IN-HOSPITAL GROWTH OF VERY LOW BIRTH WEIGHT PRETERM INFANTS: COMPARATIVE EFFECTIVENESS OF TWO HUMAN MILK FORTIFIERS

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DECLARATION

I, Johanna Elizabeth Kemp, declare that the thesis is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution. This was confirmed by my Turnitin® submission (receipt added as an Annexure).

I also declare that I did not receive any sponsorship for the study. The MIRIS™ human milk analyser was provided through a loan agreement with the University of Pretoria and the company was not involved in the study design or data interpretation.

ETHICS STATEMENT

I, Johanna Elizabeth Kemp, obtained the applicable research ethics approval for the research described in this work: University of Pretoria, Faculty of Health Sciences Research Ethics Committee: Reference no 286/2017 and amended on 28/09/2017; University of the Witwatersrand, Human Research Ethical Committee (Medical): Clearance certificate no M170546 and amended on 27/07/2017. I declare that I observed the ethical standards required in terms of the University of Pretoria's code of ethics for researchers and the policy guidelines for responsible research.

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SIGNED BY JE KEMP: DATE:

SUMMARY

The protein content of the only human milk fortifier available in South Africa was increased in 2017. The Original fortifier (OF) and the Reformulated fortifier (RF) provided similar energy. This study aimed to prospectively compare in-hospital growth during the intermediate stage of nutrition support of very low birth weight (VLBW) preterm infants receiving human milk fortified with these two formulations in a tertiary South African hospital. Intake of VLBW infants receiving exclusively human milk plus one of two fortifiers (OF 0.2gprotein/g powder; RF 0.4gprotein/g powder) was calculated. Change in Z-scores (Fenton, 2013) from start to end of fortification of weight, length and head circumference (HC) for age was calculated as primary outcomes. Additionally, weight gain velocity (g/kg/d) and gain in length and HC (cm/wk) were calculated. Fifty eight infants (52% female; gestational age: 30±2wk; birth weight: 1215±187g) received OF (2016 to 2017) and 59 infants (56% female; gestational age: 29±2wk; birth weight 1202±167g) received RF (2017 to 2018) for 15 days. Protein intake of RF (3.7±0.4g/kg/d) was significantly higher ($p<0.001$) than of OF (3.4±0.2g/kg/d). Protein-to-energy ratio of RF (2.6±0.2) was significantly higher ($p<0.001$) than of OF (2.3±0.1g/100kcal). No adverse effects were noted. In both groups Z-scores of weight and length dropped; Z-scores for HC showed slight improvements. There were no significant differences between the two groups in terms of Z-scores, weight gain velocity, length gain or HC gain. Analysed human milk from preterm infants' mothers' protein levels was higher than published values. In-hospital growth was not statistically different between groups, even though calculated protein intake and protein-to-energy ratio were significantly higher in RF group.

Key terms: Preterm infant, growth, human milk, fortifier

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LIST OF ABBREVIATIONS

AAP	American Academy of Pediatrics
AGA	Appropriate-for-gestational age
BMI	Body mass index
BUN	Blood urea nitrogen
CI	Confidence Interval
CHBAH	Chris Hani Baragwanath Academic Hospital
cm/wk	Centimetre per week
DBM	Donor breast milk
EBM	Expressed breast milk
ELBW	Extreme low birth weight
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
g	Gram
GA	Gestational age
g/kg/d	Gram per kilogram body weight per day
HC	Head circumference
HCFA	Head circumference-for-age
HCFAZ	Head circumference-for-age Z-score
HMA	Human milk analysis/analyser
HIV	Human immunodeficiency virus
IV	Intravenous
kcal	Kilocalorie
kcal/kg/d	Kilocalorie per kilogram body weight per day
kg	Kilogram

kJ	Kilojoule
KMC	Kangaroo mother care
LBW	Low birth weight
LFA	Length-for-age
LFAZ	Length-for-age Z-score
LGA	Large for gestational age
mL	Millilitre
mL/kg/d	Millilitre per kilogram body weight per day
mmol/L	Millimole per litre
MUAC	Mid-upper arm circumference
N	Nitrogen
NCR	Nutrition care record
NEC	Necrotising enterocolitis
NICU	Neonatal intensive care unit
NPO	Nil per os
OF	Original fortifier
PMA	Postmenstrual age
PN	Parenteral nutrition
RF	Reformulated fortifier
SABR	South African Breast Milk Reserve
SD	Standard deviation
SGA	Small for gestational age
VLBW	Very low birth weight
WHO	World Health Organization
WFA	Weight-for-age

WFAZ	Weight-for-age Z-score
USA	United States of America

CHAPTER 1:
BACKGROUND TO THE STUDY

1.1 Introduction

In South Africa eight out of every 100 babies are born prematurely.[1] Despite many advances in the nutrition care of preterm infants, poor in-hospital growth and extra-uterine growth restriction remain a problem in South Africa[2,3], as in other countries.[4-8] Poor growth in these infants can be attributed to many different factors, including exposure to the human immune deficiency virus (HIV)[7,9,10-12] and may affect their later growth and development.[13-15] Fortification of human milk, the feed of choice for preterm infants, is one of the strategies implemented to improve preterm infants' nutrient intake.[6-18] The growth of preterm infants receiving fortified human milk in low- and middle-income countries has been under-researched. This study subsequently compared the in-hospital growth of very low birth weight (VLBW) preterm infants receiving exclusive human milk fortified with two different human milk fortifiers. The next section expounds the rationale for the study.

1.2 Rationale for the study

For preterm infants, an external source of nutrients becomes important soon after birth as they are born with poor nutrient reserves coupled with high nutrient needs for growth and development. Adequate delivery of nutrients forms an integral part of their treatment, but due to many challenges associated with feeding these infants, optimal delivery is often not achieved. Nutrient delivery through parenteral and/or enteral routes should be commenced soon after birth, but it is often delayed due to the infant's medical condition and complications related to preterm birth. Once enteral feeding has been started, it may take some time to reach recommended volumes of intake due to, among other factors, the infant's immature gastrointestinal tract. Even if the recommended volume of intake is achieved, nutrient intake may still be inadequate when human milk is given.[17,19-21]

Nutrition support of preterm infants can be divided into three stages, namely the acute stage (early aggressive nutrition during the first days to weeks of life); the intermediate stage when infants are advanced to full enteral feeding and growth is the main objective; and the post-discharge stage. During all of these stages, human milk plays an important role, with trophic

feeding of expressed breast milk (EBM) and colostrum during the acute stage; fortification of EBM during the intermediate stage; and breastfeeding with possible continued fortification after discharge from hospital.[22,23] Human milk is the feed of choice for all infants, including preterm infants.[16,24] The advantages of human milk, especially if the infant's own mother's milk is used, are numerous and include a reduction in the incidence of necrotising enterocolitis (NEC), late-onset sepsis and retinopathy, better feeding tolerance and improved neurodevelopmental outcomes.[14,17-20,24] An important limitation is, however, that human milk does not meet the nutritional requirements of most preterm infants.[17,18,25,26] This is especially a problem among extreme low birth weight (ELBW) and VLBW infants, in small for gestational age (SGA) infants, and in those with fluid restrictions and co-morbidities that increase nutrient requirements.[9, 17-20]

Different interventions have been proposed to overcome the challenge of inadequate nutrient delivery by human milk. These include using the mother's own milk (unpasteurised) rather than donor milk (which usually comes from mothers who gave birth at term); increasing the volume of milk; using more hind milk than foremilk (which has a lower nutrient density); and fortification.[17,19,25] Fortification with commercially manufactured human milk fortifiers is now considered standard practice in most neonatal units in South Africa.[27] Although standard fortification – that is the addition of fortifier in amounts per volume as prescribed by the manufacturer – increases nutrient intake, it may still not meet the protein requirements of the very small, very immature infant. Any shortfall in protein supply is considered to be growth-limiting.[17,19,28]

Two South African studies[2,3] where fortification was done with a human milk fortifier[3] and a preterm formula[2] reported on growth in preterm infants. In a cohort of VLBW preterm infants in Johannesburg, South Africa, MacKay et al[2] found a high rate of early growth failure. In this study, corrected age was used, and weight, length and head circumference were evaluated based on Z-scores. In the study by Lang et al[3] in ELBW infants, in-hospital weight gain was 14(±2.9) gram/kilogram body weight/day (g/kg/d), which when compared to current recommendations[29,30] would not be adequate. Unfortunately, neither of these studies reported on the infants' nutrient intakes. It is difficult to compare and evaluate results from these studies since different fortification strategies were used, human milk was not given exclusively, and

different growth indices were reported. Furthermore, these studies used retrospective data ranging from 2006 to 2010, and the treatment of preterm infants may have improved since then.

At the time of the present study only one fortifier was commercially available in South Africa, namely FM85 (Nestlé, South Africa).[31] It contained extensively hydrolysed cow's milk protein in a powdered form and the addition thereof to human milk was done in most neonatal units. Using an old formulation of FM85[31] (available until March 2017), it was difficult to meet the protein requirements of preterm infants, especially those with a VLBW and ELBW. The composition of FM85[32] changed (available from April 2017), with a higher protein content being one of the most important changes. Other changes in the new formulation[32] included a lower carbohydrate but higher fat content and a change in protein hydrolysis from extensively hydrolysed to partially hydrolysed. Once added to EBM, the two formulations yielded similar energy per millilitre (mL) of human milk.[31,32] With the new formulation, protein requirements should theoretically be met and better in-hospital growth be achieved. It was therefore important to prospectively assess the growth of preterm infants on the new formulation[32] (Reformulated fortifier - RF) and to compare it to growth on the old formulation[31] (Original fortifier - OF). The study was conducted in the current neonatal environment in preterm infants fed exclusive human milk using growth indices as recommended by consensus literature.[18,33] The next section delineates the aim and objectives of the study.

1.3 Aim and objectives

1.3.1 Aim

The aim of this study was to compare the in-hospital growth of VLBW preterm infants receiving exclusive human milk fortified with two different formulations during the intermediate stage of nutritional support in the CHBAH, Gauteng, South Africa.

1.3.2 Objectives

The aim formulated above gives rise to the following objectives, namely to do the above comparison in terms of:

1.) **Primary objective:** the difference in the changes in Z-scores from initiation of fortification to exit for the following indices:

- Weight: Weight for age Z-score (WFAZ)
- Length: Length for age Z-score (LFAZ)
- Head circumference (HC): HC for age Z-score (HCFAZ)

2.) **Secondary objective:** the difference in anthropometric gains from initiation of fortification to exit for the following indices:

- Weight: Weight gain velocity in g/kg/d
- Length: Length gain in centimetres per week (cm/wk)
- HC: HC gain in cm/wk)

1.3.2.1 Supplementary objective

In addition to the objectives above, the study also aimed to determine the energy and macronutrient content of the breast milk of mothers of preterm infants.

In order to achieve the aim and objectives, the researcher worked with a number of assumptions and delimitations. These follow in the next section.

1.4 Delimitations and assumptions

1.4.1 Delimitations

- The study took place in a single tertiary hospital in urban South Africa which can be considered a resource-limited setting.
- The focus was on in-hospital growth during the intermediate stage of nutrition support and not on growth during the acute stage of nutrition support.
- The focus was on intake of enteral protein, energy and fluid, and not on parenteral intake. The study did not focus on micronutrient intake.
- The study did not include infants who received formula milk exclusively or those who were partially fed human milk (so called “mixed feeders”).

- Growth was expressed in terms of weight, length and HC indices and body composition was not measured.

1.4.2 Assumptions

It was assumed that:

- birth data, for example gestational age (GA), were accurate;
- birth anthropometric measurements were taken and recorded accurately;
- intake and output data were reported accurately in the nursing files;
- information included in the medical files and dietitians' records (nutrition care records (NCRs) (Annexure 1)) was accurately described;
- nutritional composition of the fortifiers used in the study as indicated on product information sheets accurately reflected the content;
- screening and referral procedures to start fortification were the same for the two groups;
- environmental factors such as room temperature, positioning and care of infants were the same across groups; and
- any possible difference in growth between groups could reasonably be ascribed to protein intake.

1.5 Conceptualisation and operationalisation

Table 1 explains how key terms were conceptualised and operationalised for the purpose of this study. The terms are grouped together based on their meaning.

Table 1: Conceptualisation and operationalisation of key terms

Key term	Conceptualisation	Operationalisation (a * indicates that it was the same as conceptualisation)
Preterm birth	Born before 37 completed weeks of gestation.[34]	*
Gestational age (GA)	Age of the infant at birth as determined by the length of the pregnancy. This could be calculated using the number of weeks since the last menstrual period, a clinical assessment (e.g. Ballard scale), or be determined by ultra-sound.[34]	The GA as indicated in the infant's medical notes was used. This GA was based on the neonatologist's clinical judgement and may or may not have included Ballard scale estimation.
Postmenstrual age (PMA)	The time that has elapsed from the first day of the last menstrual period (GA at birth) plus the time that has elapsed after birth (chronological age) described as a number of weeks.[34]	The time described as GA at birth plus the time that has elapsed after birth described as a number of weeks and days.
Small for gestational age (SGA)	Birth weight for GA less than the 10th percentile on a foetal-infant growth chart.[36]	Fenton 2013[35] growth chart was used.
Appropriate for gestational age (AGA)	Birth weight for GA between the 10th and the 90th percentile on a foetal-infant growth chart.[36]	Fenton 2013[35] growth chart was used.
Large for gestational age (LGA)	Birth weight for GA above the 90th percentile on a foetal-infant growth chart.[36]	Fenton 2013[35] growth chart was used.
Low birth weight (LBW)	Birth weight below 2500g.[34]	*
Very low birth weight (VLBW)	Birth weight below 1500g.[34]	*
Extreme low birth weight (ELBW)	Birth weight below 1000g.[34]	*
Preterm growth chart	Foetal-infant growth chart for preterm infants.[35]	Fenton 2013[35]growth chart was used.
Growth index	Combination of two or more growth measurements e.g. Body mass index (BMI).	Single growth measurement e.g. weight, length and HC

Key term	Conceptualisation	Operationalisation (a * indicates that it was the same as conceptualisation)
		and Growth indices e.g. BMI, weight gain in g/kg/d
Anthropometric gains	Changes in growth indices over specified time periods.	For weight: weight gain velocity expressed as g/kg/d. For length and HC: weekly gains expressed as cm/wk.
In-hospital growth	Growth (i.e. change over time) during hospitalisation in terms of weight, length and HC.[35]	Growth during intermediate stage of nutrition support. Primary indicator for weight: weight-for-age reported as change (end minus beginning) in WFAZ on Fenton 2013[35] growth chart. Secondary indicator for weight: g/kg/d. Primary indicator for length: length-for-age reported as change (end minus beginning) in LFAZ on Fenton 2013[35] growth chart. Secondary indicator for length: cm/wk. Primary indicator for HC: HC-for-age reported as change (end minus beginning) in HCFAZ on Fenton 2013[35] growth chart. Secondary indicator for HC: cm/wk.
Acute stage of nutrition support in preterm infants	Early aggressive nutrition including parenteral nutrition and trophic feeding of EBM during the first weeks of life.[22]	*
Intermediate stage of nutrition support in preterm infants	Stage when growth is the main objective and fortified EBM or preterm formula is given.[22]	Referring to human milk only. From the first day of fortification of human milk until the infant weighed 1.65 kilogram (kg) (the discharge weight at CHBAH at that point in time) *or* was discharged from

Key term	Conceptualisation	Operationalisation (a * indicates that it was the same as conceptualisation)
		hospital/transferred to another hospital *or* was taking ≥ 50% of feeds directly from the breast *or* was changed to formula feeds (whichever occurred first).
Bolus feeds	Feeds given at specific time intervals.[37]	Feeds that were given at two- or three-hourly intervals with a cup, a syringe or a feeding tube.
Continuous feeds	Feeds given hourly, usually via a feeding pump.[37]	Any feeds (hourly or two hourly) that were given via a feeding pump or syringe pump.
Human milk/Breast milk (terms to be used synonymously)	Own mother's breast milk and donor milk.	*
Donor milk	Human breast milk donated to a donor milk bank.	Human breast milk donated to the South African Breast Milk Reserve (SABR) used in CHBAH.
Preterm milk	Human milk from mothers who delivered prematurely (up to a certain PMA).	Human milk from mothers who delivered prematurely: up to day 14 of life; containing 1.5g protein and 65kcal energy per 100mL.[33]
Mature milk	Human milk from mothers who delivered at term.	Human milk from mothers who delivered prematurely: form 15 of life onwards; containing 1.2g protein and 72kcal energy per 100mL.[33]
Human milk fortifier	Multi-component fortifier specifically designed to be added to human milk in order to meet the nutritional requirements of preterm and LBW infants.[17]	FM85 powder (Nestlé South Africa): The only product that was available in South Africa on state tender at the time of the study.
Original fortifier (OF)	-	Product that was used in CHBAH until March 2017: "Old' FM85 powder (Nestlé South Africa): containing 0.2gram (g) protein in 1g of powder.[31]
Reformulated fortifier (RF)	-	Product that was used in CHBAH from April 2017: "New" FM85 powder (Nestlé South Africa):

Key term	Conceptualisation	Operationalisation (a * indicates that it was the same as conceptualisation)
		containing 0.4g protein in 1g of powder.[32]
Standard fortification	Fortification of human milk according to the manufacturer's instructions.[17]	For OF: 1g fortifier added to 20mL human milk.[31] For RF: 1g fortifier added to 25mL human milk.[32]
Energy calculation	-	Energy presented in kilocalories (kcal) and 4.2 factor used for conversion between kilojoules (kJ) and kcal. Energy presented as total energy (thus energy form protein included). Enteral protein and energy considered as 100% bioavailable. Factors used for calculation of energy form enteral and parenteral nutrition: Protein and carbohydrate: 4kcal/g Fat: 9kcal/g
Feeding tolerance	Tolerance of enteral feeding as observed by the absence of vomiting, abnormal gastric residual volumes, abdominal distension and abnormal stool output.[37]	Vomiting recorded as episodes per day. Abnormal gastric residuals referring to aspirates that were more than 50% of the volume that was fed or bilious or haemorrhagic aspirates.[37] Abdominal distension as was clinically diagnosed by the attending doctor. Abnormal stool output referring to watery or bloody stools; recorded as episodes per day.
Nutrition care record (NCR)	Gauteng Department of Health document for dietitians to document the nutritional care of patients. These included medical and background information, anthropometric and biochemical	*

Key term	Conceptualisation	Operationalisation (a * indicates that it was the same as conceptualisation)
	data, clinical and dietary assessments, nutrition care plans and progress notes. (Annexure 1)	

The next chapter reviews the available literature on growth in preterm infants receiving fortified human milk.

CHAPTER 2:
LITERATURE STUDY

This chapter builds on the rationale for the study that was presented in Chapter 1. The **intermediate stage of nutrition care** is discussed in terms of: **Fortification of human milk** (2.1: Published article and 2.2 Macronutrient content of human milk) in order to meet **enteral macronutrient requirements** (2.3) to promote **in-hospital growth** (2.4). The emphasis of the literature study is on protein and energy intake and on in-hospital growth as an assessment of the adequacy of this intake, and not on fat and carbohydrate per se. Biochemical markers used in the assessment of protein intake is also discussed briefly (2.5)

2.1 Fortification of human milk: Published article

A review article on human milk fortification was published by the researcher in a peer-reviewed journal: Kemp JE, Wenhold FAM. Human milk fortification strategies for improved in-hospital growth of preterm infants. S Afr J Clin Nutr 2016;29(4):157-64. At the time of the publication of the article the OF ("old" FM85) was the only commercially available fortifier in South Africa. A copy of the article is presented on pages 11 to 19.

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REVIEW ARTICLE

Human milk fortification strategies for improved in-hospital growth of preterm infants

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Human milk is the preferred feed for preterm infants, yet it may need to be fortified for optimal growth and development. Standard fortification of human milk seldom meets the recommended intake of protein, leading to inadequate post-natal growth. This article aims to critically review different human milk fortification strategies with a focus on in-hospital growth of premature infants in resource-limited settings. Super, adjustable and target fortification are compared to standard fortification. Different growth outcome parameters limit comparability of findings, but super fortification and adjustable fortification present opportunities to explore. More uniform growth outcome assessment is recommended. Practical implementation and cost-effectiveness in the local setting need to be investigated.

Keywords: fortification, human milk, preterm infant

Introduction

In South Africa, eight out of every 100 babies are born prematurely.[1] Despite many advances in the nutritional care of preterm infants, poor in-hospital growth and extra-uterine growth restriction (EUGR) remain a problem in industrialised and developing countries.[2–4] In a cohort of very low birth weight (VLBW) preterm infants in Johannesburg, South Africa, a high rate of early growth failure was shown.[5] Human milk is the feed of choice for all infants,[6] yet it should be fortified to meet the nutritional requirements of preterm infants, especially the very small, very immature infant.[7,8] Standard fortification of human milk, that is the addition of fortifier in amounts per volume as specified by the manufacturer, rarely meets the recommended intake of protein, and any shortfall in protein supply is not only growth limiting, but may carry the risk of neurocognitive impairment.[8–10] This article proposes to offer an integrative review and critical analysis of fortification strategies of human milk for improved in-hospital growth of preterm infants. In particular, the emphasis is on alternatives to standard fortification. Additionally, practical challenges and implications for resource-limited settings such as South Africa are discussed, so as to inform practitioners of the current state of evidence-based neonatal nutrition care.

In this article the term human milk is used synonymously with breast milk and refers to mother's own milk and banked donor milk. Multicomponent human milk fortifiers specifically designed for use in low birth weight and preterm infants are under discussion, while fortification refers to the addition thereof to human milk.

Human milk

The advantages of human milk to premature infants are numerous, especially if the infant's own mother's milk is used. The benefits which are dependent on both the dose and the duration of breastfeeding, include the reduction in the incidence of necrotising enterocolitis (NEC), late-onset sepsis and retinopathy, better feeding tolerance and improved neurodevelopmental outcomes.[7,8] The benefits can be attributed to nutritional and non-nutritional factors in human milk, such as bioactive, growth and immunological factors. The composition of human milk is dynamic and does not only vary from mother to mother, but also from feed to feed and within a feed. The nutrients in human milk originate from synthesis in the lactocyte, from maternal stores and from her dietary intake. Despite variations in maternal intake and nutritional status, the nutritional quality of human milk is remarkably conserved. Mature human milk (from mothers who delivered at term) contains approximately 65 to 70 kcal (273 to 294 kJ), 0.9 to 1.2 g protein, 3.2 to 3.6 g fat and 6.7 to 7.8 g carbohydrates per 100 ml.[11] The biggest variations in macronutrient content occur in the fat component, with hind milk having higher concentrations of fat than foremilk. Furthermore, milk from mothers who have delivered prematurely (preterm milk) differs from mature milk. These differences include higher protein, free amino acids, fat and sodium concentrations but lower concentrations of calcium compared to mature milk. These differences are, however, only seen in the first few weeks of life. Levels of protein, fat and sodium decline over time until they are similar to those seen in mature milk.[7,11,12]

Challenges in the use of human milk for the premature infant include the availability of mother's own milk, sustainability of expressing milk when infants are not feeding on the breast, the effect of pasteurisation on the nutritional and immunological content of donor milk, and transmission of viruses, including human immunodeficiency virus. The most important challenge is probably that unfortified human milk does not meet the nutritional requirements of most preterm infants.[7,13] This is particularly problematic in those born before 34 weeks gestational age; infants with a birth weight of less than 1800 g; those who are small for their gestational age (SGA); infants with fluid restrictions; and, those with co-morbidities that increase nutrient requirements.[7,9] To illustrate the above, the protein and energy requirements of a 1 kg infant are compared to the nutritional content of mature human milk at volumes typically prescribed for preterm infants. As can be seen from Table 1, human milk at the lower fluid intake of 150 ml/kg body weight/day does not meet protein or energy requirements as recommended by the American Academy of Paediatrics (AAP)[14]

Table 1: Enteral protein and energy requirements of a 1 kg preterm infant compared to the nutritional content of unfortified and fortified mature human milk

Enteral protein and energy requirements				Nutritional content					
				Human milk, unfortified (11)			Human milk, standard fortified (1 g FM85/20 ml milk) (11,17)		
				Milk volume (ml)			Milk volume (ml)		
Nutrient	Unit	AAP (14)	ESPGHAN (15)	150	180	200	150	180	200
Protein (g/day)		3.4 to 4.2	3.5 to 4.0	1.4 to 1.8	1.6 to 2.2	1.8 to 2.4	2.9 to 3.3	3.4 to 4.0	3.8 to 4.4
Energy	kcal/day	110 to 130	110 to 135	98 to 105	117 to 126	130 to 140	124 to 131	149 to 158	165 to 175
	kJ/day*	462 to 546	462 to 567	412 to 441	491 to 529	546 to 588	521 to 550	626 to 664	693 to 735
Protein:energy ratio	g/100 kcal	2.6 to 3.8	3.2 to 3.6	1.3** to 1.8*** (1.6****)			2.2** to 2.7*** (2.4****)		
	g/100 kJ	0.6 to 0.9	0.8 to 1.0	0.3** to 0.4*** (0.37****)			0.5** to 0.6*** (0.6****)		

*4.2 kJ/kcal used in conversion.
**Lowest protein and highest energy used in calculation.
***Highest protein and lowest energy used in calculation.
****Mid-values of protein and energy used in calculation.

and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN).[15] This poses a particular problem in infants who cannot tolerate large volumes of milk and in those with fluid restrictions. At higher fluid intake, energy requirements can be met by mature human milk, but protein stays below the recommendation, even at the highest volume.

The listed challenges are far outnumbered by the advantages of using human milk. Different interventions have been proposed for overcoming the challenge of inadequate nutrient delivery by human milk. These include using mother's own milk (unpasteurised) rather than donor milk (which usually comes from mothers who gave birth at term); increasing the volume of milk; using more hind milk than foremilk; and, fortification.[7,8,12] In resource-poor settings where human milk fortifiers are not available, circumstantial evidence even proposes the addition of skim milk powder.[8] To the authors' knowledge (and confirmed by personal communication with Ziegler on 26/02/2015[8]), there are no published reports on the use of skim milk powder as fortifier, and it may not supply sufficient trace minerals. Therefore, use of skim milk powder can currently not be recommended as an alternative in a country where fortifier is commercially available.

Human milk fortification strategies

Fortification of expressed breast milk (EBM) can be done by using modular components (for example, adding a protein supplement) or by using commercially available fortifier designed specifically for use in low birth weight infants. The use of modular supplements poses many challenges, including accurate measurement of the minute amounts needed, especially if the patient is bolus fed. A further potential problem is the increased osmolality of the human milk.[16] Even though the addition of modular components may aid in meeting the preterm infant's macronutrient requirements, the micronutrient composition thereof does not "complement" that of human milk, carrying the risk of either overfeeding or underfeeding of micronutrients.

The use of human milk fortifiers is now considered standard practice in most neonatal units. Fortifiers can either be bovine or human milk based, in powder or liquid form, and may contain hydrolysed or intact protein. In South Africa, there is only one commercially available fortifier, namely FM85 (Nestle, South Africa),[17] which contains extensively hydrolysed cow's milk protein in powdered form. The nutritional analysis of FM85 used in this article was correct at the time of going to press.

Standard fortification

Standard fortification (the addition of fortifier in amounts per volume as prescribed by the manufacturer) usually starts once the intake of EBM reaches 100 ml/kg body weight/day.[8,13] As an empirical dose of nutrients is added with this type of fortification, it does not always match the nutritional needs of the individual infant. In Table 1, the nutritional requirements of a 1 kg infant are compared to different volumes of human milk fortified with FM85 at the standard dosage of 1 g/20 ml EBM. Compared to recommendations by AAP[14] and ESPGHAN,[15] energy supply will be sufficient at an intake of 150 ml/kg body weight, but it will exceed recommendations at higher volumes. In contrast, protein supply will only be adequate at volumes of 180 ml/kg body weight and higher. Protein intake of 4.5 g/kg body weight/day as recommended by ESPGHAN[15] for extremely low birth weight infants (ELBW) (recommendation not shown in Table 1), will not be met, even at an intake of 200 ml/kg body weight. Even though protein requirements of infants weighing more than 1 kg can theoretically be met at high volumes, it is rarely achievable in practice. Furthermore, the high energy intake to be given in order to meet protein requirements is controversial, as excessive energy may be stored as adipose tissue.[15] To counteract the problem of providing too much energy relative to the amount of protein, the protein to energy ratio should be considered. As can be seen from Table 1, the ratio of protein to energy recommended by ESPGHAN[15] is neither met with human milk alone, nor with the standard addition of fortifier.

Arslanoglu *et al.*[10] and Corvaglia *et al.*[18] measured actual nutrient content of human milk including standard fortification. Both groups reported protein levels below the recommended 3.5 to 4.0 g/kg bodyweight/day at intakes of 150 ml/kg body weight/day. A Cochrane review in 2004 on multicomponent fortifiers, recommended 'the evaluation of both short-term and long-term outcomes in search of the "optimal" composition of fortifiers',[19] implying that follow-up research should focus on alternatives to standard fortification so as to increase protein intake. We hence conducted a literature search in April 2015 (CINAHL, MEDLINE Ovid without revisions, Web of Science) for studies on human milk fortification published in the English language since 2004. Table 2 summarises all studies identified which met the following criteria: single-intervention studies; exclusive use of human milk (thus no preterm formula); comparison of alternative fortification strategies to standard fortification; and, in-hospital growth as a primary outcome. The table does not include studies where fortified milk was compared to unfortified milk or those

comparing different types of fortifiers (for example, liquid versus powder). The studies summarised in Table 2 are discussed under the different fortification strategies: super, adjustable and target fortification.

Super fortification

Super fortification (also called blind fortification) involves the addition of greater than standard amounts of fortifier, for example adding the standard dosage to a lower volume of milk than that recommended by the manufacturer. This alternative is a relatively simple approach and, apart from the extra amount of fortifier needed, it does not imply any additional costs or manpower for example, for the nutritional analysis of milk samples. Higher protein delivery can be achieved, but additional energy and micronutrients are also provided. This fortification strategy may therefore not change the protein to energy ratio sufficiently to promote gain in lean body mass. Hypercalcaemia may be a risk and testing serum calcium and serum phosphorous more regularly should be considered.[8]

Kanmaz *et al.*[20] (Table 2) reported two levels of blind fortification (moderate and aggressive) compared to standard fortification in a group of ELBW and VLBW infants with a gestational age of about 28 weeks. Moderate and aggressive fortification led to non-significant increases in weight and length, but head circumference increased significantly. The lack of significant increases in weight and length can possibly be explained by the estimated protein intake of only 3.3 to 3.6 g/kg body weight/day in the intervention groups, which would not be considered adequate for preterm infants with a birth weight of around 1000 g.[14,15] This is supported by the fact that the serum urea levels did not increase. It is not clear from the article what energy intake was estimated to be, but the protein to energy ratio might provide some additional explanation.

Individualised fortification: Adjustable fortification

Adjustable fortification refers to a more customised method of fortification where the metabolic response of the infant is used to guide the stepwise addition of extra protein. This extra protein is usually added in the form of a modular protein supplement and is done "on top of" the addition of standard amounts of fortifier. Blood urea nitrogen (BUN) values, which have been shown to correlate closely to enteral protein intake in infants, guide the amount of additional protein needed.[8,13,21]

Alan *et al.*[22] (Table 2) compared adjustable fortification, using an additional protein supplement, to standard fortification in preterm infants fed exclusively with their own mother's milk. The estimated median amount of daily protein intake in the intervention group of 4 g/kg body weight/day (range: 3.4 - 4.6) was within the AAP[14] and ESPGHAN[15] recommendations and significantly higher than the intake in the control group. The estimated protein to energy ratio in the intervention group was 3.3 g/100 kcal which also fall within the recommended ranges. Statistically significant increases in daily growth indices for weight, length and head circumference, as well as in length and head circumference gain velocities, were seen in the intervention group. It is important to note that these results were achieved without adjustment in volume or energy intake. The median daily volume intake in both groups was about 140 ml/kg body weight/day, making this type of fortification strategy suitable for fluid restricted preterm infants. In a similar study by Biasini *et al.*[23] (Table 2), the estimated protein intake of 4.8 g/kg body weight/day in the adjustable fortification group was higher than in the study by Alan *et al.*,[22] but the protein to energy ratio was comparable at 3.4 g/100 kcal. In the latter study, however, statistically significant increases were only reported in head circumference and length, and only in a sub-group analysis of ELBW infants. It should be kept in mind that in both studies, nutritional content of fortified milk was estimated and not measured. Furthermore, in the study by Biasini *et al.*,[23] 40% of milk was donor milk, which may have had a lower nutritional content than preterm mother's own milk.

In a randomised controlled trial by Arslanoglu *et al.*[24] (Table 2), an additional fortifier in addition to the protein supplement were added based on twice weekly BUN levels. Infants received mother's own milk as well as banked donor milk. Protein content of fortified milk, which in this study was analysed and not estimated as in the aforementioned studies, was significantly higher in the intervention group. Protein intake, but not fat or energy intake, was significantly correlated with weight gain (g/kg body weight/day) and head circumference gain (mm/day), both of which were significantly higher in the intervention group than in the standard fortification group. Even though linear growth was also somewhat faster in the intervention group, it did not reach statistical significance when compared to the standard fortification group.

Individualised fortification: Target fortification

Target fortification is tailored to the individual preterm infant's needs by analysis of maternal milk before fortification. Maternal and/or donor milk is usually analysed with infrared spectroscopy equipment that provides qualitative (macronutrients) and quantitative information of a milk sample as small as 5 mL.[8,13,19] Creamatocrit analysis can also be used. In a study by Rochow *et al.*[25] (Table 2) individualised fortification was done using a stepwise approach, starting with determining the nutrient content in pooled human milk followed by standard fortification. The last step involved the addition of monomeric supplements to reach target levels of protein, fat and carbohydrate. The target levels for macronutrients were defined based on the ESPGHAN[15] recommendations and assumed an intake of 150 mL/kg body weight/day. Weight gain in the individual fortification group was similar to infants receiving standard fortification, but feeding volume was significantly higher in the latter group and could have influenced the results. A linear relationship between milk intake and weight gain was only demonstrated in the individual fortification group.

A different approach to target fortification was reported by Hair *et al.*[26] (Table 2) where fat was the only macronutrient added in addition to standard fortification. In this study a human milk-derived fortifier and a human milk cream supplement were used to provide an exclusive human milk-based diet. In the individual fortification group, human milk cream was added to increase energy to 20 kcal/oz (20 kcal/28 mL). Compared to the standard fortification group, this group had significant increases in weight and length, but not in head circumference. Unfortunately, the level of protein and the total volume of milk consumed are not clear, making comparisons with other studies difficult.

Adverse effects of fortification

The standard addition of fortifier to human milk appears to be generally safe and well-tolerated by most infants. According to a Cochrane review[20] on multicomponent fortification of human

Table 2: Outcomes of alternative human milk fortification intervention strategies

Alternative fortification strategy	Study		Intervention				Outcomes in terms of in-hospital growth		Other outcomes, including adverse effects	Reference
	Design	Sample	Initiation of standard fortification	Initiation of alternative fortification	Volume and type of milk	Type of fortifier and supplement	Growth parameter	p-value		
Super-fortification	Randomised controlled trial:	*n* = 84	**When volume of intake at:**	**When volume of intake at:**	**Full volume (ml/kg/d):**	**Fortifier:**	W gain (g/d)	0.38	**Feeding tolerance:**	[20]
			90 to 100 ml/kg/d	150–170 mL/kg	SF: 155 ± 4.6				NS differences in feeding tolerance, residuals, abdominal distension, frequency of stooling	
			GA (weeks): SF: 31	**Day of life:**	MF: 154 ± 6		W gain (g/kg/d)	0.24		
		GA ≤32wk	MF: 30.5	MF: 12	AG: 156 ± 6.9				1 Patient in MF group developed NEC	
	Moderate (MF) and Aggressive fortification (AG) compared to Standard fortification (SF)		AG: 30.5 (p = 0.18)	AG: 10	(p = 0.59)	Eoprotin (Milupa, Germany) (Cow's milk based)	L at discharge (cm)	0.85	**Biochemistry:**	
			W (g):	**Duration:**	**Type:**				NS differences in S-urea, S- calcium, S-phosphorous, S-ALP	
		BW ≤1500 g	SF: 1106 MF: 1066 AG: 1097 (p = 0.73)	Until discharge from hospital	Human milk (no indication if donor milk was used)		HC (cm/wk)	0.001	Blood gas within normal range; no metabolic acidosis	
Adjustable fortification (AF)	Prospective observational intervention:	*n* = 58	**When volume of intake at:**	**When volume of intake at:**	**Median volume (ml/kg/d):**	**Fortifier:**	W velocity (g/kg/d)	0.053	**Feeding tolerance:**	[22]
			80 ml/kg/d	not clear from article	SF: 141 (90–160)		L velocity (mm/d)	0.008		
	SF plus additional protein supplement (based on weekly S-BUN levels) compared to SF (Historical control group)	**GA** ≤32wk	**Median age:**	**Day of life:**	AF: 143.5 (125 –163)	Aptamil Eoprotin (Milupa, Germany) (Cow's milk based)	HC velocity (mm/d)	<0.001	NS differences in "feeding interruption" (abdominal distention and/or GRV > 50% and/or vomiting)	
			Day of life: 8 (for SF and AF)	17	(p = 0.135)		Daily growth index for W (%)	0.026		
		BW ≤1500 g		**Mean W (g):**	**Type:**	**Protein supplement:**	Daily growth index for L (%)	0.027	**Clinical outcome:**	
				1501 (±252)	Exclusively fed	Protifar (Nutricia, Netherlands)	Daily growth index for HC (%)	0.003	Similar between groups: NEC, BPD, ROP requiring laser treatment	
				Duration:	mother's own milk		**Subgroup analysis of GA ≤ 28wk:**			
				At least two weeks (median duration 21d)			W velocity (g/kg/d)	0.192		
							L velocity (mm/d)	0.04		
							HC velocity (mm/d)	0.004		

(Continued)

Table 2: (*Continued*)

Alternative fortification strategy	Study		Intervention				Outcomes in terms of in-hospital growth		Other outcomes, including adverse effects	Reference
	Design	Sample	Initiation of standard fortification	Initiation of alternative fortification	Volume and type of milk	Type of fortifier and supplement	Growth parameter	p-value		
							Daily growth index for W (%)	0.09		
							Daily growth index for L (%)	0.053		
							Daily growth index for HC (%)	0.027		
Adjustable fortification	Randomised controlled trial:		**When volume of intake at:**	**When volume of intake at:**	**Full volume:**	**Fortifier:**	W gain (g/d)	< 0.01	**Feeding tolerance:**	[24]
		n = 32	90 ml/kg/d	150 ml/kg/d	150 to 160 ml/kg/d	FM85 (Nestle, Italy)	W gain (g/kg/d)	< 0.01	NS differences in feeding intolerance as defined by: emesis, withholding of feeds, abdominal distention	
	Fortifier and additional protein supplement (based on twice-weekly S-BUN levels) compared to SF			**Day of life:**			L gain (mm/d)	> 0.05	No study infant had NEC or systemic infection	
		GA ≤34wk		19		**Protein supplement:**			**Biochemistry:**	
		BW ≤1700 g		**Duration:**	**Type:**	Pro-Mix (Corpak Medsystems, USA)	HC gain (mm/d)	<0.05	S-albumin, S-creatinine and S-calcium: did not change significantly	
				Until W of 2000 g (at least 14 days)	Own mother's milk or banked donor milk				S-BUN, S-phosphorous, S-ALP: NS increased	
Adjustable fortification	Randomized controlled trial:	***n*** = 61	**When volume of intake at:**	**When volume of intake at:**	**Prescribed volume of intake:**	**Fortifier:**	W gain (g/kg/d)	NS	**Feeding tolerance:**	[25]
				Full enteral feeding	160 ml/kg/d	Aptamil			No information given	
		GA ≤32wk					L gain (cm/wk)	NS		
				Duration:	**Type:**	**Protein supplement:**	HC gain (cm/wk)	NS	**Biochemistry:**	
	Fortifier and additional protein supplement (based on S-BUN level) compared to SF		Full enteral feeding				**In ELBW sub-group (W 580–980 g; GA 23–30wk):**		Significantly higher S-urea levels	
		BW 580 to 1250 g		Until discharge or transfer to other hospital or when >50% of milk taken directly from breast	Own mother's milk and banked donor milk	Protifar (Nutricia, Netherlands)	W gain (g/kg/d)	0.05	NS lower pH levels	
							Length gain (cm/wk)	0.04	Metabolic acidosis and increased S- creatinine: not more than previously seen	
							HC gain (cm/wk)	0.02		

(Continued)

Table 2: (*Continued*)

Alternative fortification strategy	Study		Intervention				Outcomes in terms of in-hospital growth		Other outcomes, including adverse effects	Reference
	Design	Sample	Initiation of standard fortification	Initiation of alternative fortification	Volume and type of milk	Type of fortifier and supplement	Growth parameter	p-value		
Target fortification (TF)	Prospective clinical trial:	***n*** = 10 (plus 20 for matched-pairs)	**When volume of intake at:**	**When volume of intake at:**	**Feeding volume:**	**Fortifier:**	W gain similar between groups but feeding volume in SF group significantly higher than in IF group ($p < 0.001$)		**Feeding tolerance:**	[25]
		GA <32w		Step-wise introduction over a 3 day period, full amount of target fort on day 4	147 ± 5 ml/kg/d (TF)	Similac (Abbott Nutrition, USA)			No feeding intolerance seen (GRV > 50% previous feeding volume; emesis; abdominal distention; decrease/delay/discontinuation of feeds)	
		BW <1500 g			155 ± 5 mL/kg (SF)	**Supplements:**				
	Fortifier plus additional protein, fat and carbohydrate supplements (based on human milk analysis) compared to SF (matched-paired groups of infants in the same neonatal unit)	***n*** = 10 (plus 20 for matched-pairs)	Not indicated	Volume of intake not indicated		**Protein:** Beneprotein (Nestle Health Care Nutrition, USA)			**Biochemistry:**	
		GA <32w		**Day of life:** 30		**Fat:** Microlipid (Nestle HealthCare Nutrition, USA)	Linear relationship between milk intake and wt gain seen in IF group but not in SF group		S-TG, S- BUN, S- protein, S-albumin and glucose all within normal ranges .No metabolic acidosis seen	
		BW <1500 g		**Duration:** Minimum of 3 consecutive weeks	**Type:** Own mother's milk	**Carbohydrate:** Polycose (Abbott Nutrition, USA)				
Target fortification	Prospective randomised trial:	***n*** = 78	**When volume of intake at:** 100 ml/kg/day or sooner	**When volume of intake at:** Once standard fortified feeds tolerated	**Feeding volume:**	**Fortifier:**	W velocity (g/kg/d)	0.03		[26]
							L velocity (cm/wk)	0.02	No cases of NEC or death reported	
		GA **SF** 27.7 ± 2.1 **TF** 27.6 ± 1.6 (p = 0.88)		**Day of life:** Not indicated	Not indicated	Prolact+H²MF (Prolacta Bioscience, USA)	HC (cm/wk)	0.21		
	Fortifier plus additional human milk cream supplement (based on human milk analysis) compared to SF	**BW** 750 to 1250 g		**Duration:** Until 36 weeks PMA or when weaned from fortification	**Type:** Own mother's milk and pasteurised donor milk	**Supplement:** **Fat:** Prolact CR (Prolacta Bioscience, USA)	W velocity from time BW regained (g/d)	0.02	NS in number of sepsis episodes	
							W velocity from time BW regained (g/kg/d)	0.02		
							L velocity from birth (cm/wk)	0.01		
							HC from birth (cm/wk)	0.58		

Notes: AF: adjustable fortification, ALP: alkaline phosphatase, BPD: bronchopulmonary dysplasia, BUN: blood urea nitrogen, BW: birth weight, ELBW: extremely low birth weight, GA: gestational age, GRV: gastric residual volume, HC: head circumference, L: length. *n*: sample size, NEC: necrotising enterocolitis, NS: non-significant, PMA: postmenstrual age, ROP: retinopathy of prematurity, SF: standard fortification, TF: target fortification, TG: serum triglycerides, W: weight, wk: weeks.

milk, it does not appear to be associated with adverse effects, even though the limited total sample size and missing data threaten the generalisability. As expected, increased enteral protein intake may increase blood urea levels and decrease blood pH levels, but the clinical significance thereof is unclear.[20]

In the studies summarised in Table 2, adverse effects of the alternative fortification strategies were mostly reported in terms of feeding intolerance and in changes in biochemical markers. No study reported significant differences in feeding intolerance, usually defined as abdominal distention, vomiting, abnormal gastric residuals and feeding interruption. Alan *et al.*[22], Arslanoglu *et al.*[24] and Hair *et al.*[26] specified that no NEC was reported in the intervention groups in their respective studies; however, Kanmaz *et al.*[20] reported NEC in one patient in the moderate fortification group. With the exception of increased serum urea levels in one study,[23] all changes in biochemical markers reported in the studies in Table 2, were not statistically significant. Kanmaz *et al.*,[20] Biasini *et al.*[23] and Rochow *et al.*[25] are the only studies that reported on the incidence of metabolic acidosis, which were either not seen or did not occur more than prior to fortification.

A study by Moltu *et al.*,[27] on the other hand, was discontinued due to an increase in late-onset septicaemia and electrolyte disturbances in the intervention group. This disconcerting outcome needs further investigation. In this study, the intervention group received additional enteral amino acids, long chain polyunsaturated fatty acids and vitamin A in addition to standard fortification. The multi-component nature of the study, which also included different types and amounts of total parenteral nutrition and preterm formula, limits conclusions with regards to the fortification strategy per se. Furthermore, the estimated enteral energy intake of 166 kcal/kg body weight/day in the intervention group far exceeded the recommendations of both ESPGHAN[15] and AAP.[14]

Conclusion and recommendations

Different strategies have been proposed to improve in-hospital growth in preterm infants fed human milk. The studies cited in Table 2, where these strategies were compared to standard fortification, were comparable in terms of inclusion and exclusion criteria, the gestational age of the infants and the use of exclusive human milk. They differed in terms of birth weight of the participants, timing of standard fortification, total volume of human milk received, duration of study and type of fortifier and modular supplements used. Despite this heterogeneity, it seems noteworthy that the most promising results were seen in terms of improved growth in head circumference[20,22–24] and length[22,23,26], and primarily in the smaller, more immature[22,23] preterm infants. The significance of this needs to be investigated further because, firstly, head circumference and length may be indicators of growth in lean body mass and, secondly, the smaller, more immature preterm infants are also the most vulnerable to impaired neurocognitive development.

An important difference between these studies relates to the parameters in which in-hospital growth was reported, ranging from growth in units/body weight/day to growth indices and velocities. This makes comparisons between the studies difficult and for future research uniformity in this regard should be aimed at. In this regard the recently published proceedings of a Consensus Development Conference, may be a useful starting point. They stated that "...the aim of postnatal growth is not to lose more than 1 SDS [standard deviation] in weight and head circumference from birth to discharge".[28] This recommendation implies a preference for growth indices that are expressed in terms of Z-scores.

A further recommendation by the aforementioned Consensus Development Conference[28] is that standard fortification should be initiated for all infants with a birth weight of less than 1800 g and, if this does not lead to appropriate growth, individualised fortification (target or adjustable) should be considered. For application in a resource-poor setting like South Africa, a lower birth weight of 1500 g may be considered as the cut-off for standard fortification, as this is the weight recommended by other authors, including the AAP.[6] In this regard, neonatal practitioners in South Africa should reach consensus as well.

For preterm infants where standard fortification does not lead to sufficient in-hospital growth, adjustable and super fortification may be strategies to consider. Due to the high cost and manpower needed for the implementation of target fortification, it would not be a suitable option in a resource-limited setting. Super fortification is currently practised in some units in South Africa where the amount of additional fortifier is based on theoretical calculations of the nutrient content of breast milk. These calculations should be tested against the measured nutrient content of milk from South African mothers of preterm infants. The effect on in-hospital growth should be evaluated as well, as the protein content may not be increased sufficiently given the current composition of FM85. The focus should be on attaining the recommended protein to energy ratio. Since serum urea levels are tested routinely in preterm infants in South African hospitals, adjustable fortification could be implemented if appropriate protocols are set in place. Such protocols should be designed taking into consideration the current status of neonatal units where overcrowding and insufficient staffing are often a reality. Essential to any fortification strategy should be the promotion of the use of breast milk, especially mother's own milk for preterm infants.

Declaration of conflict of interest: No conflict of interest. Currently there is no link between the authors and the manufacturers (Nestle, South Africa) of the fortifier referred to in the article. The manufacturers were also not involved in any stage of the conceptualisation or writing of the article. On two occasions, JEK was sponsored by the NNIA to attend NNIA workshops.

Supplementary information

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References

1. Blencowe H, Cousens S, Oestergaard MZ et al. National, regional and worldwide estimates of preterm birth rates in the year 2010 with time trends for selected countries since 1990: a systematic analysis and implications. Estimates for World Health Organization; 2012.
2. Clark R, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. Pediatrics. 2003;111:986–90. http://dx.doi.org/10.1542/peds.111.5.986
3. Cooke R, Ainswoth S, Fenton A. Postnatal growth retardation: a universal problem in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2004;89:F428–F430. http://dx.doi.org/10.1136/adc.2001.004044
4. Mukhopadhyay K, Mahajan R, Louis D, et al. Longitudinal growth of very low birth weight neonates during first year of life and risk factors for malnutrition in a developing country. Acta Paediatr. 2013;102:278–81. http://dx.doi.org/10.1111/apa.2013.102.issue-3
5. Mackay CA, Ballot DE, Cooper PA. Growth of a cohort of very low birth weight infants in Johannesburg, South Africa. BMC Pediatr. 2011;11:504. http://dx.doi.org/10.1186/1471-2431-11-50

6. Eidelman AI, Schanler RJ. Breastfeeding and the use of human milk. Pediatrics. 2011;129(3):600–3.
7. Underwood MA. Human milk for the premature infant. Pediatr Clin North Am. 2013;60(1):189–207. http://dx.doi.org/10.1016/j.pcl.2012.09.008
8. Ziegler EE. Meeting the nutritional needs of the low-birth-weight infant. Ann Nutr Metab. 2011;58(s1):8–18. http://dx.doi.org/10.1159/000323381
9. Corpeleijn WE, Vermeulen MJ, van den Akker CH, et al. Feeding very-low-birth-weight infants: our aspirations versus the reality in practice. Ann Nutr Metab. 2011;58(s1):20–9. http://dx.doi.org/10.1159/000323384
10. Arslanoglu S, Moro GE, Ziegler EE. Preterm infants fed fortified human milk receive less protein than they need. J Perinatol. 2009;29:489–92. http://dx.doi.org/10.1038/jp.2009.50
11. Ballard O, Morrow AL. Human milk composition. Pediatr Clin North Am. 2013;60(1):49–74. http://dx.doi.org/10.1016/j.pcl.2012.10.002
12. Kim JA, Chan CS, Vaucher YE, et al. Challenges in the practice of human milk nutrition in the neonatal intensive care unit. Early Hum Dev. 2013;89:S25–S38.
13. Di Natale C, Coclite E, Di Ventura L, et al. Fortification of maternal milk for preterm infants. J Matern Fetal Neonatal Med. 2011;24(sup1):41–3. http://dx.doi.org/10.3109/14767058.2011.607569
14. American Academy of Pediatrics Committee on Nutrition. Nutritional needs of the preterm infant. In: Kleinman RE, Greer FR, editors. Pediatric nutrition. 7th ed. Elk Grove Village (IL): American Academy of Pediatrics; 2014. p. 83–121.
15. Agostoni C, Buonocore G, Carnielli VP, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2010;50(1):85–91. http://dx.doi.org/10.1097/MPG.0b013e3181adaee0
16. Fenton TR, Belik J. Routine handling of milk fed to preterm infants can significantly increase osmolality. J Pediatr Gastroenterol Nutr. 2002;35:298–302. http://dx.doi.org/10.1097/00005176-200209000-00011
17. FM85 Product information leaflet for healthcare professionals. Nestle South Africa.
18. Corvaglia L, Aceti A, Paoletti V, et al. Standard fortification of preterm human milk fails to meet recommended protein intake: bedside evaluation by near-infrared-reflectance-analysis. Early Hum Dev. 2010;86:237–40. http://dx.doi.org/10.1016/j.earlhumdev.2010.04.001
19. Kuschel CA, Harding JE. Multicomponent fortified human milk for promoting growth in preterm infants. Cochrane Database Syst Rev. 2004;(1):CD000343.
20. Kanmaz HG, Mutlu B, Canpolat FE, et al. Human milk fortification with differing amounts of fortifier and its association with growth and metabolic response in preterm infants. J Hum Lact. 2012;29(3):400–5.
21. Roggero P, Gianni ML, Morlacchi L, et al. Blood urea nitrogen concentrations in low-birth-weight preterm infants during parenteral and enteral nutrition. J Pediatr Gastroenterol Nutr. 2010;51:213–5. http://dx.doi.org/10.1097/MPG.0b013e3181cd270f
22. Alan S, Atasay B, Cakir U, et al. An intention to achieve better postnatal in-hospital-growth for preterm infants: adjustable protein fortification of human milk. Early Hum Dev. 2013;89:1017–23. http://dx.doi.org/10.1016/j.earlhumdev.2013.08.015
23. Biasini A, Marvulli L, Neri E, et al. Growth and neurological outcome in ELBW preterms fed with human milk and extra-protein supplementation as routine practice: do we need further evidence? J Matern Fetal Neonatal Med. 2012;25(S4):72–4.
24. Arslanoglu S, Moro GE, Ziegler EE. Adjustable fortification of human milk fed to preterm infants: does it make a difference? J Perinatol. 2006;614–21. http://dx.doi.org/10.1038/sj.jp.7211571
25. Rochow N, Fusch G, Choi A, et al. Target fortification of breast milk with fat, protein, and carbohydrates for preterm infants. J Pediatr. 2013;163:1001–7. http://dx.doi.org/10.1016/j.jpeds.2013.04.052
26. Hair AB, Blanco CL, Moreira AG, et al. Randomized trial of human milk cream as a supplement to standard fortification of an exclusive human milk-based diet in infants 750-1250 g birth weight. J Pediatr. 2014;165:915–20. http://dx.doi.org/10.1016/j.jpeds.2014.07.005
27. Moltu SJ, Blakstad EW, Strømmen K, et al. Enhanced feeding and diminished postnatal growth failure in very-low-birth-weight infants. J Pediatr Gastroenterol Nutr. 2014;58:344–51. http://dx.doi.org/10.1097/MPG.0000000000000220
28. Moro GE, Arslanoglu S, Bertino E, et al. Human milk in feeding premature infants. Proceedings of a Consensus Development Conference-EXPO 2015, May 15–16, Milan, Italy. J Pediatr Gastroenterol Nutr. 2015;61(S1):S1–S19. http://dx.doi.org/10.1097/MPG.0000000000000897

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Supplementary information

Studies excluded from table 2

STUDY	RATIONALE FOR EXCLUSION
Berseth CL, Harris CL, Wampler JL, Hoffman DR, Dierse-Schade DA. Liquid human milk fortifier significantly improves docosahexaenoic and arachidonic acid status in preterm infants. Prostaglandins Leukot Essent Fatty Acids 2014; 97-103.	Comparison of two types of fortifiers In-hospital growth not reported
Corvaglia L, Aceti A, Paoletti V et al. Standard fortification of preterm human milk fails to meet recommended protein intake: bedside evaluation by near-infrared-reflectance-analysis. Early Hum Dev 2010; 86: 237-240.	Comparison of two types of fortifiers at standard and lower levels of fortification In-hospital growth not reported
De Halleux V, Rigo J. Variability in human milk composition: benefit of individualized fortification in very-low-birth-weight infants. Am J Clin Nutr 2013: 98(Suppl): 529S-535S.	In-hospital growth not reported
Maas C, Wiechers C, Bernhard W, Poets C, Franz AR. Early feeding of fortified breast milk and in-hospital-growth in very premature infants: a prospective cohort analysis. BMC Pediatr 2013; 13: 178-801.	Preterm infant formula used
Martins EC, Krebs VL. Effects of the use of fortified raw maternal milk on very low birth weight infants. J Pediatr (Rio J) 2009; 85(2): 157-162.	Fortified milk compared to unfortified milk
Moltu SJ, Blakstad EW, Strommen K et al. Enhanced feeding and diminished postnatal growth failure in very-low-birth-weight infants. J Pediatr Gastroenterol Nutr 2014; 58: 344-351.	Multi-component intervention Premature infant formula used in certain cases
Mukhopadhyay K, Mahajan R, Louis D, Narang A. Longitudinal growth of very low birth weight neonates during the first year of life and the risk factors for malnutrition in a developing country. Acta Paediatr 2013; 102: 278-281.	Fortified milk compared to unfortified milk
Olsen IE, Harris CL, Lawson ML, Berseth CL. Higher protein intake improves length, not weight, z scores in preterm infants. J Pediatr Gastroenterol Nutr 2014; 58: 409-416.	Comparison of two types of fortifiers Multi-component intervention
Reali A, Greco F, Fannaro S et al. Fortification of maternal milk for very low birth weight (VLBW) pre-term neonates. Early Hum Dev 2010; 86: S33-S36.	Only preliminary data presented
Sparks BB, Radmacher PG, Lewis SL, Serke LA, Adamkin DH. Human milk analysis contributes to nutritional management of very low birth weight infants. Infant, Child, & Adolescent Nutrition 2014; 6(5): 295-300.	Comparison of two types of fortifiers
Thoene M, Hanson C, Lyden E, Laura D, Ruybal L, Anderson-Berry A. Comparison of the effect of two human milk fortifiers on clinical outcomes in premature infants. Nutrients 2014; 6: 261-275.	Comparison of two types of fortifiers

The values referred to in the preceding article for the macronutrient content of human milk[38] need further exploration in light of three recently published systematic reviews.[39,40,41] This follows in the next section.

2.2 Macronutrient content of human milk

The protein, fat and carbohydrate content of human milk are largely assumed when human milk is fortified. The only exception is target fortification where addition of fortifier and macronutrient supplements are individualised based on the analysed content of human milk (refer to 2.1 for fortification strategies). Even though the nutritional quantity of human milk is remarkably conserved, there may be variations from mother to mother, from day to day, from feed to feed and within a feed. Differences in the milk of mothers of preterm infants in comparison to those who delivered at term have also been shown.[38,39,40] Factors that may affect the macronutrient content of analysed human milk are presented in Table 2. All of these factors should be taken into consideration when appraising studies on human milk analysis.

Table 2: Factors that may affect the macronutrient content of analysed human milk

Innate factors		**Methodological factors**	
Maternal factors	**Infant factors**	**Sampling**	**Analysis**
• Age [38,40] • Parity [39] • Hormonal changes post-delivery[42] • Return to menses [38] • Volume of milk produced [38] • Diet [38,39,43] • Infection [39] • Smoking [44] • BMI/Weight-for-height [38,44]	• GA [38, 41] • PMA[38,39,41] • Nursing frequency [38,44]	• Day versus night versus 24-hour sample[38] • Hind milk versus foremilk and inclusion of colostrum[38] • Hand versus pump expression[45]	• Storage and pasteurisation of milk before analysis[38] • Type of chemical analysis[38,41] • Measured versus calculated values for protein, energy[38,41]

Abbreviations: BMI Body mass index; GA gestational age; PMA postmenstrual age

Three recent systematic reviews[39,40,41] (including two meta-analyses) reported on the macronutrient and energy content of human milk. In all three reviews, studies were only included if analysis was done on 24-hour milk samples. The protein and energy content of the human milk studied in these three reviews are summarised in Tables 3 and 4 respectively. As can be seen from Table 3 protein content of human milk declines over time with values as high as 2.7g/100mL in preterm milk/colostrum in the first few days of life. Energy content (Table 4), which is lower in the first few days of life, seems to vary more. This may be due to differences in the fat content, the most variable macronutrient component of human milk.[38,39,40,41] The question arises as to which of these values to use when calculating preterm infants' nutritional intake. When quantifying the intake of infants receiving standard or adjustable fortification, an estimation of the nutrient content of human milk is needed. In order to standardise reporting of neonatal research, Cormack et al[33] recommended using preterm milk values (1.5g protein and 65kcal per 100mL) up to day fourteen of life and mature milk values (1.2g protein and 72kcal per 100mL) from day 15 onwards. Cormack et al[33] note that "although the precise nutritional content of the breast milk administered in each baby in each study is unknown, the use of standardized figures for breast milk composition would improve the comparability of studies and the likelihood of finding optimal protein and energy intakes for preterm babies".

For target fortification, "bedside" analysis of human milk is required, and this is usually done by infrared spectroscopy. It generally falls into two types, namely near-infrared and mid-infrared. Mid-infrared spectroscopy is the certified method for milk macronutrient analysis. There are two commercially available human milk analysers (HMA), Calais™ (Bedford Heights, United States of America (USA)) and MIRIS™ (Uppsala, Sweden). Both of these devices have been tested against laboratory methods and were found to be suitable for clinical use.[46]

In the next section the enteral macronutrient and energy requirements for preterm infants are discussed.

Table 3: Protein content of human milk from mothers of preterm infants

Systematic review/Meta-analysis	Method	Unit	Protein content of human milk from mothers of preterm infants (g/100mL)						
			Day 1-3	Day 4-7	Week 2	Week 3-4	Week 5-6	Week 7-9	Week 10-12
Mimouni et al[40]	Calculated and measured	mean±SD	2.57±1.44	2.11±0.44	1.98±0.68	1.6±0.5	1.43±0.25	1.34±0.2	1.26±0.2
Gidrewicz and Fenton[41]	Measured	mean±SD	2.7±1.5	1.7±0.5	1.5±0.4	1.4±0.4	1.1±0.2	1.1±0.2	1.0±0.2
			Week 1			Week 2-8			
Boyce et al[39]	Calculated	mean/median	1.9/1.88			1.27/1.24			

Abbreviations: g/100mL gram per 100 millilitres; SD standard deviation

Table 4: Energy content of human milk from mothers of preterm infants

Systematic review/Meta-analysis	Method	Unit	Energy content of human milk from mothers of preterm infants (kcal/100mL)						
			Day 1-3	Day 4-7	Week 2	Week 3-4	Week 5-6	Week 7-9	Week 10-12
Mimouni et al[40]	Calculated and measured	mean±SD	58.8±7.91	67.9±14.1	69.1±10.1	70.87±9.34	73.97±9.1	74.24±8.77	74.53±8.71
Gidrewicz and Fenton[41]	Calculated	mean±SD		65±13	70±14	68±8	67±6.9	66±8.9	66±14
	Measured	mean±SD	49±7	71±9	71±12	77±8	70±5	76±8	
			Week 1			Week 2-8			
Boyce et al[39]	Calculated	mean/median	57.11/-			65.5/65.7			

Abbreviations: kcal/100mL kilocalorie per 100 millilitres; SD standard deviation

2.3 The enteral macronutrient and energy requirements of preterm infants

The nutrient recommendations for most nutrients for preterm infants are based on accretion rates of protein, fat and minerals derived from analysis of foetal body composition at various stages of gestation. If these requirements are met, the infant should be able to grow at the same rate as it would have in utero.[17] As most placental transfer of nutrients takes place during the last trimester of pregnancy, the foetus would have been growing rapidly during that time. A very high intake of energy, macronutrients and micronutrients is therefore needed in preterm infants to mimic this growth. The energy and nutrient deficits that preterm infants are born with increase as GA and birthweight decrease.[20] According to Harding et al[20], a 24-week-old preterm infant would have to double its birth weight by 30 weeks PMA and increase it by more than five times at 40 weeks PMA to match foetal growth. In practice, this is seldom achieved[2-8] and the cumulative deficits experienced by preterm infants after birth are described by Corpeleijn et al[21] as something that "lies in wait" and is hard to recover from. Corpeleijn et al[21] further describe preterm birth as a nutritional emergency and warns that, when left untreated, it will have serious detrimental consequences for the short- and long term. It is therefore important to prioritise feeding as an important part of the medical treatment of these infants.

The fact that foetal growth is seldom achieved in preterm infants adds to the debate about whether foetal body composition and in utero growth should be used to establish nutrient requirements and extra-uterine growth rates. However, since poor in-hospital growth is associated with poor outcomes[13-15], the current consensus is to aim for intrauterine growth rates *and* to obtain a functional outcome comparable to that of infants born at term.[18,21] In order to achieve this, enteral macronutrient recommendations have recently been updated by several scientific societies.[34,47,48] As can be seen from Table 5, the recommendations by the American Academy of Pediatrics (AAP)[34] and Tsang[49] are almost identical, as well as those by Koletzko[48] and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN).[47] Literature often refer to these recommendations, including recent reviews[22,33,29] and consensus development conferences.[18,50]

Protein and energy supply to preterm infants should not be viewed in isolation since a high protein-to-energy ratio is needed in preterm infants to approximate intrauterine growth. [48,51,52] Caution should be taken to not just increase energy at the cost of protein as excessive caloric

intake “will do to a preterm infant what it does to everyone – produces excess fat”.[51] Excessive weight gain in infancy, which may include preterm infants if excessive weight gain continues beyond six months to one year of age, has been associated with an increased risk of non-communicable diseases in later life. Harding et al[20] refer to a “trade-off” in preterm infants between the need for enhanced nutrition for brain growth and cognition versus the risk of metabolic and cardiovascular disease at a later stage in life. This makes the balance between protein and energy supply to preterm infants all the more important.

Even though fluid is not a nutrient, Table 5 also features fluid requirements, which for the enterally fed preterm infant could solely come from human or formula milk. In Section 2.1 (Table 1) nutrient intakes at typical volumes of intake of human milk (fortified and unfortified) in preterm infants were compared to the recommendations of the AAP[34] and ESPGHAN[47]. It is difficult to meet these recommendations with unfortified human milk, yet a good opportunity exists for catch-up growth during the intermediate stage of nutrition care when the infant is stable and on full feeds. This offers a unique opportunity for dietitians, as important members of the multi-disciplinary team,[53-55] to contribute to improving preterm infants’ nutrition care and growth.

The next section discusses in-hospital growth as a measure of the adequacy of nutrition care, specifically in terms of enteral protein and energy intake.

Table 5: Published enteral energy, macronutrient and fluid requirements of preterm infants

	AAP[34]		ESPGHAN[47]	Koletzko et al[48]	Tsang et al[49]	
	ELBW	**VLBW**			**ELBW**	**VLBW**
Energy, kcal/kg/d	130 -150	110 -130	110 - 135	110 - 130	130 - 150	110 - 130
Protein, g/kg/d	3.8 – 4.4	3.4 – 4.2	ELBW: 4 - 4.5g VLBW (up to1.8kg): 3.5 – 4.0g	3.5 – 4.5	3.8 – 4.4	3.4 – 4.2
Carbohydrate, g/kg/d	9.0 – 20.0	7.0 – 17.0	11.6 – 13.2	11.6 – 13.2	9.0 – 20.0	7.0 – 17.0
Fat, g/kg/d g/kg/d	6.2 – 8.4	5.3 – 7.2	4.8 – 6.6	4.8 – 6.6	6.2 – 8.4	5.3 – 7.2
Protein: Energy ratio, g/100kcal		2.6 – 3.8	3.2 – 3.6	3.2 – 3.6	-	-
Fluid, mL/kg/d	-	-	135 - 200	135 - 200	160-200	135-190

Abbreviations: AAP American Academy of Pediatrics; ELBW extreme low birth weight; ESPGHAN European Society for Paediatric Gastroenterology, Hepatology and Nutrition; g/kg/day gram per kilogram body weight per day; g/100kcal gram per 100 kilocalories; kcal/kg/d kilocalorie per kilogram body weight per day; mL/kg/day millilitre per kilogram body weight per day; VLBW very low birth weight

2.4 In-hospital growth

Growth, a sensitive indicator of postnatal health,[56] can be used to assess the adequacy of nutrition care in preterm infants. In-hospital growth is usually assessed by measuring weight, length and head circumference. Skinfold thickness, mid-upper arm circumference (MUAC) and other body dimensions can also be measured.[29,57] These measurements can, for example, be used to calculate the MUAC-to-HC ratio and estimate body fat percentage, and thus, add valuable information with respect to body composition. Where weight does not distinguish between growth in terms of fat and fat free components, there is an argument to be made for using these outside of the research domain, even in resource-limited settings.[57] However, even though skinfold and body dimension measurements are inexpensive and non-invasive, they may be time consuming and it should be kept in mind that the delicate skin of the preterm infant may be easily bruised, skinfold thickness is affected by hydration status, and that it needs a trained professional to be done accurately.[57] Therefore, for day-to-day care of preterm infants, especially in units with a high patient-to-staff ratio, weight, length and HC remain the currently preferred method to evaluate in-hospital growth.[18,29,33]

For the evaluation of weight, length and HC measurements, different indices, formats for reporting and growth charts are used. The "ideal" indicator to use, as well as the "ideal" growth reference or standard to compare it with, remains under debate. The AAP[12] recommends that after an initial period of weight loss, extra-uterine growth should approximate intrauterine growth. However, since the extra-uterine environment differs markedly from that experienced in-utero, it is questionable whether preterm infants can *and* should grow according to their foetal counterparts.[29,33,58] This question goes hand-in-hand with the question whether foetal accretion rates of protein, fat and minerals should be used to estimate nutritional requirements. If it is accepted that intrauterine growth is not the ideal, is there something that can be used as the "gold standard"? Even though there may not always be agreement on what this "gold standard" is, longitudinal monitoring and early identification of growth faltering should be prioritised.[30,59,60]

2.4.1 Growth indices

Weight, length and HC for GA are the growth indices usually reported in preterm infants. For these, accurate estimation of GA is needed, which may in some cases be problematic. Some

authors[36,61] suggest using weight-for-length, BMI (weight/length^2) and the Ponderal index (weight/length^3). Since these indices are used to assess body proportionality, Olsen[36] makes an appeal for including a weight-for-length index in addition to weight-for-age in routine growth monitoring in the NICU; an important notion to consider in light of the concerns regarding excess fat gain in preterm infants.[51] All of the aforementioned indices are, however, dependent on an accurate length measurement, which may affect the reliability. Where weight and HC measurements are relatively easy to obtain, the accurate measurement of preterm infants' length is often problematic in a clinical setting.[36,62] Pereira-da-Silva and Fusch[62] note that an inaccurate length measurement would be further exacerbated when used in the BMI (where it is squared) and Ponderal index (where it is cubed).

Other growth indices that can be used without the need for an accurate GA include units/body weight/day (e.g. changes in weight in g/kg/d) or units/week (e.g. changes in HC or length in cm/wk). These indices are relatively easy to use and to calculate, except for weight gain velocity (g/kg/d). In a recent review, Cormack et al[33] found three different methods used for this calculation, making comparisons among studies very difficult. In order to standardise reporting of neonatal growth outcomes, this review[33] recommends the use of the validated formula by Patel[63] for calculation of weight velocity: Growth velocity = $[1{,}000 \times \ln(W_n/W_1]/(D_n-D_1)$ where W=weight in grams; D=day; 1=beginning of time interval; n=end of time interval.

The following targets are suggested based on estimates of foetal growth and observed growth of preterm infants: weight gain of 15 to 20g/kg/d, length and HC gains of 1.1 to 1.4cm/wk and 0.9 to 1.1cm/wk, respectively.[17,29,30] Roelants and co-workers[64] recommend using the aforementioned weight targets only after the initial drop in weight has been regained. They found it to be an attainable goal in the first month of life. This corresponds with the AAP's[34] goal of approximating extra-uterine growth after the initial weight loss. However, these targets do not "match" growth evaluated by plotting on growth charts, and Fenton et al[65] show that they only fit current growth references for limited time periods. The Fenton group[65] conclude that 15 to 20g/kg/d can be seen as a reasonable goal for preterm infants from 23 to 36 weeks, but not beyond this age. Furthermore, they recommend that when weight gain velocity is used, it should be calculated over a time interval of five to seven days or more, but not for shorter periods.[66]

On the contrary, Pereira-da-Silva and Fusch[62] state that weight, length and HC gain velocities are more sensitive in identifying changes in growth than growth charts. Clark et al[30] warn against using these in isolation since they provide no frame of reference with respect to normal. They suggest that it should be combined with plotting on growth charts. This is echoed by Fenton et al[66], who note that even though weight gain velocities may have some clinical and research use, it does not provide an entire description of infant growth as plotting on growth charts does.

2.4.2 Growth charts

The growth charts most commonly used are intrauterine charts, which are constructed by plotting growth measurements at birth against GA, therefore describing observed foetal growth.[56,57] The first published birthweight growth charts by Usher and McLean were based on 300 Caucasian infants born between 1959 and 1963 in Canada. These were followed by Lubchenco et al[67] (data from 5635 Caucasian infants in the USA), who were the first to introduce the concept of birth size-for-gestational age classifications in order to identify infants at risk. Thereafter, different growth charts were constructed which include length and HC, and later on distinguished between sexes. In a systematic review in 2016, Neubauer[59] found more than 100 different publications describing neonatal anthropometric charts, and as recently as July 2019, new reference charts for singleton birth weight percentiles for the USA[68] were published. Most of the mentioned growth charts describe observed foetal growth and foetal size at birth. Infants born prematurely are smaller than those that remain in utero and therefore all birthweight curves calculated from the cross-sectional data of infants born prematurely are based on relatively growth-restricted infants. Another limitation in the creation of these charts relates to the accurate determination of GA.[29,56]

Customised birth weight charts have been published for some countries in an attempt to account for local characteristics. Adjustments were made for maternal size, ethnicity and other variables to improve the detection of intrauterine growth restriction. Their use has been debated for some time as they were not developed to assess postnatal longitudinal growth and, in some countries, were only available for weight and not for linear growth parameters (length and HC).[29,33] A Cochrane review[69] in 2011 concluded that there is not enough evidence to recommend these charts for clinical implementation. However, Neubauer[59] and Sankilampi[60]

argue that there is a strong case for genetic influences on growth, questioning whether "one size" growth chart "may fit all".

In an effort to overcome the limitations of growth references based on cross-sectional data and to have one global standard, the Preterm Postnatal Follow-up Study of the INTERGROWTH-21st Project was done. Growth standards were developed based on longitudinal data from "ideal" conditions similar to those for the World Health Organization (WHO) standards.[70] This was done by carefully selecting participants for which foetal growth and newborn size were measured using prescribed markers and standardised methods and equipment. The participants were selected from eight geographically defined urban populations in whom health and nutrition needs were met and adequate antenatal care was provided. This project allowed for international comparisons of newborn size from 33 to 42 weeks GA and gives the best possible answer to how babies should grow.[70-72] The INTERGROWTH-21st study growth standards are recommended for use in the "monitoring of postnatal growth in preterm babies, especially after 32 weeks' postmenstrual age".[70] The latter part is of importance as 80% of the study population were born at 34 weeks gestational age or later, and only 12 infants (6%) before 30 weeks of gestation. In addition to this, infants were only weighed at three time points in the first month of life and fortification was seen as "optional". Therefore, it is questionable whether these growth standards can currently be recommended for use in hospitalised preterm infants with a GA of less than or equal to 32 weeks[33], especially in those for whom human milk fortification is indicated. Sankilampi[60] comments that even though the INTERGROWTH-21st charts have some important advantages, they may not be as sensitive as population-based, genetically accustomed charts in the timely detection of deviations in growth.

Even though growth charts based on cross-sectional foetal growth data may not be the "ideal", they have the advantage when it comes to numbers of subjects. In a systematic review and meta-analysis, Fenton and Kim[35] (Fenton 2013) combined data from six large population-based surveys (representing almost four million births, of which more than 34 000 were born before 30 weeks of gestation) to revise and update previous charts by Babson and Benda, later revised as Fenton 2003. A further advantage of the Fenton 2013 charts[35] is that they are linked to the WHO post-term growth standard from birth (where it was smoothed to avoid the "dip" experienced just prior to term birth) to ten weeks post-term. In a study[59] comparing four

different growth references that found significant deviations in interpretation of postnatal growth (especially in terms of HC), the Fenton 2013[35] charts were recommended for use since they were "consistent with regard to the relationship of HC, length and weight and plausible in their temporal course". The Fenton 2013[35] charts are currently widely used internationally[56,59] as well as in South Africa and are recommended as "the best dataset currently available for babies born at moderately preterm or earlier gestations".[33]

2.4.3 Reporting format

To describe a preterm infant's growth rate precisely, the exact percentile or Z-score has to be obtained. For infants with a size outside of the normal range (thus below the 3rd or above the 97th percentile), Z-scores are considered superior to percentiles as a more precise value can be obtained. Serial Z-scores can be useful to assess growth over time and changes in Z-score rather than a single Z-score are therefore preferable to evaluate the effect of nutrition interventions. A negative Z-score change (when end point minus start is calculated) indicates a decline in growth, a zero change indicates stable growth and a positive value indicates an increase in growth. If change in Z-scores is used to report growth, it is important to define entry and exit points.[33,73]

To conclude on the anthropometric evaluation of in-hospital growth: it should be kept in mind that preterm birth is not a natural occurrence and that "an idealized population of preterm infants does not exist", [56] therefore the "ideal" way to evaluate growth may elude us. Newer developments, for example growth trajectory calculators[74,75] may provide answers of how healthy preterm infants can adjust their growth to postnatal life. Considering current evidence, the choice of growth indicator/chart is possibly less important than to monitor growth longitudinally in order to identify and address growth faltering timeously.[30,59,60]

2.5 Biochemical markers used in the assessment of protein intake

Laboratory tests can, to a much lesser extent than anthropometric measurements, be used in the assessment of nutrition care of the preterm infant. Many factors not related to nutrition can alter biochemical results and it should therefore be interpreted in context and with caution.[62,76] An example of biochemical results that should be interpreted with caution are the serum protein concentrations of albumin, transferrin, pre-albumin and retinol-binding protein.

These serum proteins, especially albumin, are often used to assess protein status, but are neither sensitive nor specific to patients' response to nutrition support.[57,62]

Conversely, blood urea nitrogen (BUN) is not a measure of protein status, but a reflection of protein intake as it is a by-product of protein degradation.[76,77] It has been shown to correlate closely with enteral (but not parenteral) protein intake in stable growing preterm infants with adequate hydration and normal renal function. In these infants, a low BUN (S-urea) level indicates inadequate protein intake, but an elevated BUN is more difficult to interpret. An elevated BUN may reflect the suboptimal use of amino acids for anabolism that may be due to an increased amino acid oxidation in the presence of insufficient energy intake or acute inflammation due to sepsis. [62,76-78]

When applying adjustable fortification, the metabolic response of the infant based on the BUN value is used in a stepwise approach to guide the addition of protein to human milk. (refer to 2.1 for a discussion on this) Arslanoglu et al[79] used a S-urea level of 3.2 to 5.0 mmoL/L as the norm for standard fortification with values of less than 3.2 mmol/L indicating that more protein is needed, and values more than 5 mmol/L indicating that protein intake can be decreased. Embleton and Van den Akker[77] recommend using S-urea values of less than 3 mmol/L as an indication that enteral protein intake is insufficient and to avoid further increase in protein with values of more than 10 mmol/L. The latter value of 10 mmoL/L is much higher than recommended by Arslanoglu et al,[79] and Embleton and Van Den Akker[77] concede that currently there is insufficient data to support such practices routinely. More research is needed in this regard since the use of the S-urea value may be an attainable measure to monitor protein intake in a country like South Africa.

This chapter reviewed the current literature on human milk fortification and the evaluation of the adequacy of protein and energy intake in preterm infants during the intermediate stage of nutrition support. In the next chapter this information will be applied in the choice of methods employed to meet the aim and objectives of the study.

CHAPTER 3:
METHODS

This chapter presents the methodological steps that were taken to achieve the aim and objectives that were outlined in Chapter 1.

3.1 Study design and setting

The study is a comparative effectiveness study reporting on the effectiveness of the new RF. Comparative effectiveness research refers to "the generation and synthesis of evidence that compares the benefits and harms of alternative methods to prevent, diagnose, treat and monitor a clinical condition, or to improve the delivery of care".[80] One important aspect of comparative effectiveness studies is the direct comparison of interventions in real-world settings that are typical of day-to-day care.[80,81] The purpose of this type of research is to "improve health outcomes by developing and disseminating evidence-based information to patients, clinicians, and other decision makers about which interventions are most effective for which patients under specific circumstances".[81]

This study primarily compared the benefits (in-hospital growth) and mentioned the harms (feeding intolerance, adverse effects) of two alternative methods (two different human milk fortifiers) in a real-world setting (CHBAH) to improve the delivery of care (nutrition support) to VLBW preterm infants. In order to directly compare the two human milk fortifiers, data prospectively collected on the OF (as part of routine nutrition care in the form of an audit by the researcher as an employee of CHBAH) were used relative to data prospectively collected on the RF. The study design is depicted in Figure 1.

The study took place in the neonatal unit of a tertiary academic hospital, CHBAH, in Gauteng South Africa. The 3 200-bed public hospital is on the periphery of Soweto and serves mostly lower income communities. A high patient load with limited physical and human resources characterise the setting. The study-setting is a 185-bedded unit that includes the Neonatal Intensive Care Unit (NICU), Transitional Intensive Care Unit and three neonatal wards, including a kangaroo mother care (KMC) unit. The KMC unit and the hospital's lodger facilities have a limited number of beds and therefore many of the preterm infants' mothers do not stay at the hospital overnight. The mothers not lodging at the hospital are encouraged to visit their infants and to take part in their daily care, which includes feeding them during the day.

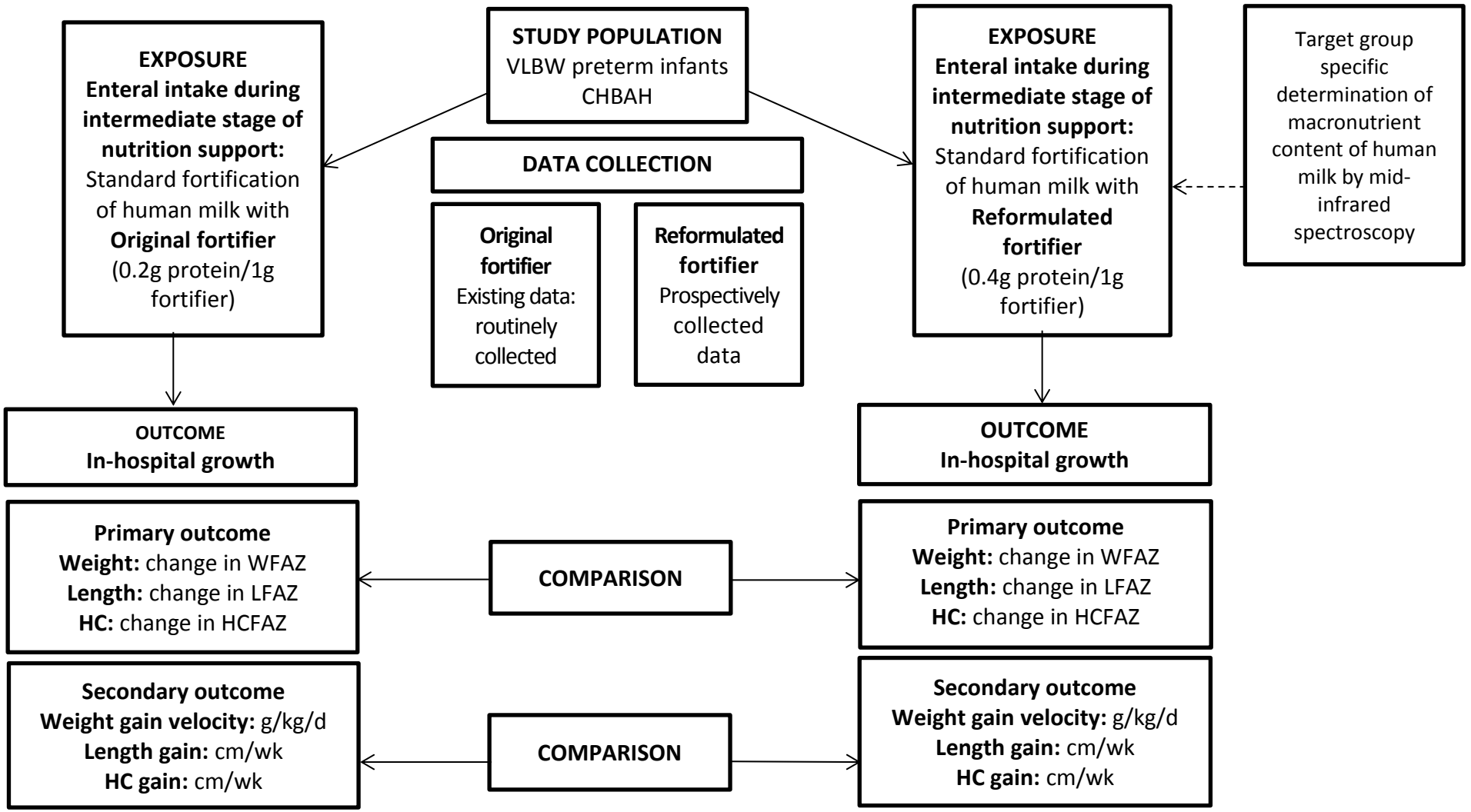


Figure 1: Study design

Abbreviations: CHBAH Chris Hani Baragwanath Academic Hospital; cm/wk centimetres per week; g gram; g/kg/d gram per kilogram bodyweight per day; HC head circumference; HCFAZ head circumference for age Z-score; LFAZ length for age Z-score; VLBW very low birth weight,; WFAZ weight for age Z-score

Three-hourly bolus feeds were administered at specific feeding times during the day (09:00, 12:00, 15:00; 18:00) and night (21:00, 24:00, 03:00, 06:00). Breast milk was manually expressed before each feeding time and additional milk was expressed and left in the ward's refrigerator for the night feeds. The hospital does not fully comply with the Baby Friendly Hospital Initiative but the ten steps are implemented as far as practically possible. In most instances where a mother did not leave sufficient milk for the night feeds or did not come in for the day, formula feeds were given. Formula feeds were prepared in one of two milk rooms, the maternity milk room or the general milk room, from where they were distributed to the wards. Infants receiving formula feeds were excluded from the study. CHBAH does not have its own donor milk bank and receives donor milk in limited quantities from the South African Breastmilk Reserve (SABR). Donor milk was not available at all times during the study and was used according to strict criteria, with only some of the VLBW infants qualifying for the first two weeks of life. Infants receiving donor milk were included in the study. At the time of the study, five dietitians, including the researcher, were working in the neonatal unit. In her capacity as a hospital dietitian, the researcher only saw infants who had gastrointestinal surgery/were awaiting surgery. These infants were excluded from the study (refer to Table 8).

Institutional birth statistics for the years 2016 to 2018 are depicted in Table 6.

Table 6: Annual birth statistics at the CHBAH for very low birth weight and preterm infants

		Number of live births per year at the CHBAH[a]		
		2016	**2017**	**2018**
According to birth weight, g	**< 500**	3	15	27
	500 - 999	268	321	397
	1000 - 1499	592	647	545
According to GA, weeks	**< 28**	260	260	307
	28 - 30	240	337	498
	31 - 34	935	1188	1445
	35 - 37	1122	1441	2445

[a] *Nakwa 24 July 2019 Personal communication*

Abbreviations: CHBAH Chris Hani Baragwanath Academic Hospital; GA gestational age

3.2 Study population and sampling

The study population consisted of all non-surgical VLBW preterm infants in the neonatal units of CHBAH who received exclusive human milk fortified with the OF from September 2016 to March 2017 and those receiving the RF from August 2017 until June 2018. During the time of the study there was only one commercially available human milk fortifier available in South Africa. The OF was available until early 2017 (in the CHBAH it was available until April 2017) after which it was discontinued and replaced by the RF. The most important difference between the two fortifiers is the higher protein content in the RF. Other changes in the RF include a lower carbohydrate but higher fat content and a change in protein hydrolysis from extensively to partially hydrolysed.[31,32] Refer to the Annexure 2 for the nutritional content of both fortifiers. An important difference between the fortifiers relates to its standard preparation: for the OF, 1g powder was added to 20mL human milk, whereas for the RF 1g powder is added to 25mL human milk. The two fortifiers could therefore not be compared on a gram to gram basis but could only be compared once fortifier had been added to human milk. Such a comparison is shown in Table 7.

Table 7: Protein and energy content of Original and Reformulated fortifiers

	Nutritional content per 1g of powder		Nutritional content per 100mL of fortified human milk[a]			
	Original fortifier [31]	Reformulated fortifier [32]	Preterm[b] human milk		Mature[c] human milk	
			Original fortifier	Reformulated fortifier	Original fortifier	Reformulated fortifier
Protein, g	0.2	0.4	2.5	3.1	2.2	2.8
Energy, kcal	3.5	4.4	82.5	82.6	87.5	87.6
Protein: Energy ratio, g/100kcal	-	-	3.0	3.8	2.5	3.2

[a]Standard fortification referring to 1g/20mL EBM (OF[31]) or 1g/25mL EBM (RF[32])

[b]Preterm human milk containing 1.5g protein and 65kcal per 100mL[33]

[c]Mature human milk containing 1.2g protein and 72kcal per 100mL[33]

Abbreviations: EBM expressed breast milk; g gram; kcal kilocalorie; kcal/100mL kilocalorie per 100 millilitres; mL millilitre; OF Original fortifier; RF Reformulated fortifier

3.2.1 Sample size calculation

During the initial planning of the research, sample size was calculated in the following manner: changes in Z-scores from entrance to exit from the study were considered a function of length of hospital stay. The mean change in the Z-scores for weight-for-age of the two fortifiers was expected to be very similar and therefore sample size determination was based on hospital stay. An increase of 2.5g/kg/d was regarded as clinically relevant and translated into a five day decrease in hospital stay for a 1kg infant. Conservative estimation of study days was expected to range from three to 50 days and standard deviation (SD) was estimated at range divided by six, that is 7.83 days. A sample of at least 53 infants per fortification group would have at least 90% power to detect a difference of five days in mean hospital stay when SD = 7.83 days and testing is two-sided at the 0.05 level of significance.

An additional way to calculate the sample size was to consider modelling of change in WFAZ, LFAZ and HCFAZ. To compare the two human milk fortifier formulations, five additional factors were taken into account, namely (i) birth WFAZ; (ii) birth weight-for-gestational-age category (SGA/AGA); (iii) birth weight classification (ELBW/VLBW); (iv) time in days human milk fortifier received; and (v) HIV-exposure. Data analysis employed regression methods and according to convention, the sample size requirement of ten to fifteen infants per parameter, would be adequate, i.e. at least 100 (50 infants per group) infants. This tied in with the initial sample size calculation of 53 infants per group.

3.2.2 Sampling: Original fortifier

In September 2016 a “Human milk fortification audit” was initiated by the Dietetic Department of the CHBAH. Permission was granted by the hospital’s authorities and it was executed by the researcher, a staff member of the CHBAH. The aim was to describe fortification practices in the neonatal units and all infants (both term and preterm) for whom fortification was started were included in the audit. During this time, the OF was used. The researcher collected the data for the audit prospectively.

All non-surgical VLBW preterm infants who were part of the audit were eligible for inclusion in the study. Once ethics approval (refer to Annexures 3 to 5 for Ethics approval documents:

University of Pretoria; University of the Witwatersrand; CHBAH) was obtained for the use of this data, the audit records were used to identify eligible infants. Consecutive sampling was done based on inclusion and exclusion criteria (Table 8). Filtering of data was done in order to comply with "RECORD" guidelines (REporting of studies conducted using Observational Routinely-collected health Data)[82] and good research practices for comparative effectiveness research.[83] In addition to the audit records, the infants' medical records and NCRs (Annexure 1) kept by dietitians were also used to check for completeness in terms of birth data, daily intake, output and anthropometric data.

Table 8: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• VLBW preterm infants who were: - Exclusively fed with human milk fed - Bolus fed	• VLBW preterm infants diagnosed with: - major congenital birth or chromosomal abnormalities e.g. Down Syndrome, gastroschisis - conditions that could have affected anthropometric measurements e.g. hydrocephalus - any condition e.g. NEC, that required the infant being kept NPO for longer than 24 hours - any condition that required gastrointestinal surgery e.g. jejunal atresia - weight > 1600g when fortification started • VLBW preterm infants with under-aged mothers (due to ethical considerations)

Abbreviations: NEC necrotising enterocolitis; NPO nil per os; VLBW very low birth weight

3.2.3 Sampling: Reformulated fortifier

Dietitians working in the neonatal units of CHBAH were the only personnel dispensing human milk fortifier. They were requested to inform the researcher of all the VLBW preterm infants for whom fortification had been started. Infants diagnosed with conditions that could have affected anthropometric measurements, feeding tolerance and those receiving continuous feeds were not considered. All non-surgical VLBW preterm infants receiving fortification and for whom informed consent had been obtained were included in the study. For ethical reasons, all infants with under-aged mothers were excluded due to the challenges of obtaining informed consent from their mothers' guardians. Teenage mothers may also be a different entity for research as they may be biologically different from mature mothers. Once infants had been included and they developed any of the conditions listed as exclusion criteria or received

formula feeds, they were considered dropouts. However, if fortification was restarted, for example after an infant was kept nil per os (NPO), infants could re-enter the study if they still met the inclusion criteria (Table 8).

3.3 Data collection

Feeds were prescribed by the attending doctor and/or the dietitian. Human milk fortification was calculated and prescribed by the dietitians and they were the only personnel who dispensed the fortifier. For both the OF and RF, the dietitians decanted the fortifier from 200g tins into smaller plastic containers. Each infant received its own individual container, a 1g scoop for measuring and an instruction sheet on the amount to be added. The 1g scoop was used to measure either 0.5g (½ scoop) or 1g (full scoop) dosages. Both the mothers of the infants and nursing staff were responsible for adding the fortifier to EBM. Before feeds were administered either orally (cup or syringe) or via a feeding tube, the fortifier was mixed with EBM. Fortification was calculated in 0.5g dosages and since fortifier was added before each feed, this meant that the dosage was not always optimal. For example, in the case of the OF a 0.75g dosage (75% of 1 gram) would be indicated for 15mL EBM (75% of 20mL) but only a 0.5g (½ scoop) would be added.

Nursing staff were responsible for charting the intake, output (urinary and stool) and tolerance of feeds (aspiration, vomiting) on intake-output charts. Dietitians were responsible for monitoring the use of the fortifier and for informing mothers and nursing staff of the amount to be added. Fortification was usually started at half strength, e.g. 0.5g (½ scoops) FM85 added to 20mL (Original fortifier) or 25mL (Reformulated fortifier) EBM and then increased to full strength if tolerated. Dietitians based their decisions on feeding intolerance on the presence of vomiting, abnormal gastric residuals, abnormal stool output and abdominal distension as described by Dutta et al.[37] Modifications to the amount of fortifier to be added was indicated in the feeding prescription and explained to both the mother and the nursing staff.

Infants entered the study on the first day that fortification was started (intermediate stage of nutrition support). Upon entrance, a data collection form containing birth and other biographic information for the infant and the mother was completed (Annexure 6). Most of these data were readily available in the infant's medical file and additional information was obtained from the mother, the attending doctor and/or the infant's Road-to-health booklet. This data

collection form was based on a position paper by ESPGHAN[84] on the core data needed for nutrition trials in infants. In addition to this, baseline anthropometry including the infant's current weight, length and HC was recorded within 24 hours of entrance to the study. All anthropometric measurements were personally taken by the researcher.

Thereafter, data were collected at specific time points. Weight, length and head circumference were measured every seven days. Medical data, for example the development of NEC, were collected as it became available and intake and output data were collected every 24 to 48 hours. The data collection forms for anthropometry as well as for intake and output information are attached (Annexures 7 and 8). During the audit, a condensed version of the aforementioned data collection forms was used (Annexure 9).

The following section describes how the anthropometric measurements for both the OF and the RF groups were done. Weight was measured on a calibrated pan-type electronic scale (Seca model 334, Hamburg, Germany), with the infant being placed in the middle of the scale. The infants were nude and all "tubes" e.g. saturation monitoring that could be removed safely for the duration of the measurement were removed. Measurement was recorded to the nearest gram and two readings were taken. If the difference between the two measurements exceeded 5g, it was repeated. If a third measurement was done, the two closest to each other were used.[85] Weight was taken in the afternoon, at least 30 minutes after the infant had been fed.

Head circumference was measured while the infant was being held up by the mother, nursing staff or one of the other dietitians. A flexible, non-stretchable measuring tape (Seca model 212, Hamburg, Germany) was used. The lower edge of the tape was positioned just above the eyebrows and ears and around the back of the head so that the maximum circumference was measured. The tape was in the same plane on both sides of the head and was pulled snugly around the head while compressing any hair. Measurement was recorded to the nearest 0.1cm and two measurements were taken. In instances where the difference between the two measurements exceeded 5mm, it was repeated. If a third measurement was done, the two values closest to each other were used and averaged.[85]

Recumbent length was measured on a special measuring device (Seca model 417, Hamburg, Germany) with the infant in the supine position. The person assisting the researcher (mother, nursing staff or fellow dietitian) was asked to keep the infant's head (placed in the Frankfort

plane) against the headboard so that the crown of the head was touching it. The researcher taking the measurement straightened the infant's legs and ensured that the feet and toes pointed upwards against the footboard. Two measurements were taken and recorded to the nearest 0.1cm. If the difference between the two measurements exceeded 7mm, it was repeated. If a third measurement was done, the two values closest to each other were used and averaged.[85]

Infants exited the study once the discharge weight of 1.65kg (official hospital policy during the time of data collection) had been reached or the infant had been discharged from the hospital or the infant had been taking more than 50% of feeds directly from the breast or the infant had started receiving formula feeds (whichever one occurred first).

3.4 Quality control

Procedures (e.g. anthropometric measurements) had been put into practice by the researcher as part of her routine work as a dietitian in the hospital. The data collection forms were tested in a pilot study on five infants before the official start of the research and thereafter a few adjustments to the forms were made. These data were not included in the main study as it did not include anthropometric measurements.

In terms of exposure, the intake of fortified human milk was recorded in a real-life setting and was based on intake records and reporting by mothers. The volume and number of feeds given as reported by the mother were used to confirm those recorded by the nursing staff in the intake records. The amount of human milk and fortifier added was also checked in terms of the feeding prescription. Since the fortifier was measured with a measuring spoon in 0.5g quantities (half a spoon for 0.5g and a full spoon for 1g), mothers were asked to indicate on the spoon the amount they were adding. If for example they indicated a 0.5g instead of a 1g, they were shown the correct way to measure. The responsible dietitian was also notified of incorrect measuring in order to educate the mothers further.

The macronutrient composition of human milk was analysed with mid-infrared spectroscopy and compared to the published values[33] that were used in calculations in the study (refer to 3.7).

In terms of outcome, anthropometric measurements were done according to standardised techniques described in the literature.[85] All anthropometric measurements for both parts of the study were taken by the researcher. In an effort to increase intra-rater reliability, all measurements were repeated twice and the average value was used. The same calibrated scale (Annexure 10) was used in both groups. Each growth parameter was evaluated in terms of two different indices, one of which was independent of an accurate estimation of GA. For example, weight was evaluated according to WFAZ and g/kg/d, with the latter being independent of GA. A validated formula[63] was used to calculate weight gain velocity (g/kg/d). The choice of Fenton 2013[35] as reference growth charts was based on recommendations in the literature.[33,56,59]

3.5 Data management and statistical analysis

A subject number was allocated to each infant in the study. A list containing the infant's name, hospital number and subject number was held separate from all other research data. Before any analysis was done, data were checked to identify outliers, errors and missing data. All data were transferred to MS Excel spread sheets. Data management according to exposure, outcome and confounding variables is discussed in Table 9.

Statistical analysis included the comparison of baseline information of the infants in the two groups to ensure comparability prior to the intervention in terms of potential confounders. Two-sample t tests were used to describe continuous variables by means, standard deviation and 95% confidence intervals (CI). Fisher's exact tests were used to report categorical variables using data frequencies and proportions. Linear regression controlling for one of the confounding variables, namely HIV exposure, was used to compare the two groups. A p-value of 0.05 was regarded as statistically significant. Data were analysed with STATA/IC 15.1 for Windows Revision 15 October 2018 (StataCorp LLC, USA) statistical software.

Table 9: Data management according to different variables

VARIABLE		DATA MANAGEMENT
Exposure variables: Protein and energy intake	OF and RF	For all calculations, the most recently recorded weight of the infant was used. For each infant total daily enteral protein (g/day) and energy (kcal/day) intake were calculated in accordance with previously published human milk composition* (preterm milk for first 14 days of life; mature milk thereafter[33]) and fortifier composition (OF;[31] RF[32]). Mature milk composition was used in cases where

VARIABLE		DATA MANAGEMENT
		donor milk was received. If the infant received glucose-containing intravenous (IV) fluids/parenteral nutrition (PN), its protein and energy contribution was added to the enteral amount for that specific day. The IV fluid used was Neonatalyte (10% glucose solution) (Adcock Ingram, Midrand) and the PN was from Fresenius Kabi, Midrand; code ITN 102 (2.1g protein, 1.2g fat, 10.5g glucose and 70kcal per 100mL). Daily mean protein (g/kg/d) and energy (kcal/kg/d) intake were then calculated for each infant based on the number of days the fortifier was administered. These values were used to calculate mean protein-to-energy ratio (g/100kcal).
Outcome variables: In-hospital growth	Weight	**Exit weight:** Since the researcher weighed the infants once a week and some infants were not discharged at exactly 1.65kg, more than one exit weight may have been available. In these instances, the weight that was numerically closest to 1.65kg was used. For example, in an infant with weights of 1.6kg and 1.75kg available, 1.6kg would have been used as exit weight. **Primary indicator:** WFAZ for entrance and exit was calculated for each infant using the Fenton clinical-exact-age-calculator[86] (PMA age in weeks and in days). Thereafter the change in WFAZ was calculated for each infant by subtracting the entrance value from the exit value. **Secondary indicator:** For each infant, weight gain velocity (g/kg/d) from entrance to exit was calculated according to the validated formula by Patel: Growth velocity (GV) = $[1{,}000 \times \ln(W_n/W_1)]/(D_n-D_1)$ where W=weight in grams; D=day; 1=beginning of time interval; n=end of time interval[63]
	Length	**Exit length:** Exit length was determined by exit weight. As far as possible, weight, length and HC of an individual were taken on the same day, therefore the exit length was the length taken on the day that the exit weight was taken. **Primary indicator:** LFAZ for entrance and exit was calculated for each infant using Fenton clinical-exact-age-calculator [86] (PMA age in weeks and days). Thereafter the change in LFAZ was calculated for each infant by subtracting the entrance value from the exit value. **Secondary indicator:** For each infant length gain (cm/wk) from entrance to exit was calculated by using the following formula: Exit value – Entrance value/days on fortifier x 7.

VARIABLE		DATA MANAGEMENT
	HC	**Exit HC:** Exit HC was determined by exit weight. As far as possible, weight, length and HC of an individual were taken on the same day, therefore the exit HC was the HC taken on the day that the exit weight was taken. **Primary indicator:** HCFAZ for entrance and exit was calculated for each infant using Fenton clinical-exact-age-calculator [86] (PMA age in weeks and days). Thereafter the change in HCFAZ was calculated for each infant by subtracting the entrance value from the exit value. **Secondary indicator:** For each infant HC gain (cm/wk) from entrance to exit was calculated by using the following formula: Exit value – Entrance value/days on fortifier x 7.
Confounding variables	GA	GA in completed weeks. If there was a discrepancy between the GA reported in the mother's obstetric history and that calculated by the Ballard score (done by the attending doctor), the average between the two was used.
	PMA when fortification started	PMA in weeks and days with birth taken as day one of life. For statistical analysis it was converted to decimal e.g. a PMA of 34 weeks and 5 days would be 34.7 weeks.
	Gender	Male/Female
	Birth weight	Birth weight was classified as either VLBW (1000 to 1500g) or ELBW (< 1000g). WFAZ was calculated by using the Fenton clinical-completed-weeks-calculator[86] (GA in completed weeks).
	Birth weight-for-gestational age	Appropriateness for gestational age was determined by using percentile values (SGA: < 10^{th} percentile; AGA: 10^{th} to 90^{th} percentile; LGA: > 90^{th} percentile) on the Fenton clinical-completed-weeks-calculator [86] (GA in completed weeks). Infants classified as LGA were excluded form Z-score analysis due to the reliance of Z-scores on accurate GA's, which may be not be the case in this population.
	Birth length	LFAZ was calculated by using the Fenton clinical-completed-weeks-calculator[86] (GA in completed weeks).
	Birth HC	HCFAZ was calculated by using the Fenton clinical-completed-weeks-calculator[86] (GA in completed weeks).
	Time receiving human milk fortifier	Number of days that the fortifier was received (exclusive of the first day and inclusive of the last day).
	HIV exposure	Binary classification according to exposed/not-exposed based on the mother's HIV status as being positive/negative.

3.6 Ethical considerations

The study was presented to the Research Committee of the Department of Human Nutrition, School of Health Care Sciences, University of Pretoria (10 February 2017) and was defended at the Research Committee of the School of Health Care Sciences, University of Pretoria on 23 May 2017. Ethics approval was obtained from the University of Pretoria, Faculty of Health Sciences Research Ethics Committee (Annexure 3a and amendment approval Annexure 3b) and the University of the Witwatersrand Health Research and Ethics Committee (Annexure 4a and amendment approval Annexure 4b). Institutional permission (Chief Executive Officer, the Head of the Neonatal Unit, the Head of Paediatrics and the Head of the Dietetic Department of the CHBAH) was granted by the Medical Advisory Committee of the CHBAH (Annexure 5a and amendment approval Annexure 5b). The ethics approval related to three components namely: (i) permission for the study of the RF; (ii) approval to use data previously collected as part of the audit (OF) for this research; and (iii) permission for the mid-infrared spectroscopy analysis of human milk (refer to 3.7).

Informed consent was obtained from the mothers of the infants receiving the RF after they were given information on the study and had the opportunity to ask questions (Annexure 11). Participation was voluntary and withdrawal from the study did not affect the routine health care that the infants received. There were no risks involved in the study, although some processes may have caused minimal discomfort for the infants, for example when the nappy was removed to weigh and measure the infant. The infants did not benefit directly from the study, but much needed information was obtained that may be used to improve nutritional care of preterm infants in future.

Confidentiality of information was addressed in the following manner: after data collection had been completed, a study number was allocated to each infant and thereafter only the study number was used for data management and analysis. The infants' names and hospital numbers will not be used in any publications.

3.7 Macronutrient analysis of human milk

As a measure to improve quality control of exposure to fortified human milk, an analysis of the macronutrient content of human milk was done. Mothers of infants in the RF group were targeted for this part of the study. In order to expand the sample size, additional mothers of preterm infants not included in the main study were recruited for inclusion. These mothers are referred to as the "Mothers in human milk analysis (HMA) designated group".

3.7.1 Study population and sampling

3.7.1.1 Mothers of infants in Reformulated fortifier group

The study population consisted of all mothers of infants included in the RF group from November 2017 to June 2018 (The shorter period of data collection was related to the logistics of obtaining the MIRIS™ equipment from Sweden). Within the first week of inclusion in the main study, mothers were asked to provide a human milk sample. Only those mothers who reported that they had sufficient milk for their infants (mothers were asked to provide a milk sample *after* they had expressed milk for their infants) and were willing to give a sample, were included.

3.7.1.2 Mothers in human HMA-designated group

The study population consisted of all mothers, of premature infants (including ELBW, VLBW and LBW) in the same neonatal wards as the main study who were expressing milk for their infants. This included infants who received formula feeding in addition to their mothers' breast milk. Mothers were recruited on eleven occasions over a three-month period from June 2018 to September 2018.

Mothers were approached in groups (in areas on the hospital premises where they expressed milk) or individually (for example in the KMC unit). Recruitment was done by the researcher and a research assistant who helped on five of these occasions. Convenience sampling was done where all mothers willing to provide their human milk samples were included.

3.7.2 Data collection

3.7.2.1 Mothers of infants in Reformulated fortifier group

After informed consent had been obtained (Annexure 12), a 10mL plastic sample collection bottle with a screw-on lid (bottles as specified by MIRIS™ for collection and storage of human milk) marked with the infant's study number and date was given to the mother. The mother was requested to provide a human milk sample (approximately 10mL) *after* she had expressed sufficient milk for her infant, thus "hind" milk. The human milk samples were expressed during the day at the 12:00 or 15:00 feeding times. The sample collection bottles were taken immediately after expression to the maternity milk room where they were frozen until the analysis was performed.

3.7.2.2 Mothers in HMA-designated group

After signing informed consent (Annexure 12), a study number was allocated to each mother and this number was written on the mother's copy of the informed consent form as well as on two sample collection bottles (same sample collection bottles used as prescribed earlier) per mother. The sample collection bottles were respectively marked as "Day" and "Night" and each was marked with the date as well. Day samples were collected at any two of three possible feeding times during the day: 9:00, 12:00 and 15:00. The two day samples were collected in the same sample collection bottle (marked "Day"). In-between collection, the bottles were kept in the ward refrigerator. Mothers were asked to express approximately 5mL at each collection time and to give the samples only *after* they have expressed sufficient milk for their infants (thus "hind" milk). Immediately after the second sample had been collected, the sample collection bottles were taken to the freezer in the maternity milk room and frozen until the time of analysis. The mothers were provided with the "Night" collection bottles only after the day collection had been completed. The mothers were asked to follow the same procedure overnight as was done during the day, that is providing two hind milk samples at two different times during the night and keeping the milk refrigerated in-between the two samples and after the second sample had been added. The mothers not lodging at the hospital took the "Night" collection sample bottles home with them. The "Night" collection sample bottles were collected the following morning by the researcher or the research assistant and taken to the maternity milk room to be frozen until the time of analysis. The researcher and the research

assistant also collected the infant's birth, anthropometric and feeding data and information on the mother's diet and health status, including the mother's mid-upper arm circumference (MUAC) measurements. Data were obtained from the infant's medical records and by interviewing the mother (Annexure 13). MUAC was taken by either the researcher or the research assistant. A flexible, non-stretchable measuring (commercial) tape was used to find the midpoint between the olecranon process and the acromion (with the arm flexed at the elbow) of each participant's non-dominant arm. With the arm being relaxed, MUAC was taken at the mid-point and recorded to the nearest 0.1cm. The average of two readings was taken.[85]

3.7.3 Analysis of the macronutrient content of human milk with MIRIS HMA™

In order to have samples representing a 24-hour collection time, "Day" and "Night" samples of the mothers in the HMA-designated group were mixed in the following manner: after defrosting, the "Mix" samples were prepared by mixing equal volumes of milk (e.g. 5mL plus 5mL) from the day and night collection bottles of each participant in a new collection bottle marked with the study number and "Mix". For those providing both day and night samples, it resulted in three sample bottles per mother. In cases where the day and/or night sample volumes were small, preference was given to the mixed sample. In some cases insufficient milk was left to analyse "Day" and/or "Night" on its own.

A detailed flow diagram of the mothers enrolled and samples included in the analysis of human milk is shown in Figure 2. Sample collection bottles were given to 94 mothers, of which 27 were in the RF group (day samples only) and 67 were in the HMA-designated group (day and night samples). Of these, a total of 147 (91 day; 56 night) sample collection bottles were returned for analysis and 53 mixed samples were generated. The volume of milk of some samples was insufficient to do the analysis and a total of 193 (87 day; 53 night; 53 mixed) human milk samples were analysed. A total of 29 (15%) out-of-range readings led to the total number of samples retained for statistical analysis being 164 (72 day, 42 night and 50 mixed).

The standard operating procedures that were followed for analysing the human milk samples with the MIRIS HMA™ (Uppsala Sweden) are depicted in Annexure 14.

3.7.4 Quality control

Human milk analysis was done using mid-infrared transmission spectroscopy with the MIRIS HMA™ (Uppsala, Sweden), which has a measurement performance of < 0.05% repeatability and < 0.1% accuracy.[87] The researcher was trained in person by a technician from the company to use all the MIRIS equipment (water bath heater, ultrasonic processor and analyser). All the consumables used (MIRIS check™, MIRIS cleaner™, MIRIS contol™, syringes and collection tubes) were used as specified by the manufacturer. A sample of known composition (MIRIS contol™) was analysed once daily/every time that the equipment was used to validate internal calibration.[87] In most cases, samples were analysed twice or thrice (depending on the volume of sample) and the average of the values was used.

Anthropometric measurements (MUAC) were done according to standardised techniques described in the literature.[85] To increase intra-rater reliability, MUAC was measured twice and the average value used for analysis.

3.7.5 Data management and statistical analysis

A subject number was allocated to each mother-and-infant pair in the study. Before any analysis was done, data were checked to identify outliers, errors and missing data. A research assistant helped with the coding of data and the assistant and the researcher crossed-checked each other's transfer of data to a MS Excel spread sheet. Data from the macronutrient analysis of human milk was only used if it did not include "out-of-range" values, even if it applied to only one of the analysed components. These components included fat, crude protein, true protein, carbohydrate, total solids and energy. For protein, the "true protein" values were used when mixed samples were compared to published data. "True protein" refers to protein nitrogen only whereas "crude protein" would also include non-protein nitrogen (e.g. oligosaccharides, urea).[87]

Data summary, by treatment, reported for continuous variables descriptive statistics including mean, standard deviation and 95% confidence intervals (two-sample t tests). For categorical variables, data frequencies and proportions were reported (Fisher's exact tests). A one-sample t-test was used to compare the analysed content of human milk to published values. Testing was done at the 0.05 level of significance. For the macronutrient analysis of human milk where day, night and mixed samples were compared with each other (paired t test), a Bonferonni

correction was done and level of significance was set at 0.0167. Data analysis was done using STATA/IC 15.1 for Windows Windows Revision 15 October 2018 (StataCorp LLC, USA) statistical software.

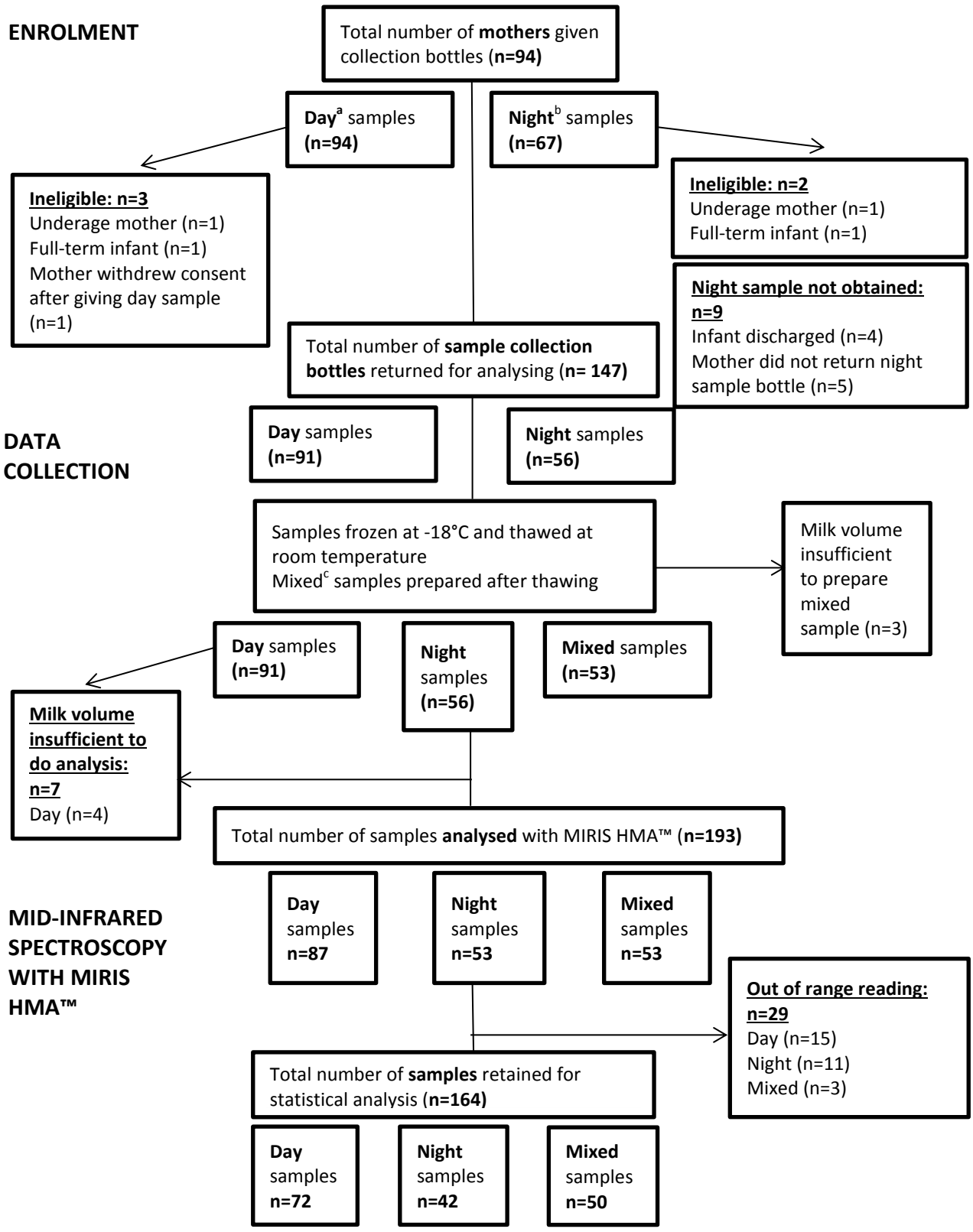


Figure 2: Flow diagram of mothers enrolled and samples included in human milk analysis

[a]Day samples from RF group (November 2017 to June 2018) and HMA-designated group (June 2018 to September 2018)

[b]Night samples were requested from HMA-designated group only (June 2018 to September 2018)

[c]Mixed samples were obtained by mixing equal volumes of Day and Night milk samples from the same mother in a new collection bottle

Abbreviations: HMA human milk analyser/analysis; RF Reformulated fortifier

CHAPTER 4:
RESULTS

This chapter presents the results of the study by offering a description of the sample followed by the exposure to fortified human milk (including the human milk analysis) and the outcome in terms of in-hospital growth.

4.1 Description of the sample

The OF group consisted of 58 VLBW preterm infants who were identified from audit records and whom met the inclusion criteria (refer to Methods 3.2.2 Table 8 for Inclusion and exclusion criteria). One hundred and eighteen audit records were screened and 60 infants were excluded, mostly for not receiving human milk exclusively. In some cases fortification was never started or was stopped. Reasons for stopping fortification included being kept NPO for feeding intolerance and suspected NEC. Infants were also excluded due to incomplete or potentially inaccurate anthropometric data and if their weight was above 1.6kg at the initiation of fortification (Figure 3).

Fifty-nine VLBW preterm infants completed the RF arm of study. Of the 122 infants who were screened, 28 were excluded due to, among other reasons, infants receiving formula feeds, cases where fortifier was not started and cases where anthropometric measurements could not be done. A further 35 infants dropped out during the course of the study, 22 (63%) of which were because of the introduction of formula feeds. Five infants were transferred to other hospitals before anthropometric measurements could be repeated. The five infants who dropped out due to being NPO included those with feeding intolerance and suspected NEC. Two infants developed hydrocephalus and were excluded as this would have affected the accuracy of anthropometric measurements. One infant passed away due to sepsis as a result of *Acinetobacter Baumanii* infection and presumed fungal sepsis for which the infant was receiving treatment. Most of the infants (74% in the OF group and 71% in the RF group) exited the study as they either reached the discharge weight of 1.65kg or were discharged from hospital (Figure 3).

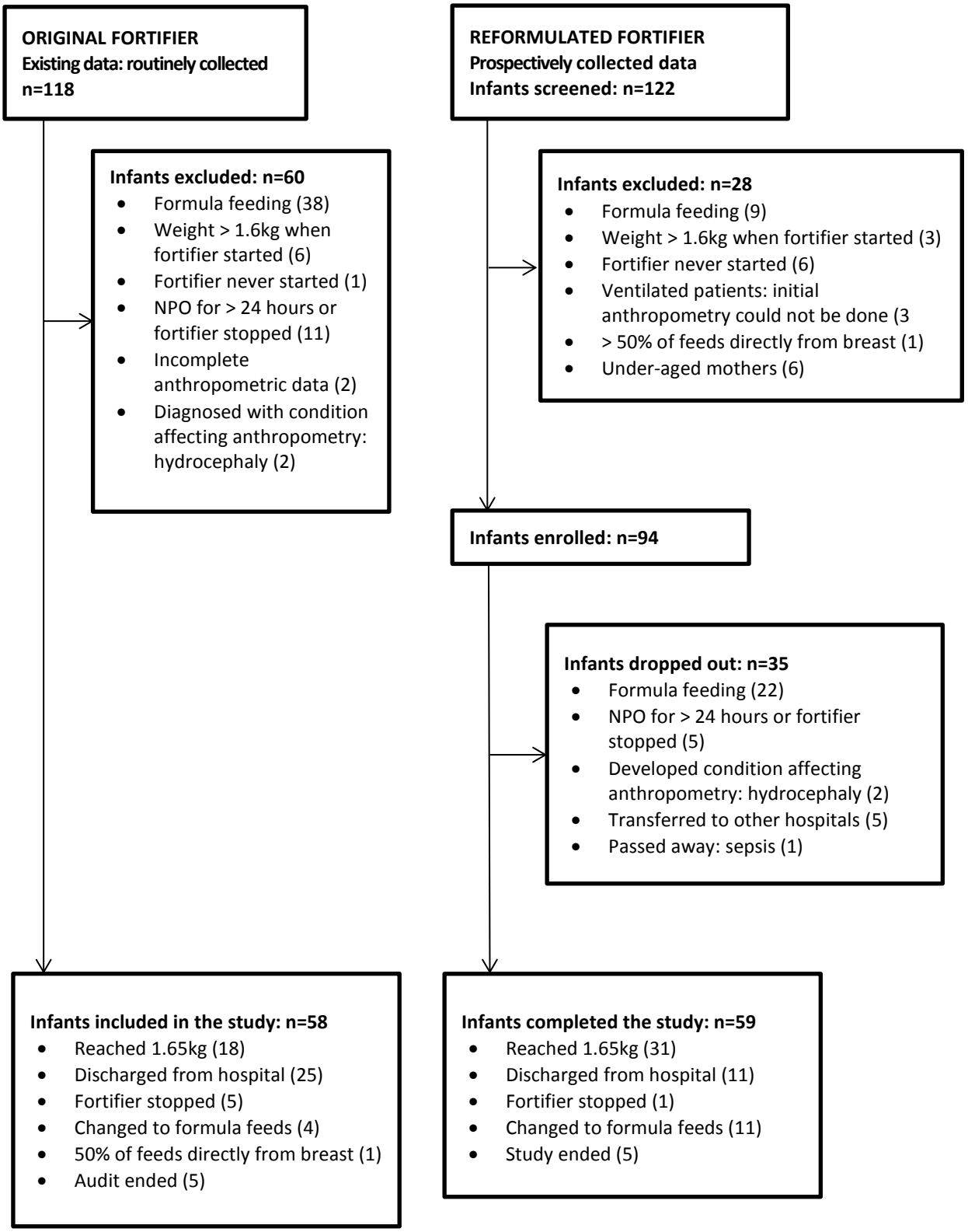


Figure 3: Flow diagram of infants included in the study

Abbreviations: NPO nil per os; kg kilogram

The birth anthropometric data of the OF and the RF groups are shown in Tables 10 and 11 respectively. These tables include within-group comparisons between genders, birth weight category and birth weight-for-gestational age. No differences were noted in either of the groups between the males and the females.

The baseline characteristics of the two groups are compared in Table 12. The two groups were comparable in all aspects presented in Table 12 including GA, birth anthropometry and exposure to HIV.

Some of the birth history and maternal information were only available for the RF group. The age of the mothers of the infants in this group ranged from 18 to 38 years with mean (±SD) 27.8 (±6.1) and 95% CI: 26.3 - 29.5 years. The majority (91%) of these mothers received antenatal care and gave birth at the CHBAH. Seventy percent of babies were born via a Caesarean section and 88% were single births.

Table 10: Description of the birth anthropometric data of the Original fortifier group

	Sex of infant							Birth weight category						Birth weight according to GA					
	Female			Male			p-value[a]	ELBW			VLBW			SGA			AGA[b]		
	n	Mean ±SD	95%CI	n	Mean ±SD	95%CI		n	Mean ±SD	95%CI	n	Mean ±SD	95%CI	n	Mean ±SD	95%CI	n	Mean ±SD	95%CI
GA, completed weeks (n=58)	30	30.1± 2.3	29.3; 31.0	28	30.0± 2.3	29.1; 30.9	0.785	10	28.1± 1.5	27.0; 29.2	48	30.5± 2.3	29.8; 31.1	11	33.1± 0.7	32.6; 33.6	47	29.3± 2.0	28.8; 29.9
Birth weight,g (n=58)	30	1182± 201	1107; 1257	28	1250± 168	1185; 1315	0.171	10	920± 85	859; 981	48	1277± 137	1237; 1316	11	1276± 126	1190; 1360	47	1201± 197	1143; 1259
Birth WFAZ (n=58)	30	-0.7± 1.1	-1.13; -0.31	28	-0.6± 1.1	-1.02; -0.17	0.662	10	-0.7± 0.8	1.3; -0.1	48	-0.7± 1.1	1.0; -0.3	11	-2.0± 0.4	-2.3; -0.8	47	-0.3± 0.9	-0.6; -0.06
Birth length, cm (n=56[c])	29	38.7± 3.1	37.5; 39.9	27	37.6± 3.4	36.3; 39.0	0.230	9	35.7± 3.3	33.1; 38.2	47	38.7± 3.1	37.8; 39.6	10	37.8± 1.9	36.4; 39.2	46	38.3± 3.5	37.2; 39.3
Birth LFAZ (n=56[c])	29	-0.3± 1.4	-0.8; 0.3	27	-0.8± 1.7	-1.4; -0.7	0.267	9	-0.5± 1.7	-1.8; 0.8	47	-0.5 ± 1.6	1.0; -0.4	10	-2.3± 0.9	-2.9; -1.6	46	-0.2± 1.4	-0.5; 0.4
Birth HC, cm (n=56[d])	29	27.2± 2.2	26.4; 28.0	27	27.9± 1.7	27.2; 28.6	0.160	9	25.6± 1.2	24.6; 26.5	47	27.9± 1.9	27.4; 28.5	10	28.1± 1.8	26.8; 29.4	46	27.4± 2.0	26.8; 28.1
Birth HCFAZ (n=56[d])	29	-0.3± 1.7	-1.0; 0.3	27	0.2± 1.4	-0.4; 0.7	0.250	9	-0.3± 1.7	-1.6; 1.0	47	-0.1± 1.5	-0.5; 0.4	10	-1.5± 1.3	-2.5; -0.6	46	0.2± 1.4	-0.2; 0.7

[a]Two-sample t test

[b]AGA include LGA infants

[c]Birth length data not available for all infants

[d]Birth HC data not available for all infants

Abbreviations: AGA appropriate for gestational age; CI confidence interval; ELBW extreme low birth weight; GA gestational age; HC head circumference; HCFAZ head circumference-for-age Z-score; LFAZ length-for-age Z-score; LGA large for gestational age, SD standard deviation; SGA small for gestational age; VLBW very low birth weight; WFAZ weight-for-age Z-score

Table 11: Description of the birth anthropometric data of the Reformulated fortifier group

	Sex of infant							Birth weight category						Birth weight according to GA					
	Female			Male			p-value[a]	ELBW			VLBW			SGA			AGA[b]		
	n	Mean ±SD	95%CI	n	Mean ±SD	95%CI		n	Mean ±SD	95%CI	n	Mean ±SD	95%CI	n	Mean ±SD	95%CI	n	Mean ±SD	95%CI
GA, completed weeks (n=59)	33	29.9± 2.2	29.1; 30.7	26	29.7± 2.0	28.9; 30.5	0.710	7	28.7± 2.1	26.8; 30.7	52	30.0± 2.1	29.4; 30.6	9	33.3± 1.6	32.1; 34.5	50	29.2± 1.5	28.8; 29.6
Birth weight, g (n=59)	33	1202± 184	1137; 1267	26	1202± 146	1143; 1261	0.999	7	927± 73	860; 995	52	1239± 139	1200; 1278	9	1230± 147	1117; 1343	50	1197± 171	1148; 1246
Birth WFAZ (n=59)	33	-0.6± 1.1	-1.0; -0.2	29	-0.7± 0.9	-1.1; -0.3	0.737	7	-0.9± 1.0	-1.8; -0.0	52	-0.6± 1.0	-0.9; -0.3	9	-2.4± 0.8	-3.0; -1.8	50	-0.3± 0.7	-0.5; -0.1
Birth length, cm (n=52[c])	30	37.5± 2.8	36.4; 38.5	22	37.7± 4.0	36.0; 39.5	0.783	6	35.0± 3.1	31.7; 38.3	46	37.9± 3.2	37.0; 38.9	6	37.2± 3.9	33.1; 41.3	46	37.6± 3.3	36.7; 38.6
Birth LFAZ (n=52[c])	30	-0.6± 1.4	-0.2; -0.1	22	-0.6± 1.7	-1.3; 0.2	0.858	6	-0.6± 1.7	-1.9; 0.6	46	-0.6± 1.6	-1.1; 0.1	6	-2.6± 0.9	-3.6; -1.7	46	-0.3± 1.4	-0.8; 0.6
Birth HC, cm (n=52[d])	30	27.6± 2.0	26.9; 28.4	22	27.7± 1.7	27.0; 28.5	0.856	6	26.7± 0.5	26.1; 27.2	46	27.8± 1.9	27.2; 28.4	6	28.5± 1.0	27.4; 29.6	46	27.6± 1.9	27.0; 28.1
Birth HCFAZ (n=52[d])	30	0.1± 1.6	-0.5; 0.7	22	0.2± 1.3	-0.4; 0.8	0.820	6	0.1± 1.5	-1.1; 1.7	46	0.3± 1.3	-0.3; 0.6	6	-1.8± 1.0	-2.9; -0.7	46	0.4± 1.3	0.0; 0.8

[a]Two-sample t test

[b]AGA include LGA infants

[c]Birth length data not available for all infants

[d]Birth HC data not available for all infants

Abbreviations: AGA appropriate for gestational age; CI confidence interval; ELBW extreme low birth weight; GA gestational age; HC head circumference; HCFAZ head circumference-for-age Z-score; LFAZ length-for-age Z-score; LGA large for gestational age, SD standard deviation; SGA small for gestational age; VLBW very low birth weight; WFAZ weight-for-age Z-score

Table 12: Comparison of the baseline characteristics of the Original fortifier and the Reformulated fortifier groups

Characteristics		Original fortifier (n=58)			Reformulated fortifier (n=59)			p-value[a]
Female, n(%)		30(52)			33(56)			0.648
Birth weight category, n(%)	ELBW	10(17)			7(12)			
	VLBW	48(83)			52(88)			0.409
Birth weight according to GA, n(%)	SGA	11(19)			9(15)			
	AGA	43(74)			48(81)			
	LGA	4(7)			2(4)			0.569[b]
HIV exposed, n(%)		23(40)			17(29)			0.216
		n	**Mean±SD**	**95%CI**	**n**	**Mean±SD**	**95%CI**	**p-value[c]**
Birth	GA, completed weeks	58	30.1±2.3	29.4;30.7	59	29.8±2.1	29.3;30.4	0.463
	Weight, g	58	1215±187	1166;1264	59	1202±167	1159;1246	0.692
	WFAZ	54[d]	-0.8±0.9	-1.1;-0.5	57[d]	-0.7±1.0	-1.0;-0.4	0.580
	Length, cm	56[e]	38.2±3.3	37.3;39.1	52[e]	37.6±3.3	36.7;38.5	0.347
	LFAZ	52[d]	-0.6±1.5	-1.0;-0.2	50[d]	-0.7±1.5	-1.1;-0.2	0.737
	HC, cm	56[e]	27.5±2.0	27.0;28.1	52[d]	27.7±1.8	27.2;28.2	0.587
	HCFAZ	52[d]	-0.3±1.4	-0.6;0.1	50[d]	0.1±1.5	-0.3;0.5	0.167
Study entry	PMA (exact age), weeks	58	32.6±2.5	31.9;33.3	59	32.5±1.9	32.0;33.0	0.808
	Day of life	58	18.4±9.4	15.9;20.9	59	18.2±7.4	15.2;21.1	0.898
	Weight, g	58	1263±182	1215;1311	59	1280±145	1242;1318	0.577
	WFAZ	54[d]	-1.8±1.1	-2.1;-1.5	57[d]	-1.6±0.9	-1.8;-1.3	0.296
	Length, cm	47[f]	38.6±1.7	38.1;39.1	59	38.9±1.5	38.5;39.3	0.337
	LFAZ	43[d]	-1.7±1.1	-2.0;-1.3	57[d]	-1.3±0.9	-1.5;-1.1	0.068
	HC, cm	52[f]	28.0±1.6	27.6±28.5	59	28.1±1.3	27.7;28.5	0.717
	HCFAZ	48[d]	-1.3±1.2	-1.7±-1.0	57[d]	-0.9±1.0	-1.2;-0.7	0.059
	Volume of milk, mL/kg/d	58	167±21	161;173	59	165±20	160;171	0.663

[a]Fisher's exact test

[b]LGA infants included with AGA infants

[c]Two-sample t test

[d]In both groups: LGA infants excluded from Z-score analysis

[e]In both groups: Birth data not available for all infants

[F]In OF group: Data not available for all infants

Abbreviations: AGA appropriate for gestational age; CI confidence interval; ELBW extreme low birth weight; GA gestational age; HC head circumference; HCFAZ head circumference-for-age Z-score; HIV Human immunodeficiency virus; LFAZ length-for-age Z-score; LGA large for gestational age, mL/kg/d millilitre per kilogram body weight per day; PMA postmenstrual age; SD standard deviation; SGA small for gestational age; VLBW very low birth weight; WFAZ weight-for-age Z-score

4.2 Exposure to fortified human milk

In both groups fortification was started at a mean (±SD) PMA of 32 weeks (OF: 32.6±2.5; RF: 32.5±1.9). The mean (±SD) day of life when fortification was started was also similar between the two groups (OF: 18.4±9.4; RF: 18.2±7.4). The mean (±SD) volume of milk at the start of fortification (study day 1) was 167±21mL/kg/d and 165±20mL/kg/d in the OF and RF groups, respectively, with no significant difference between the two groups (Table 12). In both groups fortification was started at half strength and in increased to full strength over a few days by the attending dietitian. The manufacturer recommends that in order to establish tolerance, fortification should start at lower dosages: for the OF at 20% of full strength and increased over five to seven days to full strength[31]; for the RF at 50% and increased to full strength after tolerated for 24 hours.[32] In 44 infants (75%) in the RF group, and for no apparent reason, half strength dosages were not increased after feeds were tolerated for 24 hours. Dosages were often kept at half strength for a number of days and in some instances for the entire study period.

All infants received human milk exclusively and were bolus fed via an oro-gastric tube, a syringe or a feeding cup. Infants in the OF group received only their own mothers' milk, as there was no donor milk available in the hospital during that period. In the RF group all infants, except one, received their own mothers' milk exclusively. The one infant, who received donor milk, received it for a period of five out of 13 study days. On two of these days donor milk was given for all eight feeds, whereas on the other three days it was in combination with the mother's own milk. Intra-venous (IV) fluids were administered as part of the total fluid intake in three infants in the OF group. It was given for one or two days and contributed between 10% and 26% to the specific day's energy intake. In the RF group, seven infants received IV fluids as part of their total fluid intake. They received it for a period of one to three days and it contributed between 2% and 35% to the specific day's energy intake. In both groups the same IV fluid (Neonatalyte; Adcock Ingram, Midrand) containing 10% glucose (but no fat or protein) and electrolytes was used.

Two infants (one in each group) received supplementary parenteral nutrition; both received it for two days. Both infants received parenteral nutrition code ITN 102 (Fresenius Kabi, Midrand) containing protein (2.1g/100mL), glucose (10.5g/100mL), fat (2.1g/100mL) and electrolytes. The

parenteral nutrition received by the infant in the OF group (on two out of 19 study days) contributed 27% and 43% to the energy intake and 28% to 50% to protein intake on the two respective days. The parenteral nutrition received by the infant in the RF group (on two out of 16 study days) contributed 33% and 45% to the energy intake and 30% to 45% to the protein intake on the two respective days.

All infants in both groups received daily oral vitamin and mineral supplements as per hospital protocol, namely a multivitamin [containing vitamins A (3000IU/d), B_1 (1.15mg/d), B_2 (1.25mg/d), B_3 (10mg/d), B_6 (1mg/d), C (50mg/d), D (400IU/d)]; folic acid (0.1mg/d); iron (3 to 4 mg/kg/d) and additional vitamin D (400IU/d).

Table 13 compares the calculated daily protein, energy and fluid (milk) intake of the two groups. There were no differences between the two groups in the mean daily volume of milk received. The protein intake in the RF group was significantly higher (p<0.001) and the energy significantly lower (p=0.022) than that of the OF group. The protein-to-energy ratio was also significantly higher (p<0.001) in the RF group when compared to the OF group.

Table 13: Comparison of the calculated[a] daily protein, energy and fluid intake of the Original fortifier and the Reformulated fortifier groups

	Original fortifier (n=58)		Reformulated fortifier (n=59)		p-value[b]
	Mean±SD	95% CI	Mean±SD	95% CI	
Protein, g/kg/d	3.4±0.2	3.3;3.4	3.7±0.4	3.6;3.8	<0.001
Energy, kcal/kg/d	144.8±6.9	143.0;146.6	141.8±7.1	139.9;143.7	0.022
Protein: Energy ratio, g/100kcal	2.3±0.1	2.3;2.4	2.6±0.2	2.5;2.6	<0.001
Fluid (milk) volume, mL/d	173.3±7.8	171.3;175.4	174.6±7.9	172.5;176.6	0.404

[a]Calculated by adding nutrient values for Preterm milk (up to day 14 of life: 1.5g protein and 65kcal energy per 100mL) and Mature milk (from day 15 of life onwards: 1.2g protein and 72kcal energy per 100mL)[33] plus nutrient values for OF (0.2g protein and 3.5kcal per 1g powder) or for RF (0.4g protein and 4.4kcal per 1g powder)

[b]Two-sample t test

Abbreviations: CI confidence interval; g/100kcal gram per 100 kilocalories; g/kg/d gram per kilogram body weight per day; kcal/kg/d kilocalorie per kilogram body weight per day; mL/d millilitre per day; SD standard deviation

In Table 14 the calculated protein, energy and fluid (milk) intakes in the study are compared to published recommendations for preterm infants. Since the present study was not designed to distinguish between ELBW and VLBW, the recommendations by Koletzko et al[48] were deemed

the most appropriate. In both groups the mean volume of milk intake approached 175mL/kg/d, which fell within the 135 to 200mL/kg/d as recommended by the Koletzko group.[48] Exposure to the RF led to significant increases in estimated intake of protein. Protein intake (3.7g/kg/d) in the RF group met published recommendations (3.5 to 4.5g/kg/d)[48] for preterm infants, but it was not achieved in the OF group (3.4g/kg/d).

In Table 15 the calculated protein and energy intake in the study are compared to Koletzko et al's[48] recommendations for preterm infants. In both groups, the calculated energy intake fell either within or above the recommended range, with the majority (96.5% and 96.6% for the OF and RF groups, respectively), exceeding the upper limit of 130kcal/kg/d. In contrast to this, the calculated protein intake in both the groups did not exceed the recommended range, but fell within or below it. In the OF group, 60.3% of the infant's calculated protein intake fell below and 39.7% fell within the recommended range. In the RF group, 32.2% of the infant's calculated protein intake fell below and 67.8% fell within the recommended range. The difference between the two groups for protein intake was statistically significant (p=0.003).

Table 14: Comparison of the calculated daily intake to published recommendations for preterm infants

	Intake in the study		Recommended intake					
	Original fortifier Mean(±SD)	Reformulated fortifier Mean(±SD)	AAP[34]		ESPGHAN[47]	Koletzko et al[48]	Tsang et al[49]	
			ELBW	VLBW			ELBW	VLBW
Protein, g/kg/d	3.4±0.2	3.7±0.4	3.8 – 4.4	3.4 – 4.2	ELBW: 4 - 4.5g VLBW (up to1.8kg): 3.5 – 4.0g	3.5 – 4.5	3.8 – 4.4	3.4 – 4.2
Energy, kcal/kg/d	144.8±6.9	141.8±7.1	130 -150	110 -130	110 - 135	110 - 130	130 - 150	110 - 130
Protein: Energy ratio, g/100kcal	2.3±0.1	2.6±0.2	-	2.6 – 3.8	3.2 – 3.6	3.2 – 3.6	-	-
Fluid (milk) volume, mL/kg/d	173.3±7.8	174.6±7.9	-	-	135 - 200	135-200	160 - 200	135 - 190

Abbreviations: AAP American Academy of Pediatrics; ELBW extreme low birth weight; ESPGHAN European Society for Paediatric Gastroenterology, Hepatology and Nutrition; g/kg/day gram per kilogram body weight per day; g/100kcal gram per 100 kilocalories; kcal/kg/d kilocalorie per kilogram body weight per day; mL/kg/day millilitre per kilogram body weight per day; SD standard deviation; VLBW very low birth weight

Table 15: Comparison of the calculated daily protein and energy intake to Koletzko et al's[48] recommendations for preterm infants

			Recommended range of intake per day	Calculated daily intake[a]		
			Koletzko[48]	Original fortifier (n=58)	Reformulated fortifier (n=59)	p-value[b]
Protein	**g/kg/d**		3.5 – 4.5	Mean(±SD) 3.4±0.2	Mean(±SD) 3.7±0.4	
	% of Recommended range:	Below		60.3 (n=35)	32.2 (n=19)	
		Within		39.7 (n=23)	67.8 (n=40)	0.003
		Above		0	0	
Energy	**kcal/kg/d**		110 - 130	Mean(±SD) 144.8±6.9	Mean(±SD) 141.8±7.1	
	% of Recommended range:	Below		0	0	
		Within		3.5 (n=2)	3.4 (n=2)	1.000
		Above		96.5 (n=56)	96.6 (n=57)	

[a] Preterm milk up to day 14 of life (1.5g protein and 65kcal energy per 100mL) Mature milk from day 15 of life onwards (1.2g protein and 72kcal energy per 100mL)[33]

[b] Fisher's exact test

Abbreviations: g gram; g/kg/d gram per kilogram body weight per day; kcal kilocalorie; kcal/kg/d kilocalories per kilogram body weight per day; mL millilitre; SD standard deviation

For the calculated intake of protein and energy (Tables 13, 14 and 15), the composition of human milk was based on the recommended values of Cormack et al[33] for preterm and mature milk. In order to judge the relevance of the use of these values within the South African context, analysis of human milk was done with mid-infrared spectroscopy and the results thereof are discussed in Section 4.2.1.

4.2.1 Exposure to fortified human milk: human milk analysis

The human milk samples retained for statistical analysis came from 85 mothers whose age ranged from 18 to 41 years with a mean (±SD) age 27.7 (±6.7) and 95% CI (26.3 - 29.2) years. Most of them (90%) received ante-natal care and 25% were HIV positive. Eighty seven percent of them gave birth at CHBAH, 32% were prim gravidas and 65% had a Caesarean section. A third (32%) of them stayed in the KMC unit at the hospital and the other 68% stayed at home. The mothers had a mean (±SD) MUAC of 29.8 (±4.5) cm indicating a good protein status.[85] All mothers reported consuming a traditional diet (including animal protein) and only a small number (3%) reported using vitamin and mineral supplements. Eleven percent of the mothers received one or two glasses per day of a nutritional supplement (Mom2B Pregnancy Shake®, Nativa, South Africa) provided by the hospital dietitians. This supplement was given at the dietitians' discretion for mothers struggling with human milk expression and provided 182kcal, 9g protein, 35g carbohydrate and 0.4g fat per 220mL glass, respectively.

The infants of these mothers had a mean (±SD) GA of 30.3 (±2.9) weeks and a mean (±SD) birthweight of 1310 (±402) g. The milk samples were collected when the infants had a mean (±SD) age of 25 (±15) days, PMA of 33.6 (±3.1) weeks and weight of 1461 (±376) g.

The analysed macronutrient, total solids and energy content of the day, night and mixed samples are compared in Table 16. There were no significant differences between the protein content of the three samples. The fat content of the day samples was significantly higher (p=0.006 and p=0.001, respectively) than those for both the night and the mixed samples. These differences were not apparent when the night and the mixed samples were compared with each other.

In Table 17**,** results from the mixed sample are compared to published macronutrient and energy composition of preterm and mature human milk. Apart from Cormack et al[33], whose human milk macronutrient composition was applied in this study, results were also compared

to the systematic review by Boyce et al[39] where a comparable definition for preterm and mature human milk was used. In comparison to Cormack et al[33] (Table 18), the analysed protein content of the mixed sample was significantly higher in terms of preterm ($p=0.000$) and mature ($p=0.002$) human milk whereas the analysed energy content of the mature sample ($p<0.001$) was significantly lower. Since the sample size of the preterm milk in the present study was only 13, a coefficient of variation was calculated (15.8%), which indicated that the small sample size did not threaten the estimation.

Table 16: Human milk composition: Comparison of day, night and mixed samples

	Human milk composition per 100mL						p-value[d]		
	Day[a] samples (n=72)		Night[b] samples (n=42)		Mixed[c] samples (n=50)				
	Mean±SD	95%CI	Mean±SD	95%CI	Mean±SD	95%CI	Day vs Night	Day vs Mix	Night vs Mix
Crude[e] protein, g	1.8±0.5	1.7;1.9	1.9±0.5	1.8;2.1	1.9±0.5	1.7;2.0	0.192	0.107	0.083
True[f] protein, g	1.4±0.4	1.3;1.5	1.5±0.4	1.4;1.7	1.5±0.4	1.4;1.6	0.150	0.063	0.037
Fat, g	3.8±1.2	3.5;4.0	3.2±1.3	2.8;3.6	3.5±1.0	3.2;3.7	0.006	0.001	0.022
Carbohydrate, g	7.2±0.6	7.0;7.3	7.2±0.7	7.0;7.4	7.2±0.7	7.0;7.4	0.047	0.001	0.294
Total solids[g], g	13.0±1.4	12.6;13.3	12.5±1.5	12.0;13.0	12.8±1.3	12.4;13.1	0.035	0.341	0.005
Energy, kcal	71.3±11.5	68.6;74.0	66.5±12.4	62.7;70.4	69.0±9.7	66.3;71.8	0.021	0.015	0.010
Protein[h]: Energy ratio, g/100kcal	2.1±0.6	1.9;2.2	2.3±0.6	2.1;2.5	2.2±0.6	2.0;2.4	0.006	0.003	0.308

[a]Day samples representing milk expressed at one or two collection times during the day.

[b]Night samples representing milk expressed at two collection times during the night.

[c]Mixed samples obtained by mixing equal volumes of day and night milk samples from the same mother in a new collection bottle.

[d]Paired t test. Significance set as 0.0167 (Bonferonni-correction).

[e]Includes both protein nitrogen (N) and non-protein N (e.g. oligosaccharides, urea).[87]

[f]Excludes non-protein N.[87]

[g]Total solids: Dry matter, including carbohydrate, fat, protein and minerals.[87]

[h]True protein values used in calculation.

Abbreviations: CI confidence interval; g gram; kcal kilocalorie; g/100kcal gram per 100 kilocalories; mL millilitre; N nitrogen; SD standard deviation; vs versus

Table 17: Comparison of analysed and published macronutrient and energy content of preterm and mature human milk

	Human milk composition per 100mL						
	Analysed composition of mixed[a] samples			Published composition			
				Cormack et al[33]		Boyce et al[39]	
	Total sample (n=50)	Preterm[b] milk (n=13)	Mature[c] milk (n=37)	Preterm[b] milk	Mature[c] milk	Preterm[d] milk (Including Colostrum)	Mature[e] milk
	Mean±SD	Mean±SD	Mean±SD				
Protein, g	1.5±0.4[f]	1.9±0.3[f]	1.4±0.4[f]	1.5	1.2	1.9	1.3
Carbohydrate, g	7.2±0.7	7.0±0.5	7.2±0.8	-	-	6.6	7.3
Fat, g	3.5±1.0	3.0±1.1	3.6±0.9	-	-	2.6	3.5
Energy, kcal	69.0±9.7	66.4±10.4	69.9±9.5	65	72	57	65.4
Protein: Energy ratio, g/100kcal	2.2±0.6[g]	2.9±0.5[g]	2.0±0.5[g]	2.3	1.7	3.3	2.0

[a]Mixed samples form HMA-designated group.

[b]Up to day 14 of life.

[c]Day 15 of life onwards.

[d]First week of life.

[e]Week two to eight of life.

[f]True protein values.

[g]True protein values used in calculation.

Abbreviations: g gram; g/100kcal gram per 100 kilocalories; HMA human milk analysis; kcal kilocalorie; mL millilitre; SD standard deviation

Table 18: Comparison of analysed and published protein and energy content of preterm and mature milk

	Human milk composition per 100mL					
	Preterm[a] milk			Mature[b] milk		
	Analysed composition of mixed[c] sample (n=13)	Cormack et al[33]	p-value[d]	Analysed composition of mixed[c] sample (n=37)	Cormack et al[33]	p-value[d]
	Mean±SD			Mean±SD		
Protein, g	1.9±0.3[e]	1.5	0.000	1.4±0.4[e]	1.2	0.002
Energy, kcal	66.4±10.4	65	0.318	69.9±9.5	72	<0.001

[a]Up to day 14 of life.

[b]Day 15 of life onwards.

[c]Mixed sample form HMA-designated group.

[d]One-sample t test.

[e]True protein values.

Abbreviations: g gram; HMA human milk analysis; kcal kilocalorie; mL millilitre; SD standard deviation

4.2.2 Outcome: In-hospital growth

4.2.2.1 Weight gain

Table 19 offers a comparison between the two groups in terms of their weight. The weight and WFAZ at entry to the study were comparable between the OF and the RF groups. Similarly, comparable between the two groups were the weight and WFAZ at exit from the study. As a primary outcome objective, the change in WFAZ between exit and entry was determined and no significant differences were seen between the two groups. Of interest is the significant difference (p=0.027) between the two groups when change in WFAZ between exit and birth was compared: the negative change in WFAZ form birth to exit was less pronounced in the RF.

Even though the RF group had a higher mean weight gain velocity (secondary outcome objective) than the OF group (15.1±4.7 compared to 14.5±4.3 g/kg/d), the difference was not significant (Table 19).

4.2.2.2 Length gain

Table 20 presents a comparison between the two groups in terms of their length. The length at entry to the study and the length at exit from the study were in both instances comparable between the two groups. Even though the OF group had a lower LFAZ at the entry and the exit from the study, it did not differ significantly compared to the RF group. As a primary outcome objective, the change in LFAZ between exit and entry was determined and no significant differences were seen between the two groups. Length gain calculated in cm/wk (secondary outcome objective) was similar between the two groups (Table 20).

4.2.2.3 Head circumference gain

Table 21 compares the two groups in terms of their HC. The HC at entry to the study and the HC at exit from the study were in both instances comparable between the two groups. At entry to the study, there was no significant differences in HCFAZ between the two groups, but HCFAZ at exit from the study was significantly higher in the RF group (p=0.004). In both groups, change in HCFAZ from birth to exit showed slight improvements. The change in HCFAZ between exit and entry determined as the primary outcome objective did not reach statistical significance. Gain

in HC calculated in cm/wk (secondary outcome objective) was similar for the two groups (Table 21).

4.2.3 Outcome: Feeding tolerance

Feeding tolerance is reported based on the presence of gastric residuals, vomiting, stool output and abdominal distension. Feeds were well tolerated in both groups. Gastric residuals exceeding 50% of the feeding volume was reported once in one infant in the OF group, but feeds were not omitted. No bloody or bilious gastric residuals were noted in any infant in any of the two groups. Episodes of vomiting were reported in eight and nine infants in the OF and RF groups respectively. In most cases these were isolated incidents of vomiting (one vomitus recorded on one day) and feeds were not omitted. In two infants in the OF group, vomiting was recorded on more than one day and in one of the infants two feeds were omitted on one of the days. In the RF group one infant had five episodes of vomiting over a two-day period, which was attributed to the consumption of large volumes of milk due to a mistake in the volume of the feed prescribed. In one other infant in the RF group, two episodes of vomiting on two days were reported, but no feeds were omitted.

Two infants in the OF group received an oral rehydration solution (Rehidrat®) for diarrhoea (one infant received it for one day and the other infant for two days) and one infant in the RF group received the same solution for diarrhoea for one day. Since it was used as a replacement fluid, the energy contributed by the oral rehydration solution was not included in the daily energy calculation. One infant in the RF group received Lactulose (Lacson®) for constipation for one day and one infant in the same group had one episode of a bloody stool that was attributed to an anal fissure and not NEC. Abdominal distension was noted in six infants in the OF group and in two infants in the RF group. It was described as “mildly distended” and no feeds were omitted in either group as a result.

4.2.4 Outcome: Confounding variables

Confounding variables that could have influenced the outcome of the study were identified (refer to Table 9) and included GA; gender; birth weight (ELBW vs VLBW; BWFAZ); birth weight-for-gestational age (SGA vs AGA vs LGA); birth length; birth HC; HIV-exposure; PMA and day of life on entry to study; and the time fortifier has been received. There were no significant differences between the two groups with regard to any of these factors (Tables 12, 19), and

therefore differences in growth outcomes between the two groups would most probably not have been influenced by any of the factors. In order to confirm this and due to the importance of HIV-exposure in the South African context, the growth outcomes between the two groups were adjusted for HIV-exposure in a linear regression analysis. Mean predicted effects are reported along with a 95% CI (Table 22). No significant differences were seen between the two groups, confirming that HIV exposure did not confound the growth outcome results.

Table 19: Comparison between the Original fortifier and Reformulated fortifier groups in terms of weight

			Original fortifier (n=58)			Reformulated fortifier (n=59)			p-value[a]
			n	Mean±SD	95% CI	n	Mean±SD	95% CI	
Weight, g		**Birth**	58	1215±187	1166;1264	59	1202±167	1159;1246	0.692
		Entry	58	1263±182	1215;1311	59	1280±145	1242;1318	0.577
		Exit	58	1570±123	1538;1603	59	1588±116	1557;1618	0.417
WFAZ		**Birth**	54[b]	-0.8±0.9	-1.1;-0.5	57[b]	-0.7±1.0	-1.0;-0.4	0.580
		Entry	54[b]	-1.8±1.1	-2.1;-1.5	57[b]	-1.6±0.9	-1.8;-1.3	0.296
		Exit	54[b]	-2.3±1.3	-2.6;-1.9	57[b]	-1.9±1.0	-2.2;-1.7	0.071
Entry to exit, days			58	15.9±8.8	13.6;18.2	59	15.3±8.2	13.1;17.4	0.703
Primary outcome	**Change in WFAZ**	**Birth to entry**	54[b]	-1.0±0.6	-1.1;-0.8	57[b]	-0.9±0.4	-1.0;-0.7	0.302
		Entry to exit	54[b]	-0.5±0.5	-0.6;-0.3	57[b]	-0.4±0.4	-0.5;-0.3	0.205
		Birth to exit	54[b]	-1.5±0.8	-1.7;-1.2	57[b]	-1.2±0.6	-1.4;-1.1	0.027
Secondary outcome	**Weight gain velocity, g/kg/d[c]**	**Entry to exit**	58	14.5±4.3	13.4;15.6	59	15.1±4.7	13.9;16.3	0.460

[a]Two-sample t test

[b]In both groups: LGA infants excluded from Z-score analysis

[c]Calculation done according to the method of Patel [63]

Abbreviations: CI confidence interval; g gram; g/kg/d gram per kilogram body weight per day; LGA large for gestational age; SD standard deviation; WFAZ weight-for-age Z-score

Table 20: Comparison between the Original fortifier and Reformulated fortifier groups in terms of length

			Original fortifier (n=58)			Reformulated fortifier (n=59)			p-value[a]
			n	Mean±SD	95% CI	n	Mean±SD	95% CI	
Length, cm		**Birth**	56[b]	38.2±3.3	37.3;39.1	52[b]	37.6±3.3	36.7;38.5	0.347
		Entry	47[c]	38.6±1.7	38.1;39.1	59	38.9±1.5	38.5;39.3	0.337
		Exit	47[c]	40.7±1.6	40.2;41.1	59	41.0±1.2	40.7;41.3	0.273
LFAZ		**Entry**	43[d]	-1.7±1.1	-2.0;-1.3	57[d]	-1.3±0.9	-1.5;-1.1	0.068
		Exit	43[d]	-2.0±1.2	-2.4;-1.6	57[d]	-1.6±0.9	-1.9;-1.4	0.081
Entry to exit, days			47[c]	15.1±8.8	12.5;17.7	59	15.3±8.2	13.1;17.4	0.904
Primary outcome	**Change in LFAZ**	**Entry to exit**	43[d]	-0.3±0.5	-0.5;-0.2	57[d]	-0.3±0.4	-0.4;-0.2	0.779
Secondary outcome	**Length gain, cm/wk**	**Entry to exit**	47[d]	1.1±0.5	0.9;1.2	59	1.0±0.5	0.9;1.1	0.530

[a]Two-sample t test

[b]In both groups: Birth data not available for all infants

[c]In Original fortifier group: Data not available for all infants

[d]In both groups: LGA infants excluded from Z-score analysis

Abbreviations: CI confidence interval; cm centimetre; cm/wk centimetre per week; LFAZ length-for-age Z-score**;** LGA large for gestational age; SD standard deviation

Table 21: Comparison between the Original fortifier and Reformulated fortifier groups in terms of head circumference

			Original fortifier (n=58)			Reformulated fortifier (n=59)			p-value[a]
			n	Mean±SD	95% CI	n	Mean±SD	95% CI	
HC, cm		**Birth**	56[b]	27.5±2.0	27.0;28.1	52[b]	27.7±1.8	27.2;28.2	0.587
		Entry	52[c]	28.0±1.6	27.6±28.5	59	28.1±1.3	27.7;28.5	0.717
		Exit	52[c]	29.9±1.3	29.5;30.3	59	30.3±1.2	29.9;30.6	0.095
HCFAZ		**Entry**	48[d]	-1.3±1.2	-1.7±-1.0	57[d]	-0.9±1.0	-1.2;-0.7	0.059
		Exit	48[d]	-1.3±1.1	-1.6±-0.9	57[d]	-0.7±0.8	-0.9;-0.5	0.004
Entry to exit, days			48[d]	14.1±8.8	11.6;16.5	57[d]	15.2±8.3	13.0;17.4	0.512
Primary outcome	**Change in HCFAZ**	**Entry to exit**	48[d]	0.1±0.5	-0.1;0.2	57[d]	0.2±0.5	0.1;0.4	0.056
Secondary outcome	**Head circumference gain, cm/wk**	**Entry to exit**	52[c]	1.0±0.4	0.9;1.1	59	1.0±0.4	0.9;1.1	0.639

[a]Two-sample t test.

[b]In both groups: Birth data not available for all infants.

[c]In OF group: Data not available for all infants.

[d]In both groups: LGA infants excluded from Z-score analysis.

Abbreviations: CI confidence interval; cm centimetre; cm/wk centimetre per week; HC head circumference; HCFAZ head circumference-for-age Z-score**;** LGA large for gestational age; SD standard deviation

Table 22: Linear predicted marginal means by treatment group adjusted for HIV exposure

Outcome: In-hospital growth		Original fortifier	Reformulated fortifier	Difference: Reformulated fortifier – Original fortifier	p-value[a]
		Predicted mean (95% CI)			
Weight	**Change in WFAZ**	-0.459 (-0.575; -0.343)	-0.376 (-0.490; -0.260)	0.083 (-0.804; 0.246)	0.316
	Weight gain velocity, g/kg/d	14.574 (13.42; 15.73)	15.000 (13.85; 16.15)	0.424 (-1.207; 2.055)	0.607
Length	**Change in LFAZ**	-0.313 (-0.434; -0.191)	-0.308 (-0.414; -0.202)	0.005 (-0.156; 0.166)	0.952
	Length gain, cm/wk	1.085 (0.942; 1.228)	0.996 (0.869; 1.123)	-0.089 (-0.281; 0.102)	0.358
HC	**Change in HCFAZ**	0.061 (-0.078; 0.201)	0.220 (0.091; 0.348)	0.158 (-0.032; 0.348)	0.102
	HC gain, cm/wk	0.966 (0.855; 1.077)	0.995 (0.891; 1.099)	0.029 (-0.123; 0.182)	0.706

[a]Linear prediction test

Abbreviations: CI confidence interval; cm/wk centimetre per week; g/kg/d gram per kilogram body weight per day; HC head circumference; HCFAZ head circumference-for-age Z-score**;** HIV human immunodeficiency virus; LGA large for gestational age; LFAZ length -for-age Z-score; WFAZ weight -for-age Z-score

The results as presented in this chapter are interpreted and discussed in the next chapter.

CHAPTER 5:
DISCUSSION

Dietitians, as important members of the multi-disciplinary team,[53-55] have a unique opportunity to contribute to improving preterm infants' nutrition care during their hospitalisation. At the CHBAH, dietitians are responsible for identifying preterm infants in need of fortification, calculating their nutritional requirements, dispensing fortifier and monitoring their feeding tolerance and growth. However, it is well established that preterm infants may not be growing optimally, in both industrialised[2,3,6] and low/middle income countries,[4,5,7,8] and that early growth failure may affect their later health.[13-15] Growth of preterm infants and effectiveness of fortification strategies as practiced at the CHBAH have not been investigated and published, despite it being the fourth largest hospital in the world with a 185 bed neonatal unit. The study compared the in-hospital growth during the intermediate stage of nutrition support of preterm infants receiving exclusive human milk fortified with two different human milk fortifiers.

This chapter discusses the exposure to fortified human milk and the macronutrient analysis of human milk with mid-infrared spectroscopy. The outcome in terms of in-hospital growth, specifically the lack of significant improvement with the RF, is deliberated. The strengths and limitations of the study are considered. The chapter concludes with recommendations for future research as well as for improved nutrition care of preterm infants at the CHBAH.

5.1 Exposure: Fortified human milk

The start of the intermediate stage of nutrition support, defined as the first day of fortification, was day 18 of life for both the OF and RF groups. In both groups, all infants received exclusive human milk with all infants in the OF group receiving only their own mothers' milk. In the RF group one infant received some donor milk in addition to own mothers' milk. During the study period the use of intravenous fluid (dextrose) and parenteral nutrition was minimal, but it was taken into consideration in calculation of fluid and nutrient intake.

In both study groups the body weight of the infants at the start of fortification was slightly higher than the birth weight, indicating that the (expected) initial weight loss after birth had been regained. In both groups fortification only started when infants were close to full feed volumes. In the OF group it started at 167mL/kg/d (97% of full feed volume of 173mL/kg/d) and in the RF group at 165mL/kg/d (94% of full feed volume of 175mL/kg/d). These were higher

than the starting volume of 100mL/kg/d recommended in the literature[17,22,26,37] and in the CHBAH neonatal unit's protocol book,[88] thus indicating a "late" start. The reason(s) for the late start is not clear, but it may be explained by the practice at the CHBAH of adding fortifier per individual feed and that the minimum volume for addition was 20mL (OF) and 25mL (RF) – this is discussed in more detail in 5.2.1.1 Another possible reason could be that due to the high patient load, infants were not screened timeously by dietitians or referred by doctors for fortification. Whether this late start meant that a "critical period" for protein supply had been missed, needs some consideration. Most intervention studies focusing on an early protein supply in preterm infants included parenteral amino acids and had conflicting results in terms of growth.[89-93] However, in a study[94] focusing on enteral protein supply only, standard fortification started at 100mL/kg/d (parenteral nutrition was stopped at this time) and adjustable fortification was subsequently introduced. Important increases in both length and HC (the latter statistically significant) were shown during the first week of adjustable fortification, but a lesser effect was seen during the second week. The authors concluded that this was due to the "high needs of protein being covered during the first week" and that "the benefits of human milk fortification could be improved by introducing standard fortification earlier".[94]

By contrast, a systematic review by Mimouni et al[95] came to the conclusion that "there is little evidence that early compared to late start of fortification affects important outcomes such as early growth". However, it should be noted that only two studies were included in this part of their review and more importantly that volumes for "early" and "late" introduction were 20 to 40mL/kg/d and 100mL/kg/d respectively.[95] This does not address the "very late" start of fortification at 165mL/kg/d in the present study.

In both groups the full feed volumes approached 175mL/kg/d, which fell within the 135 to 200mL/kg/d as recommended by the Koletzko group.[48] Exposure to the RF led to significant increases in estimated intake of protein. Protein intake (3.7g/kg/d) in the RF group met published recommendations (3.5 to 4.5g/kg/d)[48] for preterm infants, but it was not achieved in the OF group (3.4g/kg/d). In the RF group, 68% of infants' protein intake fell within the Koletzko group's recommendations[48] compared to only 40% in the OF group. In the case of energy, the statistically significant higher intake in the OF group was most probably not clinically significant (145kcal/kg/d in OF group versus 142kcal/kg/d in RF group). What is, however, of importance is

that 97% of infants in both groups exceeded the Koletzko et al's [48] energy recommendation (110-130kcal/kg/d). This had a negative impact on the protein-to-energy ratio. Even though the ratio in the RF group (2.6g/100kcal) was at the lower range of the AAP recommendation (2.6 to 3.8g/100kcal),[34] when compared to Koletzko et al[48], neither one of the groups met the lowest range of 3.2g/100kcal. The possible effects of protein intake and the protein-to-energy ratio on in-hospital growth are explored in 5.2.1.1 and 5.2.1.2.

In the present study, fortified human milk was well tolerated in both groups, as was expected.[96,97] Feeds were seldom omitted due to symptoms of intolerance, but in cases where this did happen, it was taken into consideration in the calculation of daily protein and energy intake. It should be kept in mind though that the study was designed to exclude infants who were kept NPO for longer than 24 hours. This was the case in eleven and five infants in the OF and RF groups respectively, with feeding intolerance and suspected NEC being some of the reasons cited in both groups.

The calculated intake of protein and energy was based on estimations for the nutritional content of preterm and mature human milk as recommended by Cormack et al[33] to standardise reporting in neonatal studies. Human milk analysis was therefore undertaken in the present study to judge the relevance of international human milk composition in the South African context. The human milk analysed came from mothers who gave birth to preterm infants with a mean GA of 30 weeks and mean birth weight of approximately 1300g. The preterm infants in both groups in the main study had a similar mean GA of 30 weeks, but a lower mean birth weight of close to 1200g.

In comparison to Cormack et al[33], the significantly higher protein content of both preterm and mature milk (from the mixed sample) needs further consideration. In a systematic review by Boyce et al[39], the protein content of mature milk (1.3g/100mL) fell in-between the values found in the present study (1.4g/100mL) and those recommended by the Cormack group[33] (1.2g/100mL). However, the protein content of preterm milk in the present study was in line with what Boyce et al[39] found (1.9g/100mL in both instances), but higher than the 1.5g/100mL as recommended by Cormack et al.[33] It should be noted though, that Boyce's[39] definition of preterm milk (week 1 of life) and mature milk (week 2 to 8 of life) differs slightly from the definitions used in the present study, which were based on Cormack et al's[33] recommendations. Comparisons to other reviews are made difficult by different classifications in terms of lactation

days, but of interest is that Mimouni et al[40] indicated protein values of 1.98g/100mL to 2.57g/100mL in the first two weeks of life compared to Gildrewicz and Fenton's[41] 1.5g/100 to 2.7g/100mL. The lowest value as indicated by Mimouni et al[40] is close to that found in the present study, whereas the lowest values indicated by Gildrewicz and Fenton[40] corresponds to the Cormack et al[33] recommendations.

The energy content of preterm milk (from the mixed sample) in the present study was comparable to the Cormack group's recommendation[33]. The significantly lower energy content of mature milk (70kcal/100mL) in comparison to the Cormack group's recommendation (72kcal/100mL),[33] is most probably not clinically significant. However, preterm milk according to Boyce et al[39] only had 57kcal/100mL compared to the 66kcal/100mL in the present study. The difference in energy is most probably related to the lower fat and carbohydrate content since the preterm milk as defined by Boyce et al[39] (only the first week of life) included colostrum. The energy content showed by both the Mimouni[40] and Gidrewicz[41] groups for the first three days of life (which included colostrum), was also 59kcal/100mL and 50kcal/100mL respectively, confirming the lower initial energy content of human milk.

These comparisons to the systematic reviews,[39-41] should however be interpreted with caution. Even though in the present study it was attempted to represent a 24-hour sample period, the mixed sample consisted of hind milk only. All three systematic reviews[39-41] only included studies with 24-hour samples and thus would have included foremilk as well. Furthermore, studies from low/middle income countries were excluded by Gidrewicz and Fenton.[41] As far as the researcher is aware, there is only one recent study[98] on the macronutrient content of human milk of South African mothers of preterm infants. In this study once-off samples of hind milk were taken on day seven of lactation (thus assuming preterm milk if lactation started on day one of life) and infrared analysis of macronutrients was performed. Protein levels in the milk of HIV-infected and uninfected mothers were 1.95g/100mL and 1.78g/100mL respectively.[98] Even though there were differences in methodology between the Fouche study[98] and the present study (for example in terms of sample collection and spectrometers used), the protein content is comparable. The energy content in the Fouche study[98] was 70kcal/100mL and 67kcal/100mL in HIV-infected and uninfected mothers, respectively.

The small sample size (n=13) of the preterm milk in the present study could be seen as a limitation, but the coefficient of variation that was calculated (15.8%) indicated that the small sample size did not threaten the estimation. Another factor that needs considering is the mixed samples coming from the mothers in the HMA-designated group only, and not from the mothers in the main study. More studies, specifically with 24-hour samples, are needed to test the results found in the present and in Fouche's study.[98] It would determine whether the Cormack group's[33] recommended values for protein and energy content of human milk are appropriate for use in research studies within the South African context.

The next section discusses the outcome in terms of in-hospital growth.

5.2 Outcome: In-hospital growth

The most important difference between the two fortifiers related to the RF being higher in protein compared to the OF; therefore better growth was expected in the RF group. In a meta-analysis by Lui et al[99], the conclusion was made that "human milk fortifiers with a higher-than-standard protein content can improve preterm infant growth". Contrary to expectation, growth in weight, length and HC in terms of both primary (change in Z-scores) and secondary (anthropometric gains) indices were not statistically different between the two groups. A discussion on the lack of improvement with the RF follows in section 5.2.1.

The drop in Z-scores (from entry to exit) for weight and length in both groups is concerning, yet the initial drop (from birth to entry) should be noted as well. For WFAZ in both groups, the drop was already close to -1SD on entry to the study with the recommendation being to not lose more than 1SD from birth to *discharge* from hospital.[18] Since the study was not designed to look at birth to entry (the acute stage of nutritional care) it is difficult to comment on reasons for this. Change in HCFAZ showed slight improvements in both groups. Even though HCFAZ was not compared to birth HCFAZ, the "slight" improvements in HCFAZ can be interpreted positively in view of the aforementioned recommendation (to aim for not *losing* more than 1 SD in Z-score from birth to discharge).[18]

In both groups weight gain velocity came close to the 15g/kg/d recommended for VLBW infants (15.1±4.7g/kg/d and 14.5±4.3g/kg/d in the RF and OF groups, respectively), but not to the 20g/kg/d recommended for ELBW infants.[17,29,30,62] Similarly, both length and HC gain of 1cm/wk in both groups came close to the recommended 1.1 to 1.4cm/wk and 0.9 to 1.1cm/wk for

length and HC respectively. [17,29,30,62] The "discrepancy" between the negative results (drop in WFAZ and LFAZ) and the positive results (coming close to recommendations for weight gain velocity and length gain) should be interpreted with caution. It is well documented that the targets for weight, length and HC gains/velocities do not "match" growth when evaluated by plotting on growth charts.[66] Both Clark et al[30] and Fenton et al[66] warned against using these in isolation and recommended their use in combination with growth charts (Z-scores). Weight gain velocity should be calculated for intervals of at least five to seven days[66] and should only be used after the initial drop in weight has been regained.[64] All of these recommendations were applied in the present study and should at least in part, be helpful for dealing with the non-linearity of early post-natal growth.

5.2.1 Outcome: Lack of significant improvement of in-hospital growth with the Reformulated fortifier

The lack of significant improvement with the RF on in-hospital growth can possibly be explained by factors related to calculated intake, the period of fortification and interference by confounding factors. Factors relating to intake include the protein intake and the protein-to-energy ratio in the RF group still being too low, the difference in intake between the two groups being too small and the infants not receiving the prescribed amount of milk and fortifier as reported.

Results are subsequently compared to other fortification studies with an emphasis on studies where a higher enteral protein intake was the primary intervention and human milk was used exclusively. Of these studies, Rigo et al [100] stands out as the same two fortifiers were compared in a multi-centre randomised controlled trial. In the Rigo et al [100] study, the OF was used in the "control" group and the RF in the "new" group. The Rigo group[100] reported significantly better in-hospital growth in the new group for weight gain (WFAZ and g/kg/d), but not for length (LFAZ or cm/wk) and HC (HCFAZ or cm/wk).

Comparison to the Rigo et al[100] study is also appropriate since the same growth reference[35] was used for evaluation of Z-scores and the same units were applied for anthropometric gains (g/kg/d for weight and cm/wk for length and HC). In should, however, be noted that in the Rigo et al[100] study, Z-scores were not reported as change (exit minus entry) as in the present study

and the formulae used for calculating g/kg/d weight gain, differed from the one used[63] in the present study.

5.2.1.1 Still too low protein intake in the Reformulated fortifier group

Even though exposure to the RF led to significant increases in calculated protein intake (3.7g/kg/d) and published recommendations (3.5 to 4.5g/kg/d)[48] were met, it was still low when compared to what it could have been. If standard fortification was applied at the volume of milk received (approximately 175mL/kg/d), infants could have received 4.9g/kg/d of protein. The "half-strength dosages" practised in the present study could have been responsible for this (refer to next paragraph for explanation of "half-strength dosages").

In a study on super-fortification by Kanmaz et al[101] with similar protein intakes (3.6g/kg/d compared to 3.3g/kg/d in the "aggressive" and "moderate" fortification groups, respectively) in-hospital growth was also similar between the two levels of protein intake. A retrospective analysis by Picaud et al[94] shows that one in three infants weighing less than 1250g at birth required protein intakes of approximately 4.2g/kg/d to achieve satisfactory growth. In the study by Rigo and co-workers[100], estimated protein intake was 3.8g/kg/d in the control group (i.e. the OF) compared to 4.5g/kg/d in the new group (i.e. the RF). This is a noteworthy observation since even in the control group (i.e. the OF) the estimated intake of protein was higher than in the RF group in the present study. Even though standard fortification was applied in both studies and fortification started at half strength, the present study included these "half-strength days", whereas Rigo et al[100] did not.

In 44 infants (75%) in the RF group, and for no apparent reason, half strength dosages were not increased after feeds were tolerated for 24 hours. Dosages were often kept at half strength for a number of days and in some instances for the entire study period. A possible explanation is that the dietitians were still applying the manufacturer's instructions for the OF (to increase over five to seven days[31]), which spans a much longer period than the 24 hours recommended for the RF.[32] Another factor that may have made the dietitians cautious to increase dosages, especially with the use of a newly formulated product, involved the high occurrence of NEC (approximately 130 infants diagnosed with NEC annually; *Nakwa 24 July 2019 personal communication*) in the unit. Even though in Cochrane reviews [96,102] no indication was given that multi-nutrient fortification increases the risk of NEC, it may still have been a factor. It should be

noted that in some instances the dosage was increased by the dietitian, but the mother and/or nursing staff may not have been compliant.

Apart from the “half-strength dosages”, another contributor to ”lower” protein intake in the RF group involved sub-optimal dosages given at some point during the study in 46 infants (78%). Since the practice at CHBAH is to add fortifier before each feed and to use 1g spoons, this meant that standard fortification (standard dosage: 1g fortifier/25mL of feed) could not be applied in infants receiving volumes not equal to or multiples of 25mL. For example, an infant receiving 36mL x 8 feeds (288mL/day) may have received 1g fortifier in each feed (8g/d), which is 30% less than the standard dosage of 11.5g/d for a volume of 288mL. In some cases, increments of 0.5g fortifier were given, for example for an infant receiving 38mL x 8 feeds, 1.5g fortifier/feed (12g/d) was prescribed, which was close to standard fortification (12.2g/d for a volume of 304mL). The smaller volume of 20mL in which 1g of the OF was added, may have made it slightly easier to avoid sub-optimal dosages in that group.

The extended use of “half-strength dosages” and high occurrence of sub-optimal fortification in the RF group also had a negative effect on the protein-to-energy ratio. Since fortifier is mainly used to add additional protein and the high volume of human milk in the present study contributed to a high energy intake, it led to an imbalance between protein and energy. Protein intake was at the lower level of intake, whereas energy intake exceeded recommendations. The protein-to-energy ratio is discussed in 5.2.1.2.

Another possible reason for the “lower” protein intake in the present study may have been an underestimation of the protein content of human milk. In the present study, preterm human milk composition values (1.5g protein per 100mL; Cormack et al[33]) were used up to day 14 of life and mature milk values (1.2g protein per 100mL; Cormack et al[33]) from day 15 onwards. As was discussed in 5.1, the mid-infrared spectroscopy analysis of human milk in our study indicated significantly higher protein content for both preterm and mature milk in comparison to Cormack et al.[33] If these actual values had been used in the calculation of intake in the present study, the argument of too low protein intake would not stand.

Conversely, in the Rigo et al [100] study preterm milk values (1.62g protein per 100mL milk[49]) were used in all calculations even though approximately 50% of their milk was donor milk (which is usually considered mature milk). Furthermore, the mean post-natal age on day one of the Rigo

et al[100] study was close to 14 days. The authors[100] conceded that the high percentage of donor milk could have led to an overestimation in both protein and energy content. However, regardless of whether intake was over- or underestimated, the Rigo group[100] still reported significantly better weight gain (WFAZ and g/kg/d) in the new group (i.e. RF) when compared to the old group (i.e. OF). This was not the case in the present study.

5.2.1.2 Still too low protein-to-energy ratio in the Reformulated fortifier group

A high protein-to-energy ratio is needed in preterm infants to approximate intrauterine growth,[48,52,103] hence this ratio may offer another explanation for the lack of improved growth in the RF group in the present study. Similar to the protein intake, the protein-to-energy ratio was significantly higher in the RF group. In the case of energy, the significantly higher intake in the OF group was most probably not clinically significant. What is, however, of importance is that 97% of infants in both groups exceeded the Koletzko et al [48] energy recommendation (110-130kcal/kg/d). This had a negative impact on the protein-to-energy ratio. Even though the ratio in the RF group (2.6g/100kcal) was at the lower range of the AAP recommendation (2.6 to 3.8g/100kcal),[34] when compared to Koletzko et al[48], neither one of the groups met the lowest range of 3.2g/100kcal. This leaves the question whether the significant difference in protein-to-energy ratio between the two groups was clinically relevant.

In a study by Arslanoglu et al[79] on adjustable fortification, protein intake, but not energy and fat intake, correlated significantly with both weight gain (g/kg/d) and HC gain (mm/d). In a study by Alan et al[104] comparing adjustable fortification to standard fortification, better growth was seen with a higher protein intake (without adjusting energy intake) indicating the importance of the protein-to-energy ratio. In the Alan et al[104] study, the protein-to-energy ratio was 3.3g/100kcal in the higher protein group. In the study by Rigo et al[100], the protein-to-energy ratio was 3.0g/100kcal and 3.6g/100kcal in the old (i.e. OF) and new (i.e. RF) groups respectively. However, in the Picaud et al[94] study better growth was seen with a much lower protein-to-energy ratio of 2.5g/100kcal, which was close to the 2.6g/100kcal in the RF group in the current study (where better growth was not seen). In the Picaud et al[94] study, the ratio was increased from 2.1 to 2.5g/100kcal, which may indicate that the difference between groups should be at a certain level to see a difference in growth. This is discussed in the next section.

5.2.1.3 Too small difference in intake between the two groups

In spite of the significantly higher protein intake in the RF group, the 0.3g/kg/d difference in protein intake between the two groups may have been too small to result in clinical differences in terms of growth. The same applies to the protein-to-energy ratio that was 0.3kcal/kg/d higher in the RF group when compared to the OF group. In the study by Kanmaz et al[101], a 0.3g/kg/d increase in protein intake between the "aggressive" and "moderate" fortification groups was not sufficient to improve growth either. The energy intake was not reported in the Kanmaz et al[101] study, therefore the protein-to-energy ratio cannot be evaluated.

In the Rigo et al[100] study, protein intake and protein-to-energy ratio between the "old" and "new" fortifier groups differed with 1.2g/kg/d and 0.6g/100kcal respectively. The same difference in protein intake (1.2g/kg/d) in the study on adjustable fortification by Alan et al[104] led to statistically significant increases in daily growth indices (calculated as percentage daily increases) for weight, length and HC, as well as in length and HC gain velocities in VLBW infants. In another study on adjustable fortification by Biasini et al[105], a 1.3g/kg/d increase in protein led to significantly better in-hospital growth in weight (g/kg/d), length (cm/wk) and HC (cm/wk), but only in a subgroup of ELBW infants. However, in a study by Reid et al[106] where the same fortifier as the OF was used (but which also included preterm formula), better growth was not seen when an extra 0.7g/kg/d of enteral protein was added. Also, in a randomised controlled trial by Bellagambia et al[92] (which included additional parenteral amino acids during the acute stage of nutrition care) an additional 1g of protein per day did not improve in-hospital weight gain (g/kg/d) in infants with a birth weight of less than 1250g. One possible explanation would be that the growth rate was already satisfactory (for example approximately 17g/kg/d in the Bellagambia et al[92] study) and that additional protein would then not be beneficial.[106] Interpretation of the different outcomes in these studies remains challenging and Van Goudoever and Moltu[103] mention some possible explanations in an invited commentary on the Bellagambia et al[92] study. These included the protein-to-energy ratio (discussed in 5.2.1.2), the quality of protein administered, and the role of micronutrients. These and other confounding factors are discussed in 5.2.1.6.

5.2.1.4 The infants not receiving the prescribed amount of milk and fortifier as reported

Intake data as documented by the nursing staff may have been inaccurate and infants may have received less milk and/or fortifier than recorded. Mothers added fortifier during the day and had to report to nursing staff, who then documented it, whereas nursing staff were responsible for both adding and recording it during the night. Even though the researcher checked these data regularly with both mothers and nursing staff, it may have been that it was incorrectly documented or that recall bias played a role. The amount of fortifier given may also have been inaccurate due to the practice of using a 1g measuring spoon, especially with the use of half gram dosages. This could have contributed to the infants receiving less milk and fortifier than what the calculated intake suggests. Supporting this argument is the fact that the infants in both groups should theoretically have gained much more weight (especially in terms of fat gain) had they received the energy as calculated that far exceeded recommendations. Inaccurate recording of milk intake and measuring of fortifier could, nevertheless, also have led to infants receiving more protein and energy than what was calculated.

5.2.1.5 Too short period of fortification

The period of fortification in the present study in both groups was approximately 15 days. This period may have been too short to see the effect of the higher protein intake in the RF group on the infants' growth. Intervention in other fortification studies[79, 100, 104] where a higher protein intake had a significant effect on in-hospital growth, lasted longer. Studies on adjustable fortification by Alan et al[104] and Arslanoglu et al[79] each lasted 21 days and showed significant effects in terms of anthropometric gains in weight,[79,104] length [104] and HC [79,104]. In the study by Rigo et al[100], significant differences in weight gain (WFAZ and g/kg/d), but not in length and HC gain, were seen after 21 days. Even though it is recognised that the period of fortification was only one of many factors in these studies, it is interesting to note that a significant gain in length was only seen in the study by Alan et al[104]. This may indicate that a 21-day intervention period may still be too short to see a difference in length, a conclusion also reached by the Rigo group[100]. The same may apply to HC, since in the Rigo et al[100] study significant improvements in HCFAZ were only seen at 40 weeks corrected age. Raghuran[107], who studied head growth trajectories and neurodevelopmental outcomes in preterm infants, reported that poor HC growth in preterm infants was often seen during NICU admission and that catch-up growth often occurred only once infants had been discharged form hospital.

The argument that a longer intervention period in the present study may have yielded better results is firstly supported by the significant difference in the change in WFAZ between birth and exit between the two groups. Even though this included the acute stage of nutrition care (which was not documented), it relates to a longer period of nutrition intervention of approximately 33 days in total. It is recommended by Cormack et al[33] that growth studies in preterm infants should report growth in relation to birth rather than from a nadir or from the time when birth weight was regained. A second argument would be that the exit weight in the present study was not 1650g as originally aimed for, but 1570±123g and 1588±116g in the OF and RF groups respectively. It is possible that better growth could have been seen if fortification had lasted longer and all infants had reached 1650g, the discharge weight at the CHBAH, at the time of the study.

5.2.1.6 Confounding factors may have played a role

The two fortification groups were very similar in terms of birth anthropometry, birth weight categories, and other baseline characteristics, including exposure to HIV. Whether being exposed to HIV (but not infected) affects growth in infancy has been debated for some time.[108-110] In the present study, growth outcomes were adjusted for HIV exposure and no significant differences were seen. Anthropometric measurements as well as the day of life and post-menstrual age on study entry were also comparable between the two groups. There may have been other confounding factors, for example co-morbidities like chronic lung disease and patent ductus arteriosus that could have negatively affected growth.[9] Asbury et al[9] indicated that both nutritional (macronutrient and energy) and non-nutritional (baseline characteristics, acuity, morbidity) factors were independently associated with growth trajectories during the different stages of hospitalisation. Of the postnatal non-nutritional factors, they found that being diagnosed with a patent ductus arteriosus had the largest effect on growth and affected weight, length and HC. Another factor that may have been different between the two groups concerns the extent to which KMC was practiced. At the CHBAH the KMC unit only has a limited number of beds and in the other neonatal wards where mothers only visited a few times a day, KMC is practiced intermittently or not at all, depending on the infants' condition. A meta-analysis by Boundy et al[111] found that KMC had a positive effect on growth in terms of HC, but not on weight or length growth.

In addition to the total protein and protein-to-energy ratio as discussed in 5.2.1 and 5.2.2, other intake factors should be considered as well. The OF and RF also differed in terms of the extent of the hydrolysis of the protein fraction, the contribution from fat and carbohydrate to total energy, the type of fat and micronutrient content. In terms of effect on in-hospital growth, these differences were assumed to be very small, especially after the addition of fortifier to human milk with a variable nutrient content itself. The intake of some micronutrients may need further consideration. Since the functions of micronutrients are interrelated, it is difficult to look at them in isolation, but Sjostrom et al[12] found some micronutrients to be independent predictors of early growth in extremely preterm infants. A low folate intake was associated with poor weight and length gain and a high iron intake with poor growth in length and HC. In the present study, both folic acid and iron supplementation was given routinely in both groups as part of the hospital's protocol, but blood transfusions may have been an additional source, especially of iron. Sjostrom et al[12] did not find any significant association between zinc intake and growth outcome, but Harris et al[112] found a positive association between enteral zinc intake and weight gain in preterm infants. Zinc was not routinely supplemented in the OF or RF groups in the present study. Another micronutrient that may be associated with growth is phosphorous, which together with calcium and vitamin D play an important role in bone mineralisation.[12,103] Vitamin D was given routinely in both groups as part of the hospital's protocol, but phosphate and calcium supplementation differed based on individual S-phosphate, S-calcium and S-alkaline-phosphatase levels. The intake of micronutrients may therefore have been different between the two groups.

The factors discussed in Section 5.2.1.1 to 5.2.1.6 may all have played a role in the lack of significant improvement in in-hospital growth with the RF and they are therefore not mutually exclusive. The strengths and limitations of the study should also be considered when interpreting results. These follow in the next section.

5.3 Strengths and limitations

The comparative effectiveness of the study implies both strengths and limitations. Its strength lies therein that data were collected in a real-life setting and that recommendations can be made to improve nutrition care of preterm infants in that specific setting. As far as the researcher is aware, it was the first study at the CHBAH where the growth of preterm infants receiving exclusive human milk was prospectively assessed. To our knowledge, it was also the

first study where the new formulation of the fortifier was prospectively evaluated in a middle/low income country.

The exclusive use of human milk in this study makes an important contribution to preterm infant growth studies since it is well established that the growth and body composition of such infants differ from those receiving formula feeds.[113-115] Furthermore, in a country where resources are limited and formula feeding use is high,[116] research can aid in promoting, protecting and supporting exclusive breastfeeding (Tshwane declaration[117]). Since practically no donor milk was given in this study, it further contributes to the understanding of the advantages and challenges associated with the use of mothers' own milk in preterm infants. The exploratory nature of the human milk analysis by mid-infrared spectroscopy creates the opportunity for more research to follow. The experience of using the human milk analyser also gave the researcher the opportunity to evaluate target fortification as a strategy to be implemented at the CHBAH.

Another strong point of the study is that all data collection and all anthropometric measurements were done by the same person (the researcher). The researcher, who is a staff member at the CHBAH, was familiar with the conditions in the neonatal unit, for example the record keeping, abbreviations used and transfer of infants between the wards. Even though the researcher works in the neonatal unit, the infants she was seeing in her capacity as a hospital dietitian (those who had/required gastrointestinal surgery) at the time of the study were excluded. Data were meticulously recorded every 24 to 48 hours and growth measurements done at least once every seven days. The same calibrated scale and length board were used in both groups to measure weight and length respectively. Growth was evaluated by using more than one index, namely changes in Z-scores and anthropometric gains. Since anthropometric gains are calculated independently of the infant's age, it would have countered the possible limitation of using inaccurate GA estimations. A validated formula[63] was used to calculate weight gain velocity. However, the high internal validity may come at the expense of the ability to generalise, with relevance of the findings possibly limited to comparable settings in South Africa and other low/middle income countries only. In these settings, an important contribution has been made to nutrition and growth studies in preterm infants.

A limitation of the comparative effectiveness design of the study lies in its non-randomisation. However, in the present study the two groups were very similar in all baseline characteristics

studied, which may have been due to “natural selection”, hence minimising this potential threat. The almost 60 infants per group also added power to the study.

The high drop-out rate in both groups is concerning and may potentially have led to bias. The infants who dropped out (but continued to be in the hospital) could have been included in an intention-to-treat analysis. However, since most of these infants received formula feeds, including them in the analysis would not have shown the best estimated effect[118] of fortification of human milk, which was the aim of the comparative effectiveness design of the study. Statistical analysis could have been employed to project growth in all infants to 1650g, the exit weight, which was not reached by all infants included in the present analysis. A longer study period and follow-up until all infants had reached 40 weeks corrected age could also have added value to the study.

The nutrition status of the preterm infants was only evaluated by anthropometry (and no body composition was done) and not by other parameters, for example biochemistry. Even though the plan initially was to include S-urea values as a measure of protein intake, it was not frequently available *and* the time points when it was done did not correspond to entrance and exit dates to the study.

Another limitation relates to the assumed composition of human milk, which was shown to be different when analysed with mid-infrared spectroscopy. Since this analysis had some limitations in itself, for example in the methodology of collecting the 24-hour “representative” sample and the use of hind milk only, the results need to be confirmed by larger studies dedicated to determining macronutrient composition of South African human milk. If the difference in composition is confirmed, the findings of the present study could be re-evaluated. Considering current evidence, the researcher did follow the guidelines by Cormack et al[33] for reporting of nutrition and growth studies in both the exposure (macronutrient composition of human milk) as well as in the outcome (growth velocity calculated with exponential method;[63] growth reported as change in Z-scores; Fenton 2013[35] growth charts used). The lack of standardised reporting limits comparisons between the present and other studies and one of the recommendations made in the following section addresses this.

5.4 Recommendations for future research

Recommendations for future research are firstly based on the reporting and evaluation of nutrition and growth in preterm infants; and secondly on improving nutrition care for these infants in South Africa and other low/middle income countries.

- In order to improve comparisons between different studies, the reporting on nutrition in preterm infants should be standardised so that "apples can be compared to apples".[33] Cormack and co-workers[33] may consider revising their recommendation for the macronutrient content of human milk (currently based on studies done between 1976 to 2009) on the more recent reviews by Boyce[39], Mimouni[40] and Gidrewicz[42]. There should also be consensus on the evaluation and reporting of growth in preterm infants. The search for the "ideal" parameters for growth evaluation in preterm infants should continue. Body composition studies could give guidance in this regard.
- The higher than expected protein content of the mothers' milk analysed in the present study indicate an urgent need for data on nutrient content of breast milk in South Africa and other low/middle income countries. Obtaining 24-hour samples from mothers of preterm infants (without affecting the infants' nutrition care) presents an ethical dilemma and collection methods to "represent" 24-hour samples should be validated. Also, in South Africa growth of preterm infants receiving fortified human milk should be investigated in a larger multi-centre trial and factors influencing growth should be identified. The "ideal" human milk fortifier and fortification strategy for use in resource limited hospital settings with a high patient-to-staff ratio should be studied. Finally, research should focus on providing the most "effective"[20] nutrition for preterm infants considering all the challenges experienced in neonatal units in low/middle income countries.

Practical recommendations for providing more effective nutrition care for preterm infants at the CHBAH are made in the next section.

5.5 Recommendations for improved nutrition care of preterm infants at the CHBAH

The recommendations that can be made based on the present study are discussed in terms of the intake of fortified human milk and growth outcomes.

5.5.1 Intake of fortified human milk

- The high drop-out rate in the study due to the early introduction of preterm formula is a concern. Measures should be put in place to improve breastfeeding rates, which may include appointing lactation management consultants or sisters, expanding on KMC beds and lodging facilities for the mothers, and establishing a donor milk bank. The efforts that have been made by the personnel of the CHBAH to become a "Baby Friendly" hospital should be expanded on and the expert group recommendations made by Nyquivist et al[119] could be useful in this regard.
- The late start (in terms of volume of milk) of fortification and use of half strength dosages for longer than recommended periods is another concern. The aim should be to start fortification sooner (at lower milk volumes) and to use half strength dosages for longer than 24h only in cases of feeding intolerance. The adoption of a screening tool may be useful in timeously identifying all preterm infants in need of fortification.
- There should be better control over the intake in order to ensure that fortified EBM is given as prescribed. One measure, to use 1g sachets instead of decanting the tinned fortifier, has been implemented since the study was done. Another measure that can be investigated would be that the fortifier is added in a controlled environment, for example in the milk kitchen and not on a ward level. In such an environment the amount of fortifier could be weighed and it could be added to a larger volume of milk (e.g. to two feeds instead of only one), which may help to solve the problem of "sub-optimal" fortification dosages.
- The use of adjustable fortification in order to individualise nutrition care should be looked into. Using the S-urea value in order to monitor protein intake is an attainable measure and a modular protein supplement is available in the hospital. S-urea is already done in these infants – it could be done more often, especially in ELBW infants and in infants not growing adequately on standard fortification. A combination of the S-urea level and weight gain (g/kg/d) during the preceding week can also be used to guide protein supplementation (as was done in the study by Picaud[94]).

5.5.2 Growth outcome

- Weight should not be the only growth parameter to be used. Length and especially HC should be measured weekly in all preterm infants to monitor brain and lean body mass

growth. Head circumference is an easy measurement to take and no specialised equipment is needed.

- Growth should not only be evaluated in terms of anthropometric gains (g/kg/d for weight and cm/wk for length and HC), but also in terms of Z-scores. Consensus should be reached on the method and the time period to be used when calculating weight gain velocity at the CHBAH.
- Using an arbitrary weight as discharge weight for all preterm infants, irrespective of PMA and in-hospital growth, should be re-evaluated. Since the study was done, the discharge weight of 1.65kg has been increased to 1.75kg. However, it would be recommended to rather look at each infant individually if weight gain has been satisfactory in terms of both g/kg/d and change in Z-scores.

5.6 CONCLUSION

In South Africa, where eight out of every 100 babies are born prematurely, the growth of these infants has been under-researched. In this study, the in-hospital growth of VLBW preterm infants receiving human milk fortified with two different formulations was described. Growth, as evaluated by weight, length and HC was inadequate in both groups and possible reasons for it were deliberated. This lack of adequate in-hospital growth urgently needs further attention, not only in terms of more research studies, but also in practical solutions to optimise the nutrition care offered to these infants. Preterm birth needs to be seen as a nutritional emergency[21] and the consequences of not acting timeously to meet nutrition requirements, should not be forgotten. In the words of the late Gabriella Mistral: *"Many of the things we need can wait. The child cannot. Now is the time his blood is made, his bones are formed, his senses developed. To him we cannot say tomorrow, his name is today".*

REFERENCES

1. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller A, Narwal R, et al. National, regional and worldwide estimates of preterm birth rates in the year 2010 with time trends for selected countries since 1990: a systematic analysis and implications. Lancet 2012;379(9832):2162-72. http://dx.doi.org/10.1016/S0140-6736(12)60820-4

2. Mackay CA, Ballot DE, Cooper PA. Growth of a cohort of very low birth weight infants in Johannesburg, South Africa. BMC Pediatr 2011;11:504. http://dx.doi.org/10.1186/1471-2431-11-50

3. Lango MO, Horn AR, Harrison MC. Growth velocity of extremely low birth weight preterms at a tertiary neonatal unit in South Africa. J Trop Pediatr 2013;59(2):79-83. http://dx.doi.org/10.1093/tropej/fms049

4. Clark R, Thomas P, Peabody J. Extra uterine growth restriction remains a serious problem in prematurely born infants. Pediatrics 2003;111:986-90. http://dx.doi.org/10.1542/peds.111.5.986

5. Cooke R, Ainswoth S, Fenton A. Post-natal growth retardation: a universal problem in preterm infants. Arch Dis Child Fetal Neonatal Ed 2004;89:F428-30. http://dx.doi.org/10.1136/adc.2001.004044

6. Mukhopadhyay K, Mahajan R, Louis D, Narang A. Longitudinal growth of very low birth weight neonates during the first year of life and the risk factors for malnutrition in a developing country. Acta Paediatr 2013;102:278-81. http://dx.doi.org/10.1111/apa.2013.102.issue-3

7. Harbor JD, Ehrenkranz RA, Badger GJ, Edwards EM, Morrow KA, Soll RF, et al. Weight growth velocity and postnatal growth failure in infants 501 to 1500g: 2000-2013. Pediatrics 2015;136(1):e84-92.

8. Zozaya C, Avila-Alvarez A, Couce ML, Rodrigo FG, Aruzza L, Fernandez-Perez C, et al. Cohort study show that growth rate increments has not been enough to prevent growth retardation of preterm infants and raised concerns about unbalanced growth. Acta Paediatr 2019;108:1793-800.

9. Asbury MR, Unger S, Kiss A, Ng DVY, Luk Y, Bando N, et al. Optimizing the growth of very-low-birth-weight infants requires targeting both nutritional and nonnutritional modifiable factors to stage of hospitalization. Am J Clin Nutr 2019;00:1-11.

10. Grover A, Khashu M, Mukherjee. Iatrogenic malnutrition in neonatal intensive care units: urgent need to modify practice. J Parenter Enteral Nutr 2008;32:140-4.

11. Sjöström ES, Öhlund I, Ahlsson F, Engström E, Fellman V, Hellström, A et al. Nutrient intakes independently affect growth in extremely preterm infants: results from a population-based study. Acta Paediatr 2013;102:1067-74. http://dx.doi.org/10.1111/apa.12359

12. Sjöström ES, Öhlund I, Ahlsson F, Domellöf M. Intakes of micronutrients are associated with early growth in extremely preterm infants. J Pediatr Gastroenterol Nutr 2016;62:885-92. http://dx.doi.org/10.1097/MPG.0000000000001085

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

14. Schneider N, Garcia-Rodenas CL. Early nutritional interventions for brain and cognitive development in preterm infants: a review of the literature. Nutrients 2017;9(187):1-20.

15. Ong KK, Kennedy K, Castaneda-Gutienez E, Forsyth S, Godfry KM, Koletzko B, et al. Postnatal growth in preterm infants and later health outcomes: a systematic review. Acta Paediatr 2015;104:974-86.

16. Eidelman AI, Schanler RJ. Breastfeeding and the use of human milk. Pediatrics 2011; 129(3):600-3.

17. Ziegler EE. Meeting the nutritional needs of the low-birth-weight infant. Ann Nutr Metab 2011;58(Suppl 1):8-18. http://dx.doi.org/10.1159/000323381

18. Moro GE, Arslanoglu S, Bertino E, Corvaglia L, Montirosso R, Picaud J, et al. Human milk in feeding premature infants. Proceedings of a consensus development conference-

EXPO 2015, May 15-16, Milan, Italy,. J Pediatr Gastroenterol Nutr 2015;61(S1):S1-S19. http://dx.doi.org/10.1097/MPG0000000000000897

19. Underwood MA. Human milk for the premature infant. Pediatr Clin North Am 2013;60(1):189-207. http://dx.doi.org/10.1016/j.pcl.2012.09.008

20. Harding JE, Cormack BE, Alexander T, Alsweiler JM, Bloomfield FH. Advances in nutrition in the newborn infants. Lancet 2017;389:1660-8.

21. Corpeleijn WE, Vermeulen MJ, Van den Akker CH, Van Goudoever JB. Feeding very-low-birth-weight infants: our aspirations versus reality in practice. Ann Nutr Metab 2011;58(Suppl 1):S20-9. http://dx.doi.org/10.1159/000323384

22. Su B. Optimizing nutrition in preterm infants. Pediatrics and Neonatology 2014;55:5-13. http://dx.doi.org/10.1016/jpedneo.2013.07003

23. Lunde D. Extrauterine growth restriction: what is the evidence for better nutritional practices in the neonatal intensive care unit? Newborn & Infant Nursing Reviews 2014;(14)92-8. http://dx.doi.org/10.1053/j.nainr.2014.06.008

24. Ahrabi AF, Schanler RJ. Human milk is the only milk for premies in the NICU! Early Hum Dev 2013;89:S51-3. http://dx.doi.org/10.1016/j.earlhumdev.2013.08.006

25. Kim JA, Chan CS, Vaucher YE, Stellwagen LM. Challenges in the practice of human milk nutrition in the neonatal intensive care unit. Early Hum Dev 2013;89:S25-S38.

26. Di Natale C, Coclite E, Di Ventura L, Di Fabio S. Fortification of maternal milk for preterm infants. J Matern Fetal Neonatal Med 2011;24(S1):41-3. http://dx.doi.org/10.3109/14767058.2011.607569

27. Raban MS, Joolay J, Harrison MC. Enteral feeding practices in preterm infants in South Africa. S Afr J CH 2013;7(1):8-12.

28. Arslanoglu S, Moro GE, Ziegler EE. Preterm infants fed fortified human milk receive less protein than they need. J Perinatol 2009;29:489-92. http://dx.doi.org/10.1038/jp.2009.50

29. Tudehope D, Gibbons K, Cormack B, Bloomfield F. Growth monitoring of low birthweight infants: What references to use? J Pediatr Child Health 2012;48:759-67.

30. Clark RH, Olsen IE, Spitzer AR. Assessment of neonatal growth in prematurely born infants. Clin Perinatol 2014;259-307. http://dx.doi.org/10.1016/j.clp.2014.02.001

31. FM85 Product information leaflet for healthcare professionals. Nestlé South Africa. ("Old" FM85) https://www.nestlenutrition-institute.org/country/za

32. FM85 Product information leaflet for healthcare professionals. Nestlé South Africa. ("New" FM85) https://www.nestlenutrition-institute.org/country/za

33. Cormack BE, Embleton ND, Van Goudoever JB,Hay WW, Bloomfield FH. Comparing apples with apples: it is time for standardized reporting of neonatal nutrition and growth studies. Pediatr Res 2016;79(6)810-20. http://dx.doi.org/10.1038/pr.2016.26

34. American Academy of Pediatrics Committee on Nutrition. Nutritional needs of the preterm infant. In: Kleinman RE, Greer FR eds. Pediatric Nutrition. 7th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2014:83-121.

35. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for premature infants. BMC Pediatrics. 2013;13:59-72.

36. Olsen IE, Lawson ML, Meinzen-Derr J, Sapsford AL, Schibler KR, Donovan EF, et al. Use of body proportionality index for growth assessment of preterm infants. J Pediatr 2009;154:486-91. http://dx.doi.org/10.1016/j.peds.2008.10.012

37. Dutta S, Singh B, Chessell L, Wilson J, Janes M, McDonald K, et al. Guidelines for feeding very low birth weight infants. Nutrients 2015;7:423-42.

38. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am. 2013; 60(1): 49-74. http://dx.doi.org/10.1016/jpcl.2012.19.002

39. Boyce C, Watson M, Lazidis G, Reeve S, Dods K, Simmer K, et al. Preterm human milk composition: a systematic literature review. Br J Nutr 2016;116:1033-45. http://dx.doi.org/10.1017/S0007114516003007

40. Mimouni FB, Lubetzky R, Yochpaz S, Mandel D. Preterm human milk macronutrient and energy composition. A systematic review and meta-analysis. Clin Perinatal 2017;44:165-72. http://dx.doi.org/10.1016/j.clp.2016.11010

41. Gildrewicz D, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. BMC Pediatr 2014;14:216. http://www.biomedcentral.com/1471-2431/14/216

42. Groh-Wargo S, Valentic J, Khaira S, Super DM, Collin M. Considering human milk variability in the nutritional management of low-birth-weight infants. ICAN: Infant, Child, & Adolescent Nutrition 2014;6(5):301-4. http://dx.doi.org/10.1177/1941406414536611

43. Bravi F, Wiens F, Decarli A, Dal Pont V, Agostoni C, Ferraroni M. Impact of maternal nutrition on breast-milk composition: a systematic review.[1,2] Am J Clin Nutr 2016;104:646-62.

44. Burianovo I, Bronsky J, Pavlikova M, Janota J, Maly J. Maternal body mass index, parity and smoking are associated with human milk macronutrient content after preterm delivery. Early Hum Dev 2019;137:104832. http://dx.doi.org/10.1016/j.earlhumdev.2019.104832

45. Hsu Y, Chen C, Lin M, Tsai C, Liang J, Wang T. Changes in preterm breast milk nutrient content in the first month. Pediatrics and Neonatology 2014;55:449-54.

46. Radmacher PG, Adamkin DH. Fortification of human milk for preterm infants. Semin Fetal Neonatal Med.2017;22:30-5. http://dx.doi.org/10.1016/j.siny.2016.08.004

47. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Durmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: Commentary from the European Society for Paediatric Gastroenterology, Hepataology, and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr 2010;50(1):85-91. http://dx.doi.org/10.1097/MPG.0b013e3181adaee0

48. Koletzko B, Poindexter B, Uauy R. Recommended nutrient intake levels for stable, fully enterally fed very low birth weight infants. In: Koletzko B, Poindexter B, Uauy R, eds.

Nutritional care of preterm infants: scientific basis and practical guidelines. Basel: Karger; 2014:297-9. http://dx.doi.org/10.1159/000360195

49. Tsang RC, Uauy R, Koletzko B, Zlotkin SH. Nutrition of the preterm infant: scientific basis and practical guidelines. 2nd ed. Cincinatti: Digital Education Publishing 2005;415-6.

50. Brennan A, Murphy BP, Kiely ME. Conference on 'Nutrition at key life stages: new finding, new approaches' Symposium 2: Nutrition in early life. Optimising preterm nutrition: present and future. Proc Nutr Soc 2016;75:154-61. http://dx.doi.org/10.1017/S002966511600136

51. Hay WW, Thureen P. Protein for preterm infants: how much is needed? How much is enough? How much is too much? Pediatr Neonatol 2010;51(4)198-207.

52. Civardi E, Tzialla C, Garofoli F, Mazzucchelli I, Bollani L, Stronati M. Nutritional needs of premature infants. J Matern Fetal Neonatal Med 2011;24(S1):27-9.

53. Sneve J, Kattelmann K, Ren C, Stevens DC. Implementation of a multidisciplinary team that includes a registered dietitian in a neonatal intensive care unit improved nutrition outcomes. Nutr Clin Pract 2008;28(6):630-4.

54. Loomsi ST, Lalonde E, Pasto V, Smith A. The impact of registered dietitian nutritionists on the nutrition management and outcomes in very low birth weight infants. Support Line 2016;38(6):11.

55. Olsen IE, Richardson DK, Schmid CH, Ausman LM, Dwyer JT. Dietitian involvement in the neonatal intensive care unit: more is better. J Am Diet Assoc 2005;105:1224-30.

56. Bhatia J. Growth curves: how to best measure growth of the preterm infant. J Pediatr 2013;162(Suppl 1):S2-6. http://dx.doi.org/10.1016/j.peds.2012.11.047

57. Strydom K, Van Niekerk E, Dhansay MA. Factors affecting body composition in preterm infants: assessment techniques and nutritional interventions. Pediatrics and Neonatology 2019;60:121-8. http://dx.doi.org/10.1016/j.pedneo.2017.10.007

58. Pereira-da-Silva L, Virella D. is intrauterine growth appropriate to monitor postnatal growth of preterm neonates? BMC Pediatr 2014;14:14.

59. Neubauer V, Fuchs T, Griesmaier E, Pupp-Peglow U, Kiechl-Kohlendorfer U. Comparing growth charts demonstrated significant deviations between interpretation of postnatal growth patterns in very preterm infants. Acta Paediatr 2016;105:268-73. http://dx.doi.org/10.1111/apa.13175

60. Sankilampi U. One size may not fit all when it comes to growth references for preterm infants. Acta Paediatr 2016;105:228-9. http://dx.doi.org/10.1111/apa.13291

61. Casey PH. Growth of very low birth weight preterm children. Semin Perinatol 2008;32:20-7.

62. Pereira-da-Silva L, Virella D, Fusch C. Nutritional assessment in preterm infants: a practical approach in the NICU. Nutrients 2019;11,doi:10.3390/nu11091999.

63. Patel AL, Engstrom JL, Meier PP, Jegier BJ, Kimura RE. Calculating postnatal growth velocity in very low birth weight (VLBW) premature infants. J Perinatol 2009;29:618-22.

64. Roelants JA, Joosten KFM, Van der Geest BMA, Hulst JM, Reiss IKM, Vermeulen MJ. First week weight dip and reaching growth targets in early life in preterm infants. Clin Nutr 2018;37:1526-33.

65. Fenton TR, Anderson D, Groh-Wargo S, Hoyos A, Ehrenkranz RA, Senterre T. J. An attempt to standardize the calculation of growth velocity of preterm infants – evaluation of practical bedside methods. Pediatr 2018;196:77-83. http://dx.doi.org/10.1016/j.jpeds.2017.10.005

66. Fenton TR, Senterre T, Griffin IJ. Time interval for preterm infant weight gain velocity calculation precision. J Arch Dis Child Fetal Neonatal Ed 2019;104:F218-9. http://dx.doi.org/10.1136/archdischild-2018-31484

67. Lubchenco LO, Hansman C, Boyd E. Intrauterine growth in length and head circumference as estimated from live births at gestational ages from 26 to 42 weeks. Pediatrics 1966;37:403-8.

68. Aris IM, Kleinman KP, Belfort MB, Kaimal A, Oken M. A 2017 US reference for singleton birth weight percentiles using obstetric estimates of gestation. Pediatrics 2019;144(1):e20190076. http://dx.doi.org/10.1542/peds.2019-0076

69. Carberry AE, Gordon A, Bond DM, Hyett J, Raynes-Greenow CH, Jeffery HE. Customised versus population-based growth charts as a screening tool for detecting small for gestational age infants in low-risk pregnant women. Cochrane Database Syst Rev. 2011;12:CD008549.

70. Villar J, Giuliani F, Bhutta ZA, Bertino E, Ohuma EO, Ismail LC, et al. Postnatal growth standards for preterm infants: the preterm postnatal follow-up study of the INTERGROWTH-21st project. Lancet Glob Health 2015;3:e681-91.

71. McCarthy EA, Walker SP. International fetal growth standards: one size fits all. Lancet 2014;384:834-6.

72. Saugstad OD. New growth charts for newborn babies. Lancet 2014;384:833-5.

73. Fenton TR, Sauve RS. Using the LMS method to calculate z-scores for the Fenton preterm infant growth chart. Eur J Clin Nutr 2007;61:1380-5. http://dx.doi.org/10.1038/sj.ejcn.1602667

74. Rochow N, Raja P, Liu K, Fenton TR, Landau-Crangle E, Gottler S, et al. Physiological adjustment to postnatal growth trajectories in healthy preterm infants. Pediatr Res 2016;79:870-979.

75. Landau-Crangle E, Rochow N, Fenton TR, Liu KL, Ali A, So HY, et al. Individualized postnatal growth trajectories for preterm infants. J Parenter Enter Nutr 2018;42:1084-92. http://dx.doi.org/10.1002/jpen.1138

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

77. Embleton ND, Van den Akker CHP. Protein intakes to optimize outcomes for preterm infants. Semin Perinatol. 2019. Article in press.

78. Rogerro P, Gianni ML, Morlacchi L, Piemontese P, Liotto N, Taroni F, et al. Blood urea nitrogen concentrations in low-birth-weight preterm infants during parenteral and enteral nutrition. J Pediatr Gastroenterol Nutr 2010;51:213-15. http://dx.doi.org/10.1097/MPG.0b013e3181cd270f

79. Arslanoglu S, Moro GE, Ziegler EE. Adjustable fortification of human milk fed to preterm infants: does it make a difference? J Perinatol 2006; 614-621. http://dx.doi.org/10.1038/sj.jp7211571

80. Sox HC, Greenfield S. Comparative effectiveness research: A report from the Institute of Medicine. Ann Intern Med 2009;151:203-5.

81. Conway PH, Clancy C. Comparative-Effectiveness Research – Implications of the federal coordinating council's report. N Engl Med 2009;361(4):328-30.

82. Benchimol EI, Smeeth L, Guttmann A. The Reporting of studies conducted using observational routinely-collected health data (RECORD) statement. PLoS Med 2015;12(10):e1001885.doi:10.1371/journal.pmed.1001885.

83. Berger ML, Mamdani M, Atkins D, Johnston ML. Good research practices for comparative effectiveness research: defining, reporting and interpreting nonrandomized studies of treatment effects using secondary data sources: the ISPOR good research practices for retrospective database analysis task force report – part I. Value Health 2009;12(8):1044-52.

84. Agget P, Agostoni C, Axelsson I, Goulet O, Hernell O, Koletzko B, et al. Core data for nutrition trials in infants: a discussion document – a commentary by the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr 2003;36(3):338-42.

85. Nieman DC. Nutritional assessment. 7th edition. Chapter 6: Anthropometry (p155-204). McGraw Hill Education. New York.2019.

86. Fenton 2013. Growth calculator for preterm infants – Peditools. https://peditools.org>fenton2013.

87. MIRIS HMA™ Human milk analyser. User manual. 2017.

88. Saloojee H, Nakwa F, Velaphi S ed. CHBAH Neonatal protocols. 4th ed. Johannesburg; 2015.

89. Cormack BE, Bloomfield F, Dezoete A, Kuschel C. Does more protein in the first week of life change outcomes for very low birthweight infants? Journal of Paediatrics and Child Health 2011;47:898-903. http://dx.doi.org/10.1111/1440-1754.2011.02106.x

90. Uthaya S, Liu X, Babalis D, Dore CJ, Warwick J, Bell J, et al. Nutritional evaluation and optimisation in neonates: a randomized, double-blind controlled trial of amino acid regimen and intravenous lipid composition in preterm parenteral nutrition. Am J Clin Nutr 2016;103:1443-52. http://dx.doi.org/10.3945/acjn.115.125138.

91. Morgan C, McGowan P, Herwitker S, Hart AE, Turner MA. Postnatal head growth in preterm infants: a randomized controlled parenteral nutrition study. Pediatrics 2014:133:e120-28.

92. Bellagambia MP, Carmenati E, D'Ascenzo R, Malatesta M, Spagnoli C, Biagetti C, et al. One extra gram of protein to preterm infants from birth to 1800g: a single-blinded randomized clinical trial. J Pediatr Gastroenterol Nutr 2016;62(6):879-884. http://dx.doi.org/10.1097/MPG.0000000000000989

93. Ng DVY, Brennan-Donnan J, Unger S, Bando N, Gibbins S, Nash A et al. How close are we to acieving energy and nutrient goals for very low birth weight infants in the first week? J Parenter Enteral Nutr 2017;41(3):500-6. http://dx.doi.org/10.1177/0148607115594674

94. Picaud J, Houeto N, Buffin R, Loys C, Godbert I, Hays S. Additional protein fortification is necessary in extremely low-birth-weight infants fed human milk. J Pediatr Gastroenterol Nutr 2016;63(1):103-5. http://dx.doi.org/10.1097/MPG.0000000000001142

95. Mimouni FB, Nathan N, Ziegler EE, Lubetzky R, Mandel D. The use of multinutrient human milk fortifiers in preterm infants. A systematic review of unanswered questions. Clin Perinatol 2017;44:173-8.

96. Brown JV, Embleton ND, Harding JE, McGuire W. Multi-nutrient fortification of human milk for preterm infants. Cochrane Database Syst Rev 2016;5:CD000343.

97. Moody GJ, Schanler RJ, Lau C, Shulman RJ. Feeding tolerance in premature infants fed fortified human milk. J Pediatr Gastroenterol Nutr 2000;30(4):408-12.

98. Fouche C. Breastmilk composition of HIV-infected mothers receiving antiretroviral therapy who gave birth to premature infants. [dissertation] Stellenbosch: University of Stellenbosch; 2016.

99. Liu T, Dang D, Lv X, Wang T, Du J, Wu H. Human milk fortifier with high versus standard protein content for promoting growth of preterm infants: a meta-analysis. J Int Med Res 2015;43(3):279-89. http://dx.doi.org/10.1177/0300060515579115

100. Rigo J, Hascoet J, Billeaud C, Picaud J, Mosca F, Rubio A, et al. Growth and nutritional biomarkers of preterm infants fed a new powdered human milk fortifier: a randomized trial. J Pediatr Gastroenterol Nutr 2017;65(4):e83-e93. http://dx.doi.org/10.1097/MPG.0000000000001686

101. Kanmaz HG, Mutlu B, Canpolat FE, Erdeve O, Oguz SS, Uras N, et al.Human milk fortification with differing amounts of fortifier and its association with growth and metabolic response in preterm infants. J Hum Lact 2012; 29(3): 400-405. http://dx.doi.org/10.1177/0890334412459903

102. Kuschel CA, Harding JE. Multicomponent fortified human milk for promoting growth in preterm infants. Cochrane Database Syst Rev. 2004; Issue1. Art.No: CD000343.

103. Van Goudoever JB, Moltu S. Have we reached the limits with regard to amino acid/protein intakes in preterm infants? J Pediatr Gastroenterol Nutr 2016;62(6):797-8. http://dx.doi.org/10.1097/MPG.0000000000001130

104. Alan S, Atasay B, Cakir U, Yildiz D, Kilic A, Kahvecioglu D, et al. An intention to achieve better postnatal in-hospital-growth for preterm infants: Adjustable protein fortification of human milk. Early Hum Dev 2013; 89: 1017-1023. http://dx.doi.org/10.1016/j.earlhumdev.2013.08.015

105. Biasini A, Marvulli L, Neri E, China M, Stella M, Monti F, et al. Growth and neurological outcome in ELBW preterms fed with human milk and extra-protein supplementation as

routine practice: do we need further evidence? J Matern Fetal Neonatal Med 2012; 25(S4): 72-74. http://dx.doi.org/10.3109/14767058.2012.715032

106. Reid J, Makrides M, McPhee AJ, Stark MJ, Miller J, Collins CT. The effect of increasing the protein content of human milk fortifier to 1.8g/100mL on growth in preterm infants: a randomised controlled trial. Nutrients 2018;10(634);doi:10.3390/nu10050634.

107. Raghuram K, Yang J, Church PT, Cieslak Z, Synnes A, Mukerji A. Head growth trajectory and neurodevelopmental outcomes in preterm neonates. Pediatrics 2017;140(1):e20170216.

108. Ramakolo V, Lombard C, Fadness LT, Doherty T, Jackson DJ, Goga AE, et al. HIV infection, viral load, low birth weight and nevirapine are independent influences on growth velocity in HIV-exposed South African infants. J Nutr 2014;144:42-48.

109. Venkatesh KK, Lurie MN, Triche EW, De Bruyn G, Harwell JI, McGarvey ST, et al. Growth of infants born to HIV infected woman in South Africa according to maternal and infant characteristics. Trop Med and Int Health 2010;15(11):1364-74.

110. Neri D, Somarriba GA, Schaefer NN, Chaparro AI, Scott GB, Mitnik GL, et al. Growth and body composition of uninfected children exposed to HIV: comparison with a contemporary cohort and United States national standards. J Pediatr 2013;163:249-52.

111. Boundy EO, Dastjerdi R, Spiegelman D, Fawzi WW, Missmer SA, Lieberman E, et al. Kangaroo mother care and neonatal outcomes: a meta-analysis. Pediatrics 2016;137(1):e20152238.

112. Harris T, Gardner F, Podany A, Kellener SL, Doheny KK. Increased early zinc intake improves weight gain in hospitalised preterm infants. Acta Paediatr 2019;108:1978-84.

113. Mol N, Zasada M, Kwinta P. Does type of feeding affect body weight composition in VLBW infants? – A prospective cohort study. Pediatrics and Neonatology 2019;60:135-40.

114. Hay WW, Hendrickson KC. Preterm formula use in the preterm very low birth weight infant. Semin Fetal Neonatal Med 2017;22:15-22.

115. Morlacchi l, Rogerro P, Gianni ML, Bracco B, Porri D, Battiato E, et al. Protein use and weight-gain quality in very-low-birth-weight preterm infants fed human milk or formula. Am J Clin Nutr 2018;107:195-200.

116. Siziba LP, Jerling J, Hanekom SM, Wentzel-Viljoen E. Low rates of exclusive breastfeeding are still evident in four South African provinces. S Afr J Clin Nutr 2015;28(4):170-9.

117. The Tshwane declaration of support for breastfeeding in South Africa. S Afr J Clin Nutr 2011;24:4.

118. Johnston BC, Guyatt CH. Best (but oft-forgotten) practices: intention-to-treat, treatment adherence and missing participant outcome data in the nutrition literature. Am J Clin Nutr 2016;104(1):197-201.

119. Nyqivist KH, Haggkvist A, Hansen MN, Kylberg E, Frandsen AL, Maastrup RM. Expansion of the baby-friendly hospital initiative ten steps to successful breastfeeding into neonatal intensive care: expert group recommendations. J Hum Lact 2013;29(3):300-9.

ANNEXURES

ANNEXURE 1:
NUTRITION CARE RECORD (GAUTENG DEPARTMENT OF HEALTH)

Name:

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

GAUTENG DEPARTMENT OF HEALTH
NUTRITION CARE RECORD

A. PERSONAL PARTICULARS

Name: _____ Classification: _____

Hospital Nr: _____ Age: _____ Sex: _____ Race: _____ DOB: _____

Contact Details: Cell: _____ Other : ( ) _____

Physical /Postal address: _____

_____ Province: _____ Country of origin: _____

B. DIETARY RESTRICTIONS

Special dietary restrictions: _____ Allergies: _____

C. REFERRAL

Source of referral: _____ Contact no: _____

Referral date & time: _____ First contact date & time (RD): _____

D. ADMISSION

DATE	ADMISSION PARTICULARS	TREATING DIETITIAN	WARD & BED
	1. Reason for referral: 2. Diagnosis: 3. Other Illnesses: 4. Tests: 5. Precautions: DR / TB / Pregnancy / Isolation		
	1. Reason for referral: 2. Diagnosis: 3. Other Illnesses: 4. Tests: 5. Precautions: DR / TB / Pregnancy / Isolation		
	1. Reason for referral: 2. Diagnosis: 3. Other Illnesses: 4. Tests: 5. Precautions: DR / TB / Pregnancy / Isolation		

Nov-17

F. MEDICAL PARTICULARS

History:

Past Treatment:

G. GENERAL INFORMATION

Home language: **Have you seen a dietitian before?**

Present Occupation: **Educational level:**

Sources of income / Grants: **Type:** **Amount:**

How many members in the household? **Adults:** **Children:**

Type of housing and facilities:

	Inside	Outside	Communal
Water:			
Electricity:	Yes	No	
Fridge:	Yes	No	
Freezer:	Yes	No	
Other:			

	Brick	Corrugated Iron		Other
Type of Housing:				
Cooking facilities:	Electric	Gas	Paraffin	Outside

Who prepares the food?

Who buys the food?

	Yes	No	
Exercise:			**Comments:**
Smoking:			**Comments:**
Alcohol:			**Comments:**

H. NUTRITIONAL ASSESSMENT

H1. CLINICAL EVALUATION

		CLINICAL SIGN	POSSIBLE NUTRIENT METABOLIC ASSOC.		CLINICAL SIGN	POSSIBLE NUTRIENT METABOLIC ASSOC.
General		LOW, muscle mass & fat stores	Protein-energy		Fatigue, anaemia	Iron
		Excess fat stores	Excess energy		Emmaciated	PEM
Nails		Koilonychia	Iron		Pale, mottled	Vit A/ Vit C
		Dull, lackluster	Protein/ Iron		Blood splintering	Vit C
		Brittle	Vit A, Protein		White transverse lines	Protein
		Hyperpigmented	Vit B_3			
Hands		Interossei muscle wasting	PEM		Thenar wasting	PEM
		Palmar erythema	Liver failure		Yellow palms	Excess Vit A
Arms		Muscle wasting	Protein-energy		Follicular hyperkeratosis	EFA/ Vit A
		Hyperpigmentation	Vit B_3			
Face		Diffuse depigmentation, swollen	Protein		Moon face	Protein, energy
		No zygomatic fat pad	PEM		Tophi	Uric acid excess
		Pallor	Iron/ Vit B_{12}/ B_3		Bilat. temporal wasting/ sunken orbits	Protein, energy
Hair & Scalp		Craniotabes/ open anterior fontanel	Vit D		Easily pluckable	Protein
		Lack of shine & luster, thin, sparse	Prot/ Zn/ Ω 6		Flag sign	Protein
Eyes		Pale conjunctive	Iron/ Vit B_{12}/ B_3		Corneal/ conjunctival xerosis	Vit A
		Night blindness	Vit A		Keratomalacia	Vit A
		Bitot's spots	Vit A		Angular palpebritis	Riboflavin/ Niacin
		Corneal arcus	Excess cholesterol		Kayser-Fleischer ring	Excess Copper
Nose		Nasolabial seborrhea	$B_2/B_3/B_{12}$		Inflamm. redness of sinus tract, discharge, obstruction or polyp	Reconsideration of NGT placement
Oral cavity, lips & mouth		Angular stomatitis	Vit B_2, B_3, B_{12}		Edentulous	Excess sugar
		Cheilosis	Vit B_2, B_3		Teeth: missing, caries, loose	Excess sugar
		Spongy, bleeding, receding gums	Vit C		White/ Brownish patches	Excess fluoride
		Candidiasis	Low immunity			
Tongue		Magenta colour	$B_2/B_3/B_{12}$		Beefy red colour, atrophied taste buds	Zinc
		Atrophic filiform papillae	$B_2/B_3/B_6/B_9/B_{12}$/Fe		Hypoguesia	Vit C
Neck		Enlarged thyroid	Iodine deficiency		Parotid gland bilateral enlargement	Protein
		Casals necklace	Vit B3			
Thorax		Beading of ribs	Vit D/ Calcium		Scaphoid appearance	Protein/ energy
Abdomen		Generalized symmetric distention	Obesity		Ascites	Prot., Sodium, fluid
Legs & Feet		Bowed legs	Vit D/ Calcium		Muscle wasting	Protein-energy
		Oedema	Inflammation/ Protein			
Skin		Poor healing, ulcers	Prot/ Vit C/ Zn		Yellow pigmentation	Carotene excess
		Xerosis	EFA/ Vit A		Poor skin turgor	fluid loss
		Follicular hyperkeratosis	EFA/ Vit A		Petechiae, ecchymoses	Vit K/ Vit C
		Pallor	Iron/ folic acid		Pelagrous dermatitis	Niacin/ tryptophan
Neurological		Low/ absent mental alertness	Influences feeding		Peripheral neuropathy	Vit B_1, B_6, B_{12}
		Psychomotor changes, confusion	Protein		Tetany	Calcium, Magnesium
		Irritability	Protein-energy			

H. NUTRITIONAL ASSESSMENT												
H2. ANTHROPOMETRICS		NORMAL VALUES	DATE									
ADULTS & PAEDIATRICS	Age											
	Height (m) / Length (cm)											
	Weight (Kg)											
	BMI (Kg/m^2)											
	MUAC (cm)											
ADULT	Triceps Skinfold (mm)											
	Arm muscle area (mm^2)											
	Arm fat area (mm^2)											
	Knee height (cm)											
	Middle circumference (cm)											
	Hip circumference (cm)											
	Ulna length (cm)											
	Calf circumference (cm)											
PAEDIATRICS	Weight for Age (z-score)											
	Height for Age (z-score)											
	BMI for Age (z-score)											
	Weight for Height (z-score)											
	Head circumference (cm)											
	MUAC for age (z-score)											
	Triceps Skinfold (z-score)											
	DOB:		GA(birth)weeks:					Corrected Age:				
	Birth-weight (Kg):		Length:					HC:				

* All babies & children must have a RtHC & WHO growth chart/ disease specific growth chart
* RtH Booklet up to date? Y / N

INTERPRETATION OF NUTRITIONAL ASSESSMENT (H1 & H2)

DATE	REMARKS

Patient Name: Hosp nr/ ID: Pg:

I. PROGRESS REPORT / DISCHARGE PLAN

DATE	TIME	MONITORING & INTERVENTION	DIETITIAN (Surname,Designation & HPCSA nr printed)

ANNEXURE 2:

NUTRITIONAL CONTENT OF ORIGINAL AND REFORMULATED FORTIFIERS

NUTRIENT	CONTENT PER 100g OF POWDER	
	ORIGINAL FORTIFIER[31]	REFORMULATED FORTIFIER[32]
Energy, kcal	347.6	434.5
Protein, g	20	35.5
Fat, g	0.4	18.1
Medium chain triglycerides, g	-	11.7
Arachidonic acid, mg	-	13.8
Docosahexaenoic acid, mg	-	157
Eicosapentaenoic acid, mg	-	36.7
Linoleic acid, mg	-	958
Alpha-linolenic acid, mg	-	417
Carbohydrates, g	66	32.4
Maltodextrin, g	60	32.4
Natrium, mg	520	918
Potassium, mg	1320	1210
Chloride, mg	460	803
Calcium, mg	1500	1890
Phosphorous, mg	900	1095
Magnesium, mg	80	100
Manganese, mg	126	202
Iron, mg	34.4	45
Iodine, µg	260	423
Copper, mg	1	1.3
Zinc, mg	18	23.5
Selenium, µg	50	93
Chromium, µg	19	23
Molybdenum, µg	20	20
Fluoride, µg	60	60
Vitamin A, IU	23664.3	29583.33
Vitamin D, IU	3000	3760
Vitamin E, IU	119.2	149.25
Vitamin K, µg	160	200
Vitamin C, mg	350	500
Vitamin B_1, mg	3	3.75
Vitamin B_2, mg	4	5
Niacin, mg	30	37.5
Vitamin B_6, mg	2.6	3.25
Folic acid, µg	800	1000
Pantothenic acid, mg	14	17.5
Vitamin B_{12}, µg	2.25	5
Biotin, µg	70	87.5
Choline, mg	171	215
Inositol, mg	78	111
Taurine, mg	36	50
Carnitine, mg	70	66

ANNEXURE 3A:

RESEARCH ETHICS APPROVAL CERTIFICATE: UNIVERSITY OF PRETORIA

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

23/08/2017

Approval Certificate
New Application

Ethics Reference No: 286/2017

Title: In-hospital growth of very low birth weight preterm infants: comparative effectiveness of two human milk fortifiers

Dear Mrs Johanna Kemp

The **New Application** as supported by documents specified in your cover letter dated 20/07/2017 for your research received on the 21/07/2017, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 23/08/2017.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year
- Please remember to use your protocol number (**286/2017**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers, MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

☎ 012 356 3084 deepeka.behari@up.ac.za / fhsethics@up.ac.za http://www.up.ac.za/healthethics
✉ Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4, Room 60, Gezina, Pretoria

ANNEXURE 3B:

RESEARCH ETHICS APPROVAL CERTIFICATE: UNIVERSITY OF PRETORIA (AMENDMENT)

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

28/09/2017

Approval Certificate
Amendment
(to be read in conjunction with the main approval certificate)

Ethics Reference No: 286/2017

Title: In-hospital growth of very low birth weight preterm infants: comparative effectiveness of two human milk fortifiers

Dear Mrs Johanna Kemp

The **Amendment** as described in your documents specified in your cover letter dated 30/08/2017 received on 31/08/2017 was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 27/09/2017.

Please note the following about your ethics amendment:
- Please remember to use your protocol number (**286/2017**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committe may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics amendment is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed; PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

☎ 012 356 3084 deepeka.behari@up.ac.za / fhsethics@up.ac.za http://www.up.ac.za/healthethics
✉ Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4, Room 60, Gezina, Pretoria

ANNEXURE 4A:

RESEARCH ETHICS APPROVAL CERTIFICATE: UNIVERSITY OF THE WITWATERSRAND

R14/49 Ms Johanna Kemp

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170546

NAME: (Principal Investigator)	Ms Johanna Kemp
DEPARTMENT:	Human Nutrition Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	In-hospital Growth of very Low birth Weight Preterm Infants: Comparative Effectiveness of two Human milk Fortifiers
DATE CONSIDERED:	26/05/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr D Wenhold and Dr F Nakwa
APPROVED BY:	Professor P. Cleaton-Jones Chairperson, HREC (Medical)
DATE OF APPROVAL:	31/07/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review May and will therefore be due in the month of May each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 21/8/2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

ANNEXURE 4B:

RESEARCH ETHICS APPROVAL CERTIFICATE: UNIVERSITY OF THE WITWATERSRAND (AMENDMENT)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

27 September 2017

Ms Johanna Kemp
92 Maluti Street
Northcliff ext 25
Johannesburg
2195

Sent by email to:kemridge@absamail.co.za

Dear Ms Kemp

Re: **Protocol Ref no: M170546**
Protocol Title: In-hospital Growth of very Low Birth Weight Preterm Infants: Comparative Effectiveness of two Human Milk Fortifiers
Principal Investigator: Ms Johanna Kemp
Protocol Amendment: Request to add Human Milk Samples

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has approved the protocol amendment on the abovementioned protocol, as detailed in your letter dated 07 September 2017.

The following documents were received:

- Cover Letter dated 07 September 2017.
- Study Proposal.
- Signed Declaration dated 21 August 2017.
- Approval Certificate University of Pretoria.
- Informed Consent document.
- Human Milk Analyzer (User Manual).

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Thank you for keeping us informed and updated.

Yours Sincerely,

L. Moeng

Mr Lebohang Moeng
Administrative Assistant
Human Research Ethics Committee (Medical)

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
27 SEP 2017
RESEARCH OFFICE HREC (Medical)
JOHANNESBURG

Research Office Secretariat: Faculty of Health Sciences, Phillip Tobias Building, 3rd Floor, Office 302, Corner York Road and 29 Princess of Wales Terrace, Parktown, 2193 Private Bag 3, Wits 2050 I T+27 (0)11-717-1234/2656/2700/1252 E: Lebo.Moeng@wits.ac.za I **Office E** HREC-MedicalResearchOffice@wits.ac.za IWebsite: www.wits.ac.za/research/about-our-research/ethics-and-research-integrity/

ANNEXURE 5A:

CHBAH: PERMISSION TO CONDUCT RESEARCH

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 15 May 2017

TITLE OF PROJECT: In-hospital growth of very low birth weight preterm infants: comparative effectiveness of two human milk fortifiers

UNIVERSITY: Pretoria

Principal Investigator: JE Kemp

Department: Dietetics

Supervisor (If relevant): FA Wenhold, F Nakwa

Permission Head Department (where research conducted): Yes

Date of start of proposed study: May 2017
Date of completion of data collection: 2020

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Hospital. The CEO /management of Chris Hani Baragwanath Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Human Research Ethics Committee of the University of the Witwatersrand.
- the Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- the MAC will be informed of any serious adverse events as soon as they occur
- permission is granted for the duration of the Ethics Committee approval.

.....
Recommended
(On behalf of the MAC)
Date: 15 May 2017

.....
Approved/Not Approved
Hospital Management
Date: 17/05/17

ANNEXURE 5B:

CHBAH: PERMISSION TO CONDUCT RESEARCH (AMENDMENT)

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 9 October 2017

TITLE OF PROJECT: In-hospital growth of very low birthweight preterm infants: comparative effectiveness of two human milk fortifiers

UNIVERSITY: Pretoria

Principal Investigator: JE Kemp

Department: Dietetics and Human Nutrition

Supervisor (If relevant): F Nakwa

Permission Head Department (where research conducted): Yes

Date of start of proposed study: October 2017
Date of completion of data collection: Dec 2020

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Hospital. The CEO /management of Chris Hani Baragwanath Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Human Research Ethics Committee of the University of the Witwatersrand.
- the Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- the MAC will be informed of any serious adverse events as soon as they occur
- permission is granted for the duration of the Ethics Committee approval.

.....
Recommended
(On behalf of the MAC)
Date: 09 October 2017

.....
Approved/Not Approved
Hospital Management
Date: 19/10/17

ANNEXURE 6:

DATA COLLECTION SHEET FOR BIRTH INFORMATION

NAME…….. HOSPITAL NO…….
DOB …… GENDER: Male/Female
WARD…… CUBICLE……..
STUDY NO……

INFANT

Place of birth	CHBAH	Other hospital		BBA
GA (weeks)		Ballard	Ultra-sound	Dates
Birth weight (g)		VLBW	ELBW	
Birth length (cm)				
Birth head circumference (cm)				
Mode of delivery	NVD		C/S	
If C/S: reason for preterm delivery				
Apgar scores (out of 10)	1 min	5 min	10 min	Unknown
Perinatal complications				
Initial diagnosis				
Additional information				

MOTHER

Age (years)			
Para/Gravida	P	G	
Antenatal care received	Yes	No	
Marital status	Married/Living with partner	Unmarried	Unknown
RVD status	Positive	Negative	Unknown
Complications during pregnancy			
Medication during pregnancy			
Vitamin/mineral supplements during pregnancy			
Educational level			
Socio-economic status			
Additional information			

ANNEXURE 7: DATA COLLECTION SHEET FOR ANTHROPOMETRIC DATA

NAME……………………………… HOSPITAL NO…………………………… DOB………………………………

GENDER: Male/Female WARD…………. CUBICLE……………. STUDY NO……………..

Date	Time	Weight (g)		Length (cm)		Head circumference (cm)		Comments
		Reading 1	Reading 2	Reading 1	Reading 2	Reading 1	Reading 2	

ANNEXURE 8: DATA COLLECTION SHEET FOR INTAKE AND OUTPUT

NAME……. HOSPITAL NO……. DOB…….....

WARD…… CUBICLE…….. STUDY NO……..

INTAKE AND OUTPUT

Date	Time	Intake: Enteral				Intake: IV fluids (mL/24h)		Output and Feeding tolerance				Comments
		Route	Type and volume of feed received (mL/24h or g/24h)			TPN	Other IV fluids*	Vomiting	Stool output	GRV's Reflux Aspiration	Abdomen	
		OGT/ NGT/ Per os	MOM: EBM/BF	DBM	FM85							

* Excluding IV fluids for medici

ANNEXURE 9: CHBAH DIETETIC DEPARTMENT HUMAN MILK FORTIFICATION AUDIT 2016: DATA COLLECTION SHEET

NAME…….. HOSPITAL NO……. WARD…… CUBICLE…… BED…….

DOB…….. GENDER: Male/Female GESTATIONAL AGE……..

BIRTH WEIGHT……..g BIRTH LENGTH……cm BIRTH HEAD CIRCUMFERENCE…….cm

DATE OF INITIATION OF FORTIFICATION…… DATE OF END OF FORTIFICATION/DISCHARGE……

Date	Anthropometry						Biochemistry	Intake: Enteral				Intake: IV fluids	Output and Feeding tolerance				Comments e.g. Adverse effects
	Weight (g)		Length (cm)		Head= circumference (cm)		S-urea (mmol/L)	Route		Type and volume of feed received (mL or g/24h)		TPN/ Other IV fluids received (mL/24h)	Vomiting	Stool output	GRV's Reflux Aspiration	Abdomen	
	1	2	1	2	1	2		OGT/ NGT/ Per os	MOM: EBM/ BF	DBM	FM85						

ANNEXURE 10:

SANAS CERTIFICATE FOR CALIBRATION WEIGHTS USED IN THE CALIBRATION OF SECA SCALE (MODEL 334, HAMBURG GERMANY)

weighing instrument services (PTY) LTD.
Reg. No. 1982/002054/07

63 EARP STREET, OPHIRTON
P.O. BOX 38122, BOOYSENS 2016
TEL: +27 - 11 - 493-6075
FAX: +27 - 11 - 493-2706
E-Mail: wis@wissa.co.za

sanas Calibration Laboratory
No. 1407

University of Pretoria
Faculty of Health Sciences, Dept. Human Nutrition

CALIBRATION CERTIFICATE No. 376/17 Page 1 of 1

Calibration of : A set of weights

Procedure : The weights have been calibrated against standards of known value, traceable to the national standard, in accordance with our procedure WIS (1)

Traceability : Certificate of Calibration No: M16-182 Dated : March 2016

Results :

Identification Number	Nominal Value (g)	Actual Value (g)	Uncertainty of Calibration (g)+ or -
SET HN 1	2 000	1 999,99	0,05
	1 000	1 000,00	0,03
	500	500,006	0,010
	50	50,001	0,002
	5	5,000 5	0,000 5
	2	2,000 2	0,000 5
	1	1,000 3	0,000 5

Uncertainty : The uncertainties of calibration were calculated and expressed in accordance with the BIPM, IEC, ISO, IUPAP, OIML document entitled "Guide to the Expression of Uncertainties in Measurement" (International Organisation for Standardisation, Geneva, Switzerland, 1993) and are based on a standard uncertainty multiplied by a coverage factor of k=2, which provides a level of confidence of approximately 95%

Validity : The values given in this certificate are correct at the time of calibration. Subsequently the accuracy will depend on such factors as the care exercised in handling and use of the instrument as well as the frequency of use. Recalibration should be performed after such a period which is chosen to ensure that the accuracy remains within the desired limits.

Calibrated By : G Bekker

Graeme Brolly
TECHNICAL SIGNATORY

Date Calibrated : 14 March 2017

Date Issued : 15 March 2017

ANNEXURE 11:

PARENT INFORMATION AND INFORMED CONSENT DOCUMENT

TITLE OF STUDY: IN-HOSPITAL GROWTH OF VERY LOW BIRTH WEIGHT PRETERM INFANTS: COMPARATIVE EFFECTIVENESS OF TWO HUMAN MILK FORTIFIERS

Dear Parent

1) INTRODUCTION

Hannelie Kemp (a dietitian at the hospital and a PhD student at the University of Pretoria) invites you and your baby to participate in a research study. This information leaflet will help you to decide if you want your baby to participate. Before you agree that your baby takes part, you should fully understand what is involved. If you have any questions that this leaflet does not fully explain, please do not hesitate to ask the investigator Hannelie Kemp or Dr Firdose Nakwa (co-supervisor).

2) THE NATURE AND PURPOSE OF THIS STUDY

The aim of this study is to see how babies receiving breastmilk with FM85 powder added to it, grow.

3) EXPLANATION OF PROCEDURES TO BE FOLLOWED

This study will use the information written about you and your baby in your baby's hospital file. We may ask you some additional questions about your baby's birth. We will weigh your baby and measure your baby's length and head circumference at the beginning and at the end of the study as well as once a week during the study. We will also ask you about the amount of FM85 powder that you are adding to your breastmilk. The study will not alter the feeding or treatment or procedures that your baby would be receiving normally.

4) RISK AND DISCOMFORT INVOLVED

There are no risks in participating in the study. Some of the processes may cause minimal discomfort for your baby for example when we weigh and measure your baby we have to take the nappy off.

5) POSSIBLE BENEFITS OF THIS STUDY

Although your baby will not benefit directly from the study, the results of the study will ensure that preterm babies grow at their best.

6) WHAT ARE YOUR RIGHTS AS A PARTICIPANT?

You and your baby's participation in this study are entirely voluntary. You can refuse to participate or stop at any time during the study without giving any reason. Your withdrawal will not affect your baby's treatment in any way.

7) HAS THE STUDY RECEIVED ETHICAL APPROVAL?

This study has received written approval from the Research Ethics Committee of the Faculty of Health Sciences at the University of Pretoria (Reference no 286/2017), telephone numbers 012 356 3084 / 012 356 3085 and from the University of Witwatersrand Health Research and Ethics Committee (Reference no M170546) and the Medical Advisory Committee of CHBAH (Approval letter dated 15 May 2017).

8) INFORMATION AND CONTACT PERSON

The contact person for the study is Hannelie Kemp. If you have any questions about the study please contact her at the following telephone number 083 755 2692. Alternatively you may contact my supervisors Dr Firdose Nakwa at 011 933 1000 or Dr Friede Wenhold at 012 356 3202.

9) COMPENSATION

You and your baby's participation are voluntary. No compensation will be given for your baby's participation.

10) CONFIDENTIALITY

All information that you give will be kept strictly confidential. Once we have analysed the information no one will be able to identify you. Research reports and articles in scientific journals will not include any information that may identify you or your baby or the hospital.

CONSENT TO PARTICIPATE IN THIS STUDY

I confirm that the person asking my consent to take part in this study has told me about nature, process, risks, discomforts and benefits of the study. I have also received, read and understood the above written information (Information Leaflet and Informed Consent) regarding the study. I am aware that the results of the study, including personal details about me and my baby, will be anonymously processed into research reports. I am participating willingly. I have had time to ask questions and have no objection to my baby participating in the study. I understand that there is no penalty should I wish to discontinue with the study and my withdrawal will not affect my baby's treatment in any way.

I have received a signed copy of this informed consent agreement.

Participant's name .....(Please print)

Participant's signature: ..... Date.....

Investigator's name Hannelie Kemp

Investigator's signature ..... Date.....

Witness's Name .....(Please print)

Witness's signature .....Date.....

VERBAL INFORMED CONSENT

I, the undersigned, have read and have fully explained the participant information leaflet, which explains the nature, process, risks, discomforts and benefits of the study to the participant whom I have asked to participate in the study.

The participant indicates that she understands that the results of the study, including personal details about herself and her baby will be anonymously processed into a research report. The participant indicates that she has had time to ask questions and has no objection to participate in the study. She understands that there is no penalty should she wish to discontinue with the study and her withdrawal will not affect her baby's treatment in any way. I hereby certify that the client has agreed that her baby can participate in this study.

Participant's Name .....(Please print)

Person seeking consent .....(Please print)

Signature .....Date.....

Witness's name .....(Please print)

Signature .....Date.....

ANNEXURE 12:

PARENT INFORMATION AND INFORMED CONSENT DOCUMENT: HUMAN MILK SAMPLING

TITLE OF STUDY: IN-HOSPITAL GROWTH OF VERY LOW BIRTH WEIGHT PRETERM INFANTS: COMPARATIVE EFFECTIVENESS OF TWO HUMAN MILK FORTIFIERS

Dear Parent

1) INTRODUCTION

Hannelie Kemp (a dietitian at the hospital and a PhD student at the University of Pretoria) has invited you and your baby to participate in a research study for which you have given informed consent on…….(date). She would now like to invite you to take part in an additional part of the same study. This information leaflet will help you to decide if you want to participate in this part of the study. Before you agree to take part, you should fully understand what is involved. If you have any questions that this leaflet does not fully explain, please do not hesitate to ask the investigator Hannelie Kemp or Dr Firdose Nakwa (co-supervisor).

2) THE NATURE AND PURPOSE OF THIS STUDY

The aim of this part of the study is to see how much goodness there is in your milk.

3) EXPLANATION OF PROCEDURES TO BE FOLLOWED

This study will use the information written about you and your baby in your baby's hospital file. We will ask you to give us two samples (about two teaspoons each time) of your milk. We will put your milk into a machine which will then tell us how much goodness there is in your milk. The machine will only test for the goodness (the protein, fat and sugar in your milk) and cannot test for anything else. The study will not alter the feeding or treatment or procedures that your baby would be receiving normally.

4) RISK AND DISCOMFORT INVOLVED

There are no risks in participating in the study. It may cause you minimal discomfort to express your milk into a test tube. We will only be asking you for milk if you have expressed enough milk for your baby.

5) POSSIBLE BENEFITS OF THIS STUDY

Although you or your baby will not benefit directly from the study, the results of the study will help us to know how much goodness there is in the milk of mothers who gave birth to preterm babies.

6) WHAT ARE YOUR RIGHTS AS A PARTICIPANT?

Your participation in this study is entirely voluntary. You can refuse to participate or stop at any time during the study without giving any reason. Your withdrawal will not affect your baby's treatment in any way.

7) HAS THE STUDY RECEIVED ETHICAL APPROVAL?

This study has received written approval from the Research Ethics Committee of the Faculty of Health Sciences at the University of Pretoria (Reference no 286/2017; Amendment approved 28 September 2017), telephone numbers 012 356 3084 / 012 356 3085 and from the University of Witwatersrand Health Research and Ethics Committee (Reference no M170546; Amendment approved 27 September 2017) and the Medical Advisory Committee of CHBAH (Approval letters dated 15 May 2017 and 9 October 2017).

8) INFORMATION AND CONTACT PERSON

The contact person for the study is Hannelie Kemp. If you have any questions about the study please contact her at the following telephone number 083 755 2692. Alternatively you may contact my supervisors Dr Firdose Nakwa at 011 933 1000 or Dr Friede Wenhold at 012 356 3202.

9) COMPENSATION

Your participation is voluntary. No compensation will be given to you for providing us with milk samples.

10) CONFIDENTIALITY

All information that you give will be kept strictly confidential. Once your milk samples have been analysed, no one will be able to identify you. Research reports and articles in scientific journals will not include any information that may identify you or your baby or the hospital.

CONSENT TO PARTICIPATE IN THIS STUDY

I confirm that the person asking my consent to take part in this study has told me about nature, process, risks, discomforts and benefits of the study. I have also received, read and understood the above written information (Information Leaflet and Informed Consent) regarding the study. I am aware that the results of the study, including personal details about me and my baby, will be anonymously processed into research reports. I am participating willingly. I have had time to ask questions and have no objection to my baby participating in the study. I understand that there is no penalty should I wish to discontinue with the study and my withdrawal will not affect my baby's treatment in any way.

I have received a signed copy of this informed consent agreement.

Participant's name ……(Please print)

Participant's signature: …… Date.....

Investigator's name Hannelie Kemp

Investigator's signature …… Date.....

Witness's Name .....(Please print)

Witness's signature .....Date.....

VERBAL INFORMED CONSENT

I, the undersigned, have read and have fully explained the participant information leaflet, which explains the nature, process, risks, discomforts and benefits of the study to the participant whom I have asked to participate in the study.

The participant indicates that she understands that the results of the study, including personal details about herself and her baby will be anonymously processed into a research report. The participant indicates that she has had time to ask questions and has no objection to participate in the study. She understands that there is no penalty should she wish to discontinue with the study and her withdrawal will not affect her baby's treatment in any way. I hereby certify that the client has agreed that her baby can participate in this study.

Participant's Name ..... (Please print)

Person seeking consent ..... (Please print)

Signature .....Date.....

Witness's name ..... (Please print)

Signature .....Date.....

ANNEXURE 13:

DATA COLLECTION SHEET FOR HUMAN MILK SAMPLING

SURNAME……..... HOSPITAL NO……

DOB …… GENDER: Male/Female

WARD……..CUBICLE…….. STUDY NO……..

DATE……..

INFANT's NAME:

Place of birth	CHBAH	Other hospital		BBA
GA (weeks)		Ballard	Ultra-sound	Dates
Birth weight (g)				
Birth length (cm)				
Birth head circumference (cm)				
Mode of delivery	NVD		C/S	
If C/S: reason				
Multiple birth	Yes		No	
Diagnosis				
Type of feeding	EBM	EBM + DBM		EBM + Formula
Mode of feeding	OGT/NGT	Per os (cup/syringe/bottle)		Breastfed
FM85 fortification	Yes		No	
Most recent weight	Date:		Weight (g):	

MOTHER's NAME:

Age (years)				
Gravida/Para	G		P	
Antenatal care received	Yes		No	
Lodging	Staying at home		Lodger	KMC
RVD status	Positive		Negative	Unknown
Co-morbidities				
Medication				
Diet	Traditional	Vegetarian		Vegan
Supplements	Mom2B	Vitamin/minerals	Other	
MUAC (cm)	Reading 1:		Reading 2:	

HUMAN MILK SAMPLING

	Date and time of collection	Volume of sample
Day sample		
Night sample		

ANNEXURE 14:
MIRIS™ HUMAN MILK ANALYSER: SUMMARY OF STANDARD OPERATING PROCEDURES

SET-UP
Water bath and HMA turned on to warm up to 40°C
Defrosting of frozen samples at room temperature; warmed in water bath
Working solutions of Miris™ Check and Miris™ Cleaner prepared according to manufacturer's instructions; warmed in water bath
Distilled water warmed in water bath
Miris™ Control warmed in water bath

↓

ZERO-SETTING CHECK
3mL Miris™ Check injected; "Check" function chosen in the "Analysis" menu
If no adjustment was necessary: proceeded to Instrument validation
If adjustment was necessary: Check procedure was repeated

↓

VALIDATION OF INSTRUMENT
Miris™ Control homogenised
3mL Miris™ Control injected in the inlet of the HMA
"Start" function chosen in the "Analysis" menu
Values compared to established target values
(From August 2018: two different control samples were analysed and compared to two different sets of target values)

↓

PREPARATION OF SAMPLE
Samples individually homogenised at 1.5seconds/mL with the Miris™ Ultrasonic Processor
If sample not analysed immediately: put back in water bath for a maximum of 20 minutes
If foam formed in the sample: the sample bottle left to stand for a few minutes until foam had disintegrated/put back in water bath
Milk mixed thoroughly by gently swirling the bottle before withdrawing a 3mL sample with a 2.5 or 5mL syringe from the centre of the bottle

↓

ANALYSIS
3mL Sample injected in the inlet of the Miris™ HMA with about 0.5mL left in the syringe "Start" function chosen in the "Analysis" menu
Results for fat, crude protein, true protein, carbohydrate, total solids and energy shown on the display
Above steps repeated for replicate analysis/subsequent samples
Ten samples analysed before cleaning

↓

CLEANING
HMA cleaned after every tenth analysis
Zero-check repeated before more milk samples analysed

ANNEXURE 15:

POSTER PRESENTED AT USANA CONGRESS 2018

HUMAN MILK FORTIFICATION PRACTICES in a tertiary hospital and WEIGHT GAIN of preterm infants: *An audit*

Kemp JE[1], Wenhold FAM[1], Nakwa F[2] (2017)

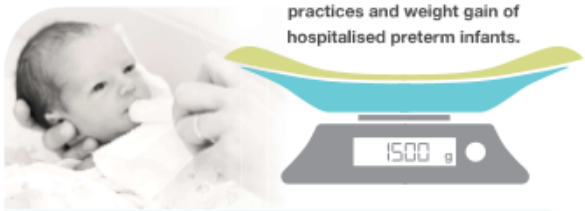
[1] Department of Human Nutrition, Faculty of Health Sciences, University of Pretoria
[2] Department of Paediatrics and Child Health, Faculty of Health Sciences, University of the Witwatersrand

BACKGROUND

In South Africa 8 out of 100 babies are born prematurely. Despite many advances in the nutrition care of preterm infants, in-hospital growth has not been documented locally. Human milk is the feed of choice for all infants, yet it should be fortified to meet internationally recommended nutrient intakes of the preterm infant.

OBJECTIVE

To describe human milk fortification practices and weight gain of hospitalised preterm infants.



METHODS

Design
Prospective audit.

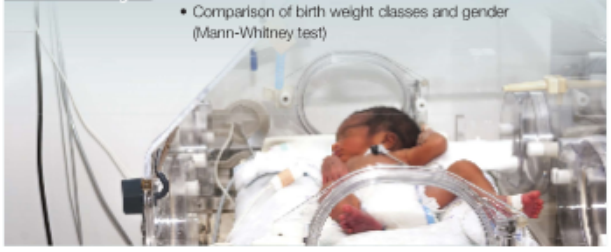
Setting
Urban, tertiary 3200-bed-hospital serving a resource-limited community in Gauteng, South Africa.

Population
- All preterm infants receiving fortified human milk in four neonatal units where approximately 3300 LBW infants are admitted annually.
- September 2016 to March 2017.

Data collection
Hospital dietitians referred preterm infants on the day of fortification initiation. The auditor
- documented birth, medical and feeding history, intake and output within 24 hours
- weighed the infants on entry and exit from the audit, and at weekly intervals in-between on a pan-type electronic scale (Seca: 334) according to standardised techniques.

Exit criteria
- Discharge from hospital or
- Body weight: 1.65kg or
- Receiving 50% of feeds directly from breast

Data analysis
- Descriptive statistics
- Comparison of birth weight classes and gender (Mann-Whitney test)



RESULTS

FORTIFICATION PRACTICES

Fortification of exclusive human milk feeding initiated in 145 preterm infants

ELBW	VLBW	LBW
13% (n=19)	79% (n=115)	8% (n=11)

Fortifier
- Only one extensively hydrolysed cow's milk protein fortifier was used.
- 0.2g protein and 3.5kcal (15kJ) per gram powder.
- Started at half strength and increased as tolerated to full strength.
- Added directly before feeding by mother or nursing staff.

Human milk
- Own mother's milk only: no donor milk available during audit.
- Bolus feeds via feeding tube, cup or syringe.
- Volume of milk: 140mL/kg/day to 200mL/kg/day.

Fortification stopped in 23 (16%) of the infants due to:
- Feeding intolerance
- Suspected NEC.

In a subgroup of ELBW and VLBW infants (n=110):

Exclusive human milk for duration of audit:	Formula feeds given:
• n=58 (53%) • 48 VLBW; 10 ELBW • 30 Female; 28 Male	• n=52 (47%) • Mixed feeding • Eventually formula only

WEIGHT GAIN

Description and weight gain of preterm infants on fortification of exclusive human milk (n=58)

Mean gestational age (weeks)	30 (SD=2.3)	
Mean birth weight (kg)	1.215 (SD=0.2)	
Day of life at fortification initiation	Range: 8-42	
Duration of fortification	4-55 days	
Mean weight at fortification initiation (kg)	1.258 (SD=0.2)	
Mean weight at discharge from audit (kg)	1.573 (SD=0.12)	
Mean weight gain (g/kg/d)	Total group: 15.2 (SD=5.5)	
	VLBW (n=48): 14.4 (SD=5.3)	P=0.011
	ELBW (n=10): 19.4 (SD=4.9)	
	Male (n=28): 14.9 (SD=5.1)	P=0.876 (NS)
	Female (n=30): 15.6 (SD=6.0)	

CONCLUSIONS AND RECOMMENDATION

- Weight gain of ELBW infants was significantly more than of VLBW infants. Weight gain of both groups approximated the recommended values of 15g/kg/d (VLBW) and 20g/kg/d (ELBW).
- Weight gain of boys and girls did not differ significantly.
- The high occurrence of mixed feeding is of concern and should be investigated.

ABBREVIATIONS

Abbreviation	Meaning	Abbreviation	Meaning
ELBW	Extreme low birth weight (<1.0kg)	NEC	Necrotising enterocolitis
LBW	Low birth weight (<2.5kg)	VLBW	Very low birth weight (<2.5kg)
		g/kg/d	gram/kilogram bodyweight/day

Ethical approval
Institutional permission to use patient records: Medical Advisory Committee of CHBA (15-05-2017)
The University of Witwatersrand Health Research and Ethics Committee (Ref: M170546)
Faculty of Health Sciences Research Ethics Committee University of Pretoria (Ref: 238/2017)

Faculty of Health Sciences

ANNEXURE 16:

POSTER PRESENTED AT THE UNIVERSITY OF PRETORIA FACULTY DAY 2019

ENERGY and MACRONUTRIENT CONTENT of breast milk from South African mothers of preterm infants: *An exploratory study*

Kemp JE[1], Wenhold FAM[1], Becker P[2] (2019)

[1] Department of Human Nutrition, Faculty of Health Sciences, University of Pretoria
[2] Office of the Dean, Faculty of Health Sciences, University of Pretoria

BACKGROUND

Human milk is the feed of choice for all infants, including preterm infants. Milk from mothers who delivered prematurely has a different macronutrient composition during the first weeks of life (i.e. preterm milk) when compared to mature milk. In calculating preterm infants' nutritional intake, the energy and macronutrient composition of mothers' milk is largely assumed to be similar to published figures. Little is known about the actual content of South African mothers' milk.

OBJECTIVE

To analyse the macronutrient content of human milk from mothers who gave birth to preterm infants in a tertiary South African hospital (2017 - 2018).

METHODS

Design Prospective, exploratory study.

Setting Urban, tertiary 3200-bed-hospital serving a resource-limited community in Gauteng, South Africa.

Population Mothers of preterm infants (ELBW, VLBW, LBW) in 4 neonatal wards who expressed milk for their infants.

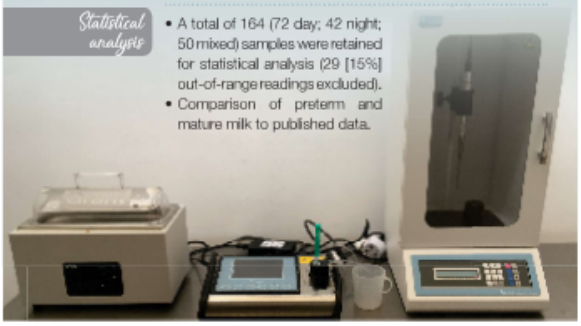
Data collection Demographic and anthropometric data of a convenience sample of mothers and infants were collected in person.

Human milk samples

Collection:
- Mothers were requested to provide day and night milk samples after manually expressing milk for their infants (→ "hind" milk).
- Day samples: two feeding times (9:00; 12:00 and/or 15:00) per day.
- Night samples: twice per night.

Chemical analysis:
- Equal parts of day and night milk were mixed to approximate a 24h sample.
- Warmed to 40°C in a waterless bath (Grant™, Uppsala Sweden).
- Homogenised with MIRIS™ Ultrasonic Processor (Uppsala Sweden).
- Analysis of 193 (87 day; 53 night; 53 mixed) human milk samples for protein, fat and carbohydrate content (Mid-infrared spectroscopy) (MIRIS HMA™, Uppsala Sweden).

Statistical analysis
- A total of 164 (72 day; 42 night; 50 mixed) samples were retained for statistical analysis (29 [15%] out-of-range readings excluded).
- Comparison of preterm and mature milk to published data.



RESULTS

Description of mother-infant pairs

Mothers	Infants
• n=85 • Mean age: 27.7±6.7 years • 90% received ante-natal care • 25% HIV-positive • Mean MUAC: 29.7±4.5 cm	• Mean GA: 30.3±2.9 weeks • Mean birth weight: 1310±401 g • At time of sample collection: - Mean PMA: 33.6±3.1 weeks - Mean weight: 1461±376 g

Macronutrient and energy content of human milk

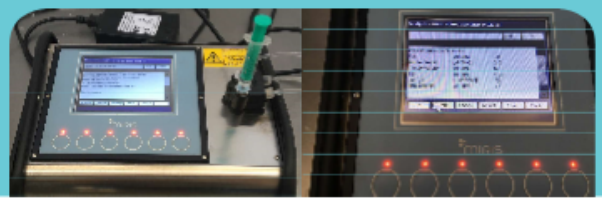
Nutrient	Analysed composition per 100mL of mixed sample		
	Total sample (n=50)	Preterm[1] milk (n=13)	Mature[2] milk (n=37)
Protein[3], g	1.5±0.4	1.9±0.3	1.4±0.4
Carbohydrate, g	7.2±0.7	7.0±0.5	7.2±0.8
Fat, g	3.5±1.0	3.0±1.1	3.6±0.9
Energy, kcal	69.0±9.7	66.4±10.4	69.9±9.5

[1] Preterm milk: up to day 14 of life
[2] Mature milk: day 15 of life onwards
[3] True protein values

Comparison of analysed to published (Cormack, 2016) protein and energy composition of preterm and mature milk

Nutrient	Human milk composition per 100mL					
	Preterm[1] milk			Mature[2] milk		
	Analysed composition (n=13) Mean±SD	Cormack (2016)	P-value[5]	Analysed composition (n=37) Mean±SD	Cormack (2016)	P-value[5]
Protein[4], g	1.9±0.3	1.5	0.002	1.4±0.4	1.2	0.0022
Energy, kcal	66.4±10.4	65	0.3181	69.9±9.5	72	<0.001

[1] Preterm milk: up to day 14 of life
[2] Mature milk: day 15 of life onwards
[5] One sample t-test
[4] True protein values



CONCLUSION AND RECOMMENDATION

Macronutrient, specifically protein content of human milk from South African mothers differs from published data. This should be taken into consideration when assessing nutritional intake of preterm infants. Results could form the foundation for further studies of human milk composition of South African mothers.

ABBREVIATIONS

Abbreviation	Meaning	Abbreviation	Meaning
ELBW	Extreme low birth weight (<1.0kg)	MUAC	Mid-upper arm circumference
GA	Gestational age	PMA	Post-menstrual age
HIV	Human immunodeficiency virus	VLBW	Very low birth weight (<1.5kg)
LBW	Low birth weight (<2.5kg)		

Ethical approval
Faculty of Health Sciences Research Ethics Committee University of Pretoria (Ref: 238/2017)
The University of Witwatersrand Health Research and Ethics Committee (Ref: M170546)
Institutional permission: Medical Advisory Committee of CHBAH (9 October 2017)

Acknowledgement MIRIS (Uppsala Sweden) for in-kind and technical support

Faculty of Health Sciences

ANNEXURE 17: TURNITIN® RECEIPT

turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

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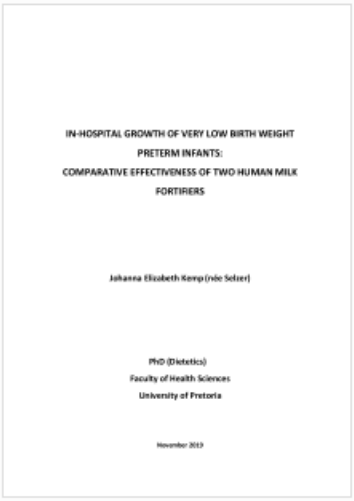
Submission title:	Thesis
File name:	Verhandeling_Hkemp_28_Nov_201…
File size:	4.26M
Page count:	143
Word count:	37,085
Character count:	194,626
Submission date:	10-Feb-2020 04:07PM (UTC+0200)
Submission ID:	1254749308



IN-HOSPITAL GROWTH OF VERY LOW BIRTH WEIGHT PRETERM INFANTS: COMPARATIVE EFFECTIVENESS OF TWO HUMAN MILK FORTIFIERS

Johanna Elizabeth Kemp (née Selzer)

PhD (Dietetics)
Faculty of Health Sciences
University of Pretoria

November 2019

ANNEXURE 18:
DECLARATION OF LANGUAGE EDITING

cumlaude
languagepractitioners

Director: CME Terblanche - BA (Pol Sc), BA Hons (Eng), MA (Eng), TEFL
22 Strydom Street
Baillie Park, 2531
Tel 082 821 3083
cumlaudelanguage@gmail.com

DECLARATION OF LANGUAGE EDITING

I, Christina Maria Etrecia Terblanche, hereby declare that I edited the research study titled:

IN-HOSPITAL GROWTH OF VERY LOW BIRTH WEIGHT PRETERM INFANTS: COMPARATIVE EFFECTIVENESS OF TWO HUMAN MILK FORTIFIERS

for **Johanna Elizabeth Kemp** for the purpose of submission as a postgraduate research study. Changes were indicated in track changes and implementation was left to the author.

Regards,

CME Terblanche

Cum Laude Language Practitioners (CC)

South African Translators Institute accr nr: 1001066

Full member of the Professional Editors Guild