The impact of early nutrition on extremely preterm infants

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To André and Annie

"Everyone can rise above their circumstances and achieve success if they are dedicated and passionate about what they do"

Nelson Mandela

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Abstract

Background Modern neonatal care has improved the survival rate of extremely preterm infants. These infants are at high risk of malnutrition and growth failure during 3-4 months of hospital care. The objectives of this study was to investigate nutritional intakes during hospitalization and explore associations between nutritional intakes, postnatal growth and retinopathy of prematurity (ROP). Perioperative nutrition in infants undergoing surgery for patent ductus arteriosus (PDA) was also investigated.

Methods This is a population-based study of Swedish extremely preterm infants (<27 weeks) born during 2004-2007 (n=602). Detailed data on nutritional supply and anthropometric measurements during hospitalization were retrospectively retrieved from hospital records. Comprehensive data on cohort characteristics, neonatal morbidity and infant mortality were obtained from the Extremely Preterm Infants in Sweden Study (EXPRESS).

Results During the first 70 days of life, intakes of energy, protein and several micronutrients, with the exception of iron and some vitamins, were less than estimated requirements, and infants showed severe postnatal growth failure. Energy and protein intake predicted growth in all anthropometric outcomes even when adjusting for severity of illness, and fat intake was positively associated with head growth. Low folate intake was positively correlated with poor weight and length gain while high iron intake, mainly explained by blood transfusions, was negatively associated with poor length gain. Furthermore, a low energy intake was associated with severe ROP (stage 3-5). An increased energy intake of 10 kcal/kg/d was associated with 24% decrease in severe ROP (p=0.01). During the first month, 99% of the infants were exclusively fed human milk. Infants who underwent surgery for PDA (n=140) were malnourished, with energy and macronutrient intakes below minimum estimated requirements before, during and after surgery.

Conclusions The severe postnatal growth failure observed in Swedish extremely preterm infants may be prevented by improved intakes of energy, protein, fat and folate and a reduction of the number of blood transfusions. Human milk is the main enteral food source and analyses of human milk macronutrient contents facilitates individualized fortification. Provision of adequate energy intakes during the first four weeks of life may be an effective way to reduce the risk of severe ROP. Perioperative nutrition in infants undergoing PDA surgery needs to be improved. The study results have important implications for nutritional regimens, postnatal growth and health outcome in this new generation of survivors.

Abbreviations

AEI   Adjusted enteral intakes
AGA   Appropriate for gestational age
ANOVA Analysis of variance
BPD   Bronchopulmonary dysplasia
CRIB   Clinical risk index for babies
cPVL   Cystic periventricular leucomalacia
ΔSDS   Delta standard deviation score
DHM   Donor human milk
ELBW   Extremely low birth weight (<1000 gram)
ELGA   Extremely low gestational age (<28 gestational weeks)
EPO   Erythropoietin
EXPRESS Extremely Preterm Infants in Sweden Study
GA   Gestational age
HC   Head circumference
HM   Human milk
IGF-1   Insulin growth factor 1
IVH   Intraventricular hemorrhage
LGA   Large for gestational age
MOM   Mother's own milk
MV   Mechanical ventilation
NICU   Neonatal intensive care unit
NEC   Necrotizing enterocolitis
OR   Odds ratio
PDA   Patent ductus arteriosus
pDHM   Pooled donor human milk
ROP   Retinopathy of prematurity
SD   Standard deviation
sDHM   Single donor human milk
SDS   Standard deviation score
SGA   Small for gestational age
VEGF   Vascular endothelial growth factor
VLBW   Very low birth weight (<1500 gram)

Keywords

Energy intake, enteral intake, extremely preterm infants, folate, growth failure, human milk, iron, macronutrients, malnutrition, micronutrients, nutrient intake, patent ductus arteriosus, protein, retinopathy of prematurity, surgery

Original papers

This thesis is based on following papers, which are referred to in the text by their Roman numerals (I-V)

I. Stoltz Sjöström E, Öhlund I, Ahlsson F, Engström E, Fellman V, Hellström A, Källén K, Norman M, Olhager E, Serenius F, Domellöf M. Nutrient intakes independently affect growth in extremely preterm infants: results from a population-based study. Acta Paediatr. 2013; 102: 1067-74.

II. Stoltz Sjöström E, Öhlund I, Ahlsson F, Domellöf M. Intakes of micronutrients is associated with early growth in extremely preterm infants – a population-based study. Umeå 2014. *Manuscript.*

III. Stoltz Sjöström E, Lundgren P, Öhlund I, Holmström G, Hellström A, Domellöf M. Low energy intake during the first four weeks of life increases the risk for severe retinopathy of prematurity in extremely preterm infants. Umeå 2014. *Submitted.*

IV. Stoltz Sjöström E, Öhlund I, Tornevi A, Domellöf M. Intake and macronutrient content in human milk given to extremely preterm infants. Umeå 2014. *Submitted.*

V. Westin V, Stoltz Sjöström E, Ahlsson F, Domellöf M, Norman M. Perioperative nutrition in extremely preterm infants undergoing surgical treatment for patent ductus arteriosus is suboptimal. Acta Paediatr. 2014; 103: 282-8.

Populärvetenskaplig sammanfattning

Bakgrund

I Sverige föds varje år cirka 100 000 barn och 0.2 % av barnen föds extremt underburna, d.v.s. före 28 fullgångna gestationsveckor. Den medicinska utvecklingen har gjort att allt fler av de allra minsta barnen överlever, men trots en förbättrad medicinsk vård är undernäring och tillväxthämning allt för vanligt hos denna patientgrupp.

Tidigt näringsintag

Bröstmjölk rekommenderas till alla nyfödda barn, vilket även inkluderar prematura barn. När barnet föds avslutas näringstillförseln abrupt, tarmen är dessutom omogen och kan enbart ta emot små mängder enteral näring under de första levnadsdagarna. Av den anledningen är det en nödvändighet att tillföra parenteral näring så snabbt som möjligt efter födelsen för att förhindra svälttillstånd hos barnet. Behovet av protein är som störst hos det extremt underburna barnet och minskar när barnet närmar dig fullgången ålder. Extremt underburna barn får alltid en kombination av enteral och parenteral nutrition, för de allra flesta barn så övergår näringstillförseln till total enteral nutrition vid 3-4 veckors postnatal ålder. Den enterala näringen är företrädelsevis bröstmjölk från barnets mamma eller från donator. Tidig tillförsel av små mängder bröstmjölk påskyndar även mognaden av tarmen. Innehållet i bröstmjölken räcker inte för att täcka de höga näringsbehoven som barnen har och därför behövs enterala tillskott och berikning för att öka innehållet av energi, protein och mikronutrienter.

Bröstmjölkens innehåll av makronutrienter varierar mellan mödrar och även över tid, därför bör bröstmjölken analyseras för att på så sätt möjliggöra en skräddarsydd berikning för det enskilda barnet. Ny teknik och nya näringsprodukter kan användas för att optimera näringstillförseln men kunskap och erfarenheter att utnyttja detta saknas till stora delar.

Tillväxt

Extremt underburna barn sjukhusvårdas oftast till fullgången ålder (ca tre till fyra månader) och bör under denna period flerdubbla sin vikt (från t.ex. 500 gram till 2500 gram). Det är lätt att inse att detta ställer höga krav på adekvat näringstillförsel. Målet är att näringstillförseln fram till fullgången ålder skall bidra till tillväxt, kroppssammansättning och organutveckling

motsvarande den intrauterina fosterutvecklingen. Optimal nutrition av det nyfödda barnet är troligen av stor betydelse för hjärnans tillväxt och utveckling. Många av barnen uppvisar dålig tillväxt långt upp i skolåldern och är både lättare och kortare jämfört med jämnåriga barn som fötts i fullgången tid.

Neonatal sjuklighet

Att födas prematur innebär en ökad risk för sjuklighet därför att organen är omogna när barnet föds. Eftersom lungorna är omogna vårdas många barn i respirator och behandling av steroider och antibiotika är vanligt. Dessa medicinska åtgärder är starkt förknippade med dålig tillväxt hos barnen.

Låg födelsevikt och låg gestationsålder har visats sig vara bidragande orsaker till ökad risk för prematuritetsretinopati (ROP), vilket är den vanligaste orsaken till synnedsättning och blindhet hos extremt prematura barn. När barnen föds är näthinnan inte färdigutvecklad och den ökade syretillförseln i miljön utanför livmodern gör att tillväxtfaktorer nödvändiga för ögats utveckling avtar och blodkärlen i ögat slutar att växa. Detta brukar kallas för den första fasen av ROP utveckling. I den andra fasen återgår miljön i ögat till att vara syrefattig och då ökar mängden tillväxtfaktorer i ögat, det sker nu en snabb tillväxt av blodkärl, men dessa blodkärl är svaga, brister lätt och är inte funktionella för en normal utveckling av näthinnan, fortgår detta förlopp så kan det leda till näthinneavlossning med blindhet som följd. Dessa två faser av ROP utveckling sker inom den första levnadsmånaden. ROP klassificeras i fem olika grader utifrån lätt eller svår grad av sjukdomen, där 3 till 5 räknas som svår grad och är oftast behandlingskrävande. Alla prematurfödda barn i riskzon för att utveckla ROP undersöks i förebyggande syfte för att man ska kunna sätta in behandling i tid.

Ductus arteriosus är ett blodkärl som förbinder lungartären med aorta under fostertiden och när barnet föds sluts detta blodkärl normalt inom de två första levnadsdygnen. Hos prematura barn är detta förlopp fördröjt, detta kallas persisterande ductus arteriosus (PDA). Mer än hälften av alla barn som föds före gestationsvecka 28 har PDA, vilket kan ge bland annat ökad andnings- och hjärtfrekvens och försämrad lungfunktion. Om ductus inte sluts spontant så behandlar man detta med läkemedel (Ibuprofen eller Indometacin) eller genom operation.

Att ge mycket vätska till extremt underburna barn har associerats med risk för ökad sjuklighet, tex PDA, men även ökad risk för dödlighet. Av medicinska orsaker kan det därför vara nödvändigt med vätskerestriktion vilket ytterligare försvårar tillräcklig näringstillförsel.

Det behövs ytterligare vetenskapliga underlag för att kunna rekommendera näringsintag och lämpliga näringsprodukter till den här patientgruppen, de underlag som finns är i många fall bristfälliga. Epidemiologiska studier har visat samband mellan låg födelsevikt och en alltför snabb tidig tillväxt s.k. "catch-up" med en ökad risk för diabetes, övervikt och hjärt-kärlsjukdom senare i livet. Därför finns det ett stort behov av att undersöka hur tidigt näringsintag påverkar den långsiktiga hälsan hos extremt underburna barn.

Syfte

Det övergripande syftet med studien var att undersöka betydelsen av det tidiga näringsintaget och dess samband med tillväxt och korttidssjukdom hos extremt underburna barn i Sverige.

Mer specifika syften var att undersöka hur mycket av energi, protein, fett, kolhydrater samt vitaminer och mineraler som barnen fick och jämföra det med det uppskattade behovet. Vi ville även undersöka vilken näring som barnen fick under sjukhusvistelsen med fokus på enteralt intag och bröstmjölk. Eftersom ROP utvecklas under den första levnadsmånaden var det angeläget att undersöka det tidiga näringsintaget och eventuellt samband med utveckling av ROP. Många extremt underburna barn drabbas av PDA och eftersom kirurgiskt ingrepp är vanligt förekommande som åtgärd är det viktigt att belysa näringsintaget under operationsveckan och jämföra det med det uppskattade näringsbehovet.

Studie population och metod

Detta är en retrospektiv nationell studie av 602 extremt underburna barn (<27 gestationsveckor) födda i Sverige mellan åren 2004 och 2007. Detaljerade uppgifter om den tidiga nutritionen och tillväxtmätningar insamlades från barnens sjukhusjournaler.

Dagligt intag av all enteral och parenteral näringstillförsel, berikningsprodukter, vitaminpreparat, mineraltillskott och elektrolyter samlades in för de första 28 levnadsdagarna. Därefter samlades data för en dag per vecka (dag 35, 42, 49 etc.) under resterande sjukhusvistelse. Information om givna blodprodukter, läkemedel och andra intravenösa infusioner samt alla uppgifter om barnens vikt, längd och huvudomfång inhämtades även från barnens sjukhusjournaler.

Omfattande bakgrundsuppgifter som till exempel neonatal sjuklighet, behandlingsinsatser och dödlighet har samlats prospektivt för barnen i den

ursprungliga kohorten Extremely Preterm Infants in Sweden Study (EXPRESS).

Resultat

Det första och andra arbetet visade ett samband mellan tidigt näringsintag och barnens tillväxt under de första 70 levnadsdagarna. Det var 531 barn som ingick i respektive arbete, i medeltal var barnen födda i gestationsvecka 25 + 2 dagar och medelvikten var 765 gram. Barnen visade dålig postnatal tillväxt, men en viss catch-up tillväxt för vikt och huvudomfång kunde ses efter de första 35-40 levnadsdagarna. Längdtillväxten visade ett annat mönster än vikt och huvudomfång och ingen direkt catch-up tillväxt var uppenbar. Under de första 70 levnadsdagarna fick barnen mindre energi och protein än uppskattat behov. Intaget av kalcium, fosfor, magnesium, zink, koppar, jod, vitamin D och folat var lägre än uppskattat behov, medan intag av järn, vitamin K och flertalet av vattenlösliga vitaminer var högre. Det höga intaget av järn berodde till stor del av givna blodtransfusioner. Oberoende av en rad riskfaktorer sågs samband mellan ett högre energi- och proteinintag med en bättre tillväxt av vikt, längd och huvudomfång. Ett högre intag av folat var associerat med bättre viktutveckling och längdtillväxt medan ett högt intag av järn visade samband med sämre längdtillväxt.

I det tredje arbetet utvärderades sambandet mellan tidigt näringsintag med risk att drabbas av svår ROP. Av inkluderade 498 barn hade 34.5 % diagnostiserats med svår ROP, av dessa behandlades 56 %. Resultat från detta arbete visade att en ökning av energiintaget med 10 kcal/kg/dag var kopplat till en 24 % minskning av risken att utveckla svår ROP hos dessa extremt underburna barn.

I det fjärde arbetet undersökte vi i vilken omfattning extremt underburna barn får bröstmjölk under sjukhusvistelsen. Arbetet omfattade 586 barn och det enterala intaget under de första 12 levnadsveckorna undersöktes. Under den första levnadsmånaden fick 99 % av barnen bröstmjölk, en mycket låg andel av barnen fick modersmjölksersättning. Nästan tre fjärdedelar av barnen fick bröstmjölk från sin mamma. Innehållet av protein, fett och kolhydrater i bröstmjölken från barnens egna mammor analyserades. Våra resultat visade att innehållet i bröstmjölk varierar stort mellan mammor och över tid. Proteininnehållet i mödrarnas bröstmjölk sjönk från 2.2 till 1.2 gram per 100 ml under de första 100 dagarna efter barnets födelse, medan innehållet av kolhydrater i bröstmjölken ökade under de första tre veckorna för att sedan stabiliseras.

I det femte arbetet undersöktes näringstillförsel hos extremt underburna barn som opererades för PDA. Det var 140 barn som behandlades med kirurgi. Näringsintaget under operationsveckan analyserades (operationsdag, tre dagar innan och tre dagar efter). Under samtliga dagar var intaget av energi, protein och fett lägre än uppskattat behov. Intag av energi, protein och fett var allra lägst under operationsdagen samt hos de barn som opererades under den första levnadsveckan.

Slutsats

Extremt underburna barn hade ett lägre intag av energi och näringsämnen än uppskattat behov och uppvisade en svår postnatal tillväxthämning Ett högre energi- och proteinintag under de första 70 dagarna hade starkt samband med bättre tillväxt av vikt, längd och huvudomfång. Intaget av flera mikronutrienter var lägre än uppskattat behov. Ett lågt intag av folat hade samband med sämre vikt- och längdutveckling medan ett högt järnintag hade samband med sämre längdtillväxt. Optimerat intag av energi, protein och folat samt reducerad mängd av blodtransfusioner kan minska tillväxthämning hos extremt underburna barn.

Svenska extremt underburna barn får till mycket stor del bröstmjölk som huvudsaklig enteral näring. Innehåll av näringsämnen i mödrars bröstmjölk visar stor variation, både mellan mödrar och över tid. Analyser av bröstmjölk, förslagsvis varje vecka under den första månaden, skulle möjliggöra en mer individanpassad näringstillförsel till dessa barn.

En ökad tillförsel av energi under de första fyra levnadsveckorna, genom både enteral och parenteral näringstillförsel kan vara ett effektivt sätt att minska risken för svår ROP hos extremt underburna barn.

De barn som genomgick operation för PDA fick för lite energi, protein och fett under operationsdagen, tre dagar innan och tre dagar efter operation. I dagsläget är det svårt att veta vilken påverkan för barnens tillväxt och hälsa denna undernäring kan medföra. Men det finns ett tydligt behov att förbättra näringsintaget vid ductus operation för dessa barn.

Klinisk betydelse

Resultaten från denna studie kommer att få en stor klinisk betydelse och har redan resulterat i underlag till bättre nutritionsrutiner inom neonatalvården i Sverige (Socialstyrelsen, 2013). Vi hoppas att ett förbättrat näringsintag kommer att ge bättre tillväxt och hälsa på kort och lång sikt till dessa små barn.

Introduction

The extremely preterm infant

In Sweden, there are 9.5 million inhabitants. There are about 100 000 newborn babies every year which are delivered at 42 obstetric departments. Preterm birth is defined as infants born before 37 completed weeks of gestation.[1] There are three sub-categories of preterm birth: moderate to late preterm (32 to <37 weeks), very preterm (28 to <32 weeks) and extremely preterm (<28 weeks).[2] Approximately 0.2 % of all infants in Sweden are born extremely preterm[1]; these infants are also commonly referred to as extremely low gestational age (ELGA) infants.

In Sweden, there are seven University hospitals with high-qualified perinatal care, to which high-risk pregnancies and care of extremely preterm infants are centralized. These University hospitals are suited in Umeå, Uppsala, Stockholm, Örebro, Linköping, Gothenburg and Lund (**Figure 1**).
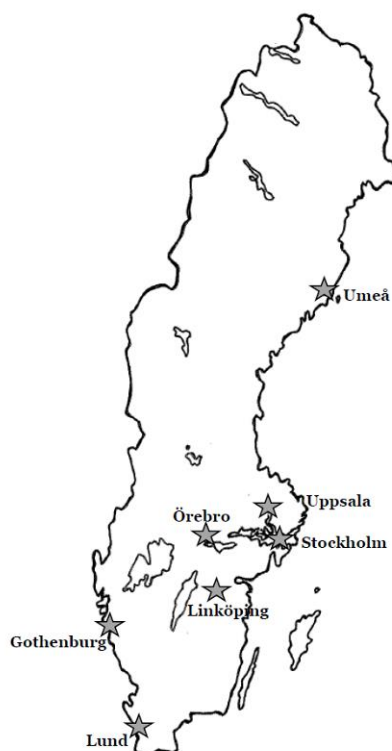

Figure 1. Map of Sweden and the seven included University hospitals.

Thanks to medical advances during the last decades, the survival rates of extremely preterm infants have improved considerably.[3-5] However, there are still concerns about the risks for morbidity and later disability, not least impaired neurodevelopment.[6, 7] Adverse developmental programming may increase both disease susceptibility in childhood and the risk of chronic diseases in adult life.[8, 9]

Nutritional supply

There are still a lack of evidence-based data on the requirements of energy and nutrients for the extremely preterm infant. Nutritional requirements for extremely preterm infants are often estimated using consensus recommendations of nutrition experts on specific nutrients.[10-14] It has been shown that preterm infants accumulate nutritional deficits during initial hospital stay.[15] Insufficient intakes of energy and macronutrients results in poor postnatal growth, which has been associated with poor neurodevelopmental outcome.[16] The preterm gastrointestinal tract is immature, leading to impaired motility, digestion and absorption of nutrients and may limit tolerance to enteral feeds.[17]

Because of fetal growth being more rapid than infant growth, the extremely preterm infant have extraordinarily high nutrient requirements and the stores of nutrients at birth are very limited.[18] Nutrients are supplied via a combination of enteral and parenteral routes during the first weeks of life[19], a gradual transition to solely enteral nutrition is often established within the first three to four weeks of life, at least in those infants with less morbidity. **Figure 2** demonstrate the transition state between parenteral and enteral nutritional routes in an infant born at 25 completed gestational weeks with a birth weight of 714 gram.

Enteral nutrition

Human milk (HM) is the preferable standard for infant feeding and nutrition, including those born extremely preterm.[11, 20] Early intakes of HM stimulates maturation of the gastrointestinal tract[21] and enteral feeding is recommended to start as soon as possible after birth. Early enteral feedings, often referred to as trophic feeding, reduces intestinal permeability[22] and may reduce the risk of necrotizing enterocolitis (NEC).[23, 24] Furthermore, early enteral feedings facilitate earlier achievement of enhanced enteral intakes in preterm infants.[25] HM compared to formula feeding, reduces the incident of NEC[26] and has been associated with neurodevelopmental advantages.[27]
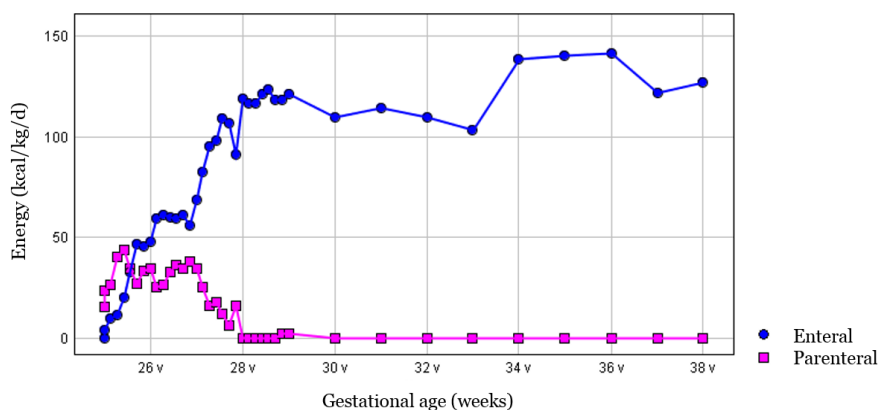
Figure 2. Supply of energy through enteral and parenteral routes.

Mother's own milk (MOM) is the first hand choice, in those cases when the mother cannot provide sufficient amounts of HM, donor human milk (DHM) is recommended to be used.[28] A human milk donor is a woman who is not the biological mother of the recipient.

Sweden has a long tradition to feed preterm infants with HM, and there is a well-developed system regarding milk banks.[29] Since the nutrient content in HM changes over time and differs between mothers[30, 31] information of macronutrient content is necessary to avoid risk of undernutrition or overnutrition of the preterm infant. Macronutrient content in DHM is also lower compared to HM from mother's giving birth to preterm infants.[32]

In order to meet nutritional requirements in extremely preterm infants it is necessary to add human milk fortifiers (HMF) to HM.[32] Several Swedish neonatal intensive care units (NICUs) routinely analyze HM for macronutrient content in order to establish the optimal amount of HMF.[29] The most common way to analyze HM in Sweden is to use mid-infrared spectrophotometry.[33, 34] All DHM are routinely heat-treated using the Holder pasteurization (62.5∘C for 30 minutes). This technique is often used to eliminate potential viral and bacterial contaminants that might occur in DHM.[35, 36]

Infants with a birth weight below 1500 grams receive enteral fluids through tube feeding, usually nasogastric feeding, because of their inability to coordinate sucking, swallowing and breathing.[19, 37] There are different methods for tube feeding; intermittent bolus feeding is one conventional method that is used. Prescribed volume of milk is then given through nasogastric tube feeding during 10 to 20 minutes every two to three hours.

Another feeding method is continuous nasogastric feeding. There is not enough evidence that either of the methods is preferable than the other.[38] Bolus feeding was the most common method in Sweden during the time of our study.

Parenteral nutrition

To meet nutritional needs of the extremely preterm infant, parenteral nutrition is required. Starvation for just one day can be detrimental in the smallest preterm infant, parenteral nutrition should therefore be introduced as soon as possible after birth.[12] There are no evidence that early provision of parenteral nutrition increases morbidity or mortality in preterm infants.[39] There are still questions about beneficial effects from early supply of parenteral nutrition on postnatal growth.

Parenteral nutrition is administrated mainly through central venous catheters; this access is required to administer the high osmolality mixture to cover adequate nutrient supply directly after birth.[12] Peripheral route is mainly used for partial or supplemental parenteral nutrition and peripheral veins are only used for short-term nutritional support.

Catheters have been shown as a significant risk factor for infections and late-onset sepsis, which often is caused by gram-positive bacteria.[40] Late-onset sepsis is a common complication in preterm infants and is associated with mortality and neurodevelopmental impairment. Most cases of infants requiring prolonged total or near total parenteral support are often due to either gastrointestinal malformations or NEC.[12]

Nutritional needs of the preterm infant

Problems that may prevent clinicians from feeding extremely preterm infants sufficient amounts of nutrients includes feeding intolerance, concurrent morbidity and metabolic problems such as hyperglycemia.[41, 42] A more active nutritional approach has been proposed to prevent catabolism in the infant during the first days of life.[43] A few intervention studies have suggested that improved nutritional intakes reduces postnatal growth faltering in preterm infants with a birth weight less than 1500 grams.[44] Postnatal growth failure in preterm infants is associated with increased motor and cognitive impairment at seven years of age.[45] However, it is still unclear if early active nutritional support of extremely preterm infants may reduce the incidence of cognitive impairments.

Requirements of energy and nutrients for extremely preterm infants are divided in three phases according to Tsang et al.[10] The first phase includes the day of birth with administration of parenteral nutrition as soon as possible after birth and adding small volumes of enteral feeds if appropriate. After birth a period of physiologic and metabolic instability occur, this condition may last several days, this is called the transition phase. When the infant is more stable, requirements adjust to the growing phase.

Energy

The energy supply to the extremely preterm infant should cover the nutritional needs e.g. basal metabolic rate, growth and compensate any previous malnutrition.

Energy can be divided into protein and non-protein energy; non-protein energy includes only energy from fat and carbohydrates. But in clinical use, all macronutrients are included in the total calculations of energy. Energy is often calculated in kilocalories (kcal), using energy factors by Atwater's: 4 kcal per gram for protein and carbohydrates, respectively and 9 kcal per gram for fat. Macronutrients may also be calculated as energy percent (E %), which is the proportion of total energy.

During the first days of life energy requirements are less defined. According to Tsang et al[10], energy supply via enteral nutrition are recommended to be 90-100 kcal/kg/day while energy from parenteral nutrition are recommended to be 75-85 kcal/kg/day. In a recent published guideline from The National Board of Health and Welfare in Sweden[14], a recommended energy intake of 105-125 kcal/kg/day at day four of life is proposed. This recommendation is calculated with consideration using a combined supply of parenteral and enteral nutrition, 50 % respectively.

Minimal enteral energy requirements of growing extremely preterm infants below 1000 grams are 110-130 kcal/kg/day[10, 11, 13] and 105-110 kcal/kg/day[10, 12, 13] for parenteral nutrition. The Swedish guideline recommend 115-135 kcal/kg/day for full enteral supply and 105-115 kcal for full parenteral supply.[14]

It may not be suitable to increase more energy than recommended if the infant's growth is poor, since it is more likely that protein or other nutrients are limiting factors. A protein to energy ratio are therefore appropriate to use in order to determine suitable intakes. The recommended share is 3.2 to 4.1 gram protein per 100 kcal.[11]

Protein

Protein synthesis in the body exceeds the protein intake; this means that proteins are persistently broken down in the body and resynthesized, generally called protein turnover.[46] To increase the lean body mass it is necessary that the rate of protein synthesis exceeds the protein breakdown, this is referred to net protein accretion.

The need of protein is higher at the beginning of the third trimester and decline slightly towards the end of gestation. The protein turnover results in a loss of about 1.0 gram/kg/day in extremely low birth weight (ELBW) infants[47], this will lead to detrimental protein deficits if infants only receive supplemental intravenous glucose during the first days of life. Early negative protein balance should be avoided if possible. It has been shown that as little as 1.0-1.5 gram of protein/kg/day can prevent a catabolic state during the first days of life in ELBW infants.[48]

According to Ziegler et al., the reference fetus at 24-28 gestational weeks grows approximately 17 gram/kg/day and has a protein accretion nearly 2 gram per/kg/day.[18] To achieve postnatal growth and protein accretion in accordance to intrauterine growth it is necessary to provide at least 3 gram/kg/day of protein and an energy intake of 90 kcal/kg/day.[49] However, this amount of protein and energy may not be enough to eradicate loss of lean body mass that occurred before the infant regained birth weight. Supply of 3.5 gram/kg/day of protein in combination of 3 gram/kg/day of lipids resulted in a protein retention of 4 gram/kg/day in preterm infants with a gestational age between 24 to 32 weeks.[50] While intakes of 2.5 gram/kg/day resulted in a protein retention of 2.0 gram/kg/day.[47]

All current nutritional recommendations regarding protein intakes are based from studies on healthy preterm infants. Neonatal illness such as infection diseases, NEC and bronchopulmonary dysplasia (BPD) contribute to a need of additional protein intakes.[15, 47]

Extremely preterm infants should receive at least 1.5-2.0 gram protein per kg and day from the first day of life.[10, 12] Swedish guidelines recommend an intake of 2.0-2.4 gram/kg/day during the first day of life.[14] Minimum enteral requirements of protein for growing extremely preterm infants are 3.8-4.5 gram/kg/day.[11, 10, 13] Swedish guidelines recommend an protein intake of 3.4-4.5 gram/kg/day on the fourth day of life and forward.[14] Minimum parenteral requirements of protein for growing extremely preterm infants are 3.5 gram/kg/day[10, 12, 13], while Swedish guidelines recommend an protein intake of 3.5-4.0 gram/kg/day.[14]

Fat

Protein synthesis is an energy-demanding process and because protein are the driving force for growth, sufficient energy supply is necessary to optimize protein synthesis. Fat (lipids) are effective as energy source since the energy content in one gram fat is more than double than in one gram of carbohydrates. Fat also supply essential fatty acids, which is necessary for the structural composition of the brain and its myelination.[51] According to guidelines, intravenous lipid emulsions should be started no later than the third day of life but may be started on the first day.[12] Failure to provide lipids at an early stage of life may contribute to suboptimal calorie intakes and proteolysis. Supply of 3 gram/kg/day of lipids and 3.5 gram/kg/day of proteins immediately after birth was well tolerated in preterm infants with a gestational age below 32 weeks, without metabolic or respiratory complications.[50] A meta-analysis showed that initiation of lipids within the first two days of life in very low birth weight (VLBW) infants seems to be safe and well tolerated, and lipids emulsions that are not purely from soybeans might be associated with a lower incidence of sepsis.[52] Early lipid supply is associated with improved neurological developmental outcome at one year of age in VLBW infants.[53]

The quality of fat intake may be important because there is some evidence that increased omega-3 long chain polyunsaturated fatty acids (ω-3 PUFAs) reduces the risk of retinopathy of prematurity (ROP).[54, 55] Lipid emulsions supplied by parenteral nutrition can be based on oils from soybean, fish, or olives.[56] A new lipid emulsion, SMOFlipid, is based on a mixture of soybean (30 %), medium-chain triglycerides (30 %), olive oil (25 %) and fish oil (15 %). This lipid emulsion provides a good source of energy, essential fatty acids, ω-3 PUFAs and monounsaturated fatty acids and is also supplemented with vitamin E. SMOFlipid has been shown to reduce oxidative stress by reducing lipid peroxidation in preterm infants.[56] During the years 2004 to 2007, Swedish extremely preterm infants received only a purely soybean-based lipid emulsion.

The upper limit of fat content in HM is approximately 5.7-6.0 gram/100 kcal[57], proportion of fat of total energy are therefore proposed to be 51-54 E %. A reasonable range for most preterm infants are 40-55 E %. Although, some infants with restricted fluid intakes may need a higher intake of fat to meet the energy needs.

Minimum estimated enteral and parenteral requirements of total fat during the first day of life according to international guidelines are 1.0 gram/kg/day while Swedish guidelines recommend 1.0-1.5 gram/kg/day.[10, 14]

For growing extremely preterm infants estimated enteral requirements are 4.8-6.6 gram/kg/day[11] or 6.2-8.4 gram according to Tsang guidelines.[10] Parenteral requirements for fat are 3.0-4.0 gram/kg/day,[10, 12, 13] and in order to prevent essential fatty acid deficiency a minimum linoleic acid intake of 0.25 g/kg/day should be given. Swedish guidelines recommend 5.0-8.0 gram/kg/day for enteral nutrition and 3.0-4.0 gram/kg/day for parenteral nutrition for growing extremely preterm infants.[14]

Carbohydrates

The minimum carbohydrate limit is based on energy requirements of glucose-dependent organs such as the brain. Excessive intakes of carbohydrates (glucose) may contribute to hyperglycemia. In animal models, hyperglycemia reduces the capacity to fight infections.[58] Hyperglycemia is a well-known problem in preterm infants and the most common cause is too much intravenous glucose infusion. Stress in the preterm infant releases a number of undesirable hormonal changes. Stress-reactive hormones such as adrenalin and nor-adrenaline increases. These hormones inhibit insulin secretion and insulin action which promote glycogen breakdown, leading to hyperglycemia.[59] The best way of treating infants with hyperglycemia is by reducing the glucose infusion rate but insulin can also be used.

Minimum estimated enteral as well as parenteral carbohydrate requirements during the first day of life are 7.0 gram/kg/day.[10] Swedish guidelines recommend 7.0-10.0 gram/kg/day increasing to 11.0-16.0 gram/kg/day on the fourth day of life.[14]

Enteral estimated requirements of carbohydrates for growing extremely preterm are 11.6-13.2 gram/kg/day or 9.0-20.0 gram/kg/day according to Tsang et al., while parenteral requirements of carbohydrates are 13.0-17.0 gram/kg/day.[10] Swedish guidelines recommend 9.0-15.0 gram/kg/day for enteral nutrition and 13.0-17.0 gram/kg/day for parenteral nutrition for growing extremely preterm infants.[14]

Micronutrients

The provision of an adequate amount of micronutrients is an important part of all nutritional support by both enteral and parenteral regimens. The optimal parenteral requirements of micronutrients for extremely preterm infants are not known due to lack of adequate evidence. Nonetheless, estimated requirements are presented in international as well as Swedish guidelines.[10-14]

There are limited information on the importance of micronutrients for the extremely preterm infant, especially regarding growth outcome. Several micronutrients are important for brain growth, these includes iron, zinc, copper, iodine, selenium, folate and vitamin A.[60] Some micronutrients are more essential for bone growth, e.g. calcium, phosphorus and vitamin D.[61] Water-soluble vitamins have a key role as cofactors for enzymes in the energy metabolism.[62] Minerals, such as iron, zinc, copper, manganese, iodine and selenium are essential for the human and play an important role in many metabolic pathways. Premature birth is associated with low stores of nutrients and infants are therefore at high risk for deficiencies. Three objectives have been taking into consideration when recommending intakes of minerals to infants born preterm: prevention of deficiencies, intakes that will allow for accretion similar that in utero, avoidance from toxicity from excessive intakes.[10]

It is crucial to provide micronutrients in parenteral solutions as well as enteral fluids to avoid deficits in micronutrients. Extremely preterm infants often receive oral supplements, e.g. multi-vitamins, iron drops and capsules or mixtures of calcium and phosphorus in order to meet estimated requirements. Yet, by adding vitamins, minerals and HMF in enteral feeds there will be changes in the osmolality and the osmolarity to consider.[63, 64]

The content of micronutrients changes over time in HM. Some micronutrients in HM, e.g. iodine and vitamin B_{12}, are reflected of the nutritional intakes by the mother.[65, 66] Enteral feedings are usually enriched with HMF in order to meet the nutritional requirement of the infant. The micronutrient content differs among HMF, some contains iodine while some lack this mineral.

It is important to take all sources of micronutrients into account in order to ensure that intakes reaches the estimated requirements.

Growth of the preterm infant

Extremely preterm infants usually require three to four months of hospital care, during which time they are at high risk of malnutrition and growth restriction. The smallest infant may increase their weight five-fold (e.g. from 500 g to 2500 g) during hospitalization (**Figure 3**). This rapid growth places high demands on adequate nutrient supply.

Figure 3. Extremely preterm girl, weighing 545 gram at birth, holding her father's thumb.
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There are different ways of defining premature infants due to birth weight; VLBW is defined as a birth weight below 1500 gram and ELBW is defined as a birth weight below 1000 gram.[67] Depending on birth weight, infants can also be classified as appropriate for gestational age in weight (AGA), they can be small for gestational age (SGA) or they can be large for gestational age (LGA).[68] Being born SGA can be defined as birth weight less than 10th percentile.[69] In Sweden and in our study, SGA is defined as a birth weight of at least two standard deviations (SD) below normal growth curve.[70]

Weight

After birth, an initial decrease in weight reflect changes in cellular fluid compartments.[71-73] The typical growth pattern in healthy newborn infants is usually a weight loss within 5 to 10 % of the birth weight. The nadir in weight often occurs at three days and birth weight is often regained at 5 to 10 days of life. The extremely preterm infant may lose even more than 10 % of birth weight; this exceeded weight loss can be caused by dehydration but may also be influenced by nutrition. It has been observed that extremely preterm infants with a gestational age below 26 weeks lost 16 % in weight, with a nadir in weight on the sixth day of life.[74] Infants regained birth weight at 18 days of life in this study. A probable cause is the change of the nutritional supply that abruptly ends at birth and the preterm infant become in a

catabolic state.[42] Weight is the most frequent anthropometric measurement within NICUs. Medical treatments and total fluids are prescribed per kilogram and day therefore; infants are often weighed every day or several times per week. Weight gain is the parameter that is measured in response to changes in nutritional intakes of the infant. An energy intake of 110-130 kcal/kg/day in healthy, growing premature infants assumes a weight gain of 16-20 gram/kg/day.[10, 11, 13]

Mimicking intrauterine growth is frequently used as golden standard which implies that the target is to achieve postnatal growth similar to a fetus in the same gestational age.[74] The recovery after early growth deficits is called catch-up growth, which refers to accelerated growth that mainly occurs after discharge.[75, 76] Growth charts used within neonatal care are based on the birth size of all newborns in a certain area or country and are presented separately for boys and girls. For Northern European countries, the Swedish reference is most appropriate to use.[77] This goal of postnatal growth may be difficult to achieve due to various diseases and adverse conditions these infants experience during the NICU stay.[78, 79] Many extremely preterm infants are exposed to postnatal steroids for treatment of severe lung disease and growth failure has been identified as an adverse side effect of this treatment strategy.[80] Eherenkranz et al showed that infants born before 29 gestational weeks did not achieve growth corresponding to the reference fetus at the same gestational age.[81] It is well known that extremely preterm infants often experience substantial growth failure, which may even sustain through school age.[82]

Length

Measuring length of extremely preterm infants can be quite problematic due to infant health status, and measuring length is more invasive compared to measuring weight and head circumference (HC). Length of the preterm infant can be measured either inside or outside the incubator (**Figure 4**).

When measuring length, one person is holding the infant's head in a vertical position with the crown of the head touching the fixed headboard. A second person extend the legs and firmly place the movable footboard against the infant's heels. Accurate measurement technique is vital for evaluating longitudinal growth and it is preferable if the same persons measure the infant.

Figure 4. Flexible measuring tape inside an incubator.
© Elisabeth Stoltz Sjöström

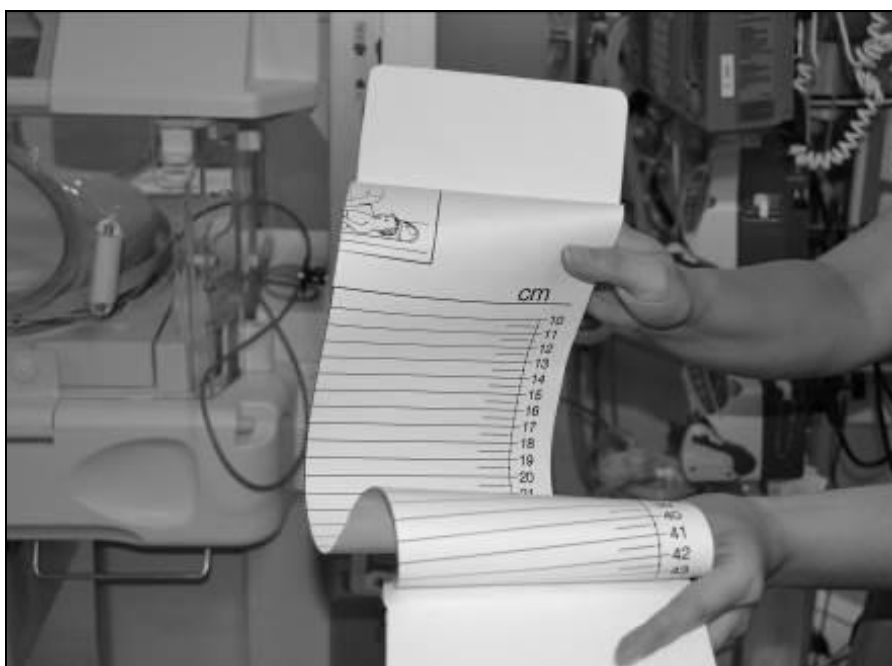Flexible cloth or plastic measuring tape (**Figure 5**) or standard measuring length boards (**Figure 6**) are used.

Figure 5. Flexible measuring tape. ©Elisabeth Stoltz Sjöström

Data on longitudinal growth in extremely preterm infants are quite scarce. Poor longitudinal growth has been associated with neurological disadvantages at two years of age in VLBW infants.[83] Saigal et al showed that preterm infants with a birth weight below 1000 grams had a significantly shorter stature in young adulthood compared with term born peers.[84] Both boys and girls were shorter than their control subjects were at 8, 14 and 23 years of age.

Figure 6. Standard measurement board inside an incubator.
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Head circumference

Growth of the HC implies brain growth. During the third trimester, brain growth accelerate and disturbance in nutritional supply during this period can be devastating for neurodevelopmental outcomes.[60] Recently, Morgan et al showed in a randomized controlled trail that postnatal head growth in preterm infants can be improved with early nutritional interventions.[85] In other studies, early provision of energy and protein has been associated with increased postnatal head growth and enhanced neurological outcomes.[86, 87] When measuring the HC, a standard nonstretchable measuring tape is used; HC is measured in centimeter and to the nearest millimeter.

Early nutrition and growth

Still, randomized clinical trials is relatively scarce concerning early nutrition and its causal effects on growth in extremely preterm infants. Nevertheless, new insights of beneficial effects of early nutritional supply on growth outcomes of the preterm infant has been accumulating during the last decade.

Initiating parenteral nutrition soon after birth has been associated with enhanced growth at 36 weeks gestational age and improved HC at 18 month corrected age.[86] It has recently been shown that enhanced nutritional supply to VLBW infants during initial hospital stay promotes postnatal catch-up growth in weight and HC.[88]

Christmann et al observed that infants receiving higher supplementations of parenteral amino acids and energy combined with more enhanced enteral feedings still developed cumulative protein and energy deficits during the first two weeks of life.[89] Postnatal growth was then compared with another group of infants who received nutrients according standard protocol. The group with enhanced feeding protocol achieved full enteral feeding sooner but both groups showed a decline in weight development, even if the decline in weight was less among infants with the enhanced feeding protocol. On the other hand, protein intakes did not reach the assumed optimal intake for many of the infants in the study. However, active nutritional support in this study was well tolerated by the infants suggesting that increased fortification of enteral feedings at an early stage might be one solution. One should keep in mind that infants in this latter study included preterm infants born before 34 gestational weeks, with very few extremely preterm infants included.

In a previous study, growth outcome were evaluated after optimizing the energy and protein intakes in extremely preterm infants.[90] This study showed that cumulative deficits of energy and protein can be reduced, resulting in optimized growth during the first week of life. A ready-to-use standardized parenteral solution was initiated as soon as possible after birth contributing to achieve a more appropriate protein supply close to estimated requirements. However, this study included only a small number of infants below 26 gestational weeks and this nutritional approach may therefore be invalid for the most immature and sick infants.

There are still unsolved questions regarding early postnatal growth in extremely preterm infants. It is still unclear if nutrition have similar effects on growth during different periods after birth, and there may be either protective or harmful effects left to define.

Neonatal morbidity

Extremely preterm infants admitted to the NICU often face complications of preterm birth. Morbidities mentioned below are meant to be a useful guide for the reader of this thesis who are not familiar with neonatal care.

Neonatal severity of illness is often established by using the clinical risk index for babies (CRIB).[91] This neonatal scoring index system includes birth weight, gestational age, presence of congenital malformations, maximum and minimum inspired oxygen and maximum base excess during the first 12 hours after birth. A high CRIB score has been associated with increased risk for cerebrovascular complications in extremely preterm infants.[6] Another well used system is the Apgar, developed by Virgina Apgar in the beginning of 1950:s.[92] At 1, 5 and 10 minutes after birth the following characteristics are scored: heart rate, respiratory effort, muscle tone, irritability, and color. Each of these characteristics is assigned a value of 0 to 2. The total sum is the score of combined characteristics and a score of seven or higher indicates that the infant's condition is good or excellent.

Patent ductus arteriosus

A common neonatal morbidity is patent ductus arteriosus (PDA), in the Extremely Preterm Infants in Sweden Study (EXPRESS) cohort, 61 % (n=303) of infants required treatment for PDA.[6] Ductus arteriosus is a blood vessel that connects the main pulmonary artery to the descending aorta during the fetal period; usually the ductus closes within the first 24 to 48 hours after birth.[93] However, spontaneous closure of ductus arteriosus is often delayed in preterm infants and closure rate also declines at low gestational ages.[94] If no closure occurs, conditions such as pulmonary over circulation and left ventricular volume overload might lead to pulmonary edema, loss of lung compliance, and deterioration of respiratory status.[93] Interventions for PDA includes both pharmacological treatment (Ibuprofen and Indomethacin) and surgical closure, where pharmacological treatment is less effective in infants born at very low gestational age.[94] Moreover, PDA has been associated with increased risk for BPD, intraventricular hemorrhage (IVH) and NEC.[95] Restricted fluid intake during the first days after birth has been reported to reduce the risk of PDA.[96]

Bronchopulmonary dysplasia

BPD is a respiratory disorder that usually evolves after respiratory distress syndrome (RDS).[97] Premature infants born between 23 to 30 gestational weeks are at the greatest risk for BPD. Some infants have severe lung disease

requiring supplemental oxygen for several months or years after birth.[98] Severe BPD is defined as the need for at least 30 % oxygen at 36 weeks of gestation.[99] There were in total 25 % of the infants in the EXPRESS cohort who were diagnosed with severe BPD.[6] This pulmonary condition leads to concomitant treatment regimens of e.g. mechanical ventilation (MV) and postnatal steroids, which are known to effect early growth.[100]

Retinopathy of prematurity

ROP is a common complication in infants born preterm, leading to high risk of visual impairment or blindness and most vulnerably are infants born extremely preterm. Prematurity in itself, low birth weight and oxygen exposure are well known risk factors for ROP development.[101] Another risk factor that has been associated with development of ROP is poor weight gain during the first weeks of life.[102, 103] This suggests that early adequate nutritional supply might be an important factor for preventing ROP.

The pathogenesis of ROP development include two phases.[104] Phase one occurs during the first weeks of life, vascularization in the retina is inhibited because of hyperoxia which leads to a down regulation of growth factors such as insulin growth factor 1 (IGF-1), erythropoietin (EPO) and vascular endothelial growth factor (VEGF). During phase two, oxygen supply is depressed which in turn leads to an up-regulation of growth factors. An overgrowth of damaged and leaky blood vessels in the retina occurs, which leads to scar formations and finally retinal detachment. ROP are classified into five different stages, and stage 3 to 5 are classified as severe ROP.[105] Preterm infants are screened for ROP according to newly revised guidelines.[106] According to these new guidelines, preterm infants with birth weight of ≤ 1500 grams or a gestational age of 30 weeks or less, are examined within the first four to five weeks postnatal age, depending on gestational age at birth. The timing for the first eye examination should be carried out before 31 postnatal weeks. This guideline is consistent with Swedish recommendations. During the years 2004 to 2007 in Sweden, screening for ROP was performed weekly or biweekly from the fifth postnatal week until the retina was fully vascularized, or regression of ROP was observed.[107] ROP stage were classified for each infant and for treatment decision, The International Classification of Retinopathy of Prematurity Revisited was used.[108]

Intraventricular hemorrhage

Brain injury, such as IVH usually evolves during the first week of life and the first three days after birth are the most vulnerably period for acquiring

IVH.[109] Extremely preterm infants are at particular high risk of brain injury, whereas these complications seldom occurs in infants born after 28 gestational weeks. IVH are graded into four different grades, grade 3 to 4 are classified as severe IVH.[110] IVH grade 3 are IVH with ventricular dilatation, infants with IVH grade 3 may acquire progressive hydrocephalus. IVH grade 4 means that, in addition to the intraventricular bleeding, there is also an infarction in the brain parenchyme.

Periventricular leucomalacia (PVL) has classical been described as the formation of cysts in the deep periventricular cerebral white matter.[111] These focal necrotic lesions are termed cystic PVL (cPVL). Cerebral ischemia, infections and cytokines are factors that influence the pathogenesis of cPVL. Higher risk of diminished developmental scores and elevated risk of cerebral palsy are associated with cPVL.[112] In the EXPRESS cohort, 14 % of the infants acquired severe brain injury (IVH≥3 and cPVL combined).[6] A lower gestational age was associated with increased risk of severe brain injury.

Necrotizing enterocolitis

NEC is an inflammatory bowel necrosis, and the most common gastrointestinal emergency and major cause of morbidity and mortality in extremely preterm infants.[113] Any segment of the small or large intestine may be involved but the terminal ileum and the proximal colon are the most commonly affected sites. The severity of the disease is classified according staging from 1 to 3 by Bells et al.[114] Briefly, stage 1 includes suspect cases with abnormal distention and ileus, stage 2 includes definitive gastrointestinal bleeding, pneumatosis intestinalis, or portal venous gas, stage 3 comprises both stage 1 and 2 and in addition septic shock and pneumperitoneum. ELBW infants undergoing surgical NEC has been associated with significant growth delay and adverse neurodevelopment outcome compared with non-NEC peers.[115] Infants with serious NEC are significantly more likely to be complicated by nosocomial sepsis and progression to neonatal lung disease.[116] The occurrence of NEC have serious short- and long-term consequences for extremely preterm infants.

The incidence of NEC in Sweden is 5.8 % among infants with a gestational age less 27 weeks[6] while an incidence of 11 % have been reported among infants with gestational age less than 29 weeks in USA.[4] Early, low-volume of enteral feedings of HM may reduce the incidence of NEC in VLBW infants.[117, 118]

Objectives

The general objectives of this study was to investigate the impact of nutritional intakes and its relation to early growth and short-term morbidity in a population-based cohort of Swedish extremely preterm infants.

The specific objectives were (respectively paper in parenthesis):

- To explore the associations between energy and macronutrient intakes and early growth (I).

- To investigate intakes of micronutrients during the first 70 days of life and its correlation to growth (II).

- To evaluate the effect of energy and macronutrient intakes during the first four weeks of life on the risk for severe retinopathy of prematurity (III).

- To describe enteral intakes and macronutrient content in human milk given to extremely preterm infants during hospitalization (IV).

- To evaluate perioperative nutrition in extremely preterm infants undergoing surgery for patent ductus arteriosus (V).

Methods

Study design and study population

This is a retrospective population-based study. All extremely preterm infants in Sweden, born between 2004-04-01 and 2007-03-31 (n= 707 live-born infants) constitute the EXPRESS cohort.[3] Most infants were born at one of the seven University hospitals in Sweden, also referred to as a level III hospital, 17 % of infants were born at a level I or level II hospital and later transported and admitted to a level III hospital.[3]

For the current nutritional study, all available data of infant's nutritional intakes, anthropometric measurements and laboratory data during hospital stay were retrospectively collected from hospital records. Data were entered and stored by using a computer-based tool Nutrium (www.nutrium.se) developed within the research group. Comprehensive prospective data on cohort characteristics, neonatal morbidity and infant mortality were obtained from the EXPRESS cohort.[3, 6]

Data collection

Nutritional and growth data were mainly collected by a registered dietitian (ESS) but also by trained local staff. Detailed data of nutritional intake for 21 332 individual days were obtained for the study-cohort. In total, weight data were registered 31 111 times, length data were registered 6 086 times and HC data were registered 8 592 times.

Each University hospital were visited by ESS and data were obtained and registered into Nutrium at site. Data collected by trained local staff were double entered during these visits in order to validate data collection. All data were continuously controlled and double-checked against original records during the entire process of data collection.

Infants were treated at 37 different Swedish hospitals, including the seven University hospitals. For infants transferred to county hospitals (45 %), data collection was continued using records from these hospitals.

Exclusions

This current nutritional study excluded infants who did not survive 24 hours after birth (15 %) because nutrition is not likely to have affected the outcome in these infants (**Figure 7**). Only 16 infants (3 %) of the entire study-cohort were excluded because of unobtainable nutritional data.
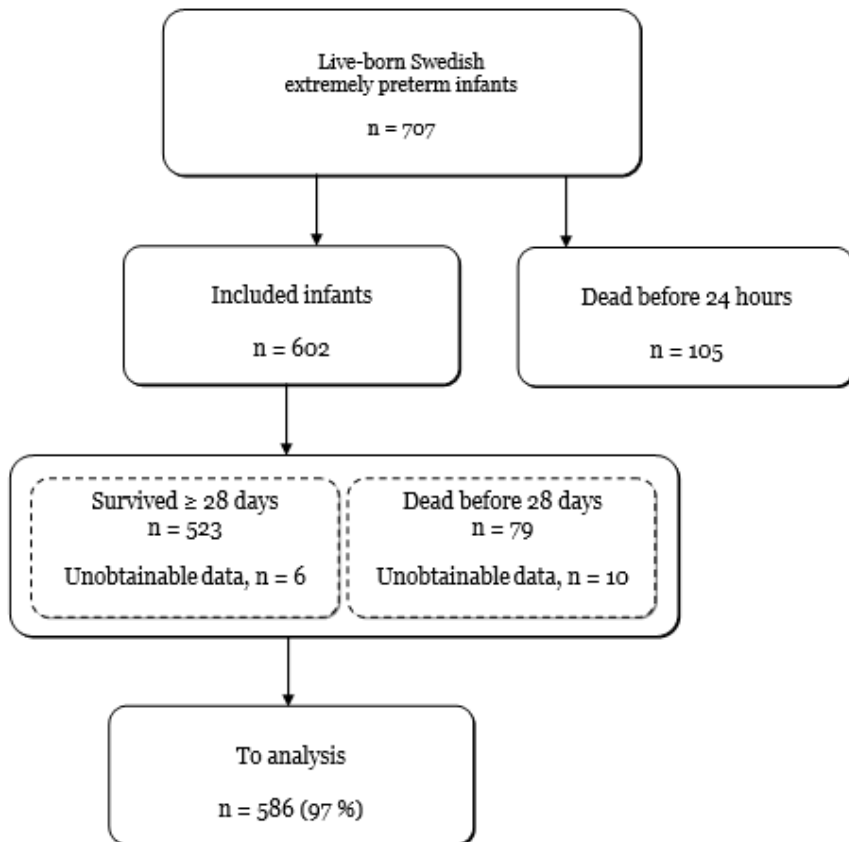
Figure 7. Schematic diagram to demonstrate the data collection.

When investigating nutritional intakes and correlations to growth outcomes, infants with following diagnoses were excluded: hydrocephalus, severe cholestasis, major congenital anomalies or chromosomal anomalies and NEC surgery (paper I-II). In paper III, infants with major congenital or chromosomal anomalies were excluded. In paper IV and V, no exclusions were made, except infants with missing nutritional data.

Nutritional data

Because of centralized neonatal care of extremely preterm infants in Sweden, most data for the first 28 postnatal days were obtained. Daily data of nutritional intakes were collected for the first 28 days and thereafter for one day per week (day 35, 42, 49 etc.) until discharge or until data no longer was obtainable (paper I, II, III, IV). When investigating perioperative nutrition in infants undergoing surgery (n=141) for PDA, daily nutritional intakes were recorded on the day of surgery and for three days before and three days after surgery (paper V).

Since macronutrient composition in HM, especially protein, changes during the first weeks postpartum, samples from MOM were divided into early HM (≤28 postpartum days) and mature HM (>28 postpartum days) based on production day. Macronutrient content in HM were determined using mid-infrared spectrophotometry (paper IV). If MOM were not analyzed, content of macronutrients were assumed to equal the average content of the analyzed early or mature HM samples depending of postpartum day of feeding (**Table 1**). Not analyzed DHM were assumed to equal the average content of analyzed mature HM samples.

Table 1. Content of macronutrients in early and mature mother's own milk.

Macronutrient per 100 mL	Early ≤28 d	Mature >28 d
Samples, *n*	*380*	*441*
Energy, kcal	73 ± 9	70 ± 7
Protein, gram	1.8 ± 0.4	1.4 ± 0.2
Fat, gram	4.0 ± 0.9	3.9 ± 0.7
Carbohydrates, gram	6.8 ± 0.4	7.0 ± 0.2

Mean ± SD

Micronutrient content in HM were calculated from published values and divided into early and mature HM since content of some micronutrients changes during the first weeks postpartum. A summary of micronutrient content in 100 mL HM is displayed in **Table 2**.

In many cases, breastfeeding occurred towards the end of hospital stay. When ingested enteral volumes from HM decreased more than 10 % during a seven day interval (e.g. between days 63 to 70), nutritional intakes were stated as unreliable and were therefore not registered.

Table 2. Content of micronutrients in early and mature human milk.

Micronutrient per 100 mL		Early ≤28 d	Mature >28 d
Sodium[119]*	mmol	0.8	0.7
Potassium[119]*	mmol	1.3	1.1
Chloride[120]*	mmol	1.6	1.3
Calcium[119]*	mg	28.0	24.0
Phosphorus[119]	mg	14.0	14.0
Magnesium[119]*	mg	2.9	3.2
Selenium[120]	μg	1.2	1.2
Manganese[57]	μg	0.5	0.5
Copper[119]*	μg	37.0	27.0
Iron[120]	mg	0.055	0.04
Zinc[119]*	mg	0.28	0.19
Iodine[120]	μg	9.0	9.0
Vitamin A (RE)[#]	μg	217.0[121]	57.0[119]
Vitamin D[119]	μg	0.125	0.125
Vitamin E[119] (TE)	mg	0.4	0.4
Vitamin K[119]*	μg	0.45	1.15
Vitamin C[120]^	mg	5.3	5.3
Thiamin[119]	μg	20.0	20.0
Riboflavin[119]	μg	50.0	50.0
Vitamin B_6[119]	μg	20.0	20.0
Vitamin B_{12}[120]	μg	0.05	0.05
Folate[122]	μg	6.6	6.6
Niacin[119] (NE)[¥]	mg	0.47	0.47
Biotin[119]	μg	0.49	0.49
Pantothenic Acid[119]	mg	0.35	0.35

*Mean values in early HM are calculated from those studies investigating content in HM only during the first month postpartum.
#Vitamin A calculated as retinol μg + (beta-carotene μg /12) = RE
^Studies including high intakes of supplements of vitamin C in mothers were excluded in calculations.
¥Calculated from content of tryptophan in human milk ((60 mg tryptophan = 1 mg niacin) + niacin= NE)

The following data was collected from each infant's hospital record:

- Enteral diet (MOM, DHM and different kinds of formula)
- Analyzed HM (content of macronutrients and energy)
- Enrichments mixed with HM or formula e.g. HMF, fat supplements.
- Enteral supplements e.g. vitamin drops, iron drops, calcium and phosphorus.

- Parenteral nutrition (different parenteral nutritional preparations with glucose, amino acids, fat, vitamins and minerals)
- Parenteral additives such as electrolytes and trace elements.
- Other intravenous fluids e.g. glucose, drug infusions, crystalloids.
- Blood products e.g. erythrocytes, plasma and albumin.

On average, eight different nutritional products were registered on a daily basis for each infant during the entire hospital stay.

Nutrient intakes from non-human sources were calculated using data from manufacturers and nutritional content in blood products were calculated from published values.[123, 124] Nutritional intakes on the day of birth were recorded based on birth time of each infant. All nutritional intakes included transfused blood products.

For the sake of simplicity, intakes from enteral and parenteral sources were added in final analyses of macronutrients and micronutrients. Amino acids was counted as protein. Parenteral intakes were adjusted for the enteral absorption of each micronutrient (enteral intakes + (parenteral intakes/absorption rate)). This calculation is referred to adjusted enteral intakes (AEI). The enteral absorption rate of each micronutrient are based on published values, which are listed in paper II.

Growth data

Each measurement of weight, length and HC during the whole hospital stay was obtained from infant's growth charts and from bedside charts. Since anthropometric measurements not were standardized at even seven days intervals, growth data for each seventh day (day 7, 14, 21 etc.) was calculated by interpolating between the two nearest actual measurements.

Standard deviation scores (SDS) for weight, length and HC were calculated by using a Swedish gender-specific growth reference[77] and for infants born before 24 weeks of gestation a Canadian growth reference was used.[125] SGA was defined as birth weight more than 2 SD below mean.

Laboratory data

Since we plan to investigate nutrient intakes with biochemical markers in an up-coming project, all available laboratory data were collected during the same time as nutritional and growth data. Following data were collected from hospital records: hemoglobin, base excess, blood glucose, and serum chloride, sodium, potassium, phosphate, ionized calcium, urea, ferritin and albumin.

Statistical analyses

In paper I-IV, data were analyzed by using SPSS Statistical software (Version 21.0 for Windows, SPSS, Inc. Chicago, IL, USA), in paper IV we also used R (Version 3.01)[126] with the R-package: Mixed Generalized additive models Computation Vehicle (MGCV).[127] In paper V, JMP Statistical software (Version 10.0; SAS Institute Inc., 2012, Chicago, IL, USA) was used.

Continuous variables are presented as mean ± 1 SD, except those variables that were not normally distributed which are presented as median, range and 10th - 90th or 25th -75th percentile.

For paper I-II, univariate linear regression analyses were performed for energy and nutrient intake as well as for possible confounders for each investigated time interval and for each growth outcome. Significant variables were then entered in a multivariate linear regression using a stepwise procedure.

In paper III, logistic regression analyses were used to assess the effects of each risk factor on the risk for severe ROP. Significant risk factors were then further analyzed in multivariate logistic regression using a forward conditional approach. Data was stated as mean and 95 % confidence interval (CI). Since risk factors may vary between hospitals, a categorical hospital variable was included in the final analysis.

In paper IV, independent sample t-test was used in comparison of nutrient content in different types of HM. To evaluate time trends within macronutrient and energy contents in MOM, general additive regression models were used, controlling for repeated measurement on subjects (mothers) and laboratory. Each nutrient was analyzed in a mixed effect model to analyze changes in nutrient composition in MOM over time using postpartum days as time variable.

In paper V, mean and 95 % CI were used to describe point estimates and analysis of variance (ANOVA) was used to test for group differences. Additional details on data analyses are further described in each paper (I-V).

All tests were 2-sided and the level of significance for all analyses was set to a p value < 0.05.

Ethical considerations

All studies in this thesis were approved by the regional research Ethic Committee in Lund, Sweden (Dnr 138-2008) and were carried out in line with the Declaration of Helsinki – ethical principles for medical research involving human subjects.

For the EXPRESS study, parents gave oral informed consent for data acquisition and the study was approved by the regional research Ethic Committee in Lund, Sweden.

Results

Characteristics of the cohort

Weight at birth was measured in every infant in the cohort (n=602). Length at birth was measured in 465 infants (77 %) and HC was measured in 477 infants (79 %). Of the 602 included infants, 79 died within the first 28 days and 11 died between days 29 to 70. There were 477 singletons, 111 were twins and 14 were triplets, 15 % (n=88) were born SGA. Baseline and background characteristics of included infants in the different studies are presented in **Table 3**.

Table 3. Infant characteristics at birth.

	Paper I-II	Paper III	Paper IV	Paper V
Time interval, days	70	28	84	7
Number of infants	531[*]	498[*]	586[*]	140
Gestational age, wks.	25.3 ± 1.1[δ]	25.4 ± 1.1	25.3 ± 1.1	24.8 ± 1.0
Birth weight, g	765 ± 170	776 ± 167	763 ± 170	723 ± 157
Birth length, cm	32.7 ± 2.5 (n=415)	32.8 ± 2.5 (n= 392)	32.7 ± 2.5 (n=457)	32 ± 2.5 (n=102)
Birth HC, cm	23.2 ± 1.6 (n=426)	23.3 ± 1.5	23.2 ± 1.6 (n=469)	22.8 ± 1.6 (n=108)
Sex, male %	54	55	55	59
Apgar score				
1 minute	5.2 ± 2.5	5.4 ± 2.5	5.2 ± 2.5	4.8 ± 2.6
5 minute	7.2 ± 2.1	7.3 ± 2.1	7.2 ± 2.1	7.1 ± 2.0
10 minutes	8.3 ± 1.6	8.3 ± 1.5	8.3 ± 1.6	8.2 ± 1.5
CRIB score	6.5 ± 3.6 (n=523)	6.2 ± 3.5 (n=490)	6.6 ± 3.6 (n=576)	7.7 ± 3.5 (n=136)

[*]Included infants at baseline of each study (I-IV)
[δ]Mean ± SD (all such values)

The range of gestational age at birth was 22 weeks + 1 day to 26 weeks + 6 days.

The lowest birth weight was 348 gram and the highest birth weight was 1315 gram. Birth weights (n=602) are plotted against gestational age in **Figure 8**.
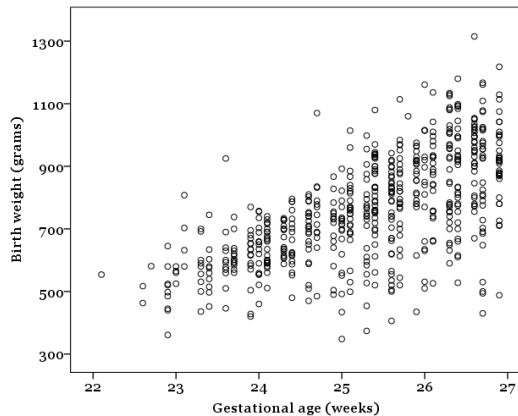

Figure 8. Birth weight in grams by gestational age in weeks.

Intakes of energy and nutrients

Nutrient intakes were calculated for each calendar day. Since infants were born at different times of the day, the average duration of the day of birth (day 0) was 12.3 hours. Seventy-five infants (14 %) did not receive any protein/amino acids on the day of birth. The fact that day 0 was not a full 24-hour period has been taken into account in all calculations of nutritional intakes.

Intakes of energy (kcal/kg/day) and macronutrients (g/kg/day) during the first 70 days of life are shown in **Figure 9**.
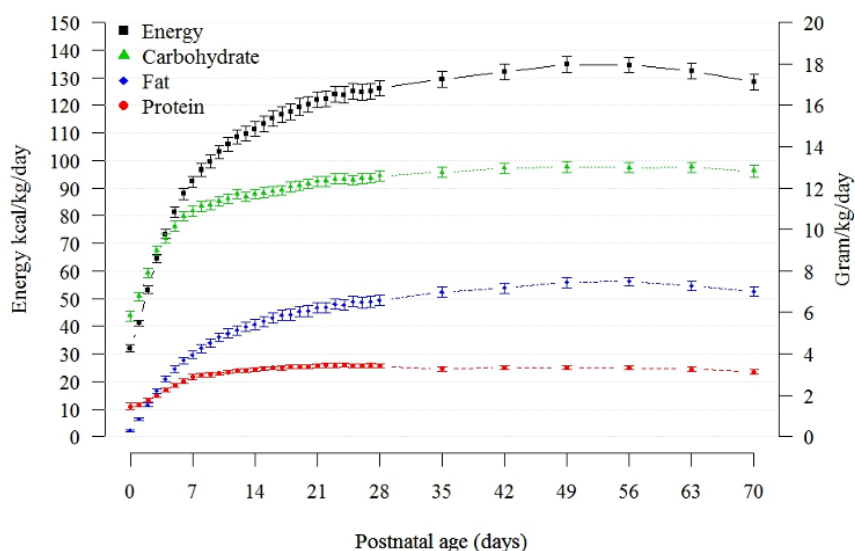

Figure 9. Intakes of energy and macronutrients (mean and 95% CI) during the first 70 days of life (n=531 to 394).

On average, during the first 70 days of life, infants (n=394) had an energy intake of 120±11 kcal/kg/day, protein intake was 3.2±0.4 gram/kg/day, fat intake was 6.2±1.0 gram/kg/day and intake of carbohydrates was 12.2±1.0 gram/kg/day (paper I).

During the first 28 days of life, infants received a combination of enteral and parenteral nutrition (paper I-II). A few infants received parenteral nutrition after day 29 of life (paper IV). During the first week of life, the proportion of enteral feeds was 27 % among infants and increased to 90 % during the fourth week of life (paper IV). During the first 28 days of life, 99 % of the

infants received only HM and at four weeks of life 70 % of the infants received only MOM as enteral feeding.

From birth to 28 days of life (n=466), the overall intake of energy was 102±14 kcal/kg/day, protein was 3.0±0.4 gram/kg/day, fat was 4.8±1.3 gram/kg/day and intake of carbohydrates was 11.2±1.1 gram/kg/day.

Clinical practice differed between hospitals; this was noted during data acquisition. **Figure 10** shows a box-and-whiskers plot of energy intakes (median, 25th-75th percentile) in 466 extremely preterm infants during the first 28 days of life, divided by hospital (A to G).

The number of treated extremely preterm infants at each University hospital varied: Hospital A = 55 infants, Hospital B = 99 infants, Hospital C = 80 infants, Hospital D = 103 infants, Hospital E = 36 infants, Hospital F = 82 infants and Hospital G = 11 infants.

Significant differences between hospitals were observed in a crude ANOVA analysis without consideration of severity of illness (not shown).
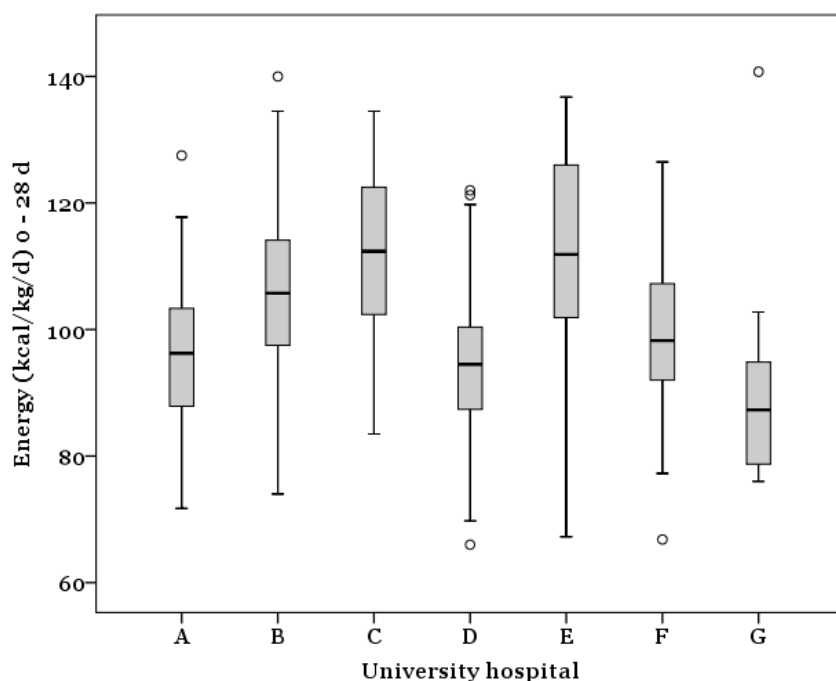

Figure 10. Energy intakes during the first 28 days of life in 466 extremely preterm infants

Figure 11 shows a box-and-whiskers plot of protein intakes (median, 25th-75th percentile) in 466 extremely preterm infants during the first 28 days of life. Significant differences between hospitals were seen. Protein from blood products are included in total protein intakes.
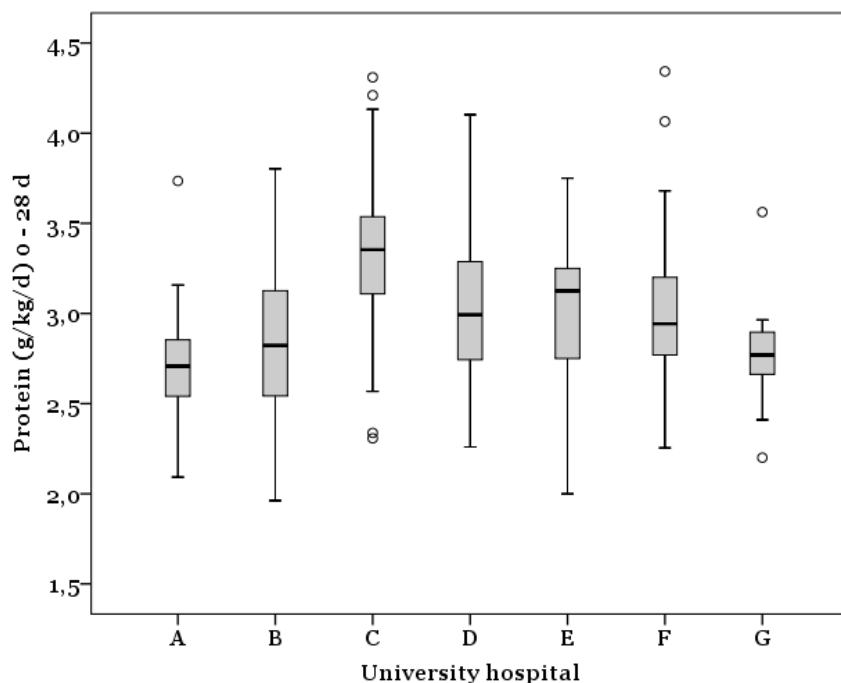

Figure 11. Protein intakes during the first 28 days of life in 466 extremely preterm infants.

During the time period 2004 to 2007, all newborns in Sweden, including extremely preterm infants, received a dose of 1 mg of vitamin K (Konakion® Novum, F.Hoffman-La Roche Ltd Basel, Switzerland).

In accordance to clinical routines at all participating hospitals, infants received MOM as a first hand choice of enteral feeds or DHM if MOM was not available (paper IV). HMF was added gradually in enteral feeds as soon as infants reached full enteral volume. However, many infants did not receive full dose HMF despite having reached full enteral volumes.

The most frequently used HMF was Enfamil® powder (Mead Johnson, Mediq Sweden AB, Kungsbacka, Sweden) and at a minority of hospitals Nutriprem® (Cow & Gate, Nutricia, Mediq Sweden AB, Kungsbacka, Sweden) was used (paper II). Enfamil® contains all vitamins and minerals

with a notable exception of iodine and Nutriprem® contains all vitamins and most of minerals with the exception of iron.

Oral vitamin supplements were started approximately at two to four weeks of age depending on local practice within each NICU (paper II). Infants received oral iron drops after 28 days of life and oral capsules or mixture of calcium and phosphorus was often administrated from approximately two to three weeks postnatal age.

During the first 28 days of life, intakes of several micronutrients were higher than estimated enteral requirements, these included iron, vitamin K, thiamin, riboflavin, vitamin B_6 and biotin (paper II). Additionally, intakes of several other micronutrients were lower than estimated enteral requirements; these were calcium, phosphorus, magnesium, zinc, copper, selenium, iodine and vitamin D. Most micronutrients originated from enteral feedings, HMF and oral supplements. In contrast, the high iron intake was mainly explained by blood transfusions. Infants received a median (25^h-75th percentile) of 6 (3-9) blood transfusions during the first 28 days of life, resulting in 75 (44-120) mL/kg of blood.

Growth of the preterm infant

Infants showed severe postnatal growth failure during hospitalization (paper I). **Figure 12** shows changes in SDS from birth to day 28, the green line on the top of the graph represents the average intrauterine growth.
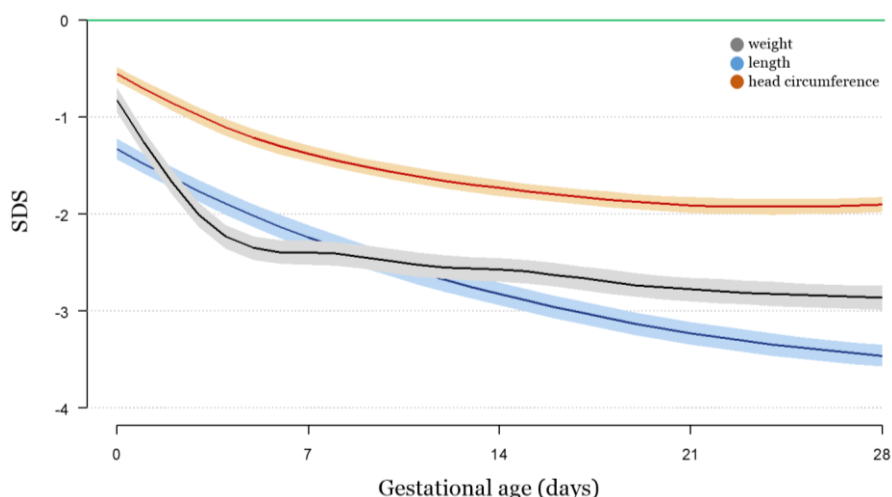

Figure 12. Growth in extremely preterm infants shown as standard deviation scores (SDS) during the first 28 days of life.

Between birth and day 28, SDS decreased with 2.1 for weight (n=465), 2.3 for length (n=357) and 1.5 for HC (n=368). No catch-up growth was seen in any anthropometric measurements during this period. Weight and HC showed similar growth patterns (paper I).

Figure 13 show a box-and-whiskers plot of weight change in SDS (median, 25th-75th percentile). Significant differences were seen between hospitals.
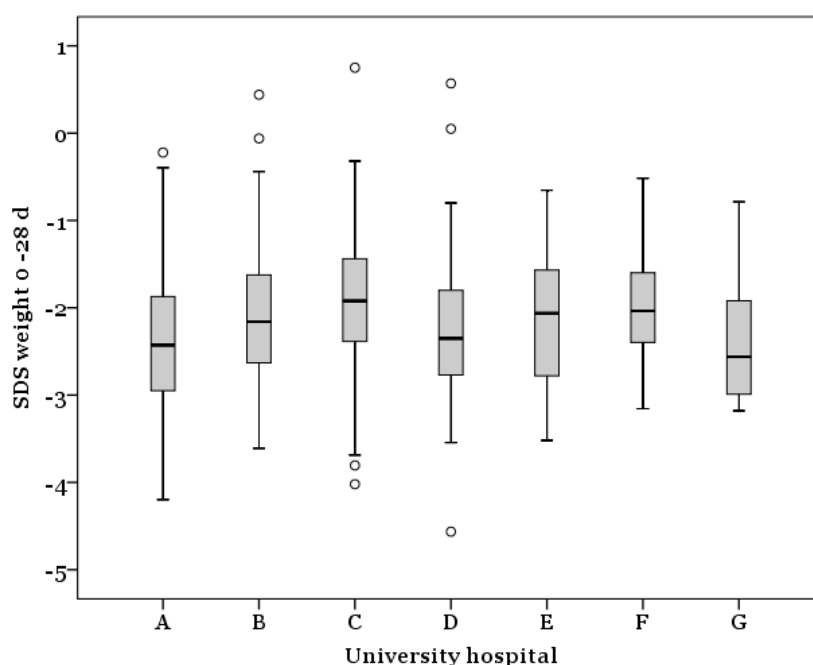
Figure 13. Weight change in standard deviation score (SDS) during the first 28 days of life.

Nutrient intake and growth outcome

Stepwise multivariate regression analyses showed that energy and protein intakes were positively and significantly associated with weight, length and HC growth during the first 70 days of life (paper I), even when considering disease-related variables in the analyses. The strongest significant association was between a higher energy intake and weight gain (**Figure 14**). No consideration of severity of illness or other confounders has been taken into account in the figure.
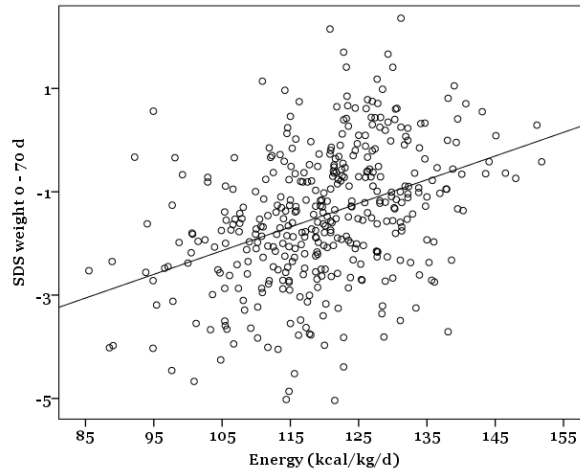
Figure 14. Energy intake in extremely preterm infants and correlation to weight change from birth to day 70.

A higher fat intake was positively associated with improved HC growth (p=0.018), paper I. Length gain was less affected by energy and macronutrient intakes compared to other growth outcomes, at least during the first 28 days of life (paper I).

Intakes of micronutrients and their associations with growth were explored in paper II. There was a significant negative association between iron intakes (AEI) and longitudinal growth during the first 28 days of life (**Figure 15**). No potential confounders has been taken into account in the figure. A negative correlation between high iron intakes and poor HC growth during the first 28 days was observed (p=0.027, paper II).
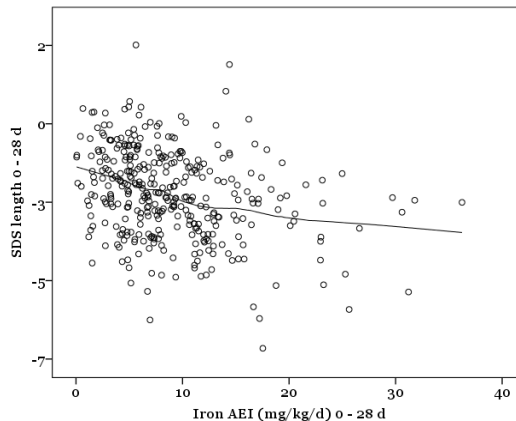
Figure 15. Adjusted enteral intakes (AEI) of iron and association with length gain during the first 28 days.

Folate intake (AEI) was positively associated with weight gain (**Figure 16**), and length gain (p=0.003, paper II) during the first 70 days. Sources of folate was mainly enteral feedings and HMF (paper II). At two University hospitals, oral capsules of folate was routinely given to the infants.
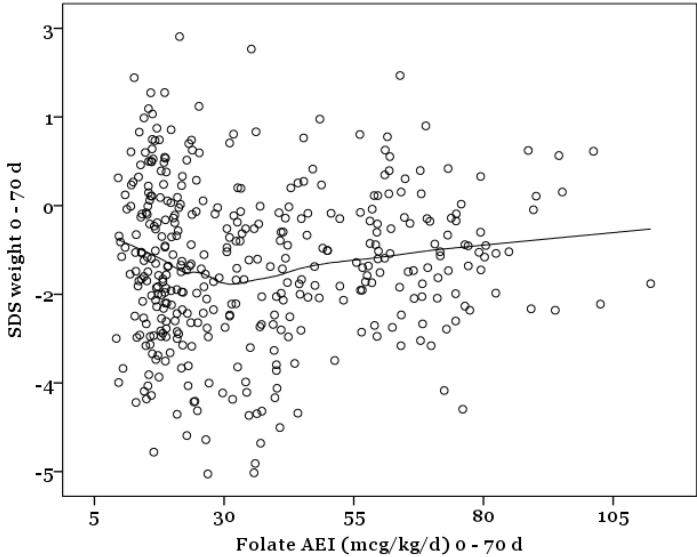
Figure 16. Adjusted enteral intakes (AEI) of folate and weight gain during the first 70 days.

Retinopathy of prematurity

One hundred seventy-two infants had severe ROP (stage 3-5); 56 % of these infants were treated. Distribution of stages of ROP in the cohort are displayed in **Table 4**.

Table 4. Distribution of stages of ROP in 498 extremely preterm infants.

ROP stages	*n*	%
No ROP	137	27.5
Stage 1	76	15.3
Stage 2	113	22.7
Stage 3	166	33.3
Stage 4	3	0.6
Stage 5	3	0.6
Total	498	100

Poor intakes of energy and fat were significantly associated with risk for severe ROP within each week during the first four weeks of life (**Table 5**). Intakes of protein were significant during the fourth week but not significant during the whole period of 28 days. Poor intakes of energy, fat and carbohydrates during the whole period of four weeks were significantly associated with the risk for developing severe ROP.

Table 5. Associations between energy and macronutrient intakes and severe retinopathy of prematurity.

Intakes	Energy[δ]		Protein[^]		Fat[^]		Carbohydrates[^]	
(kg/day)	kcal	p	gram	p	gram	p	gram	p
Day 0-6	66 (10)[*]	0.007	2.2 (0.6)	ns	2.2 (0.8)	0.031	9.1 (1.3)	ns
Day 7-14	102 (17)	0.001	3.0 (0.5)	ns	4.7 (1.5)	0.009	11.3 (1.4)	0.029
Day 15-21	116 (21)	<0.001	3.3 (0.6)	ns	5.8 (1.8)	0.001	12.0 (1.6)	ns
Day 22-28	124 (21)	0.002	3.4 (0.7)	0.033	6.4 (1.9)	0.017	12.4 (1.7)	0.019
Day 0-28	102 (14)	<0.001	3.0 (0.4)	ns	4.8 (1.2)	0.001	11.2 (1.1)	0.004

Logistic regression model adjusted for gestational age and birth weight
[*]Mean ± (SD), all such values
[δ]Energy: 10 kcal/kg/day increment
[^]Protein, fat and carbohydrates: 1 gram/kg/day increment

When investigating associations between nutrient intakes and the risk for severe ROP, several confounders were taken into account (paper III). Poor postnatal growth, gestational age, birth weight, CRIB score, duration of MV, blood transfusions, proportion of enteral fluids, treatment of steroids and antibiotics and co-morbidities such as PDA, IVH were included together with energy and nutrient intake in a multiple logistic regression analysis. The following variables remained significant: gestational age, birth weight, energy intake and blood transfusions (paper III).

In a second multivariate model, a categorical hospital variable was included. In this model, energy intake, blood transfusions, birth weight, gestational age and hospital remained significant. In both these models, an energy increase of 10 kcal/kg/day during the first four weeks was associated with a 24 % decrease in the risk for developing severe ROP (p=0.01) in extremely preterm infants.

Macronutrient content in human milk

In total, macronutrient contents of 1175 samples of HM were analyzed using mid-infrared spectrophotometry (paper IV).

Two hundred fifty-six mothers of extremely preterm infants delivered 821 milk samples. In addition, 354 analyses of DHM samples were analyzed. Two different laboratories analyzed the milk samples. Steins laboratory in Jönköping, Sweden (here referred to the central laboratory) analyzed 63 % of the HM samples and 37 % of the samples were analyzed at the Milk Bank at Sahlgrenska University hospital (paper IV).

The content of protein in MOM decreased significantly over time and reached the lowest level at 85 postpartum days (paper IV). During postpartum days four to seven the highest value was noted (2.2 gram per 100 mL). The lowest value was noted during postpartum days 85 to 112 (1.2 gram per 100 mL). Carbohydrate content significantly increased during the first three weeks postpartum.

The inter-individual variation in macronutrient contents were high in MOM samples, especially fat content, which also resulted in similar variability of energy content (paper IV).

There was no significant correlation between gestational age and protein content in MOM.

Of analyzed 1175 samples of HM (MOM and DHM combined); the central laboratory showed a higher content of protein and lower content of fat, carbohydrates and energy compared to analyzed HM samples at Sahlgrenska University hospital (**Table 6**).

Table 6. Energy and macronutrient contents in human milk analyzed at two different laboratories.

	HM Central Laboratory	HM Sahlgrenska	P^*
Samples, n	*737*	*438*	
Content per 100 mL			
Energy, kcal	68.5 ± 7.5	73.7 ± 8.8	<0.001
Protein, gram	1.6 ± 0.4	1.5 ± 0.4	0.014
Fat, gram	3.7 ± 0.8	3.9 ± 0.8	<0.001
Carbohydrates, gram	6.9 ± 0.3	7.1 ± 0.4	<0.001

HM, human milk
*Independent sample t-test to detect differences between analyses in each laboratory.

Perioperative nutrition

In total, 141 infants of the entire study cohort underwent surgical treatment of PDA; one infant was excluded in this sub-cohort due to missing nutritional data. Of the included infants (n=140) the postnatal age at surgery varied between three and 93 days with a median of 20 days (paper V).

During the seven days included in the perioperative week, infants had an average daily energy intake of 95±18 kcal/kg, protein intake was 2.9±0.6 gram/kg, fat intake was 4.2±1.6 gram/kg and intake from carbohydrates was 11.0±1.9 gram/kg (paper V).

On the day of surgery (stated as day 0), mean intake of energy (78 kcal/kg/day) reached the lowest levels during the perioperative week (**Figure 17**). This was significantly lower than minimal requirements (paper V). The lowest mean intake of protein (2.8 gram/kg/day) was on the second day after surgery (Day+2).
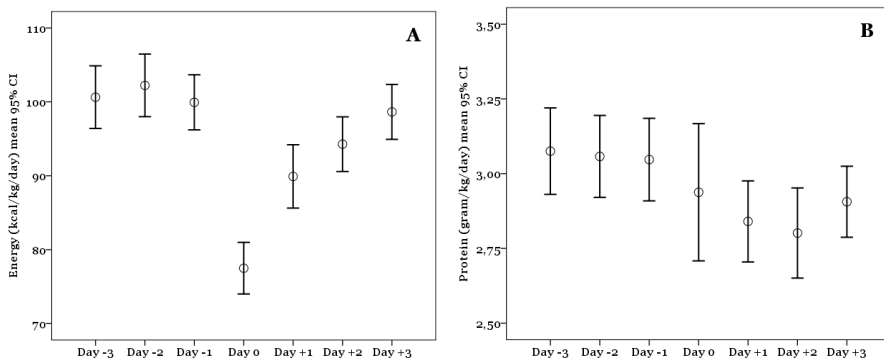

Figure 17. Intakes of energy (A) and protein (B) during the perioperative week in infants undergoing surgery for patent ductus arteriosus.

On the day of surgery, mean intake of fat (2.5 gram/kg) reached the lowest levels during the perioperative week (**Figure 18**). This was lower than estimated minimal requirements (paper V).
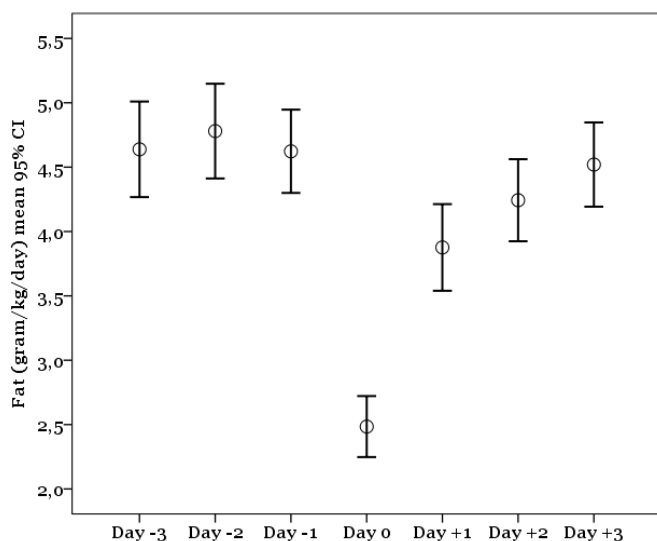
Figure 18. Intakes of fat during the perioperative week in infants undergoing surgery for patent ductus arteriosus.

Blood and plasma transfusions were administrated to the infants during the perioperative week. Median intake (10th-90th percentile), were 3.7 (1.1-6.4) mL/day for blood transfusions and 1.4 (0.0-5.7) for plasma transfusions. Nine infants did not receive any blood transfusions and 58 infants did not receive any plasma transfusions during the perioperative week.

Neonatal morbidity

Neonatal morbidity stratified by gestational age at birth (completed weeks) of included 602 infants is presented in **Table 7**. The most common complications due to preterm birth were PDA, ROP and BPD. Only a small proportion of infants developed cPVL or NEC.

Correlations between intakes of each nutrient (including energy) and each of the three growth outcomes (weight, length, HC) were explored in univariate regression analyses. Similar analyses were performed also for non-nutritional possible confounders, including maternal characteristics, delivery data, severity-of-illness related variables and morbidities that occurred at an early stage of life (paper I). Since we did not have access to data on date at diagnosis of PDA and BPD, and since these morbidities theoretically would only affect growth after that date, these morbidities were not included in our calculations.

Table 7. Neonatal morbidity, stratified by gestational age at birth in 602 infants.

	Gestational age (completed weeks) at birth						
	22	23	24	25	26	<27	
Number of infants at birth	13	67	124	192	206	602	
	n/N*	n/N*	n/N*	n/N*	n/N*	n/N*	%
PDA, any treatment	9	48	82	115	92	346	57.5
PDA, surgery	2	29	47	39	23	140	23.3
ROP, any stage	4/4	47/54	87/102	125/167	103/184	366/511	71.6
ROP, stage 3-5	3/4	32/54	53/102	53/165	38/186	179/511	35
BPD<30% O_2@36wks.	2/4	30/54	53/99	81/168	94/183	260/508	51.2
BPD>30% O_2@36wks.	2/4	16/54	31/99	49/168	41/183	139/508	27.4
IVH, any grade	9	33	54	80	64	240	39.9
IVH, grade 3-4	2	15	19	33	18	87	14.5
cPVL	0	7	8	10	8	33	5.5
NEC, any	0	2	9	14	10	35	5.8
NEC, surgery	0	2	5	8	4	19	3.2

N*number of infants for whom data was accessible, denominator shown only if different from *n*. PDA, patent ductus arteriosus; ROP, retinopathy of prematurity; BPD, bronchopulmonary dysplasia; IVH, intraventricular hemorrhage; cPVL, cystic periventricular leucomalacia; NEC, necrotizing enterocolitis.

Variables that were significant in the univariate analyses were then further analyzed in stepwise multivariate regression analyses (paper I-II). These analyses showed that prominent markers of severity of illness that influenced growth outcomes were duration of MV, postnatal steroid treatment, antibiotics and CRIB score (paper I).

In this context, the proportion of enteral fluids was also used as a marker of severity of illness since the sicker babies tend to have less tolerance of enteral feeds and therefore need more parenteral nutrition. This variable was also significantly affecting growth in the multivariate models.

Discussion

Major findings

Comprehensive nutritional data were available for 97 % of this large Swedish population-based cohort of extremely preterm infants born at a gestational age less than 27 weeks.

Infants received considerably less energy and protein during the early postnatal life compared to estimated requirements. During the first 70 days of life, infants showed severe postnatal growth failure in weight, length and HC. In addition to macronutrient intakes, we also present intakes of 25 micronutrients during the first 70 days of life. Intakes of several micronutrients, such as iron, vitamin K and water-soluble vitamins were higher than estimated requirements while intakes of other micronutrients e.g., calcium, phosphorus, zinc, magnesium, iodine and vitamin D were lower than estimated requirements. Intakes of energy, protein, fat, iron and folate were significantly associated with growth even when adjusting for possible confounders.

Another interesting finding was that extremely preterm infants in Sweden received almost exclusive intakes of HM during the first four weeks of life. Already during the second week of life, 92 % of the infants received breast milk from their own mother. Protein content in MOM decreased rapidly during the first four weeks postpartum and macronutrient content in MOM were variable within and between mothers.

Furthermore, we found that low intakes of energy during the first four weeks of life were strongly associated with increased risk for developing severe ROP.

Extremely preterm infants undergoing surgery for PDA were highly malnourished during the perioperative week. Infants who underwent surgery during the first week of life faced the largest deficits in nutritional intakes.

Intakes of energy and nutrients

During the first 28 days of life, infants received on average 102 kcal/kg/day.[128] Compared with estimated requirements of enteral energy intakes according to international and Swedish guidelines[10, 11, 14] these infants received 15-18 % less energy than recommended. Protein intakes during the same time period was on average 3.0 gram/kg/day, which is considerably

less than estimated enteral requirements of 3.5-4.5 gram/kg/day for growing infants. Nutritional guidelines recommend 1.5-2.4 grams/kg/day of protein (or amino acids) during the first day of life.[10, 14] Yet, 14 % of the infants did not receive any amino acids during the day of birth, inevitably leading to detrimental protein deficits at this early stage of life. Mean intake of carbohydrates was 11.2 gram/kg/day, which is within estimated requirements according to Tsang guidelines[10] as well as the Swedish guidelines.[14] Mean intake of fat was 4.8 gram/kg/day during the first 28 days of life; this is in accordance with estimated minimum enteral requirements. However, to facilitate protein synthesis and cover early energy needs, sufficient intakes from protein (amino acids) and fat (lipids) are necessary. Even considering fluid restriction, which probably was required, not least in infants with PDA, an improved protein and fat intake may be achieved by using more concentrated parenteral nutrition solutions and a more active fortification of HM.

Main sources of micronutrient intakes were enteral nutrition, including oral supplements such as fat- and water-soluble vitamins, capsules or mixture of calcium and phosphorus, iron drops and HMF. Adding micronutrients and electrolytes together with HMF into enteral feeds increases the risk of hyperosmolarity in the intestine,[63] which might lead to osmotic diarrhea. Our observation of both high and low intakes of micronutrients illustrates the importance of micronutrient content in multivitamin supplements and HMF, which most likely can be further improved in the future.

It is well established that HM is the preferred enteral food source for infants, including those born extremely preterm. We observed that Swedish preterm infants with a gestational age less than 27 weeks received HM to a large extent during hospitalization (paper IV). We also observed that the content of macronutrients and energy in HM is highly variable between and within lactating women and changes over time; this finding is supported by others.[30, 129]

Protein content in MOM decreases rapidly during the first 28 days postpartum, and to achieve the estimated enteral requirements of protein (3.8-4.5 gram/kg/day) it is necessary to add HMF. To avoid the risk of undernutrition or overnutrition an individualized approach has been suggested.[32] Individualization can be achieved by two different strategies: "targeted fortification" means adjusting HMF according to HM analyses, while "adjustable fortification" means adjusting HMF according to the metabolic response of the infant, most commonly blood urea nitrogen (BUN).[130] The latter strategy has some advantages compared to targeted fortification: actual protein status is measured in each infant and it does not

require HM analyzers. However, by using this strategy, important information on energy and fat intakes might be lost and waiting for a decrease in BUN means allowing infants some extent of malnutrition before HMF is added or increased.

Infants undergoing surgical treatment for PDA had fat intakes of 2.5 gram/kg/day during day of surgery; this is half of the recommended intake, which also contributed to the poor energy intake that was observed for the infants.[131] Associations between PDA surgery and white matter injury and brain damage has recently been reported, no nutritional intakes were reported in that study.[132] The significance of undernourishment for outcome in extremely preterm infants undergoing PDA surgery is still unclear and requires further investigation. Nonetheless, this group of infants might be at a particular increased risk of adverse neurodevelopmental outcome. Interruption of nutritional support to these infants should be avoided and nutritional guidelines for extremely preterm infants undergoing PDA surgery are warranted.

It is noteworthy that evidence for nutritional requirements of infants weighing less than 750 grams are scarce. Most estimated nutritional requirements are for healthy, growing preterm infants with a weight of >1000 grams and most formal studies of nutrient requirements have been performed in infants with birth weights about 1500-2000 grams.[57, 133] Although a lot of progress regarding neonatal nutritional needs during the last decades has been made, suboptimal nutrition is frequently present at NICUs, despite the knowledge of increased risk of neonatal diseases and poor neurodevelopmental outcomes.[134]

Clinical practices varied between hospitals, which was noted by ESS during data acquisition. We found a significant difference between study hospitals regarding nutritional intakes as well as growth among infants. Based on our results from paper I[128] and paper II, Swedish national guidelines for nutrient intakes of extremely preterm infants have recently been proposed by The National Board of Health and Welfare.[14]

Nutritional impact on growth

Extremely preterm infants in Sweden showed severe postnatal growth failure during hospital stay.[128] During the first 28 days, SDS decreased by 2.1 for weight, 2.3 for length and 1.5 for HC. Total intakes of calories and protein were the strongest predictors of growth, suggesting that many of these infants did not reach minimum nutritional intakes required for growth even at several weeks of postnatal age.

Energy and macronutrients

Initial weight loss due to changes in body fluid compartments is likely one inevitable contributor.[72] However, this initial fluid loss ranges approximately over the first three days of life[135] while infants in our study continued to decrease in SDS at least until day 28.[128] This suggests that fluid loss is not the major cause of long-term weight change in this group of patients. We observed that total energy intake was the strongest predictor of weight gain during the first 70 days of life, including different time intervals that were investigated.[128] Other studies support our results, even though investigated time periods were shorter.[90, 136] Protein intakes were also an independent predictor of weight gain, which has also been shown in previous studies.[44, 137]

The growth of HC showed similar growth pattern as weight, with a loss of 1.5 SD (compared to -2.1 SD in weight) during the first 28 days of life (Figure 12). This observed growth failure in HC indicates that brain growth may be suboptimal and this might lead to impaired neurodevelopmental outcome.[87] During the first 70 days of life, intakes of energy, protein (E %) and fat (E %) were independent predictors of HC growth.[128] A recent randomized study showed that it is possible to improve HC growth by optimizing protein and calorie intakes in preterm infants with a gestational age less than 29 weeks.[85] Whether this enhanced nutrient intake leads to improved neurodevelopmental outcomes in extremely preterm infants needs to be further investigated.

Length gain seems to be more affected in extremely preterm infants compared to changes in weight and HC growth[128], with a different growth pattern and with less notable catch-up during hospital stay. Poor longitudinal growth has been associated with impaired neurodevelopmental outcomes.[83] Total intakes of energy and protein (E %) were positively associated with length gain during the first 70 days. A weakness in our study was that measurements of length at birth were lacking for 22 % of the infants.

Micronutrients

Low intakes of folate (AEI) during the first 70 days of life were associated with poor growth in weight and length. Intakes of folate between days 29 to 70 only reached 63 % of minimal estimated requirements. Folate sources originated mainly from enteral nutrition. However, folate in HM does not meet folate needs in extremely preterm infants[122] and for that reason, HMF is required. Insufficient intakes of folate have been associated with impaired growth.[10] The majority of infants in this study did not receive full dose

fortification with HMF. If they had received full dose fortification, folate intakes would have reached the recommended level. We conclude that extremely preterm infants should receive either full dose HMF or a separate folate-containing supplement.

Excessive iron intake (AEI) was associated with impaired longitudinal growth and poor head growth; these observations are supported by a randomized study, even if that study included only term babies.[138] Iron intakes via enteral nutrition were relatively low with sources such as iron drops and iron-containing HMF. The main source of iron was blood transfusions and we observed that NICUs in Sweden have liberal policies regarding blood transfusions given to extremely preterm infants. Since blood transfusions are given to the sickest babies, it is impossible to know whether it is the iron intake or the illness that causes the poor longitudinal growth. Nevertheless, we can speculate that a more restrictive blood transfusion policy in Sweden may promote longitudinal growth in extremely preterm infants.

It is well known that calcium, phosphorus, magnesium and vitamin D are essential for bone growth and bone mineralization.[139] We observed an overall low intake of calcium, phosphorus, magnesium and vitamin D. Even if all of these micronutrients were significantly associated with growth outcome in univariate analyses, neither of those stayed significant in final multivariate models. It is difficult to establish the clear relationship between insufficient intakes and growth outcome, since many of these micronutrients interact with each other. It has been suggested that calcium retention of 60-90 mg/kg/day would facilitate appropriate bone mineralization in VLBW infants[140], an enteral intake of 120-140 mg/kg/day would meet this needs. Intakes of calcium (AEI) during the first 28 days of life was 63 mg/kg/day, this is clearly lower than estimated requirements of 120-140 mg/kg/day.[11, 14] A deficit of calcium and other micronutrients may contribute to impaired bone mineralization, but this was not investigated in our study.

Nutritional impact on retinopathy of prematurity

ROP development occurs during the first few weeks of life. In this study, low intakes of energy, fat and carbohydrates during the first four weeks of life were associated with increased risk for severe ROP (paper III). Surprisingly, no significant association between protein intake and severe ROP was observed during the period of four weeks. In multivariate analyses, low energy intake during the first four weeks of life remained as a significant risk factor even when considering other illness-related variables.

The energy intake was 102 kcal/kg/day during the first four weeks of life; this is clearly below estimated requirements. Results from our study do not give a clear cut-off limit below which the risk for severe ROP increases, but our data indicate that the lowest estimated requirements of 105-110 kcal/kg/day[10, 12] may be too low.

Low fat intake was associated with increased risk for developing severe ROP. There is some evidence that ω-3 PUFAs reduces the risk for ROP[55], we did not attempt to calculate fatty acid intakes in this study. During this time (2004-2007), Swedish infants received only a pure soybean-based lipid emulsion, which has a very low content of ω-3 PUFAs. Further, fatty acids content in HM depend on the nutritional intakes of the mother[141], and we did not analyze the quality of fat in HM.

There is only one similar study exploring the associations between nutritional intakes during the first month of life and the risk for developing ROP.[142] Similarly, to our results, they concluded that improved nutritional intakes with special target on lipids and calories might reduce the incidence for severe ROP. A strength of our study is that we included 28 days of complete data compared to only four days in the other study[142] and that we included more possible confounders in our analyses.

Severity of illness

In the analyses of the nutritional impact on growth and ROP, we included several non-nutritional variables that are known to affect postnatal growth as well as development of severe ROP. As expected, severity-of-illness related variables such as high CRIB score, duration of MV, infections (reflected by antibiotic treatment) and treatment of postnatal steroids were significant predictors of poor growth outcome. However, it is noteworthy that the effect of energy intake on weight development during the first 70 days of life was three time larger than the effect of postnatal steroids.[128]

Since we lack the information of exact date of diagnosis of PDA and BPD, those disease variables were not included in our papers regarding growth outcome (paper I-II).[128] It is well known that infants with severe lung disease have poor growth and delayed catch-up growth, which may persist well into childhood.[143, 144] However, we did include other variables such as total fluid intakes, duration of MV and postnatal steroid therapy, which are known to be closely correlated with PDA and BPD.[98,96]

We observed significant differences regarding nutritional intakes and postnatal weight gain between included University hospitals. Differences of

mortality rate within the first 12 hours has been reported within the EXPRESS cohort.[145] Even though Swedish neonatal care are centralized, there were considerable variations in mortality rates among infants born between 22 to 24 weeks of gestational age; this may be related to differences in perinatal practices at each University hospital. Improved survival in Swedish extremely preterm infants is not associated with an increase in morbidity rates.[146, 147] However, the high risk of suboptimal neurodevelopmental outcome calls for improved neonatal care, such as improved nutritional management.

Strength and limitations of the studies

Strength of this study include the comprehensive collection of nutritional data on all extremely preterm infants in Sweden during a three-year period. Nutritional and growth data was available for 97 % of the cohort and nutritional intake data for 21 332 individual days was collected. Moreover, macronutrient content of 1175 HM samples were retrieved, including data of 821 HM samples from mother's giving birth to extremely preterm infants.

Finally, the cohort was prospectively characterized according to predefined criteria, including information of ROP, PDA and other morbidities.

Limitations of the study include the retrospective collection of nutritional data and the fact that the nutritional content of flush solutions were not systematically noted in hospital records. The lack of standardized anthropometric measurements and the limited reliability of clinical length and HC measurements is another limitation. Moreover, micronutrients and fatty acids in HM were not analyzed and two different laboratories yielded slightly different results for the macronutrient content in HM. There were no available information of direct oxygen exposure in those infants with severe ROP and detailed information on hemodynamics and surgical ligation is lacking.

Conclusions

Swedish extremely preterm infants received less energy and macronutrients than estimated requirements and showed postnatal growth failure during hospital stay. Intakes of energy, protein and fat were independent predictors of growth even when considering disease-related variables. Total intakes of energy and protein were independently correlated with all growth outcomes (weight, length and HC) during the first 70 days of life, while total fat intake was positively associated with HC growth. Our results suggest that improved energy and protein intakes may reduce postnatal growth failure in these infants.

Intakes of several micronutrients were considerably lower than estimated requirements while intakes of some micronutrients were higher than recommended. Several micronutrients were independent predictors of growth outcomes. The strongest association was shown with regard to folate and iron: low intakes of folate predicted poor weight and length gain while high iron intakes were associated with poor length and HC growth. It may be beneficial to increase folate intake and limit iron supply from blood transfusions in this specific population of infants.

Low energy intake during the first four weeks of life was an independent risk factor for developing severe ROP (stage 3-5). Provision of adequate energy from parenteral and enteral sources early in life may therefore be an effective method for reducing the risk of severe ROP in extremely preterm infants.

During the first four weeks of life, 99 % of the infants received exclusively human milk as enteral feeding. Swedish extremely preterm infants receive high amounts of mother's own milk during hospitalization. The content of energy, protein and carbohydrates in mother's own milk changed significantly with time. Weekly analyses of mother's own milk may therefore allow a more individualized nutritional support to this vulnerable group of infants.

Perioperative nutrition in extremely preterm infants undergoing PDA surgery is suboptimal, and infants are at high risk of malnutrition.

Finally, our findings from these studies suggest that optimized nutritional supply during early postnatal life may prevent growth failure and severe ROP in extremely preterm infants. Study results may also have important implications for health in adult life for this new generation of survivors.

Future perspectives

Cognitive and behavioral problems are commonly observed at pre-school and school age in children born extremely preterm. We will have the opportunity to investigate the impact of early nutritional intakes on cognitive and behavioral outcomes at 2½ years corrected age and 6 years of age in this population of infants. Follow-up data on cognitive and behavioral functions have already been collected within the EXPRESS cohort.

Since we presented the first preliminary data from this study in 2009, changes in nutritional practices have been made at most Swedish NICUs. These changes have included the introduction of routine calculations of nutrient intakes, more concentrated parenteral nutrition solutions and earlier fortification of HM. Moreover, at most hospitals, the purely soybean based parenteral nutrition solutions have been changed to lipid sources with higher omega-3 fatty acid content. It remains to be seen if these changes also will improve growth and reduce the incidence of severe ROP and other morbidities. We plan to compare nutrient intakes as well as growth and health outcomes (including ROP) between the EXPRESS cohort (2004-2007) with more recent cohorts (e.g. Umeå and Stockholm 2009-2012).

Baby girl born at 24 weeks + 6 days with a birth weight of 545 gram. Photo taken at 5 weeks postnatal age. © Elisabeth Stoltz Sjöström

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