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The impact of nutrition on growth, biomarkers, and health outcomes in preterm infants

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To all infants born preterm

There is no absolute knowledge. And those who claim it, whether they are scientists or dogmatists, open the door to tragedy. All information is imperfect. We have to treat it with humility.

Jacob Bronowski, Mathematician

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Abstract

Introduction Nutrients play a crucial role for growth and brain development after preterm birth. Meeting the nutritional needs of preterm infants is challenging. Particularly, the most immature infants have a high risk of malnutrition and poor growth during hospital care. To meet recommended energy and nutrient intakes during early postnatal life, a concentrated parenteral nutrition (PN) regimen was implemented in clinical use in 2012 at the neonatal intensive care unit at Umeå University Hospital (Umeå, Sweden). However, electrolyte homeostasis is labile after preterm birth and infants require an electrolyte supply that corresponds to their energy and protein intakes to avoid electrolyte disturbances. Although sodium imbalances such as hyper- and hyponatremia are common in the most immature preterm infants, there is limited knowledge to what extent these imbalances are affected by fluid volume and sodium supply. Furthermore, it is unclear whether the early high sodium concentrations lead to any adverse effects, including intraventricular hemorrhage, or simply reflect immediate adaptive processes after preterm birth.
Aim This thesis investigates the impact of nutrition on growth, nutritional biomarkers, and health outcomes in preterm infants born with a birth weight below 1500 g.
Methods We used data form two study populations. First, we collected data for all very low birth weight infants (< 1500 g) born between 2010 and 2013 and treated at Umeå University Hospital (Umeå, Sweden; n = 134). Second, we used data from the EXtremely PREterm infants in Sweden Study (EXPRESS). We incluced all infants born before 27 gestational weeks in Sweden between 2004 and 2007 who survived the first 24 h (n = 602). Data collection for both study populations included a) intakes of all parenteral and enteral nutritional products and other fluids during the first 28 postnatal days, b) all anthropometric measurements during hospital stay, c) perinatal data, and d) neonatal morbidity.
Results The concentrated PN regimen improved early energy and macronutrient intakes in very low birth weight infants. Furthermore, weight and length growth from postnatal week two to a postmenstrual age of 36 weeks improved in very low birth weight infants who received the concentrated PN regimen compared with infants who received the previous original PN regimen (Paper I). Increased parenteral energy and protein intakes provided by the concentrated PN regimen, did not induce a higher occurrence of electrolyte imbalances as electrolytes were supplied according to the current recommendations (Paper II). In the EXPRESS cohort, the majority of extremely preterm infants had hypernatremia during the first and hyponatremia during the second postnatal week. Gestational age and supply of sodium, rather than fluid volume, were the major factors determining the risks of hyper- and hyponatremia (Paper III). High total supply of sodium was significantly correlated with severe intraventricular hemorrhage if mostly mediated by blood product transfusions (Paper IV).
Conclusions Our results suggest that in very immature preterm infants a concentrated PN regimen improves early nutrient intakes and postnatal growth without causing electrolyte disturbances. Hyper- and hyponatremia are common and the supply of sodium is a major predictor. The impact of sodium on severe intraventricular hemorrhage needs further investigation.

Sammanfattning på svenska

Introduktion Att möta energi- och näringsbehovet hos barn som föds för tidigt är utmanande, framförallt för de allra minsta barnen som på grund av omogenhet har hög risk för undernäring och dålig tillväxt. Med syfte att möta det rekommenderade energi- och näringsbehovet direkt efter födelsen hos för tidigt födda barn implementerades därför en koncentrerad parenteral näringslösning (PN) på den neonatala intensivvårdsavdelningen vid Umeå universitetssjukhus i februari 2012. Även elektrolytobalans är vanligt hos för tidigt födda barn. För att undvika denna obalans behöver för tidigt födda barn en elektrolytförsörjning som motsvarar energi- och proteinintaget. Både högt och lågt natrium (hyper- och hyponatremi) är vanliga tillstånd hos de mest omogna barnen men det finns begränsad kunskap i vilken utsträckning dessa obalanser påverkas av vätskevolym och natriumintag. Vidare är det oklart om höga natriumkoncentrationer leder till negativa konsekvenser, som exempelvis intraventrikulär blödning, eller om natriumobalanser enbart återspeglar en omedelbar anpassning efter för tidig födelse.
Syfte Denna avhandling undersöker energi- och näringsintag och dess samband med tillväxt, näringsrelaterade biomarkörer och hälsoutfall hos för tidigt födda barn med en födelsevikt under 1500 gram.
Metoder Två studiepopulationer användes. Alla barn som föddes med en födelsevikt under 1500 gram mellan 2010 och 2013, vårdade på Umeå universitetssjukhus utgjorde den ena studiepopulationen (n = 134). Den andra studiepopulationen utgjordes av data från Extremely PREterm infants in Sweden Study (EXPRESS) där alla barn födda före 27 graviditetsveckor mellan 2004 och 2007 ingick. Från EXPRESS inkluderades barn som överlevde de första 24 timmarna (n = 602). Datainsamlingen för båda studiepopulationerna inkluderade a) intag av alla parenterala och enterala näringsprodukter samt andra vätskor under de första 28 levnadsdagarna, b) alla antropometriska mätningar under sjukhusvistelsen, c) perinatal data och d) neonatal sjuklighet.
Resultat Koncentrerad PN förbättrade det tidiga energi- och makronutrientintaget hos barn med en födelsevikt under 1500 gram. Vikt- och längdtillväxten förbättrades från andra levnadsveckan till 36 veckor postmenstrual ålder hos barn som fick koncentrerad PN jämfört med barn som fick ursprunglig PN (Delarbete I). Det ökade parenterala energi- och proteinintaget var inte associerat med högre förekomst av elektrolytobalanser eftersom elektrolyter gavs enligt aktuella rekommendationer (Delarbete II). I EXPRESS kohorten hade majoriteten av barnen hypernatremi under den första levnadsveckan och hyponatremi under den andra levnadsveckan. Ålder vid födelse och natriumtillförsel, snarare än vätskevolym, var faktorer som bidrog till hyper- och hyponatremi (Delarbete III). Hög total natriumtillförsel var associerat med svår intraventrikulär blödning men detta samband var främst medierat av natrium från transfusioner av blodprodukter (Delarbete IV).
Slutsatser En koncentrerad PN förbättrar tidigt energi- och näringsintag samt tillväxt hos för tidigt födda barn utan att orsaka elektrolytobalans. Hyper- och hyponatremi är vanligt och tillförseln av natrium är en viktig faktor. Samband mellan högt natriumintag och svår intraventrikulär blödning behöver ytterligare undersökas.

Abbreviations

EPT	Extremely preterm
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology And Nutrition
EXPRESS	EXtremely PREterm infants in Sweden Study
HC	Head circumference
IVH	Intraventricular hemorrhage
NICU	Neonatal intensive care unit
PN	Parenteral nutrition
PUMPA	Premature in UMeå PArenteral nutrition
SD	Standard deviation
SDS	Standard deviation score
VLBW	Very low birth weight

Original papers

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

I. Späth C, Zamir I, Stoltz Sjöström E, Domellöf M. Use of Concentrated Parenteral Nutrition Solutions Is Associated With Improved Nutrient Intakes and Postnatal Growth in Very Low-Birth-Weight Infants. JPEN J Parenter Enteral Nutr. 2019 Feb 12. doi: 10.1002/jpen.1522. [Epub ahead of print]

II. Späth C, Stoltz Sjöström E, Domellöf M. Effects of two different parenteral nutrition regimen on plasma electrolyte concentrations in very low birth weight infants. Umeå 2019. Manuscript.

III. Späth C, Stoltz Sjöström E, Ahlsson F, Ågren J, Domellöf M. Sodium supply influences plasma sodium concentrations and the risks of hyper- and hyponatremia in extremely preterm infants. Pediatr Res. 2017; 81: 455-460.

IV. Späth C, Stoltz Sjöström E, Ahlsson F, Ågren J, Domellöf M. Associations between sodium supply, sodium imbalances and severe intraventricular haemorrhage in extremely preterm infants: A nested case-control study. Umeå 2019. Manuscript.

.

Introduction

Preterm birth

Preterm birth, birth before 37 completed weeks of gestation, is typically divided into three sub-categories: moderate to late preterm birth (32 to 36 weeks), very preterm birth (28 to 31 weeks), and extremely preterm (EPT) birth (< 28 weeks) (1). Accounting for about 16% of deaths globally, complications linked to preterm birth are among the leading causes of death in children younger than five years old (2). Over the last few decades, rates of preterm birth have been increasing worldwide (3, 4). In 2014, the estimated global preterm birth rate was 10.6%, equating to an estimated 14.8 million live preterm births (4). Of these births, more than 80% occurred in Asia and Sub-Saharan Africa and only about 5% in Europe. Among preterm births Europe, 11 and 5% were very preterm and EPT, respectively (4).

In low- and middle-income countries the mortality of preterm infants is high, in particular among infants born at a lower gestational age (5). In contrast, in developed counties the survival rate of the most immature preterm infants has been increasing over the last several decades. However, surviving infants have a high risk for developing short- and long-term morbidities, even in high-income countries (6, 7). In Sweden, the one-year survival rate among live-born EPT infants born with a gestational age < 27 weeks between 2014 and 2016 was 77% (8). Of these infants, 50% suffered from at least one major neonatal morbidity. Long-term follow-up data obtained from the nationwide EXtremely PREterm infants in Sweden Study (EXPRESS), including all EPT infants born < 27 weeks of gestation between 2004 and 2007, showed that 34% of the infants had either moderate or severe neurodevelopmental disabilities at 6.5 years of age (9). Therefore, studies that examine how to improve both short- and long-term outcome, especially among EPT infants, are of high importance.

The importance of nutrition for the preterm infant

Provision of energy and nutrients is essential for growth and development after preterm birth (10). Particularly in very immature preterm infants, parenteral nutrition (PN) is necessary to ensure nutritional needs during the first weeks after birth (11). In addition, enteral nutrition using preferably fortified mother's own or donor human milk should be initiated as soon as the infant's medical condition allows (12, 13). Maintaining at least minimal enteral feeding is of major importance for gastrointestinal maturation.

During the first weeks of life, low energy and nutrient intakes and poor weight gain may be involved in the development of morbidities (14), including the pathogenesis of retinopathy of prematurity (15, 16) and bronchopulmonary dysplasia (17). Furthermore, many nutrients and growth factors regulate brain development during fetal and early postnatal life (18). Thus, malnutrition and growth failure during hospital stay may have important and long-lasting effects on cognitive function (19, 20). Certain nutritional components might also have both a neuroprotective role against white matter injury and beneficial effects for the microbiome-gut-brain axis (21). Enhance nutritional intakes and/or postnatal growth have been associated with improved neurodevelopmental outcome in immature preterm infants (22-27). In contrast, studies also suggest that faster postnatal weight gain and higher nutrient intakes increase the risk for later cardiovascular disease potentially caused by programming effects during sensible neonatal periods (28-30).

Meeting nutritional needs in preterm infants

Although malnutrition and postnatal growth restriction in the neonatal intensive care unit (NICU) has decreased during the last decades, it is still a common condition (31-33). In particular, the most immature infants are at high risk for poor nutritional intakes and growth during hospital care (34, 35). There are several reasons for either not starting, slowly increasing, or stopping parenteral and/or enteral nutrition, including metabolic toxicities, feeding intolerance, or the desire to minimize morbidities such as bronchopulmonary dysplasia, patent ductus arteriosus, and/or necrotizing enerocolitis (36, 37).

There are several major goals for enteral and parenteral nutrition recommendations for preterm infants: a) to achieve a growth rate similar to the normal healthy fetus of the same gestational age, b) to maintain normal concentrations of blood and tissue nutrients, and c) to achieve satisfactory functional development (10). However, the composition of the optimal diet to achieve these goals is unknown and meeting nutritional needs of preterm infants is challenging. Many preterm infants born at low gestational age or with low birth weight suffer from neonatal morbidities with differing nutritional needs. The effect of any nutritional intervention further depends on timing, dose, and duration of the supplied nutrients/nutritional regimen (18).

Parenteral nutrition

Since 2005, the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends the start of PN as soon as possible after preterm birth and a rapid increase within the first postnatal days, a strategy intended to ensure an anabolic state (38-41). This concept is often called "early aggressive PN". However, it has been proposed to call this concept "adequate PN", as it represents the standard of care (42). In 2018, ESPGHAN updated the guidelines for pediatric PN. In brief, ESPGHAN now recommends an energy intake of 90-120 g/kg/d (38) and a protein intake of 2.5-3.5 g/kg/d (39) from the second postnatal day for immature preterm infants. These energy and protein intakes lead to a minimum of 30-40 kcal per 1 g amino acids to guarantee amino acid utilization (39). ESPGHAN also recommends a parenteral lipid intake not exceeding 4 g/kg/d (40), and an adjusted amount of glucose (41). Standard PN solutions are recommended for the majority of preterm infants and the use of individually-tailored solutions when nutritional requirements cannot be met by standard PN formulations such as for very sick and metabolically unstable infants (43).

However, clinical practice in care of preterm infants frequently does not allow energy and nutrient intakes recommended by the ESPGHAN (44, 45). For example, clinical practice often restricts fluid volume when treating bronchopulmonary dysplasia or patent ductus arteriosus (46, 47). The latter condition and associated fluid restriction has recently been linked to decreased energy and macronutrient intakes during early postnatal life (48).

Optimized parenteral nutrient intakes

Several studies have assessed the impact of optimized and more concentrated PN protocols and/or increased parenteral intakes of single nutrients on postnatal growth in populations of very immature preterm infants (Table 1) (49-58). In the majority of these studies, optimized PN was associated with improved growth outcome without showing adverse effects (51, 52, 55, 57, 58). At the NICU at Umeå University Hospital (Umeå, Sweden), a more concentrated PN regimen was implemented in clinical use in 2012, to enhance nutritional intakes without increasing fluid volume. Studies that address the effect of optimized PN on brain volume or later neurodevelopmental outcome of preterm infants are rare and results are inconsistent (49, 59-61).

Table 1. Effect of improved parenteral nutrition on postnatal growth in preterm infants.

Reference	Study type Population	Type of change	Time of change	Effect on growth	Adverse effects
Balakrishnan et al. 2018 (49)	RCT < 1250 g n = 168	Start with 1-2 g AAs/kg/d at day 1 and increase by 0.5 g/kg/d until 4 g/kg/d vs. Start with 3-4 g AAs/kg/d and increase to 4 g/kg/d by day 1	From birth until maximum day 6	↓ weight, ↓ length, ↓ HC growth in the intervention group	No
Tan et al. 2008 (50)	RCT < 29 weeks n = 142	Hyperalimented vs. standard PN+EN protocol	Within 7 days until discharge	No difference in weight, length, and HC growth	No
Senterre et al. 2011 (51)	OS < 1250 g n = 102	Optimized PN according recommended intakes	From birth to discharge	↑ weight growth ↓ growth restriction	-
Fischer et al. 2013 (52)	OS < 1000 g n = 121	↑ Parenteral lipid intakes	First postnatal week	↑ weight growth	-
Vlaardingerbroek et al. 2013 (53)	RCT < 1500 g n = 144	2.4 g AAs/kg/d vs. 2.4 g AAs/kg/d + 2-3 g lipids/kg/d vs. 3.6 g AAs/kg/d +2-3 g lipids/kg/d	Within 6 hours after birth until day 3	No difference in time to regain BW and growth in weight, length, and HC	No
Moltu et al. 2014 (54)	RCT < 1500 g n = 50	PN with ↑ energy, ↑ AAs, ↑ lipids; Change of lipid source; EN supplementation with AAs, DHA + arachidonic acid, vitamin A	Within 24 hours after birth until discharge	↑ regain of BW ↑ weight and HC growth ↓ growth restriction	↑ Electrolyte disturbances ↑ Septicemia
Morgan et al. 2014 (55)	RCT < 1200 g < 29 weeks n = 150	PN with ↑ energy, ↑ macronutrients	Within 120 hours after birth until day 28	↑ HC growth ↑ weight growth	No
Uthaya et al. 2015 (56)	RCT < 31 weeks n = 168	1. High vs. low AA intake 2. Lipid source	Within 24 hours after birth until day 3	1. ↓ HC growth 2. No effect	No
Izquierdo et al. 2016 (57)	OS ≤ 1500 g ≤ 32 weeks n = 142	Change of PN protocol with ↑ nutrient intakes	Within 48 hour after birth until discharge	↑ weight growth at 14 days (not thereafter)	No
Miller et al. 2017 (58)	OS <2000 g < 32 weeks n = 193	PN protocol for optimal nutrient intakes	EN of 20 mL/kg until full feeds	↑ weight growth	↑ Septicemia in controls

AA(s), amino acid(s); BW, birth weight; DHA, docosahexaenoic acid; EN, enteral nutrition; HC, head circumference; PN, parenteral nutrition; OS, observational study; RCT, randomized controlled trial.

Adaptation of fluid and electrolyte homeostasis after preterm birth

During intrauterine life, the fetus receives a constant supply of water and electrolytes via placental exchange, which suddenly is interrupted at birth. Because of this discontinuation, immediate adaptive processes affect the metabolism of fluid and electrolytes (62-64). This process can be divided into three major phases:

Phase 1:	Period of transition with the loss of body weight.
Phase 2:	Intermediate period with the introduction of full fluid volume and nutrition.
Phase 3:	Period of stable growth.

Phase 1, the immediate postnatal phase, is characterized by an initial oliguria and insensible water loss via the immature skin. The first urine is hypertonic to plasma with increased concentrations of urea, potassium, and phosphate, but not of sodium and chloride. Initial oliguria lasts between hours and days and is followed by diuresis. Body compartments are rearranged by contraction of the extracellular fluid compartment. Natriuresis, as present during fetal life, continues. Phase 1 starts at birth and usually ends when maximum weight loss has occurred. In phase 2, transcutaneous water loss usually decreases, urine volume falls below 1-2 ml/kg/h, and electrolyte excretion in urine is low. In very immature preterm infants, such as EPT infants, urinary output including electrolyte excretion might still be high in phase 2. Intestinal ability to digest oral feedings increases during phase 2 and usually this phase ends when infants return to their birth weight. Phase 3 is characterized by stable weight gain, positive fluid and electrolyte balance, and accretion of body tissue mass. Furthermore, kidney function has fully adapted to extra uterine conditions.

It is important to replace water and electrolyte losses that might occur during this postnatal adaptive process to maintain electrolyte homeostasis and normal plasma/serum electrolyte concentrations. Recent studies have shown that phosphorus, potassium, and calcium homeostasis after preterm birth are influenced by the recommended PN approach (65, 66).

The refeeding-like syndrome

In adults, refeeding syndrome is described as the potentially fatal shifts in fluids and electrolytes that may occur in malnourished patients when re-introducing food after a period of starvation (67, 68). In preterm infants, the response to early nutrition is often called refeeding-like syndrome, which is briefly explained

below. Due to the high supply of energy and protein, the cell maintains an anabolic state promoting uptake of phosphorus and potassium into the cell for energy production and tissue accretion (69, 70). About 80% of the body phosphate content and 98% of body calcium is stored in the skeleton (71, 72). In the absence of adequate phosphorus supply, plasma phosphate concentration decreases, initiating the release of phosphate and calcium from bone tissue. Thus, the refeeding-like syndrome is characterized by hypophosphatemia, hypokalemia, and hypercalcemia (66, 73) (Figure 1).

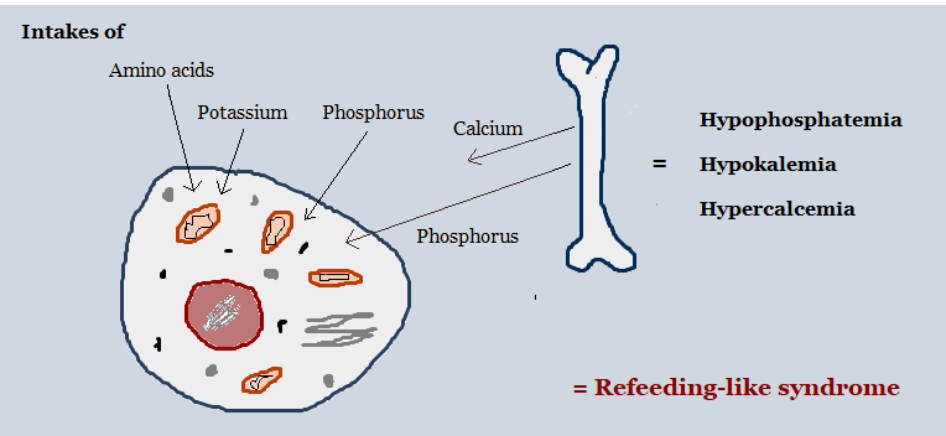

Figure 1. Possible mechanism of the refeeding-like syndrome. Figure adapted from Bonsante et al. (66).

Since phosphorus plays a critical role in maintaining energy balance in the cell, hypophosphatemia can basically affect every organ in the body. Clinical manifestations of hypophosphatemia include respiratory failure, myocardial impairment, red cell dysfunction, and/or rhabdomyolysis, while hypokalemia is known to cause a variety of arrhythmias (68, 74).

Randomized controlled studies show that an increase in early intakes of energy and protein without adequate provision of phosphorus and potassium induce the refeeding-like syndrome in low birth weight piglets and in very low birth weight (VLBW, < 1500 g) infants (75, 76). These studies found that subsequent increase of phosphorus and potassium supply restores the electrolyte imbalances. Further studies suggest that the parenteral electrolyte intakes recommended by the ESPGHAN in 2005 induce electrolyte imbalances, resulting in the refeeding-like syndrome in preterm infants who received adequate amounts of energy and protein (77, 78). Therefore, the authors suggest a revision of these previous guidelines. Updated ESPGHAN PN guidelines, published in 2018, include increased recommended intakes for phosphorus, potassium, and calcium and

therefore an increase in the calcium:phosphorus ratio compared to the 2005 PN recommendations (Table 2) (62, 72, 79).

Table 2. Recommended parenteral electrolyte intakes for preterm infants.

Electrolyte	ESPGHAN 2005	ESPGHAN 2018
Sodium, mmol/kg/d	0-3 (5)[a]	0-2 (3) / 0-5 (7) / 2-5 (7)[b]
Potassium, mmol/kg/d	0-2[a]	0-3 / 0-3 / 2-3[b]
Calcium, mg/kg/d	40-93	32-80[c] / 64-140
Phosphorus, mg/kg/d	31-71	31-62[c] / 50-108
Calcium:phosphorus ratio	0.8-1: 1	1.3-1.7: 1

ESPGHAN, European Society of Paediatric Gastroenterology, Hepatology and Nutrition; Recommendations are valid for the growing stable preterm infant if not otherwise noted.
[a]Recommended for the first postnatal week.
[b]Recommended for postnatal days 1-2 / 3 / 4-5.
[c]Recommended for the first days after birth.

Sodium imbalances after preterm birth

Sodium, the major cation in the extracellular fluid, determines the extracellular fluid volume and is contained in the bone and several other tissues (80). Furthermore, sodium is necessary for growth and the development and function of the central nervous system (80-82). After EPT birth, sodium imbalances such as hyper- and hyponatremia or large changes in serum sodium concentrations occur frequently and have been associated with impaired brain health and increased mortality (83-88). Infants often develop hypernatremia within the first days after birth, followed by hyponatremia a few days later. This course of plasma/serum sodium concentrations could be explained by the earlier described adaptive process after preterm birth, including contraction of the extracellular fluid compartment, high initial insensible water loss, and immature renal function (63, 64)

Electrolytes, including sodium, enter the human body via parenteral or enteral nutrition (63), and other intravenous fluids and medications. No endogenous production or release of electrolytes exist. Urinary output is the only way the body regulates active electrolytes, but this regulation is physiological limited and depends on the infant's gestational age and postnatal age. Therefore, sodium imbalances after preterm birth might be somewhat iatrogenic. However, little is known about how fluid and electrolyte management interact with the described postnatal adaptive process and to what extent sodium imbalances such as hyper- and hyponatremia can be affected by sodium supply and/or fluid volume.

Intraventricular hemorrhage and sodium imbalances

Intraventricular hemorrhage (IVH), the most common type of intracranial hemorrhage, occurs primarily in preterm infants (89). Its development and severity is inversely related to an infant's gestational age and birth weight (90, 91). In 95% of infants, onset of IVH is between 24 hours after birth and postnatal day 4, and between 20 and 40% of infants show a progression within 3 to 5 days after initial diagnosis (92, 93). Compared to more mature infants, both onset and progression of IVH occur earlier in immature preterm infants (91).

Consequences of IVH depend on the severity of the bleeding and include impaired neurodevelopmental outcome along with cerebral palsy, periventricular hemorrhagic infarction, and increased risk of death (94, 95). IVH lesions, routinely diagnosed with cranial ultrasound, are typically the result of bleeding into the subependymal germinal matrix (96). In the majority of studies, including this thesis, the classification system of Papile et al. is used to categorize IVH and severe IVH is defined as an IVH of grade 3 to 4 (97) (Table 3). In the previously mentioned EXPRESS cohort, 10% of EPT infants developed severe IVH (98).

Table 3. Classification of intraventricular hemorrhage according to Papile et al. (97).

Grade	Description
1	Isolated germinal matrix hemorrhage
2	Intraventricular hemorrhage with normal ventricle size
3	Intraventricular hemorrhage of sufficient severity to dilate the ventricles with blood
4	Intraparenchymal hemorrhage

Sodium imbalances such as hypernatremia or large fluctuations in serum sodium concentrations as well as high sodium supply have been associated with IVH in a few retrospective observational studies (Table 4) (83, 99-101). For example, Dalton et al. found that IVH of any grade was more frequent in hypernatremic infants. However, their study found that hypernatremia was not associated with severe IVH (83). Lim et al. showed that sodium fluctuations > 13 mmol/l within the first three postnatal days were associated with severe IVH (99). Other studies found associations between total sodium supply and IVH, although these studies found that sodium imbalances were not associated with IVH (100, 101). In summary, data regarding associations between sodium supply, sodium imbalances, and severe IVH in EPT infants are sparse and partly conflicting.

Table 4. Observational studies on associations between sodium intake and/or sodium imbalances and intraventricular hemorrhage in preterm infants.

Reference	Population	Result	Further information
Dalton et al., 2015 (83)	< 1000 g < 29 weeks n = 216	P-Na ≥ 150 mmol/l was associated with any IVH grade	No association with IVH grades 3 to 4; No association between Na fluctuations and IVH in hypernatremic infants
Lim et al., 2010 (99)	≤ 1000 g ≤ 26 weeks n = 72	Na fluctuations < 13 mmol/l were associated with IVH grades 3 to 4	Case-control design; No associations between P-Na and IVH grades 3 to 4
Barnette et al., 2010 (100)	≤ 1500 g n = 722	Na intake > 4.5 mmol/kg/d was associated with IVH grades 2 to 4	No associations between P-Na and IVH grades 2 to 4 or IVH grades 3 to 4
Lee et al., 2014 (101)	< 1000 g n = 169	Na intake other than BPT was associated with IVH grades 3 to 4	Adjustment only for GA; No associations between P-Na and IVH grades 3 to4

BPT, blood product transfusions; GA, gestational age; IVH, intraventricular hemorrhage; Na, sodium; P-Na, plasma sodium.

Aims

This thesis investigates the impact of nutrition on growth, nutritional biomarkers, and health outcomes in VLBW infants and in EPT infants.

The specific aims of the included papers (I to IV) are as follows:

I. To investigate if a change to a concentrated PN regimen improves intakes of energy and macronutrients as well as postnatal growth in VLBW infants.

II. To investigate the effect of a concentrated PN regimen on electrolyte intakes and plasma electrolyte concentrations during the first postnatal week in VLBW infants.

III. To investigate the prevalence of hyper- and hyponatremia and possible associations with predisposing factors in EPT infants.

IV. To investigate the associations between sodium supply, sodium imbalances, and severe IVH in EPT infants.

Study cohorts and Methods

Overview of selected study design

This thesis includes four observational studies (Paper I to IV) (Table 5).

Table 5. Study designs and cohorts used for this thesis.

Paper	Study design	Study cohort
I	Observational study Retrospective before-and-after design (single-center)	PUMPA cohort
II	Observational study Retrospective before-and-after design (single-center)	PUMPA cohort
III	Observational study Population-based prospective design including retrospectively collected data (multi-center)	EXPRESS and Nutrium-EXPRESS cohort
IV	Observational study Population-based nested matched case-control design including retrospectively collected data (multi-center)	EXPRESS and Nutrium-EXPRESS cohort

PUMPA, Premature in UMeå PArenteral nutrition; EXPRESS, EXtremely PREterm infants in Sweden Study.

The PUMPA study cohort

For Paper I and II, we collected data to establish the Premature in UMeå PArenteral nutrition (PUMPA) cohort. The cohort consists of all VLBW infants born between February 1, 2010 and September 30, 2013 who were admitted to Umeå University Hospital (Umeå, Sweden) within the first 24 postnatal hours and who were treated at the hospital for at least one week. On February 19, 2012, the NICU introduced a concentrated PN regimen to satisfy the recommended PN intakes. As reflected in our a priori conducted power analyses, our primary intention was to collect data two years before and after the concentrated PN regimen was introduced to compare differences associated with the change of the PN regimen. However, data collection ended earlier due to further alteration of the main PN bag, which was mainly based on the high magnesium content of the solution. Included and excluded infants in Paper I and II are shown in Figure 2.

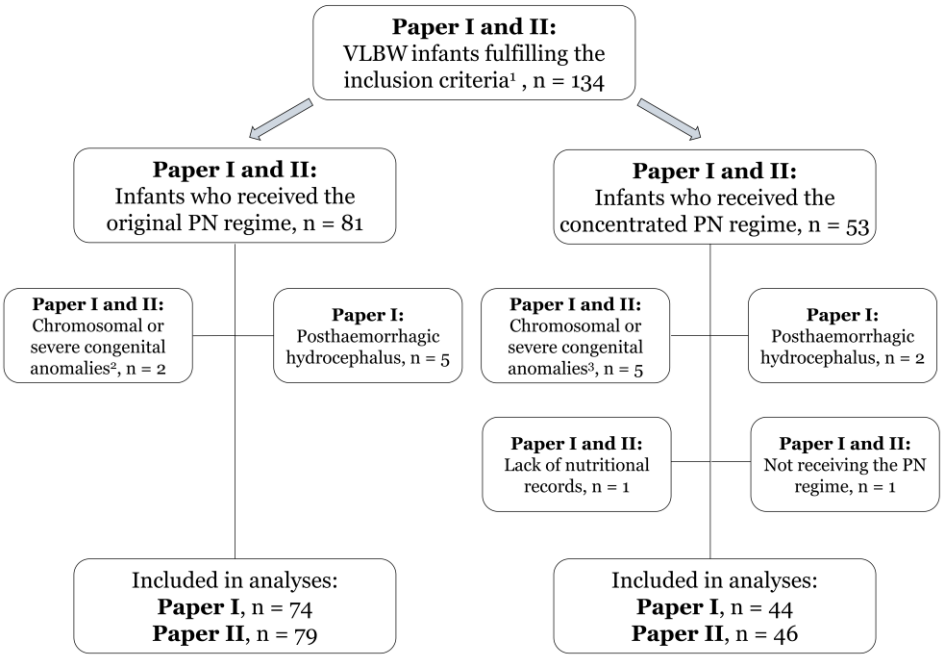

Figure 2. Included and excluded infants in Paper I and II. VLBW, very low birth weight (< 1500 g); PN, parenteral nutrition. [1]Admission to Umeå University Hospital < 24 hours after birth; Treatment duration ≥ 1 week. [2]Gastrointestinal malformation, n = 1; Skeletal malformation, n = 1. [3]Gastrointestinal malformation, n = 1; Congenital heart disease, n = 2; Down syndrome, n = 2.

The EXPRESS cohort and the Nutrium-EXPRESS cohort

For Paper III and IV, we used perinatal data from the EXPRESS cohort. As previously indicated, EXPRESS includes all live-born EPT infants with a gestational age of 22 weeks 0 days to 26 weeks 6 days in Sweden between April 1, 2004 and March 31, 2007. All obstetric and pediatric units in Sweden participated in EXPRESS and each unit was assigned to one of seven health care regions. Each health care region was served by a university hospital. The general policy was to centralize EPT deliveries to more specialized health care centers.

Data regarding nutritional intakes, postnatal growth, and nutritional biomarker concentrations were retrieved from the Nutrium-EXPRESS cohort. Nutrium-EXPRESS serves primarily as an extension of the data collection of the EXPRESS.

Of 707 live-born infants in the EXPRESS cohort, 70% survived at least one year (102). Among one-year survivors, 45% had no major neonatal morbidity, which was defined as absence of IVH grade > 2, absence of retinopathy of prematurity stage > 2, absence of periventricular leukomalacia, absence of necrotizing enterocolitis, and/or absence of severe bronchopulmonary dysplasia (102). Included and excluded infants in Paper III and IV are shown in Figure 3.

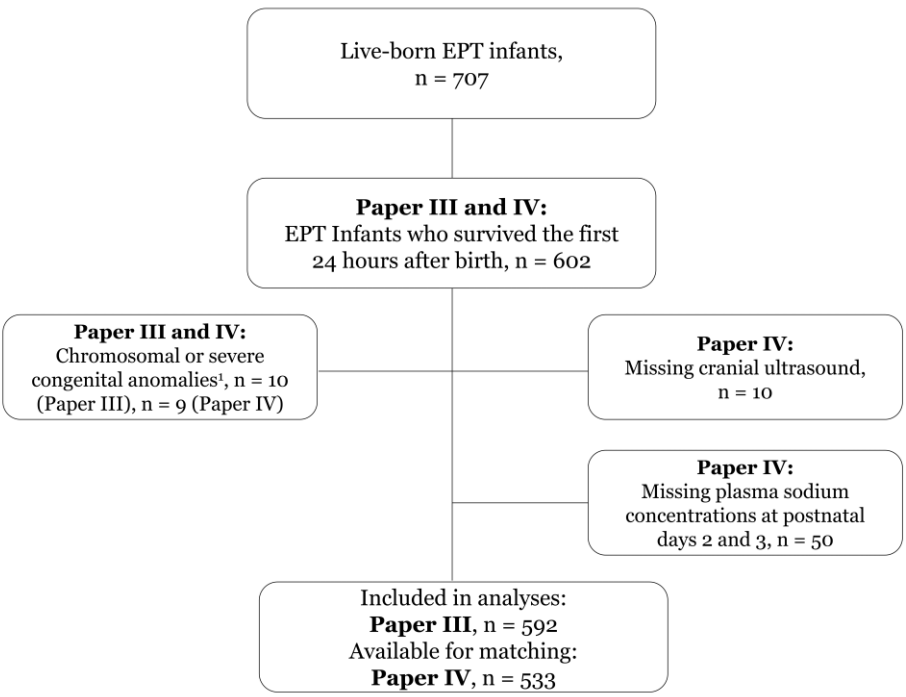

Figure 3. Included and excluded infants in Paper III and IV. EPT, Extremely preterm. [1]Multiple congenital anomalies, n = 3 (Paper III) and n = 2 (Paper IV); Gastrointestinal malformation, n = 2; Limb reduction defects, n = 2; Chromosomal anomalies, n = 3.

All infants with missing plasma sodium concentrations at postnatal days 2 and 3 were excluded from analyses in Paper IV since results from Paper III showed that plasma sodium concentrations increased steeply until postnatal day 3 and decreased thereafter. Thus, missing sodium concentrations during postnatal days 2 and 3 would have impaired our ability to diagnose sodium imbalances. Paper IV was conducted as a case-control study in which each infant with an IVH grade 3 or 4 was matched with one IVH-free infant by day of birth, hospital, sex, gestational age, and birth weight. The analyses included 70 matched case-control pairs.

Data collection and management

The following data were collected retrospectively from clinical charts for the PUMPA and the Nutrium-EXPRESS cohort (34) and used in this thesis:

1) Daily parenteral and enteral nutritional intakes and other fluids during the first 28 postnatal days.
2) All available postnatal weight, length, and head circumference (HC) measurements during hospital stay.
3) The first available laboratory result each day for the following nutritional biomarkers: pH, base excess, glucose (highest concentration each day), sodium, potassium, phosphate, and ionized calcium during the first 28 postnatal days.

These data were collected using the nutrition calculation program Nutrium (Nutrium software by Nutrium AB, Umeå, Sweden).

Furthermore, perinatal and morbidity data were prospectively collected from the Swedish Neonatal Quality Register for the PUMPA cohort (Paper I and II) and from the EXPRESS cohort (Paper III and IV) (98, 102). Severe IVH was defined as grades 3 to 4 hemorrhage according to the classification system of Papile et al. (97) as previously described. No time point for IVH diagnosis was recorded. Because the vast majority of infants develop IVH within the first three postnatal days, we limited the exposure to this period (92, 93). We used retinopathy data collected from the Swedish Retinopathy of Prematurity Register.

For infants who were transferred between hospitals, data collection continued using records from the respective hospitals. When total weekly fluid volume decreased by 10% or more as mothers started breastfeeding, data collection was stopped as the amount of breastmilk the infants received was unknown. In this case and in the case of partially unobtainable data and/or infant death, infants were included in the analyses as far as possible.

Nutrient intakes

Nutrient intakes were analyzed and presented as total, parenteral, and enteral intakes and included flush solutions, saline infusions, and transfused blood products. Blood product transfusions covered transfusions of erythrocytes, plasma, thrombocytes, and albumin. Energy and macronutrient content of breast milk samples were routinely analyzed using midinfrared spectrophotometry analyses. If analyses were missing, average contents were assumed. Furthermore, we adjusted for the low estimated intestinal absorption rate of enteral calcium intakes as described by Hicks et al. (103).

Nutrient intakes were calculated between 6am and 6am the following day using manufacturer-supplied information and/or published nutrient contents for each nutritional product (104-106). Intakes at the day of birth (day 0) were corrected for time of birth using the following formula:

$$Intakes\ of\ day\ 0 = Intakes\ during\ the\ first\ 24\ h \times \frac{h\ from\ birth\ until\ 6\ am}{24}$$

Nutritional treatment during the two regimen (Paper I and II)

Both the original and concentrated PN regimen consisted of a) two separate PN bags, which were always available at the NICU, and b) an adjustable parenteral glucose, lipid, and/or amino acid bag containing minerals (Table 6). Parenteral nutrient sources, not included in Table 6, were blood product transfusions, flush solutions, and saline infusions.

Table 6. Energy and nutrient content of the two parenteral nutrition regimen.

Nutrients	Original PN	Concentrated PN
Bag 1 (main bag)	Pharmacy-prepared all-in one solution	Commercially available solution[a]
Energy, kcal/100ml	54.0	83.0
Macronutrients		
Glucose, g/100ml	6.84	16.7
Lipids, g/100ml	1.66	-
Amino acids, g/100ml	2.72	3.90
Micronutrients		
Na, mmol/100ml	1.05	2.70
K, mmol/100ml	1.05	2.60
Cl, mmol/100ml*	-	3.90
Ca, mg/100ml	22.5	63.0
P, mg/100ml	47.3	41.5
Ca:P ratio	0.5:1	1.5:1
Magnesium, mg/100ml*	4.86	12.6
Iron, mg/100ml*	-	-
Zinc, mg/100ml*	0.10	-
Copper, µg/100ml*	8.00	-
Selenium, µg/100ml*	0.80	-
Manganese, µg/100ml*	0.40	-
Iodine, µg/100ml*	0.40	-
Further minerals added to bag 1**	No	Yes[c]
Vitamin A, µg/100ml*	41.4	-
Vitamin D, µg/100ml*	0.60	-
Vitamin E, mg/100ml*	0.38	-
Vitamin K, µg/100ml*	12.0	-
Vitamin B1, µg/100ml*	150	-
Vitamin B2, µg/100ml*	216	-
Vitamin B6, µg/100ml*	240	-
Vitamin B12, µg/100ml*	0.30	-
Niacin, mg/100ml*	2.40	-
Pantothenic acid, mg/100ml*	0.90	-
Biotin, µg/100ml*	3.60	-
Folic acid, µg/100ml*	24.0	-
Vitamin C, mg/100ml*	6.00	-
Bag 2	Glucose bag with individually added minerals	Lipid bag (SMOF) with phosphorus and vitamins[b]
Adjustable glucose, lipid and/or amino acid bag containing minerals[c]	yes	yes

Ca, calcium; Cl, chloride; K, potassium; Na, sodium; P, phosphorus; PN, parenteral nutrition; SMOF, based on Soybean oil, Medium chain triglycerides, Olive oil, and Fish oil.
[a]Contains further the individually added minerals zinc, copper, selenium, manganese, and iodine.
[b]Contains vitamin A, vitamin D, vitamin E, vitamin K, vitamin B1, vitamin B2, vitamin B6, vitamin B12, niacin, pantothenic acid, biotin, folic acid, and vitamin C.
[c]Partially administrated to the very low birth weight infants according to clinical decision.
*Not included in the analyses of Paper II. **Partly included in the analyses of Paper II.
Parenteral nutrient sources not included in the table are blood product transfusions, flush solutions, and saline infusions. Table adapted and extended from Paper I and II.

In addition to the implementation of the concentrated PN regimen, no changes in parenteral and enteral nutrition routines were made at the NICU during the study period. Per standard practice, enteral nutrition was introduced as soon as possible after birth. Enteral nutrition consisted of mothers' own or, if not available, banked donor human milk. A human milk fortifier was added and individually adjusted according to breast milk analysis when enteral fluid volume was around 70 to 100 ml/kg/d. In mid-2011, the manufacturer, unrelated to our intervention, slightly changed contents of protein, carbohydrates, sodium, potassium, and phosphorus in the human milk fortifier.

Growth measurements and definitions

If daily growth measurements were missing, the infants' weight, length, and/or HC values were calculated by linear interpolation. If length and/or HC measurement at the day of birth were missing, the measurement on the first or second postnatal day was used as birth length and/or birth HC so these infants could be included in the analyses (Paper I). To allow for comparisons of different gestational age and different sex, we converted absolute weight, length, and HC measurements into standard deviation scores (SDS). SDS calculations were based on a Swedish growth reference for infants born after 24 gestational weeks (107). Because the Swedish growth reference does not include reference values for infants born with a gestational age below 24 weeks, a Canadian reference was used for these infants (108). Small for gestational age and/or postnatal growth restriction were defined as a birth weight or a weight at a certain postnatal day of more than two standard deviations (SD) below the mean.

Nutritional biomarkers

The majority of nutritional biomarker analyses were performed on plasma samples using different point-of-care blood gas analyzers and are therefore referred to as plasma concentrations. Since universally agreed definitions are lacking, we a priori defined electrolyte imbalances according to definitions used by ESPGHAN and in the scientific literature (62, 72, 76).

Quality control

All data were collected by trained staff and internal controls were performed continuously and double-checked against the original records. In EXPRESS, one obstetric and one pediatric study coordinator were responsible for data collection and quality control of provided data in each of the seven health care regions. In addition, one internal and one external data control were performed on a randomly selected subset of infants. We also performed descriptive statistics to detect potentially unreasonably high or low values. These values were double-checked against the original records.

Statistical methods

Differences between groups were analyzed using the Independent samples t-test and the Fisher's exact test for continuous and binary outcome variables, respectively (Paper I and II). Differences between matched case-control pairs were analyzed using the paired sample t-test and the McNemar's test for continuous and binary outcome variables, respectively (Paper IV). If any of the background factors differed significantly between the investigated groups ($P < .05$), appropriate multivariable models were performed to at least partially compensate for these factors.

To investigate risk determinants of abnormal plasma sodium concentrations, we applied both linear regression analyses and binary logistic regression analyses for continues and binary outcome variables, respectively (Paper III). Significant risk factors in univariable analyses were analyzed using the respective multivariable regression models.

All data were analyzed using SPSS (IBM, SPSS Version 23.0, 24.0, and 25.0 for Windows, Armonk, NY, USA). All tests were two-sided and a $P < .05$ was considered statistically significant (Paper I to IV).

Ethical approval

All studies in this thesis were carried out in line with the Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects, developed by the World Medical Association.

The Regional Ethical Committee at Umeå University (Umeå, Sweden) approved the PUMPA study (Dnr 2011-417-31 M, 2012-458-31 M, and 2017-35-32 M). The EXPRESS and the Nutrium-EXPRESS were approved by the Regional Ethical Review Board, Lund University (Lund, Sweden) (Dnr 42-2004 and Dnr 138-2008, respectively). The parents provided oral informed consent for data acquisition in the EXPRESS. The respective ethical committee declared waiver of consent for the additional data acquisition in the Nutrium-EXPRESS as well as for data acquisition in the PUMPA study.

Results

PUMPA study (Paper I and II)

Cohort characteristics and infant morbidity

VLBW infants who received the concentrated PN regimen (concentrated PN group) were significantly shorter at birth, adjusted for gestational age and sex, compared with infants who received the original PN regimen (original PN group), as indicated by a significantly more negative birth length SDS in the concentrated PN group. Other growth data at birth and the infants' gestational age did not differ significantly between the groups (Table 8).

Significantly more infants were born after multiple pregnancy in the concentrated compared with the original PN group (Paper I: 39 vs. 15%, $P = .006$; Paper II: 39 vs. 18%, $P = .011$). In addition, fewer infants were treated for patent ductus arteriosus in the concentrated PN group (Paper I: 27 vs. 46%, $P = .052$; Paper II: 28 vs. 49%, $P = .025$). No further differences in baseline characteristics or infant morbidity were identified between the two PN groups (Paper I and II).

Nutrient intakes

All VLBW infants received nutrients from parenteral and enteral sources. During the first postnatal week, infants obtained 70% of the total fluid volume via parenteral sources, whereas in the second, third, and fourth postnatal week the percentages were 32, 18, and 12%, respectively. Neither parenteral, enteral, nor total fluid volumes differed significantly between the groups during the first four postnatal weeks (Table 7 and Figure 4a) (Paper I and II).

Postnatal week 1

First week total intakes of energy, macronutrients, and electrolytes were significantly higher in the concentrated PN group compared with the original PN group. The higher total intakes were mainly based on significantly increased parenteral nutrient intakes in the concentrated PN group, whereas enteral intakes, with the exception of carbohydrates and potassium, did not differ significantly between the two groups (Table 7) (Paper I and II).

Table 7. Mean daily parenteral, enteral, and total intakes of fluid, energy, and nutrients during the first postnatal week in very low birth weight infants who received either an original or a concentrated parenteral nutrition regimen.

Nutrients	Parenteral intakes			Enteral intakes			Total intakes		
	Original PN n = 79 Mean ± SD	**Concentrated PN n = 46 Mean ± SD**	***P*[b]**	**Original PN n = 79 Mean ± SD**	**Concentrated PN n = 46 Mean ± SD**	***P*[b]**	**Original PN n = 79 Mean ± SD**	**Concentrated PN n = 46 Mean ± SD**	***P*[b]**
Fluid, ml/kg/d[a]	93 ± 32	89 ± 27	.534	38 ± 20	47 ± 25	.054	131 ± 19	136 ± 15	.110
Energy, kcal/kg/d	45 ± 14	56 ± 16	< .001	26 ± 14	30 ± 18	.169	72 ± 8.0	87 ± 7.0	< .001
Macronutrients									
CHO, g/kg/d	7.7 ± 2.0	8.3 ± 2.1	.102	2.7 ± 1.5	3.4 ± 1.9	.045	10.3 ± 1.1	11.7 ± 1.0	< .001
Fat, g/kg/d	0.7 ± 0.5	1.5 ± 0.7	< .001	1.4 ± 0.8	1.5 ± 0.9	.473	2.1 ± 0.7	3.0 ± 0.5	< .001
Protein, g/kg/d	2.2 ± 0.9	2.6 ± 0.8	.008	0.6 ± 0.4	0.7 ± 0.4	.236	2.7 ± 0.7	3.3 ± 0.5	< .001
Electrolytes									
Na, mmol/kg/d	2.6 ± 1.4	3.8 ± 1.5	< .001	0.4 ± 0.4	0.5 ± 0.5	.213	2.9 ± 1.3	4.3 ± 1.4	< .001
K, mmol/kg/d	0.7 ± 0.3	1.3 ± 0.4	< .001	0.4 ± 0.3	0.5 ± 0.3	.044	1.1 ± 0.2	1.8 ± 0.3	< .001
Ca, mg/kg/d	22 ± 7	41 ± 12	< .001	10 ± 8 5.6 ± 4.5[c]	14 ± 11 7.5 ± 6.2[c]	.075	32 ± 7 27 ± 5[c]	55 ± 9 49 ± 8[c]	< .001
P, mg/kg/d	19 ± 8	37 ± 10	< .001	5.9 ± 5.0	7.8 ± 6.5	.090	25 ± 7	45 ± 7	< .001
Ca:P ratio	1.2: 1	1.1: 1		1.7: 1 0.9: 1[c]	1.8: 1 1: 1[c]		1.3: 1 1.1: 1[c]	1.2: 1 1.1: 1[c]	

Ca, calcium; CHO, carbohydrates, K, potassium, Na, sodium; P, phosphorus; PN, parenteral nutrition; SD, standard deviation.
[a]Fluid volume is taken from Paper I and is similar in Paper II.
[b]Independent samples t-test.
[c]Enteral calcium intakes are adjusted for the estimated intestinal absorption rate (Adjusted enteral calcium intakes = enteral calcium intakes*0.54; Adjusted total calcium intakes = parenteral calcium intakes + (enteral calcium intakes*0.54)) (103).
Table adapted and extended from Paper I and II.

Parenteral nutrient sources were a) the respective PN regimen, b) blood product transfusions, c) flush solutions, and d) saline infusions. On average, the PN regimen contributed 56% of the total parenteral sodium intake and between 98 and 99% of the total parenteral intakes of potassium, phosphorus, and calcium. Compared with the original PN regimen, the concentrated PN regimen provided the double amount of electrolytes to the VLBW infants (Paper II).

Postnatal weeks 2 to 4

During the second postnatal week, infants who received the concentrated PN regimen had significantly higher mean daily total intakes of energy, fat, protein, and parenteral fat compared with infants who received the original PN regimen (Figure 4a and 4b; Parenteral fat intake, mean ± SD [g/kg/d]: 1.1 ± 1.0 vs. 0.6 ± 0.8, P = .014). During the third postnatal week, total protein intake was still significantly increased in infants who received the concentrated PN regimen (Figure 4b). Enteral energy and macronutrient intakes did not differ significantly between the groups during postnatal weeks 2 to 4 (Paper I).

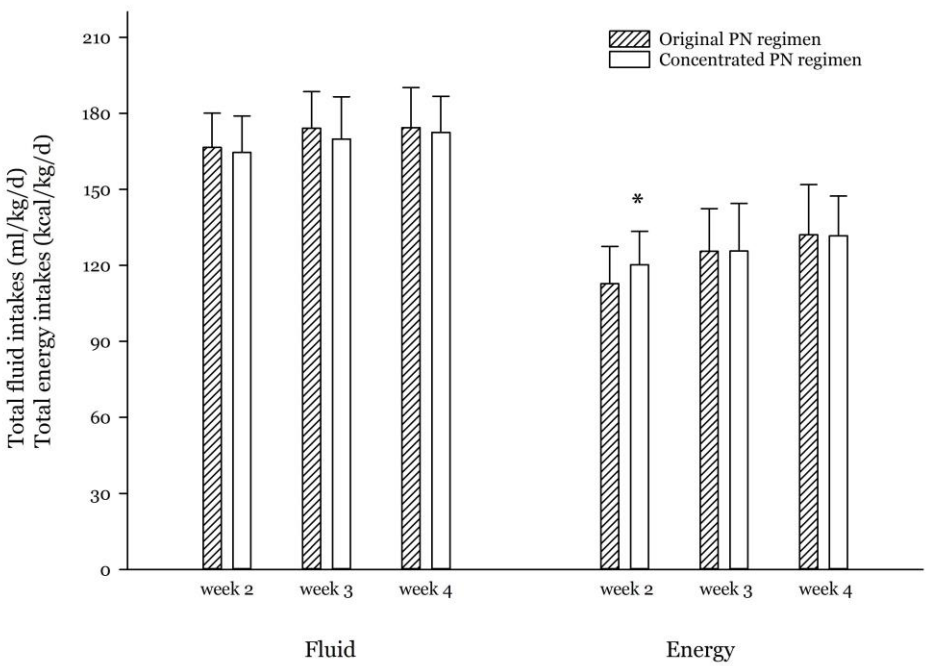

Figure 4a. Mean daily total intakes of fluid and energy during postnatal weeks 2 to 4 in very low birth weight infants who received either an original (n = 68 to 71) or a concentrated (n = 43 to 44) parenteral nutrition (PN) regimen. Different infant numbers were due to start of breastfeeding, infant death, and unobtainable data. Values are expressed as mean ± standard deviation. Independent samples t-test. *P < .01.

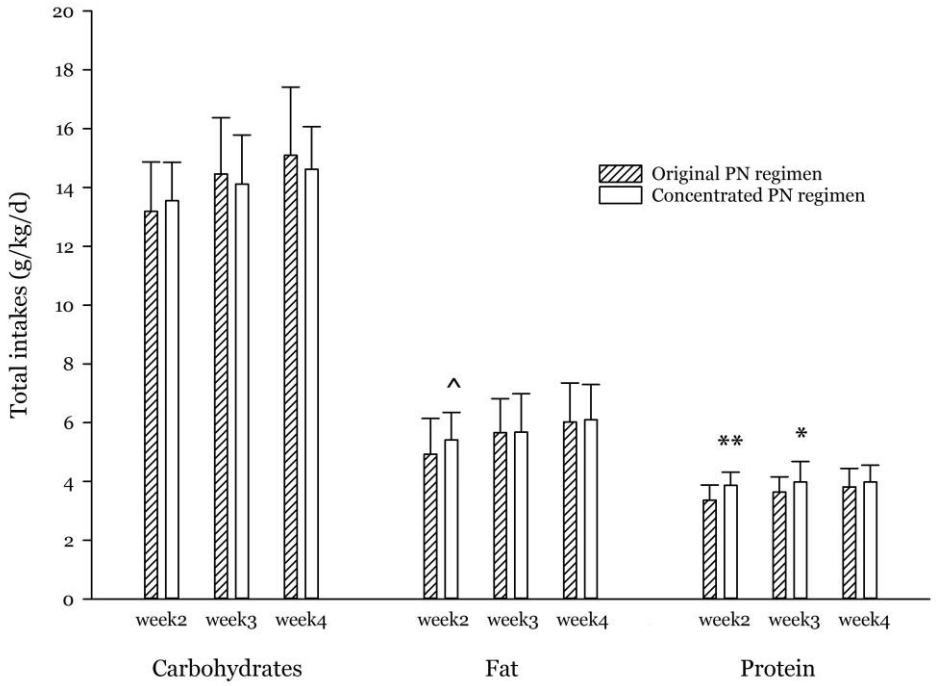

Figure 4b. Mean daily total intakes of macronutrients during postnatal weeks 2 to 4 in very low birth weight infants who received either an original (n = 68 to 71) or a concentrated (n = 43 to 44) parenteral nutrition (PN) regimen. Different infant numbers were due to start of breastfeeding, infant death, and unobtainable data. Values are expressed as mean ± standard deviation. Independent samples t-test. ^$P < .05$, *$P < .01$, **$P < .001$.

Postnatal growth

Infants who received the concentrated PN regimen had significantly improved changes in weight and length SDS from birth to postnatal days 7 to 28 compared with infants who received the original PN regimen. These improved changes in weight and length SDS were still significant at a postmenstrual age of 36 weeks. In contrast, change in HC SDS was improved in infants who received the original PN regimen, although this change was not statistically significant at any of the investigated time-points (Figure 5) (Paper I).

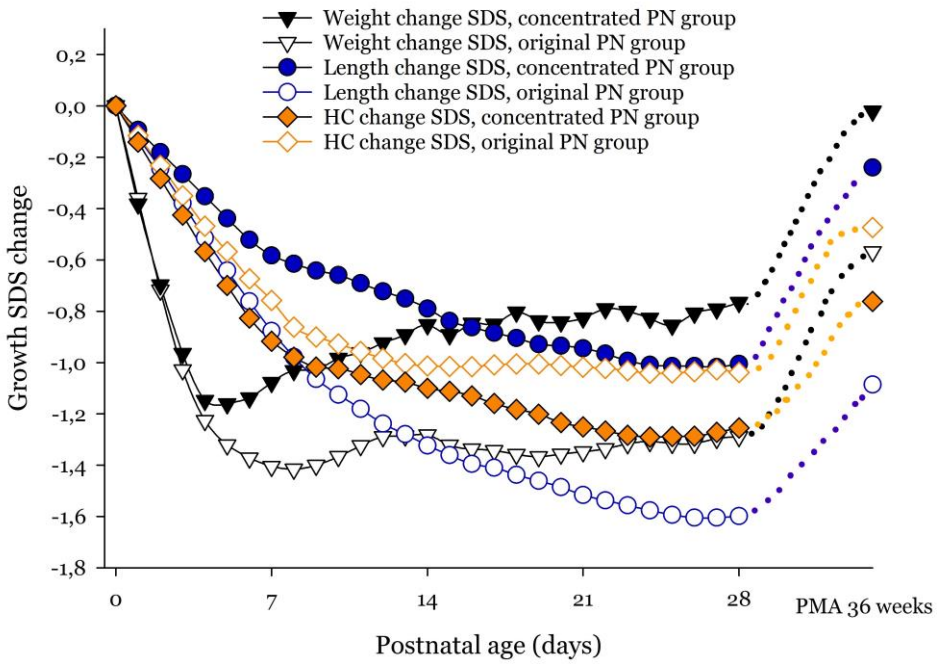

Figure 5. Mean changes in weight, length, and head circumference standard deviation scores from birth to postnatal days 1 to 28 and a postmenstrual age of 36 weeks in very low birth weight infants who received either an original (n = 64 to 74) or a concentrated parenteral nutrition regimen (n = 41 to 44). Different infant numbers were due to infant death and unobtainable data. HC, head circumference; PN, parenteral nutrition; PMA, postmenstrual age; SDS, standard deviation score. Figure adapted and extended from Paper I.

Absolute growth data of VLBW infants who received either an original or a concentrated PN regimen are shown in Table 8. Infants in the concentrated PN group had a significantly less weight loss from birth to postnatal day 7 but not thereafter compared with infants in the original PN group (Paper I).

Table 8. Growth data in very low birth weight infants who received either an original or a concentrated parenteral nutrition regimen.

Growth data	Orig. PN	Conc. PN	*P*[d]	Orig. PN	Conc. PN	*P*[d]
	Birth			Day 7		
	n = 70-74[c]	n = 44		n = 69-74[c]	n = 44	
	n (%)	**n (%)**		**n (%)**	**n (%)**	
SGA/PNGR[a]	20 (27.0)	14 (32)	.675	50 (68)	33 (75)	.414
	Mean ± SD	**Mean ± SD**		**Mean ± SD**	**Mean ± SD**	
GA, weeks	26.9 ± 2.7	27.4 ± 2.3	.369	-	-	-
Weight, g	919 ± 318	920 ± 303	.986	853 ± 274	899 ± 279	.387
Weight gain, g/kg/d	-	-	-	-8.7 ± 15.2	-2.2 ± 10.3	.006
Weight, SDS	-1.30 ± 1.41	-1.74 ± 1.64	.128	-2.71 ± 1.23	-2.82 ± 1.48	.665
Length, cm	34.9 ± 4.2	34.1 ± 3.7	.353	35.2 ± 4.0	34.7 ± 3.4	.562
Length, SDS	-1.42 ± 1.70	-2.35 ± 1.78	.007	-2.30 ± 1.44	-2.93 ± 1.63	.033
HC, cm	24.3 ± 2.8	25.0 ± 2.3	.180	24.3 ± 2.6	24.7 ± 2.4	.409
HC, SDS	-0.81 ± 0.86	-0.69 ± 0.84	.435	-1.58 ± 0.72	-1.60 ± 76	.869
	Day 28			**PMA 36 weeks**		
	n = 69-70[c]	n = 44		n = 66-69[c]	n = 41	
	n (%)	**n (%)**		**n (%)**	**n (%)**	
SGA/PNGR[a]	50 (71)	27 (61)	.307	27 (39)	15 (37)	.841
	Mean ± SD	**Mean ± SD**		**Mean ± SD**	**Mean ± SD**	
Weight, g	1303 ± 454	1348 ± 420	.592	2227 ± 363	2253 ± 392	.727
Weight gain, g/kg/d[b]	18.4 ± 4.7	19.1 ± 2.9	.325	16.6 ± 2.4	16.6 ± 1.7	.869
Weight, SDS	-2.59 ± 1.18	-2.50 ± 1.33	.710	-1.83 ± 1.31	-1.78 ± 1.44	.844
Length, cm	37.9 ± 4.6	37.9 ± 3.8	.960	43.9 ± 2.6	43.7 ± 2.7	.790
Length, SDS	-3.10 ± 1.64	-3.35 ± 1.76	.430	-2.55 ± 1.77	-2.65 ± 1.86	.779
HC, cm	26.8 ± 3.0	26.9 ± 2.7	.799	31.4 ± 1.6	31.1 ± 1.5	.436
HC, SDS	-1.83 ± 0.87	-1.94 ± 0.93	.529	-1.26 ± 1.22	-1.45 ± 1.12	.405

GA, gestational age; HC, head circumference; PMA, postmenstrual age; PN, parenteral nutrition; PNGR, postnatal growth restriction; SDS, standard deviation score; SGA, small for gestational age.
[a]Defined as a weight of less than two standard deviations below the mean.
[b]From postnatal day 7 to 28 respective from postnatal day 7 to postmenstrual age of 36 weeks (growing stable infant).
[c]Different infant numbers were due to infant death and/or unobtainable data at the day of birth.
[d]Fisher's exact test and Independent samples t-test for binary and continuous outcome variables, respectively.

Plasma electrolyte concentrations

During the first postnatal week, plasma concentrations of sodium and ionized calcium did not differ significantly between infants who received the two PN regimen. Infants who received the original PN regimen had significantly lower mean and maximum phosphate concentrations compared with infants who received the concentrated PN regimen, but there was no difference in the occurrence of hypophosphatemia (< 1.40 mmol/l) between both groups (45 vs. 40%, $P = .692$) (Table 9). Plasma concentrations of potassium were significantly lower and the prevalence of hypokalemia (< 3.50 mmol/l) was higher in the original PN group compared with the concentrated PN group (76 vs. 30%, $P < .001$). None of the infants in the total study cohort had hyperkalemia (> 6 mmol/l) (Table 9) (Paper II).

Table 9. Electrolyte concentrations in the first postnatal week in very low birth weight infants who received either an original or a concentrated parenteral nutrition regimen.

Plasma electrolyte concentrations		Original PN n = 79 Mean ± SD	Concentrated PN n = 46 Mean ± SD	*P*[b]
Na, mmol/l	Mean 1st week	138.0 ± 4.6	138.0 ± 4.8	.967
	Minimum 1st week	131.4 ± 3.9	131.0 ± 4.5	.605
	Maximum 1st week	144.9 ± 6.8	145.1 ± 7.1	.846
K, mmol/l	Mean 1st week	3.94 ± 0.41	4.32 ± 0.44	< .001
	Minimum 1st week	3.24 ± 0.36	3.55 ± 0.27	< .001
	Maximum 1st week	4.95 ± 0.84	5.27 ± 0.80	.044
iCa, mmol/l	Mean 1st week	1.33 ± 0.06	1.35 ± 0.05	.112
	Minimum 1st week	1.13 ± 0.11	1.16 ± 0.09	.077
	Maximum 1st week	1.47 ± 0.09	1.48 ± 0.10	.393
P, mmol/l n = 60/45[a]	Mean 1st week	1.51 ± 0.47	1.76 ± 0.29	.001
	Minimum 1st week	1.42 ± 0.48	1.57 ± 0.36	.093
	Maximum 1st week	1.60 ± 0.54	2.01 ± 0.31	< .001

iCa, ionized calcium; K, potassium; Na, sodium; P, phosphate; PN, parenteral nutrition.
[a]Different infant numbers were due to unobtainable data.
[b]Independent samples t-test.

EXPRESS (Paper III and IV)

Prevalence of sodium imbalances

The Mean ± SD gestational age and birth weight of 592 included EPT infants were 25.3 ± 1.1 weeks and 765 ± 169 g respectively. Plasma sodium concentrations steeply increased from birth to day 3, decreased then to a nadir on day 10, and thereafter remained stable at a plasma concentration at around 135 mmol/l (Figure 8). Hypernatremia was common in EPT infants during the first postnatal week (50%). In contrast, during the second postnatal week hyponatremia occurred in 79% of EPT infants (Table 10) (Paper III).

Table 10. Prevalence of hyper- and hyponatremia in extremely preterm infants.

Sodium imbalances	n (%)
Hypernatremia days 0 to 6, > 145 mmol/l, n = 556[a]	282 (50)
Mild hypernatremia, 146-150 mmol/l	163 (29)
Moderate hypernatremia, 151-155 mmol/l	90 (16)
Severe hypernatremia, > 155 mmol/l	29 (5)
Hyponatremia days 7 to 13, < 135 mmol/l, n = 515[a]	404 (79)
Mild hyponatremia, 130-134 mmol/l	199 (39)
Moderate hyponatremia, 125-129 mmol/l	164 (32)
Severe hyponatremia, < 125 mmol/l	41 (8)

[a]Different infant numbers were due to unobtainable data and infant death.

Predisposing factors of sodium imbalances

Major risk determinants for the development hyper- and/or hyponatremia were the infants' gestational age at birth and the provision of sodium the preceding days (Figure 6, Table 11) (Paper III).

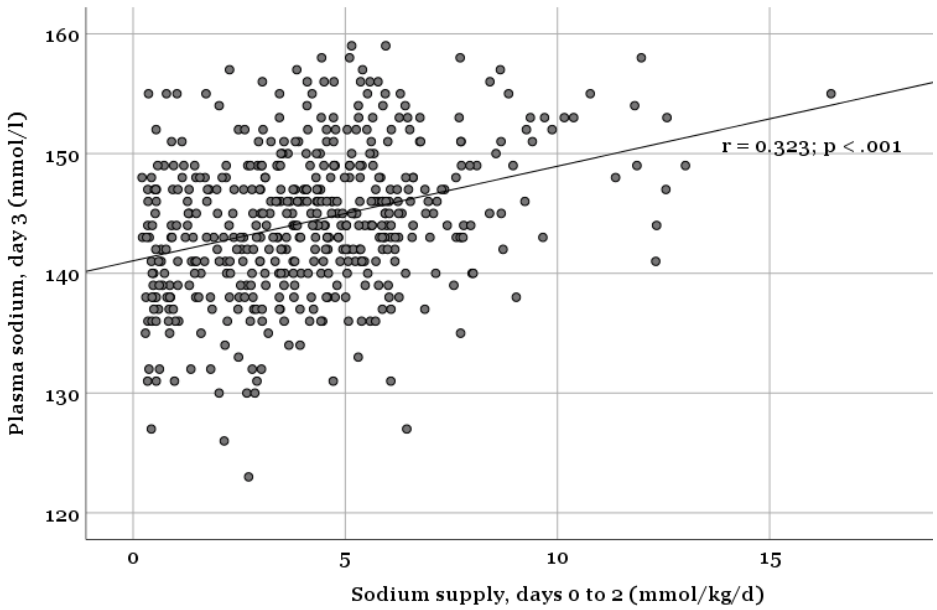

Figure 6. Plasma sodium concentration at postnatal day 3 and mean daily sodium supply for the preceding days in extremely preterm infants (n = 518).

Fluid volume was associated with percentage weight change ($P < .001$) and had a minor impact on the risk of hypernatremia (Table 11).

Table 11. Factors associated with hyper- and hyponatremia in extremely preterm infants.

Risk factor	Hypernatremia, day 2 n = 534/337[a]		Hypernatremia, day 3 n = 518/514[a]	
	OR	**aOR**	**OR**	**aOR**
Na supply, mmol/kg/d[b]	1.22**	1.31**	1.19**	1.16**
Gestational age, weeks	0.56**	0.48**	0.66**	0.69**
BW, 100 g increment	0.77**	ns	0.79**	ns
Percentage weight change[c]	0.95^	0.92*	ns	-
CRIB score	1.08*	ns	1.10**	ns
Apgar score, 5 minutes	0.90^	ns	ns	-
	Hypernatremia, day 4 n = 505/501[a]		**Hyponatremia, day 10 n = 416/413[a]**	
	OR	**aOR**	**OR**	**aOR**
Na supply, mmol/kg/d[b]	1.36**	1.33**	0.85**	0.85**
Fluid volume, ml/kg/d[d]	1.24^	0.74*	ns	-
Gestational age, weeks	0.69**	ns	ns	-
BW, 100 g increment	0.80*	ns	ns	-
CRIB score	1.11**	ns	ns	-
Apgar score, 1 minute	0.90^	ns	ns	-
MV treatment[e]	ns	-	0.92^	ns

BW, birth weight; CRIB, clinical risk index for babies; MV, mechanical ventilation; Na, sodium; ns, not significant; OR, odds ratio; aOR, adjusted odds ratio.
[a]Different infant numbers were due to unobtainable data and infant death.
[b]For the preceding days (days 0-1, 0-2, 0-3, and 4-9) from all enteral and parenteral sodium sources.
[c]Until the respective postnatal day; n = 337 due to unobtainable weight data.
[d]For the preceding days from all enteral and parenteral fluids and corrected for sodium.
[e]Number of days during postnatal days 0-3 (hypernatremia) and postnatal week 1 (hyponatremia).
Univariable and multivariable logistic regression analyses. ^$P < .05$, *$P < .01$, **$P < .001$.
Table adapted from Paper III.

During the first days after birth, major sodium sources were saline injections/infusions and blood product transfusions. At the end of the first and during the second postnatal week, sodium sources shifted towards parenteral and enteral nutrition (Paper III).

Sodium imbalances and severe intraventricular hemorrhage

Gestational age (mean ± SD) was the same for the 70 EPT infants with severe IVH (cases, 25.0 ± 1.0 weeks) and the 70 IVH-free EPT infants (controls, 25.0 ± 1.1 weeks). Furthermore, birth weight (mean ± SD) did not differ between cases and controls (741 ± 144 and 736 ± 140 g, respectively). Infants suffering from severe IVH had a higher clinical risk index for babies score compared with IVH-free infants (8.7 ± 4.0 vs. 6.8 ± 3.0, $P < .001$). In addition, twice as many infants with severe IVH received mechanical ventilation between birth and postnatal day 3 (81 vs. 43%, $P < .001$). No further differences in potential confounding factors and/or matching factors were identified (Paper IV).

When performing analyses adjusting for clinical risk index for babies score and mechanical ventilation treatment, we found no significant associations between the occurrence of severe IVH and plasma sodium concentration, sodium fluctuation, or hypernatremia, all assessed within the first three postnatal days. However, a higher total supply of sodium and a higher total fluid volume significantly increased the risk of severe IVH. These associations did not remain when excluding the amount of sodium delivered from blood product transfusions from the total sodium supply and the total fluid volume (Figure 7) (Paper IV).

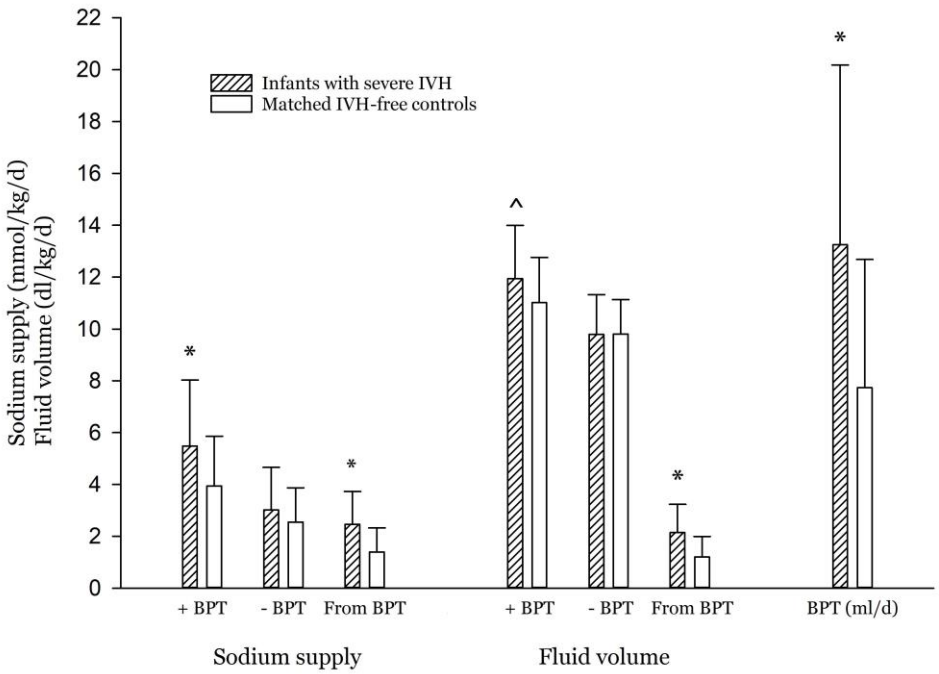

Figure 7. Mean daily sodium supply, fluid volume, and blood product transfusions during postnatal days 0 to 2 in extremely preterm infants with severe intraventricular hemorrhage (IVH; n = 61) and in the matched IVH-free control group (n = 61). Nine case-control pairs were excluded due to unobtainable nutritional intake data. Values are expressed as mean ± standard deviation. Multivariable conditional logistic regression analyses. Adjustment for clinical risk index for babies score and mechanical ventilation treatment. ^$P < .05$, *$P < .01$. +/-BPT, with and without sodium delivered from blood product transfusions. Figure adapted and extended from Paper IV.

Discussion

Discussion of the methods

Ethical considerations when choosing the study design and what were potential limitations and advantages of the used design?

Original research studies are categorized into observational and interventional studies (109). The strongest study design to investigate causal inference is the randomized controlled design. However, observational study designs are used when it might not be logistically feasible or ethical to use an interventional design. Ethical considerations were crucial in the selection of the study design of the research presented in Paper I and II. At the time of introduction of the concentrated PN regimen, it was known that EPT infants in Sweden receive far too low amounts of energy and macronutrients, leading to poor postnatal growth (34). Both low nutritional intakes and poor postnatal growth have been associated with impaired neurological outcome (24-27). Therefore, it would have been ethically questionable to delay the introduction of the concentrated PN regimen in parts of the study population.

Similar to this situation, a randomized controlled design would not have been ethically acceptable for the studies presented in Paper III and IV. The studied outcomes of Paper III and IV (i.e., sodium imbalances and severe IVH) might significantly affect neurological outcome and lead to infant death (85, 94). However, the nested matched case-control design, as used in Paper IV, is particularly suitable for exploring risk factors for rare diseases while controlling for multiple confounders (109).

Observational studies have important limitations in terms of their predisposition to bias and confounding, restricting their ability to investigate causality (109, 110). A key question in observational studies is the selection of variables that need to be controlled for to remove confounders (111). In this thesis, we adjusted or matched the analyses for confounding variables defined as variables associated with both exposure and outcome (112). However, we cannot exclude the possibility of unknown confounding influencing the presented results (113).

Advantages of observational studies, on the other hand, include lower costs, often resulting in longer study periods and a higher number of study participants. Participants in these studies often display greater diversity, including the higher presence of common comorbidities compared with randomized controlled studies (114, 115). Consequently, large and well-designed observational studies have the potential to provide important information about safety and efficacy of

treatments in clinical practice. Furthermore, a before-and-after design, such as used in the PUMPA study, which included almost all VLBW infants admitted to the NICU at Umeå University Hospital, has the ability to more closely reflect daily clinical practice. Therefore, such studies have the potential to provide more clinically relevant data, which might not be necessarily the case in randomized controlled studies (110, 114).

Are the retrospectively collected clinical data reliable?

Data regarding nutritional intakes, postnatal growth, laboratory results, and perinatal and infant morbidity are documented in detail at NICUs in Sweden. Nevertheless, some bias may have been introduced due to misclassification of neonatal morbidities and indistinct or erroneous data documentation in clinical charts. Other variables of potential interest such as urinary electrolyte output, daily phosphate concentrations, or blood pressure measurements are lacking as they either have not been assessed in clinical routine or were not considered in the data collection. Thus, these variables were not available for the present data analyses.

Was the power appropriate within the performed studies?

In the PUMPA study, an a priori power calculation was based on the primary outcome change in weight SDS from birth to postnatal day 28 (Paper I). To detect a difference of 0.3 SD in weight SDS, the number of required study pairs was estimated to be 60, allowing for a dropout rate of 15%. These calculations were based on a power of 0.8, a significance level of .05, and 2-tailed biostatistical testing (116). Studying the main outcome (i.e., change in weight SDS), differences between groups were observed based on slightly changed numbers of participants (n = 46 and n = 79), implying this study had sufficient power (Paper I). However, the study was not powered to detect differences in secondary outcomes such as infant morbidity. In particular, we lacked power to detect differences in rare morbidities such as necrotizing enterocolitis or severe IVH.

Similarly, post hoc power calculations in Paper II and IV showed sufficient power to detect differences in the respective main outcome, but not in secondary outcomes.

Discussion of the results

Paper I and II

Is it possible to achieve recommended parenteral nutrient intakes?

During the first postnatal week, VLBW infants in the PUMPA cohort received most of their fluid volume from PN. Infants who received the concentrated standard ready-to-use PN achieved currently recommended energy and nutrient intakes during the first postnatal week (ESPGHAN 2018 PN recommendations) (117). In contrast, infants who received the less concentrated PN regimen (original PN group) generally did not reach these recommended intakes (Table 12). Our results were confirmed by a randomized controlled study of Moltu et al. that compared two nutritional protocols for VLBW infants (54). This study demonstrated that adherence to a strict nutritional protocol, including the use of standardized bags, makes it possible to achieve the currently recommended energy and nutrient intakes during the first four postnatal weeks.

Energy and nutrients provided to the original PN group were below ESPGHAN PN recommendations used during the study period (2010 to 2013) (ESPGHAN 2005 PN recommendations) (68). During the same period, parenteral fluid volume provided to VLBW infants did not change. Consequently, restriction of fluid volume in combination with the use of a less concentrated PN regimen might largely contribute to insufficient energy and nutrient intakes during the first postnatal week. Therefore, concentrated PN that provides the recommended parenteral energy and nutrient intakes is of particular importance for the sickest and therefore most often fluid-restricted infants.

Table 12. Total fluid, energy, and nutrient intakes and weight gain of very low birth weight infants who received either an original or a concentrated parenteral nutrition regimen and the current parenteral nutrition recommendations.

Characteristic	Original PN n = 79	Concentrated PN n = 46	ESPGHAN 2018 (117)
	Week 1	**Week 1**	**Day 1 / Days 2+**
Fluid, ml/kg/d	131±19	136±15	70-100 (day 1) to 160-180 (day 6)
Energy, kcal/kg/d*	72±8.0	87±7.0	45-55 / 90-120
Weight gain, g/kg/d[a]	18.4 ± 4.7	19.1 ± 2.9	17-20
Macronutrients			
CHO, g/kg/d	10.3±1.1	11.7±1.0	5.8-11.5 / 11.5-14.4
Fat, g/kg/d*	2.1±0.7	3.0±0.5	4[d]
Protein, g/kg/d	2.7±0.7	3.3±0.5	1.5[e] / 2.5-3.5
Electrolytes			
	Days 0-3 / 4-6	**Days 0-3 / 4-6**	**Days 1-2 / 3 / 4-5**
Na, mmol/kg/d	2.6±1.6 / 3.4±1.5	3.8±1.3 / 4.9±2.1	0-2(3) / 0-5(7) / 2-5(7)
K, mmol/kg/d*	0.7±0.2 / 1.6±0.4	1.5±0.3 / 2.2±0.4	0-3 / 0-3 / 2-3
Ca, mg/kg/d*	27±6 / 38±12 24±6[c] / 30±9[c]	47±8 / 64±15 44±8[c] / 54±13[c]	32-80[f] / 64-140[a]
P, mg/kg/d*	17±6 / 34±12	37±7 / 54±8	31-62[f] / 50-108[a]
Ca:P ratio[b]	1.5: 1 / 0.9: 1	1.2: 1 / 1: 1	0.8-1: 1

Ca, calcium; CHO, carbohydrates; ESPGHAN, European Society of Paediatric Gastroenterology, Hepatology and Nutrition; K, potassium, Na, sodium; P, phosphorus; PN, parenteral nutrition.
*Most prominent differences that deviate from the current parenteral nutrition recommendations.
[a]Growing stable preterm infant; Counted from postnatal day 7 to 28 in Paper I.
[b]Of the respective parenteral nutrition regimen.
[c]Enteral calcium intakes are adjusted for the estimated intestinal absorption rate (Adjusted total calcium intakes = parenteral calcium intakes + [enteral calcium intakes*0.54]) (103).
[d]Not exciding 4 g/kg/d and starting as soon as possible after birth and no later than postnatal day 2.
[e]Start as soon as possible after birth.
[f]During the first days of life.
Table adapted and extended from Paper I and II.

What are the effects of an energy and nutrient provision according to recommendations?

Initial loss in weight SDS was reduced and ended earlier in infants who received currently recommended energy and nutrient intakes (ESPGHAN 2018 PN recommendations) (117) during the first postnatal week compared with infants not receiving this recommended intakes (Paper I). Reduced weight loss appeared from postnatal day 4, suggesting that weight loss during the first three postnatal days was solely caused by shifts in fluid balance. After initial weight loss, infants in both groups remained within their growth trajectories until postnatal day 28 (Figure 5). This might be explained by the fact that both groups received an appropriate supply of energy and macronutrients after the first postnatal week until postnatal day 28 (13). Accordingly, weight gain during this period was within the recommended range in both groups (Table 12). Similar to this pattern,

we observed a difference in length growth between the groups during the first postnatal week but not thereafter. In addition, both VLBW infant groups showed equal increase in weight and length SDS between postnatal day 28 and a postmenstrual age of 36 weeks (Figure 5). However, nutritional intakes during this period were not analyzed and both groups did not show catch up growth up to a postmenstrual age of 36 weeks.

Our observed patterns for growth in weight and length (Paper I) are similar to the results of Moltu et al. (54). In line with our data, Moltu et al.'s intervention group displayed first week energy and macronutrient intakes according to ESPGHAN 2018 PN recommendations (117). These intakes might have led to reduced weight loss that ended earlier compared with the control group, which did not receive recommended intakes of energy (38) and carbohydrates (41). In our study, nutritional intakes of both groups were within ESPGHAN 2010 enteral nutrition recommendations (13) between the first postnatal week and postnatal day 28. Furthermore, no major changes in enteral nutrition were introduced during our study. In contrast, Moltu et al.'s intervention group received enteral supplementation with amino acids, long chain polyunsaturated fatty acids, and vitamin A, and the group's nutritional intakes were above ESPGHAN 2010 enteral nutritional recommendations (13). However, change in weight and length SDS from birth to a postmenstrual age of 36 weeks were almost identical in our study compared with the study of Moltu et al.

Optimal growth pattern in preterm infants, including catch up growth to achieve good long-term health outcome in these infants, is still debated. Some studies report associations between increased early growth and/or catch up growth and risk of later metabolic and cardiovascular disease (28, 29, 118). In contrast, a review assessing the effect of growth rate on body adiposity concluded that catch up growth during the first 15 months of life is not linked with adiposity in preterm infants (119). Another review suggested that risk of metabolic and cardiovascular disease is influenced by growth in later childhood and adolescence rather than by early postnatal growth (42). However, there is an accepted consensus that rapid infant weight gain after preterm birth is beneficial for the infants' later neurological outcome, which supports the promotion of growth for preterm infants after birth (120, 121).

Our study and other studies demonstrated that initial weight loss after preterm birth determines growth outcome (Paper I) (51, 54). Based on recent investigations of preterm infants receiving adequate nutritional supply starting at birth (51, 54, 122, 123), ESPGHAN recommends an initial weight loss between 7 and 10% of birth weight for VLBW infants (62). Weight loss of less than 3% and more than 12% was associated with increased mortality in infants with a birth weight < 1000 g (124). A recently published study by Andrews et al. describes a

single neonatal unit's experience of longitudinal growth in a cohort of preterm infants born before 32 gestational weeks (125). This study showed no initial absolute weight loss in any of the gestational age-based subgroups. Data regarding the prevalence of morbidity and/or mortality were not published. Even though, the study demonstrates that questions regarding both what can and should be achieved in terms of initial weight loss of preterm infants still remain.

We observed decreased HC in infants who received currently recommended parenteral energy and nutrient intakes (117) compared with infants who generally did not reach these recommended intakes (Paper I). This finding is not in accordance with the results of Moltu et al. (54) and another randomized controlled study (55). Both these studies observed increased HC growth in infants who received improved nutritional protocols. The non-significantly decreased HC growth observed in our concentrated PN group could have been influenced by measurement errors in particular in the retrospective study design.

Single nutrient interventions or interventions applied during very limited periods (49, 53, 56) often did not result in nutrient intakes consistent with current ESPGHAN recommendations (except for intervened nutrient or period). Such interventions had less or no impact on infant growth compared with comprehensive adjustments of the PN protocol (54, 55, 57, 58).

In our study, VLBW infants who received electrolytes during the first postnatal week according to current ESPGHAN 2018 PN recommendations (62, 72) generally displayed no electrolyte imbalances (Paper II). This might not be true for the recommended potassium intake during postnatal days 1 to 3. Despite a potassium intake according to the current ESPGHAN 2018 PN recommendations (Table 12) (62), infants in the original PN group compared with infants in the concentrated PN group had a significantly higher prevalence of hypokalemia already at postnatal day 3. Therefore, an increase of the minimum recommended potassium intake during the first three postnatal days to 1 mmol/kg/d might be useful to prevent hypokalemia during this time period. However, our study and other studies found that infants who received electrolytes below the ESPGHAN 2018 PN recommendations exhibited increased electrolyte disturbances such as hypokalemia, hypophosphatemia, and hypercalcemia (Paper II) (76, 78).

Are other factors than protocol change responsible for increased nutritional intakes?

Along with the change of the PN protocol, several other factors might have influenced nutritional practice at the NICU at Umeå University Hospital during the study period. General practice at the NICU is to individually adapt nutritional support, including using additional nutritional products and flexible introduction

of human milk fortification. The manufacturer of the human milk fortifier changed its protein and electrolyte content during the study period. In addition, at the beginning of 2012 a practical manual regarding nutritional care of preterm infants was introduced and between 2010 and 2011 a computer-aided nutrition calculation program was introduced. Because of these changes, and because the nutrition calculation program improves postnatal growth (126), we cannot exclude that other factors than change of the PN regimen influenced nutritional intakes before and after February 2012.

We did not observe significant differences between the groups in parenteral, enteral, or total fluid volume during the first four postnatal weeks (Paper I and II). Furthermore, the main differences in energy and nutrient intakes were mainly caused by parenteral sources (Table 7). Therefore, it is reasonable to conclude that the change to the concentrated PN regimen was the major factor contributing to improved total intakes of energy and nutrients (Paper I and II).

Paper II, III, and IV

Is sodium harmful to preterm infants?

EPT infants show an increase in plasma sodium concentration after birth similar to healthy term-born infants (127). Typically, in healthy term-born infants, sodium concentration stabilizes at about 140 mmol/l after 24 hours. However, in our EPT cohort (Paper III), we observed increasing plasma sodium concentration, peaking at a mean of 144.3 mmol/l at postnatal day 3 and decreasing thereafter to a mean of 134.4 mmol/l at postnatal day 10 (Figure 8). Consequently, EPT infants are more likely to be affected by sodium imbalances than healthy term-born infants. These sodium imbalances after preterm birth could be caused by immature adaptive processes (63). Initial hypernatremia in EPT infants might be explained by low urinary sodium excretion, high insensible water loss, and/or contraction of the extracellular fluid compartment immediately after preterm birth. The following hyponatremic phase might be related to high urinary sodium losses occurring after initial oliguria.

Gestational age and preceding provision of sodium were major factors determining plasma sodium concentration and the risk of hyper- and hyponatremia in our EPT cohort (Paper III). Furthermore, we showed that daily sodium intakes were significantly higher during the first postnatal week in VLBW infants who received the concentrated PN regimen compared with infants who received the original PN regimen. However, plasma sodium concentration was only slightly increased in infants who received the concentrated PN (Figure 8), illustrating a relatively weak effect of sodium supply on plasma sodium concentration (Paper II).

Therefore, initial sodium imbalances in EPT infants seem to largely reflect immature adaptive processes after birth. This hypothesis is supported by a higher increase in plasma sodium in the EPT cohort compared with more mature VLBW infants who received concentrated PN despite lower daily provision of sodium to the EPT cohort during the first postnatal week (Mean ± SD [mmol/l]: 4.05 ± 2.35 vs. 4.26 ± 1.41). Furthermore, a recent study by Monnikendam et al. demonstrated significantly lower serum sodium concentrations in infants born with a gestational age of 27 to 29 weeks compared with infants born with a gestational age of both 23 to 24 weeks and 25 to 26 weeks (88).

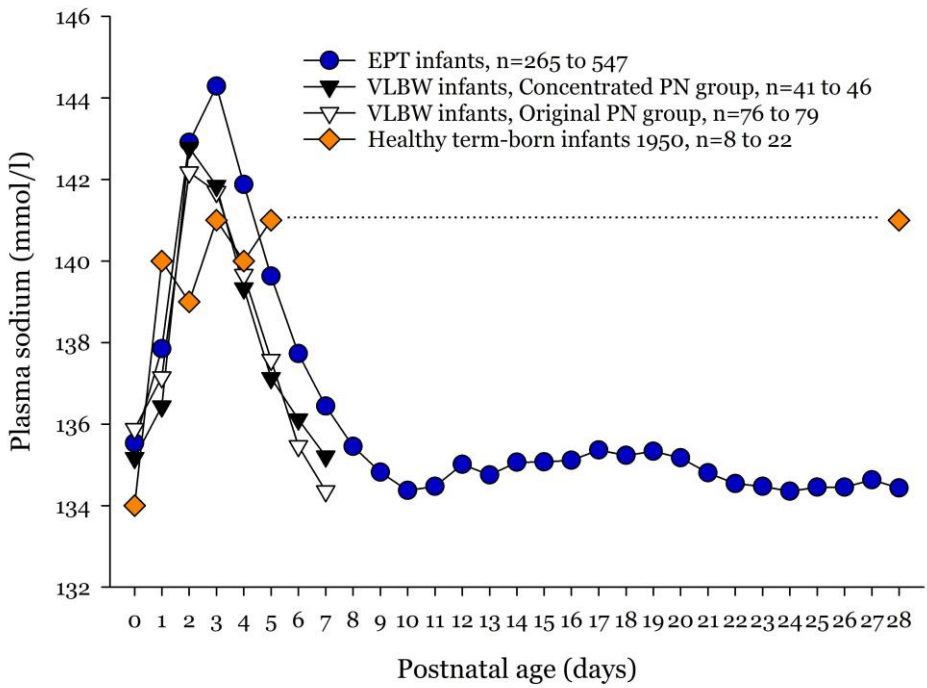

Figure 8. Mean daily plasma sodium concentrations of extremely preterm infants (EPT), very low birth weight (VLBW) infants, and healthy term-born infants during the first 28 postnatal days. Different infant numbers were due to unobtainable data and infant death. Figure adapted and extended from Paper II and III.

In addition, we investigated the associations between sodium supply, sodium imbalances and severe IVH (Paper IV). A few retrospective observational studies have investigated these associations, but their results are conflicting (83, 99-101) (Table 4). Two of these studies observed an association between sodium imbalances and IVH of any severity or severe IVH in infants with a birth weight ≤ 1000 g (83, 99). In contrast, we did not observe an association between risk of severe IVH and occurrence of sodium imbalances (Paper IV). This incongruity of

results might partially be explained by the relatively low frequency of sodium imbalances in our EPT cohort (e.g., 20% of infants had a plasma sodium concentration > 150 mmol/l) compared with the aforementioned studies (e.g., 58% of infants had a serum sodium concentration ≥ 150 mmol/l (83)).

However, other studies as well as our study showed that early total sodium intake including sodium delivered from transfused blood products was increased in infants with severe IVH (100, 101) (Paper IV). Of note, neither Barnette et al. (100) nor we (Paper IV), could confirm the association between severe IVH and sodium supply after excluding the amount of sodium delivered from blood product transfusions. Whether the effect of total sodium supply on severe IVH is mediated via sodium load or hemodynamic changes remains to be investigated.

In summary, our results (Paper II to IV) suggest that sodium imbalances in EPT infants are mainly mediated by the immaturity of infants and only to a lesser extent by the amount of supplied sodium. To reduce both hypernatremia and the risk of severe IVH, the use of sodium rich products including blood product transfusions should be restrictively used during the first three postnatal days. In addition, randomized controlled studies show that sodium restriction during the first few days after preterm birth allow for normal postnatal adaptation, beneficially affect oxygen requirement, lower the occurrence of bronchopulmonary dysplasia, and do not compromise growth (128-130). However, supplementation with sodium after the first few days is required to counter hyponatremia (Paper III) (131, 132).

Conclusions

Paper-specific (I to IV) conclusions:

I. Concentrated PN can improve early intakes of energy and macronutrients as well as postnatal growth up to a postmenstrual age of 36 weeks in VLBW infants. This improvement can be achieved without causing additional fluid load.

II. A PN regimen containing less sodium does not prevent early hypernatremia in VLBW infants. An increase of the recommended potassium intake to 1-3 mmol/kg/d during the first three postnatal days might be necessary to prevent hypokalemia.

III. Hyper- and hyponatremia are common electrolyte imbalances in EPT infants during the first and second postnatal week, respectively. Major risk determinants for these imbalances are gestational age and sodium supply.

IV. Total sodium supply, largely provided by blood product transfusions, is associated with severe IVH in EPT infants. Whether this association is mediated by sodium load or hemodynamic changes remains to be investigated. However, neither hypernatremia nor fluctuation in plasma sodium concentration is associated with severe IVH.

Future perspectives

There is consensus about the fact that nutrition and postnatal growth affect short- and long-term health outcome, including neurodevelopmental function in preterm infants (121). However, little is known about how specific nutrients, nutrient combinations, and the timing of nutritional intakes influences short- and long-term health outcome. Similarly, the optimal postnatal growth pattern to achieve best possible health outcome is unclear, especially when it comes to selected populations such as infants born small for gestational age.

Most EPT infants and VLBW infants can be managed successfully with standardized feeding protocols (133). However, more studies are needed that address the specific nutritional needs of critically ill infants. Furthermore, the majority of studies assess the effects of preterm nutrition and growth during hospitalization. That is, few studies evaluate the effects of nutritional intakes and growth after hospital discharge on long-term outcome of preterm infants. Moreover, there are no well-designed controlled studies that assess the risk of high postnatal sodium intakes for the development of IVH and later neurodevelopmental disabilities in infants born EPT or with a VLBW.

Finally, an increasing number of studies have highlighted the importance of sufficient parenteral and enteral provision of energy, macronutrients, and micronutrients to counteract accumulated intrauterine nutrient deficits. In many NICUs, however, postnatal nutrient deficits and growth restriction are still present and contribute to impaired short- and long-term outcome of preterm infants. More research is needed on how to facilitate nutritional treatment in daily clinical practice such as determining accurate parameters and reference values to monitor efficacy and safety of parenteral and enteral nutrition.

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