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FEEDING THE VERY LOW BIRTH WEIGHT INFANT

SHORT AND LONG TERM EFFECTS OF TUBE FEEDING METHODS IN EARLY POSTNATAL LIFE

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Stockholm 2012

Published by Karolinska Institutet. Printed by [Universityservice-AB]

ISBN 978-91-7457-798-3

ABSTRACT

The overall aim of this thesis was to investigate short term effects of enteral feeding methods, continuous versus intermittent bolus feeding in very low birth weight infants on gastro intestinal tolerance and growth (I) and, behavioural responses of stress during feeding (II) in early postnatal life. Furthermore, to compare and follow up of longer term effects of these enteral feeding methods on later breastfeeding capability (III).

Methods: Seventy very low birth weight infants, gestational age (GA) 24-29 weeks and birth weight <1200grams, were randomly assigned within 30 hours of birth to one of three feeding methods: continuous nasogastric feeding, bolus nasogastric feeding and bolus orogastric feeding, the intervention last to 32 weeks postmenstrual age. At this time point continuous fed infants were transitioned to intermittent bolus feeding and, bolus orogastric infants transitioned to bolus nasogastric. The follow-up phase continued up until to six month corrected age or as long as the infants were breastfed. The assessments comprised gastrointestinal tolerance, assessed by time to achieved full enteral feeding and, bone growth velocity from birth to 32 and 36 weeks was assessed by knemometer. Behavioural stress responses to feeding were assessed by video-recording at seven and 15 days of postnatal age and at 32 weeks PMA. Follow-ups of the infants' feeding and breastfeeding behaviour were assessed during hospital stay and by structured interviews with mothers at four and six months corrected age. Total length of breast feeding was followed up by telephone contact.

Results:

The continuous fed infants achieved full enteral feeding significantly faster than the intermittently fed infants (hazard ratio (HR) 1.86; 95 % CI 1.07 - 3.22). In stratified analysis according to birth weight, the improvement was more pronounced in the smallest infants < 850 grams, (adjusted HR 4.13; 95 % CI 1.48 - 11.53). Growth rate was significantly faster among the continuously fed infants (p 0.002) (I).

Bolus fed infants showed a significantly higher risk of a behavioural stress response to feeding compared with continuous-fed infants at 15 days of age, adjusted odds ratio 4.1 (95% CI: 1.1 - 15.4) with a similar result at 32 weeks, adjusted OR 4.2 (95% CI: 1.0 - 17.8). Bolus-fed infants showed greater need of behavioural and physiological stabilisation during feeding (II). In follow-up of infants breast feeding capability, continuous fed infants had a significantly higher probability of feeding themselves directly from their mothers' breast compared with bolus fed infants, at discharge from hospital prevalence ratio (PR) 1.7 (95% CI=1.1 - 2.5), at two months corrected age PR

2.1 (95% CI =1.2 - 3.6) and at four months corrected age 2.3 (95% CI=1.02 - 4.3). Continuous fed infants were also breastfeeding for a significantly longer time period, adjusted HR 6.5 (95% CI 2.8-15.4) (p= < 0.0001) and achieved exclusive breastfeeding to a higher extent (p=0.02) compared to bolus fed infants. In infants compromised with severe broncopulmonary dysplasia (BPD) requiring oxygen treatment after discharge from hospital, capability to transition to exclusive breastfeeding between study groups remained significant (p=0.03) (III).

Conclusion

The results presented in this thesis suggest that feeding methods used in early postnatal life influence both short term and indicates an influence of longer term effects in infants with birth weight below 1200grams and GA at birth 24 to 29 weeks. Continuous nasogastric feeding was found to enhance gastrointestinal tolerance, growth and decrease behavioural stress in early postnatal life. The results of the follow-up study showed effects on later feeding capability. These results indicate that continuous nasogastric feeding may serve as beneficial for this specific group of very preterm infants. An increased nutritional status is supported by the findings concerning improved enteral tolerance and better growth. The findings concerning reduced stress with less need for direct nursing to ameliorate discomfort may also have influenced improved growth and development. These results during early postnatal life may indicate that continuous nasogastric feeding has a protective and supportive function regarding infants' later feeding behaviour, and thus may explain the findings revealed in the follow up of the infants' breast feeding capability.

Key words: tube feeding, very low birth weight infants, gastrointestinal tolerance, stress, video recording, breastfeeding.

LIST OF PUBLICATIONS

I. Ann Dsilna, Kyllike Christensson, Lars Alfredsson, Hugo Lagercrantz, Mats Blennow. Continuous feeding promotes gastrointestinal tolerance and growth in Very Low Birth Weight Infants. Journal of Pediatrics 2005;147:43-9.

II. Ann Dsilna, Kyllike Christensson, Ann-Sofi Gustafsson, Hugo Lagercrantz, Lars Alfredsson. Behavioral stress is affected by the mode of tube feeding in Very Low Birth Weight infants. Clinical Journal of Pain 2008;24:447-455.

III. Ann Dsilna, Lars Alfredsson, Henrik Källberg, Kyllike Christensson. Tube feeding in early postnatal life influence later breastfeeding capability in Very Low Birth Weight infants: Follow-up of a randomised controlled trial. Submitted.

CONTENTS

LIST OF ABBREVIATIONS

GA	Gestational age
PMA	Post menstrual age
HR	Hazard ratio
CI	Confidence interval
OR	Odds ratio
PR	Prevalence ratio
BPD	Broncopulmonary dysplasia
NIDCAP	Newborn Individualized Care and Assessment Program
VLBW	Very low birth weight
TPN	Total parenteral nutrition
PPN	Partial parenteral nutrition
a/A ratio	Arterial alveolar oxygen tension
CPAP	Continuous positive airway pressure
IPPV	Intermittent positive pressure ventilation
HFOV	High frequency oscillatory ventilation
CNG	Continuous nasogastric feeding
BNG	Bolus nasogastric feeding
BOG	Bolus orogastric feeding
NEC	Necrotizing enterocolitis
IPPV	Intermittent positive pressure ventilation
HFOV	High frequency oscillatory ventilation
SGA	Small for gestational age

INTRODUCTION

Starting point

This research started with a NIDCAP observation. The year was april 1996, and we were running the NIDCAP study at the neonatal intensive care unit at The Karolinska Hospital, and I was one of the trained NIDCAP observers. On this specific day I was observing one of these immature infants randomised for the study. It was a boy struggling feeding difficulties related to gastrointestinal intolerance, still on parenteral nutrition six weeks after birth. During the NIDCAP observation, which took place before, during and after a tube feeding session, it stood out very clearly that the bolus feeding itself, had an extremely negative influence on all of the neurobehavioural subsystems observed, specifically the autonomic system. The tube feeding given very slowly by the nurse caused immediate irregular breathing pattern with decreased oxygen saturation and heart rate. The feeding situation for this child seemed to drain his energy. In order to minimize these negative effects, specifically on the autonomic system I wanted to try to give feeds extremely slowly as a continuous intragastric infusion drip around the clock. Continuous feeding was not used in this group of infants in Sweden at that time so I discussed the proposal with my NIDCAP colleagues and the physician in charge who gave the permission to test it since it was a rescue situation. The effect was immediate and the infant reached full enteral feeds within 3 days. We could also lower both the CPAP pressure and the oxygen support. And when I looked around at our unit watching all these infants struggling with feeding problems I wondered to myself if we have been applying the feeding by intermittent bolus on a subgroup of VLBW - infants who have not the physiological and developmental capacity to handle it. The method was replicated on two more cases suffering from feeding intolerance with similar results. For me these “rescue cases” raised a number of questions: What kind of infant will benefit from this enteral feeding method? What knowledge is available about intragastric continuous versus bolus feeding in this new group of very immature survivors? Can this method be harmful in any way? What do we really know about these infants’ capabilities to handle enteral feedings? In searching for answers it was obvious a gap in knowledge in this area.

BACKGROUND

Improved knowledge during the last decades has lead to major advancements in perinatal care with concurrently increasing survival rates among very low birth weight infants[1] . This improvement has switched the focus in neonatal care, from simply decrease mortality to attaining decreased morbidity as well[2, 3 4, 5]. In normal foetal growth and development there is a continuous interaction between the genetic regulators on the chromosomes, the genome and the environment. These two are often distinguished as nature, the genome, and nurture the environment. Changes in the environment during early gestation may have deleterious influence on foetal development.

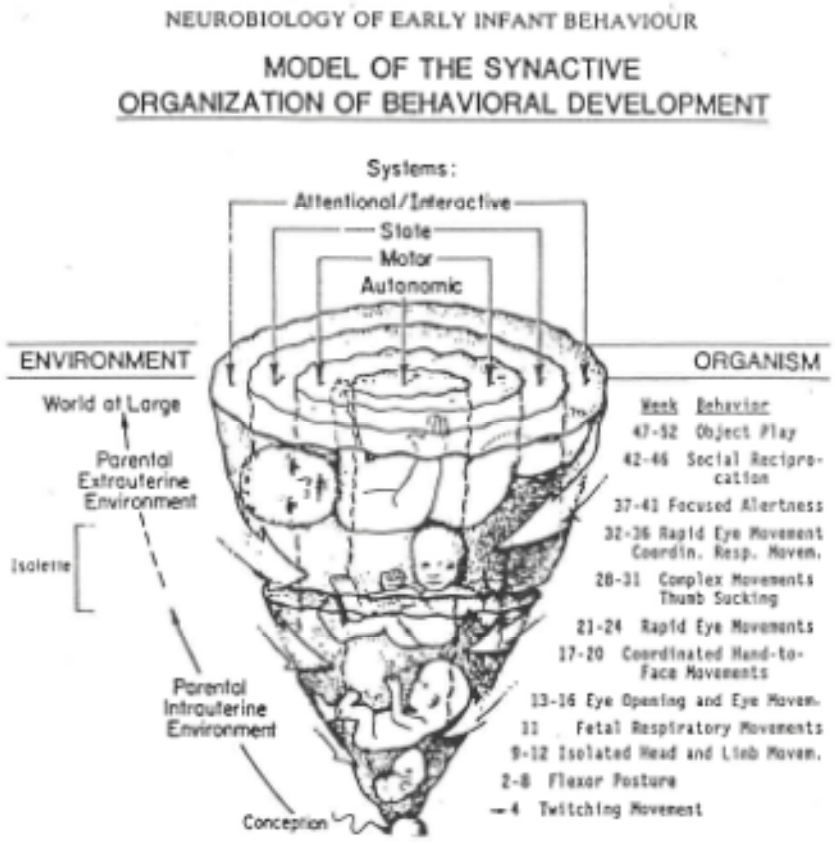

Figure 1. This picture provides a schematic sketch of infant development and environment which may influence infant development during early gestation (Published with permission H. Als.)

In utero the foetus is nurtured parenterally from the mother through the placenta via the umbilical cord. When very preterm birth occurs the infant is ceased from this natural environmental support, and forced to adapt to an environment they are not yet ready for. (Fig. 1). Nutritional support is fundamental for life and development. Providing

nutritional support in these infants is challenging as they do not only need to balance the high energy requirements for survival, but also need optimal nutrition and an environmental context that support growth and development [2, 6]

Physiological developmental preconditions to enteral feeding

Foetal development

During the early weeks after conception, structure of the gut is formed, and by the end of the first trimester, villi and mucosal digestive enzymes are present. Foetal swallowing and sucking appears in the beginning of the second trimester and is important in the regulation of amniotic volume. Initially the foetus swallow 2-7 mL/ day, which increase to 300-700 mL/day about term[7]. Parietel cells are functionally mature at 13 weeks gestation[8]. Newborn preterm infants at 24 weeks of gestation demonstrate gastric secretion of gastric acid at pH <2 within the first day of life, and its presence act as a barrier to the entry of micro-organisms into the small intestine[9]. At about 20 weeks gestation the foetal gut structurally and histological resembles that of an adult[10]. The pre-programmed step for gastrointestinal function occurs later.

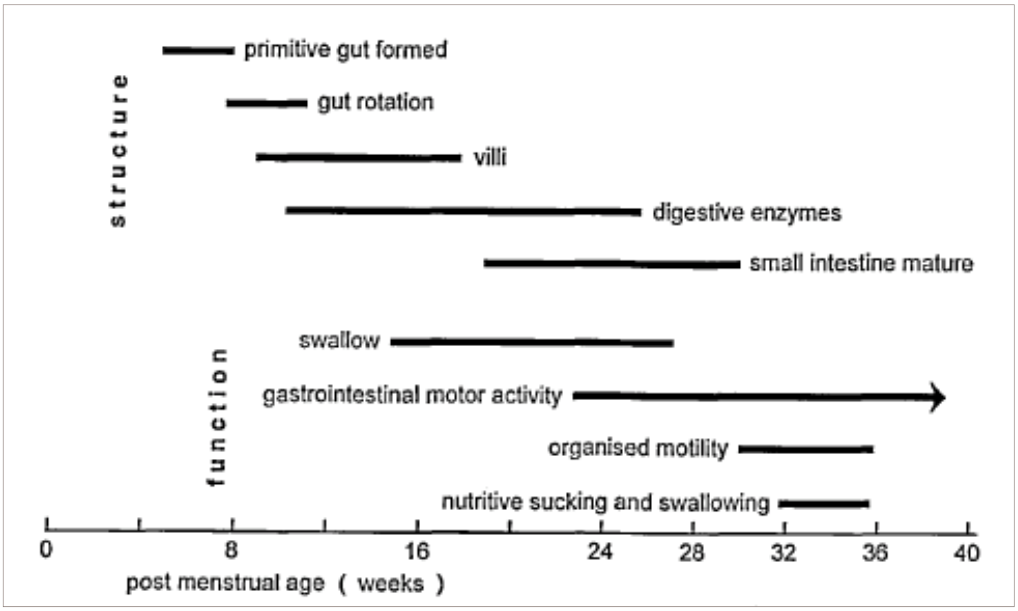

Figure 2 Ontogeny of gut development and function

(Published with permission Newell SJ 1996)

Gastro intestinal tolerance and function

Gastrointestinal motor activity is present at about 23 weeks, but migrating motor complex is immature, showing disorganised pattern of motor activity[11]. Studies on both human foetuses [10] animal studies [12] , and on preterm infants show little or no

gastrointestinal motor activity before 30 weeks of gestation. At 30 weeks an increase in maturation occur by increased organised motor activity[11, 13] .The infants, born before 30 weeks gestation, show a functional gastrointestinal immaturity which leads to increased risk of enteral feeding intolerance (Figure 2).

Parenteral nutrition, total or partial is needed to support optimal nutrition[6]. However, intraluminal nutrition is essential to avoid the risk for gastrointestinal atrophy and dysfunction[14, 15]. Parenteral nutrition is also followed by increased risk delayed growth, chronic lung disease[16], and nosocomial infections[17 18, 19]. Several studies have shown that enteral nutrients promote maturation of motoractivity [20]and that slow rate infusion promotes maturation of duodenal motor activity[21, 22].

Feeding in VLBW infants

Even though foetal swallowing is present at very early gestation [23] synchronised suckle, swallowing and breathing does not occur until 32 weeks gestation.[24-26] Infants born before this stage of development need to be fed by intragastric feeding tube. Different feeding methods can be used; bolus feeding in various intervals, slow rate infusion intermittently or by continuous feeding around the clock.

Another feeding strategy used is minimal enteral feeding[27] also named trophic feeding[28], which is very small amounts, up to 24 ml/kg/day administrated as continuous or bolus, without increase in feeding volume. The first report of continuous feeding was in 1972 by Valman [29] who described this method as an option when interval feeding did not work. Few randomised trials have been performed on intermittent bolus versus continuous feeding[30-35]. In the latest updated Cochrane review the meta analysis couldn't detect differences between the two feeding strategies, regarding enteral tolerance, measured by time to achieve full enteral feeding.[36] Although, in subgroup analysis of infants with birth weight less than 1000grams fewer days on parenteral nutrition and increased growth velocity were detected in the continuous feeding method compared the bolus feeding method.[36]

Indeed, interval feeding has been suggested to be preferred to continuous feeding since it causes pattern of cyclical burst in secretion of insulin, which have been proposed to leading to improved mucosal growth[37, 38] In contrast during continuous feeding insulin secretion is presence at a higher base level but without cyclical surges compare

to the preprandial levels before a bolus feeding[38]. However, positive effects on subsequent growth velocity due to cyclical surges in studies of continuous versus bolus feeding have not been detected. However, is difficult to detect differences in growth by weight during early postnatal time period in this population which is due to the fact that weight gain reflects differences in fat and fluid deposition rather than real growth.[39] Despite that weight loss occur after birth, linear growth still exist and can be measured during this period, methods suggested in newborns are crown –heel length. Measure of real bone growth by kne-heel length by use of a handheld knemometer is another efficient method validated method. This method has been proposed as a gentle and instrument, easy to use within the incubator with little disturbance on the infant, it is also validated in preterm infants' [40 41].

Long term effects of tube feeding

VLBW infants are very vulnerable to all kind of external stressors [2, 42], including environmetal exposure to bright light[43-45], noise[46] and also to caregiving procedures[47 48, 49] in the neonatal intensive care unit. There is a growing concern of that frequent physical stress caused by invasive procedures exert noxious long-term effects on the wiring of the neuronal networks and alter subsequent development[2, 42, 50]. Invasive procedures may have long term noxious or negative influences on the neuronal network[3, 42, 50]. Interoceptive stressors due to feeding may be as important. Previous studies in healthy human adults exposed to interoceptive stressors have shown significant immediate effects on gastrointestinal function by inhibition of gastric emptying and alterations in gastric secretion[51, 52]. Stangelini et al proposed that elevations in plasma levels of catecholamines and ß-endorphine due to stress may be involved as mediators of the central effects of the gut [53] . Feeding in VLBW infants with birth weight <1200grams is often followed by symptoms of abdominal distention, increasing gastric residual volumes and vomiting. General illness like compromised pulmonary function, environmental stressors as well as interoceptive stress due to tube feeding itself may also negatively influence enteral tolerance. In addition the need for a feeding tube is followed by frequent invasive insertions which cause negative vagal respons [54]. However, if left the tube in place between feedings it cause nasal resistance, and compromise pulmonary function[55] Both method acts as stressors, although research on which it's preferable is not known.

However not to feed is not on option. Optimal nutrition by reaching full enteral feeding is of major importance and if delayed, it is associated with increased risk of morbidity and mortality[18, 19]. Several studies have demonstrated positive effects of high human milk intake on brainstem maturation[56], and cognitive development in preterm infants[57] [58]. Human milk, in particular the mother's own milk is important in this group of vulnerable infants[59, 60]. Because it's numerous nutrients, hormones and host defence factors, with capacity to prevent inflammation and stimulate optimal growth[61]. Human milk has also been proved to enhance gastric emptying compare to formula feeding[62]

With reference to questions raised in the end of the introduction part of this thesis it became obvious that these problems has to be studied in an interdisciplinary randomised control trial, focusing on physiological, behavioural and nursing perspective.

AIM

The overall aim of this thesis was to investigate and compare effects of different enteral feeding methods in very low birth weight infants to gain further knowledge and understanding regarding consequences of enteral feeding on the developing very immature infant.

The specific aims were:

- To investigate and compare short term effects of tube feeding methods, continuous versus intermittent bolus feeding, and to evaluate placement of the feeding tube, nasal or oral, on gastrointestinal tolerance and growth in very low birth weight infants.(I)

- To investigate and compare short term effects of tube feeding methods, continuous versus intermittent bolus feeding, and to evaluate placement of the feeding tube, nasal or oral, on behavioural stress response during tube feeding in a population of very low birth weight infants. (II)

- To follow up and evaluate effects of tube-feeding methods, continuous versus bolus and evaluate placement of the feeding tube nasal or oral, on later breastfeeding capability in a population of very-low-birth-weight infants. (III)

METHODS

Study I, II and III included in this doctorial thesis relate to studies performed in Sweden, and based on a randomised control trial conducted 1998 - 2004. Participants were recruited to the study between February 1998 and November 2001, and the last follow-up was done in February 2004.

Table 1 Overview study samples, data collection and statistical analysis

	Study I	**Study II**	**Study III**
Outcome	Gastro intestinal tolerance	Behavioural stress	Breastfeeding capability
Time at data collection	From birth to discharge from hospital Birth to 32 weeks PMA Birth to 36 weeks PMA	At 7 days after birth At 15 days after birth At 32 weeks PMA	From start of oral feeding to end of breastfeeding. At Discharge At 2 months corrected age At 4 months corrected age At 6 months corrected age
Setting	In hospital	In hospital	In hospital and Home visit
Data collection methods **Type of Data**	Derived prospectively from medical records Gastrointestinal tolerance: Time to achieve full enteral feeds Growth: Time to regain birth weight Knee heel length: Birth to 32 weeks PMA Birth to 36 weeks PMA	Video recording Behavioral stress response Nursing interaction	Derived from medical records. Interviews: face to face interview and by telephone with mother of the infant. Infants breastfeeding capability, prevalence and duration
Methods for analysis	ANOVA, Student T-test. Kruskal Wallis, Mann-Whitney, Pearson Chi square, Fisher exact test Hazard Ratio according to Cox proportional regression models	ANOVA, Student Ttest Kruskal Wallis, Mann-Whitney, Pearson Chi square, Fisher exact test Logistic regression Cohen's κappa	ANOVA, Student T-test. Kruskal Wallis, Mann-Whitney, Pearson Chi square, Fisher exact test Prevalence ratio Mantel Haenszel Hazard Ratio Cox proportional Regression models

SETTING I – III

The setting was three neontatal units in Stockholm, capital city of Sweden. The three neonatal units are located at Karolinska Hospital, Danderyd Hospital and Huddinge Hospital, 75% of the geographical catchment area of Stockholm, County, Sweden. According to the general recommendation that pregnancies with expected preterm delivery at 27 weeks less should, if possible be delivered at a hospital with access to

neonatal intensive care with mechanical ventilation treatment. In this trial infants about 80%, were delivered at this level at Karolinska Hospital in Solna. After stabilisation, and when need for mechanical ventilation was no longer the case, infants were referred to their neonatal units, according to their geographical belonging within the Stockholm County. Referrals to intensive care at Karolinska hospital could also take place if required during hospitalisation. Follow-up was performed within hospital and in the home environment of each infant and its family.

PARTICIPANTS I – III

Identification of study sample – pre studies

Prognostic targeting was used to define infants with risk of enteral intolerance. Medical records of VLBW infants born in 1995 were checked for a) number of days to full enteral feeding, and b) number of days with parenteral nutrition (lipid and aminoacid solutions) in relation to birth weight and GA at birth. Infants with birth weight less than 1200grams and, GA at birth below 29 weeks were found to require intravenous alimentations for an increased number of days.

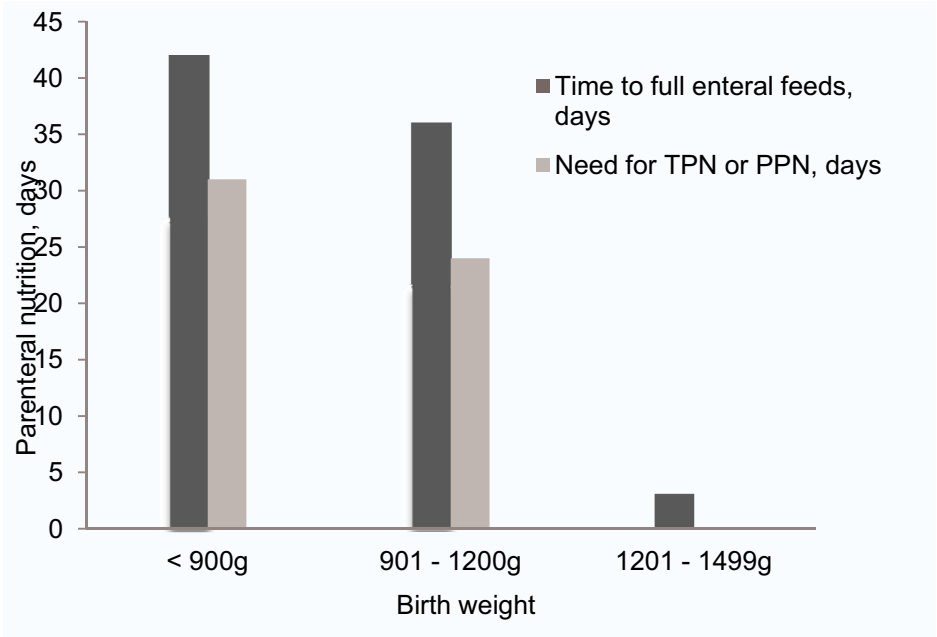

Figure 3. Time to full enteral feeds and need for total and/or partial parenteral nutrition in relation to birth weight in infant' born at Karolinska Hospital 1995

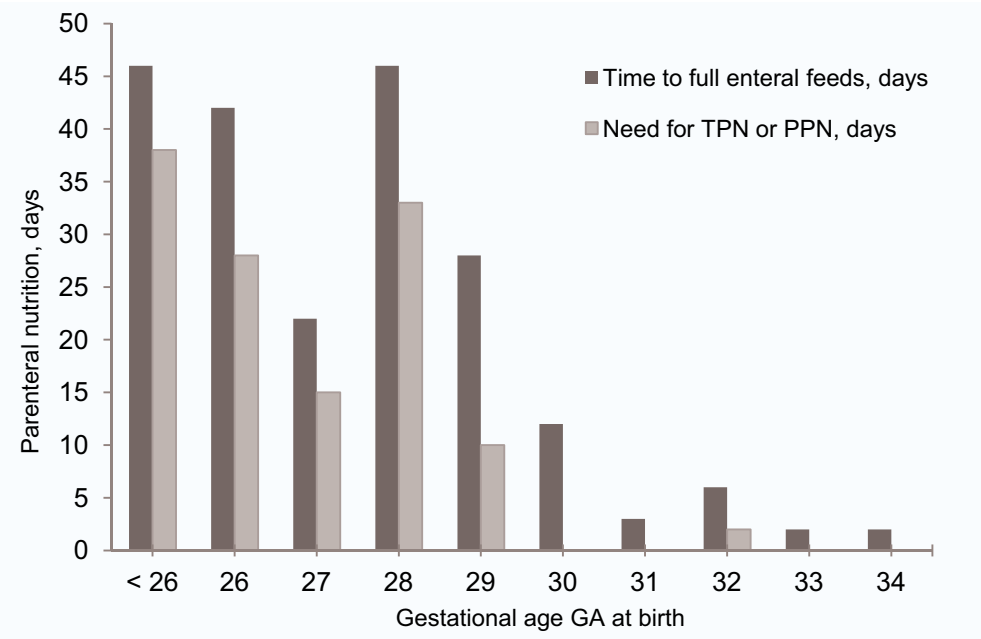

Figure 4. Time to full enteral feeds and, need for total and/or partial parenteral nutrition in relation to gestational age at birth, in VLBW infants, Karolinska Hospital 1995

The results of the pre-study for identifying a study population at risk were also found to correlate with the ontogenic timetable for gastrointestinal function. As presented in the background (figure 2), gastro intestinal motor activity is present at about 22 weeks PMA, but organised motility pattern is seen first at a PMA about 30 weeks[11].

Recruitment of study sample

Inclusion criteria

On the basis of our first cases, prestudies and the ontogenic timetable for functional gastrointestinal motor activity, the inclusion criteria for the study sample in this randomised control trial were defined to infants with GA at birth below 29 weeks, and with birth weight less than 1200grams. It was of importance to have control of other known factors that may influence outcome. One such factor is, timing i.e., start of enteral feeding within 30 hours after birth. Maternal inclusion criteria, was reading comprehension, in the Swedish language, and a residence within the geographical catchment area of the three neonatal units.

Exclusion criteria

Exlusion criteria were based on severity of illness. Arterial alveolar oxygen tension ratio (a/A ratio)[63] was decided to be used as measurement for severity of respiratory

and circulatory status. Independently of the need of respiratory assistance, CPAP and/or mechanical ventilation (IPPV or HFOV), the limit was set to a/A ratio 0.18, that is with normal arterial O2 partial pressure and CO2 partial pressure, is the equivalent to a fraction of inspired O2 (FiO2) of 0.55 to 0.60. Other exclusion criteria were congenital malformations.

Estimation of sample size

The sample size, 22 infants in each of the 3 groups (one index group and two control groups) was chosen to permit detection of a 40% difference in the primary outcome, the time to achieve full enteral feeding, with a type I error of 0.05 and a power of 0.80. Regarding sample size calculation when planning the study II and III, it was difficult to ensure a sufficient sample size for the current purpose. This was due to the lack of knowledge regarding enteral feeding, continuous versus bolus, on frequency of behavioural expressions indicating stress (study II), and on follow-up of breastfeeding capability (study III). However, regarding study II, we found that given the observed frequencies of behavioural stress response, the sample size of the present study was sufficient (power above 0.80) to detect an odds ratio in the order of about 3.9.

Randomisation

Randomisation was done the main study centre at Karolinska Hospital, using opaque envelopes in blocks of 12, randomly ordered in sequence by a person unconnected to the study. In the case of multiple births, the first-born infant was considered for allocation if he/she fulfilled the inclusion criteria, if not, the second-born infant was considered. Due to potential demographic differences in the population in Stockholm County, blocking was performed for the two geographical regions, Stockholm North (Karolinska Hospital and Danderyd Hospital) and Stockholm South (Huddinge Hospital). All newborns admitted were assessed for eligibility by the paediatrician in charge, who then telephoned the study coordinator to randomise the patient. Parental informed consent was obtained before the allocated treatment was started. Masking allocated intervention during the trial was not possible in the clinical setting.

STUDY DESIGN

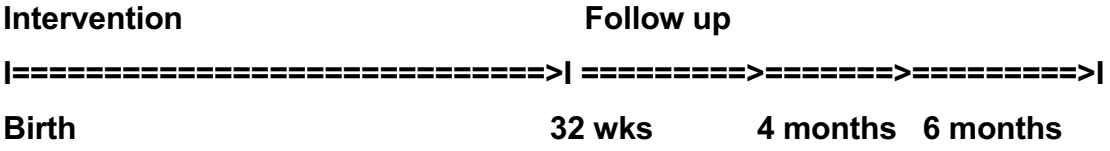

Figure 5 Overview study design

Intervention phase

Infants who fulfilled the inclusion criteria were enrolled within 30 hours of birth and randomly assigned to one of the following tube feeding methods: continuous feeding with nasogastric feeding tube remaining in place (index group, CNG), bolus feeding every three hours with nasogastric feeding tube remaining in place (control group 1 BNG), and bolus feeding every three hours with intermittently inserted orogastric feeding tube (control group 2, BOG). Two control groups were chosen to detect any differences due to the placement of the feeding tube (oral or nasal) on gastrointestinal tolerance, infants' behavioural response and later feeding capability. All infants remained in their assigned group from the time of randomisation to a postmenstrual age of 32 weeks (± 1 week). The timing was set according to the ontogenetic timetable for structure and function of upper foregut.

Follow-up phase

The follow-up phase started at 32 weeks PMA (± 1) week. Infants in the index group were gradually weaned from continuous feeding to intermittent feeding every three hours over a period of 10 to 14 days. At the same time point (32 weeks PMA (± 1), infants belonging to BOG, the feeding tube was change from intermittent orogastric feeding tube placement to nasogastric feeding tube remaining in place between feeds. During this period, oral feeding was introduced as soon as the infants were stable and off mechanical ventilation.

PROCEDURES I – III

Start of nutrition - enteral and parenteral

During the first hours of life an intravenous glucose infusion (10g/100ml) 60-80 ml/kg/d was commenced. Enteral feeding was started before 30 hours of postnatal age. Only human milk was used, according the pre-established protocols of the neonatal hospital units. If available, the mother's own milk was administered as soon as lactation was established. If the milk production of the mother was insufficient, frozen pasteurized human milk from the local milk bank was added. In the control groups, BNG and BOG, the attending nurse gave feedings every third hour during a period of 15 to a maximum of 40 minutes. According to clinical routines, the duration of feeds was adjusted to the volumes given, as well as if feeding difficulties were encountered. CNG was administered by an electric infusion pump (Terufusion Syringe Pump model STC – 521, Terumo). To minimise loss of nutrients due to flotation of lipids within the syringe, a technichal device was development to enable feeding infusion at an angel of 40 degrees, with the tubing at the higher end. In the BOG group, a 4F feeding tube was inserted before each feeding and immediately removed afterwards. In the CNG and BNG, a 3 - 4 F feeding tube remaining in place between feeds was used. The attending nurse inserted the feeding tube and the position of the tip of the tube was checked by aspirates. The total amounts of enteral feeds were gradually increased by 10-15 ml/kg/day (in infants with birth weight < 1000 grams) and 15-20 ml /kg/day (in infants with birth weight 1000-1199grams) during the first two days. Starting on the third day of feeding, if no clinical signs of enteral intolerance and / or severe medical instability were noted, the amounts were increased by 15-20 ml/kg/day in all infants.

Enteral intolerance

Gastric residuals were checked every six hours (BNG, BOG) and every eighth hour (CNG), or more frequently if signs of enteral intolerance appeared. If clinical signs of enteral intolerance and / or severe medical instability were noted, the feeding volume was reduced or the feeds were temporarily withheld according to clinical routines. Enteral intolerance was defined as showing signs of possible necrotizing enterocolitis (NEC), such as abdominal distension, visible bowel loops, bile stained pre-aspirates or emesis, increased gastric residuals > 50% of the previous meal (BNG, BOG), an exceeded hourly infusion rate (CNG), and / or hemepositive stools (Bell stage I)[64] .

Partial and total parenteral nutrition

Partial parenteral nutrition (PPN) with lipids (Intralipid, Fresenius Kabi AG, Germany) and amino acid solutions, (EvaLac extempore, Apoteket AB, Sweden, or Vaminolac, Fresenius Kabi AG, Germany) was started before 72 hours of postnatal age, according to clinical routines. Sodium, potassium, calcium, trace elements and vitamins were given according to clinical routines and at the discretion of the attending physician. The total volume prescription volume was increased by 10 ml /kg/d to a target volume of 140 – 160 ml/kg/day. When enteral feeds reached 75 % of the total target volume PPN was discontinued.

Fortification of human milk

As soon as PPN/TPN was discontinued, additional fortification of human milk was performed for all infants. According to clinical routines the content of protein, fat, lactose and energy in human milk was analysed. Based on the individual breast milk analysis, fortification with addition of supplemental protein (HMF Presemp, Semper AB, Sweden) calculated to a protein content of 3-4g/kg/day and fat emulsions (Calogen or Liquigen, SHS International Ltd, United Kingdom) to a caloric content of 120-150 kcal/kg/day.

Support of infants feeding behaviour

According to clinical routines pacifiers were used in all infants to support non-nutritive-sucking. Skin-to-skin contact was introduced when the respiratory and circulatory status of the infant was stable, according to the physician in charge for the infant. That implied that this was allowed irrespective of respiratory support i.e., it was also permitted for infants on CPAP and on mechanical ventilator, as long as their condition was physiological stable. However, at the time for the data collection, it was not possible for the infant to be skin to skin with the mother or father if treated in HFOV. Initiation of oral feeding was approved when infants were stable and off mechanical ventilation. According to clinical practice, mothers who intended to breastfeed were supported by the nurses to look for their infant's cues regarding pre-feeding behaviour[65]. When the infant showed hand to mouth movements, areola grasps, licking or rooting behaviour, they were encouraged to initiate breastfeeding. Two strategies were used during the transition from tube feeding to breastfeeding, the need for supplementation was assessed by test-weighing before and after breastfeeding, and/or

supplements were gradually decreased and control weight was tested on pre-scheduled days.

SPECIFIC PROCEDURES STUDY II

Time periods of data collection

Data collection was performed by video recording the infants' behavioural responses to feeding at three prescheduled times: postnatal age of 7 (±2) days, postnatal age of 15 (±2) days and PMA of 32 (±1) weeks. The time period when video recording was performed were standardised to the first or second meal after 12 noon for bolus fed infants and in the same time period for continuous fed infants. Index infants were recorded at a period of 45-65 minutes; control infants were recorded 10-15 minutes before feeding, during feeding, and 10-15 minutes after feeding, giving a total recording period of 45-65 minutes. The length of the recording period was adjusted to the feeding volume given in both index and control infants.

Procedures in connection to video recordings

Procedures included taking temperatures, changing nappies, and placing the infant in a right-side position, supported by blanket rolls. Room lighting was put to semidarkness, and the blanket covering the incubator was removed. The infant was then left undisturbed for 30 minutes before video recording was started. A camera system was developed in order to give simultaneous pictures from two angles. Two cameras capable of taking pictures in a semi-dark environment with high humidity level were attached. Camera 1, with a zoom lens, was placed in the front of the incubator, and focusing on the infant's face and throat, provided detailed pictures of the infant's facial expression; camera 2, with a wide-angle lens, was placed above the incubator, and provided detailed pictures of the infant's full body movements. The cameras were synchronized using a character generator for automatically imprinting a time code, and then connected to two separate videocassette recorders. A microphone recorded the infant's sounds. The principal investigator (AD) set up all technical devices throughout the period of data collection. The nurse in charge of the infant provided all care giving and was instructed to interact with the infant only if: 1) the feeding method required it (defined as indirect nursing interaction), which included feeding-tube insertion, control of feeding-tube placement and bolus feeding without physical contact with the infant; or 2) the infant showed signs of medical instability or signs of stress and discomfort and a need for physiological and/or behavioural stabilisation by the nurse (defined as

direct nursing interaction). Protocols were used to measure care giving interactions and the level of sound, light and activity in the nursery.[66] In the later phase during coding of the films, the coders measured infants bedding support.

Development of a tool for assessment of behavioural response

In order to transcribe behavioural expressions from the video recordings to figures, a tool for the assessment of behaviours was developed. The development of this tool was done in a systematic order similar to that presented by Lobo M [67]. This was done before the coding of the material was started. The first step was to a) to determine the behavioural expressions to be used. This was done in the following order: a) literature review and naturalistic observations (NIDCAP) of behavioural response to enteral feeding; b) selection of behaviours reflecting the phenomenon of interest and; c) recurring meetings and seminars with experts in the field to reach consensual validity for the behaviours selected from the NIDCAP manual[68]. To be as close as possible to the original naturalistic NIDCAP observational method one-zero sampling i.e., occurrence or non-occurrence during each sample interval of each selected behaviour, was used.

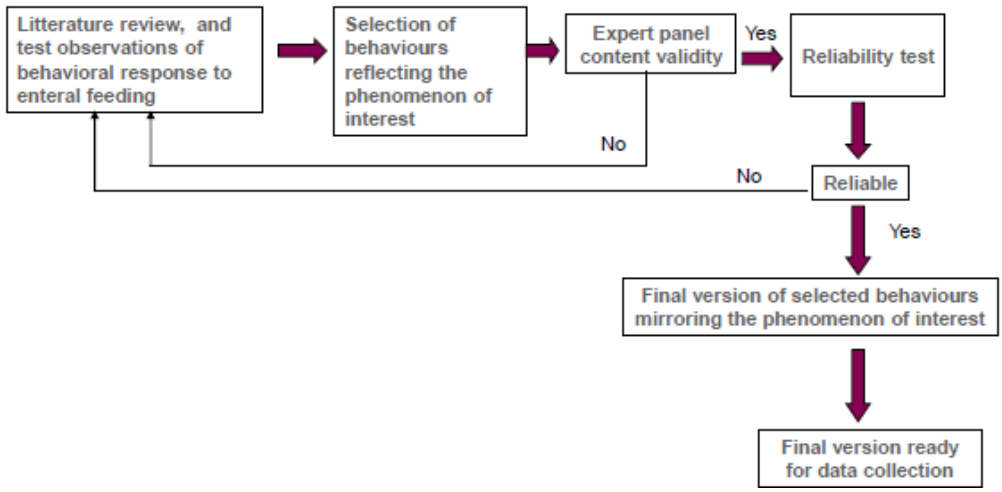

Figure 6. Systematic scheme of selection of behavioural expressions

Systematic order of coding process

All coding was performed by two independent coders, trained in the NIDCAP educational programme, and also specifically pre-trained for the purpose of this research project. Coding was done simultaneously from two TV screens, one showed

the infant's full body and, the other simultaneously showed close-up picture of the infants face and throat. Before coding was started, the coder synchronised the two films. Within our "coding design", a standardised detailed procedure protocol for the coding of the material was used. In this, the coders sampled identified behaviours on a sheet that comprising five one-zero intervals. At the beginning of the coding, and at the start of every new observational sheet, they were instructed to freeze the two TV screens to check the infant's position and behavioural expressions selected for the study. Coders also checked and assessed the infants bedding support (scale 0-2) during the five minute period and noted their assessments on the sheet at the end of each period. The coders were allowed, if required, to review the recording and, if necessary, a specific structure protocol was used for that purpose.

Test of the tool

In the process of the construction of the tool, test and re-test of inter rater reliability between coders was carried out by coding: 1) each selected behaviour separately; 2) sets of behaviours related within each NIDCAP subsystems and, 3) test of final version of selected behaviours mirroring the phenomenon of interest. During this process, improved reliability was achieved by adding a more distinct definition to some of the behaviours in the subsystems of state and motor activity. In order to create as optimal tool as possible for the identification of potential differences between study groups, as short sample interval as possible in the one-zero sampling was the goal. Therefore, test and retest reliability of different length within the sample interval, was carried out. In the final test version of the tool, a zero-one sampling with sample interval of one-minute periods was used. The inter-rater reliability kappa value achieved prior to the start of coding the material was at excellent level interpreted according to Fleiss 0.77 (95% CI: 0.74-0.81) [69].

The collected data, 174 video recordings, were equally divided into two, one half for each observer. During the process of coding the material, 12% of the video recordings were tested for inter-rater and intra-rater reliability. According to the reliability test, the inter-rater reliability was satisfactory with a kappa value of 0.66 (95 % CI: 0.62-0.70), as was the intra-rater reliability with kappa value 0.81 (95% CI: 0.74-0.84) and 0.69 (95% CI: 0.63-0.75) for observer 1 and 2, respectively. Coding was conducted blind to all clinical information and to the point in time for reliability tests.

Twenty-four of the coded behaviours (n=49) defined as expressions indicating stress were selected for the purpose of this study. To evaluate which behaviours were affected by feeding, sequence analyses of the mean frequency per minute were carried out among the control infants for observations before, during and after feeding.

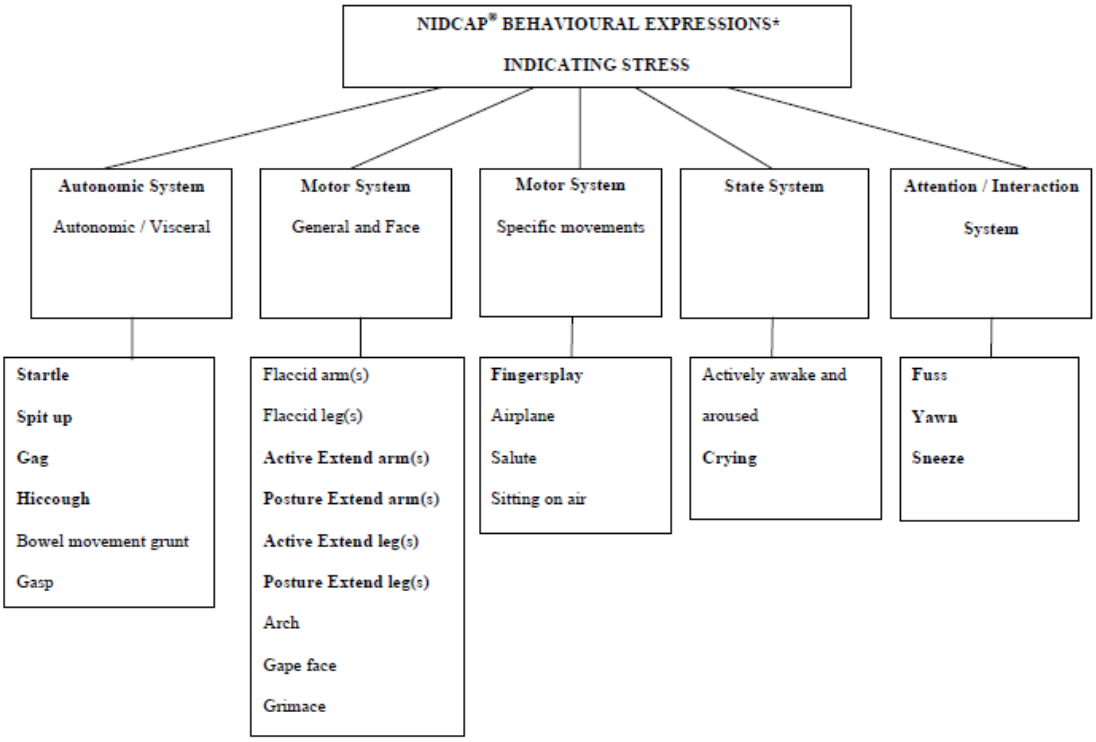

Figure 7 NIDCAP behavioural expressions indicating stress and discomfort

Thirteen behaviours were found to be negatively or positively affected by feeding. These behaviours represented four of the five behavioural subsystems as described by Als H, in the NIDCAP model. The behavioural expressions, n=11, which were not affected positively or negatively by bolus feeding, did not differ with regard to occurrence among index and control groups.

SPECIFIC PROCEDURES STUDY III

Demographic and clinical data were obtained prospectively from medical records during the hospital stay. A follow-up of the feeding status was conducted during a home visit at four months corrected age and included: observation of a video recorded feeding session, a structured interview with the mother including detailed questions concerning the infant's feeding behaviour and, source of nutrition from discharge to the

date of the visit. At six months corrected age, the interview was repeated by telephone. The total duration of breastfeeding in infants who were still breastfed at six months was followed up via telephone contact. All follow-up interviews were conducted by the principal investigator (AD).

OUTCOME MEASURES

STUDY I

Definition of gastro intestinal tolerance and nutritional intake

Time to achieve full enteral feeding, was defined as the time period from birth to when enteral feeds fulfilled the total prescribed volume with respect to postnatal age and weight. Thus, this could be achieved at volumes less than 140-160 ml/kg/day. The primary outcome was extracted from the patient records. A minimum of 48 hours of exclusive enteral nutrition was required. Enteral and parenteral energy (kcal/kg/day) and protein (g/kg/day) intake during the intervention phase was calculated from the patient records.

Growth

Time to regain birth weight was defined as the number of days from birth until birth weight was regained. Lower leg growth rate was defined as the average growth of the lower leg, mm/day, during the period from birth to 32 weeks (intervention phase) and from birth to 36 weeks postmenstrual age. To assess lower leg growth, knee – heel length was measured to the closest mm using a knemometer[40] (Force Institute, Copenhagen, Denmark).

Clinical morbidities

Gastric residuals were defined as a gastric residual volume > 50% of the previous meal (BNG, BOG) or as exceeding the hourly feeding infusion rate (CNG). The total number of occasions with gastric residuals was calculated from the patient records. Emesis was assessed by the attending nurse and defined as the vomiting of gastric contents. NEC was defined by typical clinical signs together with the presence of pneumatosis intestinalis on abdominal radiographs according to Bell MJ[64]. The diagnosis of septicemia required clinical symptoms, elevated C-reactive protein and one positive blood culture. The duration of antibiotic treatment (days) was retrieved from the patient records. Respiratory distress syndrome was diagnosed on the basis of characteristic radiographic findings, respiratory distress and an increasing FiO2 demand. The number

of infants requiring respiratory support by intermittent positive pressure ventilation (IPPV), high frequency oscillatory ventilation (HFOV), continuous positive airway pressure (CPAP) and need for supplemental oxygenation were extracted from the patient records. Patent ductus arteriosus was suspected by presence of clinical findings and, defined by echocardiography.

STUDY II

Definition of outcome behavioural stress

For each infant and video recording, each one-minute time-period was observed with regard to the occurrence of the thirteen behaviours (range 0-13). The frequency of stress behaviours in total was then calculated as the sum of these occurrences divided by the total length (minutes) of the video recording and defined as a primary outcome. A high occurrence of behavioural stress was defined according to the distribution of the calculated frequencies among all the study infants (index and controls). For each of the three video recordings, two cut-off points were chosen, the 50th and the 75th percentile. An infant with a frequency above these cut-offs was classified a "case", i.e. experiencing stress.

STUDY III

Study endpoints collected during the follow-up phase were, initiation of breastfeeding, breastfeeding at discharge from hospital at two, four and six months corrected age and, total duration of breastfeeding.

Table 2. Relevant terms and definitions of outcome measures in study III

Terms	Definitions
Initiation of breastfeeding	Infant's first time at mother's breast
Breastfeeding	Infant actively suckles directly from mother's breast.
Partly breastfeeding	Infant actively suckles directly from mother's breast and, receives supplementary expressed breastmilk, formula or other food by tube feeding, cup, bottle and/or spoon.
Exclusive breastfeeding	Infants actively suckle all nutrients directly from mother's breast with no other supplements except for prescribed vitamins minerals and medicines.
Non nutritive sucking	Sucking on pacifier or finger.
Skin-to-skin contact	Infant is skin-to-skin with the mother or father, in upright position, with no clothing in between, except for a cap on infant's head and a nappy and covered by blanket.

STATISTICAL METHODS

STUDY I-III

In general base line characteristics and outcomes were estimated together with p values for potential differences between the study groups. For normally distributed variables parametric test was used and for non-normally distributed variables non parametric test was used. Statistical methods were one-way analysis of variance, ANOVA (including

Bonferroni post hoc test), Student T-test was used, Kruskal Wallis test and Mann-Whitney U- test. For dichotomous variables Chi^2 test (Pearson test and Fischer exact test) was used. Statistical significance was set at 0.05.

Specific statistical methods STUDY I

To compare the primary outcome, time to achievement of full enteral feeding, between the index and control groups, the hazard ratio (HR) together with a 95% confidence interval (CI) was calculated by means of Cox regression analysis. Since the two control groups (BNG and BOG) did not differ regarding primary outcome, demographic and birth-related factors and duration of feeds, the infants in the CNG group were compared with the two control groups taken together i.e., BNG +BOG. Analysis was performed crude and with adjustments for potential factors that may influence the result. In subsequent analysis investigation of primary outcome was carried out according to birth weight. The stratified analysis on birth weight was carried out with a cut- off set at the median birth weight for the whole study population (850grams).

Specific statistical methods STUDY II

For each infant and video recording, each one-minute time-period was observed with regard to the occurrence of the thirteen behaviours (range 0-13). The frequency of stress behaviours in total was then calculated as the sum of these occurrences divided by the total length of the video recording and defined as primary outcome. A high occurrence of behavioural stress was defined according to the distribution of the calculated frequencies among all the study infants (index and controls). For each of the three video recordings, two cut-off points were chosen, the 50th and the 75th percentile. An infant with a frequency above these cut-offs was classified a "case" that is experiencing stress. The primary outcome among index infants was compared with that among control infants by calculating crude and adjusted odds ratios (OR) together with a 95% CI by means of logistic regression analysis. Adjustments were made for sex, birth weight, GA, respiratory stability (a/A ratio), environmental conditions (light, sound and activity level), and bedding support. The two control groups did not differ regarding demographic factors, length of the recorded time-period, rate of feeding infusion or frequency of behavioural stress, the infants in the index group were therefore compared with the two control groups taken together. Inter-rater and intra-rater reliability were estimated by calculating Cohen's κ with 95% CI and interpreted according to Fleiss et al. SAS software (version 7.0) was used for the logistic regression

analysis, while SPSS software (version 11.5) was used for all other analyses. Statistical significance was set at 0.05.

Specific statistical methods STUDY III

Prevalence ratio (PR) of breastfeeding with corresponding 95 percent confidence interval (CI) was calculated according to Mantel-Haenszel with consideration taken to intent to treat. This was done at 4 successive time points: at discharge from hospital, at 2 months, 4 months and 6 months of corrected age. Infants with missing data during the follow-up were classified as not breastfed. In the analysis regarding total duration of breastfeeding, Cox proportional regression models were used to estimate the hazard ratio (HR) and corresponding (95% CI) for mother-infant pairs who initiated breastfeeding. Cox regression models were checked for the proportional hazard assumption; adjustments were made for GA, SGA, antenatal steroid treatment, and mothers' milk production. The analysis was carried out using SPSS version 15·0 and SAS version 9·1. Statistical significance was set at 0·05.

RESULTS

STUDY I-III

Out of seventy eight eligible VLBW infants, born between February 1998 and November 2001, 70 infants were randomly allocated to receive human milk by one of the three tube feeding methods.

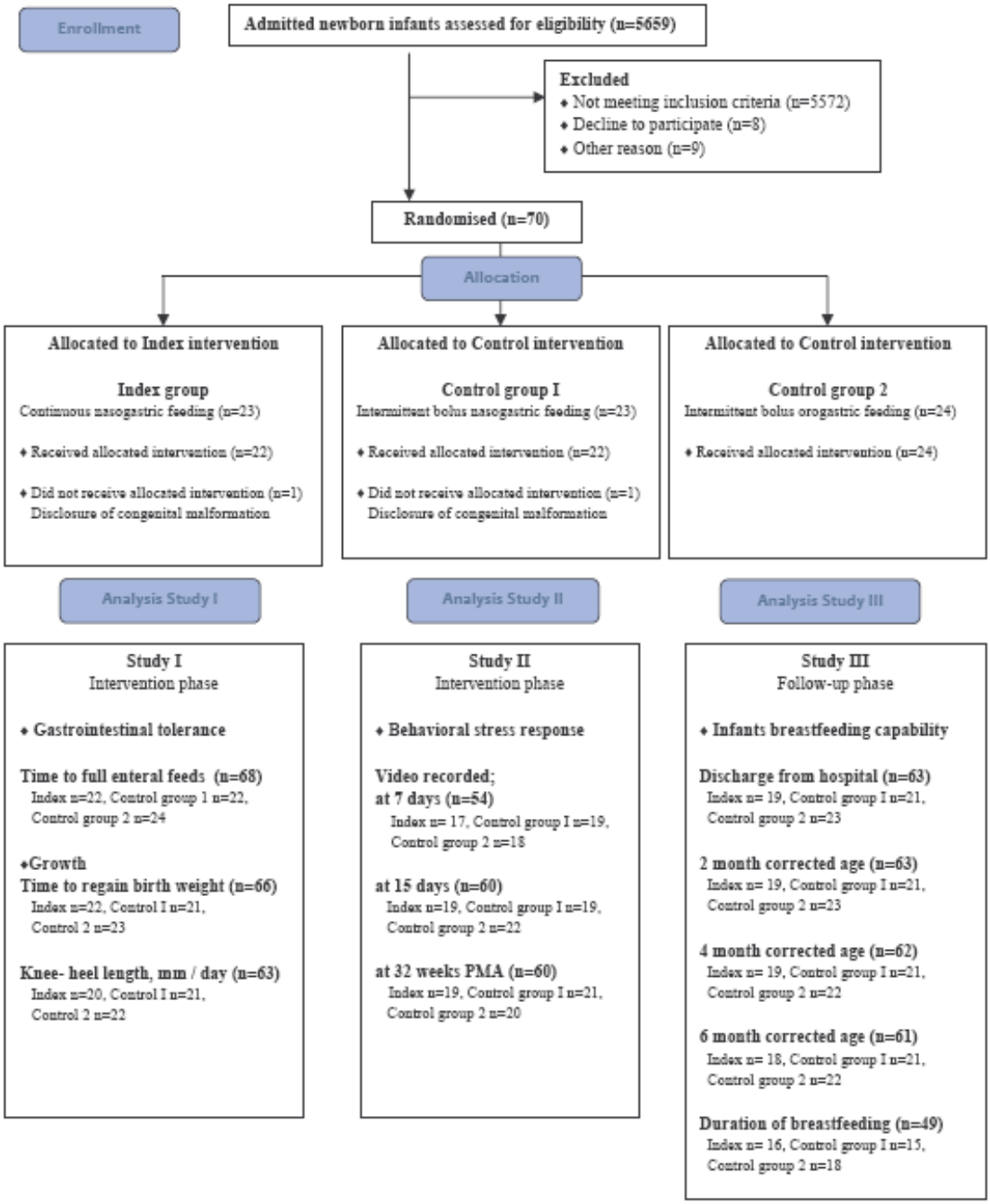

Figure 8. Trial profile and analysis Study I-III

Drop outs and post randomised exclusions

Two infants were excluded after allocation because of post randomised disclosure of congenital malformations. In the analysis of the primary outcome in study I, time to achieve full enteral feeding, all infants were included in the analysis as intent to treat i.e., all infants were analysed as allocated. Regarding analysis of growth parameters, reasons for drop-out were: death before PMA 32 weeks and, parental decline of further participation in the study. Reasons for drop outs in study II were: not on tube feeding because of death, too sick to be fed and, and parental decline of participation. In study III reasons for loss of follow- up were, death (in hospital) and not traceable at time points for follow-up.

Allocated tube feeding and intent to treat incidences

During the intervention phase, three infants, from the control groups switched feeding methods because of severe medical problems. One infant in the BOG group switched to CNG during a period of 14 days (35% of the intervention phase) due to severe apnéas and bradycardia due to gastro-oesophageal reflux. Another infant in the BOG group switched to BNG during a period of 13 days (30.2% of the intervention phase) due to severe bradycardia during tube insertions. One infant in the BNG group switched to BOG during a period of 6 days i.e. 9.5 % of the intervention phase. The reason for this was carbon dioxide retention resulting from nasal obstruction, probably caused by the feeding tube.

Infant characteristics, clinical morbidities and mortality

There were no statistically significant differences between groups regarding demographic and birth related factors, except for the expected increased oral and nasal insertions in the BOG group.

Table 2 Infant characteristics STUDY I-III, by group

	Index Group Continuous Nasogastric (n=22)	Control Group 1 Bolus Nasogastric (n= 22)	Control Group 2 Bolus Orogastric (n = 24)
Infant characteristics			
Gestational Age, weeks	26.9 (26.3-27.5)	26.6 (26.0-27.1)	26.8 (26.2-27.4)
Birth weight, grams	864 (787-940)	833 (754-911)	899 (823-975)
Sex, Male	10 (46%)	13 (59%)	13 (54%)
Apgar score, 5minutes	8.0 (5.0-10.0)	8.0 (3.0-10.0)	8.0 (3.0-10.0)
CRIB score	5.5 (4.1-6.9)	6.2 (4.7-7.6)	5.3 (3.9-6.7)
Small for Gestational Age	7 (32%)	6 (27%)	3 (13%)
Antenatal steroids	19 (86%)	18 (86%)	18 (75%)
Level of birth hospital, NICU	16 (73%)	20 (91%)	18 (75%)
Discharge from hospital, PMA weeks	39.6 (35-46)	39.9 (35-46)	39.4 (38-41)
Discharge from domiciliary care, PMA weeks	48.1 (41-56)	46.8 (37-66)	53.9 (41-71)
Postnatal diagnosis and treatment			
Mechanical ventilatory support, IPPV/HFOV	15 (68%)	18 (82%)	15 (63%)
IPPV/HFOV, days	6.0 (0-67)	4.5 (0-34)	6.5 (0-36)
CPAP support	21 (96 %)	21 (96 %)	24 (100%)
Oxygen treatment, days	84 (44-124)	93 (52-133)	73 (48-99)
Oral and nasal insertions, No	246 (161-330)	230 (152-309)	440 (358-521)
BPD any degree	14 (64 %)	12 (55 %)	11 (46 %)
Severe BPD, with oxygen treatment after discharge from hospital	6 (27%)	5 (23%)	5 (21%)
Septicaemia	16 (73%)	16 (73%)	20 (83%)
Necrotising enterocolitis	2 (9%)	0 (0 %)	1 (4 %)
Gastrooesophagal reflux disease	1 (5 %)	1 (5 %)	2 (8 %)
IVH grade III/ IV and/or PVL	3 (14 %)	1 (5 %)	0 (0%)

Data are mean, (95% CI) for normally distributed continuous variables, median (range min-max) for skewed continuous variables, or n (%) for diagnosis or treatment. CRIB Score[70], Clinical Risk Index for Babies. NICU Neonatal intensive care unit. CPAP Continuous positive airway pressure. BPD Broncho pulmonary dysplasia any degree according to Bancalari[71]. Oral and nasal insertions were: Endotracheal tube; Feeding tube insertions and suctions including nasal, oral and endotracheal.

Clinical morbidity and mortality

All study infants needed respiratory support by supplemental oxygen. The need for supplemental oxygen (days), mechanical ventilatory support IPPV / HFOV or CPAP were comparable between groups, as well as the incidence of broncho pulmonary dysplasia (BPD). Septicaemia, need for antibiotic treatment, patent ductus arteriosus and NEC was similar between groups. NEC was diagnosed in three infants. Two cases

occurred early in the intervention phase, one BOG infant at day 13 and one CNG infant at day 14. One infant in the CNG group developed NEC three weeks after the intervention phase was finished at a postmenstrual age of 35 weeks. The mortality did not differ significantly between groups. Three infants, one in each feeding group, died early in the intervention phase, their deaths were caused by respiratory and circulatory collapse. Two infants in the CNG group, died after the intervention phase at a postmenstrual age of 33 and 47 weeks, respectively. Their deaths were caused by septicemia in combination with severe respiratory and circulatory distress.

RESULTS STUDY I

Gastrointestinal tolerance

With regard to the primary outcome in this study, the index infants achieved full enteral feeding faster than the control infants (HR = 1.86 (95% CI: 1.07 – 3.22)). This result remained virtually unchanged when adjusting for demographic and birth related factors (HR = 1.84 (95 % CI: 1.03 – 3.27)) (Table 2). To investigate whether improvement differed according to birth weight, a stratified analysis on birth weight was carried out. Among infants with a birth weight of 850grams or less the continuous fed infants achieved full enteral feeding considerably faster than the control infants and also after adjustment of confounding factors relating to demographic and birth related factors (HR = 4.13 (95 % CI: 1.48 – 11.53)). Among the infants with a birth weight of > 850 grams the difference between compared groups was smaller and more uncertain in terms of the width of the confidence interval (HR = 1.89 (95 % CI: 0.80 – 4.42)) No statistically significant difference was found between the two control groups, neither crude, (HR =1.22 (CI: 0.66 – 2.24)), nor after adjustment for demographic and birth related factors (HR =1.62 (CI: 0.77 – 3.4)).

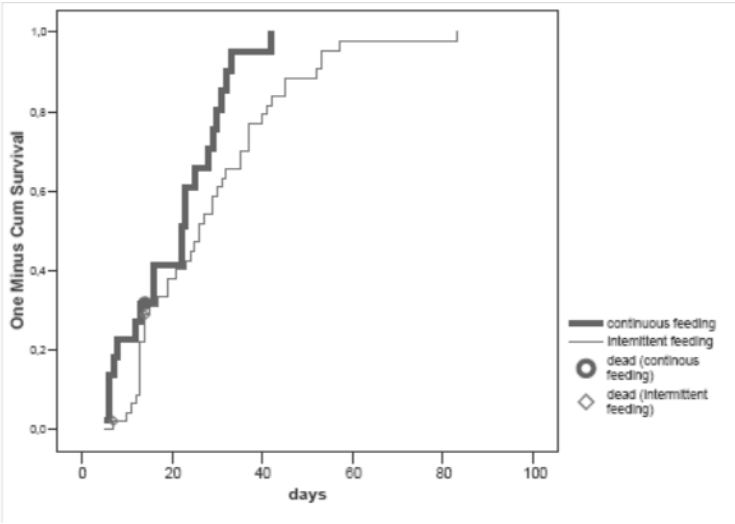

Figure 9. Kaplan Maier Curve illustrating the incidence proportion of achieved full enteral feeding among continuous (index n=22) and intermittent bolus (control 1 & 2 n=46) groups respectively, by time.

Nutrition and growth

Energy and protein intake during the intervention phase was similar for the groups as well as the time to regain birth weight. However, the growth rate of the lower leg during the intervention phase, from birth to 32 weeks PMA, and from birth to 36 weeks PMA was significantly faster among the CNG infants compared to the control groups (p 0.002 and 0.012, respectively). Feeding intolerance, gastric residuals and vomiting, were more common in the BOG group but did not differ significantly from CNG and BNG, (p-values 0.055 and 0.090, respectively). Only two infants regressed to PPN after reaching full enteral feeding, one in the CNG group and one in the BOG group.

Table 3. Gastro intestinal tolerance, growth and nutritional outcomes among study infants (n=68), by group

	Index group Continuous Nasogastric feeding (n = 22)	Control group 1 Intermittent Nasogastric Feeding (n = 22)	Control group 2 Intermittent Orogastric Feeding (n = 24)	P value
Time to achieve full enteral feeding, days†	20.1 (10.5)	26.1 (13.6)	28.8 (18.2)	0.027*
Time to regain birth weight, days†	15.5 (4.3)	16.0 (3.6)	15.1 (3.9)	NS
Growth rate of the lower leg, from birth to 32 weeks postmenstrual age, mm/day†‡	0.32 (0.12)	0.24 (0.10)	0.20 (0.12)	0.002
Growth rate of the lower leg, from birth to 36 weeks postmenstrual age, mm/day†	0.39 (0.11)	0.31 (0.08)	0.30 (0.11)	0.012
Total energy intake, kcal/kg/day †‡	99 (17)	101(23)	100 (18)	NS
Total protein intake, g/kg/day†‡	2.5 (0.5)	2.4 (0.7)	2.4 (0.5)	NS
Gastric residuals No ¶ ‡	3.5 (0.0-25.0)	3.0 (0.0-14.0)	6.0 (0.0-30.0)	NS
Vomiting No¶ ‡	1.5 (0.0-28.0)	2.0 (0.0-20.0)	4.5 (0.0-56.0)	NS

* Continuous (n=22) versus intermittent bolus (n=46); †Values are Mean (SD); ‡Time period intervention phase i.e., birth to 32 weeks, postmenstrual age; ¶Values are Median (Range); NS Not significant (p value > .05).

RESULTS STUDY II

The aim in this study was to compare the effects of continuous versus bolus feeding with regard to behavioural responses indicating stress at early postnatal age. All study infants on tube feeding were video recorded. Fifty-four infants were eligible for video recording session 1 at age 7 days, 60 infants for recording 2 at age 15 days, and 60 infants for recording 3 at 32 weeks PMA. Data comprised 174 video recordings.

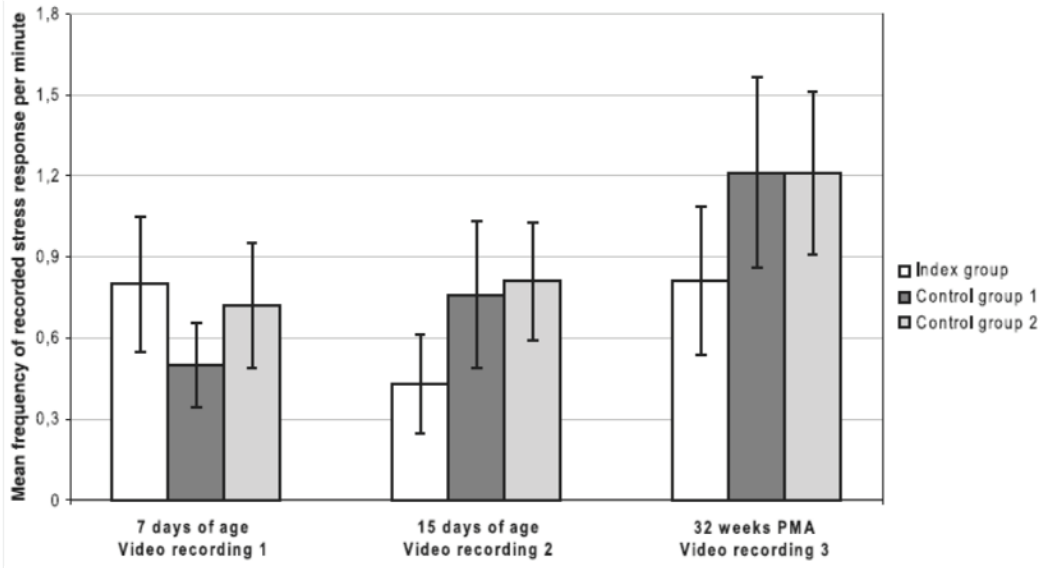

Figure 10. Frequency of behavioural stress response during tube feeding, by group Frequency of behavioural stress response per minute with corresponding 95% CI, for the index and control groups respectively, by video recording session. Index group=continuous fed infants; control group 1=bolus nasogastric fed infants; control group 2 = bolus orogastric fed infants.

With regard to the frequency of behavioural responses to stress, there were no statistically significant differences between control infants and index infants at age 7 days, when using the 50th percentile cut-off (OR=0.7 (95% CI: 0.3-2.0)) or the 75th percentile cut-off (OR=0.3 (95% CI: 0.1-1.1)). These results remained virtually unchanged after adjustment for demographic and birth-related factors, respiratory stability and environmental factors. At 15 days of age, the control infants had a significantly higher risk of behavioural stress response compared with index, adjusted OR=4.1 (95% CI: 1.1 - 15.4) when the cut-off was set according to 50th percentile, and adjusted OR=8.9 (95% CI: 1.3-62.9) when the cut-off was set according to 75th percentile. A higher frequency of stress in the control infants, compared to index infants was also observed at 32 weeks PMA. An increase in behavioural stress response over time was observed.

Table 4. Unadjusted and adjusted odds ratio for behavioural response of stress and discomfort together with 95% CI with bolus feeding compared with continuous feeding.

Bolus versus Continuous feeding	Cut-off point*	Bolus No (%) †	Continuous No (%) †	Unadjusted OR (95.0% C. I)	Adjusted ‡ OR (95.0% C. I)
Percentile 50					
Video recording 1, at postnatal age, 7 days §	0.52	18 (48.6)	10 (58.8)	0.7 (0.3 - 2.0)	0.5 (0.1 - 2.3)
Video recording 2, at postnatal age, 15 days	0.58	25 (61.0)	5 (26.3)	4.0 (1.3 - 12.8)	4.1 (1.1 - 15.4)
Video recording 3, at postmenstrual age, 32 wks	0.95	24 (58.5)	6 (31.6)	2.9 (1.0 - 8.8)	4.2 (1.0 - 17.8)
Percentile 75					
Video recording 1, at postnatal age, 7 days §	0.94	7 (18.9)	7 (41.1)	0.3 (0.1 - 1.1)	0.3 (0.1 - 1.2)
Video recording 2, at postnatal age, 15 weeks	0.96	13 (31.7)	2 (10.5)	3.9 (0.8 - 19.3)	8.9 (1.3 - 62.9)
Video recording 3, at postmenstrual age, 32 wks	1.51	13 (31.7)	2 (10.5)	4.0 (0.8 - 19.3)	6.3 (1.0 - 39.7)

* The cut of points, Median and percentile 75, are based on the frequency of stress and discomfort for all study infants, at each video recording 1, 2 and 3.

† Number (%) of cases over the cut off point in each group, bolus and continuous.

‡ Adjusted for: Birth weight, Gestational age, Sex, Arterial/alveolar oxygen tension ratio, Environmental light, sound and activity level and Bedding support. §Continuous feeding n =17, Bolus feeding n=37.

Continuous feeding n=19, Bolus feeding n=41.

During all three video recordings, there was a statistically significant higher frequency and duration of indirect nursing interaction among the control infants compared to the index infants ($p<0.0001$). The duration of direct nursing interaction among control infants was significantly longer than among the index infants at all three video recordings (at age 7 and 15 days $p=0.01$) and at 32 weeks PMA ($p=0.03$)). For frequency of direct nursing interaction, we found no significant differences at ages 7 and 15 days; however, there was a statistically significant higher frequency among controls compared to index infants at 32 weeks PMA ($p=0.03$)

RESULTS STUDY III

Infant and maternal characteristics were similar between study groups with the expeption of the significantly higher frequency of tube insertions in the bolus orogastric group. All the mothers started to express milk. The mothers who stopped milk expression before their infant's first oral feed were significantly younger, primiparous, smokers, and had less skin-to-skin contact with their infant during hospital stay, and their infants had lower weight and GA at birth.

Table 5. Maternal characteristics and parental-infant interaction

	Index Group Continuous nasogastric (n=22)	Control group 1 Bolus nasogastric (n=22)	Control group 2 Bolus orogastric (n=22)
Maternal characteristics			
Maternal age, year	32.4 (30.2-34.5)	32.4 (30.4-34.4)	30.0 (28.1-31.9)
Maternal education level			
Secondary school	3 (14 %)	3 (14 %)	5 (21 %)
Upper secondary school	11 (50 %)	10 (46 %)	9 (42 %)
University	8 (36 %)	9 (41 %)	10 (42 %)
Co-habiting with partner	21 (96 %)	22 (100 %)	23 (96 %)
Primipara	10 (46 %)	9 (41 %)	10 (42 %)
Smoking	2 (9 %)	4 (18 %)	3 (13 %)
Mode of delivery, Sectio	13 (59 %)	15 (68 %)	16 (68 %)
Multiple birth	2 (9 %)	5 (23 %)	3 (13 %)
Started express breast milk	22 (100 %)	22 (100%)	24 (100 %)
Infant feeding days with exclusive mothers milk during hospital stay, ratio	0.59 (0.44-0.74)	0.57 (0.38-0.75)	0.50 (0.35-0.66)
Parent infant interaction			
First skin to skin contact, postnatal day	13 (8-17)	10 (7-12)	13 (8-18)
Skin to skin within hospital, No times	29 (21-37)	27 (18-36)	29 (19-39)
Skin to skin within hospital, No of hours per session	2.1 (1.7-2.4)	1.7 (1.4-1.9)	1.9 (1.7-2.1)

Data are mean (95% CI) for normally distributed continuous variables, median (range min-max) for skewed continuous variables, or n (%). Number of twins where both twins in the pair survived was: Index group n=1 and Control group 2 n=1.

Initiation of breastfeeding and parent-infant interaction

No differences were found between groups regarding proportion of mother-infant pairs initiating breastfeeding mean prevalence proportion 0.72 (n=49) (p 0.87), or infants first time at mothers breast, PMA, mean weeks (95% CI) Index group, 33.7 (32.8-34.4); Control group 1, 33.8 (32.9-34.7); Control group 2, 33.9 (33.0-34.8) (p 0.89). Nor were there differences found in infant or maternal characteristics, except for a significant difference regarding length of time per session of skin to skin contact, among mother-infant pairs who initiated breastfeeding (n=49), mean (95%CI) CNG group 2.24 (1.9-2.6); BNG 1.66 (1.3-2.0) and BOG 1.85 (1.6-2.1); p=0.037.

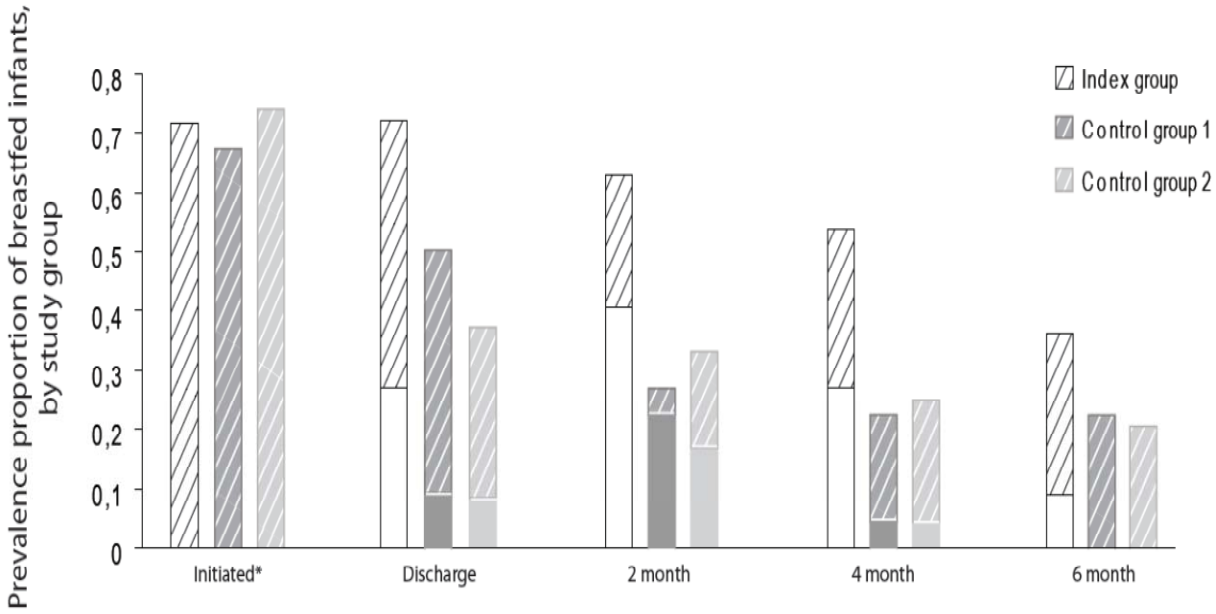

Figure 11 The prevalence proportion of breastfeeding at given time points, by group. Partly breastfeeding are given in striped pattern. Breastfeeding status at: initiation of breastfeeding, at discharge from hospital, and at two, four, and six month of age corrected for expected time of birth.

Breastfeeding, partly and exclusive at given time points

At discharge from hospital all continuous-fed infants (Index group) who initiated breastfeeding were still breastfed, n=16/16, whereas cessation of breastfeeding before discharge was observed in n=4/15 in the bolus-nasogastric group (Control group 1) and n=9/18 in the bolus-orogastric group (Control group 2). Prevalence ratio of breastfeeding at discharge from hospital, and at 2, 4 and 6 months corrected age, was higher among infants who had been exposed to continuous tube-feeding.

Table 6. Prevalence proportion and prevalence ratio, of breastfeeding infants, continuous versus bolus fed infants (n=68).

	Prevalence proportion of breastfed infants		Continuous versus Bolus		Bolus nasogastric versus Bolus orogastric	
	Continuous	Bolus	P R* (95 % C I)	p value	P R (95 % C I)	p value
Breastfeeding partly and/or exclusive						
Initiated breastfeeding	16/22 (73%)	33/46 (72%)	1.0 (0.7-1.4)	0.932	0.9 (0.6-1.3)	0.609
At discharge from hospital	16/22 (73%)	20/46 (43%)	1.7 (1.1-2.5)	0.027	1.33 (0.7-2.6)	0.394
At 2 months corrected age†	14/22 (64%)	14/46 (30%)	2.1 (1.2-3.6)	0.011	0.8 (0.3-2.0)	0.656
At 4 months corrected age	12/22 (55%)	11/46 (24%)	2.3 (1.02-4.3)	0.015	0.9 (0.3-2.6)	0.857
At 6 months corrected age	8/22 (36%)	10/46 (22%)	1.7 (0.8-3.6)	0.205	1.1 (0.7-1.3)	0.876
Exclusive breastfeeding						
At initiation of breastfeeding	0/22 (0%)	0/46 (0%)	---	---	----	--
At discharge from hospital	6/22 (27%)	4/46 (9%)	3.1 (0.99-10.0)	0.053	1.1 (0.2-7.1)	0.927
At 2 months corrected age	9/22 (41%)	9/46 (20%)	2.3 (1.1-4.7)	0.032	1.4 (0.4- 4.4)	0.606
At 4 months corrected age	6/22 (27%)	2/46 (4%)	6.3 (1.4-28.6)	0.015	1.1 (0.1 -16.4)	0.950
At 6 months corrected age	2/22 (9%)	0/46 (0%)	---	---	---	---

Duration of breastfeeding and achieved exclusive breastfeeding

The total duration, months of breastfeeding, was also significantly longer in the continuous-nasogastric group, mean (95%CI) 11.0 (6.5-15.5) compared to the bolus nasogastric group, 4.9 (2.5-7.4), and the bolus-orogastric group, 4.9 (2.1-7.8) respectively. In Cox regression models, duration of breastfeeding between the continuous group and the bolus groups was significant, HR 2.6 (95%CI 1.3-4.8), and adjusted HR 6.5 (95%CI 2.8-15.4; p= <0.0001).

In infants who survived to discharge (n=63) all except one attained full oral feeding by breast or bottle. Of the mother-infant pairs who initiated breastfeeding, transition to exclusive breastfeeding was achieved by n=21 (42.9 %), with a statistically significant difference between the groups (Continuous-nasogastric n=11/16, Bolus-nasogastric n=6/15, Bolus-orogastric n=4/18; p=0.02). Maturation at full oral feeding was found to be similar between the groups, PMA weeks, mean (95%CI) (Continuous-nasogastric 39.0 (35.6-48.3), Bolus-nasogastric 38.4 (36.0-131.0) Bolus-orogastric 38.3 (34.4-44.4); p=0.530). Total duration of exclusive breastfeeding was significantly longer in continuously fed infants compared to bolus fed infants adjusted HR 2.2 (95%CI (1.1-4.4; p=0.027)).

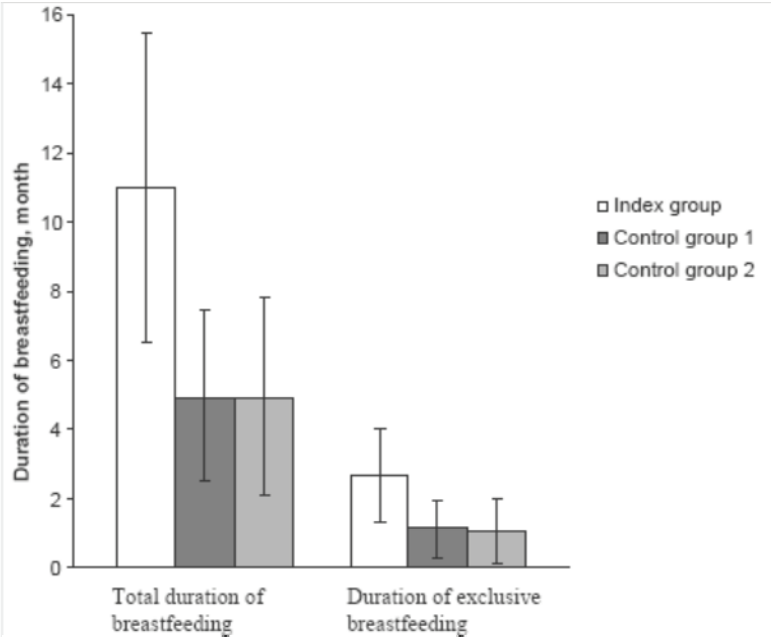

Figure 12. Duration of breastfeeding corrected age months among infants who initiated breastfeeding (n=49) by group

Breastfeeding in infants with severe bronchopulmonary dysplasia

Among the subsample of infants diagnosed with severe BPD (n=16), the CNG infants achieved exclusive breastfeeding to a significantly higher extent compared to the BNG and BOG infants. Out of 6 BPD infants in the continuous group 4 reached exclusive breastfeeding, whereas only 1/5 in the bolus nasogastric, and 0/5 in the bolus-orogastric group (p=0·03). Within this subsample of BPD infants (n=16), the mother's experience of feeding problems was significantly lower among mother-infant pairs who achieved exclusive breastfeeding (p=0.01).

DISCUSSION

METHODOLOGICAL CONSIDERATIONS

Target population, sample size and study protocol

In order to include children that may benefit from the investigated treatment, measures were taken to identify and target infants at risk for enteral intolerans prior to this randomised control trial (study I-III). This strategy of selective recruitment based on strict criteria and prognostic targeting, prior to randomisation has been proposed to increase power in randomised controlled trials with relatively small study sample and hinder dilutive effects[72]. In this study a strict study protocol was used to keep important conditions known to influence outcome, other than the intervention, constant for all groups. Such conditions in this study were early start of feeding within 30 hours of birth, solely human milk as diet and scheduled feeding advancements allowing full enteral feeds within a week. However, the use of strict criteria leads to limitation of generalisability to similar populations and similar feeding protocol as in the study sample; in addition all comparisons between groups were selected a priori. In paper I and III, sub group analyses were done, in order to evaluate potentially increased treatment effect among the most immature infants.

Demographic factors within the geographical area

To further reduce potential influence from variation in background variables, blocking for time and for geographical area was used. With the given sample size, data collection was estimated to be about 3 years. During such long time period, both demographic situations in the geographical areas of the growing capital city of Sweden, may change and probably also changes in medical treatment will occur. Regarding potential differences in demographic and socioeconomic factors between geographical areas, blocking was performed for Stockholm County, north and south.

Enteral tolerance and growth

The main outcome in study one “time to achieve full enteral feeding, was well defined and easily determined and could be measured with a high degree of precision, due to the fact that the infants were continuously monitored during their hospital stay. Misclassification regarding treatment group, was also unlikely since exposure to the different enteral feeding methods was followed by a strict study protocol bedside for

each study infant. Since of the nature of the primary outcome, time to achievement of full enteral feeds is typical “time to event data”, survival analyses was utilised to compare the outcome between the different groups.

Estimated growth velocity is depending on energy intake and energy expenditure, as well as reliable instruments for the detection of changes in growth. Information on growth was generated by two methods. Weight gain i.e., the number of days to regain birth weight and measurement of growth velocity of the lower leg, by using of an electronic knemometer. The latter technique enables measurement of linear bone growth velocity over short periods, which is specific a useful when measure growth in early postnatal life and is validated in both moderate and extremely preterm infants[40, 41]

Methodological considerations of behavioural assessments

Even though video recording as a method is frequently used in behavioural biology science, it has its limitations. In this study, one fundamental question may be formulated. ”How do internal as well as external causal factors elicit and control behaviour in the short term [73, 74]? Translating to the study included in this thesis, how do we know to what stimuli the infant reacts on? In addition to internal stress due to enteral feeding, one must be aware of the fact that there are numerous potential external causal stressors in the environment surrounding the preterm infant in the neonatal intensive care unit that may also influence the infant's behavioural response. This implies that when using source data from video-recordings focusing on the infant inside the incubator, you may overlook information regarding the interplay between the infant and its environmental context. In study II, such factors were controlled for within the study design. Strategies were; the time period during the day when the video recording took place was standardised a protocol was used to measure level of sound, light and activity in the nursery [66] Since nursing interaction itself has also been shown to have adverse effects on infants’ physiology and behaviour [47-49], direct and indirect nursing interactions was measured at the time of video recording.

Even though the behaviours in the NIDCAP manual are well defined, and have been used in previous research of preterm infants, the NIDCAP manual is, nevertheless validated for more mature preterm infants[68]. This may have influenced the results in our study, and be one reason for the increase in detected occurrences of frequency of behaviours over time.

Although, besides having the opportunity with a camera system with two focus, enable coding behavioural responses from very detailed images close on infants face and throat and simultaneous infants whole body movements One advantage of using video recording in contrast to naturalistic observations is that the coding process of the material can be carried out during a short time period; in this study during a period of three months, after a data collection which took more than 3½ year had to completed. This allow, the researcher to perform intra reliability tests, evaluating consistency collection format of behavioural assessments over time, which is not possible when using naturalistic observations. The trained coders, who were unconnected to the research group where aware of that reliability tests should be performed several times during each coding period, but time point when tests were performed was blinded to the coders.

Other methodological considerations taken in to account in relation to the process of coding, was observer fatigue during coding. However, within this "coding design", with the standardised detailed procedure protocol for the coding during the coding of each film and this might have had positive effects on the coders' ability. In addition coders were set to do a maximum of four video recordings during a "working day", with breaks in between to increase precision [74].

Follow-up of breastfeeding capability

In study III capability of breastfeeding was used as a measurement of the infants' feeding capability. One can ask, how come? Actively suckling at the mothers' breast, says more about infant's early eating behaviour and capability to feed itself than any other method. This because the infant not only must have the ability to suckle, but also to succeed in the developmental steps that lie within active suckling at the mothers breast [65] a process which needs a high level of physiological and behavioural maturation. The infant also requires the capability of infant-maternal interaction. The choice of breastfeeding as an indicator of infant feeding capability is, of course, also dependent on high rates of breastfeeding in the community, which was the case in Stockholm County at the time of this study.

RESULTS IN PERSPECTIVE

The overall aim of this thesis was to investigate and compare different enteral feeding methods used in early postnatal age in very low birth weight infants, in order to gain further knowledge and understanding regarding consequences of enteral feeding on the developing very immature infant.

The results showed that continuous slow rate intragastric feeding in infants with birth weight < 1200grams and GA of 24 to 29 weeks improved gastrointestinal tolerance and shortened the time needed to achieve relative to bolus feeding. The study also indicates that continuous feeding might be even more suitable with regard to enteral tolerance in extremely low birth weight infants ≤ 850grams, though the relatively low number of subjects may hamper a definitive conclusion. An indication of marked nutritional efficiency was the significantly better real bone growth during the critical period from birth to 32 weeks (study I). These results will be addressed in the first part of the discussion. The infants' behavioural stress response during tube feeding was increased at 15 days and at 32 weeks PMA among infants exposed to intragastric bolus feeding when compared to continuously fed infants (study II). This will be the focus in the second part of the discussion. The results of the follow-up of infants later feeding capability in terms of infants' ability to feed themselves directly at mothers breast will be addressed in the third part of the discussion (study III).

Tube feeding methods and gastro intestinal tolerance and growth

Gastrointestinal tolerance

The main reason for the better outcome regarding enteral tolerance in the continuous group, is probably related to that the continuous slow rate intra gastric infusion promotes gastro intestinal functional motor activity in the developing very immature infant. As mentioned there is evidence of a species specific programme of development with a distinct pattern of maturation which goes from disorganised to organised gastrointestinal motility at about 31 weeks of gestation[11]. However In 1998, Baker and Berseth, presented data on relative healthy preterm infants, indicating that intragastric slow rate infusion promoted gastric emptying and maturation of duodenal motor activity[21]. In a study published the same year, de Ville and Berseth[22] showed an almost paralytic pattern of duodenal motor activity as a result of intra gastric bolus feeding in stable preterm infants. When they exposed the infants to slow rate intragastric infusion, the infants showed gastric emptying and duodenal motility pattern which resembled that found in adults. These findings indicate that the rate of feeding infusion, fast or

slow, may have direct influence on the functional maturation of gastro intestinal motor activity.

The significantly better results regarding enteral tolerance in the continuous fed group compare to the bolus groups in study I, differ from previous randomised reports on this topic[75, 76 33, 77], these studies couldn't detect differences between the feeding regimes continuous versus bolus. As discussed in the Cochrane review o, possible reasons for not detecting differences between studies regarding continuous versus bolus feeding, are probably due to methodological differences [36]. In study I, we were able to control for factors that influence enteral tolerance. That is; starting time, all infants started within 30 hours of age[78], got the same diet, i.e., sole human milk[22, 62] and where exposed to similar pro active feeding advancement protocol which enabled achievement of full enteral feeds within a week after birth. To date, there is only one study that has investigated continuous versus bolus feeding in critically ill infants, with as low birth weight birth and with GA at birth as low as 24 weeks, with a similar protocol as the study included in this thesis. That study reported similar results although it was a retrospective study[79]. However one pilot study, with few infants in each group, showed the opposite result to the study included in this thesis. Although, differences in the start of feeds, mixed intervals 2 or 3 hours in the bolus group, inconsistency regarding type of diet, and inadequate sample size estimation may have influenced the results. As mentioned, another important factor regarding randomised trials with relative small sample is that has been discussed is to increase power by different strategies. As proposed by Roozenbeek et al.[72] , identification of patient at risk is one; a second is to adjust for potential confounders in the analysis.

Growth

Despite similar protein and caloric intake during the intervention phase infants randomised to continuous feeding were find to have significantly better growth velocity regarding bone growth of the lower leg, during this time period compared to the intermittent bolus groups. This result remained significant at the time period from birth to 36 weeks PMA. The increased growth velocity among the infants exposed to continuous slow rate intragastric feeding is probably due to how these enteral feeding systems act on the developing target organ of the gastrointestinal tract. Regarding the increased enteral tolerance seen in the continuous group, in which the drip of human milk, without interruptions, hence permitted a continuous presence of human milk on

the surface of the intestinal mucosa. Human milk with its unique components including: numerous of nutrients, growth factors, hormones and factors that serve as host defence, most probably provide supportive effect on the development and growth of the gastrointestinal mucosa, which is fundamental for optimal growth[61]. The increased enteral tolerance regarding feeding advancements in the continuous group compared to the intermittent bolus groups may also play an important role regarding better growth. Since, continuous enteral nutrition compared to bolus has been proved to have a positive influence on bioenergetic and metabolic response. In an experimental study on healthy adults, decreasing energy expenditure and reduce thermal losses was detected. The authors suggested, that less energy requirements are needed during continuous feeding compared to meal ingestions[80]. This is naturally of importance in all critically ill trauma patients, and in particular in the very premature infant. The results regarding energy expenditure in adults was also confirmed in a study of preterm infants by Grant et al. 1991[81]. The findings showed that continuous feeding was correlated with increased energy efficiency, when compared to feeding by intermittent boluses. Energy efficiency is essential in the very premature infants, who do not only need to reach homeostasis but also need to have resources facilitating the pre-programmed development in all organ systems. In the present thesis, as well as seen in previous reports of continuous versus intermittent bolus feeding there was no detection of any differences between groups regarding time to regain birth weight[30, 76, 77, 82]. This may be due to difficulties to indentify such differences, because of weight gain during early life in the very preterm infant, may not necessarily reflect changes in real growth, but rather differences in fat and fluid deposition[39]. Other factors that may influence both enteral tolerance and growth are reducing of stress and painful situations related to enteral feeding.

Tube feeding methods and behavioural stress

Behavioural stress and enteral tolerance

The findings of significantly higher behavioural stress response in infants' exposed to intragastric feeding by bolus, compared to those fed by intra gastric continuous feeding, may relate to the fact that continuous feeding decrease the risk of gastric distension, which minimize negative visceral sensory experiences for the infant. Previous studies have shown that feeding by bolus adversely influence respiratory function in VLBW infants[83]. The increased pressure on the very immature infant, who most often is already severely respiratory compromised, may explain such effect. As suggested in a

study by Baker and Berseth, the mechanical gastric distension caused by bolus feeding probably activates local neural reflexes which inhibit gastric duodenal motor activity[21]. This reactivity indicating visceral stress, could explain both the increased enteral intolerance and the significantly higher behavioural stress response found in the bolus fed infants in study II. In addition, activated stress, leads to increase behavioural activity i.e., body movements which also increase energy losses. In contrast, the continuous fed infants, with less activated behavioural stress, and less need for nursing interaction caused by physiological instability and/or discomfort, would have more opportunity to restful sleep, promoting growth[84].

NIDCAP behaviours as indicators for behavioural stress response

In study II, behaviours indicating stress was selected from four of the NIDCAP subsystem. The use of behaviours indicating stress from several of the subsystems, when coding the material, may have increased the ability to detect possible negative influences due to feeding in these very immature infant. Even though not statistically significant, at 7 days of age, a somewhat higher frequency of stress among the continuous fed infants was observed compared to infants fed by bolus. This might be explained by the higher total enteral volume intake mL/kg/day at early postnatal age. NIDCAP behaviours, mostly from the motor system, have been used to evaluate[50, 85-87] behavioural responses to both painful and stressful stimulus in the preterm infant. In study II, NIDCAP behaviours indicating stress was found to be activated not only from the motor system but, from the attention interaction system, autonomic visceral system and behavioural state as well. It seemed that feeding, bolus feeding in particular, has a negative effect on the entire organism of these very immature infants. As in previous publications regarding pain and stress response, we also noticed that frequencies of behavioural stress due to feeding increased over time. An explanation for this phenomenon could be visceral sensitisation to pain or stressful stimuli [88] but also as mentioned earlier the older the infant, the more robust and recognisable the response [89].

Behavioural stress response and nursing interaction

The bolus fed infants had a significantly increased need for direct nursing contact to ameliorate discomfort when compared to continuously fed infants.

The bolus fed infants also had a higher frequency and duration of indirect nursing interaction than the continuously fed infants. This could probably be due to the feeding

method itself, however, one cannot exclude that the more frequent interactions in the bolus group also could have influenced the infants' stress response [47, 49].

Behavioural stress response due to tube feeding placement

In this study we could not detect any differences between the two bolus groups regarding behavioural stress response due to feeding. However, the placement of the feeding tube, either nasal remaining in place between feeds or intermittently orogastric insertion, may act as stressors but in different ways. A nasogastric feeding tube remaining in place between feeds is known to increase nasal resistance [55], and compromise pulmonary function[83]. It may also cause discomfort by irritation and lesions of the nasal mucosa. However, intermittently inserted orogastric feeding tubes also have adverse effects. Oral insertions are known to have immediate negative effect on vagal response[90], cerebral blood flow velocity[91], and increase the risk for gastrooesophagal reflux in preterm infants [92].

Tube feeding methods and infants' breast feeding capability

The findings in the planned follow-up study (Study III) of infants exposed to continuous enteral feeding during early postnatal life was found to have higher prevalence rates, and longer duration of breastfeeding compared to intermittently bolus fed infants. The overall significantly increased breastfeeding in infants exposed to continuous tube feeding in early life, compared to infants exposed to tube feeding by intermittent bolus may relate to underlying physiological and behavioural advantages convergent with continuous slow rate intragastric infusion during early postnatal life.

Early enteral nutritional support and infants' later breastfeeding capability

In this study (III), all groups were exposed to early human milk feeding, standardised daily feeding advancements and human milk as sole diet until at least 36 weeks PMA. However, as previously discussed and in contrast to bolus-fed groups, the continuous group showed better enteral tolerance, by attaining full enteral feeding about a week earlier, and there was an even greater difference of about 10 days among the extremely immature infants with birth weight below 850grams. An indication of marked nutritional efficiency was the significantly better real bone growth during the critical growth period from birth (GA 24-29) to 32 weeks PMA[30]. This effect was also evident during the period from birth to 36 weeks[30]. In view of reported beneficial effects of

human milk feeding in preterm infants regarding brainstem maturation[56] and cognitive function [57] [58, 93] , the importance of high proportion of enteral human milk intake, in relation to infants' later breastfeeding capability cannot be underestimated. One cannot exclude the possibility that nutritional benefits in the continuous group in our study may have positively influenced neural maturation required for the complexity of coordinating suck-swallow-breathing. This might be one explanation of why all the infants who initiated breastfeeding in the continuous group were still breastfed at discharge from hospital compared to the fewer numbers in the bolus groups.

Postponed exposure to intermittent bolus feeding

Parent-infant interaction and later breastfeeding capability

The decrease in breastfeeding among the bolus fed infants prior to discharge could also be due to increased sensitisation related to visceral stress related to intermittent bolus feeding schedule during early postnatal life[84]. In contrast to previous reports comparing continuous versus bolus feeding [36], an identified maturational stage of 32 weeks PMA was used to start a gradual transition from continuous tube feeding to tube feeding by intermittent boluses. From a developmental perspective, one can hypothesise that this postponed timing of exposure to bolus feeding in early life may serve a protective role. Besides effects of probable decrease in visceral pain and stress[84], continuous slow rate infusion also provides an opportunity for individualised care according to the infant's sleep-wake pattern, compared to the interruptions for both infants and parents by the bolus feeding schedule every third hour around the clock[94]. This could be an explanation for the longer skin to skin sessions in the continuous group, which may have provided additional support for parent infant interaction, and advantages for the infant regarding accelerated autonomic and neurobehavioral maturation, as well as in the circadian systems [95].

The timing and gradual transition to intermittent bolus feeding may also have enhanced infants' developing feeding pattern. This is because exposure to intermittent feeding i.e. hunger, took place at a stage of development which correlates with wiring of neural pathways integrated in coordination of suck-swallow-breathing[11, 96, 97].

Invasive procedures and influence on later breastfeeding

The pronounced decrease in breastfeeding seen in the bolus-orogastric group in our study might also be related to the frequent feeding tube insertions. Although in-depth

analyses of infants' pre-feeding and suckling behaviour at the mother's breast were not the focus of the present follow-up, a study on healthy newborns demonstrate that invasive procedures, such as suctioning directly after birth, could adversely delay infants' early breastfeeding behaviour and alter oral sensitivity [98]. Studies on preterm infants show similar results. Dodrill and colleagues found that of prolonged duration of enteral feeding tube use displayed alter oral sensitivity, facial defensiveness and feeding delays[99]. In the present randomised cohort, significantly higher was reported behavioural stress-response was reported, as was the need for stabilisation during tube feeding in the bolus groups at 15 days after birth, and at 32 weeks PMA[84], about a week before the initiation of breastfeeding. In view of these findings, breastfeeding might be challenging since it depends on interactive sensitive communication between the mother and the baby, especially if the baby displays low and/or weak activity, or defensive behaviour at the mother's breast. Blaymore Bier and colleagues reported that prolonged orotracheal intubation and extended need for oxygen treatment negatively affected the sucking ability in very preterm infants at term and at three months[96].
In contrast to the continuous group, the negative trend in breastfeeding prevalence in the bolus groups remained at two, four and six months corrected age. Both insertions[54, 98] and feeding by bolus [22] have been shown to negatively affect vagal response. Thus the significant decline in breastfeeding prevalence in the bolus-fed infants might reflect a more pronounced maturational lag in vagal regulation, which has been proposed to be a marker for continuing feeding difficulties[97].

Severe BPD and capability to attain exclusive breastfeeding

The differences in breastfeeding discussed above are also reflected in the results relating to exclusive breastfeeding at discharge, two, four and six months. As mentioned, regarding enteral tolerance, the smallest infants with birth weight $\leq$ 850grams were those who benefitted most from continuous feeding[30].
In contrast to bolus fed infants with severe BPD, continuous fed infants with the same diagnosis were found to be more successful regarding transition to exclusive breastfeeding. Of particular interest, irrespective of group allocation was the significant correlation between the achievement of exclusive breastfeeding and less maternal experiences of feeding problems. In view of published reports raising concerns that infants born extremely preterm and/or compromised with severe BPD are at high risk for feeding difficulties and malnutrition during infancy[25 100], our results indicate that continuous tube feeding may support a more normal development of later feeding

capability in this group of infants. Exclusive breastfeeding has been shown to be especially important for this vulnerable group. Blaymore Bier and colleagues reported that, in contrast to bottle feeding, breastfeeding in extremely low birth weight infants is physiologically less stressful, increases oxygen saturation and promotes control of suckling-pausing and rhythm[96]. They also raised the issue of milk intake during breastfeeding, which was less per session compared to bottle feeding. In this follow-up it was also found that among infants with severe BPD, continuously fed infants had a higher probability of reaching exclusive breastfeeding compared to infants exposed to bolus feeding, thus indicating increased efficiency regarding milk intake among infants' in the continuous group compare to bolus groups (study III).

Duration of breastfeeding

The duration of breastfeeding in the continuous group was found to be more than twice that of the bolus groups. Breastfeeding i.e., when the infant suck directly from the mother's breast not only provides optimal nutrition[61], it also automatically gives the infant access to closeness: supporting the sensitive communication with the mother and probably enhancing neural maturation of the pre-programmed system involved in feeding and chewing during the infant's first year.[57, 58, 101]. The closeness during breastfeeding sessions also enhanced maternal bonding to the child in several ways. Hormonal influences of oxytocine increase during closeness and breastfeeding which, not only strengthen maternal feelings but may also increase maternal ability to be "open" and aware of the infant's signals. This is of course of importance for all human beings, but may be of specifically importance in the often fragile situation when infants are born very preterm [102],[103].

CONCLUSION

In the present thesis, effects of different enteral feeding methods, continuous feeding versus intermittent bolus feeding, has been investigated. This in order to gain further knowledge and understanding regarding consequences of different feeding regimes used in early life on very immature infants.

The findings presented in this thesis support the notion from previous research that infants born at very early gestational age have specific needs in treatment and care during critical periods of development in all organ systems, including the gastro intestinal tract. The result relating to the primary question in this thesis; if continuous slow rate intragastric feeding around the clock, compared to intragastric feeding by intermittent bolus every third hour, may improve gastrointestinal tolerance and promote growth - confirms this view.

The results of less expressed stress behaviours, together with less need for direct nursing contact to ameliorate discomfort, reflecting stability and indicates an increased opportunity of resting for the infant, which may be one explanation to the effects on growth. These effects, may also indicate an influence on these infant's development of feeding behaviour, and thus support the findings revealed in the follow up of infant breast feeding capability.

FUTURE DIRECTIONS

The results from this research programme need to be validated in future studies, but also open up for new research questions regarding gaining deeper insight into the mechanisms behind these effects such as:

- How do the different feeding methods influence the patterns of gut hormones as well as stress hormones during and following the intervention?

- Is later feeding behaviour and growth influenced by the different feeding methods in early life and if so, how?

- The present study indicates that early parental interaction is facilitated by continuous feeding. How do mothers experience different feeding methods during hospital stay strategies?

- A fourth interesting research question would be, if the continuous feeding method may also positively influence the interactive pattern in the family?

ACKNOWLEDGEMENTS

TACK

Jag vill tacka alla er som på olika sätt har givit mig stöd och styrka att genomföra detta arbete. Jag vill särskilt tacka alla barn och föräldrar som deltagit i detta forsknings projekt. Utan ert deltagande och ert förtroende och engagemang ni visat, under den långa sjukhusvistelsen, och senare i uppföljning ”öppna” dörren till ert hem för ännu en filmning och fler frågor. Ni har lärt mig så mycket om hur det är att få ett för tidigt fött barn.

Stort tack till Alla ni barnsköterskor och undersköterskor, sjuksköterskor och läkare på Karolinska, Danderyd och Huddinges neonatalavdelningar som, för ert engagemang i detta projekt, ni gav mig styrka och glädje att detta var något viktigt att ta reda på, något som ni gör varje dag på jobbet, och har undrat över.

Jag vill dessutom särskilt tacka:

Kyllike Christensson, professor och huvudhandledare, för din visdom och ditt oändliga tålamod med mina utvikningar åt olika håll. Men kanske framför allt för Din tillförsikt och tro på mig, att du givit mig spelrum för kreativitet i upplägget och genomförandet av detta projekt. Samt alla berikande diskussioner förstås.

Mats Blennow, professor och bihandledare för ditt otroliga stöd under de roliga men stundtals slitsamma åren under insamling av data.

Lars Alfredsson, professor och bihandledare. För din fenomenala förmåga att se vad som händer bakom de siffror som är de kommande resultaten, och din vägledning genom denna för mig ”nya” värld av metodval och analyser. Du fick mig att förstå hur mångskiftande och spännande denna del av forskningen är.

Hugo Lagercrantz, professor och bihandledare, för din excellens och för att du alltid ställer de allra mest tänkvärda frågorna, som gör att ens egna tankar ändrar riktning och nya ”fönster” öppnas.

Professor Eva Nissen, för allt Ditt stöd.

Mina medarbetare och medförfattare: Lena Vermin för din excellence vad gäller ultraljud med gott samarbete under långa kvällspass. Ulrika Smedh för att du ”räddade” blodproverna på andra sidan Atlanten och för ditt kunnande i hormonernas värld. Henrik Källberg för din excellence och närvaro när det gäller, och Ann-Sofi Gustafsson för ditt kunnande om dessa barns uttryck och rörelsemönster. Ulrika Lageblad och Lena Pettersson kodare av 174 timmar video film för givande arbete och tid för skratt mellan varven. Anna Lindh för god hjälp med data hantering.

Veronica Berggren för ditt kliniska kunnande och stöd i allt praktiskt. Kristina von Eicwald, Anne Belfrage Mårtensson, Wiwi Stefanicki nära medarbetare som höll ställningarna med insamling av data på tre sjukhus.

Susanne Abenius, chefssjuksköterska på KS NeoIVA, för ditt stora stöd och engagemang för att detta skulle kunna gå att genomföra. Och Pia Lindberg min nuvarande chef för att du inte slutat att tro på mig.

Susanne Åkerström och Kristina Sandberg för givande samtal och diskussioner på Danderyd. Vill också tacka alla ni barnsköterskor och sjuksköterskor som fungerade som kontaktpersoner på KS DS och HS och höll total koll på alla som kunde tänkas lottas in till projektet, ingen nämnd ingen glömd Stort Tack.

Mina duktiga språkgranskare, Pauline Binder och Sheila MacDonald.

Speciellt tack till Barbro Hedman och Charlotte Ovesen för praktiskt stöd under vägen – ni är ovärderliga.

Nuvarande och tidigare doktorandkollegor forskare på enheten för Reproduktiv hälsa, Karolinska Institutet, för trevlig samvaro och stöd, Ann Marie Widström, Ulla Waldenström, Emma Fransson, Anna Hjelmstedt, Cecilia Ekéus, Ann Olsson, Louise Seymyr, Mia Ahlberg, Annika Samsio, Björn Salomonsson, Anette Kaplan, Christina Westerberg, Iris Ronnevi, Sabine Blixt, Malin Bergström och Yuki Takahashi.

Ett speciellt varmt tack till min rumskamrat och mina kära vänner, Malin Söderberg Marianne Velandia, Kristin Svensson, Wibke Jonas, Eva Andersson, Sofia Zwedberg,

Ett riktigt varmt tack till mina trogna vänner och vapendragare Kristina och Lennart Bergström som finns till i alla väder värme, god mat och inte att förglömma, Malin Martin och Mattias.

Mina systrar Åsa och Lis och Mor Karin för att ni alltid finns därute.

Jag spar det bästa till sist. Tack mina älskade, numer vuxna barn, Daniel David, och Emelie och Anna och Petter för att ni finns, och för att mina barnbarn Leva och Jesper finns och håller mig vid Liv.

Denna avhandling har understötts med fondmedel från Karolinska Institutet; Fonden Mjölkdroppen; Jerringfonden; Fonden Solstickan; Vårdalstiftelsen; Frimurare barnhuset; Odd Fellow Södertörn.

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