319 (36%)
Mothers smoking	61/319 (19%)
Fathers smoking	94/294 (32%)

Information on allergic symptoms was obtained in 90% (290/324) VPI. Because of withdrawal of 7 VPI before 12 months CA, there was 283 VPI remaining at 12 months CA (ITT analysis). An overall incidence and prevalence (corrected for missing data from 4 VPI) of allergic symptoms at 12 months CA among those 283 VPI is shown in Table 2.

Information on atopic predisposition was obtained from 78% (253/324) with 25% (63/253) predisposed to allergic diseases. The number of VPI who completed in their assigned nutrition groups until 2 months CA was 205 (63%) with 199 (61%) remaining at 12 months CA (PP-group). Comparing nutrition groups (A, B, and C), no difference was found between nutrition groups as regards development of AD or RW during the first year of life.

Atopic predisposition was associated with the development of AD (ITT and PP), while gender (ITT and PP) and atopic predisposition (PP) were associated with RW using multiple regression models (Table 3). Analyzing only breastfed VPI (A plus B) early introduction to complementary feeding (16.0 ± 3.5 vs. 17.8 ± 3.7 wk) was associated with development of RW ($p = 0.006$) (OR 0.97 (95% CI 0.95–0.99)).

Specific IgE were analyzed in blood-samples from only 51% (163/320) VPI at 4 months CA. In 2 VPI, specific IgE to cow's milk was detected: In a girl (GA 31.0), specific IgE to milk was 0.38 KIU/l. She received HMF added to donor milk and mother's milk during hospitalization. She was exclusively breastfed at discharge and supplemented with mature formula and complementary food shortly before 4 months CA. She had two episodes with wheezing treated with bronchodilatator at 9 and 11 months CA and no other allergic symptoms were reported. In a boy (GA 29.6), specific IgE to milk was 0.36 KIU/l. He also received HMF added to human milk during hospitalization. He was bottlefed with preterm formula from hospital discharge until 4 months CA. He did not show any allergic symptoms before 12 months CA.

Discussion

Allergic symptoms in this age group are often unspecific and only seldom represent a true allergic disease, and the most common allergic symptoms are AD and RW. When these conditions are caused by allergy, it will most often be a food allergy e.g., to milk or egg. We found an overall incidence of possible allergic symptoms among VPI within 12 months CA to be 56.2% (159/283), while the prevalence at 12 months CA was 41.8%, most of these being RW, probably because of viral infections and small airways.

Predisposition to allergic disease and being a boy increased the risk of developing AD and RW within the first 12 months CA. Nutrition with or without CMP after hospital discharge was not associated with development of any allergic symptoms.

Studies describing allergic symptoms and studies on dietary prevention among preterm infants are very few. By the age of 18 months CA, Lucas et al. found an overall incidence of one or more allergic symptoms among 777 preterm infants (BW < 1850 g) to be 44% (wheezing 22.5% and eczema/AD

Table 2 The incidence [rate of occurrence of new cases from birth to 12 months corrected age (CA)], the median age of onset of allergic symptoms, and the prevalence (rate of cases at 12 months CA) among 283 very preterm infants (VPI)

Allergic symptoms	No of cases	Incidence (%) (birth to 12 months CA)	Median corrected age of onset of symptoms (months) (min–max)	Prevalence (%) (cases at 12 months CA)¶
Urticaria	7	2.5	7.2 (0.8 to 9.3)	0.0
Atopic dermatitis Total	51	18.0	6.1 (–2.6 to 11.9)	12.1
Treated with steroids	8	2.8	6.7 (0.2 to 11.9)	–
Treatment unknown	43	15.2	6.1 (–2.6 to 11.9)	–
Gastrointestinal symptoms	38	13.4	1.0 (–3.3 to 6.8)	1.4
Recurrent wheezing Total	111	39.2	7.1 (–0.4 to 12.0)	32.7
Treated with bronchodilatators (*)	7 (oral) 23 (inh.)	10.6	8.0 (1.9 to 11.9)	–
Treated with inhaled glucocorticosteroids†‡	31	11.0	6.1 (–0.4 to 11.1)	–
Treatment unknown	50	17.7	7.3 (–0.2 to 12.0)	–
Rhinitis§	27	9.5	2.1 (–2.7 to 11.8)	3.7
Conjunctivitis§	10	3.5	5.8 (–0.2 to 11.1)	1.8
One or more of the above symptoms	159	56.2	–	41.8

Treatment with; *beta-2-agonist oral or inhaled, †inhaled glucocorticosteroids, ‡2 VPI were also treated with leukotriene receptor antagonists. §One child was treated with oral antihistamine (Clarityn®). ¶corrected for missing data from 4 VPI at 12 months CA.

Table 3 Variables associated with development of atopic dermatitis and recurrent wheezing

			By multiple regression model	
			p-value	Odds Ratio (95% CI)
By intension to treat (n = 219)				
Atopic dermatitis (AD)	+AD	–AD		
Atopic predisposition	16/41	37/182	0.03	2.3 (1.1–5.0)
Recurrent wheezing (RW)	+RW	–RW		
Boys	76/111	80/172	0.000	3.3 (1.8–6.1)
Treated per protocol (n = 155)				
Atopic dermatitis (AD)	+AD	–AD		
Atopic predisposition	12/30	28/129	0.04	2.6 (1.0–6.4)
Recurrent wheezing (RW)	+RW	–RW		
Atopic predisposition	19/55	21/104	0.02	2.7 (1.2–6.3)
Boys	49/72	61/127	0.003	3.1 (1.5–6.5)

19.4%). They found atopic predisposition, maternal smoking, vaginal delivery and duration of ventilation to be associated with wheezing, and multiple births to be associated with AD (11). The same study-population was used for two randomized trials (A: banked donor milk vs. preterm formula and B: term vs. preterm formula) and found that CMP intake was not associated with an overall risk of developing allergic disease, but in the subgroup with atopic predisposition, early exposure to CMP increased the risk of developing allergic diseases – especially AD and CMPA (12).

Chronic respiratory morbidity has been described as common following preterm birth, particular if complicated by bronchopulmonary dysplasia (BPD) (13, 14). In our study, VPI with BPD have been excluded from the RCT. A recent but solely register- and population-based study on 'administrative claims data' found that preterm infants (born ≤32 wk) had a higher prevalence of persisting asthma later in childhood (11.7%) compared with term births (8%) [OR 1.51 (95% CI 1.40–1.63)] (15). A study from New Zealand based on parental reports at 12 months of age found an incidence of RW among very preterm infants (VPI) (< 33 wk) to be 14.5% and among term infants to be 3%. They found significant risk factors to be parental history of asthma, maternal smoking, siblings at home, neonatal oxygen supplementation at 28, 36, and 40 wk of gestation (16). A Swedish birth cohort (on preterm and term infants) study found a cumulative incidence on RW (doctors diagnosed and any wheezing) to be 27% up to 2 yr of age (17). The incidence and prevalence of RW up to 1 yr of age among 'healthy VPI' were 39.2% and 32.7%, respectively, in our study, indicating that preterm birth might increase the risk of RW even though preterm infants with BPD were excluded from our study.

A population-based birth cohort study among term infants in Denmark found an incidence of AD to be 11% (18) and the cumulative 1-yr prevalence to be 8.2% (19). An association between AD and being a boy and predisposed to atopy (maternal) was found (18). In a large Norwegian prospective cohort study, based on clinical examinations if parents reported allergic symptoms, they found a prevalence of AD at 1 yr of life to be 13% among preterm infants (GA 28–35 wk) and 10% among term infants. There was no significant difference between preterm and term infants (20). A much higher prevalence of AD at 12 months post-term of 35.8% was found in a British prospective study among preterm infants (GA ≤ 37 wk) based on questionnaires and home-visits by midwifes (21). Our study is also a population-based birth cohort of preterm infants, and the incidence and

prevalence of AD in our study were 18% and 12.1%, respectively, and seem to correlate very well with the results of the Norwegian study.

The main allergic symptoms reported in our study are RW and AD. Our results are based on standardized questionnaire-based interviews performed by the doctors and/or nurses when the VPI attended the out-patient clinics for follow-up examination at 4 and 12 months CA. Allergic gastrointestinal symptoms have been reported with an incidence of 13.4% but a prevalence of 1.4% at 1 yr of age. Symptoms from the gastrointestinal tract are unspecific and are difficult to interpret among preterm infants. A Norwegian study (22) including preterm and term infants diagnosed 27 (9 preterm) of 555 with adverse reactions to cow's milk such as pain behavior, gastrointestinal symptoms (excessive vomiting or diarrhea), respiratory symptoms, and AD. Twenty-six of these infants were diagnosed by elimination/challenge tests (all but one were exclusively breastfed). Non-IgE-mediated reactions were the most frequent and just one child with AD as the presenting symptom, had a positive SPT as well as elevated IgE level to cow's milk. No difference was found between preterm and term infants. In our study, 38 of 283 reported gastrointestinal symptoms but none had food allergy.

The allergic symptoms urticaria, rhinitis, and conjunctivitis are also difficult to interpret among preterm infants. Two children suspicious of these allergic diseases showed no sensitization at SPT and the specific IgE analysis.

In our study, 24% VPI were excluded and parents of 25% VPI refused to participate. Other studies dealing with VPI have shown similar problems with exclusion and refusals (23). The parents have often experienced their VPI critically ill and they do not have resources for participation in a study also. At 4 months CA, only 51% VPI had blood drawn for specific IgE analysis, because many parents did not want their infant to experience a *veno*-puncture. A Norwegian study found one child with elevated IgE levels to both egg and milk and one child with only elevated IgE levels to egg (22). In our study, one child was suspicious of CMPA because of AD and a positive SPT, but CMPA was not confirmed by elimination/challenge test, and further two VPI (2/163) had positive IgE reactions to cow's milk but none of them had CMPA.

Breastfeeding possibly influences the occurrence of asthma and allergic disease through different mechanisms. The infant gut is immature and may poorly exclude multiple allergens or large quantities of allergens that can react with the immune system. Human milk contains substances that may influence the micro flora in the gut and possibly protect the intestinal mucosa. Cytokines/growth factors in human milk also may promote the maturation of gastrointestinal mucosa restricting the penetration of antigenic material and contribute to the anti-inflammatory effect of human milk (24). Especially preterm infants with an even more immature intestine might have an increased risk of absorption to food allergens and development of food allergy (such as CMPA). Cases of three preterm infants, who presented signs suggestive of sensitization to CMP after supplementation of their mother's milk with a HMF, have been reported. The diagnoses were based on clinical response to elimination of the allergen from the diet only (25). In our study, 321 VPI received a cow's milk-based HMF during hospitalization, and only one was suspected but not confirmed to have food allergy after hospital discharge while he was fed a cow's milk-based preterm formula. Multicomponent fortification of human milk for preterm infants is associated with improvements in growth, and support the use of fortification as a common practice in neonatal intensive care units (26, 27). In our study, fortification was used also after hospital discharge and did not increase the risk of developing allergic diseases among VPI during the first year of life.

Conclusion

Cow's milk-based fortification of mother's milk or preterm formula for preterm infants after hospital discharge was not associated with an increased risk of developing allergic diseases until 1 yr of age among VPI. Predisposition to allergic disease and being a boy increased the risk of developing both AD and RW. No one was diagnosed with CMPA. Compared to other studies of preterm infants, wheezing was reported with a high incidence and prevalence, while AD was reported with an incidence and prevalence comparable with other studies. The infants in our study will be followed according to allergic symptoms until 6 yr of age.

References

1. Halken S. Prevention of allergic disease in childhood: clinical and epidemiological aspects of primary and secondary allergy prevention. *Pediatr Allergy Immunol* 2004: **15** (Suppl. 16): 4–32.
2. Muraro A, Dreborg S, Halken S, et al. Dietary prevention of allergic diseases in infants and small children. Part III: Critical review of published peer-reviewed observational and interventional studies and final recommendations. *Pediatr Allergy Immunol* 2004: **15**: 291–307.
3. Host A, Halken S. Primary prevention of food allergy in infants who are at risk. *Curr Opin Allergy Clin Immunol* 2005: **5**: 255–9.
4. Reali A, Greco F, Fanaro S, et al. Fortification of maternal milk for very low birth weight (VLBW) pre-term neonates. *Early Hum Dev* 2010: **86**: 533–6.
5. Mead Johnson Nutritionals. History Enfamil Human Milk Fortifier. http://www.mead-johnson.com Latest access date. 2010 Aug 21.
6. Aggett PJ, Agostoni C, Axelsson I, et al. Feeding preterm infants after hospital discharge: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2006: **42**: 596–603.
7. Griffin IJ, Cooke RJ. Nutrition of preterm infants after hospital discharge. *J Pediatr Gastroenterol Nutr* 2007: **45** (Suppl. 3): S195–203.
8. Zachariassen G, Faerk J, Grytter C, Esberg B, Juvonen P, Halken S. Factors associated with successful establishment of breastfeeding in very preterm infants. *Acta Paediatr* 2010: **99**: 1000–4.
9. The Danish National Centre For Social Research. Oversigt over de 5 socialgrupper. Available at: http://www.sfi.dk Latest access date. 2010 Feb 2.

10. Niklasson A, bertsson-Wikland K. Continuous growth reference from 24th week of gestation to 24 months by gender. *BMC Pediatr* 2008: **8**: 8.
11. Lucas A, Brooke OG, Cole TJ, Morley R, Bamford MF. Food and drug reactions, wheezing, and eczema in preterm infants. *Arch Dis Child* 1990: **65**: 411–5.
12. Lucas A, Brooke OG, Morley R, Cole TJ, Bamford MF. Early diet of preterm infants and development of allergic or atopic disease: randomised prospective study. *BMJ* 1990: **300**: 837–40.
13. Greenough A. Long-term pulmonary outcome in the preterm infant. *Neonatology* 2008: **93**: 324–7.
14. Greenough A. Late respiratory outcomes after preterm birth. *Early Hum Dev* 2007: **83**: 785–8.
15. Dombkowski KJ, Leung SW, Gurney JG. Prematurity as a predictor of childhood asthma among low-income children. *Ann Epidemiol* 2008: **18**: 290–7.
16. Elder DE, Hagan R, Evans SF, Benninger HR, French NP. Recurrent wheezing in very preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1996: **74**: F165–71.
17. Lannero E, Wickman M, Pershagen G, Nordvall L. Maternal smoking during pregnancy increases the risk of recurrent wheezing during the first years of life (BAMSE). *Respir Res* 2006: **7**: 3.
18. Johnke H, Norberg LA, Vach W, Host A, Andersen KE. Patterns of sensitization in infants and its relation to atopic dermatitis. *Pediatr Allergy Immunol* 2006: **17**: 591–600.
19. Johnke H, Vach W, Norberg LA, Bindslev-Jensen C, Host A, Andersen KE. A comparison between criteria for diagnosing atopic eczema in infants. *Br J Dermatol* 2005: **153**: 352–8.
20. Kvenshagen B, Jacobsen M, Halvorsen R. Atopic dermatitis in premature and term children. *Arch Dis Child* 2009: **94**: 202–5.
21. Morgan J, Williams P, Norris F, Williams CM, Larkin M, Hampton S. Eczema and early solid feeding in preterm infants. *Arch Dis Child* 2004: **89**: 309–14.
22. Kvenshagen B, Halvorsen R, Jacobsen M. Adverse reactions to milk in infants. *Acta Paediatr* 2008: **97**: 196–200.
23. O'Connor DL, Khan S, Weishuhn K, et al. Growth and nutrient intakes of human milk-fed preterm infants provided with extra energy and nutrients after hospital discharge. *Pediatrics* 2008: **121**: 766–76.
24. Oddy WH. The long-term effects of breastfeeding on asthma and atopic disease. *Adv Exp Med Biol* 2009: **639**: 237–51.
25. Vlieghe V, Roches AD, Payot A, Lachance C, Nuyt AM. Human milk fortifier in preterm babies: source of cow's milk protein sensitization? *Allergy* 2009: **64**: 1690–1.
26. Kuschel CA, Harding JE Multicomponent fortified human milk for promoting growth in preterm infants. *Cochrane Database Syst Rev* 2004;CD000343.
27. Maggio L, Costa S, Gallini F. Human milk fortifiers in very low birth weight infants. *Early Hum Dev* 2009;**85**(10 Suppl.):S59–61.

Appendix 1. Nutritional products for preterm infants

Composition of Enfamil Human Milk Fortifier and Enfalac Premature Formula on the following pages

Comparison chart of human milk, human milk fortifier, and formulas for post discharge nutrition of preterm infants.

	Human milk (Mean 8 weeks after preterm birth)	Enfamil ® Human Milk Fortifier (MJN) 1 packet	Human milk and 1 packet Enfamil (*)	Enfalac ® Preterm Formula (MJN)	PreNAN Discharge ® (Nestlé) (#)	Enfamil EnfaCare ® Discharge (MJN in US only)	Term Formula (e.g. Allomin 1 ®, Semper) (&)
Energy KJ and Kcal /100 ml	273 and 65.3	14.8 and 3.5	287.8 and 68.8	280 and 68	310 and 75	309 and 74	287 and 66
Protein g/100 ml	1.3	0.3	1.6	2.0	2.0	2.1	1.4
Carbohydrate g/100 ml	7.0	<0.1	~ 7.0	7.4	7.7	7,8	7.2
Fat g/100 ml DHA/ARA (¤)	3.5 +/+	0.3 +/+	3.8 +/+	3.4 +/+	3.8 +/+	4.0 +/+	3.5 +/+
Calcium mg/100 ml	?	22.5	?	81	80	90	50
Phosphor mg/100 ml	?	12.5	?	44	48	50	30

(*) Assuming the infant gets 500 ml of mothers milk and 5 packets of Enfamil during 24 hours (5 packets/500ml human milk = 1 packet/100ml human milk). (#) Partially hydrolyzed and with probiotics. (&) With prebiotics. (¤) Long-chain polyunsaturated fatty acids: DHA= docosahexaenoic acid and ARA= arachidonic acid.

Enfamil HMF

Ingredienser: Vegetabiliska oljor (MCT, soja (genetiskt modifierad)), natriumkaseinat, hydrolyserat vassleprotein, emulgeringsmedel sojalecitin (genetiskt modifierat), **mineraler** (kalciumfosfat, kalciumglycerolfosfat, kalciumglukonat, natriumcitrat, kaliumklorid, kaliumcitrat, kaliumfosfat, järnsulfat, zinksulfat, magnesiumfosfat, kopparsulfat), **vitaminer** (askorbinsyra, DL-alfa-tokoferylacetat, niacinamid, retynylpalmitat, kalciumpantotenat, riboflavin, tiaminhydroklorid, pyridoxinhydroklorid, folsyra, fyllokinon, kolekalciferol, biotin, cyanokobalamin), majssirap (genetiskt modifierad).

Näringsdeklaration: Normalt tillsätts 4 portionsförpackningar (2,84 g) Enfamil HMF till 100 ml bröstmjölk varvid bröstmjölken förstärks med:		
Energi	kJ/Kcal	59/14
Protein	g	1,1
Kolhydrater	g	<0,4
...varav laktos	g	<0,01
Fett	g	1
...varav linolsyra	mg	140
...varav α-linolensyra	mg	17
Vitamin A	µg RE	290
Vitamin D	µg	3,8
Vitamin K	µg	4,4
Vitamin C	mg	12
Tiamin	mg	0,15
Riboflavin	mg	0,22
Niacin	mg NE	3,0
Vitamin B_6	mg	0,12
Folacin	µg	25
Pantotensyra	mg	0,73
Vitamin B_{12}	µg	0,18
Biotin	µg	2,7
Vitamin E	mg α-TE	3,1
Natrium	mg	16
Klorid	mg	13
Kalium	mg	29
Kalcium	mg	90
Fosfor	mg	50
Magnesium	mg	1
Järn	mg	1,44
Zink	mg	0,72
Koppar	µg	44
Mangan	µg	10
Osmolalitet	35	mOsm/kg

Enfalac Premature

Ingredienser: Glukossirup, vegetabiliska oljor (MCT, soja, solros, kokosnöt), skummjölkspulver, vassleprotein, laktos, "single cell-olja"*(AA från Mortierella alpine och DHA från Crypthecodinium cohnii), **mineraler** (kalcium, fosfat, natrium, magnesium, klorid, järn, zink, koppar, jod), emulgeringsmedel sojalecitin, **vitaminer** (E, niacin, C, pantotensyra, A, B_1, B_2, B_6, folsyra, K_1, D_3, biotin, B_{12}), inositol, kolin, L-karnitin, antioxidationsmedel (natriumaskorbat, askorbylpalmitat).
*olja med arakidonsyra (AA) och dokosahexaensyra (DHA) producerad av encelliga organismer.

Näringsdeklaration			
Per 100 ml färdig lösning		68 kcal	81 kcal
Energi	kJ/kcal	280/68	340/81
Protein	g	2	2,4
Kolhydrater	g	7,4	8,9
Fett	g	3,4	4,1
…varav linolsyra	g	0,62	0,74
…varav α–linolensyra	mg	74	89
…varav arakidonsyra	mg	23	28
…varav dokosahexaensyra	mg	11,5	13,7
…varav MCT	g	1,3	1,6
Vitamin A	µg RE	104	124
Vitamin D	µg	1,7	2
Vitamin K	µg	5,4	6,5
Vitamin C	mg	13,5	16,2
Tiamin	mg	0,14	0,16
Riboflavin	mg	0,2	0,24
Vitamin B_6	mg	0,1	0,12
Niacin	mg NE	2,7	3,2
Folacin	µg	27	32
Vitamin B_{12}	µg	0,17	0,2
Pantotensyra	mg	0,81	0,97
Biotin	µg	2,7	3,2
Vitamin E	mg α-TE	2,9	3,4
Natrium	mg	39	46
Klorid	mg	58	69
Kalium	mg	68	81
Kalcium	mg	81	97
Fosfor	mg	44	53
Magnesium	mg	6,1	7,3
Järn	mg	1,2	1,4
Zink	mg	0,68	0,81
Koppar	µg	71	85
Jod	µg	17	20
Selen	µg	0,74	0,89
Mangan	µg	4,3	5,1
Kolin	mg	12,2	14,6
Inositol	mg	30	36
Taurin	mg	4,1	4,9
Karnitin	mg	1,4	1,6
Osmolaritet	mOsm/l	227	273

Appendix 2. Results using random effect models on growth (tables)

Comparing nutrition groups for Weight differences.

Explanation and calculation of weight-difference on the next page.

Random effect model (ITT).

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	-0.04	-0.64	0.57	0.91	
	252d	0.12	-0.47	0.70	0.70	
	280d	0.40	-0.23	1.03	0.21	
	2m	0.93	0.05	1.81	0.04	*
	4m	1.33	0.21	2.44	0.02	*
	6m	1.58	0.24	2.92	0.02	*
	12m	1.52	-0.59	3.64	0.16	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	0.31	-0.31	0.93	0.32	
	252d	0.26	-0.34	0.86	0.40	
	280d	0.23	-0.42	0.88	0.49	
	2m	0.48	-0.43	1.39	0.30	
	4m	1.00	-0.16	2.16	0.09	
	6m	1.64	0.24	3.03	0.02	*
	12m	2.99	0.79	5.19	0.008	*

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Girls	238d	0.35	-0.24	0.94	0.25	
	252d	0.14	-0.43	0.71	0.63	
	280d	-0.17	-0.79	0.45	0.59	
	2m	-0.45	-1.31	0.41	0.31	
	4m	-0.32	-1.43	0.78	0.57	
	6m	0.05	-1.28	1.39	0.94	
	12m	1.47	-0.62	3.56	0.17	

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	-0.11	-0.68	0.47	0.72	
	252d	0.03	-0.51	0.58	0.91	
	280d	0.25	-0.35	0.85	0.41	
	2m	0.54	-0.30	1.38	0.21	
	4m	0.62	-0.44	1.67	0.25	
	6m	0.57	-0.70	1.83	0.38	
	12m	0.17	-1.85	2.19	0.87	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	0.30	-0.24	0.84	0.27	
	252d	0.42	-0.09	0.94	0.11	
	280d	0.68	0.12	1.24	0.02	*
	2m	1.26	0.47	2.05	0.002	*
	4m	1.80	0.80	2.80	0.000	*
	6m	2.24	1.04	3.44	0.000	*
	12m	2.51	0.61	4.41	0.01	*

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Boys	238d	0.40	-0.15	0.96	0.15	
	252d	0.39	-0.14	0.92	0.15	
	280d	0.43	-0.16	1.01	0.15	
	2m	0.72	-0.09	1.54	0.08	
	4m	1.18	0.15	2.21	0.02	*
	6m	1.67	0.44	2.90	0.008	*
	12m	2.33	0.38	4.29	0.02	*

Random effect model (PP).

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	-0.08	-0.90	0.73	0.84	
	252d	0.11	-0.67	0.89	0.78	
	280d	0.45	-0.40	1.29	0.30	
	2m	0.94	-0.23	2.11	0.12	
	4m	1.16	-0.30	2.62	0.12	
	6m	1.16	-0.58	2.90	0.19	
	12m	0.41	-2.34	3.16	0.77	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	0.46	-0.29	1.21	0.23	
	252d	0.42	-0.30	1.14	0.25	
	280d	0.45	-0.33	1.23	0.26	
	2m	0.86	-0.20	1.93	0.11	
	4m	1.58	0.24	2.93	0.02	*
	6m	2.42	0.81	4.03	0.003	*
	12m	4.12	1.60	6.64	0.001	*

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Girls	238d	0.54	-0.27	1.36	0.19	
	252d	0.31	-0.47	1.09	0.44	
	280d	-0.00	-0.85	0.85	1.00	
	2m	-0.08	-1.24	1.09	0.90	
	4m	0.43	-1.04	1.90	0.57	
	6m	1.26	-0.50	3.02	0.16	
	12m	3.71	0.94	6.48	0.009	*

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	0.04	-0.76	0.83	0.93	
	252d	0.07	-0.69	0.83	0.85	
	280d	0.10	-0.72	0.92	0.81	
	2m	0.01	-1.12	1.15	0.98	
	4m	-0.21	-1.63	1.20	0.77	
	6m	-0.52	-2.21	1.17	0.55	
	12m	-1.44	-4.14	1.26	0.30	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	0.21	-0.44	0.86	0.53	
	252d	0.32	-0.30	0.95	0.31	
	280d	0.56	-0.12	1.24	0.11	
	2m	1.08	0.14	2.01	0.02	*
	4m	1.53	0.35	2.71	0.01	*
	6m	1.88	0.47	3.29	0.009	*
	12m	1.92	-0.28	4.13	0.09	

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Boys	238d	0.17	-0.58	0.92	0.66	
	252d	0.25	-0.47	0.97	0.49	
	280d	0.46	-0.31	1.24	0.24	
	2m	1.06	0.00	2.12	0.05	*
	4m	1.75	0.42	3.07	0.01	*
	6m	2.40	0.81	3.98	0.003	*
	12m	3.36	0.83	5.89	0.009	*

Comparing gender for Weight differences.

Random effect model (ITT).

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. A	238d	-0.20	-0.80	0.40	0.51	
	252d	0.30	-0.27	0.88	0.30	
	280d	1.16	0.54	1.78	0.000	*
	2m	2.44	1.57	3.31	0.000	*
	4m	3.06	1.96	4.17	0.000	*
	6m	3.21	1.89	4.53	0.000	*
	12m	2.21	0.11	4.31	0.04	*

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. B	238d	-0.27	-0.86	0.32	0.37	
	252d	0.22	-0.35	0.78	0.45	
	280d	1.01	0.40	1.63	0.001	*
	2m	2.05	1.19	2.90	0.000	*
	4m	2.35	1.28	3.43	0.000	*
	6m	2.19	0.91	3.48	0.001	*
	12m	0.86	-1.18	2.90	0.41	

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. C	238d	-0.21	-0.78	0.35	0.46	
	252d	0.47	-0.08	1.02	0.09	
	280d	1.61	1.02	2.21	0.000	*
	2m	3.22	2.39	4.05	0.000	*
	4m	3.86	2.80	4.92	0.000	*
	6m	3.81	2.53	5.09	0.000	*
	12m	1.72	-0.28	3.73	0.09	

Random effect model (PP).

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. A	238d	-0.21	-0.93	0.52	0.58	
	252d	0.31	-0.39	1.00	0.39	
	280d	1.20	0.45	1.96	0.002	*
	2m	2.62	1.58	3.67	0.000	*
	4m	3.43	2.12	4.74	0.000	*
	6m	3.76	2.20	5.31	0.000	*
	12m	3.06	0.61	5.50	0.01	*

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. B	238d	-0.09	-0.97	0.80	0.85	
	252d	0.27	-0.58	1.11	0.54	
	280d	0.86	-0.05	1.76	0.07	
	2m	1.70	0.44	2.95	0.008	*
	4m	2.06	0.50	3.62	0.01	*
	6m	2.08	0.22	3.93	0.03	*
	12m	1.20	-1.77	4.18	0.43	

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. C	238d	-0.46	-1.14	0.22	0.18	
	252d	0.21	-0.45	0.87	0.54	
	280d	1.32	0.61	2.03	0.000	*
	2m	2.84	1.87	3.80	0.000	*
	4m	3.37	2.15	4.60	0.000	*
	6m	3.21	1.75	4.68	0.000	*
	12m	0.86	-1.43	3.14	0.46	

Explanation and calculation of weight-difference

Since weight was transformed by taking the square root before it was entered into the REM, the unit of Coef and CI are in “square root of gram”. To calculate a weight-difference in absolute terms expressed by a coefficient and a CI in real gram, the equation:

$$((\sqrt{W})+C)^2 - W$$

has to be used, where W is the actual weight of an infant in gram and C is Coef or CI from the tables above.

Ex.: The weight difference comparing boys and girls at term (280 days) in group A.
If the weight of the girl is 3200 gram, a boy at the same age will be
$(\sqrt{3200}+1.16)^2 - 3200 = 133$ *gram* heavier (the weight-difference) with CI min and max
$(\sqrt{3200}+0.54)^2 - 3200 = 61$ *gram* and $(\sqrt{3200}+1.78)^2 - 3200 = 205$ *gram* respectively.

Comparing nutrition groups for Length differences. Unit: Coef and CI in mm.

Random effect model (ITT).

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	-0.29	-4.23	3.66	0.89	
	252d	0.21	-3.52	3.94	0.91	
	280d	1.18	-2.62	4.98	0.54	
	2m	3.11	-1.64	7.86	0.20	
	4m	4.68	-0.91	10.27	0.10	
	6m	5.77	-0.53	12.06	0.07	
	12m	5.54	-3.69	14.77	0.24	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	1.09	-2.97	5.14	0.60	
	252d	1.89	-1.96	5.74	0.34	
	280d	3.47	-0.45	7.40	0.08	
	2m	6.78	1.87	11.70	0.007	*
	4m	9.69	3.85	15.53	0.001	*
	6m	12.00	5.38	18.63	0.000	*
	12m	14.21	4.60	23.82	0.004	*

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Girls	238d	1.38	-2.42	5.17	0.48	
	252d	1.68	-1.93	5.28	0.36	
	280d	2.29	-1.37	5.96	0.22	
	2m	3.67	-0.91	8.25	0.12	
	4m	5.01	-0.47	10.49	0.07	
	6m	6.24	-0.00	12.48	0.05	
	12m	8.68	-0.31	17.66	0.06	

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	0.47	-3.30	4.23	0.81	
	252d	0.78	-2.75	4.30	0.67	
	280d	1.29	-2.32	4.90	0.48	
	2m	2.00	-2.57	6.57	0.39	
	4m	2.31	-3.03	7.65	0.40	
	6m	2.38	-3.62	8.37	0.44	
	12m	2.20	-6.78	11.18	0.63	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	1.37	-2.16	4.90	0.45	
	252d	2.33	-1.00	5.66	0.17	
	280d	4.19	0.79	7.59	0.02	*
	2m	7.88	3.59	12.17	0.000	*
	4m	10.94	5.86	16.02	0.000	*
	6m	13.21	7.47	18.95	0.000	*
	12m	14.65	6.22	23.09	0.001	*

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Boys	238d	0.91	-2.74	4.55	0.63	
	252d	1.55	-1.88	4.99	0.38	
	280d	2.90	-0.59	6.39	0.10	
	2m	5.88	1.51	10.25	0.008	*
	4m	8.63	3.51	13.75	0.001	*
	6m	10.84	5.07	16.60	0.000	*
	12m	12.46	3.93	20.98	0.004	*

Random effect model (PP).

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	-1.73	-6.70	3.24	0.49	
	252d	-0.04	-4.68	4.60	0.99	
	280d	2.77	-1.95	7.50	0.25	
	2m	6.64	0.64	12.63	0.03	*
	4m	8.00	1.01	14.99	0.02	*
	6m	7.53	-0.28	15.34	0.06	
	12m	0.54	-11.10	12.19	0.93	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	0.20	-4.42	4.83	0.93	
	252d	1.44	-2.92	5.80	0.52	
	280d	3.79	-0.61	8.18	0.09	
	2m	8.29	2.82	13.75	0.003	*
	4m	11.85	5.40	18.31	0.000	*
	6m	14.42	7.12	21.71	0.000	*
	12m	15.96	5.31	26.61	0.003	*

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Girls	238d	1.94	-3.04	6.92	0.45	
	252d	1.48	-3.19	6.16	0.53	
	280d	1.02	-3.70	5.74	0.67	
	2m	1.65	-4.26	7.56	0.58	
	4m	3.85	-3.13	10.83	0.28	
	6m	6.89	-0.99	14.77	0.09	
	12m	15.42	3.82	27.01	0.009	*

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	-1.39	-6.25	3.46	0.57	
	252d	-0.41	-4.92	4.10	0.86	
	280d	1.11	-3.46	5.68	0.63	
	2m	2.68	-3.11	8.47	0.36	
	4m	2.51	-4.24	9.26	0.47	
	6m	1.27	-6.30	8.83	0.74	
	12m	-3.82	-15.29	7.66	0.51	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	0.40	-3.64	4.44	0.84	
	252d	1.59	-2.21	5.38	0.41	
	280d	3.80	-0.05	7.66	0.05	
	2m	7.89	3.03	12.75	0.001	*
	4m	10.92	5.19	16.65	0.000	*
	6m	12.87	6.43	19.31	0.000	*
	12m	12.47	3.04	21.89	0.01	*

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Boys	238d	1.79	-2.74	6.33	0.44	
	252d	1.99	-2.22	6.21	0.35	
	280d	2.69	-1.54	6.92	0.21	
	2m	5.21	-0.08	10.50	0.05	
	4m	8.41	2.24	14.59	0.008	*
	6m	11.61	4.65	18.56	0.001	*
	12m	16.28	5.72	26.84	0.003	*

Comparing gender for Length differences. Unit: Coef and CI in mm.

Random effect model (ITT).

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. A	238d	-1.12	-5.05	2.82	0.58	
	252d	1.08	-2.63	4.79	0.57	
	280d	4.89	1.09	8.69	0.01	*
	2m	10.85	6.05	15.65	0.000	*
	4m	14.16	8.52	19.79	0.000	*
	6m	15.43	9.09	21.76	0.000	*
	12m	12.30	2.92	21.68	0.01	*

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. B	238d	-0.36	-4.15	3.43	0.85	
	252d	1.64	-1.93	5.21	0.37	
	280d	5.00	1.37	8.63	0.007	*
	2m	9.74	5.20	14.28	0.000	*
	4m	11.79	6.48	17.09	0.000	*
	6m	12.04	6.07	18.00	0.000	*
	12m	8.96	0.13	17.79	0.05	*

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. C	238d	-0.83	-4.50	2.83	0.66	
	252d	1.52	-1.97	5.01	0.39	
	280d	5.61	2.06	9.15	0.002	*
	2m	11.95	7.53	16.38	0.000	*
	4m	15.40	10.09	20.71	0.000	*
	6m	16.63	10.58	22.69	0.000	*
	12m	12.74	4.05	21.43	0.004	*

Random effect model (PP).

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. A	238d	-0.86	-5.39	3.67	0.71	
	252d	1.23	-3.02	5.47	0.57	
	280d	4.88	0.55	9.22	0.03	*
	2m	10.75	5.25	16.24	0.000	*
	4m	14.21	7.78	20.63	0.000	*
	6m	15.78	8.60	22.96	0.000	*
	12m	13.64	3.05	24.23	0.01	*

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. B	238d	-0.51	-5.83	4.80	0.85	
	252d	0.86	-4.07	5.80	0.73	
	280d	3.22	-1.78	8.22	0.21	
	2m	6.79	0.48	13.10	0.03	*
	4m	8.71	1.39	16.04	0.02	*
	6m	9.52	1.32	17.72	0.02	*
	12m	9.28	-3.20	21.76	0.14	

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. C	238d	-0.66	-4.83	3.51	0.76	
	252d	1.37	-2.57	5.32	0.49	
	280d	4.90	0.95	8.84	0.01	*
	2m	10.35	5.51	15.19	0.000	*
	4m	13.28	7.50	19.06	0.000	*
	6m	14.24	7.65	20.83	0.000	*
	12m	10.15	0.64	19.65	0.04	*

Comparing nutrition groups for HC differences. Unit: Coef and CI in mm.

Random effect model (ITT).

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	-0.04	-2.26	2.18	0.97	
	252d	0.83	-1.24	2.90	0.43	
	280d	2.16	0.04	4.29	0.05	*
	2m	3.49	0.79	6.19	0.01	*
	4m	3.18	0.07	6.29	0.04	*
	6m	1.83	-1.58	5.24	0.29	
	12m	-3.98	-8.99	1.03	0.12	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	-0.39	-2.64	1.87	0.74	
	252d	0.66	-1.45	2.77	0.54	
	280d	2.30	0.13	4.48	0.04	*
	2m	4.16	1.37	6.96	0.003	*
	4m	4.22	0.99	7.46	0.01	*
	6m	3.08	-0.48	6.63	0.09	
	12m	-2.75	-7.88	2.37	0.29	

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Girls	238d	-0.35	-2.51	1.81	0.75	
	252d	-0.17	-2.19	1.85	0.87	
	280d	0.14	-1.96	2.24	0.90	
	2m	0.68	-2.04	3.39	0.63	
	4m	1.04	-2.10	4.17	0.52	
	6m	1.25	-2.20	4.70	0.48	
	12m	1.23	-3.82	6.28	0.63	

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	-0.87	-2.96	1.22	0.41	
	252d	-0.27	-2.21	1.67	0.79	
	280d	0.67	-1.32	2.66	0.51	
	2m	1.69	-0.85	4.24	0.19	
	4m	1.68	-1.25	4.61	0.26	
	6m	1.03	-2.18	4.25	0.53	
	12m	-1.67	-6.43	3.08	0.49	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	-0.59	-2.53	1.36	0.56	
	252d	0.02	-1.79	1.84	0.98	
	280d	1.01	-0.87	2.89	0.29	
	2m	2.25	-0.17	4.67	0.07	
	4m	2.48	-0.31	5.28	0.08	
	6m	1.98	-1.10	5.05	0.21	
	12m	-1.73	-6.23	2.77	0.45	

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Boys	238d	0.29	-1.77	2.34	0.79	
	252d	0.29	-1.62	2.20	0.77	
	280d	0.34	-1.62	2.30	0.73	
	2m	0.56	-1.94	3.06	0.66	
	4m	0.81	-2.06	3.67	0.58	
	6m	0.94	-2.19	4.08	0.56	
	12m	-0.06	-4.71	4.59	0.98	

Random effect model (PP).

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	-0.02	-2.87	2.84	0.99	
	252d	0.77	-1.90	3.45	0.57	
	280d	2.06	-0.68	4.80	0.14	
	2m	3.68	0.23	7.12	0.04	*
	4m	4.01	0.08	7.95	0.05	*
	6m	3.45	-0.85	7.74	0.12	
	12m	-0.62	-6.79	5.55	0.84	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Girls	238d	-0.47	-3.09	2.15	0.73	
	252d	0.79	-1.68	3.25	0.53	
	280d	2.81	0.28	5.34	0.03	*
	2m	5.28	2.11	8.46	0.001	*
	4m	5.69	2.06	9.31	0.002	*
	6m	4.66	0.71	8.61	0.02	*
	12m	-1.86	-7.50	3.78	0.52	

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Girls	238d	-0.45	-3.32	2.43	0.76	
	252d	0.01	-2.68	2.71	0.99	
	280d	0.75	-2.02	3.52	0.60	
	2m	1.61	-1.89	5.10	0.37	
	4m	1.67	-2.32	5.67	0.41	
	6m	1.21	-3.16	5.58	0.59	
	12m	-1.23	-7.56	5.09	0.70	

Group B vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	-0.08	-2.85	2.69	0.96	
	252d	0.09	-2.49	2.67	0.95	
	280d	0.21	-2.42	2.84	0.88	
	2m	-0.27	-3.59	3.04	0.87	
	4m	-1.40	-5.19	2.39	0.47	
	6m	-2.76	-6.90	1.39	0.19	
	12m	-5.39	-11.43	0.65	0.08	

Group C vs. A

	Time	Coef	CI min	CI max	p-value	
Boys	238d	-0.02	-2.28	2.25	0.99	
	252d	0.50	-1.63	2.63	0.64	
	280d	1.29	-0.91	3.49	0.25	
	2m	2.02	-0.77	4.80	0.16	
	4m	1.71	-1.49	4.90	0.29	
	6m	0.71	-2.78	4.20	0.69	
	12m	-3.65	-8.61	1.32	0.15	

Group C vs. B

	Time	Coef	CI min	CI max	p-value	
Boys	238d	0.06	-2.56	2.68	0.96	
	252d	0.42	-2.02	2.85	0.74	
	280d	1.08	-1.40	3.56	0.39	
	2m	2.29	-0.83	5.41	0.15	
	4m	3.11	-0.43	6.65	0.09	
	6m	3.47	-0.39	7.33	0.08	
	12m	1.74	-3.93	7.42	0.55	

Comparing gender for HC differences. Unit: Coef and CI in mm.

Random effect model (ITT).

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. A	238d	1.19	-0.96	3.34	0.28	
	252d	2.49	0.48	4.49	0.02	*
	280d	4.67	2.61	6.72	0.000	*
	2m	7.82	5.20	10.45	0.000	*
	4m	9.27	6.23	12.31	0.000	*
	6m	9.55	6.21	12.89	0.000	*
	12m	7.67	2.82	12.53	0.002	*

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. B	238d	0.36	-1.83	2.54	0.75	
	252d	1.39	-0.65	3.42	0.18	
	280d	3.18	1.09	5.26	0.003	*
	2m	6.02	3.38	8.67	0.000	*
	4m	7.76	4.74	10.78	0.000	*
	6m	8.75	5.45	12.06	0.000	*
	12m	9.98	5.05	14.91	0.000	*

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. C	238d	0.99	-1.08	3.06	0.35	
	252d	1.85	-0.09	3.79	0.06	
	280d	3.38	1.36	5.39	0.001	*
	2m	5.91	3.30	8.51	0.000	*
	4m	7.53	4.52	10.54	0.000	*
	6m	8.45	5.14	11.76	0.000	*
	12m	8.70	3.90	13.49	0.000	*

Random effect model (PP).

Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. A	238d	0.34	-2.17	2.84	0.79	
	252d	1.58	-0.77	3.93	0.19	
	280d	3.76	1.34	6.17	0.002	*
	2m	7.24	4.19	10.30	0.000	*
	4m	9.29	5.79	12.79	0.000	*
	6m	10.23	6.40	14.05	0.000	*
	12m	9.17	3.74	14.59	0.001	*

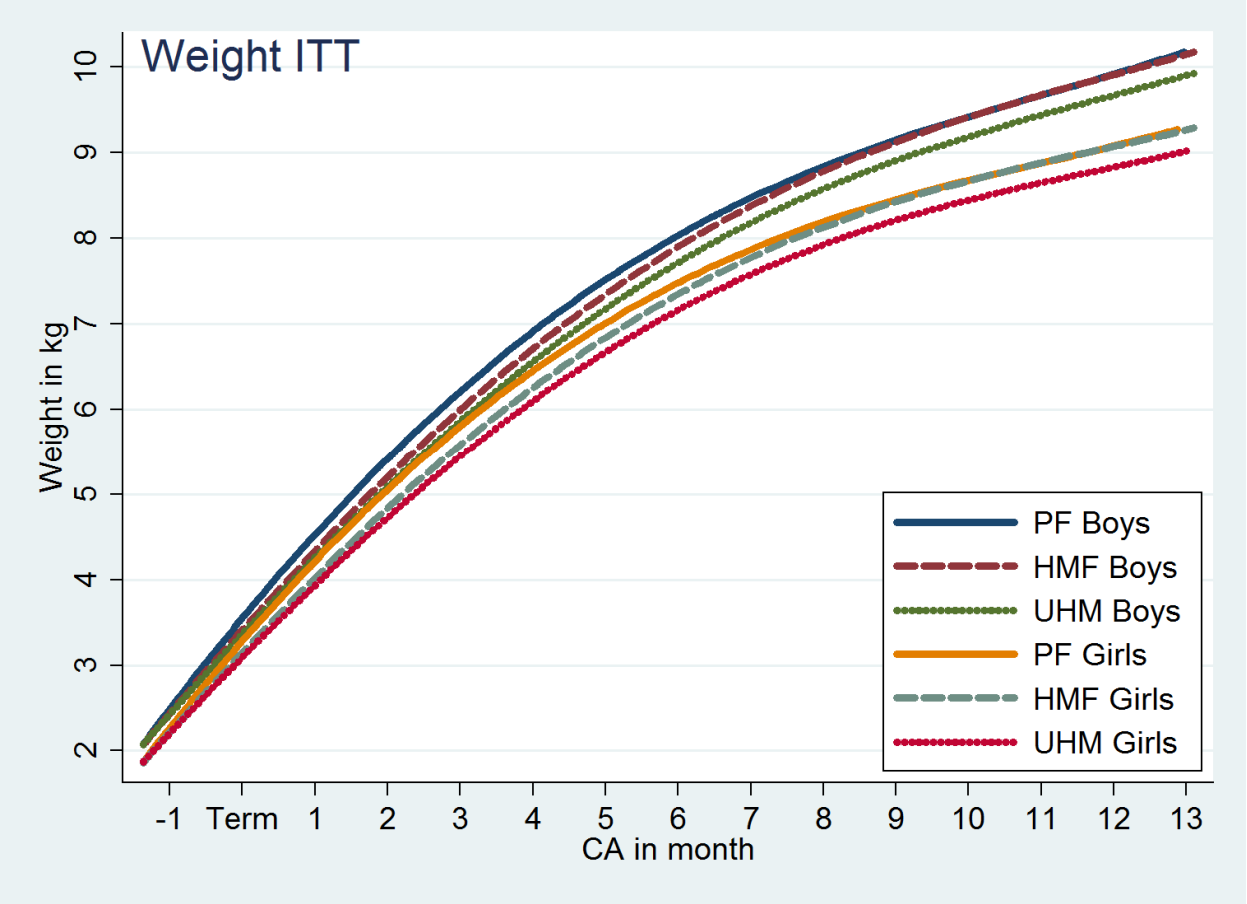
Boys vs. Girls

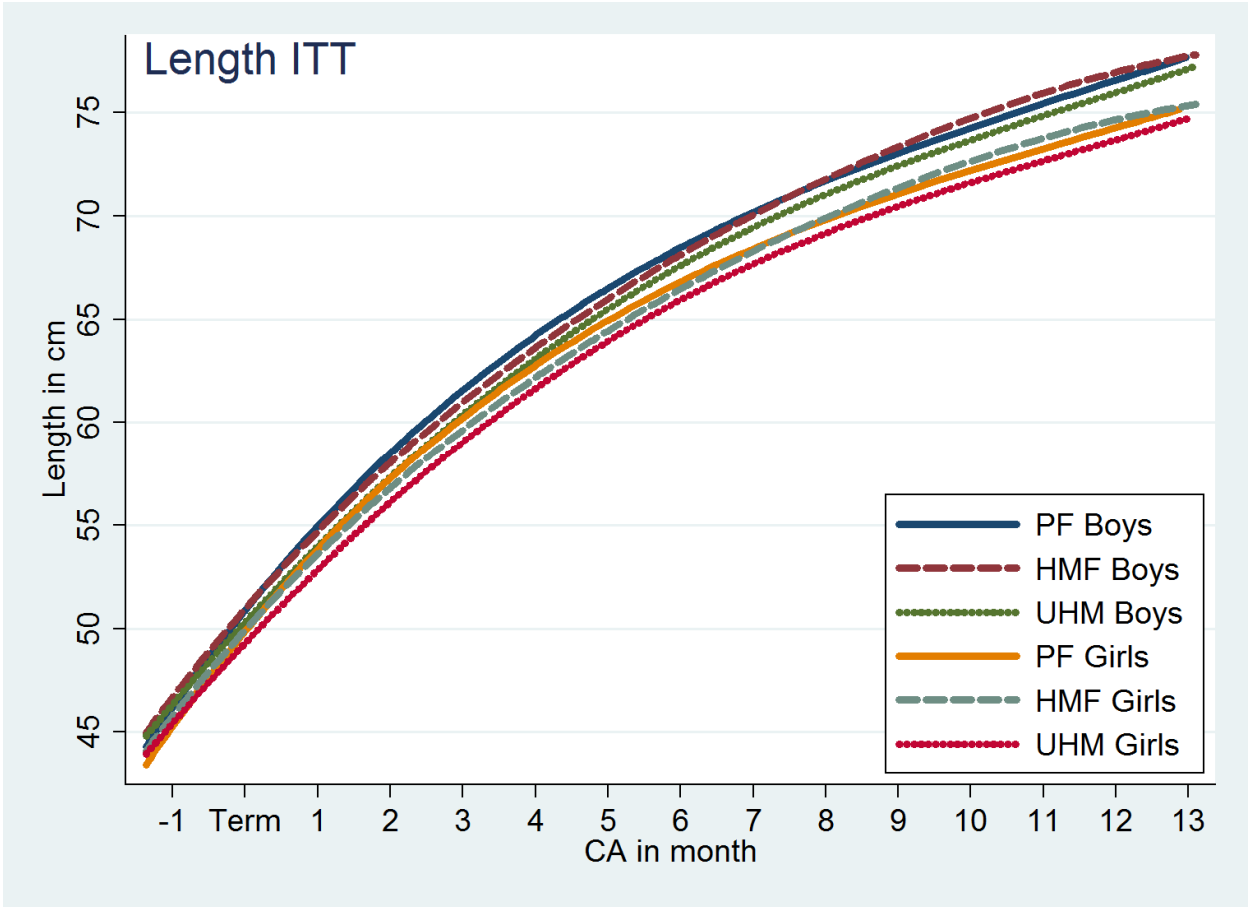
	Time	Coef	CI min	CI max	p-value	
Gr. B	238d	0.28	-2.83	3.39	0.86	
	252d	0.89	-2.01	3.79	0.55	
	280d	1.91	-1.05	4.86	0.21	
	2m	3.29	-0.40	6.99	0.08	
	4m	3.88	-0.33	8.09	0.07	
	6m	4.02	-0.57	8.62	0.09	
	12m	4.40	-2.33	11.13	0.20	

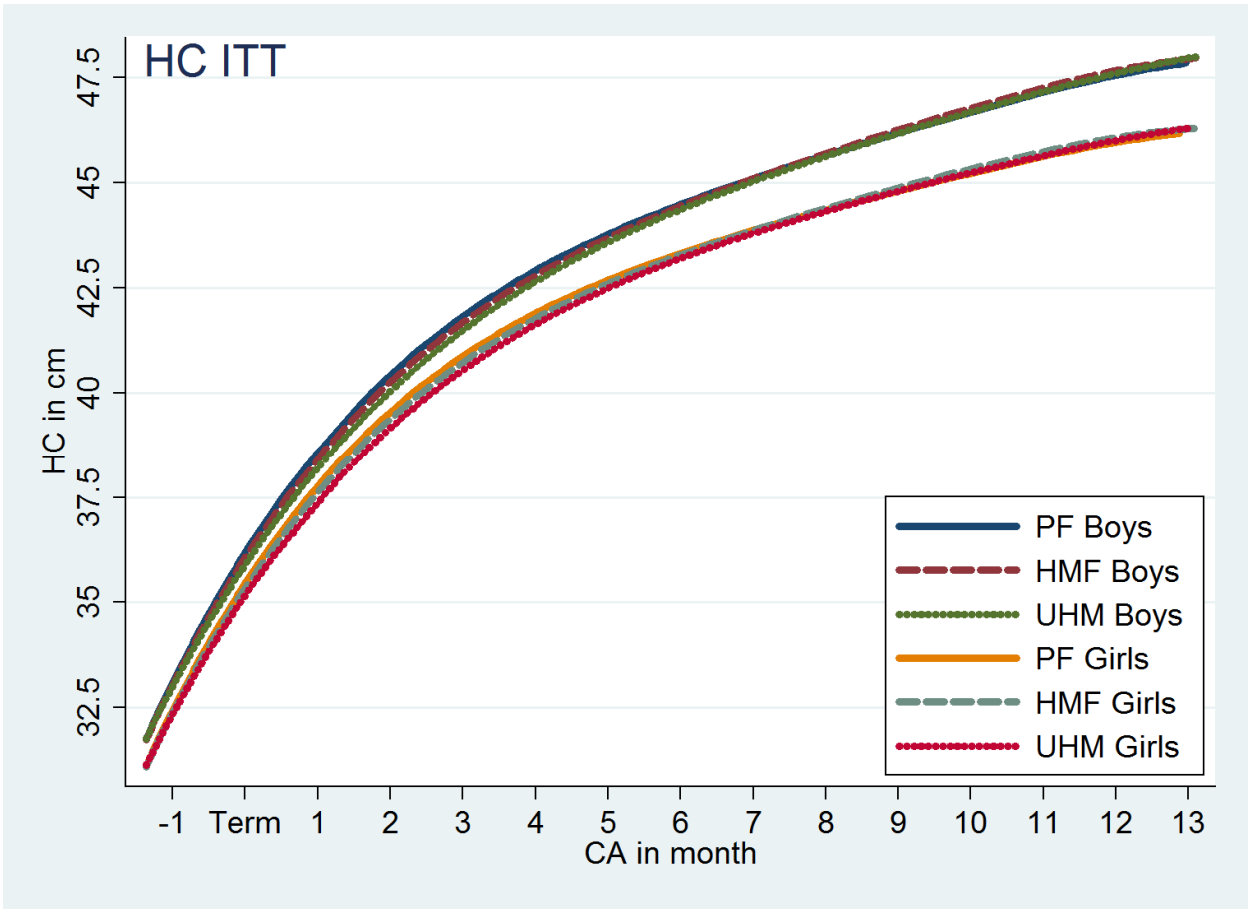
Boys vs. Girls

	Time	Coef	CI min	CI max	p-value	
Gr. C	238d	0.79	-1.62	3.19	0.52	
	252d	1.29	-0.97	3.56	0.26	
	280d	2.24	-0.10	4.58	0.06	
	2m	3.98	1.05	6.91	0.008	*
	4m	5.31	1.97	8.66	0.002	*
	6m	6.28	2.64	9.92	0.001	*
	12m	7.38	2.17	12.59	0.006	*

Appendix 3. Growth-charts in absolute terms using REM (ITT).




Length ITT
Length in cm
45
50
55
60
65
70
75
-1 Term 1 2 3 4 5 6 7 8 9 10 11 12 13
CA in month
PF Boys
HMF Boys
UHM Boys
PF Girls
HMF Girls
UHM Girls


HC ITT
HC in cm
32.5
35
37.5
40
42.5
45
47.5
-1 Term 1 2 3 4 5 6 7 8 9 10 11 12 13
CA in month
PF Boys
HMF Boys
UHM Boys
PF Girls
HMF Girls
UHM Girls

Appendix 4. Z-scores and number of infants with Z-score < -2 SDS (tables)

Number of infants with Z-score < -2 SDS according to **weight** and nutrition (ITT).
Abbreviations: d = days, m = months

Weight (ITT)

	Birth	238d	252d	Term	2m	4m	6m	12m
Group A: n:	102	102	102	102	99	98	98	89
Mean Z score all:	-1.02	-1.53	-1.15	-1.09	-0.68	-0.61	-0.58	-0.83
Delta Z score from birth:	.	-0.51	-0.13	-0.07	0.35	0.41	0.44	0.19
Z score < -2 SDS (n):	20	28	16	18	11	12	15	18
Z score < -2 SDS %:	19.6	27.5	15.7	17.6	11.1	12.2	15.3	20.2
SGA at birth (n):	20	20	20	20	20	19	19	18
Mean Z score SGA:	-2.82	-2.86	-2.31	-2.04	-1.57	-1.23	-1.19	-1.41
Z score < -2 SDS (SGA n):	20	17	11	10	6	6	6	7
Group B: n:	105	105	105	105	104	104	102	92
Mean Z score all:	-1.03	-1.52	-1.12	-0.95	-0.53	-0.46	-0.34	-0.67
Delta Z score from birth:	.	-0.50	-0.10	0.08	0.50	0.57	0.68	0.36
Z score < -2 SDS (n):	21	28	18	13	12	12	10	11
Z score < -2 SDS %:	20.0	26.7	17.1	12.4	11.5	11.5	9.8	12.0
SGA at birth (n):	21	21	21	21	21	21	20	19
Mean Z score SGA:	-2.68	-2.75	-2.23	-1.98	-1.69	-1.76	-1.68	-1.72
Z score < -2 SDS (SGA n):	21	17	13	9	8	8	7	7
Group C: n:	113	113	111	111	107	106	103	96
Mean Z score all:	-1.23	-1.45	-0.98	-0.64	-0.25	-0.23	-0.29	-0.58
Delta Z score from birth:	.	-0.22	0.25	0.59	0.99	1.00	0.94	0.65
Z score < -2 SDS (n):	27	34	17	11	7	9	9	17
Z score < -2 SDS %:	23.9	30.1	15.3	9.9	6.5	8.5	8.7	17.7
SGA at birth (n):	27	27	27	27	24	24	24	23
Mean Z score SGA:	-2.73	-2.76	-2.19	-1.67	-1.29	-1.23	-1.30	-1.47
Z score < -2 SDS (SGA n):	27	25	16	10	6	5	4	8
Total: n:	320	320	318	318	310	308	303	277
Mean Z score all:	-1.10	-1.50	-1.08	-0.89	-0.48	-0.43	-0.40	-0.69
Delta Z score from birth:	.	-0.40	0.01	0.21	0.62	0.67	0.69	0.40
Z score < -2 SDS (n):	68	90	51	42	30	33	34	46
Z score < -2 SDS %:	21.3	28.1	16.0	13.2	9.7	10.7	11.2	16.6
SGA at birth (n):	68	68	68	68	65	64	63	60
Mean Z score SGA:	-2.74	-2.79	-2.24	-1.87	-1.50	-1.40	-1.39	-1.53
Z score < -2 SDS (SGA n):	68	59	40	29	20	19	17	22

CHI-square test comparing number of infants with Z score < -2 SDS

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	207	207	207	207	203	202	200	181
	p-value:	1.00	1.00	0.85	0.33	1.00	1.00	0.29	0.16
Group C vs. A:	n:	215	215	213	213	206	204	201	185
	p-value:	0.51	0.76	1.00	0.11	0.32	0.49	0.19	0.71
Group C vs. B:	n:	218	218	216	216	211	210	205	188
	p-value:	0.52	0.65	0.85	0.67	0.24	0.50	0.81	0.31

t-test comparing change in z score from birth to ...

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	207	207	207	207	203	202	200	181
	p-value:	.	0.84	0.72	0.21	0.26	0.23	0.13	0.31
Group C vs. A:	n:	215	215	213	213	206	204	201	185
	p-value:	.	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.02*
Group C vs. B:	n:	218	218	216	216	211	210	205	188
	p-value:	.	0.00*	0.00*	0.00*	0.00*	0.01*	0.10	0.12

Number of infants with Z-score < -2 SDS according to **weight** and nutrition (PP).
Abbreviations: d = days, m = months

Weight (PP)

	Birth	238d	252d	Term	2m	4m	6m	12m
Group A: n:	73	73	73	73	73	73	73	68
Mean Z score all:	-0.93	-1.48	-1.11	-1.05	-0.71	-0.66	-0.63	-0.88
Delta Z score from birth:	.	-0.56	-0.18	-0.12	0.22	0.27	0.30	0.05
Z score < -2 SDS (n):	13	18	11	12	7	9	12	15
Z score < -2 SDS %:	17.8	24.7	15.1	16.4	9.6	12.3	16.4	22.1
SGA at birth (n):	13	13	13	13	13	13	13	13
Mean Z score SGA:	-2.74	-2.80	-2.30	-1.95	-1.50	-1.17	-1.11	-1.40
Z score < -2 SDS (SGA n):	13	12	7	7	3	4	4	5
Group B: n:	51	51	51	51	51	51	49	42
Mean Z score all:	-1.09	-1.62	-1.20	-1.07	-0.76	-0.72	-0.57	-0.89
Delta Z score from birth:	.	-0.53	-0.10	0.02	0.33	0.37	0.52	0.20
Z score < -2 SDS (n):	11	15	10	6	8	8	7	7
Z score < -2 SDS %:	21.6	29.4	19.6	11.8	15.7	15.7	14.3	16.7
SGA at birth (n):	11	11	11	11	11	11	10	9
Mean Z score SGA:	-2.85	-2.93	-2.32	-2.11	-1.91	-2.06	-1.97	-1.89
Z score < -2 SDS (SGA n):	11	9	8	5	4	5	5	4
Group C: n:	87	87	87	87	87	87	84	78
Mean Z score all:	-1.20	-1.45	-0.97	-0.60	-0.14	-0.10	-0.18	-0.57
Delta Z score from birth:	.	-0.25	0.23	0.60	1.06	1.10	1.02	0.64
Z score < -2 SDS (n):	19	26	12	8	4	5	7	14
Z score < -2 SDS %:	21.8	29.9	13.8	9.2	4.6	5.7	8.3	17.9
SGA at birth (n):	19	19	19	19	19	19	19	18
Mean Z score SGA:	-2.78	-2.78	-2.19	-1.64	-1.20	-1.20	-1.32	-1.47
Z score < -2 SDS (SGA n):	19	17	11	7	4	3	4	6
Total: n:	211	211	211	211	211	211	206	188
Mean Z score all:	-1.08	-1.50	-1.07	-0.87	-0.49	-0.44	-0.43	-0.75
Delta Z score from birth:	.	-0.42	0.01	0.21	0.59	0.64	0.65	0.33
Z score < -2 SDS (n):	43	59	33	26	19	22	26	36
Z score < -2 SDS %:	20.4	28.0	15.6	12.3	9.0	10.4	12.6	19.1
SGA at birth (n):	43	43	43	43	43	43	42	40
Mean Z score SGA:	-2.78	-2.82	-2.25	-1.85	-1.47	-1.41	-1.41	-1.54
Z score < -2 SDS (SGA n):	43	38	26	19	11	12	13	15

CHI-square test comparing number of infants with Z score < -2 SDS

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	124	124	124	124	124	124	122	110
	p-value:	0.65	0.68	0.63	0.61	0.40	0.61	0.80	0.63
Group C vs. A:	n:	160	160	160	160	160	160	157	146
	p-value:	0.56	0.48	0.83	0.23	0.23	0.17	0.14	0.54
Group C vs. B:	n:	138	138	138	138	138	138	133	120
	p-value:	1.00	1.00	0.47	0.77	0.03*	0.07	0.38	1.00

t-test comparing change in z score from birth to ...

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	124	124	124	124	124	124	122	110
	p-value:	.	0.80	0.57	0.39	0.55	0.64	0.46	0.65
Group C vs. A:	n:	160	160	160	160	160	160	157	146
	p-value:	.	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.01*
Group C vs. B:	n:	138	138	138	138	138	138	133	120
	p-value:	.	0.00*	0.01*	0.00*	0.00*	0.00*	0.01*	0.06

Number of infants with Z-score < -2 SDS and SGA according to **length** and nutrition (ITT).
Abbreviations: d = days, m = months

Length (ITT)

		Birth	238d	252d	Term	2m	4m	6m	12m
Group A:	n:	91	101	102	101	99	98	98	89
	Mean Z score all:	-0.37	-1.09	-1.02	-0.98	-0.69	-0.20	-0.19	-0.21
	Delta Z score from birth:	.	-0.72	-0.66	-0.61	-0.32	0.16	0.18	0.16
	Z score < -2 SDS (n):	12	26	23	24	20	14	11	10
	Z score < -2 SDS %:	13.2	25.7	22.5	23.8	20.2	14.3	11.2	11.2
	SGA at birth (n):	19	20	20	20	20	19	19	18
	Mean Z score SGA:	-2.51	-3.26	-2.81	-2.45	-2.04	-1.18	-0.78	-0.69
	Z score < -2 SDS (SGA n):	9	15	12	11	10	7	4	2
Group B:	n:	100	105	105	105	104	104	102	92
	Mean Z score all:	-0.18	-0.88	-0.74	-0.71	-0.30	0.01	0.05	0.07
	Delta Z score from birth:	.	-0.69	-0.56	-0.53	-0.12	0.19	0.23	0.25
	Z score < -2 SDS (n):	12	20	20	16	12	9	8	5
	Z score < -2 SDS %:	12.0	19.0	19.0	15.2	11.5	8.7	7.8	5.4
	SGA at birth (n):	19	21	21	21	21	21	20	19
	Mean Z score SGA:	-2.19	-2.60	-2.51	-2.13	-1.50	-1.30	-1.16	-0.70
	Z score < -2 SDS (SGA n):	11	12	13	11	7	6	5	2
Group C:	n:	104	113	111	110	106	105	102	95
	Mean Z score all:	-0.53	-1.28	-1.02	-0.84	-0.07	0.17	0.13	-0.00
	Delta Z score from birth:	.	-0.75	-0.50	-0.31	0.45	0.70	0.66	0.52
	Z score < -2 SDS (n):	14	32	22	19	8	8	5	4
	Z score < -2 SDS %:	13.5	28.3	19.8	17.3	7.5	7.6	4.9	4.2
	SGA at birth (n):	24	27	27	27	24	24	24	23
	Mean Z score SGA:	-2.27	-3.04	-2.56	-2.03	-1.11	-0.67	-0.67	-0.52
	Z score < -2 SDS (SGA n):	11	22	17	12	4	4	2	2
Total:	n:	295	319	318	316	309	307	302	276
	Mean Z score all:	-0.36	-1.09	-0.93	-0.84	-0.35	-0.00	-0.00	-0.05
	Delta Z score from birth:	.	-0.73	-0.57	-0.48	0.01	0.36	0.36	0.31
	Z score < -2 SDS (n):	38	78	65	59	40	31	24	19
	Z score < -2 SDS %:	12.9	24.5	20.4	18.7	12.9	10.1	7.9	6.9
	SGA at birth (n):	62	68	68	68	65	64	63	60
	Mean Z score SGA:	-2.32	-2.97	-2.62	-2.18	-1.52	-1.03	-0.86	-0.63
	Z score < -2 SDS (SGA n):	31	49	42	34	21	17	11	6

CHI-square test comparing number of infants with Z score < -2 SDS

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	191	206	207	206	203	202	200	181
	p-value:	0.83	0.32	0.61	0.16	0.12	0.27	0.47	0.18
Group C vs. A:	n:	195	214	213	211	205	203	200	184
	p-value:	1.00	0.76	0.74	0.30	0.01*	0.17	0.12	0.10
Group C vs. B:	n:	204	218	216	215	210	209	204	187
	p-value:	0.84	0.12	1.00	0.72	0.36	0.81	0.57	0.74

t-test comparing change in z score from birth to ...

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	191	206	207	206	203	202	200	181
	p-value:	.	0.37	0.37	0.40	0.13	0.44	0.46	0.29
Group C vs. A:	n:	195	214	213	211	205	203	200	184
	p-value:	.	0.61	0.27	0.06	0.00*	0.00*	0.01*	0.07
Group C vs. B:	n:	204	218	216	215	210	209	204	187
	p-value:	.	0.68	0.86	0.34	0.02*	0.03*	0.05	0.41

Number of infants with Z-score < -2 SDS according to **length** and nutrition (PP).
Abbreviations: d = days, m = months

Length (PP)

		Birth	238d	252d	Term	2m	4m	6m	12m
Group A:	n:	65	73	73	73	73	73	73	68
	Mean Z score all:	-0.29	-0.97	-0.85	-0.85	-0.65	-0.33	-0.32	-0.28
	Delta Z score from birth:	.	-0.68	-0.56	-0.56	-0.36	-0.04	-0.03	0.01
	Z score < -2 SDS (n):	9	15	14	15	14	11	9	8
	Z score < -2 SDS %:	13.8	20.5	19.2	20.5	19.2	15.1	12.3	11.8
	SGA at birth (n):	13	13	13	13	13	13	13	13
	Mean Z score SGA:	-2.23	-3.06	-2.54	-2.27	-2.03	-1.35	-0.95	-0.83
	Z score < -2 SDS (SGA n):	6	9	8	7	7	5	3	1
Group B:	n:	48	51	51	51	51	51	49	42
	Mean Z score all:	-0.55	-1.15	-1.04	-0.88	-0.52	-0.14	-0.11	-0.16
	Delta Z score from birth:	.	-0.60	-0.50	-0.33	0.03	0.40	0.44	0.39
	Z score < -2 SDS (n):	7	12	10	8	6	5	5	3
	Z score < -2 SDS %:	14.6	23.5	19.6	15.7	11.8	9.8	10.2	7.1
	SGA at birth (n):	9	11	11	11	11	11	10	9
	Mean Z score SGA:	-2.72	-2.99	-2.83	-2.19	-1.58	-1.21	-1.03	-0.85
	Z score < -2 SDS (SGA n):	6	8	7	5	3	2	2	1
Group C:	n:	80	87	87	87	87	87	84	78
	Mean Z score all:	-0.53	-1.27	-1.04	-0.86	0.00	0.28	0.20	-0.00
	Delta Z score from birth:	.	-0.74	-0.51	-0.33	0.53	0.80	0.73	0.52
	Z score < -2 SDS (n):	12	23	17	17	6	5	3	3
	Z score < -2 SDS %:	15.0	26.4	19.5	19.5	6.9	5.7	3.6	3.8
	SGA at birth (n):	16	19	19	19	19	19	19	18
	Mean Z score SGA:	-2.45	-2.89	-2.55	-2.05	-0.94	-0.61	-0.67	-0.63
	Z score < -2 SDS (SGA n):	9	14	12	10	3	3	2	2
Total:	n:	193	211	211	211	211	211	206	188
	Mean Z score all:	-0.45	-1.14	-0.97	-0.86	-0.35	-0.03	-0.06	-0.14
	Delta Z score from birth:	.	-0.68	-0.52	-0.41	0.10	0.42	0.40	0.31
	Z score < -2 SDS (n):	28	50	41	40	26	21	17	14
	Z score < -2 SDS %:	14.5	23.7	19.4	19.0	12.3	10.0	8.3	7.4
	SGA at birth (n):	38	43	43	43	43	43	42	40
	Mean Z score SGA:	-2.44	-2.96	-2.62	-2.15	-1.43	-0.99	-0.84	-0.75
	Z score < -2 SDS (SGA n):	21	31	27	22	13	10	7	4

CHI-square test comparing number of infants with Z score < -2 SDS

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	113	124	124	124	124	124	122	110
	p-value:	1.00	0.83	1.00	0.64	0.33	0.43	0.78	0.53
Group C vs. A:	n:	145	160	160	160	160	160	157	146
	p-value:	1.00	0.46	1.00	1.00	0.03*	0.06	0.07	0.11
Group C vs. B:	n:	128	138	138	138	138	138	133	120
	p-value:	1.00	0.84	1.00	0.65	0.36	0.50	0.14	0.42

t-test comparing change in z score from birth to ...

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	113	124	124	124	124	124	122	110
	p-value:	.	0.26	0.41	0.15	0.06	0.09	0.12	0.14
Group C vs. A:	n:	145	160	160	160	160	160	157	146
	p-value:	.	0.68	0.65	0.22	0.00*	0.00*	0.00*	0.07
Group C vs. B:	n:	128	138	138	138	138	138	133	120
	p-value:	.	0.43	0.66	0.66	0.11	0.20	0.28	0.88

Number of infants with Z-score < -2 SDS according to **HC** and nutrition (ITT).
Abbreviations: d = days, m = months

HC (ITT)

		Birth	238d	252d	Term	2m	4m	6m	12m
Group A:	n:	81	101	102	101	99	98	97	86
	Mean Z score all:	-0.39	-0.66	-0.15	-0.01	0.15	0.24	0.28	0.15
	Delta Z score from birth:	.	-0.27	0.24	0.38	0.55	0.63	0.67	0.54
	Z score < -2 SDS (n):	5	7	2	1	0	1	1	1
	Z score < -2 SDS %:	6.2	6.9	2.0	1.0	0.0	1.0	1.0	1.2
	SGA at birth (n):	17	20	20	20	20	19	19	18
	Mean Z score SGA:	-1.47	-1.65	-1.01	-0.68	-0.59	-0.41	-0.49	-0.48
	Z score < -2 SDS (SGA n):	4	6	2	1	0	0	0	0
Group B:	n:	89	103	105	105	104	103	98	81
	Mean Z score all:	-0.46	-0.67	-0.08	0.14	0.31	0.33	0.40	0.12
	Delta Z score from birth:	.	-0.21	0.37	0.59	0.77	0.78	0.85	0.58
	Z score < -2 SDS (n):	6	4	1	0	0	2	2	0
	Z score < -2 SDS %:	6.7	3.9	1.0	0.0	0.0	1.9	2.0	0.0
	SGA at birth (n):	21	21	21	21	21	20	18	14
	Mean Z score SGA:	-1.54	-1.49	-0.75	-0.52	-0.55	-0.64	-0.47	-0.37
	Z score < -2 SDS (SGA n):	5	4	1	0	0	1	1	0
Group C:	n:	98	113	111	109	105	103	100	91
	Mean Z score all:	-0.47	-0.63	-0.06	0.16	0.36	0.37	0.27	0.03
	Delta Z score from birth:	.	-0.16	0.41	0.63	0.83	0.84	0.74	0.50
	Z score < -2 SDS (n):	7	12	2	1	2	5	5	3
	Z score < -2 SDS %:	7.1	10.6	1.8	0.9	1.9	4.9	5.0	3.3
	SGA at birth (n):	22	27	27	27	24	24	24	22
	Mean Z score SGA:	-1.40	-1.60	-0.86	-0.44	-0.57	-0.62	-0.70	-0.69
	Z score < -2 SDS (SGA n):	6	9	2	1	1	3	3	2
Total:	n:	268	317	318	315	308	304	295	258
	Mean Z score all:	-0.44	-0.65	-0.10	0.10	0.28	0.31	0.31	0.10
	Delta Z score from birth:	.	-0.21	0.34	0.54	0.72	0.75	0.76	0.54
	Z score < -2 SDS (n):	18	23	5	2	2	8	8	4
	Z score < -2 SDS %:	6.7	7.3	1.6	0.6	0.6	2.6	2.7	1.6
	SGA at birth (n):	60	68	68	68	65	63	61	54
	Mean Z score SGA:	-1.47	-1.58	-0.87	-0.54	-0.57	-0.56	-0.57	-0.54
	Z score < -2 SDS (SGA n):	15	19	5	2	1	4	4	2

CHI-square test comparing number of infants with Z score < -2 SDS

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	170	204	207	206	203	201	195	167
	p-value:	1.00	0.37	0.62	0.49	.	1.00	1.00	1.00
Group C vs. A:	n:	179	214	213	210	204	201	197	177
	p-value:	1.00	0.47	1.00	1.00	0.50	0.21	0.21	0.62
Group C vs. B:	n:	187	216	216	214	209	206	198	172
	p-value:	1.00	0.07	1.00	1.00	0.50	0.45	0.44	0.25

t-test comparing change in z score from birth to ...

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	170	204	207	206	203	201	195	167
	p-value:	.	0.60	0.36	0.24	0.08	0.16	0.23	0.28
Group C vs. A:	n:	179	214	213	210	204	201	197	177
	p-value:	.	0.22	0.08	0.06	0.01*	0.07	0.48	0.75
Group C vs. B:	n:	187	216	216	214	209	206	198	172
	p-value:	.	0.50	0.40	0.48	0.36	0.62	0.62	0.48

Number of infants with Z-score < -2 SDS according to **HC** and nutrition (PP).
Abbreviations: d = days, m = months

HC (PP)

		Birth	238d	252d	Term	2m	4m	6m	12m
Group A:	n:	56	73	73	73	73	73	73	67
	Mean Z score all:	-0.31	-0.66	-0.15	-0.11	0.11	0.21	0.25	0.11
	Delta Z score from birth:	.	-0.35	0.16	0.20	0.43	0.52	0.57	0.42
	Z score < -2 SDS (n):	3	4	0	0	0	1	1	1
	Z score < -2 SDS %:	5.4	5.5	0.0	0.0	0.0	1.4	1.4	1.5
	SGA at birth (n):	11	13	13	13	13	13	13	13
	Mean Z score SGA:	-1.34	-1.55	-0.87	-0.69	-0.65	-0.46	-0.53	-0.48
	Z score < -2 SDS (SGA n):	2	3	0	0	0	0	0	0
Group B:	n:	47	51	51	51	51	51	48	40
	Mean Z score all:	-0.59	-0.81	-0.28	-0.02	0.10	0.09	0.19	-0.01
	Delta Z score from birth:	.	-0.22	0.31	0.57	0.69	0.68	0.78	0.58
	Z score < -2 SDS (n):	5	3	1	0	0	1	2	0
	Z score < -2 SDS %:	10.6	5.9	2.0	0.0	0.0	2.0	4.2	0.0
	SGA at birth (n):	11	11	11	11	11	11	10	9
	Mean Z score SGA:	-1.77	-1.57	-0.79	-0.36	-0.47	-0.59	-0.42	-0.34
	Z score < -2 SDS (SGA n):	4	3	1	0	0	0	1	0
Group C:	n:	78	87	87	87	87	87	84	78
	Mean Z score all:	-0.47	-0.64	-0.04	0.18	0.48	0.51	0.37	0.05
	Delta Z score from birth:	.	-0.17	0.42	0.64	0.95	0.98	0.83	0.51
	Z score < -2 SDS (n):	5	9	1	0	0	2	3	3
	Z score < -2 SDS %:	6.4	10.3	1.1	0.0	0.0	2.3	3.6	3.8
	SGA at birth (n):	15	19	19	19	19	19	19	18
	Mean Z score SGA:	-1.56	-1.73	-0.92	-0.44	-0.36	-0.45	-0.56	-0.69
	Z score < -2 SDS (SGA n):	4	7	1	0	0	1	2	2
Total:	n:	181	211	211	211	211	211	205	185
	Mean Z score all:	-0.45	-0.69	-0.14	0.03	0.26	0.31	0.29	0.06
	Delta Z score from birth:	.	-0.23	0.31	0.48	0.71	0.76	0.74	0.51
	Z score < -2 SDS (n):	13	16	2	0	0	4	6	4
	Z score < -2 SDS %:	7.2	7.6	0.9	0.0	0.0	1.9	2.9	2.2
	SGA at birth (n):	37	43	43	43	43	43	42	40
	Mean Z score SGA:	-1.56	-1.63	-0.87	-0.49	-0.48	-0.49	-0.52	-0.54
	Z score < -2 SDS (SGA n):	10	13	2	0	0	1	3	2

CHI-square test comparing number of infants with Z score < -2 SDS

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	103	124	124	124	124	124	121	107
	p-value:	0.46	1.00	0.41	.	.	1.00	0.56	1.00
Group C vs. A:	n:	134	160	160	160	160	160	157	145
	p-value:	1.00	0.39	1.00	.	.	1.00	0.62	0.62
Group C vs. B:	n:	125	138	138	138	138	138	132	118
	p-value:	0.50	0.53	1.00	.	.	1.00	1.00	0.55

t-test comparing change in z score from birth to ...

		Birth	238d	252d	Term	2m	4m	6m	12m
Group B vs. A:	n:	103	124	124	124	124	124	121	107
	p-value:	.	0.27	0.29	0.07	0.16	0.34	0.43	0.07
Group C vs. A:	n:	134	160	160	160	160	160	157	145
	p-value:	.	0.05	0.02*	0.00*	0.00*	0.01*	0.16	0.36
Group C vs. B:	n:	125	138	138	138	138	138	132	118
	p-value:	.	0.67	0.39	0.51	0.09	0.13	0.69	0.35

Appendix 5. Results on glycated haemoglobin and fetal haemoglobin

Poster (EAP Nice 2008)

The Impact of Prematurity on Fetal Haemoglobin and how that can bias measurement of Glycated Haemoglobin

Gitte Zachariassen[1], Gitte Hass Esberg[2], Carl Grytter[3], Mads Nybo[4]

[1] Dept. of Paediatrics, Odense University Hospital, Odense, Denmark

[2] Dept. of Paediatrics, Skejby University Hospital, Aarhus, Denmark

[3] Dept. of Paediatrics, Kolding Hospital, Denmark

[4] Dept. of Biochemistry, Pharmacology, and Genetics, Odense University Hospital, Odense, Denmark

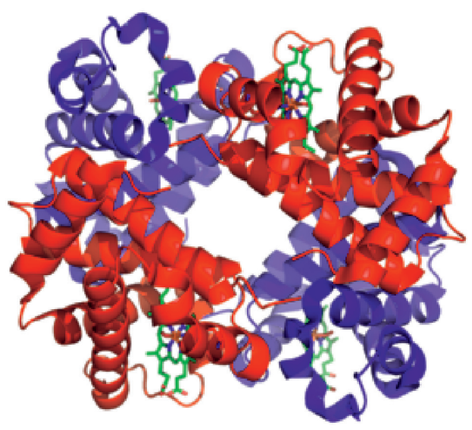
The Impact of Prematurity on Fetal Haemoglobin and how that can bias measurement of Glycated Haemoglobin

Gitte Zachariassen[1], Gitte Hass Esberg[2], Carl Grytter[3], Mads Nybo[4]

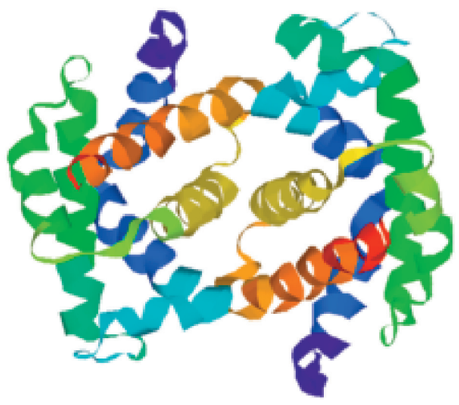
[1] Dept. of Paediatrics, Odense University Hospital, Odense, Denmark
[2] Dept. of Paediatrics, Skejby University Hospital, Aarhus, Denmark
[3] Dept. of Paediatrics, Kolding Hospital, Denmark
[4] Dept. of Biochemistry, Pharmacology and Genetics, Odense University Hospital, Odense, Denmark

Introduction

The extent to which fetal haemoglobin (HbF) concentrations are increased in premature infants at the age of six to eight months is only sporadically described. The influence of HbF on measurement of glycated haemoglobin (HbA_{1c}) has not been investigated among children born premature.



HbA



HbF

Background

Changes in globin chain synthesis in the fetus and newborn: HbF (α2γ2) is produced from early in gestation (4-5 weeks) and is the predominant haemoglobin until after birth. Adult haemoglobin (HbA) (α2β2) is also produced from an early stage (6-8 weeks gestation) but remains at low levels (10-15%) until 30-32 weeks. After this time, the rate of HbA production increases while the HbF production falls resulting in an average HbF-level at term of 70-80% and HbA of 25-30%. After birth HbF decrease to <10% at 4 months of age, to <4% at 6 months of age and to <2% at the age of 12 months with a corresponding increase in HbA.

Premature infants have sporadically been described with a delay in decreasing the level of HbF and corresponding increasing the level of HbA during their first 6 months of life.

Methods

As part of a nutritional follow-up study on premature children, HbF and HbA1c were measured in 46 premature infants at the age of six to eight months (4 months from term, post-conceptional age (PCA) of 56 weeks). Only healthy preterm infants with a gestaional age (GA) <32 weeks have participated in the study and they have been fed three different diets from discharge until 4 months from term = PCA of 56 weeks (the time for blood-sample).
HbA1C and other parameters are planned to be repeated at the age of 6 years.

University of Southern Denmark

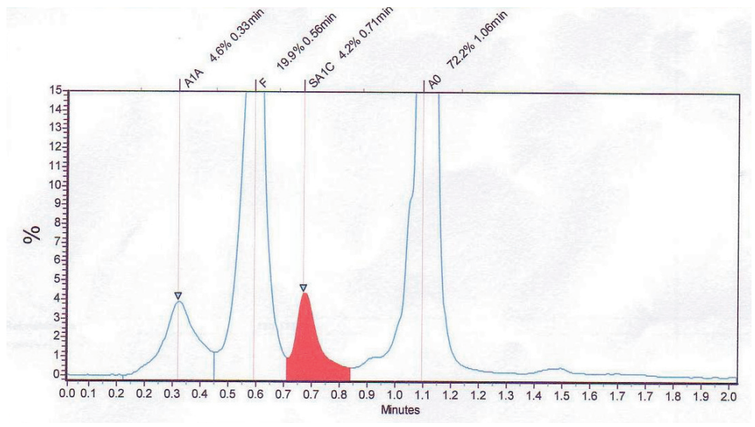
Results

The impact of Gestational age (GA), Birth weight (BW), age at blood-sampling and post-conceptional age (PCA) on HbF and HbA1C.

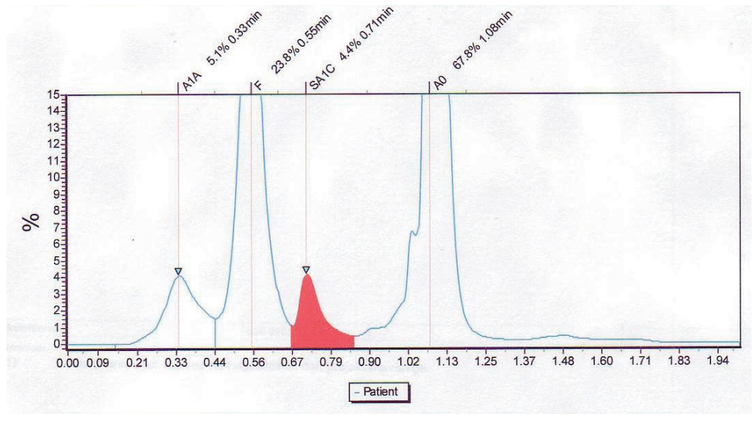
	Mean ± SD	Median (range)
Gestational age at birth (weeks)	29.4 ± 2.1	30.3 (24.4-31.9)
Birth weight (grams)	1209 ± 371	1193 (590-2020)
Age at blood sampling (weeks)	28.1 ± 3.3	28.6 (13.9-33.7)
Post-conceptional age at blood sampling (weeks)	57.4 ± 3.3	57.7 (40.3-62)
HbA1C adjusted (%)	4.9 ± 0.2	5.0 (3.2-5.1)
Fetal haemoglobin (%)	12.0 ± 7.1	10.3 (2.0-39.2)

Results

HbA1c measurement could not be performed in 52% of the 46 infants due to HbF concentrations > 10%, and only 13% of the HbA1c results were reliable without corrections or validation of the chromatogram. Median HbF percentage was 10.3% (range 2.0 to 39.2%).
In a multiple regression model only birth weight (BW) (P = 0.002) and PCA (P < 0.001) were significantly negatively correlated to HbF. Adjusted HbA1c measurements (4.9±0.2%) differed significantly from unadjusted values (4.4±0.4%), (P < 0.0001) with bias for unadjusted values ranging from 1.9 to 33.3%.



Ex. Chromatogram 1:
Premature child born GA 25+6, BW 1026g. Three Blood transfusions and 5 days with mechanical ventilation. Hb 7.3 mmol/l at 4 months corrected age / PCA 56 weeks.

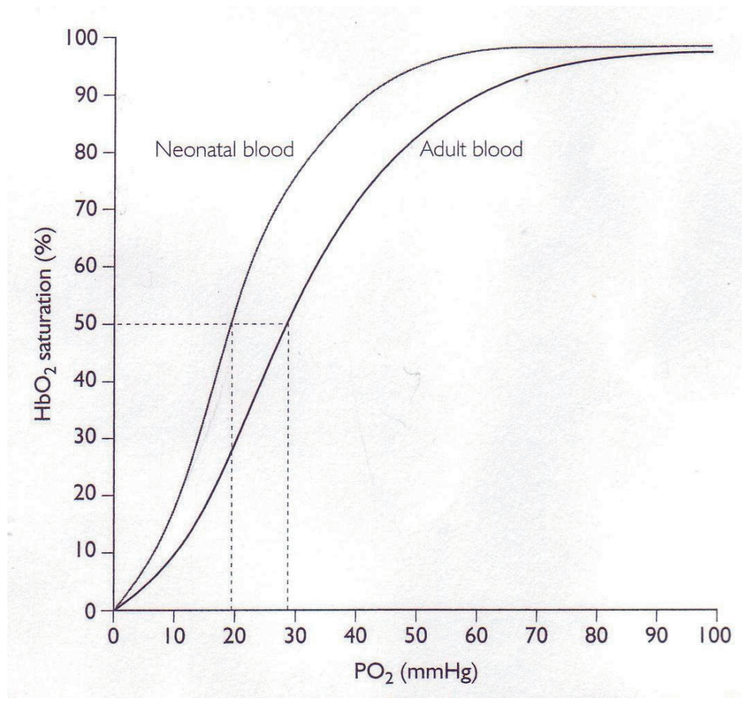


Ex. Chromatogram 2:
Premature child born GA 30+3, BW 1493g. No Blood transfusions and no mechanical ventilation. Hb 7.0 mmol/l at 4 months corrected age / PCA 56 weeks.

In our study 22% (5 unknown, 9 of 41) of the premature infants had one or more blood transfusions. We found no correlation between receiving blood transfusion(s) and the level of HbF, Hb and HbA1c at the age of six to eight months.

Discussion

Increased fetal HbF synthesis has been shown to occur during fetal hypoxemia and severe anaemia.
Studies have found an inverse correlation between the Hb-level and HbF-synthesis.
Premature infants who have received blood-transfusions during the first few days of life had a mean HbF that was lower (around 50-55%) compared to the mean HbF among infants who did not receive blood-transfusions (around 85-90%) before discharge (GA 36 weeks).
Very preterm infants are born at a time when more than 90% of their red blood cells contain HbF and their blood has high affinity for oxygen. When transfusions are performed with adult blood red cells containing HbA the HbO2 affinity will decrease. The shift in HbO2 affinity can expose the immature tissues of these infants to higher levels of oxygen.



The decreased oxygen affinity after transfusion with adult red cells increases the amount of oxygen available to the immature tissues of preterm infants. Lowering oxygen saturation after a transfusion may be protective against hyper oxygenation – and may especially decrease the risk of retinopathy of prematurity (ROP).

Conclusions

The HbF concentration remains high in premature infants at six to eight months of age. The clinical implication of this work is a renewed attention on the prolonged HbF expression in premature infants and the hereof following complications in measuring different analytes, e.g. HbA1c. The issue of HbA1c measurement can be solved with awareness on the condition, while the reason for the prolonged HbF expression and the possible clinical implications of this warrants further investigations.

Abbreviations

PCA, postconceptional age
GA, gestational age
BW, birth weight
Hb, haemoglobin
HbF, fetal Hb
HbA, adult Hb
HbA1c, glycated haemoglobin

References

Halleux, Truttmann, Gagnon and Bard. The effect of blood transfusion on the hemoglobin oxygen dissociation curve of VLBW-infants during the first week of life. Sem. In Perinat. Vol 26, No 6, 2002 (411-415).
Bard, Lachance, Widness and Gagnon. The reactivation of fetal hemoglobin synthesis during anemia of prematurity.
Ped Res Vol 36, No 2 1994 (253-256).
Cochran-Black, Cowan, Neas. The relation between newborn HbF and risk factors for SIDS. Arch Pat Lab Med Vol 125, 2001 (211-217).
Roberts and Murray, Chap. 30 Haematology.

EAP October 2008

Appendix 6. Results on blood pressure

Results on blood pressure among very preterm infants according to nutrition after hospital discharge.

ITT	Mean arterial blood pressure (MAP) ±1SD		
	Group A	Group B	Group C
W36 (PMA) (n=89)	51.1 ± 6.9 (n=28)	51.7 ± 6.9 (n=32)	50.7 ± 7.5 (n=29)
Term CA (n=187)	58.9 ± 8.4 (n=62)	63.7 ± 9.9 (n=67)*#	59.6 ± 9.1 (n=58)
2 months CA (n=203)	65.8 ± 11.6 (n=66)	70.4 ± 11.6 (n=75)*	69.3 ± 14.0 (n=62)
4 months CA (n=204)	71.0 ± 13.3 (n=63)	74.7 ± 11.1 (n=74)	71.1 ± 14.0 (n=67)
6 months CA (n=184)	75.2 ± 14.4 (n=58)	77.0 ± 14.2 (n=62)	72.5 ± 11.6 (n=64)
12 months CA (n=137)	76.3 ± 12.1 (n=41)	75.3 ± 18.9 (n=45)	74.4 ± 10.6 (n=51)

Significant difference ($p<0.05$) (*) B > A (#) B > C.
At 36 weeks PMA girls had a significant (p=0.043) higher MAP compared with boys (coef. 3.1 95% CI 0.1-6.0).

PP	Mean arterial blood pressure (MAP) ±1SD		
	Group A	Group B	Group C
W36 (PMA) (n=54)	52.2 ± 6.6 (n=10)	50.0 ± 7.2 (n=13)	51.5 ± 8.0 (n=22)
Term CA (n=132)	58.9 ± 8.4 (n=48)	63.3 ± 10.8 (n=31)	59.8 ± 9.5 (n=53)
2 months (n=150)	66.9 ± 11.6 (n=51)	69.5 ± 12.2 (n=37)	69.3 ± 14.0 (n=62)
4 months (n=154)	70.9 ± 12.8 (n=50)	73.5 ± 11.3 (n=37)	71.1 ± 14.0 (n=67)
6 months (n=141)	74.1 ± 13.5 (n=47)	75.3 ± 13.3 (n=30)	72.5 ± 11.6 (n=64)
12 months (n=101)	74.8 ± 11.6 (n=32)	70.9 ± 18.9 (n=18)	74.4 ± 10.6 (n=51)

No significant difference between nutrition groups or gender.

The infants in the intervention study were supposed to have their blood pressure measured 3 times and the mean-value of the MAP's to be registered. However, measuring a blood pressure on an infant or child occurred to be difficult and many infants and children only had one measurement done.
The blood pressures have also been measured with infants sitting, lying, screaming, sleeping, just relaxed, or before or after activity / examination. It is unknown if this is of any relevance when measuring blood pressure among infants and children, but it is very difficult to standardize the circumstances when measuring blood pressure among infants and especially active children.

Unfortunately, blood pressure measurement instruments were not the same and standardized ahead of study-start.

The blood pressure measurements in this study are probably not fully reliable due to lack of standardization. A more standardized set up will be necessary in order to produce results that are more reliable.

The infants in the intervention study will be followed and examined at 6 years of age, and the blood pressure measurements and equipment will be standardized.