

EARLY FEEDING TYPE, NUTRIENT INTAKE,
AND LONGITUDINAL GROWTH OF PRETERM
INFANTS AT A TERTIARY NEONATAL UNIT
IN KUALA LUMPUR, MALAYSIA:
PROSPECTIVE COHORT STUDY

BADMUS SUAD MAZIDATUL-KHAYR

UNIVERSITI KEBANGSAAN MALAYSIA

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DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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ABSTRAK

Dengan kemajuan dalam penjagaan neonatal, kelangsungan hidup bayi pramatang semakin meningkat. Walau bagaimanapun, kegagalan pertumbuhan pascanatal dan defisit pemakanan masih kerap berlaku. Ketidakpastian masih wujud sama ada pemberian susu ibu difortifikasi secara predomanan (predominantly fortified breast milk, PBM) atau susu formula secara predomanan (predominantly formula milk, PFM) semasa kemasukan ke unit rawatan intensif neonatal (neonatal intensive care unit, NICU) dikaitkan dengan perbezaan dalam penyampaian nutrien, trajektori pertumbuhan atau hasil komposisi badan, khususnya di NICU Malaysia yang masih kekurangan data longitudinal tempatan. Kajian ini menilai pengambilan nutrien semasa kemasukan ke NICU, trajektori pertumbuhan sehingga 6 bulan umur terkoreksi (corrected age, CA), serta hasil komposisi badan dan mineralisasi tulang dalam kalangan bayi pramatang yang menerima PBM atau PFM di sebuah unit neonatal tertuari di Kuala Lumpur, Malaysia. Kajian kohort longitudinal prospektif telah dijalankan di NICU Hospital Canselor Tuanku Muhriz, Kuala Lumpur, antara September 2024 hingga November 2025. Bayi pramatang yang lahir pada usia gestasi ≤ 34 minggu dan/atau berat lahir ≤ 1800 g, tanpa anomali kongenital utama atau keadaan perubatan yang diketahui mempengaruhi pertumbuhan, serta berada di NICU sekurang-kurangnya 14 hari, telah direkrut. Pengambilan nutrien harian dan pencapaian pemakanan diperoleh daripada rekod klinikal. Skor-z berat menurut umur (WAZ), skor-z panjang menurut umur (LAZ), dan skor-z lilitan kepala menurut umur (HCZ) dinilai semasa lahir, semasa discaj, serta pada 1, 3 dan 6 bulan CA. Bayi diklasifikasikan sebagai PBM atau PFM berdasarkan sumber utama pengambilan enteral semasa kemasukan ke NICU, iaitu jenis susu yang menyumbang bahagian terbesar daripada jumlah pengambilan enteral terkumpul. Klasifikasi ini ditetapkan semasa discaj tanpa mengambil kira perubahan pemakanan selepas discaj. Penilaian komposisi badan seluruh tubuh menggunakan absorptiometri dwi-tenaga sinar-X (dual-energy X-ray absorptiometry, DXA) dijalankan pada 6 hingga 9 bulan CA dalam sub-sampel yang tersedia bagi menilai kandungan mineral tulang, ketumpatan mineral tulang, jisim tanpa lemak dan peratus lemak badan. Seramai 76 bayi diklasifikasikan sebagai PBM ($n = 34$) atau PFM ($n = 42$). Bayi dalam kumpulan PBM mempunyai usia gestasi yang lebih rendah (median 32 berbanding 33 minggu; $p = 0.01$), kadar resusitasi yang lebih tinggi (58.8% berbanding 35.7%; $p = 0.04$), kadar sindrom gangguan pernafasan yang lebih tinggi (85.3% berbanding 61.9%; $p = 0.02$), serta tempoh kemasukan hospital yang lebih lama (median 30 berbanding 21 hari; $p = 0.01$). Walaupun terdapat perbezaan klinikal asas ini, purata pengambilan nutrien sepanjang Minggu 1 hingga 3 adalah setara antara kumpulan bagi tenaga (101.8 berbanding 102.4 kcal/kg/hari; $p = 0.87$) dan protein (2.78 berbanding 2.87 g/kg/hari; $p = 0.51$). Kedua-dua kumpulan kekal di bawah julat sasaran minimum ESPGHAN bagi pengambilan tenaga dan protein, dengan pencapaian 88.5–89.0% daripada sasaran minimum tenaga dan 79.4–82.0% daripada sasaran minimum protein. Semasa discaj, perubahan WAZ (Δ WAZ) tidak berbeza antara bayi PBM dan PFM (-1.27 ± 0.35 berbanding -1.13 ± 0.55 ; $p = 0.21$), dan kegagalan pertumbuhan pascanatal berlaku masing-masing dalam 47.1% dan 45.2% bayi ($p = 0.87$). Kumpulan pemakanan dan pengambilan nutrien awal tidak berkait secara bebas dengan Δ WAZ semasa discaj dalam model terlaras. Semasa susulan selepas discaj ($n = 57$), WAZ menurun secara progresif dari 1 hingga 6 bulan CA dalam kedua-dua kumpulan. Pada lawatan klinik susulan pertama selepas discaj, 28.1% bayi (16/57) kekal menerima susu

ibu secara eksklusif, 33.3% (19/57) kekal menerima susu formula secara eksklusif, 8.8% (5/57) telah beralih daripada susu formula kepada susu ibu, 10.5% (6/57) telah beralih daripada susu ibu kepada susu formula, dan 19.3% (11/57) menerima suapan campuran. Dalam model terlaras, bayi PFM mempunyai WAZ yang lebih tinggi sepanjang tempoh susulan (anggaran = 1.00, 95% CI 0.15 hingga 1.85; $p = 0.03$). Walau bagaimanapun, dapatan ini tidak konsisten dalam analisis sensitiviti dan mungkin mencerminkan faktor pengeliruan residual akibat perbezaan klinikal asas. WAZ semasa lahir merupakan peramal terkuat bagi trajektori WAZ selepas discaj dalam semua model ($p = 0.003$ hingga $p < 0.001$), mencadangkan bahawa status pertumbuhan semasa lahir lebih kuat dikaitkan dengan pertumbuhan selepas discaj berbanding jenis pemakanan predomanan semasa kemasukan ke NICU. DXA telah dijalankan pada purata CA 6.8 ± 1.0 bulan dalam 12 bayi. Purata kandungan mineral tulang ialah 169.2 ± 29.2 g dan purata ketumpatan mineral tulang ialah 0.25 ± 0.02 g/cm², setanding dengan kohort pramatang yang telah diterbitkan dan mencadangkan mineralisasi tulang secara umumnya terpelihara dalam kohort kecil ini. Purata jisim tanpa lemak ialah 4887 ± 786 g, manakala purata peratus lemak badan ialah $39.4 \pm 6.7\%$, yang kelihatan lebih tinggi daripada nilai yang dilaporkan dalam bayi cukup bulan yang sihat pada umur yang setara. Dapatan ini mencadangkan profil komposisi badan yang relatif cenderung kepada adipos dan memerlukan pemantauan lanjut selepas discaj. Di bawah protokol unit yang dipiawaikan, bayi PBM dan PFM mencapai penyampaian makronutrien awal yang secara umumnya setara serta hasil pertumbuhan semasa discaj yang serupa, walaupun terdapat perbezaan klinikal asas antara kumpulan. Dapatan ini menunjukkan bahawa bayi yang menerima PBM masih boleh mencapai pertumbuhan yang setanding dengan PFM apabila pengambilan nutrien dicapai secara mencukupi melalui amalan fortifikasi dan protokol pemakanan yang tersusun. Trajektori pertumbuhan selepas discaj pula kelihatan lebih rapat dikaitkan dengan status pertumbuhan asas dan kematangan gestasi, seperti yang dicerminkan oleh WAZ semasa lahir dan usia gestasi, berbanding jenis pemakanan predomanan semasa kemasukan ke NICU atau pengambilan nutrien yang dicapai. Walau bagaimanapun, penurunan WAZ yang progresif sehingga 6 bulan CA dalam kedua-dua kumpulan menunjukkan kebimbangan terhadap pertumbuhan selepas discaj dan menyokong keperluan susulan pemakanan berstruktur selepas discaj hospital. Kajian ini turut menyumbang data kontemporari tempatan mengenai komposisi badan dan mineralisasi tulang menggunakan DXA dalam kalangan bayi pramatang pada 6 hingga 9 bulan CA. Dapatan DXA yang bersifat eksploratori mencadangkan bahawa pemantauan pertumbuhan bayi pramatang perlu mempertimbangkan komposisi badan dan kesihatan tulang, bukan pertambahan berat badan semata-mata. Secara keseluruhan, dapatan ini menyediakan data tempatan awal untuk membimbing kajian masa hadapan yang mempunyai kuasa sampel mencukupi mengenai pemakanan selepas discaj, kualiti pertumbuhan dan hasil metabolik jangka panjang dalam populasi ini.

ABSTRACT

With improved neonatal care, preterm infant survival has increased; however, postnatal growth failure and nutritional deficits remain common. Uncertainty persists regarding whether predominantly fortified breast milk (PBM) or predominantly formula milk (PFM) feeding during neonatal intensive care unit (NICU) admission is associated with different nutrient delivery, growth trajectories, or body composition outcomes, particularly in Malaysian NICUs where local longitudinal data remain limited. This study examined nutritional intake during NICU admission, longitudinal growth trajectories to 6 months corrected age (CA), and body composition and bone mineralisation outcomes among preterm infants receiving PBM or PFM at a tertiary neonatal unit in Kuala Lumpur, Malaysia. A prospective longitudinal cohort study was conducted in the NICU of Hospital Canselor Tuanku Muhriz, Kuala Lumpur, between September 2024 and November 2025. Preterm infants born at ≤ 34 weeks' gestation and/or ≤ 1800 g, without major congenital anomalies or medical conditions known to affect growth, and who remained in the NICU for at least 14 days were enrolled. Daily nutrient intake and feeding milestones were extracted from clinical records. Weight-for-age z-score (WAZ), length-for-age z-score (LAZ), and head circumference-for-age z-score (HCZ) were assessed at birth, discharge, and at 1, 3, and 6 months CA. Infants were classified as PBM-fed or PFM-fed according to the predominant source of enteral intake during NICU admission, defined as the milk type contributing the largest proportion of cumulative enteral intake. This classification was fixed at discharge regardless of subsequent post-discharge feeding transitions. Whole-body dual-energy X-ray absorptiometry (DXA) was performed at 6–9 months CA in an available subsample to assess bone mineral content, bone mineral density, lean mass, and body fat percentage. Seventy-six infants were classified as PBM-fed ($n = 34$) or PFM-fed ($n = 42$). PBM infants had lower gestational age (GA) (median 32 vs 33 weeks; $p = 0.01$), higher rates of resuscitation (58.8% vs 35.7%; $p = 0.04$), higher rates of respiratory distress syndrome (85.3% vs 61.9%; $p = 0.02$), and longer length of stay (median 30 vs 21 days; $p = 0.01$). Despite these baseline differences, mean nutrient delivery during Weeks 1–3 was comparable between groups for energy (101.8 vs 102.4 kcal/kg/day; $p = 0.87$) and protein (2.78 vs 2.87 g/kg/day; $p = 0.51$). Both groups remained below the lower ESPGHAN target ranges for energy and protein intake, achieving 88.5–89.0% of the minimum energy target and 79.4–82.0% of the minimum protein target. At discharge, Δ WAZ did not differ between PBM and PFM infants (-1.27 ± 0.35 vs -1.13 ± 0.55 ; $p = 0.21$), and postnatal growth failure occurred in 47.1% and 45.2%, respectively ($p = 0.87$). Feeding group and early nutrient intake were not independently associated with Δ WAZ at discharge in adjusted models. During post-discharge follow-up ($n = 57$), WAZ declined progressively from 1 to 6 months CA in both groups. At the first post-discharge clinic visit, 28.1% of infants (16/57) remained exclusively on breast milk, 33.3% (19/57) remained exclusively on formula milk, 8.8% (5/57) had transitioned from formula milk to breast milk, 10.5% (6/57) had transitioned from breast milk to formula milk, and 19.3% (11/57) were receiving mixed feeds. In adjusted models, PFM infants had higher WAZ across follow-up (estimate = 1.00, 95% CI 0.15 to 1.85; $p = 0.03$), although this finding was not consistent across sensitivity analyses and may reflect residual confounding by baseline clinical differences. Birth WAZ was the strongest predictor of post-discharge WAZ trajectory in all models ($p = 0.003$ to $p < 0.001$), suggesting that birth growth status was more strongly associated with post-

discharge growth than NICU feeding type. DXA was performed at a mean CA of 6.8 ± 1.0 months in 12 infants. Mean bone mineral content was 169.2 ± 29.2 g and mean bone mineral density was 0.25 ± 0.02 g/cm², comparable to published preterm cohorts and suggesting generally preserved bone mineralisation within this small cohort. Mean lean mass was 4887 ± 786 g, and mean body fat percentage was $39.4 \pm 6.7\%$, appearing higher than values reported in healthy term-born infants at comparable age. This suggests a relatively adipose-predominant body composition profile that warrants further post-discharge monitoring. Under a standardised unit protocol, PBM and PFM infants achieved broadly comparable early macronutrient delivery and similar growth outcomes at discharge despite baseline clinical differences between groups. These findings suggest that infants receiving PBM can achieve growth outcomes comparable to those receiving PFM when nutrient delivery is adequately supported through fortification and structured feeding protocols. Post-discharge growth trajectory appeared to be more closely associated with baseline growth status and gestational maturity, reflected by birth WAZ and GA, than with predominant NICU feeding type or achieved nutrient intake. However, the progressive decline in WAZ through 6 months CA in both groups indicates an ongoing post-discharge growth concern and supports the need for structured nutritional follow-up beyond hospital discharge. This study also contributes contemporary local DXA-derived data on body composition and bone mineralisation among preterm infants at 6–9 months CA. The exploratory DXA findings suggest that growth monitoring in preterm infants should consider body composition and bone health, not weight gain alone. Overall, these findings provide preliminary Malaysian data to guide future adequately powered studies on post-discharge nutrition, growth quality, and longer-term metabolic outcomes in this population.

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
ARA	Arachidonic Acid
ATM	Adipose Tissue Mass
ATEA	Appropriate for Term Equivalent Age
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BM	Breast Milk
BMF	Breast Milk Fortifier
BMR	Basal Metabolic Rate
BW	Bone Width
CA	Corrected Age
CLD	Chronic Lung Disease
CoN	Committee on Nutrition
DBM	Donor Breast Milk
DEXA/ DXA	Dual-Energy X-ray Absorptiometry
DHA	Docosahexaenoic Acid
DOL	Day of Life
EBM	Expressed Breast Milk
ELBW	Extremely Low Birth Weight
EN	Enteral Nutrition
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FFM	Fat-Free Mass
FM	Formula Milk / Fat Mass
FMM	Fortified Mother's Milk
GA	Gestational Age
G	Gram
HC	Head Circumference

HCZ	Head Circumference-for-Age Z-score
HCTM	Hospital Canselor Tuanku Muhriz
HPKK	Hospital Pakar Kanak-Kanak
IQR	Interquartile Range
IVH	Intraventricular Hemorrhage
Kg	Kilogram
LCZ	Length-for-Age Z-score
MD	Mean Difference
MEF	Minimal Enteral Feeding
MBD	Metabolic Bone Disease
NEC	Necrotizing Enterocolitis
NICU	Neonatal Intensive Care Unit
PDF	Post-Discharge Formula
PDA	Patent Ductus Arteriosus
PBM	Predominantly Fortified Breast Milk
PFM	Predominantly Formula Milk
PGF	Postnatal Growth Failure
PN	Parenteral Nutrition
PMA	Postmenstrual Age
PUFA	Polyunsaturated Fatty Acids
REE	Resting Energy Expenditure
RDAs	Recommended Dietary Allowances
ROP	Retinopathy of Prematurity
SD	Standard Deviation
SEM	Standard Error of the Mean
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
UKM	Universiti Kebangsaan Malaysia
VLBW	Very Low Birth Weight

WAZ	Weight-for-Age Z-score
WHO	World Health Organization



CHAPTER I

INTRODUCTION

1.1 BACKGROUND OF STUDY

A full-term pregnancy typically lasts between 37 and 42 weeks. An infant born alive before 37 completed weeks of gestation is classified as preterm (WHO 2023). Gestational age (GA), measured in complete weeks from the first day of the mother's last menstrual period, categorises preterm births into three distinct groups (MOH 2023): Extremely preterm (<28 weeks), Very preterm (28 to <32 weeks), Moderate to late preterm (32 to <37 weeks).

Globally, an estimated 13.4 million infants were born preterm in 2020, representing approximately 9.9% of all live births (Ohuma et al. 2023). The burden is highest in low and middle income settings, with South Asia and sub Saharan Africa contributing the greatest proportions (Ohuma et al. 2023; WHO 2023). In Malaysia, approximately 500,000 babies are born each year, with a preterm birth rate of approximately 12.3% (MOH 2023). Prematurity is the second leading cause of under-five mortality in Malaysia (MOH 2023). Hospital-based registry data also suggest an increasing incidence of preterm birth over time, adding pressure to neonatal services (Damanhuri et al. 2024; Sutan et al. 2018).

Malaysia's National Neonatal Registry 2016 data, cited in the national guideline indicate that among preterm infants, 23.3% were born before 32 weeks' GA and 25.5% had birth weight of 1500 g or less (MOH 2023). Survival increases steeply across GA, from 17.1% at 24 weeks and 34.8% at 25 weeks to 79.1% at 28 weeks and 90.5% at 30 weeks (Boo et al. 2020). The guideline also reports that 77.3% of infants born before 32 weeks experienced at least one major morbidity, such as confirmed sepsis,

necrotising enterocolitis, oxygen dependency at 36 weeks or discharge, severe retinopathy of prematurity, or patent ductus arteriosus requiring surgical ligation (MOH 2023).

With advances in neonatal intensive care resulting in evidently improved survival, clinical attention has shifted toward the quality of survival particularly the nutritional management and growth outcomes of preterm infants during and after neonatal intensive care unit (NICU) admission (Cheah et al. 2021; Horbar et al. 2015). Preterm infants are born with limited nutrient reserves, have substantially elevated metabolic demands relative to term counterparts, and are often unable to establish full oral feeding for weeks. How this nutritional vulnerability is addressed specifically through the type of feeding provided during NICU admission has important consequences not only for in-hospital growth but for growth trajectories and body composition throughout infancy (Cerasani et al. 2020; Johnson et al. 2012).

Breast milk is universally recognised as the gold standard for infant nutrition due to its immunological and bioactive components and associations with improved clinical outcomes (Embleton et al. 2023; WHO, 2023). However, preterm infants have higher nutritional requirements than term infants, and unfortified breast milk alone does not meet the protein, energy, calcium, and phosphorus needs of extremely preterm, very preterm, and very low birth weight (VLBW) infants (Embleton et al. 2023; WHO, 2023). Moderate and late preterm infants can also experience feeding difficulties and growth faltering; guidance therefore recommends close monitoring with consideration of nutrient-enriched feeds rather than treating them equivalently to term infants (Boies et al. 2016; Lapillonne & Griffin 2013).

The nutritional gap between unfortified breast milk and recommended intake targets is substantial. Unfortified preterm breast milk provides approximately 1.0 to 1.4 g protein per 100 mL, declining from early lactation values as high as 2.7 g/100 mL in the first week of life (Boyce et al. 2016; Gidrewicz & Fenton 2014). Recommended protein intake for stable growing preterm infants is at least 3.5 to 4.0 g/kg/day, rising to 4.5 g/kg/day where growth is poor (Agostoni et al. 2010; Embleton et al. 2023). The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition

(ESPGHAN) recommends a total enteral energy intake of 115 to 140 kcal/kg/day for most stable growing preterm infants, with higher intakes up to 160 kcal/kg/day considered only when growth remains poor after protein and other nutrient intakes are adequate (Embleton et al. 2023). Calcium and phosphorus targets of 120 to 200 mg/kg/day and 70 to 115 mg/kg/day respectively also substantially exceed what unfortified breast milk can provide at standard feeding volumes, with direct implications for bone mineralisation and metabolic bone disease risk (Agostoni et al. 2010; Embleton et al. 2023). As breast milk protein declines over the course of lactation, infants receiving unfortified milk are at progressively increasing risk of falling below protein targets if fortification is delayed, insufficient, or discontinued (Gidrewicz & Fenton 2014). For these reasons, routine multi-nutrient breast milk fortification and/or use of preterm formula are standard practice for preterm infants, particularly those with VLBW (Agostoni et al. 2010; Embleton et al. 2023).

In routine NICU care, feeding practices vary by GA, clinical stability, tolerance of enteral feeds, and the availability of mother's milk (Abdul Hamid et al. 2021; De Rose et al. 2024; Embleton et al. 2023). Barriers to sustained mother's own milk provision including physical separation of mother and infant, immature sucking and swallowing, and the need for prolonged pump-dependent milk expression vary considerably by GA and institutional context (Heller et al. 2021; Patel et al. 2025; Quitadamo et al. 2023). Many infants therefore receive mixed feeding, with changing proportions of fortified breast milk, formula, and parenteral nutrition across admission (Abdul Hamid et al. 2021; De Rose et al. 2024).

The World Health Organization defines predominant breastfeeding in community settings as feeding breast milk as the primary source of nourishment, with water-based liquids permitted but no non-human milk or formula (WHO 2021). In the NICU context, this community-based definition cannot be directly applied, as many preterm infants receive parenteral nutrition and mixed enteral feeds during clinical instability. For this study, infants were classified according to the milk type contributing the largest proportion of cumulative enteral intake during NICU admission. This approach is consistent with prior neonatal feeding studies that classified feeding exposure according to proportional or prevalent milk intake, although exact thresholds

differ across studies (Abdul Hamid et al. 2021; Quitadamo et al. 2023). Predominantly fortified breast milk fed (PBM) refers to infants for whom fortified breast milk contributed the largest proportion of cumulative enteral intake, while predominantly formula milk fed (PFM) refers to infants for whom formula milk contributed the largest proportion. This classification reflects the dominant enteral nutritional exposure and does not imply exclusive feeding of either type.

Even when protocols recommend fortified breast milk or preterm formula, achieved intake can differ from intended targets because of illness severity, feed interruptions, fasting for procedures, and day to day variation in volumes delivered (Cormack et al. 2016; Embleton et al. 2023). Grouping infants by predominant feeding type therefore does not guarantee that energy, protein, and mineral targets are met consistently, and assessment of delivered intake is necessary to evaluate nutritional adequacy under routine NICU conditions (Cormack et al. 2016; Embleton et al. 2023).

Optimising early nutrition is vital to reducing postnatal growth failure (PGF) in preterm infants, as early growth faltering has been associated with later neurodevelopmental and metabolic consequences, including poorer cognitive and motor outcomes and altered cardiometabolic risk (Bell et al. 2022; Embleton et al. 2023). However, discharge growth alone does not fully capture the nutritional course of preterm infants, because growth deficits may persist or worsen after NICU discharge. Longitudinal assessment at 1, 3, and 6 months corrected age (CA), defined as chronological age adjusted for prematurity, is therefore needed to evaluate whether early nutritional exposure during admission is associated with later growth trajectory.

Despite this, Malaysian longitudinal data comparing predominantly fortified breast milk and predominantly formula milk during NICU admission remain limited. It is also unclear whether feeding type alone, or the actual energy and macronutrient intake achieved during admission, is more closely related to postnatal growth outcomes. In addition, conventional anthropometry cannot distinguish between lean mass, fat mass, and bone mineralisation, leaving uncertainty about the quality of growth achieved after discharge.

This study therefore examined nutrient intake during NICU admission, growth trajectories from birth to 6 months CA, and exploratory DXA-derived body composition and bone mineralisation outcomes among preterm infants predominantly fed fortified breast milk or formula milk in a Malaysian tertiary NICU.

1.2 PROBLEM STATEMENT

Despite routine use of fortified breast milk and preterm formula in NICUs, uncertainty remains regarding how predominant feeding strategies relate to achieved nutrient delivery, growth trajectories, and body composition in preterm infants (Cormack et al. 2016; Suganuma et al. 2021). Internationally, PGF remains common among VLBW infants, although reported prevalence varies according to definition, population, and discharge criteria. In a large North American cohort, PGF at discharge decreased from 64.5% in 2000 to 50.3% in 2013, but approximately half of VLBW infants still had PGF at discharge (Horbar et al. 2015). In Southeast Asia, severe PGF was reported in 28.8% of VLBW infants and 36.5% of extremely low birth weight infants in a Singapore-Malaysia cohort, with insufficient protein and energy intake identified as contributing factors (Lee et al. 2020). This issue remains relevant in Malaysia, where 52% of preterm infants in one local NICU cohort had a WAZ decline of ≥ 1.28 SD by discharge despite high breast milk fortifier use and apparently adequate calculated protein intake (Abdul Hamid et al. 2021).

The interpretation of feeding type is complicated by the difference between prescribed feeding and achieved nutrient intake. Evidence comparing breast milk and preterm formula feeding remains inconclusive because of heterogeneous feeding definitions, variable study quality, and inconsistent reporting of actual nutrient delivery (Suganuma et al. 2021). In the Malaysian and wider Asia-Pacific context, sustained mother's own milk provision and consistent PBM nutrient delivery may be affected by service-related and sociocultural barriers, such as postpartum confinement practices, short maternity leave, milk kinship concerns affecting donor milk banking, and limited access to specialised nutritional care products (Cheah et al. 2021). In addition, breast milk composition varies between mothers and across lactation, while fortification practices differ across settings. Recommended energy, protein, mineral, and

micronutrient targets may not always be achieved even when fortification is prescribed (Arslanoglu et al. 2019; Gidrewicz & Fenton 2014).

Beyond conventional anthropometry, early feeding and nutrient delivery may affect growth quality, including lean mass, fat mass, and bone mineralisation. This is clinically relevant because preterm infants miss part of the third-trimester period of calcium, phosphorus, protein, and fat accretion, increasing their risk of altered body composition and impaired skeletal mineralisation. Early macronutrient intake has been associated with bone mineral content at 6 months CA (Calor et al. 2021). Assessing body composition alongside growth trajectories extends evaluation beyond weight, length, and head circumference alone and helps describe the quality of post-discharge growth.

Local DXA-derived data on body composition and bone mineralisation among Malaysian preterm infants remain scarce. The only published Malaysian DXA study was reported in 2007 and noted the absence of local comparative data at that time (Rohana et al. 2007). Since then, neonatal care, nutritional protocols, and medical support for preterm infants have changed. Although findings from the present study are not directly comparable with the earlier Malaysian study because of differences in age at assessment and study design, updated local data are needed to describe body composition and bone mineralisation after NICU discharge.

In current Malaysian NICU practice, it is often assumed that fortified breast milk and preterm formula, when prescribed according to unit protocols, are sufficient to meet nutritional targets and support adequate post-discharge growth. This assumption has not been adequately tested using local longitudinal data based on actual delivered nutrient intake rather than prescribed intake alone. It remains unclear whether PBM and PFM provide comparable achieved energy and macronutrient delivery under routine clinical conditions, whether feeding type and achieved intake during NICU admission are associated with growth trajectories from discharge to 6 months CA, and what body composition and bone mineralisation profiles are observed in this population at 6–9 months CA.

This study therefore compared achieved nutrient intakes during NICU admission and longitudinal growth trajectories up to 6 months CA between preterm infants fed PBM and those fed PFM at Hospital Canselor Tuanku Muhriz (HCTM). In an available subset, whole-body DXA at 6–9 months CA provided preliminary local data on body composition and bone mineralisation. DXA will give an extended the evaluation beyond anthropometry alone and allowed the observed growth profile to be interpreted in relation to published international preterm cohorts.

1.3 STUDY JUSTIFICATION

Despite improvements in neonatal survival, PGF and nutritional shortfalls remain common among preterm infants in Malaysian NICUs. Local data comparing PBM and PFM remain limited, particularly in relation to achieved nutrient intake, post-discharge growth, and body composition outcomes. This study is justified by four main gaps in the current literature.

The first gap is the lack of Malaysian data comparing achieved energy and macronutrient intake between PBM and PFM groups under routine NICU conditions. Prescribed nutritional targets do not always reflect actual intake because illness severity, feed interruptions, delayed fortification, and fasting for procedures may reduce delivered nutrition (Cormack et al. 2016). Sukanuma et al. (2021) reported that evidence comparing growth outcomes between breast milk and formula feeding in VLBW infants remains inconclusive, partly because of inconsistent feeding definitions and limited reporting of achieved intake. Abdul Hamid et al. (2021) examined nutritional intake and growth at HCTM but did not stratify outcomes by predominant milk type during NICU admission. Objective 1 addresses this gap by comparing achieved energy and macronutrient intakes between PBM and PFM groups during defined weekly periods of NICU admission.

The second gap is the absence of Malaysian post-discharge growth trajectory data stratified by predominant NICU feeding type. Existing local studies mainly report growth up to discharge (Abdul Hamid et al. 2021; Lee et al. 2019). Whether early exposure to PBM or PFM is associated with growth from discharge to 1, 3, and 6 months CA remains unknown. This matters because post-discharge feeding in Malaysia may be

influenced by maternal milk availability, family feeding decisions, cultural practices, and access to follow-up nutrition care (Cheah et al. 2021). Objective 2 addresses this gap by describing growth trajectories up to 6 months CA according to NICU feeding group.

The third gap is the limited availability of recent local data on body composition and bone mineralisation among Malaysian preterm infants. The only published DXA study from HCTM was reported in 2007 and focused mainly on bone mineral content near term-equivalent age (Rohana et al. 2007). Since then, neonatal care, nutritional protocols, and medical support for preterm infants have changed. Weight gain alone does not fully describe growth quality because infants with similar anthropometry may differ in lean mass, fat mass, bone mineral content, and bone mineral density. Objective 3 addresses this gap by providing updated local DXA-derived data on body composition and bone mineralisation at 6 to 9 months CA in an available subset.

The fourth gap is the lack of local multivariable evidence on whether NICU feeding type and achieved nutrient intake are associated with growth outcomes after accounting for clinical factors. In observational studies, feeding type may be influenced by GA, birth size, illness burden, maternal milk availability, and feeding tolerance. Without adjustment for these factors, crude comparisons may wrongly attribute growth differences to milk type alone. Objective 4 addresses this gap by examining whether feeding type and achieved nutrient intake are associated with growth outcomes after adjustment for key clinical confounders such as GA, birth weight z-score, and morbidity burden.

Addressing these gaps has relevance for clinical care and future research. At the infant level, identifying early protein and energy shortfalls may help guide nutritional review during admission. At the family level, local data on PBM and PFM may support more balanced feeding counselling after discharge. At the healthcare level, findings may help identify infants who require closer nutritional follow-up, continued fortification, or structured growth monitoring. The exploratory DXA data also provide preliminary local information on growth quality beyond weight gain alone.

Overall, this study provides local longitudinal data on achieved nutrient intake, growth trajectory, and exploratory DXA-derived body composition and bone mineralisation among preterm infants in a Malaysian tertiary NICU. By comparing PBM and PFM using actual delivered intake rather than feeding label alone, the study addresses a local data gap and provides a basis for future multicentre work on preterm nutrition and post-discharge growth monitoring in Malaysia.

1.4 SIGNIFICANCE OF THE RESEARCH

This study is important because it provides local data on preterm infant feeding, growth, body composition, and bone mineralisation in a Malaysian NICU. Previous Malaysian studies have described nutrient intake and short-term growth outcomes, but local data that combine achieved nutrient intake, longitudinal growth follow-up, and DXA-derived body composition remain limited.

The main contribution of this study is the combined assessment of three outcome domains: achieved nutrient intake during NICU admission, growth trajectories up to 6 months CA, and DXA-derived body composition and bone mineralisation at 6 to 9 months CA. This approach moves beyond weight gain alone, which may not fully reflect nutritional adequacy in preterm infants. Infants with similar weight gain may differ in lean mass, fat mass, and bone mineral status. Therefore, assessing these outcomes together provides a broader evaluation of growth quality in this population (Johnson et al. 2012; Embleton et al. 2023).

A second contribution is the provision of contemporary Malaysian DXA-derived data on body composition and bone mineralisation among preterm infants. The only previous local DXA study from this institution was reported by Rohana et al. (2007), used earlier DXA technology, and assessed infants at a near-term time point. The present study extends local data to 6 to 9 months CA and allows body composition and bone mineralisation findings to be interpreted relative to published international preterm cohorts. Although the DXA subset is exploratory, it provides preliminary evidence on whether current nutritional care is associated with lean mass, fat mass, and bone mineral outcomes comparable to those reported in other preterm populations.

The third contribution is the week-by-week measurement of achieved macronutrient and energy intake during NICU admission according to predominant feeding type. This is important because prescribed intake may not reflect actual nutrient delivery, especially when feeds are interrupted, fortification is delayed, or clinical instability affects feeding progression. By comparing PBM and PFM groups using achieved intake, this study provides local data on whether the two feeding strategies deliver comparable energy and macronutrients under routine clinical conditions.

These findings may support neonatal nutrition care in several ways. For clinicians and dietitians, the study provides local information on nutrient delivery, growth trajectory, and growth quality among preterm infants. For discharge planning, it may help identify infants who require closer nutritional review after discharge. For families, locally generated data may support clearer counselling about breast milk fortification, formula use, and post-discharge feeding follow-up. At the health system level, the findings provide an evidence base for future Malaysian studies and for refinement of local preterm nutrition protocols.

Overall, this study contributes Malaysia-specific longitudinal data on how predominant NICU feeding exposure relates to achieved nutrient intake, growth up to 6 months CA, and exploratory DXA-derived body composition and bone mineralisation outcomes. This extends local evaluation beyond discharge weight and provides a basis for future larger studies on preterm nutrition and growth quality in Malaysia.

1.5 RESEARCH QUESTIONS

1. Are there differences in energy and macronutrient intake achieved during NICU admission between preterm infants receiving PBM and those receiving PFM?
2. Are there differences in growth outcomes of preterm infants at discharge and at 1, 3, and 6 months CA between those who received PBM and those who received PFM during NICU admission?
3. Are NICU feeding type and nutrient intake associated with growth outcomes at discharge and at 1, 3, or 6 months CA among preterm infants?

1.6 RESEARCH OBJECTIVES

1.6.1 Main Objective

To examine differences in nutritional intake during NICU admission and subsequent growth outcomes among preterm infants receiving predominantly fortified breast milk (PBM) or predominantly preterm formula milk (PFM).

1.6.2 Specific Objectives

1. To compare energy and macronutrient intake achieved during NICU admission between PBM and PFM fed preterm infants.
2. To compare growth outcomes of preterm infants by feeding type at discharge, and at 1, 3, and 6 months CA.
3. To describe body composition and bone mineralisation at 6 months CA using available DXA data.
4. To examine whether NICU feeding type and nutrient intake are associated with growth outcomes at discharge and at 1, 3, and 6 months CA.

1.6.3 Hypothesis

- H1: Energy and macronutrient intakes achieved during NICU admission will differ between PBM- and PFM-fed preterm infants.
- H2: Preterm infants who received PBM during NICU admission will have different growth outcomes at discharge and at 1, 3, and 6 months CA compared with those who received PFM.
- H3: NICU feeding type and nutrient intake will be associated with growth outcomes at discharge and at 1, 3, and 6 months CA.

1.7 CONCEPTUAL FRAMEWORK

The conceptual framework guiding this study was developed from the four research objectives and the evidence reviewed in Chapter 2. It organises the primary exposure, achieved nutrient intake pathway, growth outcomes, exploratory DXA outcomes, covariates, and unmeasured post-discharge factors considered in this study.

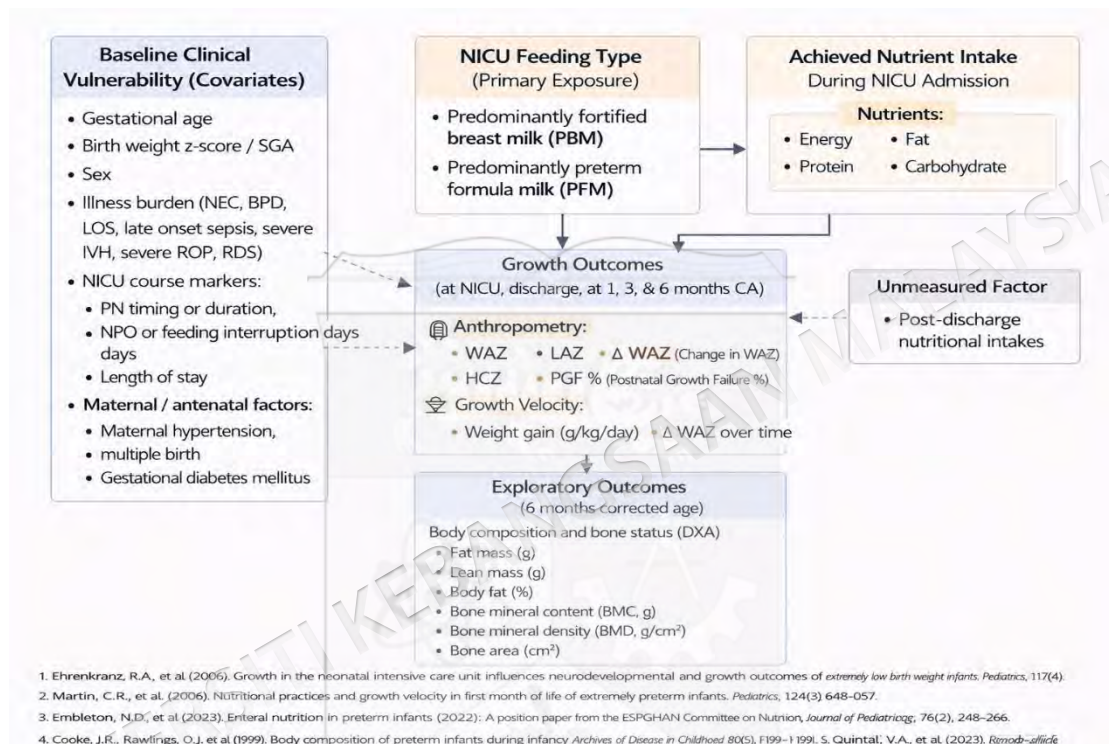


Figure 1.1 Conceptual framework of NICU feeding type, achieved nutrient intake, clinical covariates, and growth and body composition outcomes in preterm infants.

The primary exposure of interest is NICU feeding type, classified as PBM or PFM during NICU admission. Feeding type is hypothesised to be associated with differences in achieved nutrient delivery during hospitalisation, particularly energy, protein, fat, and carbohydrate intake. Achieved nutrient intake is therefore treated as a central pathway through which NICU feeding exposure may relate to early and later growth outcomes.

Baseline clinical vulnerability factors were included in the framework because they may influence both feeding exposure and growth outcomes. These factors include GA, birth weight z-score or small-for-gestational-age status, sex, illness burden, NICU

course markers, and maternal or antenatal factors. More clinically vulnerable infants may have slower feeding advancement, longer parenteral nutrition exposure, more feeding interruptions, and higher risk of poor growth. These variables were therefore considered potential confounders in the relationship between feeding type and growth. However, not all variables shown in the framework were entered simultaneously into the multivariable models. Final model covariates were selected based on clinical relevance, data completeness, sample size, and model stability.

Growth outcomes form the primary endpoint of the framework and were assessed during NICU admission, at discharge, and after discharge at 1, 3, and 6 months CA. Growth was evaluated using weight-for-age z-score, length-for-age z-score, head circumference-for-age z-score, change in weight-for-age z-score, PGF percentage, and weight gain velocity. These measures allow assessment of both attained size and change in growth status over time.

Exploratory outcomes at 6 to 9 months CA were assessed using DXA. These outcomes included body composition measures, namely fat mass, lean mass, and percentage body fat, as well as bone measures including bone mineral content, bone mineral density, and bone area where available. These DXA outcomes extend the evaluation beyond conventional anthropometry by describing growth quality in terms of tissue accretion and bone status (Cormack et al. 2016; Embleton et al. 2023).

Post-discharge nutrition was included in the framework as an unmeasured factor because later growth may be affected by feeding after hospital discharge. In this study, post-discharge feeding category was recorded for available infants, but detailed post-discharge nutrient intake was not available. This limits the ability to assess how nutrient intake after discharge may have influenced growth trajectories from 1 to 6 months CA.

Overall, the framework shows that NICU feeding type may relate to growth through achieved nutrient delivery, while baseline clinical vulnerability may confound this relationship. It also clarifies that DXA outcomes were examined exploratorily and that detailed post-discharge nutrient intake was acknowledged as a limitation rather than directly modelled.

CHAPTER II

LITERATURE REVIEW

2.1 NUTRITIONAL CHALLENGES AND RATIONALE FOR OPTIMISING GROWTH IN PRETERM INFANTS

Preterm infants are at increased risk of postnatal growth failure (PGF) because birth interrupts the rapid nutrient accretion that normally occurs during late gestation. This transition eliminates the continuous placental supply of energy, amino acids, minerals, and micronutrients at a time when the infant has limited endogenous nutrient stores and immature gastrointestinal, metabolic, and feeding systems (De Rose et al. 2024; Embleton et al. 2023). Fetal weight gain varies considerably by gestational age (GA), decreasing from slightly above 20 g/kg/day at 23 to 25 weeks of gestation to approximately 13 to 17 g/kg/day at 30 to 34 weeks and around 10 g/kg/day near term explaining why nutritional targets for preterm infants must be individualised by GA and postnatal growth trajectory rather than applied uniformly (Embleton et al. 2023).

The aim of postnatal nutritional management is to support growth and body composition that approximate those of a normally growing fetus of the same postmenstrual age, while avoiding both nutrient deficiency and excessive catch-up growth (Embleton et al. 2023). In practice, this target is difficult to achieve because neonatal intensive care unit (NICU) nutrition must replace placental nutrient delivery while the infant is clinically unstable and often unable to tolerate full enteral feeding. This mismatch between nutrient requirement and achieved nutrient delivery is a major driver of PGF in preterm infants.

PGF remains common among very low birth weight (VLBW) infants despite improvements in neonatal care. In a large North American cohort of infants weighing 501 to 1500 g across 736 hospitals, average growth velocity increased from 11.8 to 12.9 g/kg/day between 2000 and 2013, while PGF at discharge decreased from 64.5% to 50.3% and severe PGF decreased from 39.8% to 27.5% (Horbar et al. 2015). Despite these improvements, half of infants still had PGF at discharge by 2013, and hospital-level variation remained wide with the interquartile range for PGF covering 41.1% to 61.7% across hospitals indicating that improved survival and reduced morbidity do not automatically translate into adequate growth. More recent evidence confirms that PGF and its associated neurodevelopmental consequences remain clinically significant concerns in contemporary neonatal practice (De Rose et al. 2024; Embleton et al. 2023).

Nutrient deficits may accumulate during several phases of neonatal care: the early postnatal period when parenteral nutrition is being established; the transition from parenteral to enteral nutrition, identified by European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) as a critical period for cumulative energy and protein deficits and poor growth; and periods of clinical deterioration when feeds are interrupted while metabolic demand increases (De Rose et al. 2024; Embleton et al. 2023). ESPGHAN recommends standardised feeding protocols defining feed advancement, feeding intolerance management, fortification strategy, and the threshold for full enteral feeding to minimise these cumulative deficits (Embleton et al. 2023). Recent reviews also emphasise that nutrition should be tailored from the first days of life, particularly in infants with fetal growth restriction or high clinical vulnerability (De Rose et al. 2024).

A further challenge is that neonatal nutrition studies do not always measure or report intake in consistent ways, making it difficult to compare findings or aggregate evidence. Cormack et al. (2016) reviewed studies and found substantial variation in how nutrient intake was calculated whether prescribed or achieved intake was reported, which human milk composition values were assumed, and which growth references were applied. The Standardised Reporting Of Neonatal Nutrition and Growth (StRONNG) checklist therefore recommends standardised reporting of neonatal nutrition and growth, including achieved nutrient intakes rather than prescribed targets,

use of validated preterm growth references such as the Fenton 2013 charts, and reporting of z-scores and z-score change over time. This approach is clinically important because prescribed intake overestimates what infants actually receive and tolerate, particularly when feeding interruptions, feed intolerance, or delayed fortification occur (Cormack et al. 2016).

The challenges of meeting these nutritional targets under routine NICU conditions are further compounded by gastrointestinal immaturity, clinical instability, and practical constraints of NICU care factors that are examined in detail in the context of NICU feeding practices in Section 2.3. The definitions of PGF and the growth trajectories used to evaluate nutritional adequacy are addressed in Section 2.5.1.

This literature review covers studies on preterm nutrition with four aims: (i) nutritional requirements; (ii) breast milk fortification approaches; (iii) feeding types (fortified breast milk versus formula); and (iv) how these exposures relate to growth outcomes at discharge and during follow-up.

2.2 NUTRITIONAL REQUIREMENTS OF PRETERM INFANTS

Preterm infants require higher energy and protein intakes per kilogram of body weight than term infants to approximate fetal growth rates and support neurodevelopment in the extrauterine environment (Embleton et al. 2023). Nutrient requirements vary with postnatal age, clinical stability, and the balance between parenteral and enteral nutrition routes. Understanding these requirements is essential for establishing the intake benchmarks used throughout this thesis to assess nutritional adequacy and growth outcomes in preterm infants. This section summarises international guidance on energy, protein, fat, micronutrients, and essential fatty acids, and explains how these recommendations define the intake benchmarks used in this thesis.

2.2.1 Energy and Macronutrient Requirements

Total energy intake in preterm infants must meet resting energy expenditure (REE), thermoregulation, minimal physical activity, and, critically, the energy cost of tissue accretion. The energy cost of growth accounts for approximately 35% of total energy

requirements at one month of postnatal age, declining progressively to approximately 3% by 12 months of life (Joosten et al. 2018). This declining proportion reflects the transition from rapid growth-phase metabolism to a more metabolically mature state.

For enteral nutrition, energy intakes of 115–140 kcal/kg/day are recommended for stable, growing preterm infants (Embleton et al. 2023). This range provides adequate energy to meet REE and growth requirements whilst accounting for faecal energy losses inherent to enteral feeding. When growth remains below the recommended target of 15–20 g/kg/day despite adequate protein and micronutrient provision, enteral energy intakes may be cautiously increased up to 160 kcal/kg/day (Embleton et al. 2023).

Parenteral energy requirements are substantially lower than enteral requirements because parenteral nutrition bypasses splanchnic losses and stool energy losses associated with gastrointestinal transit. Initial parenteral energy intakes are typically 45–55 kcal/kg/day on the first day of life, advancing toward approximately 90–120 kcal/kg/day during the stable phase of parenteral nutrition (Joosten et al. 2018). Early parenteral nutrition is supported to limit cumulative nutrient deficits during the transition phase when parenteral support is being reduced before enteral volumes and fortification are adequate (Embleton et al. 2023).

During the transition from predominantly parenteral to enteral nutrition, a period characterised by decreasing parenteral volumes whilst enteral feeds advance, total nutrient intakes may temporarily fall below recommended targets, placing infants at particular risk for suboptimal growth (Embleton et al. 2023).

Endogenous glucose production averages approximately 6 mg/kg/minute (≈ 8.6 g/kg/day), and maximal glucose oxidation capacity is approximately 8 mg/kg/minute (≈ 11.5 g/kg/day); glucose infusion above oxidative capacity increases hyperglycaemia risk (Joosten et al. 2018). For enteral nutrition, carbohydrate intakes are typically 11–15 g/kg/day, with adjustments to maintain euglycemia and prevent excessive glucose excursions (Embleton et al. 2023).

2.2.2 Protein Requirements

Protein is the principal determinant of lean tissue accretion when non-protein energy is adequate (Embleton et al. 2023). A recent systematic review demonstrated that for each gram per kilogram per day increase in enteral protein intake, weight gain increases by approximately 5.7 g/kg/day (Sanchez-Holgado et al. 2025).

For stable, growing preterm infants receiving enteral nutrition, ESPGHAN 2022 recommends enteral protein intakes of 3.5–4.0 g/kg/day together with sufficient macro- and micronutrients (Embleton et al. 2023). When growth lags despite adequate energy and micronutrient provision, protein intakes may be cautiously increased up to 4.5 g/kg/day, provided that protein quality is high, concomitant micronutrient delivery is optimal, and no other causes for poor growth have been identified (Embleton et al. 2023).

For preterm infants receiving parenteral nutrition, amino acids are initiated at 1.5–2.0 g/kg/day within the first 8 hours of the decision to commence parenteral nutrition, with advancement toward 3.0–4.0 g/kg/day as clinical tolerance permits and non-protein energy intakes are optimised (Mihatsch et al. 2018; van Goudoever et al. 2018). Early amino acid provision, within 24 hours of birth when feasible, has been associated with reduction in sepsis risk and faster return to birth weight.

Serial measurement of blood urea nitrogen (BUN) provides clinical guidance for assessing protein intake adequacy in preterm infants. Persistently low BUN concentrations in the second and third weeks of life may indicate that protein intakes can be safely increased, whereas BUN concentrations persistently exceeding approximately 5.7 mmol/L (≈ 34 mg/dL), in the absence of renal dysfunction or dehydration, may signal protein oversupply and necessitate reassessment of the protein-to-energy balance (Embleton et al. 2023).

Unfortified breast milk typically provides approximately 1.0–1.5 g of protein per 100 mL; at typical feeding volumes of 150–180 mL/kg/day, this delivers only approximately 1.5–2.7 g/kg/day of protein, substantially below the recommended target of 3.5–4.0 g/kg/day (Embleton et al. 2023; Gidrewicz & Fenton 2014). Therefore,

fortification of expressed breast milk is essential to achieve guideline-recommended nutrient intakes and support optimal growth and neurodevelopment.

2.2.3 Fat and Essential Fatty Acids

Fat provides approximately 45–55% of total non-protein energy in preterm feeding and serves as the essential vehicle for delivery of fat-soluble vitamins and critical polyunsaturated fatty acids. Recommended total fat intake is 4.8–8.1 g/kg/day, with the important caveat that medium-chain triglycerides should comprise no more than 40% of total fat to ensure adequate provision of long-chain polyunsaturated fatty acids (Embleton et al. 2023).

In preterm infants receiving parenteral nutrition, lipid emulsions are advanced progressively toward approximately 3.0–4.0 g/kg/day; total parenteral lipid intake should not exceed 4.0 g/kg/day (Mihatsch et al. 2018). Lipid emulsion tolerance varies considerably with clinical condition; preterm infants with conditions associated with systemic inflammation such as sepsis, bronchopulmonary dysplasia, or other critical illness may require slower advancement or temporary restriction of lipid provision to minimise metabolic and immunological complications (Mihatsch et al. 2018). Biochemical monitoring of serum triglyceride concentrations supports safe advancement when clinical tolerance is uncertain.

Essential fatty acids include linoleic acid and α -linolenic acid which must be provided exogenously as preterm infants cannot synthesise these fatty acids. Long-chain polyunsaturated fatty acids (LC-PUFAs) specifically docosahexaenoic acid (DHA) and arachidonic acid (ARA) are conditionally essential in preterm infants because endogenous synthesis from essential fatty acids is often insufficient to meet the accelerated demands of brain and retinal development (Embleton et al. 2023).

DHA and ARA are actively transferred across the placenta during the third trimester and accumulate substantially in the developing brain, retina, and other neural tissues. Many preterm infants demonstrate reduced plasma DHA and ARA concentrations after birth despite adequate essential fatty acid provision, suggesting insufficient endogenous conversion (Embleton et al. 2023). Low circulating

concentrations have been epidemiologically associated with increased risk of retinopathy of prematurity, septicaemia, and severe bronchopulmonary dysplasia.

ESPGHAN 2022 recommends DHA intake of 30–65 mg/kg/day and ARA intake of 30–100 mg/kg/day, assuming adequate co-supplementation of ARA to maintain an ARA:DHA ratio of 0.5–2:1 (Embleton et al. 2023). These intake targets aim to achieve and maintain plasma concentrations approximating those achieved in the intrauterine environment.

2.2.4 Micronutrient, Mineral, and Vitamin Requirements

Recommended calcium and phosphorus intakes are 1.3–1.4 g/kg/day and 0.5–0.7 g/kg/day respectively, with the recommended calcium-to-phosphorus ratio (by weight) maintained at approximately 1.7:1 to support optimal skeletal mineral accretion (Mihatsch et al. 2021). Early mineral provision is critical; deficits in the first weeks of life are associated with development of postnatal metabolic bone disease of prematurity (Faienza et al. 2019). Early initiation of multi-component fortification from 40–100 mL/kg/day is therefore essential to ensure adequate mineral provision from the initiation of enteral feeding advancement (Embleton et al. 2023).

Magnesium intake of approximately 0.08 g/kg/day (approximately 0.3–0.4 mmol/kg/day) supports neuromuscular function and participation in numerous enzymatic reactions critical to metabolism and growth (De Rose et al. 2024). Breast milk and standard fortifiers generally provide adequate magnesium; additional supplementation is rarely required in stable, growing preterm infants.

Recommended zinc intake is 450–500 µg/kg/day in parenteral nutrition to match intrauterine growth rate (De Rose et al. 2024). Standard neonatal trace element preparations do not supply zinc in sufficient quantity, and additional zinc must be added to parenteral nutrition fluid to achieve adequate intakes. For enterally fed infants, zinc fortification through multi-component fortifiers is essential. Zinc supplementation for more than two weeks has been associated with improvements in head circumference growth and correlates with better motor development scores at 12 months of life (De Rose et al. 2024).

Recommended copper intake is 0.1–0.15 mg/kg/day and selenium intake is 1.3–3.0 µg/kg/day (De Rose et al. 2024). Both elements are essential components of antioxidant systems and metalloproteins critical to immune function and metabolic regulation. Copper deficiency can lead to hypocupraemia, neutropenia, and bone disease, whilst selenium deficiency impairs immune function and increases risk of sepsis and bronchopulmonary dysplasia. These elements are typically provided through human milk, fortifiers, and parenteral trace element preparations.

Vitamin A (Retinol) at 700–1500 IU/kg/day supports retinal development, immune function, and cellular differentiation (De Rose et al. 2024). Preterm infants are at heightened risk of vitamin A deficiency due to limited transplacental transfer, minimal tissue stores at birth, and accelerated utilisation during critical illness and rapid growth. Vitamin A deficiency has been associated with increased risk of retinopathy of prematurity, bronchopulmonary dysplasia, and impaired immune response. Human milk vitamin A concentration is highly variable and often insufficient; fortified preterm formulas typically provide adequate amounts, though additional supplementation may be necessary in the most premature infants.

Vitamin D at 400–1000 IU/kg/day is critical for calcium-phosphorus homeostasis, skeletal mineralisation, immune function, and neurodevelopment (De Rose et al. 2024). Vitamin D deficiency in preterm infants is associated with rickets of prematurity, delayed skeletal mineralisation, impaired immune function, and respiratory complications. Regular biochemical assessment of serum 25-hydroxyvitamin D is essential throughout hospitalisation.

Vitamin E (α-tocopherol) at 2.8–3.5 mg/kg/day provides potent lipid-soluble antioxidant protection, shielding cellular membranes from oxidative damage (De Rose et al. 2024). Preterm infants have immature antioxidant defence systems and heightened oxidative stress, placing them at particular risk of vitamin E deficiency, which may contribute to bronchopulmonary dysplasia and retinopathy of prematurity.

Vitamin K at 10 µg/kg/day supports synthesis of coagulation factors and bone-resident proteins (De Rose et al. 2024). All newborns receive intramuscular vitamin K prophylaxis at birth to prevent haemorrhagic disease of the newborn. Exclusively breastfed preterm infants may receive inadequate vitamin K postnatally, particularly following hospital discharge, necessitating consideration of oral vitamin K supplementation (approximately 50 µg/day) to prevent late vitamin K deficiency bleeding.

Folic acid (Vitamin B₉) at 56 µg/kg/day supports rapid cell division and proliferation critical for growth and development, whilst additional B vitamins including thiamine, riboflavin, niacin, and pyridoxine support energy metabolism and neural development (De Rose et al. 2024). These vitamins are provided through breast milk, fortifiers, and standard parenteral vitamin preparations.

In critically ill preterm infants, withholding parenteral macronutrients (carbohydrate and lipids) for one week while continuing parenteral micronutrient provision intravenously has been shown in clinical trials to reduce infection rates, mechanical ventilation duration, and intensive care unit length of stay without adverse nutritional effects (Joosten et al. 2018). This selective nutrient withholding approach may be considered in infants with severe systemic inflammation or septicaemia.

Parenteral micronutrient formulations specifically designed for neonates is recommended to be used when available, as these provide micronutrient compositions optimised for the unique physiological requirements of preterm infants and reduce the burden of individual micronutrient supplementation (De Rose et al. 2024).

2.2.5 Clinical Considerations for Glucose and Lipid Provision

Glucose provision in parenteral nutrition must remain within the physiological oxidation limits of the preterm infant to minimise hyperglycaemia risk. As noted above, maximal glucose oxidation capacity averages 8 mg/kg/minute (approximately 11.5 g/kg/day); exceeding this threshold results in glucosuria and metabolic complication (Joosten et al. 2018). Dextrose infusion rates should be adjusted serially in response to frequent plasma glucose measurements, aiming to maintain glucose concentrations

within the normal fetal range of $> 3 \text{ mmol/L}$ ($> 54 \text{ mg/dL}$) and $< 6 \text{ mmol/L}$ ($< 108 \text{ mg/dL}$) to the extent possible.

Lipid emulsion tolerance varies considerably with clinical condition and inflammatory state. Preterm infants with conditions associated with enhanced inflammation such as sepsis, bronchopulmonary dysplasia, necrotising enterocolitis, or other systemic illness may require slower advancement or temporary restriction of lipid provision to minimise metabolic complications and lipid-induced immunosuppression (Ghavam et al. 2024). Biochemical monitoring of serum triglyceride concentrations supports safe advancement when tolerance is uncertain, with triglyceride concentrations typically maintained below 200 mg/dL during acute illness phases.

2.2.6 International Guideline Comparison and Clinical Implementation

Current international guidelines differ in their specific nutrient recommendations for preterm infants, reflecting variations in evidence interpretation, expert consensus processes, and clinical contexts. Key distinctions between widely cited guidelines are summarised below.

Koletzko et al. (2021) recommends enteral energy of $110\text{--}140 \text{ kcal/kg/day}$, protein of 3.5 g/kg/day , fat of $4.55\text{--}8.1 \text{ g/kg/day}$, and linoleic acid of $385\text{--}1540 \text{ mg/kg/day}$. However, this guideline does not explicitly specify required intakes for DHA and ARA.

ESPGHAN Committee on Nutrition 2022 (Embleton et al. 2023) recommends enteral energy of $115\text{--}140 \text{ kcal/kg/day}$ (extendable to 160 kcal/kg/day when growth is below the recommended range), protein of $3.5\text{--}4.0 \text{ g/kg/day}$ (up to 4.5 g/kg/day under specified conditions), fat of $4.8\text{--}8.1 \text{ g/kg/day}$, carbohydrate of $11\text{--}15 \text{ g/kg/day}$, and explicitly specifies DHA at $30\text{--}65 \text{ mg/kg/day}$ and ARA at $30\text{--}100 \text{ mg/kg/day}$.

The key distinctions between these guidelines are: (1) ESPGHAN 2022 defines specific, evidence-based targets for LC-PUFAs (DHA and ARA), reflecting the enhanced understanding of their importance for neural and retinal development; (2) protein recommendations in ESPGHAN 2022 are narrowed to a specific range of 3.5--

4.0 g/kg/day with restricted, well-defined conditions for increases to 4.5 g/kg/day; (3) ESPGHAN 2022 permits higher maximum energy intakes (160 kcal/kg/day) when clinically indicated for growth support; and (4) ESPGHAN 2022 explicitly recommends carbohydrate intake targets (11–15 g/kg/day) to guide clinical practice.

These recommendations establish the evidence-based nutrient intake targets used throughout this thesis to assess nutritional adequacy, monitor growth and body composition outcomes, and interpret the nutritional intake data collected from preterm infants enrolled in the research cohort.

Table 2.1 Recommended enteral nutrient intakes for preterm infants: Koletzko et al. (2021) vs Embleton et al. (2023)

	Koletzko et al. (2021)	Embleton et al. (2023)
Fluid, mL/kg/d	135–200	150–180 (135–200)
Energy, kcal/kg/d	110–135	115–140 (–160)
Protein, g/kg/d	3.5–4.5	3.5–4.0 (–4.5)
Fat, g/kg/d	4.8–6.6	4.8–8.1
Linoleic acid, mg/kg/d	385–1540	385–1540
α -Linolenic acid, mg/kg/d	> 55	≥ 55
DHA, mg/kg/d	12–0	30–65
ARA, mg/kg/d	18–42	30–100
EPA, mg/kg/d		< 20
Carbohydrate, g/kg/d	11.6–13.2	11–15 (–17)
Sodium, mmol/kg/d	3.0–5.0	3.0–5.0 (–8.0)
Chloride, mmol/kg/d	3.0–5.0	3.0–5.0 (–8.0)
Potassium, mmol/kg/d	1.7–3.4	2.3–4.6
Calcium, mmol/kg/d	3.0–3.5	3.0–5.0
Phosphorus, mmol/kg/d	1.9–2.9	2.2–3.7
Magnesium, mmol/kg/d	0.3–0.6	0.4–0.5
Iron, mg/kg/d	2.0–3.0	2.0–3.0 (–6.0)
Zinc, mg/kg/d	1.1–2.0	2.0–3.0
Copper, µg/kg/d	100–132	120–230
Selenium, µg/kg/d	5–10	7–10
Manganese, µg/kg/d	< 27.5	1–15
Iodine, µg/kg/d	11–55	11–55
Chromium, µg/kg/d	0.03–1.23	0.03–2.25
Molybdenum, µg/kg/d	0.3–5	0.3–5.0
Thiamine (B1), µg/kg/d	140–300	140–290
Pantothenic acid, mg/kg/d	0.33–2.1	0.6–2.2

to be continued...

...continuation

Biotin, µg/kg/d	1.7–16.5	3.5–15
Niacin, µg/kg/d	380–5500	1100–5700
Ascorbic acid (vitamin C), mg/kg/d	11–46	17–43
Riboflavin (B2), µg/kg/d	200–400	200–430
Pyridoxine, µg/kg/d	45–300	70–290
Pyridoxine, µg/kg/d	35–100	23–100
Cobalamin (B12), µg/kg/d	0.1–0.77	0.1–0.6
Vitamin A, IU/kg/d	1333–3300	1333–3300
Vitamin D, IU/kg/d	800–1000 IU/d	400–700 IU/kg/d (< 1000)
Vitamin E, mg/kg/d	2.2–11	2.2–11
Vitamin K, µg/kg/d	4.4–28	4.4–28

ARA = arachidonic acid; CoN = Committee of Nutrition; DHA = docosahexaenoic acid; EPA = Eicosatetraenoic acid, IU = International units. Figures in brackets represent ranges or upper intakes that might occasionally be needed in routine clinical practice under certain conditions.

Source: Koletzko et al. 2021, Embleton et al. 2023.

2.3 PRETERM FEEDING IN THE NICU: BREAST MILK AND FORMULA MILK

The central unresolved question in preterm NICU nutrition is not whether breast milk is biologically superior to formula in principle that is well established but whether exposure to predominantly fortified breast milk (PBM) versus predominantly formula milk (PFM) produces meaningfully different patterns of nutrient delivery and growth in routine clinical practice. Breast milk is the preferred substrate because of its unique immunological and bioactive properties, yet unfortified breast milk cannot reliably meet the elevated protein, mineral, and energy demands of very preterm infants at conventional feeding volumes without fortification (Arslanoglu et al. 2019; Embleton et al. 2023). Preterm formula provides more standardised macronutrient delivery and has been associated with faster short-term weight gain when compared with donor breast milk in the absence of maternal milk, but it confers none of the morbidity protection that characterises breast milk feeding, including the reduction in necrotising enterocolitis (NEC) risk (Quigley et al. 2019). The clinical and scientific challenge is therefore not one of preference but of measurable consequence: does the predominant feeding substrate received during NICU admission translate into differences in achieved nutrient intake, and do those variances persist as different growth trajectories through early infancy? This question frames the evidence reviewed in this section and motivates the study design described in subsequent chapters.

Preterm infants require enteral nutrition support during hospitalisation (Embleton et al. 2023), with available substrates including mother's own milk (MOM), pasteurised donor breast milk (PDBM), and preterm formula milk (Embleton et al. 2023). The choice of feeding substrate significantly influences early growth trajectories (Zhang et al. 2020), morbidity outcomes, and long-term neurodevelopmental trajectories in this vulnerable population (Embleton et al. 2023).

Preterm feeding practice is strongly shaped by gastrointestinal maturity. Preterm birth interrupts late gestational development of the gastrointestinal tract, immune system, and gut microbiota, leaving infants with immature motility, digestive enzyme activity, mucosal barrier function, immune defence, and microbial development (Henderickx et al. 2021; Joosten & Vermeulen 2024). Henderickx et al. (2021) study demonstrated that gastrointestinal and microbial proteins related to gut maturity are associated with gestational and postnatal age, and that preterm infants had lower levels of gastrointestinal barrier-related proteins during the first six postnatal weeks compared with term infants. These findings suggest that digestive and barrier function remains immature during early postnatal life, so prescribed or delivered feeds may not fully represent the nutrients available for growth.

Early enteral feeding remains important because use of the gut supports intestinal development and maturation. Thoene and Anderson-Berry (2021) reported that early enteral feeding in preterm infants supports micronutrient delivery, intestinal development, microbiome development, inflammatory control, brain growth, and neurodevelopment. Their review also noted that delayed feeding, prolonged trophic feeding, and slow feed advancement may increase the time needed to reach full enteral feeding. Current ESPGHAN recommendations support starting small-volume enteral feeds as soon as possible after birth in most preterm infants and advancing feeds as clinically tolerated; in stable infants, a daily increment of 18 to 30 mL/kg/day is recommended when clinicians judge that feeds can be increased (Embleton et al. 2023; Joosten & Vermeulen 2024).

Gastrointestinal immaturity also explains why many preterm infants require parenteral nutrition before full enteral feeding is established. Joosten and Vermeulen

(2024) state that most preterm infants cannot tolerate full enteral feeding immediately after birth because of gastrointestinal immaturity, so parenteral nutrition is needed while enteral feeds are increased. The transition from parenteral to enteral nutrition is a recognised period of risk for cumulative energy and protein deficits and poor growth (Embleton et al. 2023; Joosten & Vermeulen 2024), and ESPGHAN recommends standardised NICU feeding protocols covering minimal enteral feeding, daily feed advancement, gastric residuals, feeding intolerance, fortification strategy, and the definition of full enteral feeds (Embleton et al. 2023).

Feeding intolerance further complicates nutrient delivery. It occurs in approximately 75% of VLBW infants and is commonly described using signs such as increased gastric residual volume, abdominal distension, emesis, apnea, bradycardia, and desaturation (Ortigoza 2022). However, these signs are non-specific and feeding-related cardiorespiratory occurrences that are common in preterm infants but are not reliable indicators of feeding intolerance (Ortigoza 2022). This matters because overdiagnosis of feeding intolerance may lead to unnecessary feed interruption, slower feed advancement, longer parenteral nutrition exposure, and lower achieved nutrient intake during a period of high growth demand (Embleton et al. 2023; Ortigoza 2022).

Therefore, NICU feeding exposure cannot be interpreted by milk type alone. Whether an infant receives fortified breast milk or formula, the nutritional effect depends on GA, clinical stability, gut tolerance, timing of feed advancement, parenteral nutrition duration, feed interruptions, and achieved nutrient intake (Cormack et al. 2016; Joosten & Vermeulen 2024). This provides the rationale for examining feeding type together with achieved nutrient intake and feeding milestones, rather than relying only on feeding category, when assessing postnatal growth trajectories in preterm infants.

The rationale for prioritising MOM is supported by substantial evidence demonstrating its unique protective properties. MOM is the preferred foundation for enteral nutrition in preterm infants (Embleton et al. 2023) because it offers unique protection against major neonatal morbidities. A systematic review of 42 studies (n = 89 638) comparing formula feeding with MOM in preterm and low birth weight infants found that formula feeding increased the risk of NEC by threefold (odds ratio [OR]

2.99, 95% confidence interval [CI] 1.75–5.11) (Strobel et al. 2022). In contrast, no significant differences were demonstrated between feeding types for all-cause mortality (OR 1.26, 95% CI 0.91–1.76), severe infection (OR 1.52, 95% CI 0.98–2.37), growth, or neurodevelopment at discharge. However, these analyses included predominantly observational studies; the certainty of evidence was low for mortality and NEC, and very low for growth and neurodevelopmental outcomes, indicating that whilst NEC protection is substantive, mortality reduction is not definitively demonstrated at the level of randomised trials (Strobel et al. 2022).

Preterm maternal milk differs substantially from term milk, containing higher concentrations of proteins, lipids, calories (approximately 80 kcal/100 mL), and immunological factors that persist throughout the first four weeks of lactation (Embleton et al. 2023; Gidrewicz & Fenton 2014). Breast milk provides multiple bioactive components, including immunoglobulin A (IgA), lactoferrin, lysozymes, breast milk oligosaccharides, and antimicrobial peptides (Yi & Kim 2021), that enhance intestinal maturation, support immune function, and reduce bacterial translocation. Clinical practice therefore prioritises early initiation of expressed breast milk, beginning within 24 hours of life when the infant is clinically stable, commencing with trophic feeding (10–20 mL/kg/day) and advancing incrementally at 20–30 mL/kg/day toward full enteral nutrition (150–180 mL/kg/day) (Embleton et al. 2023; Yi & Kim 2021).

When MOM is unavailable or insufficient, PDBM is recommended over preterm formula, particularly for very low birth weight infants (≤ 1500 grams), to reduce the risk of necrotising enterocolitis (Parker et al. 2021; Embleton et al. 2023). Donor breast milk is intended as a short-term bridge to establish feeding and is typically transitioned once maternal milk supply becomes adequate to meet the infant's nutritional needs or when the infant is clinically stable and no longer at high risk of NEC (Parker et al. 2021). However, PDBM presents its own nutritional limitations. Pasteurisation by the Holder method reduces specific proteins, enzymes, and growth-promoting factors. Additionally, PDBM is predominantly obtained from mothers of term or older infants and therefore has a lower macronutrient profile, particularly protein, than preterm MOM (Ong et al. 2025). A randomised controlled trial from Singapore's KK Women's and Children's Hospital found that even with standard

fortification, the protein content of PDBM frequently fell below ESPGHAN recommended intakes for very low birth weight infants, and that an enhanced fortification strategy selecting high-fat PDBM with additional protein supplementation of 0.67 g/100 mL resulted in smaller declines in linear and head circumference z-scores at discharge, though it did not significantly reduce impaired weight gain (Ong et al. 2025). This finding, from a regional tertiary NICU using a similar care model to Malaysia, illustrates that even active fortification of donor milk may be insufficient to fully close the growth gap when the underlying substrate has nutritionally inferior composition.

Preterm formula is used when both MOM and donor breast milk are unavailable or insufficient to meet nutritional targets despite fortification and lactation support (Embleton et al. 2023). Preterm formulas are specifically designed to meet the elevated nutritional demands of preterm infants, with higher protein content (2.0–2.4 g/100 mL), increased energy density (approximately 80 kcal/100 mL), and elevated concentrations of calcium, phosphorus, and other minerals to support lean tissue accretion and bone mineralisation (Embleton et al. 2023). ESPGHAN recommends that medium chain triglycerides should not exceed 40% of total fat, given their advantage in absorption independent of bile-mediated emulsification. Recommended intakes of docosahexaenoic acid are 30 to 65 mg/kg/day with adequate arachidonic acid, and arachidonic acid is recommended at 30 to 100 mg/kg/day (Embleton et al. 2023).

A 2019 Cochrane review comparing formula (term or preterm) with donor breast milk (12 trials, n = 1871 infants) found that when maternal breast milk is not available, formula feeding resulted in faster rates of weight gain, linear growth, and head circumference gain during hospitalisation (Quigley et al. 2019). However, this growth advantage came with a substantially increased risk of NEC (RR 1.87, 95% CI 1.23-2.85), raising a critical clinical question: whether faster early growth at the cost of higher morbidity represents an appropriate trade-off in very preterm infants, especially given that long-term neurodevelopmental and growth benefits remain uncertain.

Standard preterm formulas (80 kcal/100 mL) are typically used for infants < 1800 grams or < 34 weeks gestation (Embleton et al. 2023). Transitional post-discharge

formulas (72 kcal/100 mL) are introduced for growing preterm infants approaching discharge (typically > 1800 grams or > 34 weeks), and standard term formula (68 kcal/100 mL) with appropriate vitamin and mineral supplementation is used for infants approaching term-equivalent age and discharge weight (Embleton et al. 2023).

Unfortified MOM, whilst providing irreplaceable bioactive protection, may not provide sufficient protein and energy to meet the elevated nutrient demands of very preterm infants at conventional feeding volumes (150–180 mL/kg/day). Multi-nutrient fortification of breast milk, where additional protein, carbohydrate, fat, minerals, and vitamins are added to increase nutrient density while maintaining osmolality within safe limits (typically < 450 mOsm/kg), serves to bridge this nutritional gap (Embleton et al. 2023). Whether the combination of MOM with fortification achieves growth outcomes equivalent to those seen with preterm formula, and whether any differences persist beyond discharge through 6 months CA, remains an open question that this study is designed to address.

Overall, preterm feeding in the NICU cannot be interpreted by milk type alone. Whether an infant receives fortified breast milk or formula, the nutritional effect depends on GA, clinical stability, gastrointestinal tolerance, timing of feed advancement, duration of parenteral nutrition, feed interruptions, and achieved nutrient intake. This is important when comparing PBM and PFM, because PBM may retain the biological benefits of breast milk but has more variable nutrient delivery, while PFM provides a more standardised nutrient profile but lacks human milk bioactive components. Therefore, Section 2.5 examines how these predominant feeding types may relate to nutrient adequacy, anthropometric growth, body composition, and bone outcomes in preterm infants.

2.4 BREAST MILK FORTIFICATION IN PRETERM INFANTS: RATIONALE AND STRATEGIES

2.4.1 Rationale for Breast Milk Fortification

Breast milk remains the optimal nutritional source for preterm infants due to its immunological properties, digestive advantages, and neurodevelopmental benefits (Robinson et al. 2023). However, unfortified breast milk consistently falls short of the elevated nutritional requirements of VLBW, < 1500 g and extremely low birth weight (ELBW, < 1000 g) infants (Arslanoglu et al. 2019).

Milk composition varies over lactation, with higher protein in colostrum and transitional milk, followed by a marked decline during the first month (Gidrewicz & Fenton 2014). This variability adds further uncertainty when fixed assumptions are used for nutrient delivery in NICU care.

Mature breast milk provides approximately 67 kcal/100 mL and 1.0–1.3 g protein/100 mL, 28 mg/dL of calcium, and 14 mg/dL of phosphorus (Bzikowska et al. 2018). At standard enteral volumes of 150–180 mL/kg/day, unfortified breast milk delivers only 100–120 kcal/kg/day and 1.5–2.3 g protein/kg/day substantially below consensus recommendations of 115–160 kcal/kg/day and 3.5–4.0 g protein/kg/day required to approximate intrauterine growth rates (Embleton et al. 2023). This shortfall is particularly pronounced for protein, where delivery commonly falls below 2.0 g/kg/day without fortification, creating the conditions for progressive protein-energy deficits (Arslanoglu et al. 2019; Embleton et al. 2023).

Beyond macronutrients, mineral provision proves equally limiting. Unfortified breast milk supplies insufficient calcium and phosphorus to match the third-trimester bone mineralisation rate of approximately 100–120 mg/kg/day calcium and 60–70 mg/kg/day phosphorus, predisposing infants to metabolic bone disease of prematurity (Embleton et al. 2023). Similarly, native concentrations of sodium, zinc, and fat-soluble vitamins frequently fail to meet the enhanced demands imposed by immature regulatory mechanisms and rapid tissue accretion in extremely preterm populations (Arslanoglu et al. 2019; Embleton et al. 2023).

The physiological intolerance of large enteral volumes in the immediate postnatal period compounds nutritional vulnerability. Gastric capacity limitations, delayed gastric emptying, and feeding interruptions for clinical procedures restrict achievable volumes during the critical first weeks when extrauterine growth failure typically becomes established (Embleton et al. 2023). Even when full enteral feeds are attained, volumes exceeding 180–200 mL/kg/day risk feed intolerance, increased gastric residuals, and potentially higher aspiration risk, creating a ceiling effect that cannot be overcome simply by increasing milk volume (Rochow et al. 2016). Fortification addresses this constraint by augmenting nutrient density within tolerable volume limits.

The consequence of sustained inadequate intake manifests as progressive accumulation of nutrient deficits relative to expected foetal accretion. Longitudinal studies demonstrate that unfortified feeding results in weight-for-age z-scores declining by 1.0–1.5 standard deviations from birth to term-equivalent age, with disproportionate loss of lean body mass reflecting chronic protein insufficiency (Rochow et al. 2013). This extrauterine growth restriction associates with adverse neurodevelopmental outcomes, including reduced cognitive scores and increased risk of cerebral palsy in ELBW cohorts (Fabrizio et al. 2020). Similarly, inadequate mineral provision during the period of maximal skeletal growth establishes osteopenia that may persist beyond hospital discharge and increase fracture susceptibility (Embleton et al. 2023).

Fortification emerged from the recognised imperative to bridge the gap between what unfortified milk provides and what preterm metabolism demands to support organ maturation and approximate intrauterine growth trajectories (Arslanoglu et al. 2019). The theoretical framework posits that by enriching breast milk to deliver nutrients at concentrations matching or exceeding estimated requirements, fortification enables infants to benefit from breast milk's immunological and developmental advantages while simultaneously achieving nutritional intakes consistent with their unique physiological state. This dual objective preserving the bioactive components and anti-infective properties of breast milk while correcting its quantitative nutrient deficiencies constitutes the fundamental rationale underpinning contemporary fortification practice (Arslanoglu et al. 2019; Embleton et al. 2023; Fusch et al. 2021).

2.4.2 Types of Breast Milk Fortifiers

Commercial fortification products vary considerably in formulation, nutrient composition, and manufacturing origin, necessitating informed selection based on institutional resources, population characteristics, and clinical priorities. Contemporary fortifiers typically employ a multinutrient approach, incorporating protein, carbohydrate, lipid, minerals (particularly calcium, phosphorus, sodium, and zinc), and vitamins within a single product to address the multiple simultaneous deficiencies present in unfortified milk (Arslanoglu et al. 2019; Embleton et al. 2023). Protein components may derive from intact casein or whey fractions, partially hydrolysed proteins, or extensively hydrolysed peptides, with varying degrees of bioavailability and allergenic potential. Energy supplementation most commonly employs maltodextrin or corn syrup solids for carbohydrate and medium-chain triglyceride oil or vegetable oils for lipid, though products differ considerably in their specific fat blends and inclusion of LC-PUFAs (Embleton et al. 2023).

The mineral profile of fortifiers aims to replicate foetal bone accretion rates while accounting for the lower bioavailability of calcium and phosphorus from milk compared with placental transfer. Standard formulations typically target calcium-to-phosphorus ratios of approximately 1.7:1 to optimise skeletal mineralisation while preventing hypercalciuria or hyperphosphatemia (Arslanoglu et al. 2019). Micronutrient inclusion encompasses fat-soluble vitamins (A, D, E, K) and water-soluble vitamins (B-complex, C), with dosing calibrated to prevent both deficiency states and toxicity from excessive supplementation in the context of renal and hepatic immaturity (Embleton et al. 2023).

2.4.3 Bovine Milk-Derived Versus Human Milk-Derived

Fortifiers are principally classified by protein source into bovine milk-based multinutrient fortifiers (BMBF) and human milk-derived fortifiers (HMBF). BMBFs, manufactured from cow milk components, represent the most widely utilised category globally due to lower cost, established manufacturing infrastructure, and extensive clinical experience supporting safety and efficacy (Arslanoglu et al. 2019). These products contain bovine proteins, which theoretically introduce non-human antigens

that might stimulate inflammatory responses or contribute to feeding intolerance, though clinical significance remains debated (Jensen et al. 2024).

HMBFs, produced from pooled pasteurised donor human milk through ultrafiltration and concentration processes, were developed to facilitate an exclusive human milk diet free from bovine proteins. Proponents hypothesised that exclusive human milk feeding might further reduce necrotising enterocolitis and other inflammatory complications beyond the benefits already conferred by breast milk as the base diet (Embleton et al. 2023).

LC-PUFA content represents another critical dimension of variation. While some fortifiers include preformed DHA and ARA to support retinal and cerebral membrane development, others lack these components or provide them only in trace amounts, necessitating separate supplementation if target intakes are to be achieved (Embleton et al. 2023). Mineral composition also differs meaningfully, particularly regarding sodium content, which ranges from minimal to > 30 mg per gram of fortifier; this variation has implications for infants at risk of hyponatremia or those with chronic lung disease requiring sodium restriction (Arslanoglu et al. 2019).

Osmolality constitutes an additional consideration, as fortified milk osmolality may approach or exceed 450 mOsm/kg depending on product choice and concentration used, potentially influencing feeding tolerance and theoretically affecting gut mucosal integrity, though clinical data directly linking fortifier osmolality to necrotising enterocolitis risk remain limited (Embleton et al. 2023; Fusch et al. 2021).

Comparative safety data between BMBF and HMBF have been strengthened by the Swedish N-forte trial, which definitively demonstrated clinical equivalence for major neonatal outcomes. The trial provided robust evidence that HMBF does not reduce NEC, sepsis, or mortality compared with BMBF in the context of an exclusive breast milk base diet and targeted fortification (Jensen et al. 2024).

Tolerability concerns including feeding intolerance, increased gastric residuals, and metabolic derangements appear similar across fortifier types when implemented according to standard protocols, though individual product characteristics (such as protein hydrolysis degree or osmolality) may influence tolerance in specific clinical contexts (Embleton et al. 2023). Long-term neurodevelopmental and metabolic outcomes following different fortification approaches remain incompletely characterised, highlighting an important knowledge gap requiring ongoing investigation through extended follow-up studies (Fusch et al. 2021).

2.4.4 Fortification Strategies

Implementation of fortification practice employs several distinct methodological approaches that differ fundamentally in their precision, resource requirements, and capacity to address inter-individual and intra-individual variability in milk composition and infant metabolic response.

Standard (Fixed-Dose) Fortification is the most widely employed method globally, characterised by the addition of a predetermined, fixed quantity of multinutrient fortifier per unit volume of milk (typically per 100 mL) once enteral feeds reach a threshold volume, commonly 50–100 mL/kg/day (Arslanoglu et al. 2019). This approach is predicated on population-average assumptions about breast milk composition and infant requirements, applying uniform supplementation regardless of actual milk nutrient content or individual infant characteristics beyond GA and birth weight (Embleton et al. 2023).

The principal advantages of standard fortification include operational simplicity, minimal equipment requirements, low staff training burden, and applicability in resource-limited settings where sophisticated monitoring or laboratory infrastructure is unavailable (Brown et al. 2020). Implementation typically follows straightforward protocols that nursing staff can execute reliably with minimal error risk, facilitating widespread adoption and consistency across shifts and providers (Arslanoglu et al. 2019).

However, this approach possesses inherent limitations stemming from its inability to account for the substantial biological variability in breast milk composition. Protein content in expressed milk varies markedly between mothers (coefficient of variation 20%–30%), changes over the course of lactation (declining from early to mature milk), differs between fore- and hind-milk, and fluctuates across pumping sessions even in the same individual (Fusch et al. 2021). Consequently, fixed-dose fortification may systematically under-fortify when native milk protein is low or over-fortify when endogenous content is high, potentially resulting in persistent protein deficits in a substantial proportion of infants despite fortification (Rochow et al. 2013).

Adjustable (Individualised) Fortification: Adjustable fortification, also termed individualised or biochemical-guided fortification, builds upon standard fortification by incorporating metabolic feedback to titrate nutrient delivery according to individual infant response (Arslanoglu et al. 2019; Embleton et al. 2023). This method initiates with standard-dose fortification and subsequently adjusts protein (and sometimes energy) supplementation upward or downward based on biochemical markers reflecting protein–energy status, most commonly blood urea nitrogen (BUN) (Khorana et al. 2024).

The physiological rationale derives from BUN's relationship to protein intake and utilisation. At adequate protein-to-energy ratios, BUN typically ranges between 2.5 and 5.0 mmol/L (7–14 mg/dL), reflecting appropriate protein anabolism. Values below this range suggest insufficient protein delivery relative to energy, while substantially elevated concentrations may indicate protein excess, inadequate energy provision, or impaired renal function (Embleton et al. 2023). By monitoring BUN at regular intervals (typically weekly or biweekly) and adjusting fortifier dose or adding modular protein when BUN falls below target thresholds, the approach attempts to optimise individual protein intake dynamically over the hospitalisation course (Khorana et al. 2024).

Despite these benefits, adjustable fortification imposes operational requirements beyond standard practice. These include regular phlebotomy for BUN monitoring (approximately weekly), which demands phlebotomy capability, laboratory access with reasonable turnaround time, and established protocols defining target ranges and dose-

adjustment algorithms (Embleton et al. 2023; Khorana et al. 2024). Additionally, BUN reflects protein–energy balance rather than absolute protein intake, potentially missing protein deficits in infants with concomitant energy insufficiency, and may be influenced by clinical factors such as dehydration, renal immaturity, or critical illness independent of nutritional status (Fusch et al. 2021).

Targeted fortification represents the most analytically precise approach. It employs direct measurement of macronutrient content in expressed breast milk samples to calculate exact nutrient provision and guide individualised supplementation with modular protein, fat, and carbohydrate additives (Fusch et al. 2021). Milk analysis is typically performed two to three times weekly using mid-infrared spectroscopy analysers originally developed for the dairy industry and adapted for human milk analysis, providing rapid quantification of protein, fat, carbohydrate, and energy content in small sample volumes (Rochow et al. 2013).

The conceptual advantage of targeted fortification lies in its capacity to compensate directly for the substantial inter-individual and intra-individual variability in breast milk composition that limits fixed-dose approaches. By measuring actual nutrient content rather than relying on population averages, targeted fortification enables calculation of precise supplementation needs for each infant's milk supply, theoretically eliminating systematic under- or over-fortification (Fusch et al. 2021). This approach addresses the biological reality of milk variability and reduces cumulative nutrient deficits that persist even with standard fortification (Fusch et al. 2021).

However, subsequent trials have highlighted implementation challenges that may limit broader applicability. A Polish randomised controlled trial originally designed to enrol 220 infants ≤ 32 weeks' gestation was terminated prematurely after randomising only 39 infants due to feasibility concerns and high rates of feeding intolerance in the targeted arm (Seliga-Siwecka et al. 2023). The study found no significant difference in weight gain velocity between targeted and standard fortification (15.1 ± 4.4 vs. 13.9 ± 3.1 g/kg/day, $p = 0.34$) and noted greater feeding intolerance in the targeted group, though the limited sample size precludes definitive

conclusions (Seliga-Siwecka et al. 2023). This experience underscores that successful implementation does not only require analytical equipment. It demands meticulous attention to modular mixing technique, staff training in complex calculations and additives handling, quality assurance processes to prevent errors, and clinical protocols for managing tolerance issues that may arise with higher nutrient density (Seliga-Siwecka et al. 2023).

Beyond the three principal methods, alternative fortification strategies have been explored to address specific clinical scenarios or resource constraints. One such approach involves using post-discharge formulas designed for preterm infants as fortifiers by adding them to breast milk rather than employing purpose-manufactured fortifier products (Arslanoglu et al. 2019). This practice, termed "formula-as-fortifier", offers cost advantages in settings where commercial fortifiers are prohibitively expensive or unavailable, and may provide more consistent nutrient delivery than standard fortification since formula composition is standardised (Embleton et al. 2023). However, it necessarily introduces bovine proteins, precludes exclusive breast milk feeding, and may achieve less precise nutrient targets than purpose-designed fortifiers, limiting its application primarily to resource-limited environments where the alternative is unfortified milk (Arslanoglu et al. 2019).

Another alternative involves selective fortification, where only specific nutrients (most commonly protein or minerals) are supplemented rather than using comprehensive multnutrient products, guided either by biochemical monitoring or population-based risk assessment (Embleton et al. 2023). This approach may be appropriate when specific deficiencies are anticipated (e.g., protein in late preterm infants receiving predominantly mature milk, or minerals in infants with metabolic bone disease risk factors) but requires careful attention to maintain appropriate nutrient ratios and avoid iatrogenic imbalances (Embleton et al. 2023).

Practical considerations and resource requirements vary substantially across these strategies. Standard fortification requires minimal resources beyond the fortifier product itself, making it suitable for diverse clinical settings. Adjustable fortification necessitates laboratory access for BUN monitoring and staff trained in interpreting

results and adjusting fortification accordingly. Targeted fortification demands significant capital investment in milk analysers, specialised staff training, quality control systems, and ongoing maintenance, representing the highest resource requirement among the approaches.

International recommendations and indications for fortification such as ESPGHAN, the European Milk Bank Association (EMBA), and the World Health Organization (WHO) endorse multinutrient fortification for very preterm and extremely preterm infants (typically < 1800 g birth weight or < 32 weeks' gestation) (Embleton et al. 2023; Arslanoglu et al. 2019; WHO 2023). Consensus recommendations specify energy targets of 115–160 kcal/kg/day and protein targets of 3.5–4.0 g protein/kg/day to approximate intrauterine growth rates while accounting for individual variation in metabolic demands and milk composition (Embleton et al. 2023). These guidelines generally emphasise multicomponent fortification over single-nutrient supplementation, recognising that simultaneous deficiencies in protein, energy, minerals, and vitamins are characteristics of unfortified milk and require coordinated correction (Embleton et al. 2023; Arslanoglu et al. 2019).

Fortification is typically initiated when infants tolerate 100 mL/kg/day of enteral feeds (or 50–100 mL/kg/day in some protocols) and is continued until postmenstrual age approaches 36–40 weeks, infants reach discharge body weight targets (commonly 1800–2000 g), or discharge from hospital occurs (Embleton et al. 2023; WHO 2023).

2.5 IMPACT OF PREDOMINANT FEEDING TYPE ON GROWTH, BODY COMPOSITION, AND BONE OUTCOMES IN PRETERM INFANTS

The feeding comparison central to this thesis is predominantly fortified breast milk (PBM) versus predominantly formula milk (PFM), rather than fortified breast milk versus unfortified breast milk. This distinction is important because PBM and PFM differ in nutrient consistency, biological properties, and nutrient delivery during routine NICU care. PBM preserves the bioactive and immunological properties of breast milk but depends on breast milk composition, fortification timing, feed volume, and feeding tolerance. PFM provides a more standardised nutrient profile but lacks the bioactive components of breast milk.

Breast milk is widely recommended as the preferred enteral feed for preterm infants because of its immunological and bioactive properties (Embleton et al. 2023). However, unfortified breast milk does not consistently meet the increased nutritional requirements of very preterm and VLBW infants, particularly for protein, energy, calcium, phosphorus, and other micronutrients required for growth and bone mineralisation (Arslanoglu et al. 2019; Embleton et al. 2023). Fortification is therefore required to increase nutrient density. Even with fortification, achieved nutrient delivery from PBM may remain variable because breast milk composition differs between mothers and changes across lactation, especially in protein and energy content (Gidrewicz & Fenton 2014; Cormack et al. 2016).

PFM provides a more standardised nutrient profile because preterm formula is manufactured to defined concentrations of macronutrients, minerals, and micronutrients (Embleton et al. 2023). This may allow more predictable nutrient delivery when full enteral volumes are tolerated. However, PFM does not provide the same breast milk bioactive components, and nutrient adequacy still depends on feed tolerance, clinical stability, and achieved volume. Therefore, any apparent difference between PBM and PFM cannot be interpreted from milk type alone.

Studies comparing fortified breast milk-based feeding and preterm formula feeding in VLBW and very preterm infants remains difficult to interpret. Suganuma et al. (2021), in a systematic review and meta-analysis of 44 studies, found that evidence on short-term growth differences was largely inconclusive because of low-certainty evidence, small sample sizes, heterogeneous feeding definitions, and variable fortification practices. The review reported possible small advantages in weight gain with lower breast milk exposure and possible small increases in head circumference gain among infants receiving exclusive preterm formula compared with any breast milk, but the authors noted that the clinical significance of these differences was unclear. Regional and Malaysian studies also suggest that growth outcomes are shaped by achieved protein and energy delivery, illness burden, and NICU feeding practice, not feeding type alone (Lee et al. 2020; Abdul Hamid et al. 2021).

The impact of PBM and PFM may also extend beyond weight gain. Protein intake is important for lean tissue accretion, while calcium and phosphorus are central to bone mineralisation in preterm infants (Embleton et al. 2023). Weight, length, and head circumference cannot distinguish fat mass, lean mass, and bone status. Therefore, studies comparing PBM and PFM should consider not only anthropometric growth, but also growth quality.

Body composition and bone outcomes provide additional information on growth quality after preterm birth. Dual-Energy X-ray Absorptiometry (DXA)-derived measures such as fat mass, lean mass, percentage body fat, bone mineral content, bone mineral density, and bone area can provide exploratory information on tissue and bone outcomes not captured by weight-for-age-z score (WAZ), length-for-age-z score (LAZ), or head circumference-for-age-z score (HCZ) alone. In this thesis, DXA outcomes are interpreted through contextual comparison with published preterm cohorts, not as definitive reference values.

Overall, the literature suggests that PBM and PFM may affect growth through different pathways. PBM provides breast milk bioactive benefits but may have variable nutrient delivery even after fortification. PFM provides a more standardised nutrient profile but lacks breast milk biological components. Existing evidence does not clearly establish whether one predominant feeding type is associated with better growth, body composition, or bone outcomes, especially in Malaysian NICU practice. This uncertainty supports examination of PBM and PFM in relation to achieved nutrient intake, longitudinal growth trajectories, and exploratory DXA-derived growth quality outcomes.

2.5.1 Short-Term In-Hospital Growth Outcomes between PBM and PFM

Short-term in-hospital growth is commonly used to assess whether preterm infants are receiving adequate nutritional support during NICU admission. When growth is interpreted by feeding type, it is necessary to distinguish between studies comparing fortified breast milk with unfortified breast milk, studies comparing formula with donor human milk, and studies comparing fortified breast milk with formula-based supplementation. These categories are related but not interchangeable. This distinction

is important for the present thesis because the exposure of interest is PBM versus PFM, not fortified versus unfortified breast milk.

The strongest evidence supporting fortification comes from the Cochrane review by Brown et al. (2020), which included 18 randomised or quasi-randomised trials involving 1456 preterm infants. The review found that multinutrient fortification of breast milk improved short-term in-hospital growth compared with unfortified breast milk. Fortified milk increased weight gain by 1.76 g/kg/day, length gain by 0.11 cm/week, and head circumference gain by 0.06 cm/week. These findings support routine fortification in breast milk-fed preterm infants. However, this review does not directly answer whether PBM and PFM produce different growth outcomes because the comparison was fortified versus unfortified breast milk. Formula milk was not used as a parallel predominant feeding group in the main comparison.

This distinction matters because Brown et al. (2020) supports the claim that unfortified breast milk is nutritionally insufficient for many preterm infants, but it does not prove that fortified breast milk produces growth equivalent to preterm formula. The included trials were generally small and varied in fortifier composition, feeding protocols, infant maturity, and outcome measurement. Longer-term growth and developmental outcomes were also limited.

Comparing breast milk and preterm formula more directly remains difficult to interpret. Sukanuma et al. (2021) conducted a systematic review and meta-analysis of short-term growth in preterm and VLBW infants, including 44 studies, with 37 studies and 9963 infants included in meta-analyses. Importantly, the review focused on studies comparing fortified breast milk with preterm formula and excluded studies comparing unfortified breast milk with term formula, making it more relevant to the PBM versus PFM question than fortification-only reviews (Sukanuma et al. 2021). Comparisons included exclusive breast milk versus exclusive preterm formula, any breast milk versus exclusive preterm formula, and higher versus lower breast milk exposure. Overall, the evidence was limited by study quality, heterogeneity in feeding definitions, and inconsistent reporting of achieved nutrient intake. Lower breast milk exposure was associated with a small increase in weight gain during hospital admission, while

exclusive preterm formula compared with any breast milk was associated with a small increase in head circumference growth. However, these findings came from different comparisons within the review, the certainty of evidence was low, and the clinical meaning of the differences was unclear (Suganuma et al. 2021). This supports the need to examine achieved nutrient intake rather than relying on feeding category alone.

Formula-based feeding has also been associated with faster in-hospital growth when compared with donor breast milk. In the Cochrane review by Quigley et al. (2019), formula-fed preterm or low birth weight infants had higher rates of weight gain, linear growth, and head growth than infants receiving donor breast milk, but formula feeding was associated with higher necrotising enterocolitis risk. This creates an important clinical trade-off: formula may provide higher and more consistent nutrient density, but breast milk has biological benefits that are not captured by growth velocity alone. For the present thesis, this evidence suggests that faster growth with formula in some studies should not be interpreted as simple superiority of PFM over PBM.

Studies evaluating formula powder as a fortifier provide additional evidence relevant to PBM and PFM interpretation. Chinnappan et al. (2021) conducted a randomised non-inferiority trial comparing preterm formula powder with commercial human milk fortifier for fortifying expressed breast milk in preterm neonates. This study is useful because it examined whether a formula-based fortification approach could support growth comparable to standard fortifier. However, it was still not a direct PBM versus PFM comparison because both groups received breast milk as the base feed. Its main value for this thesis is that it separates the question of fortifier composition from the broader question of predominant feeding substrate.

Kumar et al. (2021) similarly reviewed the use of infant formula to fortify breast milk in VLBW infants. This evidence is relevant to low- and middle-income settings where commercial fortifier availability, cost, and local feeding practice may differ from high-income settings. However, the evidence was limited and rated as very low certainty. These findings suggest that formula-based fortification may be a possible alternative where standard fortifier access is constrained, but they do not establish that PFM and PBM have equivalent effects on growth under routine NICU conditions.

Pedaveeti et al. (2025) provides a closer comparison because it evaluated three feeding regimens in VLBW appropriate-for-gestational-age infants: expressed breast milk alone, expressed breast milk with human milk fortifier, and expressed breast milk with preterm formula supplementation. By discharge or 6 weeks, infants receiving fortified milk showed better growth than those receiving expressed breast milk alone, while preterm formula supplementation produced intermediate growth outcomes. This finding reinforces two points relevant to the present thesis. First, unfortified expressed breast milk is less likely to meet the growth needs of VLBW infants. Second, formula supplementation may improve nutrient delivery, but growth response depends on the full feeding regimen, clinical condition, and achieved intake rather than feeding label alone.

The distinction between prescribed feeding and achieved nutrient intake is therefore central. PBM combines breast milk with added fortifier, but nutrient delivery may vary according to expressed breast milk composition, timing of fortifier initiation, feed volume, feeding tolerance, and feed interruptions. PFM provides a more standardised nutrient profile and may deliver more predictable energy, protein, calcium, and phosphorus when full enteral volumes are achieved. However, formula-fed infants may still fail to reach nutrient targets if feed advancement is delayed, feeds are interrupted, or illness limits tolerance. Therefore, short-term growth differences between PBM and PFM cannot be interpreted without examining the actual energy and macronutrient intake received.

Timing and consistency of intake are also important. Gomez-Pomar et al. (2025) reported that earlier achievement of target enteral feeding and fortification during the first week of life was associated with improved discharge growth z-score patterns in preterm infants. However, these associations may be influenced by GA, birth weight, and clinical stability. This is relevant to PBM and PFM comparisons because infants in the two groups may differ in how quickly they reach full feeds, begin fortification, or tolerate consistent intake. As a result, apparent feeding-group differences may reflect clinical case-mix and feeding progression rather than milk type alone (Gomez-Pomar et al. 2025).

Evidence from fortification strategy trials also suggests that increasing protein intake alone does not always produce better short-term growth. Sanchez-Holgado et al. (2024) reported that adjusted fortification guided by blood urea nitrogen achieved higher protein intake than targeted fortification in extremely low birth weight infants, yet weight gain and z-score change at discharge did not differ between groups. This suggests that growth response depends not only on protein dose but also on energy balance, illness burden, feed tolerance, timing of delivery, and other nutrient requirements. For PBM versus PFM comparisons, nutrient adequacy must therefore be interpreted as a whole rather than reducing the question to milk type or protein intake alone.

Regional evidence also supports the importance of fortification but does not fully resolve the PBM versus PFM question. Handayani et al. (2019) reported higher short-term weight gain velocity among preterm infants receiving bovine milk fortifier compared with unfortified breast milk over a 14-day observation period. This supports the argument that fortification improves short-term growth in breast milk-fed preterm infants. However, because the comparison was fortified versus unfortified breast milk, it does not establish whether PBM achieves similar in-hospital growth outcomes to PFM.

Overall, the literature indicates that fortification improves short-term growth compared with unfortified breast milk, while formula feeding may support faster early growth in some human milk comparison studies. However, direct evidence comparing PBM and PFM remains limited. Randomised comparisons are difficult because mother's own milk is recommended when available, and observational comparisons are vulnerable to differences in GA, birth size, illness burden, maternal milk availability, feed tolerance, and duration of admission. Apparent differences in short-term in-hospital growth may therefore reflect achieved energy and protein intake, timing of fortification, feeding progression, and clinical case-mix rather than feeding type alone. This uncertainty provides the rationale for examining PBM and PFM in relation to achieved nutrient delivery and discharge growth outcomes in the present Malaysian NICU cohort.

2.5.2 Influence of PBM and PFM on Achieved Energy and Protein Adequacy

A meaningful comparison of PBM and PFM feeding in preterm infants requires a clear distinction between feeding type and achieved nutrient delivery. Feeding type describes the primary milk substrate received during NICU admission, whereas achieved nutrient delivery refers to the actual amounts of energy and macronutrients received after accounting for feed interruptions, tolerance, fortification practice, and feeding volumes. This distinction matters because a feeding label alone does not indicate whether an infant has met recommended nutritional targets.

PBM has compositional features that make nutrient delivery variable. Even when breast milk fortifier is added, the protein and energy content of the underlying milk differs between mothers and changes across lactation. Gidrewicz and Fenton (2014), in a systematic review and meta-analysis of breast milk composition, included 26 preterm milk studies involving 843 mothers and showed that preterm milk protein content is highest in colostrum and declines with advancing postnatal age. The authors also reported wide variability in breast milk composition and showed that protein estimates differed by 1–37% depending on whether true protein or total nitrogen-based methods were used. This means that two infants receiving the same volume of fortified breast milk may still have different estimated protein intakes, depending on maternal milk composition, timing of fortifier initiation, and the nutrient values used for intake calculation. In settings where individual human milk composition is not routinely measured, this variability introduces uncertainty into estimates of achieved PBM intake.

PFM provides a more standardised nutrient profile because preterm formula is manufactured to defined concentrations of energy, protein, fat, carbohydrate, minerals, and micronutrients. Many preterm formulas are designed to provide higher energy and protein density than standard term formula, commonly around 80 kcal/100 mL with higher protein content to support the needs of preterm infants. This may make nutrient delivery more predictable per unit volume than fortified breast milk (Embleton et al. 2023). However, standardised formula composition does not guarantee adequate intake. PFM-fed infants may still fail to meet nutrient targets if enteral volumes are restricted

by feeding intolerance, clinical instability, slow feed advancement, or feed interruptions.

ESPGHAN recommends that most stable growing very preterm infants receive approximately 3.5 to 4.0 g/kg/day of protein and 115 to 140 kcal/kg/day of energy enterally (Embleton et al. 2023). Meeting these targets can be difficult in both PBM and PFM groups. Cormack et al. (2016) noted substantial inconsistency in how neonatal nutrition studies estimate and report nutritional intake, particularly where breast milk composition is assumed rather than measured. This affects interpretation of PBM and PFM comparisons because estimated intake may not reflect the nutrients actually delivered to the infant.

Local evidence from Malaysia supports the need to measure achieved intake directly. Abdul Hamid et al. (2021) reported high exposure to breast milk and breast milk fortifier among Malaysian infants, with 98% receiving breast milk and 86% receiving fortified breast milk during admission. Despite this, recommended protein intake was not consistently achieved; mean protein intake during weeks 1 to 4 was 3.0 ± 0.5 g/kg/day in the Malaysian cohort, below the lower ESPGHAN recommended protein range of 3.5 g/kg/day. This shows that high fortifier use does not automatically translate into adequate protein delivery. In PBM-fed infants, protein adequacy depends on the protein content of the milk, timing and consistency of fortifier use, and volumes actually received. In PFM-fed infants, nutrient delivery may be more predictable per unit volume, but adequacy still depends on whether sufficient feed volume is tolerated.

The literature therefore indicates that PBM and PFM differ in the consistency of nutrient delivery, but neither feeding type guarantees that energy or protein targets are met under routine NICU conditions. PBM offers human milk biological benefits but has variable nutrient content even after fortification. PFM offers greater nutrient consistency per unit volume but remains dependent on feed tolerance and achieved volume. Identifying differences in achieved energy and protein delivery between PBM and PFM therefore requires week-by-week quantification of actual intake rather than reliance on feeding category alone.

2.6 BODY COMPOSITION AS A GROWTH-QUALITY OUTCOME

Body composition describes the relative contribution of fat mass and fat-free mass to body weight. It provides information that cannot be obtained from weight and length measurements alone, because two preterm infants with similar weight gain may accrue different proportions of lean tissue and fat tissue during recovery growth (Johnson et al. 2012). Weight gain alone is therefore an incomplete indicator of nutritional quality in the preterm period.

At term-equivalent age, preterm infants have been reported to have lower fat-free mass and a higher percentage of body fat than term-born infants, even when body weight appears clinically acceptable (Hamatschek et al. 2020). This difference may not be apparent from routine anthropometry and has been discussed as relevant to later metabolic health. Body composition assessment therefore provides additional context for interpreting whether postnatal growth reflects lean tissue accretion, fat deposition, or both.

The relationship between nutrition and body composition is important in preterm infants because nutrient delivery may affect the quality of tissue accretion, not only the rate of weight gain. Protein intake is particularly relevant for fat-free mass accretion, while imbalance between energy and protein delivery may contribute to growth characterised by relatively greater fat deposition (Hamatschek et al. 2020; Parat et al. 2020). This matters when comparing feeding strategies, because fortified breast milk and preterm formula may support similar anthropometric growth but differ in their effects on lean and fat tissue accretion, although available evidence comparing body composition outcomes by feeding type remains limited and inconclusive (Suganuma et al. 2021).

Studies from predominantly high-income settings suggest that nutritional strategies designed to reduce variability in delivered macronutrients may influence body composition outcomes in preterm infants. Parat et al. (2020) compared targeted protein fortification with standard fortification in VLBW infants receiving breast milk. The targeted group received higher protein intake, but fat-free mass in grams did not differ clearly between groups in the full sample. In a small post-hoc subgroup of infants

who received more than 30 days of intervention, the targeted group had a higher fat-free mass percentage and lower fat mass percentage than the standard group. These findings suggest that improved protein delivery may influence growth quality, but the evidence remains preliminary because of the small sample size, short intervention duration, and post-hoc subgroup analysis.

Evidence comparing breast milk-based feeding and preterm formula feeding further shows that body composition outcomes remain uncertain. Suganuma et al. (2021) reported that few studies assessed body composition, and the available evidence on fat mass and fat-free mass differences between exclusive preterm formula and exclusive human milk feeding was generally inconclusive. This supports interpreting body composition as part of a broader assessment of growth quality rather than as a stand-alone marker of feeding superiority.

In Malaysia and the wider Southeast Asian region, directly comparable body composition data for preterm infants remain limited. Most published evidence on DXA-derived or other body composition outcomes comes from high-income settings where feeding protocols, access to milk analysis, fortifier availability, formula composition, and post-discharge follow-up practices may differ from local NICU care. Local body composition data are therefore needed for contextual comparison with published preterm cohorts and to describe whether current growth patterns are accompanied by lean tissue accretion, fat deposition, and preserved bone mineralisation.

Taken together, the literature supports the use of body composition assessment as an exploratory growth-quality outcome in preterm infant nutrition research. DXA-derived measures, including fat mass, lean mass, percentage body fat, bone mineral content, bone mineral density, and bone area, can complement anthropometric indices by describing tissue and bone outcomes that are not captured by weight, length, and head circumference alone. In this thesis, these measures are used to provide preliminary local data and contextual comparison with published preterm cohorts, rather than to define local reference values or make confirmatory conclusions.

2.6.1 Methods Used to Assess Body Composition

Quantifying body composition in preterm infants requires specialised methods that are suitable for small and clinically fragile infants. Four methods are commonly used in neonatal research and selected clinical settings: air-displacement plethysmography, dual-energy X-ray absorptiometry, bioelectrical impedance analysis, and magnetic resonance imaging. Each method differs in technical requirements, feasibility, assumptions, and the body compartments measured (Nagel et al. 2020 ;Yumani et al. 2023).

Air-displacement plethysmography (ADP) estimates body composition by measuring body volume and body density, allowing calculation of fat mass and fat-free mass using a two-compartment model. ADP is non-invasive, rapid, does not involve ionising radiation, and does not usually require sedation, which makes it suitable for infant body composition research (Mazahery et al. 2018 ;Yumani et al. 2023). Compared with impedance-based methods, ADP is less dependent on hydration-based prediction equations. However, ADP requires specialised equipment, is not available in many clinical settings, and requires the infant to be stable enough for transfer and measurement outside usual incubator or cot-based care. Its estimates also depend on assumptions about the density and composition of fat-free mass, which may vary in preterm infants (Demerath & Fields 2014; Mazahery et al. 2018).

Dual-energy X-ray absorptiometry (DXA) estimates bone mineral content, bone mineral density, lean mass, and fat mass at whole-body or regional levels. Its main advantage is that it provides information on both soft tissue and bone status in a single assessment, which is relevant for preterm infants because they are vulnerable to altered tissue accretion and reduced skeletal mineralisation (Ahmad et al. 2010; Demerath & Fields 2014). DXA involves low radiation exposure and has been used in neonatal body composition research. However, scan quality can be affected by infant movement, positioning, small body size, and reduced bone mineralisation in very preterm infants. These limitations mean that DXA-derived findings in preterm infants should be interpreted cautiously and, where appropriate, through contextual comparison with

published preterm cohorts rather than as definitive reference values (Yumani et al. 2023).

Bioelectrical impedance analysis (BIA) estimates body composition from the resistance of body tissues to a small electrical current. It has the advantages of portability, speed, low cost, and suitability for repeated bedside measurements (Nagel et al. 2020; Yumani et al. 2023). However, BIA estimates in neonates depend on prediction equations, and the validity of these equations may vary according to GA, illness status, and body water distribution. Accuracy is also affected by hydration status, which is highly variable in preterm infants receiving intensive medical support. This introduces measurement uncertainty when estimating fat-free mass in this population (Nagel et al. 2020; Yumani et al. 2023).

Magnetic resonance imaging (MRI) provides detailed assessment of body tissues and can distinguish adipose tissue depots, such as subcutaneous and visceral fat. This allows more detailed evaluation of fat distribution than methods that provide only whole-body fat and fat-free mass (Nagel et al. 2020). MRI does not involve ionising radiation, but it is expensive, less accessible, and often requires longer acquisition times. In preterm infants, transport, monitoring, temperature control, and motion artefact limit its routine use in acute NICU settings. Whole-body MRI has therefore been used mainly in research cohorts to characterise adipose tissue compartments and non-adipose tissue mass at term-equivalent age (Nagel et al. 2020; Yumani et al. 2023).

Overall, no single method is ideal for all neonatal settings. ADP is rapid and radiation-free but mainly provides two-compartment body composition. DXA provides both soft tissue and bone measurements but requires cautious interpretation in small and immature infants. BIA is suitable for bedside use but is sensitive to hydration status and prediction-equation validity. MRI provides detailed tissue distribution but is less feasible for routine NICU use. The choice of method therefore depends on the study objective, infant stability, available equipment, and whether the focus is whole-body composition, bone mineralisation, or tissue distribution (Nagel et al. 2020; Yumani et al. 2023).

2.6.2 Impact of Feeding Type and Macronutrient Delivery on Body Composition

The type and composition of nutrition provided to preterm infants during NICU admission may influence body composition, not only anthropometric growth. Protein and energy delivery are particularly relevant because adequate protein supply supports fat-free mass accretion, while imbalance between energy and protein delivery may favour relatively greater fat deposition (Lingwood et al. 2020). This is important when comparing PBM and PFM, because the two feeding exposures may differ in protein density, energy delivery, fortification timing, and consistency of nutrient intake.

Evidence linking feeding type to body composition remains limited and is mostly based on measurements at term-equivalent age rather than later post-discharge follow-up. In a secondary analysis of a randomised trial, Li et al. (2019) examined 133 infants born at less than 31 weeks' gestation who underwent whole-body magnetic resonance imaging at term-equivalent age. Compared with infants fed exclusively breast milk, predominantly formula-fed infants had 257.4 g greater non-adipose tissue mass and greater increase in weight z-score from birth to term. However, adipose tissue mass and percentage body fat did not differ between groups. The authors suggested that lower non-adipose tissue mass among exclusively breast milk-fed infants may reflect lower protein intake or inconsistent fortification rather than an adverse effect of breast milk itself.

More recent evidence from the PREMFOOD trial also suggests that body composition may differ by in-hospital milk exposure, although interpretation remains cautious. Mills et al. (2025) analysed very preterm infants according to intake of exclusive unfortified breast milk, predominantly unfortified breast milk, predominantly fortified breast milk, or predominantly preterm formula from birth to 35 weeks postmenstrual age. At term, infants in the predominantly preterm formula group had 274.3 g greater non-adipose tissue mass than infants in the exclusive unfortified breast milk group. However, the predominantly formula group was small, and the comparison included unfortified, fortified, and formula exposures rather than a simple PBM versus PFM contrast. These findings suggest that formula exposure may be associated with

greater non-adipose tissue mass at term, but they do not establish that PFM is superior to PBM for body composition outcomes.

Other studies suggest that breast milk-based feeding may be associated with body composition closer to that of term-born infants, but findings remain limited. Mól et al. (2019) assessed 34 VLBW preterm infants and 19 term controls using bioelectrical impedance analysis at estimated term age. Formula-fed preterm infants had higher percentage fat mass and lower percentage fat-free mass than term-born controls, whereas breast milk-fed preterm infants did not differ clearly from term-born controls in these measures. In that study, breast milk was supplemented with standard human milk fortifier once feeding volume exceeded 140 mL/kg/day. However, the breast milk-fed group was small, and the study was observational. Nevertheless, the finding is consistent with the hypothesis that breast milk-based feeding may support a body composition profile closer to term-born infants, pending confirmation in larger studies.

Fortified breast milk studies also show that body composition may differ even when routine anthropometric measurements appear similar. Piemontese et al. (2018) assessed 73 VLBW infants using air-displacement plethysmography at term-corrected age (CA). Infants receiving at least 50% target-fortified breast milk during hospitalisation had higher fat-free mass percentage than those receiving less than 50%, despite no clear differences in weight, length, or head circumference at discharge or term-CA. This supports the view that conventional growth measures may miss differences in tissue accretion related to feeding exposure and fortification adequacy.

Evidence on individualised breast milk fortification and body composition remains preliminary. Parat et al. (2020) compared targeted protein fortification with standard fortification in VLBW infants receiving breast milk. The targeted group received higher protein intake, but fat-free mass in grams did not differ clearly between groups in the full sample. In a small post-hoc subgroup of infants who received more than 30 days of intervention, the targeted group had higher fat-free mass percentage and lower fat mass percentage than the standard group. These findings suggest that improved protein delivery may influence growth quality, but the evidence remains

limited because of the small sample size, short intervention duration, and post-hoc subgroup analysis.

A study protocol by Cardoso et al. (2021) illustrates the ongoing clinical interest in whether fortification based on measured breast milk composition can improve energy and macronutrient intake and growth quality in very preterm infants. However, because that publication is a protocol and not an outcomes paper, it can only be used only to support the rationale for ongoing research on measured composition fortification, not as evidence of effects on adiposity or lean mass.

Taken together, the literature suggests that feeding type and macronutrient delivery may influence body composition in preterm infants, particularly through effects on fat-free mass and adiposity. Formula exposure has been associated with greater non-adipose tissue mass in some MRI-based studies, while higher fortified human milk exposure has been associated with higher fat-free mass percentage in some ADP-based cohorts. However, available studies differ in feeding definitions, fortification practices, measurement methods, timing of assessment, and sample size. Evidence specific to post-discharge body composition remains limited, and data from Malaysian or wider Southeast Asian cohorts are scarce. This supports the use of body composition outcomes in the present thesis as exploratory measures of growth quality when comparing PBM and PFM.

2.7 BONE MINERALISATION AND THE IMPACT OF FEEDING TYPE

Bone mineralisation is relevant to this thesis because PBM and PFM may differ not only in energy and protein delivery, but also in calcium, phosphorus, vitamin D, and total mineral delivery. These nutrients are central to skeletal mineral accretion in preterm infants. Therefore, bone outcomes should be considered alongside anthropometric growth and body composition when comparing predominant feeding exposure during NICU admission.

2.7.1 Importance of Calcium and Phosphorus Accretion in Preterm Infants

Bone mineralisation refers to the deposition of calcium and phosphorus into the developing skeleton to form hydroxyapatite within the organic bone matrix. In the fetus, most skeletal mineral accretion occurs during the third trimester, when placental transfer of calcium and phosphorus increases rapidly (Faenza et al. 2019). Preterm birth interrupts this period of high mineral accretion and transfers the infant into an extrauterine environment where matching intrauterine mineral delivery is difficult because of fluid limits, feed tolerance, parenteral nutrition constraints, and delayed achievement of full enteral feeding (Chinoy et al. 2019; Faenza et al. 2019).

This is directly relevant to PBM and PFM comparisons. Unfortified breast milk does not meet the calcium and phosphorus requirements of very preterm and VLBW infants, so fortification is required to improve mineral delivery. PFM provides a more standardised mineral content per unit volume, but achieved mineral intake still depends on feed volume, tolerance, and interruptions. Therefore, the skeletal effect of PBM or PFM cannot be interpreted from milk type alone. It depends on the amount of calcium, phosphorus, protein, and energy actually delivered during admission.

2.7.2 Metabolic Bone Disease of Prematurity and Feeding-Related Risk

Metabolic bone disease (MBD) of prematurity is characterised by reduced skeletal mineralisation, with biochemical and radiological features related to bone demineralisation (Faenza et al. 2019). It occurs most often in very preterm, VLBW, and ELBW infants because these infants miss part of the third-trimester period of mineral accretion and are more likely to experience prolonged parenteral nutrition, delayed enteral feeding, chronic lung disease, medication exposure, and inadequate mineral intake (Chinoy et al. 2019; Faenza et al. 2019).

Reported incidence varies widely because studies use different definitions, screening markers, and imaging methods. Faenza et al. (2019) reported that MBD is often observed in infants born before 28 weeks and occurs in approximately 16% to 40% of VLBW and extremely low birth weight infants. Clinical manifestations may include biochemical abnormalities, radiological demineralisation, rickets, fractures,

respiratory compromise due to rib softening, and impaired growth in severe cases (Chinoy et al. 2019; Faienza et al. 2019).

The feeding-type question is clinically relevant because mineral delivery differs across feeding strategies. PBM depends on the mineral content of breast milk, timing of fortifier initiation, consistency of fortifier use, and achieved feed volume. PFM offers a more standardised mineral composition but may still fail to meet mineral needs if feeds are interrupted or enteral volumes are restricted. Therefore, apparent differences in bone mineralisation between PBM and PFM groups may reflect cumulative mineral delivery, illness burden, parenteral nutrition duration, and medication exposure rather than milk type alone.

2.7.3 Evidence on Early Nutrition and Bone Mineral Outcomes

International evidence suggests that early nutritional intake may affect later bone mineral outcomes in preterm infants. Calor et al. (2021) assessed extremely and very preterm infants using DXA at term corrected age and 6 months CA. The study reported that fat intake during the first four postnatal weeks was associated with bone mineral content and bone mineral density at term CA, while protein and fat intake during early life were predictors of bone mineral density at six months CA. These findings support the view that early nutrient delivery may contribute to skeletal outcomes beyond discharge.

Longitudinal DXA studies also show that bone mineralisation in preterm infants may remain lower than that of term-born infants beyond the NICU period. Quintal et al. (2014) used DXA to assess bone mineral content, bone mineral density, and whole-body lean mass in preterm and term infants from birth to six months corrected postnatal age. Mihatsch et al. (2021) followed VLBW infants from six months to three years and reported that bone mineralisation, fat-free mass, and growth remained lower than in term controls, even when in-hospital nutrition followed contemporary macronutrient recommendations. These studies support assessment of skeletal outcomes beyond discharge, but they do not provide Malaysian PBM versus PFM-specific data.

Local and regional evidence remains limited. Published Malaysian DXA data in preterm infants are scarce, and no local longitudinal DXA study comparing infants exposed to PBM and PFM through six months CA has been identified. Therefore, in this thesis, DXA-derived bone outcomes are treated as exploratory and descriptive. They are used for contextual comparison with published preterm cohorts, not to define Malaysian reference values or to make confirmatory claims about feeding-type superiority.

2.7.4 Methods for Assessing Bone Mineral Content and Density

Several methods are available for assessing bone mineralisation in preterm infants, including DXA, biochemical surveillance, and quantitative ultrasound. These methods measure different aspects of bone health and should not be treated as interchangeable.

DXA estimates bone mineral content, bone mineral density, lean mass, and fat mass at whole-body or regional sites. For this thesis, DXA is relevant because it provides both body composition and bone mineralisation data in the same assessment. DXA has been widely used in neonatal and paediatric research, but interpretation in preterm infants requires caution because scan quality can be affected by movement, positioning, small body size, and low bone mineral content (Ahmad et al. 2010; Yumani et al. 2023). Therefore, DXA-derived bone outcomes in this thesis are interpreted through contextual comparison with published preterm cohorts rather than as definitive reference values.

Biochemical surveillance uses markers such as serum phosphate, serum calcium, alkaline phosphatase, and urinary calcium or phosphorus to assess mineral status and screen for risk of MBD. Elevated alkaline phosphatase with low serum phosphate is commonly used to raise suspicion of impaired mineralisation, although cut-off values vary between studies and clinical protocols (Chinoy et al. 2019; Faienza et al. 2019). Biochemical markers are useful for screening and monitoring, but they do not directly measure skeletal mineral content. Some infants may have altered bone mineralisation without a single biochemical marker giving a complete picture.

Quantitative ultrasound is a radiation-free method that assesses bone properties through speed of sound measurements. It may be useful in neonatal settings because it is portable and avoids radiation exposure. However, QUS and DXA assess different bone properties. DXA estimates mineral mass and density, whereas QUS reflects bone characteristics influenced by elasticity, structure, and mineralisation. Tarrell et al. (2023) compared tibial QUS with DXA in preterm infants and found only weak association between the two measures, supporting the view that QUS and DXA are complementary rather than interchangeable.

Overall, bone mineralisation outcomes are relevant to PBM and PFM comparisons because mineral intake and skeletal accretion depend on more than milk type. They are shaped by calcium and phosphorus delivery, fortifier use, feed tolerance, parenteral nutrition duration, illness burden, and medication exposure.

2.7.5 Achieved Mineral Intake, Feeding Type, and Bone Mineral Outcomes

Direct evidence comparing PBM and PFM in relation to bone mineral outcomes is limited. Most available studies do not test milk type as an isolated exposure. Instead, they examine total mineral provision, fortification timing, parenteral mineral delivery, biochemical mineral markers, or DXA-derived bone outcomes. Therefore, the feeding-type question can only be interpreted through achieved calcium and phosphorus intake rather than through milk category alone.

In a small longitudinal cohort, Quintal et al. (2014) assessed whole-body bone mineral content, bone mineral density, and lean mass by DXA in 14 preterm infants born at ≤ 32 weeks' gestation and 14 full-term controls. Measurements were taken at 40 weeks corrected postconceptional age, 3 months CA, and 6 months CA. The preterm infants received breast milk during hospitalisation; half received multicomponent human milk fortifier, and infants with urinary phosphorus deficiency received additional calcium and phosphate. Preterm infants had lower bone mineral measures at 40 weeks than term controls, with catch-up by 6 months CA. Although the sample was small, this study is relevant because it shows that bone mineralisation after preterm birth may improve during infancy when mineral intake is monitored and supplemented.

Longer follow-up data suggest that bone mineral outcomes may not fully normalise in all VLBW infants. Mihatsch et al. (2021) followed VLBW infants from 6 months to 3 years and reported lower bone mineral density, fat-free mass, and growth compared with term controls, despite in-hospital nutrition that followed current macronutrient recommendations. This suggests that meeting average macronutrient targets during admission may not be sufficient to ensure skeletal mineral outcomes comparable with term-born infants. Mineral intake, illness burden, medication exposure, and post-discharge nutrition may also contribute to later bone status.

Evidence comparing fortified mother's milk with preterm formula suggests that mineral markers can be similar when fortified breast milk is supported by additional mineral supplementation. Doege and Bauer (2007) compared infants receiving high-volume mother's milk with individualised mineral and protein supplementation against infants receiving preterm formula. Serum calcium, phosphorus, alkaline phosphatase, and urinary mineral excretion did not differ clearly between groups at the end of the study. However, the study did not include DXA or other skeletal imaging, so it cannot confirm equivalence in bone mineral accretion. Its value is therefore limited to showing that fortified mother's milk with additional mineral supplementation can achieve biochemical mineral profiles comparable to preterm formula under a structured protocol.

Quality improvement studies further support the importance of early and consistent mineral delivery, but they do not isolate the effect of feeding type. Krithika et al. (2022) reported a reduction in MBD after a bundle that included earlier use of human milk fortifier, parenteral calcium and phosphorus supplementation, and early enteral mineral supplementation. Because multiple interventions were introduced together, the findings cannot be attributed to fortifier alone. However, they support the broader point that mineral delivery during early NICU care is modifiable and may reduce MBD risk.

Current nutritional guidance also supports focusing on achieved mineral intake rather than milk type alone. ESPGHAN recommendations describe enteral and parenteral calcium and phosphorus targets for preterm infants and note that parenteral

mineral delivery may be constrained by solubility and fluid limitations (Embleton et al. 2023). This is relevant to both PBM and PFM groups. PBM requires appropriate fortification to improve calcium and phosphorus delivery, while PFM provides more standardised mineral content but still depends on achieved volume and feeding tolerance. In both groups, feed interruption, delayed full enteral feeding, prolonged parenteral nutrition, diuretic exposure, and chronic lung disease may reduce effective mineral accretion.

Taken together, the literature does not provide strong direct evidence that PBM or PFM is superior for bone mineral outcomes. Instead, it suggests that skeletal mineralisation depends on cumulative calcium and phosphorus delivery, fortification timing, parenteral mineral provision, feed tolerance, and clinical morbidity.

2.7.6 Impact of Feeding Type on Post-Discharge Growth in Preterm Infants

Direct evidence comparing post-discharge growth trajectories between infants exposed to PBM and PFM during NICU admission remains limited. Most post-discharge growth studies classify infants by broad feeding categories, discharge feeding type, or post-discharge formula exposure rather than by predominant in-hospital feeding exposure. Many studies also do not report achieved nutrient intake during admission alongside post-discharge outcomes. Therefore, the available literature cannot clearly isolate PBM or PFM as an independent predictor of post-discharge growth.

This limitation is important because feeding exposure often changes after discharge. Infants classified as PBM or PFM during NICU admission may later receive exclusive breast milk, formula milk, mixed feeding, continued fortification, post-discharge formula, or standard term formula. As a result, post-discharge growth cannot be attributed to NICU feeding type alone. It must be interpreted in relation to achieved nutrient intake during admission, discharge growth status, gestational maturity, morbidity burden, and post-discharge feeding pattern.

Post-discharge growth faltering is well documented across preterm cohorts. In Southeast Asia, Rohsiswatmo et al. (2024) followed 306 Indonesian preterm infants to 24 months CA and reported that WAZ declined at 3 months CA before improving

gradually toward 24 months CA. The proportion of stunted infants also decreased across follow-up, reaching 13.4% by 24 months CA. This pattern is relevant to the present Malaysian cohort because it suggests that the first 3 to 6 months after discharge may represent a vulnerable period for detecting growth faltering after NICU discharge.

Catch-up growth after preterm birth also has timing implications. Preterm infants are vulnerable to extrauterine growth restriction during admission and after discharge, partly because of cumulative protein and energy deficits (Raaijmakers & Allegaert 2016). Early infancy therefore represents an important window for nutritional recovery. Growth status at 6 months CA can show whether early nutritional disadvantage during admission has persisted beyond the immediate post-discharge period or whether partial recovery has occurred.

Evidence from post-discharge formula trials also suggests that feeding type alone does not consistently explain later growth. Young et al. (2016) reviewed 16 trials involving 1251 preterm infants and found that nutrient-enriched post-discharge formula did not show consistent benefit for growth or developmental outcomes up to 12 to 18 months post-term when compared with standard formula. Limited evidence suggested that preterm formula may increase growth rates, but this formula is generally intended for in-hospital use rather than routine post-discharge feeding. These findings support cautious interpretation of formula exposure after discharge because formula type, nutrient density, timing, and duration differ across studies.

The quality of post-discharge growth is also relevant. Catch-up weight gain may improve anthropometric status, but rapid weight gain without adequate lean tissue accretion may contribute to disproportionate fat deposition. Conversely, poor post-discharge weight gain may reflect persistent nutritional inadequacy, unresolved morbidity, or insufficient feeding support. For this reason, post-discharge growth should be interpreted using WAZ, LAZ, HCZ, weight gain velocity, and, where available, body composition measures rather than weight alone.

In the Malaysian context, local evidence remains limited. Existing local studies have focused mainly on in-hospital growth and discharge outcomes rather than longitudinal post-discharge growth by predominant NICU feeding type. No published Malaysian cohort has clearly examined whether infants exposed to PBM or PFM during NICU admission show different WAZ, LAZ, or HCZ trajectories at 1, 3, and 6 months CA. This gap is important because Malaysian post-discharge feeding may be affected by maternal milk availability, family feeding choices, access to fortifier or formula, and follow-up care pathways.

Overall, the literature suggests that post-discharge growth in preterm infants is shaped by both NICU nutritional history and post-discharge feeding conditions. Direct evidence that PBM or PFM during NICU admission is superior for post-discharge growth is limited. Observed trajectories may reflect achieved nutrient intake, clinical maturity, morbidity burden, discharge growth status, and feeding changes after discharge rather than milk type alone. This supports the need to examine PBM and PFM in relation to longitudinal growth through 6 months CA, with cautious interpretation of feeding-type differences.

2.8 SUMMARY OF LITERATURE, EVIDENCE GAPS, AND RATIONALE FOR THE PRESENT STUDY

The literature reviewed in this chapter establishes the nutritional, physiological, and contextual basis for the present study. Preterm infants face early nutritional vulnerability because recommended energy, protein, mineral, and micronutrient targets are difficult to achieve during routine NICU care. Human milk remains the preferred enteral feed because of its biological and immunological properties, including protection against important prematurity-related morbidities such as necrotising enterocolitis. However, unfortified breast milk does not meet the nutrient requirements of very preterm and VLBW infants. Multinutrient fortification improves short-term in-hospital weight gain, linear growth, and head growth compared with unfortified human milk, but this evidence does not directly resolve whether PBM and PFM produce different patterns of nutrient delivery, growth trajectory, body composition, or bone mineralisation (Brown et al. 2020; Miller et al. 2018; Embleton et al. 2023).

A central limitation in the literature is that feeding exposure is often classified by milk type without adequate measurement of achieved nutrient intake. This is a major weakness because PBM and PFM differ in nutrient consistency, but neither feeding type guarantees adequate nutrient delivery under routine clinical conditions. PBM depends on human milk composition, timing and consistency of fortification, feeding tolerance, and achieved volume. PFM provides a more standardised nutrient profile, but intake may still be reduced by clinical instability, feed interruption, or poor tolerance. Therefore, the effect of feeding type on growth cannot be interpreted from feeding label alone. It requires measurement of achieved energy, protein, and mineral intake alongside feeding exposure (Suganuma et al. 2021; Campbell-Yeo et al. 2025; Azhar et al. 2025; Embleton et al. 2023).

Evidence on post-discharge growth further shows why feeding type alone is insufficient. In Indonesia, longitudinal follow-up of preterm infants showed a decline in WAZ by 3 months CA, followed by gradual recovery toward 24 months CA, suggesting that the early post-discharge period is a vulnerable window for growth faltering (Rohsiswatmo et al. 2024). More recent post-discharge nutrition evidence also suggests that nutrient-enriched formula may affect growth quality, not only size. In moderate-to-late preterm infants, van de Lagemaat et al. (2024) reported modest increases in length, head circumference, DXA-derived lean mass, and bone mineral content at 6 months corrected age among infants fed protein- and mineral-enriched post-discharge formula compared with standard term formula. However, this study involved moderate-to-late preterm infants in a high-income setting and compared post-discharge formula types rather than PBM and PFM exposure during NICU admission. Therefore, it supports the biological relevance of post-discharge nutrient density but cannot be directly extrapolated to Malaysian NICU feeding groups.

Growth quality is another important gap. Weight, length, and head circumference describe body size, but they do not distinguish lean mass, fat mass, or bone mineralisation. International studies using DXA and other body composition methods show that preterm infants may differ from term-born infants in body composition and bone mineral status beyond discharge. However, these data come mainly from non-Malaysian cohorts, with differences in population characteristics,

feeding protocols, scanner type, software, and timing of assessment (Quintal et al. 2014; Mihatsch et al. 2021; van de Lagemaat et al. 2024). For this reason, DXA findings from international cohorts cannot be treated as Malaysian reference values. In this thesis, DXA-derived body composition and bone outcomes are interpreted as exploratory and descriptive and are used for contextual comparison with published preterm cohorts rather than for confirmatory claims.

The key Malaysian gap is the absence of longitudinal data comparing PBM and PFM using achieved nutrient intake, post-discharge growth, and growth-quality outcomes within the same cohort. Existing local studies have mainly focused on in-hospital intake and discharge growth. No published Malaysian study has clearly examined whether infants predominantly exposed to PBM or PFM during NICU admission show different WAZ, LAZ, or HCZ trajectories through 1, 3, and 6 months CA while also measuring week-by-week nutrient intake during admission. Local DXA-derived body composition and bone mineralisation data for preterm infants beyond term-equivalent age are also scarce.

The present study addresses these gaps by prospectively comparing infants predominantly fed PBM and PFM during NICU admission in a Malaysian tertiary NICU. It quantifies achieved energy and macronutrient intakes during admission, relates feeding exposure and achieved intake to growth outcomes at discharge and at 1, 3, and 6 months CA, and includes exploratory DXA assessment of body composition and bone mineralisation in an available subset. The study therefore moves beyond feeding label alone and examines whether actual nutrient delivery and predominant feeding exposure are associated with postnatal growth trajectory and growth quality in Malaysian preterm infants.

CHAPTER III

METHODOLOGY

3.1 STUDY DESIGN

This was a prospective observational cohort study that examines nutritional intake during neonatal intensive care unit (NICU) admission and post-discharge growth outcomes among preterm infants receiving predominantly fortified breast milk (PBM) and predominantly formula milk (PFM).

3.2 STUDY POPULATION

The study population comprised preterm infants born at or before 34 weeks' gestational age (GA) and/or with a birth weight of 1800 grams or less, admitted to the NICU at Hospital Canselor Tuanku Muhriz (HCTM) within 24 hours of birth. The clinical rationale for targeting this GA and birth weight threshold, including the nutritional vulnerability, growth failure risk, and need for nutrient-enriched feeds in this group, is reviewed in Chapter 2. The preterm infants recruited during NICU admission at HCTM are the same subjects who subsequently attended Hospital Tunku Ampuan Besar Tuanku Aishah Rohani, Hospital Pakar Kanak-Kanak (HPKK) for post-discharge follow-up assessments, dietary review, and Dual-energy X-ray absorptiometry (DXA) body composition scanning.

3.3 STUDY LOCATION

The study was conducted in the NICU of HCTM and in the Dietetics Outpatient Clinic and Radiology Department at HPKK Universiti Kebangsaan Malaysia (UKM). The NICU at HCTM is a tertiary level IIIB NICU that provides on-site surgical support (excluding cardiac surgery), routinely cares for infants born at more than 25 weeks'

gestation and infants of 23–24 weeks' gestation if necessary, and has 28 cots comprising 8 intensive care cots, 8 semi-intensive care cots, 8 convalescent cots, and an additional 4 isolation cots for infectious cases. Baseline data collection occurred at HCTM and all post-discharge assessments were conducted at HPKK within the same study cohort.

The Dietetics Outpatient Clinic at HPKK comprises four independent clinics serving patients from birth to 20 years of age, with a combined average patient volume of 100–120 patients per month. The clinic provides nutrition care for routine and special dietary needs. This includes management of malnutrition (failure to thrive, obesity), very low birth weight (VLBW) infants (including term and preterm infants) or any at-risk infants, special needs children, management of children with food aversion, specialised diet, weaning and complementary feeding, and enteral nutrition or oral nutritional supplement issues. Clinic sessions run as follows: Specialist Clinic (Tuesday–Friday, 8:00 AM–12:00 PM), Obesity Clinic (first Wednesday of the month, 8:00 AM–12:00 PM), Feeding Clinic (last Tuesday of the month, 8:00 AM–12:00 PM), and VLBW Clinic (first Monday of the month, 2:00 PM–4:00 PM or as needed). Follow-up assessments for this study were scheduled on Mondays, Wednesdays, and Fridays to accommodate clinic availability.

The Radiology Department at HPKK provides imaging services including X-ray, fluoroscopy, computed tomography (CT), MRI, ultrasound, and angiography. DXA scanning is performed routinely for hip, lumbar spine, and whole-body assessments across age groups, including adult patients at the affiliated HCTM. DXA scans are conducted by appointment, typically on Thursdays, with fewer than 10 patients scanned per month. For this study, dedicated sessions were scheduled on Friday mornings from 9:00 am to 12:00 pm.

In this study, baseline recruitment and early postnatal data collection were conducted in the HCTM NICU, while follow-up dietary assessment and nutrition counselling were conducted at the HPKK Dietetics Outpatient Clinic. Body composition (including bone mineralization) was measured at the HPKK Radiology Department using DXA imaging.

3.4 SAMPLING METHOD

This study employed purposive sampling to recruit preterm infants (≤ 34 weeks GA and/or ≤ 1800 g birth weight). This method ensured focused comparison of feeding regimens in a clinically relevant group and improved feasibility by selecting infants likely to attend follow-up assessments.

3.5 SELECTION CRITERIA

3.5.1 Inclusion Criteria

1. Preterm infants born at ≤ 34 weeks' gestation (up to 34 weeks + 6 days), or any preterm infants with birth weight ≤ 1800 g.
2. Infants in (1) born and admitted within 24 hours of birth to the NICU at HCTM UKM.
3. Not transferred out for any part of their neonatal care.
4. The length of stay of at least 14 days.
5. Infants can attend and complete the whole study visits in the research setting.

3.5.2 Exclusion Criteria

1. Infants with major congenital anomalies or malformations, genetic abnormalities, and critical illness with short life expectancy, affecting growth/bone development.
2. Missing more than three days of growth or nutritional intake records during admission that could not be retrieved.
3. Discharged or death of infant at < 14 days of hospital stay.
4. Parents did not give consent for the inclusion in the study.

3.6 FEEDING GROUP CLASSIFICATION

Infants were classified by predominant enteral feeding during NICU admission as follows:

1. Predominantly fortified breast milk fed (PBM): infants for whom the greater total volume of enteral intake across the NICU admission was derived from breast milk, including fortified breast milk as the primary component. Fortification was introduced according to the unit protocol once enteral volumes reached the threshold for supplementation; any unfortified breast milk received prior to fortifier initiation was part of the same continuous breast milk-predominant feeding trajectory and did not alter the classification. Classification was based on cumulative enteral volume across the full admission rather than at any single timepoint.
2. Predominantly formula milk (PFM): infants whose enteral feeding during NICU admission consisted mostly of preterm formula milk, where the greater total volume of enteral intake was derived from preterm formula compared with breast milk sources. This classification reflects the predominant milk source received rather than exclusive formula feeding, as some infants in this group may have received a small volume of breast milk at some point during admission. The predominance of formula feeding in these infants arose from clinical and maternal circumstances, including limited or absent maternal milk supply, and was not the result of a planned feeding allocation. Classification was therefore volumetrically determined and reflects the real-world feeding patterns observed under standard NICU practice at this centre.

Feeding group allocation was fixed at the point of NICU discharge and was not revised or reclassified based on post-discharge feeding transitions. Infants classified as PBM at discharge who subsequently transitioned to formula feeding after discharge, and infants classified as PFM at discharge who subsequently established or resumed breast milk feeding, remained in their original group throughout all analyses. This approach reflects the study design, which examines the effect of predominant in-

hospital feeding exposure on subsequent growth rather than the effect of sustained post-discharge feeding type. Post-discharge feeding transitions were recorded separately at each follow-up visit through caregiver interview and were reported descriptively. These transitions were not included as covariates in the main post-discharge growth trajectory models because quantitative post-discharge nutrient intake was not measured, feeding categories changed over time, and the available follow-up sample size limited additional adjustment. Predominant feeding type was determined using the relative contribution of fortified breast milk and formula milk to total enteral intake during NICU admission, rather than a fixed percentage cut-off, to reflect routine NICU feeding patterns.

3.7 SAMPLE SIZE CALCULATION

Preterm infants with GA of ≤ 34 weeks and/or preterm infants with birth weight of ≤ 1800 g were classified by feeding regimen during NICU admission into (i) PBM and (ii) PFM. This classification supported the study objectives:

1. To compare energy and macronutrient intake achieved during NICU admission between PBM and PFM fed preterm infants.
2. To compare growth outcomes of preterm infants at discharge and at 1, 3, and 6 months CA.
3. To describe body composition and bone mineralisation at 6 months CA using available DXA data.
4. To examine whether NICU feeding type and nutrient intake are associated with growth outcomes at discharge and at 1, 3, and 6 months CA.

Sample size was calculated using G*Power 3.1 software (Faul et al. 2007) based on independent t-test statistics for comparing nutrient intake and growth outcomes between feeding groups. Based on a recent systematic review and meta-analysis by Sanchez-Holgado et al. (2025), which demonstrated a significant linear relationship between protein intake and weight gain (5.73 g/kg/day weight gain for each gram of protein/kg/day, $p = 0.001$) in preterm infants receiving fortified breast milk, the following parameters were used:

- Test statistic: Independent t-test (two-tailed)
- Effect size (Cohen's d) = 0.8 (large effect size)
- Alpha (α) = 0.05
- Power ($1-\beta$) = 0.80
- Allocation ratio = 1:1

The calculated required sample size was 52 participants (26 per group). Accounting for an anticipated 20% dropout rate, the target sample size was set at 65 participants

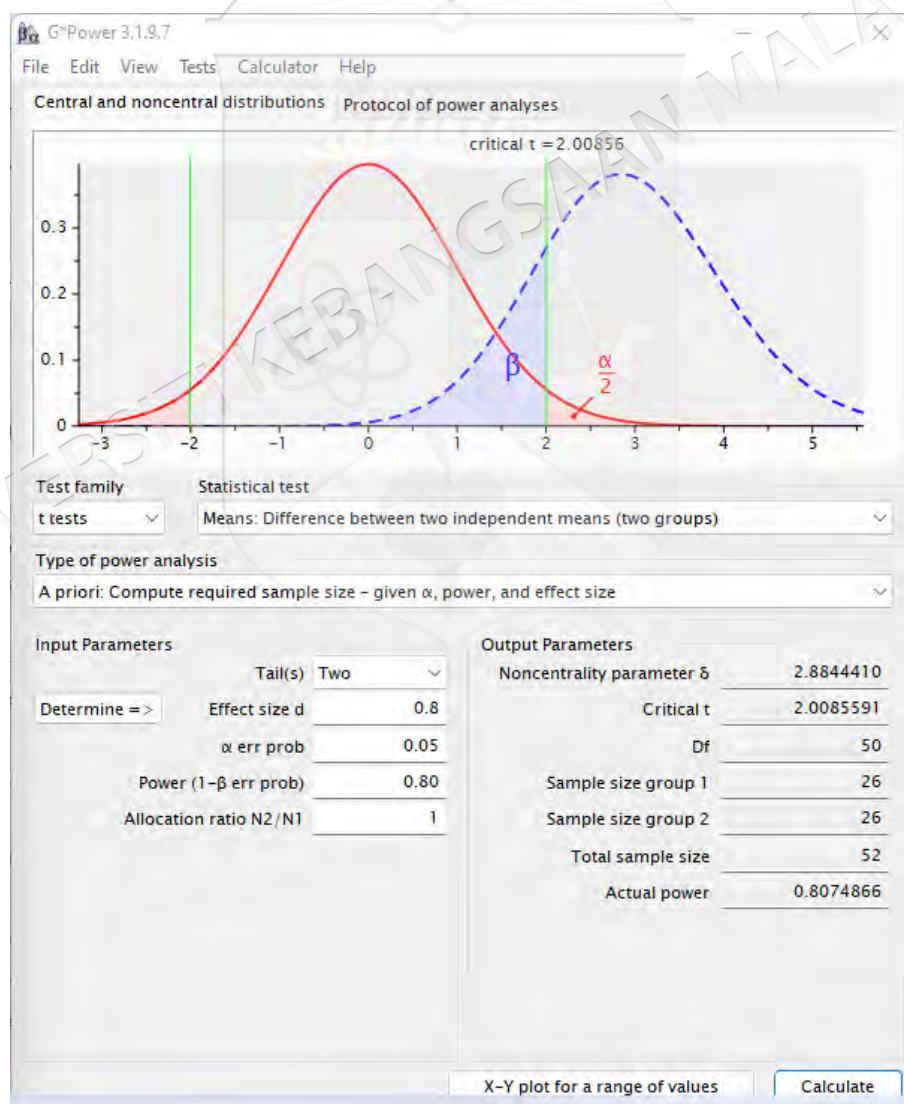


Figure 3.1 Result calculation of sample size using G*Power 3.1 software

A post hoc power analysis was conducted to contextualise the detectable effect size in the final study sample. The analysis assumed a two-group independent samples comparison, a two-tailed alpha of 0.05, and a large standardised effect size of Cohen's $d = 0.8$. This effect size was selected to represent a clinically meaningful between-group difference in nutrient intake or growth outcomes. Under these assumptions, the achieved power was approximately 0.93. This suggests that the final sample had sufficient power to detect large between-group differences, although smaller differences may not have been detected.

It is important to note that this post hoc power estimate applies specifically to the primary two-group comparisons of nutrient intake and growth outcomes. Power to detect smaller effect sizes, for example Cohen's $d = 0.5$ representing a moderate difference, would be lower, at approximately 0.60 with this sample. Therefore, clinically relevant but modest between-group differences may not have been detected, and this limitation was considered when interpreting null findings.

The DXA body composition component was limited by participant uptake, with 12 infants completing the assessment. This subsample was insufficient for comparative analysis between feeding groups and was not adequately powered for hypothesis testing. DXA findings are therefore presented descriptively and treated as exploratory. They provide preliminary local descriptive data and effect size estimates to inform future adequately powered studies, rather than supporting inferential conclusions about group differences.

Association analyses were conducted using parsimonious multivariable models, meaning that the number of predictor variables included in each regression model was deliberately limited relative to the available sample size. Model overfitting becomes a concern when too many predictors are included relative to the number of observations. With a maximum analytic sample of 76 participants, and with incomplete post-discharge follow-up reducing the effective sample size at some timepoints, inclusion of a large number of covariates would have increased the risk of unstable parameter estimates and unreliable confidence intervals.

Covariates were selected based on clinical relevance, data completeness, sample size, and sufficient variation within the cohort. Candidate variables included GA at birth, birth weight z-score or small-for-gestational-age status, sex, illness burden or major neonatal morbidity, and where appropriate, duration of parenteral nutrition. Post-discharge feeding type was included as a time-varying covariate in longitudinal models where sufficient data were available. Collinearity among candidate variables was assessed, and highly correlated variables were not entered simultaneously into the same model.

This approach prioritises statistical validity and reproducibility over the inclusion of every potentially relevant variable and is consistent with recommendations for multivariable analysis in studies with moderate sample sizes. Where results were sensitive to the choice of covariate set for example, where the PFM-PBM association changed substantially with or without adjustment for GA this is reported explicitly in the results and discussed as a limitation in the interpretation of findings.

3.8 ETHICAL APPROVAL

Ethical approval for this study was obtained from the Research Ethics Committee of UKM (Approval No: JEP-2024-519). Written parental consent was obtained for non-routine assessments of bone mineralisation and body composition using DXA scans during follow-up visits. A different consent form was provided for parents who agreed for the infants to be included for nutritional intake assessments and anthropometric measurements only, without DXA scan.

3.9 DATA COLLECTION PROCEDURE

Data collection commenced only after ethical approval and institutional research and development permissions were obtained (approval period: 2 September 2024–1 September 2026). Subsequent institutional permissions for data collection were obtained from: (1) the Director of HCTM; (2) the Director of HPKK UKM; and (3) the relevant heads of departments/units including the Neonatal Unit (HCTM), Specialist (Dietetics) Clinic (HPKK), Radiology Department (HPKK), and Nursing Department (HCTM and HPKK).

During hospital admission, eligible infants were identified from the HCTM NICU admission register and study admission sheet, which listed all preterm infants born at ≤ 34 weeks' GA and/or with birth weight ≤ 1800 g. The admission book and medical records were reviewed a minimum of twice weekly to extract feeding and nutrition information, daily/weekly anthropometry, and relevant clinical details. Each infant was assigned a unique anonymous study number. Access to nursing charts was assisted by a trained nurse (AS). Where information was incomplete, missing data were retrieved electronically via the Caring Hospital Enterprise System (CHES), and supplemented, when necessary, through paper-based records obtained from the Department of Medical Record, HCTM.

Post-discharge follow-up visits occurred according to routine clinical practice and were not mandated by the study protocol. However, attendance at follow-up was influenced by practical and logistical factors, including caregivers' preferences for follow-up at other healthcare facilities, distance from the study centre, and difficulties in post-discharge contact.

Post-discharge follow-up was conducted at the HPKK outpatient clinic. Growth assessments (weight, length, and head circumference) were scheduled at corrected age (CA) of 1, 3, and 6 months. At 6 months CA, bone status and body composition were assessed using DXA scanner. Although DXA was planned at 6 months CA, some scans were performed outside this time point due to scheduling and clinical constraints; therefore, DXA measurements obtained between 6- and 9-months CA were included in the analysis.

For post-discharge follow-up at HPKK, anthropometric measurements including weight, length, and head circumference were obtained during clinic visits at the Specialist (Dietetics) Clinic. The Arcus Air Hospital Information System (HIS) (IQVIA Corporation 2024), the electronic medical record platform implemented at HPKK, was subsequently used to retrieve missing anthropometric data and other relevant clinical information documented during follow-up visits. All extracted data were entered and organised into a structured spreadsheet using Microsoft Excel. Data accuracy and consistency were verified against source records.

Figure 3.2 presents the sequential data collection procedure for the study. Following ethical approval, preterm infants born at ≤ 34 weeks' GA and/or ≤ 1800 g birth weight were screened, assessed for eligibility, and enrolled during NICU admission. Baseline maternal and neonatal clinical data were extracted from medical records. During admission, daily enteral and parenteral intake data, fortification details, feeding interruptions, and feeding milestones were recorded from clinical charts. Body weight was recorded daily, while length and head circumference were recorded weekly where clinically feasible and at discharge.

At discharge, infants were classified into two feeding groups according to the predominant source of cumulative recorded enteral intake during NICU admission: PBM and PFM. Post-discharge feeding status was recorded descriptively at follow-up visits. Growth assessments were conducted at 1, 3, and 6 months CA, while whole-body DXA assessment for body composition and bone status was conducted at 6–9 months CA in infants who attended the assessment.

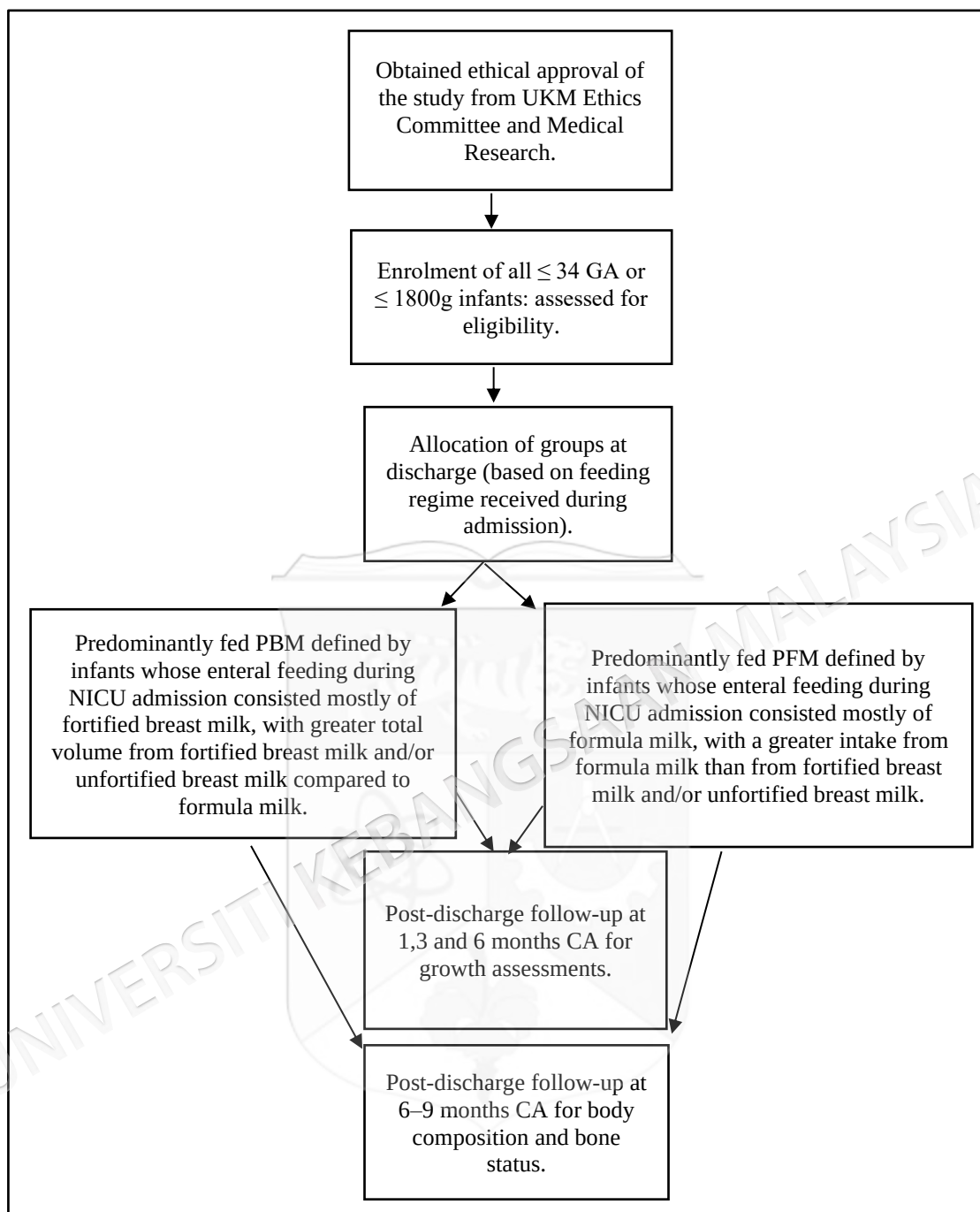


Figure 3.2 Data collection flowchart

3.10 TYPES OF DATA COLLECTED

During NICU admission, four main categories of data were collected: infant and maternal baseline data, nutritional intake data, clinical and medical data, and growth measurements. After discharge, follow-up data included feeding status, growth measurements, DXA-derived body composition and bone mineralisation parameters.

Routine clinical records at HCTM were documented in paper-based medical records and electronically through CHETs. Baseline perinatal and clinical data were extracted from the admission book, nursing charts, and medical records. Infant variables included date and time of birth, sex, singleton or multiple birth, GA at birth, birth weight, birth length, head circumference at birth, mode of delivery, Apgar score at 5 minutes, and need for resuscitation. GA was determined according to standard clinical practice at HCTM, primarily from early first-trimester ultrasound when available, or from obstetric records and maternal history when ultrasound data were unavailable. When early ultrasound was not available, GA was estimated from the last menstrual period. Maternal variables included maternal age, parity, gestational diabetes mellitus, and hypertension.

Nutritional intake data during NICU admission included daily enteral intake, parenteral nutrition, type of milk received, fortification status, feeding interruptions, and feeding milestones. Expressed breast milk and formula volumes were recorded in millilitres from nursing intake charts. These data were used to calculate achieved nutrient intake during admission and to classify infants according to predominant feeding exposure.

Clinical and medical data collected during admission included major neonatal morbidities and clinical course variables relevant to nutrition and growth. These included respiratory distress syndrome, bronchopulmonary dysplasia, necrotising enterocolitis, late-onset sepsis, intraventricular haemorrhage, retinopathy of prematurity, duration of parenteral nutrition, and length of hospital stay.

Growth data collected during admission, at discharge, and during post-discharge follow-up included weight, length, and head circumference; detailed measurement procedures are described in Section 3.10.3.

After discharge, follow-up data were collected at the Specialist Dietetics Clinic at HPKK at 1, 3, and 6 months CA. Anthropometric data collected at each visit included weight, length, and head circumference. These measurements were recorded as outpatient clinic measurements and used to calculate post-discharge growth outcomes, including WAZ, LAZ, HCZ, Δ WAZ, and growth trajectories.

Post-discharge feeding status was recorded at each clinic visit by the attending dietitian through caregiver verbal interview. Feeding status was categorised descriptively as exclusive breast milk, exclusive formula milk, mixed feeding, or transition between feeding types. Quantitative post-discharge nutrient intake was not calculated because feeding after discharge was highly variable, with feeding transitions, mixed feeding, changes between milk types, variable intake volume per feed, and direct breastfeeding sessions that could not be measured reliably under routine clinic conditions. Therefore, post-discharge feeding data were used descriptively and considered when interpreting the post-discharge growth findings, but were not included as covariates in the main longitudinal growth analyses.

For infants who attended DXA assessment, whole-body DXA was performed at 6–9 months CA. DXA-derived parameters included bone mineral content (g), bone mineral density (g/cm²), bone area (cm²), fat mass (g), lean body mass (g), total body mass (g), and percentage body fat (%). These data were used descriptively as exploratory measures of body composition and bone mineralisation.

3.10.1 Nutrient Intake

This study followed the unit's standard care protocols for preterm infants. Parenteral nutrition (PN) was provided to extreme and very preterm infants with GA below 32 weeks or birth weight below 1500 g. PN was also initiated when clinically indicated in some moderate preterm infants with birth weight below 1800 g, particularly those who were small for GA or had clinical compromise. Minimal enteral feeding was initiated

as early as day 1 of life in clinically stable infants, irrespective of PN status, according to the unit feeding advancement protocol. Transition to oral feeding by direct breastfeeding or bottle feeding was encouraged when infants reached approximately 1700 g, showed readiness cues, and were clinically stable. Discharge was planned when infants achieved a weight of at least 1800 g and postmenstrual age above 34 weeks, together with clinical stability and established oral feeding competence.

During NICU admission, daily intake data were extracted from clinical records. These included the volume of glucose solutions, PN, lipid emulsions, other intravenous fluids excluding medications and blood products, enteral feeds, breast milk fortifier, formula milk, and protein supplements such as Myotein®. Enteral feeding records included expressed breast milk, fortified breast milk, unfortified breast milk, formula milk, and fortifier use. Expressed breast milk and formula intake were recorded in millilitres from nursing intake charts.

Direct breastfeeding sessions during NICU admission were not converted into intake volume because pre-feed and post-feed test weighing was not routinely performed. Session duration alone was not considered a reliable estimate of milk transfer. This may have underestimated total breast milk exposure in infants who directly breastfed during admission. However, feeding group classification was based on cumulative recorded enteral intake across the NICU admission.

From the admission intake records, daily nutrient intakes from birth to discharge were calculated for total energy, protein, fat, carbohydrate, and fluid intake. These were expressed as kcal/kg/day for energy, g/kg/day for macronutrients, and mL/kg/day for fluids. Protein energy ratio was calculated as g protein per 100 kcal. Cumulative energy, protein, fat, carbohydrate, and fluid deficits were also calculated across admission where applicable.

The following nutrient intake variables were derived:

- a. Total daily energy intake, kcal/kg/day
- b. Total protein intake, g/kg/day

- c. Total fat intake, g/kg/day
- d. Total carbohydrate intake, g/kg/day
- e. Protein energy ratio, g/100 kcal
- f. Total fluid intake, mL/kg/day
- g. Cumulative energy deficit, kcal/kg
- h. Cumulative protein deficit, g/kg
- i. Cumulative fat deficit, g/kg
- j. Cumulative carbohydrate deficit, g/kg
- k. Cumulative fluid deficit, mL/kg

Additional feeding-related variables were extracted to describe feeding progression during NICU admission. These included day of life at initiation of PN and enteral nutrition, day of life to reach at least 120 mL/kg/day of enteral feeding, day of life to reach at least 150 mL/kg/day of enteral feeding, day of life at full enteral feeds, duration of PN, day of life when fortification began, rate of advancement to full feeds, type of milk feeding at discharge, and the proportion of cumulative energy supplied by PN, breast milk, formula milk, and glucose solutions from birth to discharge.

Post-discharge feeding information was obtained at follow-up visits through caregiver verbal interview. Caregivers were asked about the infant's current feeding type and, where available, approximate milk intake. Feeding was categorised descriptively as exclusive breast milk, exclusive formula milk, mixed breast milk and formula, or complementary foods alongside milk feeds. Quantitative post-discharge nutrient intake was not calculated because feeding after discharge was variable and difficult to measure reliably under routine clinic conditions. Many infants experienced feeding transitions, trial changes between milk types, mixed feeding, variable intake volume per feed, and direct breastfeeding sessions that could not be quantified accurately. Post-discharge feeding information was therefore used descriptively and considered during interpretation of the post-discharge growth findings, but it was not included as a covariate in the main longitudinal growth analyses.

3.10.2 Calculation of Nutrient Intakes

For daily nutritional intake calculations, birth weight was used as the reference weight until the infant's measured weight first exceeded birth weight. Thereafter, the most recent daily weight measurement was used for subsequent calculations.

Intakes were calculated per day as follows:

- a. Total fluid intake (mL/kg/day): $(\text{IV fluids} + \text{PN} + \text{milk volume}) \div \text{working weight}$
- b. Total energy intake (kcal/kg/day): $[\text{energy from IV dextrose (5\%, 10\%, 12.5\%, 15\%, 20\%), PN (Vaminolact®), lipid, breast milk/fortified breast milk, formula milk, and supplements (e.g., Myotein®)}] \div \text{working weight}$
- c. Protein intake (g/kg/day): $[\text{protein from PN (Vaminolact®), breast milk/fortified breast milk, formula milk, and supplements}] \div \text{working weight}$
- d. Fat intake (g/kg/day): $[\text{fat from lipid emulsions, breast milk/fortified breast milk, formula milk, and supplements}] \div \text{working weight}$
- e. Carbohydrate intake (g/kg/day): $[\text{carbohydrate from PN (based on dextrose \%), breast milk/fortified breast milk, formula milk, and supplements}] \div \text{working weight}$

Daily nutrient deficits were calculated as the difference between actual nutrient intake and the ESPGHAN 2022 recommended minimum enteral targets for preterm infants. The recommended targets were energy 115–140 kcal/kg/day; protein 3.5–4.0 g/kg/day for infants 1000–1800 g birth weight (or 4.0–4.5 g/kg/day for infants < 1000 g); fat 4.8–8.1 g/kg/day; and fluid 135–200 mL/kg/day. Cumulative nutrient deficit was calculated as the day-by-day summation of daily deficits across the entire hospital admission period (Embleton et al., 2001; Abdul Hamid et al., 2021).

In this neonatal unit, breast milk fortification is routinely performed using PreNAN® breast milk fortifier powder, according to a standard protocol of adding 1 g

of fortifier to 25 ml of expressed breast milk (EBM). Breast milk fortifier is typically introduced once clinically stable preterm infants reach enteral breast milk intakes of approximately 40–100 ml/kg/day, in line with Malaysian Paediatric Association guidelines (Malaysian Paediatric Association 2019) and HCTM/HPKK NICU practice.

Expressed breast milk volumes were recorded by nursing staff on daily fluid balance and feeding charts as millilitres delivered per feed via nasogastric or orogastric tube. These records were extracted at minimum twice weekly by the researcher from paper-based nursing charts and verified against the CHetS electronic record. Daily EBM volume in mL was summed and converted to mL/kg/day using the working weight denominator described in Section 3.10.2. There was no formal percentage cutoff applied to classify infants by feeding group. Classification as PBM or PFM was based on whether the greater cumulative volume of enteral intake across the NICU admission was derived from breast milk sources or from preterm formula respectively, as described in Section 3.6. This volumetric approach was chosen to reflect actual feeding patterns under routine clinical conditions rather than imposing an arbitrary threshold.

Direct breastfeeding volume was not recorded because it is not routinely measured in the NICU, and for most infants it was initiated only in the few days before discharge. The absence of direct breastfeeding volume measurement represents a recognised limitation of the present study. Direct breastfeeding was initiated under unit protocol only when infants reached approximately 1700 g and demonstrated oral feeding readiness cues, which in most infants occurred in the few days immediately before discharge. Its contribution to total enteral volume across the full admission period was therefore minimal in the majority of participants. The consequence is a potential slight underestimation of breast milk-derived energy and protein intake during the final days before discharge. This limitation was managed in three ways. First, the nutritional analysis covers the full admission period and the contribution of direct breastfeeding to cumulative intake across this period was small relative to the weeks of recorded tube-based intake preceding it. Second, the feeding group classification was fixed at the end of admission and reflects the predominant pattern across the whole stay rather than the final days alone. Third, this limitation is explicitly acknowledged in the

discussion alongside comparable methodological constraints reported in other studies examining preterm feeding in routine clinical settings (Belfort et al. 2025).

Tables 3.1–3.3 summarise the nutrient composition (per 100 ml) of the main nutrition sources used to estimate daily intakes during admission which are unfortified breast milk and fortified EBM with PreNAN® breast milk fortifier by week of lactation (Table 3.1), preterm formula options and protein supplement used (Table 3.2), and PN components including amino acids (Vaminolact®), lipid emulsions (Intralipid/SMOFlipid), and dextrose solutions of varying concentrations (Table 3.3). Values were obtained from published breast milk composition data and manufacturers' product information.

Table 3.1 — Nutrient composition of breast milk (per 100 ml)

	Breast milk	Breast milk	Fortified EBM with PreNAN® BMF	Fortified EBM with PreNAN® BMF
Used in	Week 1	Week 2 (onward)	Week 1	Week 2 (onward)
Energy, kcal	57	66	74.4	83.4
Protein, g	1.9	1.27	3.34	2.71
Fat, g	2.59	3.46	3.31	4.18
Carbohydrate, g	6.55	7.34	7.83	8.62

Breast milk composition data from Gidrewicz & Fenton, 2014. Fortifier composition from Nestlé Nutrition Institute manufacturer specifications for PreNAN® human milk fortifier powder (Nestlé S.A., Vevey, Switzerland).

The primary formula milk (preterm formula) options used in the study unit include Similac® NeoSure®, Similac® Special Care® 24, Novalac AR®, and Aptamil® which are specialised formulas designed to meet the nutritional needs of preterm infants. Additional protein supplementation was incorporated as needed and is included in the calculation of total enteral protein intake for each participant.

Table 3.2 Nutrient composition of formula milk and protein supplement (per 100 ml)

	Similac® NeoSure®	Aptamil®	Similac® Special care® 24	Novalac AR®	Myotein®
Energy, kcal	74.4	67	81	66	415
Protein, g	2.08	1.3	2.7	1.6	79.8
Fat, g	4.09	3.4	4.1	3.3	8
Carbohydrate, g	7.51	7.5	8.8	7.4	6.1

Manufacturers' literature for Similac® NeoSure® and Similac® Special Care® 24, Abbott Laboratories, Ross Products Division, Columbus, OH; Nutricia International B.V. (Danone Specialized Nutrition), Schiphol Boulevard 105, 1118 BG Schiphol, The Netherlands; Novalac AR (special anti-regurgitation infant formula): Novalac infant formulas Laboratoires Novalac/United Pharmaceuticals; Myotein®, Valens Nutrition, Bedford Business Park, Off Jalan Klang Lama, 58000 Kuala Lumpur.

Table 3.3 Nutrient composition of parenteral nutrition (per 100 ml)

	Vaminolact® 6.5%	Intralipid 20% or SMOFlipid 20%	Parenteral nutrition 10%	Parenteral nutrition 12.5%	Parenteral nutrition 15%
Energy, kcal	24	200	60	74	88
Protein, g	6.5	NA	3	3.5	4
Fat, g	NA	20	NA	NA	NA
Carbohydrate, g	NA	NA	12	15	18

Manufacturers' literature for Vaminolact®, Intralipid 20% and SMOFlipid 20%, Fresenius Kabi

Post-discharge achieved nutrient intake was not calculated in this study. At the 1-, 3-, and 6-month corrected age follow-up visits, feeding information was obtained verbally from caregivers by the attending dietitian as part of routine clinic review. The information collected described the infant's current feeding type, including exclusive breast milk, exclusive formula milk, mixed breast milk and formula feeding, or complementary foods alongside milk feeds where applicable. Detailed quantitative intake data were not collected using a standardised dietary recall, feeding diary, test weighing, or measured home milk volume record. Therefore, post-discharge feeding information was used descriptively to characterise feeding patterns and transitions during follow-up, but it was not used to calculate post-discharge energy, protein, fat, carbohydrate, or fluid intake, and was not included as a covariate in the main longitudinal growth analyses. This approach was necessary because post-discharge feeding during early infancy was highly variable, with feeding transitions, mixed feeding, changes between milk types, variable intake per feed, and direct breastfeeding

sessions that could not be quantified reliably under routine outpatient clinical conditions.

3.10.3 Growth Measurements

Growth data were collected from birth to 6 months corrected age. During NICU admission, growth was assessed using body weight, length, and head circumference. Body weight was measured daily as part of routine neonatal care, while length and head circumference were measured weekly where clinically feasible. After discharge, weight, length, and head circumference were measured at scheduled outpatient clinic visits at 1, 3, and 6 months CA. No additional growth measurements were collected between scheduled clinic visits.

Birth and admission growth data were interpreted using the Fenton 2013 preterm growth chart, which provides age- and sex-specific percentiles and z-scores for preterm infants from 22 to 50 weeks postmenstrual age (Fenton & Kim, 2013). Weight, length, and head circumference were converted to z-scores using postmenstrual age during admission. The Fenton research bulk calculator was used to calculate z-scores based on completed weeks of gestation and postmenstrual age.

In HCTM NICU, body weight was recorded daily by trained neonatal nurses. Weight was measured using routine unit weighing procedures, including tared bed-weighing where required for clinically unstable infants. Length and head circumference were recorded weekly where the infant's clinical condition allowed measurement. Length and head circumference recording was occasionally incomplete, particularly in infants requiring active respiratory support or those unable to tolerate handling at the scheduled measurement time. This was less common for body weight because weight could usually be obtained with minimal disturbance.

Where a daily weight measurement was not recorded, the most recent available weight was carried forward for that day's nutrient intake calculation. Where a weekly length or head circumference measurement was missing, the most proximate recorded measurement within that calendar week was used. Where no measurement was available within that week, the value was treated as missing, and the infant was excluded

from analyses requiring that specific time point. Missing length and head circumference values were not imputed.

Measurement variability may have occurred because anthropometric measurements were collected under routine clinical conditions. Variation in handling, infant positioning, respiratory support, oedema, head moulding, and clinical instability may have affected length and head circumference measurements. These limitations are acknowledged in Chapter 5.

Following discharge, outpatient anthropometric measurements were collected at the Specialist Dietetics Clinic at HPKK at 1, 3, and 6 months CA. Weight and length were measured by clinic nurses, while head circumference was measured by the attending dietitian using a flexible, non-stretchable measuring tape. CA was calculated using the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network Adjusted Age Calculator (NICHD 2021) and checked against the infant's date of birth, expected date of delivery, and clinic visit date. Post-discharge z-scores for weight, length, and head circumference were calculated using WHO Child Growth Standards (WHO 2006).

3.10.4 Clinical Outcome Data

Infant clinical morbidities documented during the NICU stay included respiratory distress syndrome, late-onset sepsis, necrotising enterocolitis, intraventricular haemorrhage, periventricular leukomalacia, chronic lung disease, retinopathy of prematurity, and patent ductus arteriosus, as defined below:

- a. Respiratory distress syndrome (RDS): Clinical diagnosis based on respiratory distress in the early neonatal period with supportive chest radiograph findings and need for oxygen or ventilatory support.
- b. Late-onset sepsis (LOS): Suspected sepsis occurring after 72 hours of life, for which blood cultures were taken.

- c. Necrotising enterocolitis (NEC): Suspected NEC diagnosed on clinical and/or radiological grounds, with feeds withheld and intravenous antibiotics administered for at least five days.
- d. Intraventricular haemorrhage (IVH): Any grade of IVH (Grade 1–4) recorded on cranial ultrasound or other imaging.
- e. Periventricular leukomalacia (PVL): Cystic PVL identified on cranial imaging.
- f. Chronic lung disease (CLD): Ongoing need for respiratory support or supplemental oxygen at 36 weeks PMA.
- g. Retinopathy of prematurity (ROP): Any stage of ROP diagnosed during routine ophthalmologic screening.
- h. Patent ductus arteriosus (PDA): PDA confirmed by echocardiography.

All diagnoses were taken from clinician documentation during admission and extracted from both paper charts and electronic medical records.

3.10.5 Body Composition

A subset of infants recruited into the study during NICU admission and followed through the post-discharge period underwent DXA body composition assessment at 6 months CA. Where scheduling or clinical constraints precluded attendance at the exact 6-month timepoint, measurements were obtained between 6 and 9 months CA. This was a cross-sectional, single time-point assessment and not a repeated measure.

Infants were eligible for DXA assessment if all of the following criteria were met:

- a. Written parental consent was obtained specifically for DXA scanning at enrolment.
- b. The infant had reached a CA of at least 6 months at the time of the appointment.

- c. The infant was clinically stable and did not have an active acute illness on the day of the scan.
- d. The infant was assessed as likely to tolerate the feed-and-wrap procedure described below.

Infants whose parents declined DXA-specific consent, who did not attend the scheduled DXA appointment, or whose scans could not be completed due to unresolvable movement artefact were excluded from the DXA subsample but remained in the study for anthropometric follow-up at 1, 3, and 6 months CA.

Given these eligibility and logistical constraints, the DXA subsample was smaller than the full follow-up cohort. All DXA analyses are therefore presented descriptively and are considered exploratory in nature. Body composition was measured by whole-body DXA using a Hologic Discovery A scanner (Hologic Inc., Bedford, MA, USA) in Infant Whole-Body mode with software version 12.3.3. DXA scans were performed by trained radiographers at the Radiology Department of HPKK according to standardised protocols.

3.10.6 Pre-Visit Preparation and "Feed-and-Wrap" Protocol

To obtain DXA images without pharmacological sedation, a standardised feed-and-wrap technique was used. This approach relies on feeding, swaddling, and postprandial natural sleep to reduce infant movement during image acquisition. Feed-and-wrap protocols are commonly used in infant imaging as an alternative to sedation, particularly in MRI studies, and the same principle was adapted in this study to minimise motion artefact during DXA scanning (Lollert et al. 2024; Moltoni et al. 2024).

Infants approaching 6 months CA were identified and scheduled for DXA assessment if parental consent had been obtained. Parents received a reminder text at least two days before the appointment. They were advised to avoid feeding the infant and to keep the infant awake for approximately three hours before the appointment, where feasible, to encourage feeding and natural sleep at the time of scanning. On arrival, registration was completed and anthropometric measurements were recorded.

Parents then helped settle the infant on the DXA scan bed, fed and swaddled the infant, and waited outside the scanning area during image acquisition.

Before each scan, the radiographer checked that all metal or non-biological items had been removed, including bracelets, anklets, clips, monitoring leads, and nappies with metal tabs. The infant was scanned wearing a gown or dry diaper only and was swaddled in a thin cotton wrap. The infant was positioned in the centre of the scan table according to manufacturer guidance and paediatric DXA positioning principles. A small gap was maintained between the arms and trunk, and the legs were gently straightened with the feet placed consistently. When infant size approached the scan field limits, minor centring adjustments were made to keep the head and lower limbs within the scan area. Scans with clear movement artefact or incomplete body coverage were repeated during the same visit where feasible (Binkovitz & Henwood 2007).

Daily quality control procedures were completed before participant scans. The scanner was calibrated using the manufacturer-supplied Hologic DXA quality control phantom, serial number 102565. The phantom contained reference values for bone mineral density and bone mineral content and was used for daily scanner stability checks according to manufacturer procedures.

Scans were analysed using Hologic paediatric analysis software, version 12.3.3, by trained radiographers. Regions of interest were manually adjusted where required. The neckline was placed at the mandibular border, the thoracolumbar line at the T12–L1 junction, and pelvic lines were aligned with the iliac crests and ischial tuberosities. Scans with unresolvable movement artefact were excluded from analysis.

The DXA-derived variables included fat mass, lean body mass, percentage body fat, bone mineral content, bone mineral density, and bone area. These values were generated using the system's internal algorithms. Instrument calibration was supported by the Hologic anthropomorphic quality control phantom, while body composition outputs were generated using the Hologic paediatric whole-body analysis software.

Table 3.4 Reference studies used for comparison of body composition

References	Population	DXA outcomes	Use for	Purpose
Rawlings and Cooke (1999)	Preterm infants (≤ 34 weeks GA, ≤ 1750 g BW). DXA at discharge, term, 12 weeks, 6 and 12 months CA	Whole body composition by DXA across infancy time points (fat and lean compartments are available in the full text)	FM, percent fat, total mass, lean compartment at 6 months CA	Matches your key endpoint (6 months CA) and is a large preterm cohort with repeated DXA measures

Rawlings and Cooke (1999) are the main preterm comparator for fat and lean outcomes at 6 months CA.

3.10.7 Bone Mineralisation

Bone mineralisation was assessed from the whole-body DXA scans as described above. Indices of bone mineralisation were obtained from the whole-body DXA scan performed. For each infant, the paediatric analysis software provided:

- Bone mineral content (BMC, g): total mass of mineralised bone within the scanned region.
- Areal bone mineral density (aBMD, g/cm²): BMC divided by the projected bone area.

Table 3.5 Reference studies used for comparison of bone mineral outcomes (BMC, aBMD, bone area)

References	Population	DXA outcomes	Use for	Purpose
Quintal et al. (2014)	Preterm (GA ≤ 32 weeks) and term peers. DXA at 40 weeks, 3 months, 6 months CA	Whole body BMC, aBMD, plus lean mass; aBMD defined as BMC divided by bone area	Primary comparator for BMC and aBMD at 6 months CA	It has the same CA end point and includes term peers in the same protocol
Rawlings and Cooke (1999)	Preterm repeated DXA through 6 months CA	Includes DXA bone measures in the repeated follow-up dataset	Trajectory support for bone measures across infancy	Larger cohort and repeated CA

DXA was chosen and used to evaluate bone status because it is accepted for paediatric densitometry, with published guidance supporting its use in infants and young children and noting that DXA can provide reproducible measures of BMC and aBMD. DXA uses a very low radiation exposure, commonly reported in the

microSievert range for spine and whole-body scans, which is much lower than many diagnostic radiology tests.

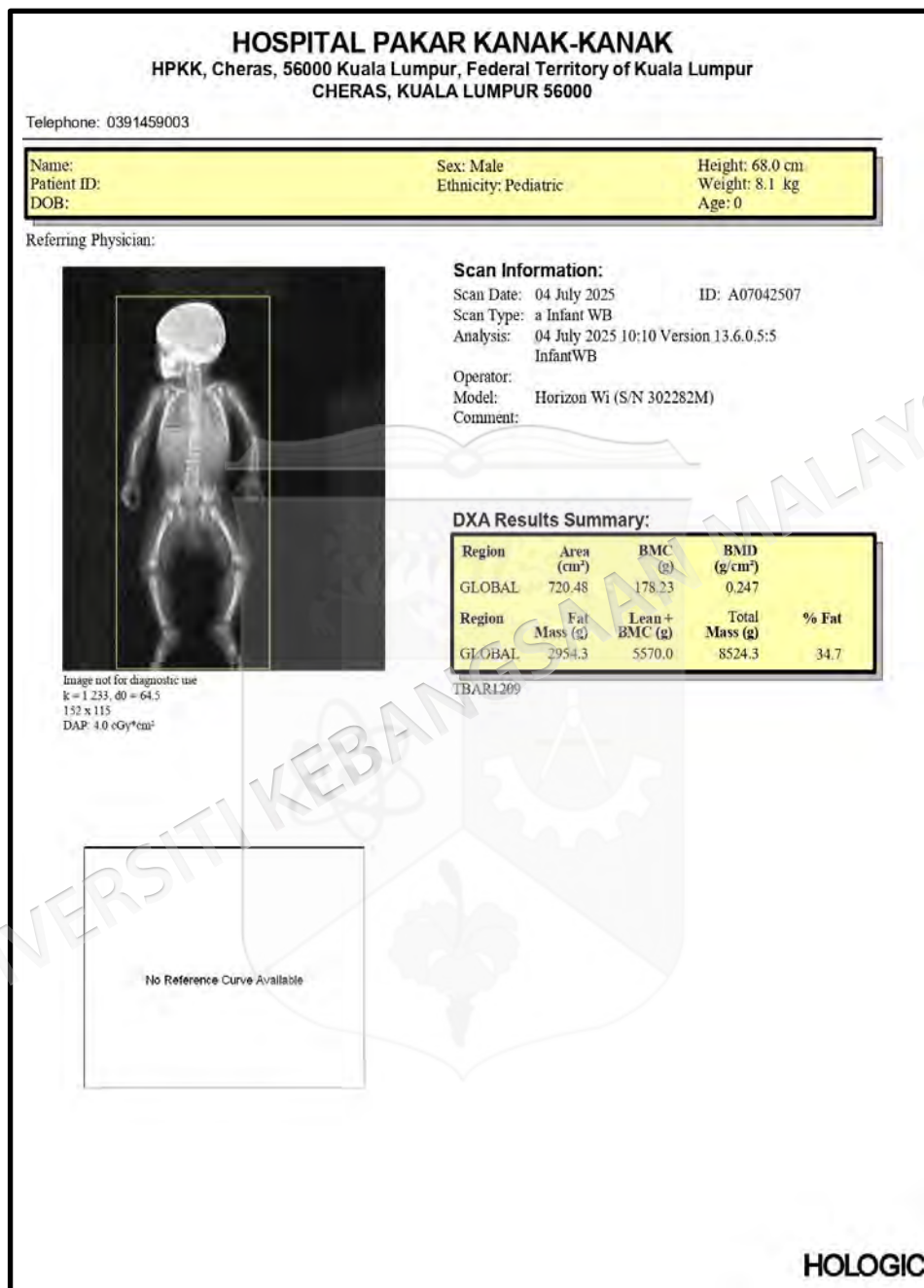


Figure 3.3 Example of DXA scan and output report in an infant participant

3.11 STATISTICAL ANALYSIS

All analyses were performed using IBM SPSS Statistics version 30.0.1.0. Descriptive statistics were used to summarise infant characteristics, feeding patterns, nutrient intakes, growth outcomes, and DXA-derived body composition and bone measures. Categorical variables were reported as n (%). Continuous variables were summarised as mean (SD) for approximately normally distributed data and median (IQR) for skewed data. Normality was assessed using the Kolmogorov–Smirnov test and inspection of histograms.

Between-group comparisons used the independent samples t-test for normally distributed continuous variables and the Mann–Whitney U test for non-normally distributed variables. Categorical variables were compared using the chi-square test or Fisher’s exact test, as appropriate. All tests were two-sided and $p < 0.05$ was considered statistically significant. P-values were reported to three decimal places.

Objective 1: Feeding practices and nutrient intakes during NICU admission

For Objective 1, feeding practices and nutrient intakes during NICU admission were examined descriptively and comparatively between infants fed PBM and those fed PFM. Daily energy and macronutrient intakes were calculated for each infant using recorded enteral and parenteral nutrition data and summarised over predefined postnatal periods, specifically Weeks 1–3 and Weeks 4–6 of admission.

For each analysis period, mean daily intakes of energy, protein, fat, carbohydrate, fluid, and PER were calculated. Cumulative energy and protein deficits were also derived by comparing achieved intakes against recommended targets over time. Nutrient intake data were analysed using available-case analysis, as the number of infants contributing data decreased over time due to discharge.

Comparisons between feeding groups were conducted separately for Weeks 1–3 and Weeks 4–6. Continuous variables were compared using independent samples t-tests when normally distributed and Mann–Whitney U tests when distributions were skewed. Categorical feeding practices were compared using Pearson’s chi-square test

or Fisher's exact test as appropriate. Mean or median differences between feeding groups were reported with corresponding 95% confidence intervals.

Weekly trends in nutrient intake and cumulative nutrient deficits from Week 1 to Week 6 were additionally examined graphically to illustrate temporal patterns and variability between feeding groups. Nutrient intakes were interpreted in relation to ESPGHAN recommendations for preterm infants to provide clinical context

Objective 2: Growth outcomes from birth to discharge and post-discharge

For Objective 2, growth outcomes were examined at multiple time points from birth through 6 months corrected age using a combination of cross-sectional, longitudinal, and velocity-based analyses.

Growth outcomes at discharge

Anthropometric measurements at discharge, including weight, length, head circumference, and corresponding z-scores, were summarised and compared between PBM and PFM feeding groups. Δ WAZ from birth to discharge and the prevalence of postnatal growth failure were also assessed.

Between-group comparisons at discharge were performed using independent samples t-tests for normally distributed continuous variables, Mann–Whitney U tests for non-normally distributed variables, and chi-square or Fisher's exact tests for categorical outcomes, as appropriate.

Growth outcomes at 1, 3, and 6 months CA: Post-discharge growth outcomes at 1, 3, and 6 months CA were analysed among infants with available follow-up data. Weight, length, head circumference, and corresponding z-scores (WAZ, LAZ, HCZ) were summarised as mean (SD) at each time point and compared between feeding groups using independent samples t-tests.

Nutritional status at each follow-up visit was classified according to WHO growth standards, with underweight, stunting, and low head circumference defined as z-scores below -2 SD. Group differences in the prevalence of these outcomes were assessed using Fisher's exact tests due to small sample size.

Growth velocity analysis: Growth velocity was calculated for weight and length between 1–3 months and 3–6 months CA by dividing the change in anthropometric measurements by the duration of the interval. Mean monthly growth velocities were compared between feeding groups using independent samples t-tests, with Welch's correction applied when variance equality was not met.

Longitudinal growth patterns: To examine overall growth patterns across time, repeated measurements were considered descriptively, and longitudinal trends were explored graphically. Subsequent longitudinal modelling approaches addressing repeated measures and covariate adjustment were undertaken under Objective 4.

Objective 3: DXA-derived body composition and bone outcomes

For Objective 3, body composition and bone mineralization outcomes measured using dual-energy X-ray absorptiometry (DXA) at 6–9 months CA were analysed descriptively. Outcomes included percentage body fat, lean mass, BMC, bone area, and aBMD. Continuous variables were summarised using means and standard deviations.

Although published reference data were available from previous studies conducted in preterm populations, formal statistical comparisons with external reference values were not undertaken due to differences in study populations, DXA devices, measurement protocols, and the small sample size in the current study. Reference values were therefore presented alongside study findings for contextual interpretation only, and all DXA analyses were considered descriptive in nature.

Objective 4: Growth outcomes and associations with feeding type and nutrient intake

For Objective 4, growth outcomes were analysed using a structured, stepwise analytical approach, guided by outcome timing, data completeness, and sample size considerations.

Growth change from birth to discharge: Δ WAZ from birth to discharge were initially summarised descriptively and compared by NICU feeding group. Univariable linear regression analyses were then conducted to assess unadjusted associations between NICU feeding type, early nutrient intake during Weeks 1–3 of admission and Δ WAZ from birth to discharge.

Given that some infants were discharged before completion of Week 3, analyses involving Week 1–3 nutrient intake were restricted to infants with complete intake data. Variables with substantial missing data beyond this time window were excluded from these analyses. Univariable analyses were considered exploratory and were used to inform subsequent multivariable modelling.

Multivariable regression models at discharge: Multivariable linear regression models were fitted to examine independent associations with Δ WAZ at discharge. Covariates were selected a priori based on established clinical relevance rather than data-driven selection methods. These included GA at birth, sex, and birthweight z-score, which are known determinants of early postnatal growth in preterm infants.

NICU feeding group and early nutrient intake variables were entered as exposures of interest. Separate sensitivity analyses were conducted by substituting protein intake with mean energy intake or protein-to-energy ratio to assess the robustness of findings. Given the exploratory nature of the analyses and the limited sample size, no automated variable selection procedures were applied, and model estimates were interpreted cautiously.

Binary growth outcomes were analysed using logistic regression, with results presented as odds ratios and 95% confidence intervals.

Post-discharge growth trajectories: Longitudinal post-discharge growth was examined using linear mixed-effects models to account for repeated WAZ measurements at 1, 3, and 6 months CA. A random intercept for infant was included to account for within-infant correlation over time.

To align nutrient exposure windows with outcome timing, nutrient intake during Weeks 4–6 of NICU admission was examined in relation to post-discharge WAZ trajectories. Due to incomplete intake data beyond discharge and reduced sample sizes, Week 4–6 nutrient intake variables were analysed in separate models for protein, energy, and protein-to-energy ratio.

Given the limited analytic sample sizes for these models, they were specified parsimoniously. Interaction terms and sex were excluded to reduce model complexity and improve stability of estimates, while key a priori covariates (GA at birth and birthweight z-score) were retained. These post-discharge analyses were considered exploratory.

All regression results were reported as effect estimates with corresponding 95% confidence intervals. Statistical significance was interpreted cautiously, recognising the potential for reduced statistical power due to small sample sizes and incomplete data. Formal adjustment for multiple comparisons was not undertaken, as the analyses were hypothesis-generating in nature and designed to explore associations rather than to test definitive causal hypotheses. For Objective 1, daily energy and macronutrient intakes were summarised for each infant over predefined periods (week 1–6) and compared between feeding groups using the tests described above. Where relevant, mean or median differences with 95% confidence intervals were reported.

3.12 CONFIDENTIALITY

All information obtained from this study was reported collectively. The results of the data analysis remain confidential, as no personal or identifying information about the study participants was disclosed.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 CHARACTERISTICS OF THE STUDY POPULATION

From September 2024 to August 2025, 76 eligible infants were recruited from the neonatal intensive care unit (NICU) at Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia. Eligibility was confirmed through clinical records following the study's predefined inclusion and exclusion criteria. Infants were classified into two feeding groups based on their predominant type of nutrient intake during admission: fortified breast milk (PBM) and formula milk (PFM).

Figure 4.1 outlines the study recruitment process, from initial screening of NICU admissions to the final analytic sample. During the recruitment period, 356 infants were screened, and 79 infants were enrolled. However, three infants were later excluded due to missing critical data (weight, feeding intake, growth parameters), which prevented their inclusion in the analysis. These exclusions were necessary to ensure data integrity and avoid compromising the validity of the statistical results. The final analytic sample therefore consisted of 76 infants.

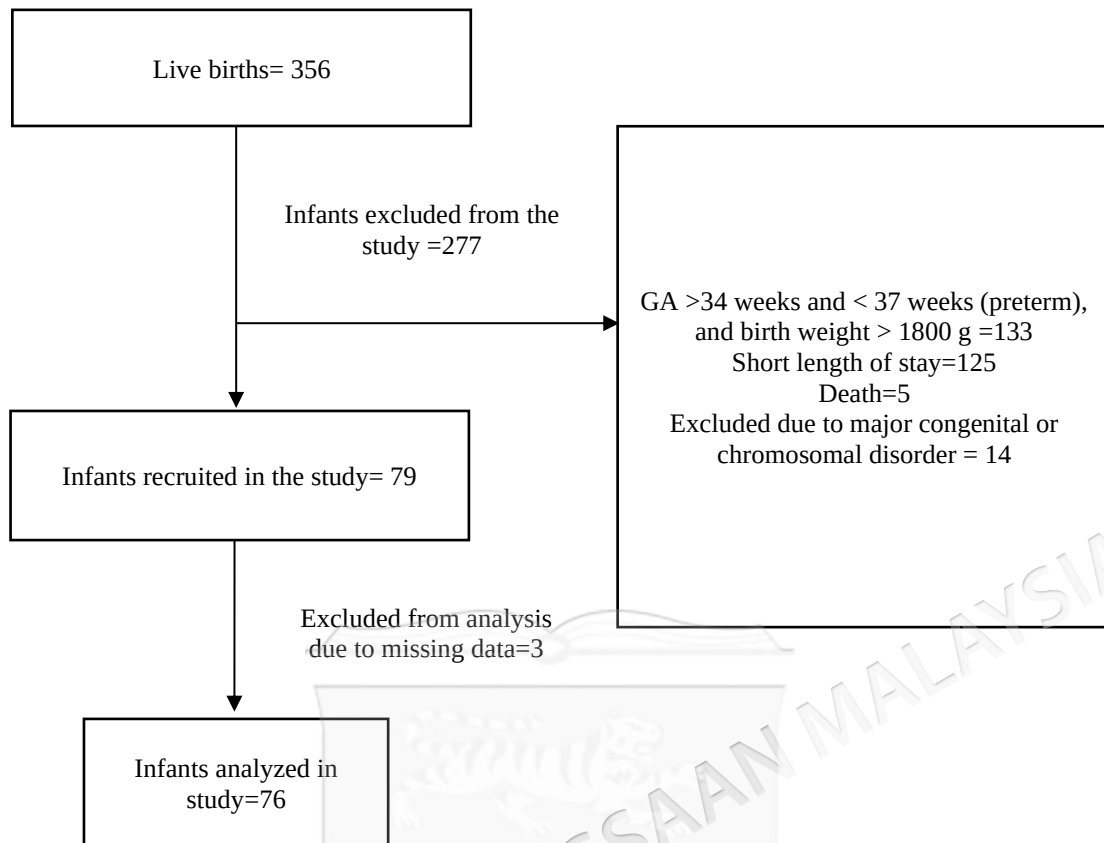


Figure 4.1 Flowchart of infant recruitment, exclusions, and reasons for exclusion

Next, Table 4.1 presents the baseline characteristics of the cohort, stratified by feeding group. The PBM group had a slightly lower median gestational age (GA) at birth compared to PFM (32 weeks vs 33 weeks, $p = 0.01$), and a higher proportion of very low birthweight (VLBW) infants (38.2% vs 14.3%).

There were no significant differences in birth weight, head circumference, length and the corresponding z-scores between the PBM and PFM groups. The overall distribution of GA categories also did not differ significantly ($p = 0.24$), although the PBM group had a numerically higher proportion of very preterm infants (41.2% vs 26.2%), whereas the PFM group had a greater proportion of moderate preterm infants (71.4% vs 52.9%). Birth status, including the proportion of twin and multiple pregnancies, did not also differ significantly between groups ($p = 0.55$) and is presented in Table 4.1.

Table 4.1 Infant characteristics of PBM and PFM feeding groups

Variable	All infants (n = 76)	PBM (n = 34)	PFM (n = 42)	p-value
Sex, n (%)				
Male	40 (52.6)	18 (52.9)	22 (52.4)	0.96
Female	36 (47.4)	16 (47.1)	20 (47.6)	
GA (weeks), median (IQR; range)	32 (30–33;24–35)	32(30–33; 24–35)	33(31–34;28–35)	0.01
GA group, n (%)				
Extremely preterm (<28 weeks)	3 (3.9)	2 (5.9)	1 (2.4)	0.24
Very preterm (28–31 weeks)	25 (32.9)	14 (41.2)	11 (26.2)	
Moderate preterm (32–34 weeks)	48 (63.2)	18 (52.9)	30 (71.4)	
Birthweight (g), mean (SD)	1719.2 (465.0)	1630.4 (471.7)	1791.1 (452.3)	0.14
Birth Weight-for-age Z score (WAZ), mean (SD)	−0.25 (0.91)	−0.11 (0.85)	−0.37 (0.96)	0.23
Birthweight category, n (%)				
Extremely low birthweight (<1000 g)	4 (5.3)	2 (5.9)	2 (4.8)	0.05
Very low birthweight (1000–1499 g)	19 (25.0)	13 (38.2)	6 (14.3)	
Low birthweight (1500–2499 g)	53 (69.7)	19 (55.9)	34 (81.0)	
Birthweight status, n (%)				
Small for GA (SGA)	11 (14.5)	3 (8.8)	8 (19.0)	0.45
Appropriate for GA (AGA)	63 (82.9)	30 (88.2)	33 (78.6)	
Large for GA (LGA)	2 (2.6)	1 (2.9)	1 (2.4)	
Head circumference at birth (cm), mean (SD)	29.57 (2.57)	29.07 (2.67)	29.96 (2.45)	0.14
Head circumference Z score (HCZ), median (IQR; range)	0.15 (−0.56–0.91; −2.14–2.96)	−0.35 (−1.60–0.91; −2.80–2.09)	0.12 (−1.32–0.84; −2.68–2.09)	0.43
Length at birth (cm), mean (SD)	40.55 (3.67)	39.72 (4.03)	41.22 (3.24)	0.08
Length-for-age Z score (LCZ), mean (SD)	−0.55 (0.97)	−0.51 (0.92)	−0.59 (1.03)	0.72
Birth status, n (%)				
Singleton	65 (86)	30 (88)	35 (83)	0.55
Twin/multiple	11 (15)	4 (12)	7 (17)	
Mode of delivery, n (%)				
Caesarean section	62 (81.6)	28 (82.4)	34 (81)	0.88
Vaginal delivery	14 (18.4)	6 (17.6)	8 (19.0)	

P-values for comparisons between the two groups were determined by the student's t-test or Mann Whitney U test for continuous variables and by Chi squared or Fisher's exact tests for categorical variables, as appropriate.

Next, Table 4.2 presents and compares the prevalence of clinical outcome at birth and throughout the admission until discharge among infants in the two feeding groups.

Table 4.2 Neonatal status at birth, clinical complications and NICU stay between PBM and PFM infants

Variable	All infants (n = 76)	PBM (n = 34)	PFM (n = 42)	p - value
Apgar score at 5 minutes, median (IQR; range)	9 (1; 2–10)	9 (1; 2–10)	9 (1; 4–10)	0.56
Resuscitation required, n (%)	35 (46.1)	20 (58.8)	15 (35.7)	0.04
Respiratory distress syndrome (RDS), n (%)	55 (72.4)	29 (85.3)	26 (61.9)	0.02
Necrotising enterocolitis (NEC, suspected), n (%)	6 (7.9)	3 (8.8)	3 (7.1)	0.79
Retinopathy of prematurity (ROP), n (%)	11 (14.5)	4 (11.8)	7 (16.7)	0.55
Intraventricular haemorrhage (IVH), n (%)	5 (6.7)	4 (12.1)	1 (2.4)	0.09
Patent ductus arteriosus (PDA), n (%)	2 (2.6)	1 (2.9)	1 (2.4)	0.88
PMA at discharge (weeks), median (IQR, range)	36 (35 - 37; 33 - 41)	36 (35 - 36; 34 - 40)	36 (36 - 37; 33 - 41)	0.45
Length of stay (days), median (IQR, range)	24 (17 - 48; 14 - 95)	30 (22 - 48; 14 - 95)	21 (17 - 34, 14-85)	0.01

P-values for comparisons between the two groups were determined by the student's t-test or Mann Whitney U test for continuous variables and by Chi squared or Fisher's exact tests for categorical variables, as appropriate.

As shown in Table 4.2, feeding groups differed significantly in a few clinical aspects. PBM infants had a significantly higher rate of resuscitation (58.8% vs 35.7%, $p = 0.04$) and a higher incidence of respiratory distress syndrome (RDS) (85.3% vs 61.9%, $p = 0.02$) compared to PFM infants. However, for other clinical complications like NEC, ROP, IVH, and PDA, the incidence rate was low and there were no statistically significant differences between groups.

Length of stay in the NICU was significantly shorter for PFM infants (21 days) compared to PBM infants (30 days, $p = 0.01$).

Next, Table 4.3 presents feeding practices and nutritional intakes during NICU admission by feeding group.

Table 4.3 Feeding practices and nutritional intake between the two feeding groups

Variable	PBM (n = 34)	PFM (n = 42)	p-value
Parenteral Feeding			
PN (TPN) used, n (%)	17 (50)	18 (42.9)	0.53
First PN day, median (IQR; range)	2 (1; 1–2)	2 (1; 1–2)	0.07
PN duration, median (IQR; range)	2(1-7;1-17)	2(1-2;1-16)	0.49
Enteral feeding			
First enteral feed day, median (IQR; range)	1 (1–3; 1–5)	1 (1–2; 1–4)	0.01
Day reach 120 mL/kg/day, median (IQR; range)	5 (4–6; 2–8)	5 (4–6; 2–6)	0.01
Day reach 150 mL/kg/day, median (IQR; range)	6 (6–9; 4–12)	5 (4–7; 2–8)	0.04
Feed advance rate to full feeds, mL/kg/day, median (IQR)	26.5 (20–30)	30 (22–35)	0.09
Milk exposure during admission			
Any breast milk, n (%)	34 (100)	41 (97.6)	1.00
Any formula, n (%)	33 (97.1)	42 (100)	0.45
Fortifier used, n (%)	34 (100)	-	-
First fortifier day, median (IQR; range)	9 (5; 0–21)	-	-
Feeding at discharge			
Any breast milk, n (%)	34 (100)	37 (88.1)	0.06
Exclusive breast milk, n (%)	26 (76.5)	7 (16.7)	< 0.001
Mixed breast milk plus formula, n (%)	8 (23.5)	30 (71.4)	< 0.001
Exclusive formula, n (%)	0 (0.0)	5 (11.9)	—
Energy source during admission, mean (SD)			
From PN, %	0.3 (2.19)	0.0 (1.03)	0.40
From breast milk, %	79.0 (14.8)	21.0 (12.7)	< 0.001
From formula, %	17.4 (14.8)	74.7 (14.3)	< 0.001

Values are n (%) or median (IQR; range), unless otherwise stated. Day variables are day of life. PN = parenteral nutrition (total parenteral nutrition, TPN). IV = intravenous. NA = not applicable. Continuous variables were compared using Student's t test (approximately normal) or Mann Whitney U test (skewed). Categorical variables were compared using chi square or Fisher's exact test, as appropriate. Energy source (%) represents the mean proportion of each infant's total recorded energy intake during admission that was derived from breast milk, formula milk, or PN.

As shown in Table 4.3, there were no significant differences in all PN-related practices between the feeding groups (all $p > 0.05$). As for EN, PBM infants started enteral feeds later and reached 120 and 150 mL/kg/day later than PFM infants ($p = 0.01$, $p = 0.01$, and $p = 0.04$, respectively). However, median values were very similar between groups (1 vs 1 day; 5 vs 5 days; 6 vs 5 days), suggesting small shifts in the

distribution of feeding milestones. After Bonferroni adjustment for the three milestone tests ($\alpha = 0.02$), the differences in first enteral feed and time to 120 mL/kg/day remained statistically significant, whereas the difference in time to 150 mL/kg/day was no longer statistically significant ($p = 0.04$).

Exclusive breast milk feeding at discharge was significantly higher in the PBM group (76.5%) compared to the PFM group (16.7%) ($p < 0.001$). All PBM infants received breast milk fortifier, which was introduced at a median of day 9. About 70% of PFM infants received mixed feeding at discharge.

Regarding nutritional intake during admission, PBM infants derived a significantly higher mean proportion of total energy from breast milk compared with PFM infants (82.4% vs 20.9%, $p < 0.001$). Conversely, PFM infants derived a higher mean proportion of energy from formula milk compared with PBM infants (74.0% vs 13.7%, $p < 0.001$).

4.2 CALORIE AND MACRONUTRIENT INTAKE BETWEEN PBM- AND PFM-FED PRETERM INFANTS DURING NICU ADMISSION FROM WEEK 1 TO 6

Tables 4.4 and 4.5 present infant macronutrient intake during weeks 1 to 3 and weeks 4 to 6, respectively, for infants fed PBM versus PFM.

Table 4.4a Mean Energy and Macronutrient Intake During Weeks 1–3 of Admission by Feeding Group

Variable (mean, SD)	PBM (n=34)	PFM (n=42)	MD (95% CI)	p- value
Energy intake (kcal/kg/day)	101.81 (12.79)	102.38 (17.15)	-0.57 (-7.63 to 6.49)	0.87
Protein intake (g/kg/day)	2.78 (0.54)	2.87 (0.66)	-0.09 (-0.37 to 0.19)	0.51
Protein: energy ratio (g/100 kcal/day)	2.92 (0.67)	2.83 (0.84)	0.03 (-0.32 to 0.39)	0.86
Fat intake (g/kg/day)	4.94 (1.03)	5.24 (1.06)	-0.30 (-0.78 to 0.18)	0.21
Carbohydrate intake (g/kg/day)	10.77 (1.61)	11.02 (1.59)	-0.25 (-0.98 to 0.49)	0.20
Fluid intake (mL/kg/day)	158.87 (18.20)	166.73 (30.43)	-7.86 (-19.22 to 3.50)	0.19

Values are presented as mean (SD). Between-group comparisons were performed using the Mann–Whitney U test for skewed continuous variables and independent samples t-tests for normally distributed variables.

Table 4.4b Energy and Macronutrient Intake During Weeks 1–3 in Relation to ESPGHAN 2022 Target Ranges

Variable (mean, SD)	PBM (n=34)	PFM (n=42)	ESPGHAN 2022 target range
Energy (kcal/kg/day)	101.81 (12.79)	102.38 (17.15)	115 – 140
Protein (g/kg/day)	2.78 (0.54)	2.87 (0.66)	3.5– 4.0
Protein: energy ratio (g/100 kcal/day)	2.92 (0.67)	2.89 (0.84)	2.8 - 3.6
Fat (g/kg/day)	4.94 (1.03)	5.24 (1.06)	4.8- 8.1
Carbohydrate (g/kg/day)	10.77 (1.61)	11.02 (1.59)	11 – 15
Fluid (mL/kg/day)	158.87 (18.20)	166.73 (30.43)	150- 180
Energy target achieved (≥ 115 kcal/kg/day), n (%)	3 (8.8)	11 (26.2)	—
Protein target achieved (≥ 3.5 g/kg/day), n (%)	2 (5.9)	4 (9.5)	—
Energy minimum target achieved (%)	88.5	89.0	—
Protein minimum target achieved (%)	79.4	82.0	—
Carbohydrate minimum target achieved (%)	97.9	100	—

Values are presented as mean (SD) unless otherwise stated. Individual target achievement was calculated using each infant's mean intake during Weeks 1 to 3, with targets defined as energy ≥ 115 kcal/kg/day and protein ≥ 3.5 g/kg/day. Percentage of ESPGHAN minimum target achieved was calculated as group mean intake divided by the lower limit of the ESPGHAN 2022 recommended range, multiplied by 100.

As shown in Table 4.4a, the groups did not differ significantly in energy intake (PBM: 101.81 kcal/kg/day vs PFM: 102.38 kcal/kg/day, $p = 0.87$) or protein intake (PBM: 2.78 g/kg/day vs PFM: 2.87 g/kg/day, $p = 0.51$). The protein-to-energy ratio, fat intake, and carbohydrate intake also showed no significant differences between groups. Fluid intake was similar between the two groups ($p = 0.19$), and carbohydrate intake was slightly lower in the PBM group ($p = 0.02$).

In terms of cumulative deficits, both groups had substantial energy and protein deficits, with no significant difference between them (energy deficit: PBM -187.26 kcal/kg vs PFM -147.65 kcal/kg, $p = 0.18$; protein deficit: PBM -9.86 g/kg vs PFM -8.06 g/kg, $p = 0.34$).

Referring to Table 4.4b, both PBM and PFM groups had mean energy and protein intakes below the ESPGHAN 2022 minimum recommended targets during Weeks 1 to 3. In the PBM group, mean energy intake was 101.81 kcal/kg/day, representing 88.5% of the minimum recommended intake of 115 kcal/kg/day, while mean protein intake was 2.78 g/kg/day, representing 79.4% of the minimum

recommended intake of 3.5 g/kg/day. In the PFM group, mean energy intake was 102.38 kcal/kg/day, representing 89.0% of the minimum energy recommendation, while mean protein intake was 2.87 g/kg/day, representing 82.0% of the minimum protein recommendation.

Mean protein-energy ratio, fat intake, and fluid intake were within the recommended ranges in both groups. Carbohydrate intake was marginally below the minimum recommended intake in the PBM group, reaching 97.9% of the minimum target, while the PFM group was within range, reaching approximately 100% of the minimum carbohydrate target.. At the individual level, target achievement was low in both groups. Only 3 of 34 PBM infants (8.8%) achieved the energy target of at least 115 kcal/kg/day, and 2 of 34 (5.9%) achieved the protein target of at least 3.5 g/kg/day. In the PFM group, 11 of 42 infants (26.2%) achieved the energy target and 4 of 42 (9.5%) achieved the protein target. These findings indicate that early energy and protein shortfalls were common in both groups, despite comparable mean intake between PBM and PFM infants.

Table 4.5a Mean Energy and Macronutrient Intake During Weeks 4–6 of Admission by Feeding Group

Variable (mean, SD)	PBM (n=20)	PFM (n=11)	MD (95% CI)	p value
Energy intake (kcal/kg/day)	119.90 (15.22)	108.06 (22.69)	11.84 (-2.09 - 25.77)	0.09
Protein intake (g/kg/day)	3.39 (0.61)	3.05 (0.76)	0.34 (-0.15 - 0.83)	0.17
Protein: energy ratio (g/100 kcal/day)	2.73 (0.58)	2.90 (0.59)	-0.17 (-0.71 - 0.37)	0.52
Fat intake (g/kg/day)	5.77 (0.70)	5.25 (1.69)	0.53 (-0.35 - 1.41)	0.23
Carbohydrate intake (g/kg/ day)	13.20 (1.97)	11.94 (2.04)	1.27 (-0.26 - 2.80)	0.10
Fluid intake (mL/kg/day)	154.77 (18.47)	162.58 (23.94)	-7.87 (-23.63 - 7.89)	0.32

Values are presented as mean (SD). For the comparison between groups in statistical tests were performed using the Mann-Whitney U test for skewed continuous variables and t-tests for normally distributed variables.

Table 4.5b Energy and Macronutrient Intake During Weeks 4–6 in Relation to ESPGHAN 2022 Target Ranges

Variable	PBM (n=20)	PFM (n=11)	ESPGHAN 2022 target range
Energy (kcal/kg/day)	119.90 (15.22)	108.06 (22.69)	115 – 140
Protein (g/kg/day)	3.39 (0.61)	3.05 (0.76)	3.5– 4.0
Protein: energy ratio (g/100 kcal/day)	2.73(0.58)	2.90(0.59)	2.8-3.6
Fat (g/kg/day)	5.77 (0.70)	5.25 (1.69)	4.8- 8.1
Carbohydrate (g/kg/day)	13.20 (1.97)	11.94 (2.04)	11 – 15
Fluid (mL/kg/day)	154.77 (18.47)	162.58 (23.94)	150- 180
Energy target achieved (≥ 115 kcal/kg/day), n (%)	14 (70.0)	6 (54.5)	—
Protein target achieved (≥ 3.5 g/kg/day), n (%)	8 (40.0)	7 (63.6)	—
Energy minimum target achieved (%)	104.3	94.0	—
Protein minimum target achieved (%)	96.9	87.1	—
Carbohydrate minimum target achieved (%)	120.0	108.5	—

Values are presented as mean (SD) unless otherwise stated. Individual target achievement was calculated using each infant's mean intake during Weeks 4 to 6, with targets defined as energy ≥ 115 kcal/kg/day and protein ≥ 3.5 g/kg/day. Percentage of ESPGHAN minimum target achieved was calculated as group mean intake divided by the lower limit of the ESPGHAN 2022 recommended range, multiplied by 100.

As Table 4.5a shows, there was no significant differences between PBM and PFM infants in energy intake or any macronutrient intake during Weeks 4–6 of admission (all $p > 0.05$).

Referring to Table 4.5b, mean energy intake during Weeks 4 to 6 was within the ESPGHAN 2022 recommended range in the PBM group but remained below the minimum recommended target in the PFM group. The PBM group achieved 104.3% of the minimum energy recommendation, while the PFM group achieved 94.0%. Mean protein intake remained below the minimum recommended target in both groups, with the PBM group achieving 96.9% and the PFM group achieving 87.1% of the ESPGHAN minimum protein recommendation. Mean carbohydrate intake was within the recommended range in both groups, reaching 120.0% of the minimum target in the PBM group and 108.5% in the PFM group. Fat and fluid intakes were within the recommended ranges in both groups, while protein-energy ratio was slightly below the recommended range in the PBM group and within range in the PFM group.

Mean fat and fluid intakes were within the ESPGHAN recommended ranges in both groups. Protein-energy ratio was slightly below the recommended range in the PBM group but within range in the PFM group. At the individual level, target achievement improved during Weeks 4 to 6 compared with Weeks 1 to 3. In the PBM group, 14 of 20 infants achieved the energy target, while 6 of 11 infants in the PFM group achieved the energy target. Protein target achievement should be interpreted using the correct available denominator for each group. Overall, these findings suggest partial improvement in nutrient delivery during Weeks 4 to 6, particularly for energy intake in the PBM group, although protein intake remained below the ESPGHAN minimum recommendation.

Cumulative nutrient deficits remained evident by Week 6. The PBM group had numerically smaller cumulative energy deficit than the PFM group (-8.48 kcal/kg vs -159.93 kcal/kg, $p = 0.42$) and a smaller cumulative protein deficit (-12.62 g/kg vs -16.62 g/kg, $p = 0.51$). However, these between-group differences were not statistically significant.

Figures 4.2 to 4.4 present weekly trends in macronutrient intake and cumulative nutrient deficits for infants fed PBM versus PFM from weeks 1 to 6.

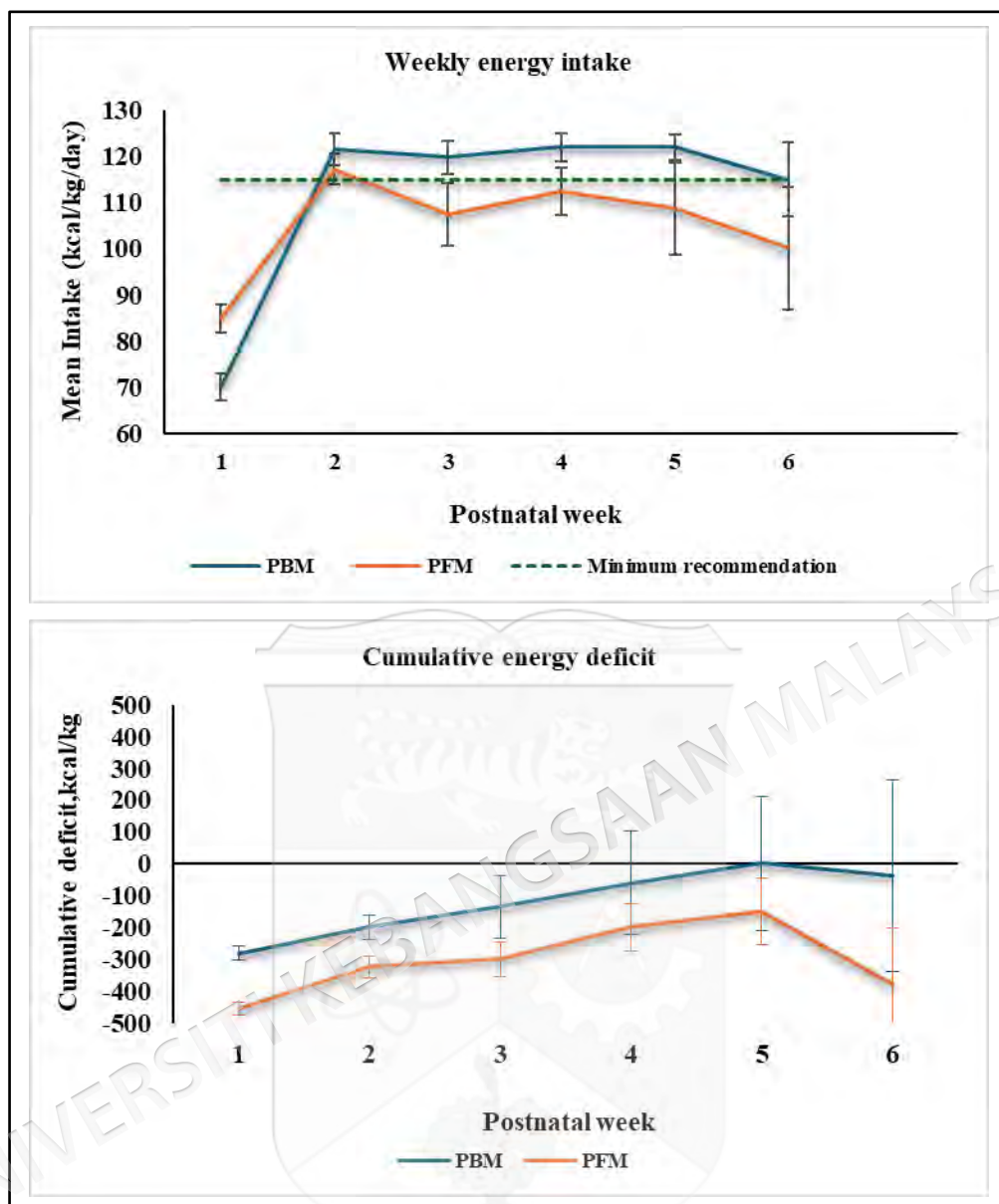


Figure 4.2 Mean weekly energy intake and cumulative energy deficits between two feeding groups (error bars represent the standard error (SE))

Figure 4.2 presents mean weekly energy intake and cumulative energy deficit for PBM and PFM across postnatal weeks 1 to 6. Weekly energy intake increased sharply from week 1 to week 2 in both groups. In week 1, PBM infants received approximately 70 kcal/kg/day and PFM infants about 85 kcal/kg/day. By week 2, mean intakes in both groups converged around the ESPGHAN minimum recommendation of 115 kcal/kg/day, and thereafter the PBM group generally remained at or slightly above this threshold (≈ 120 – 122 kcal/kg/day from weeks 2–5, decreasing by week 6).

In contrast, the PFM group showed a decline from week 3 onwards, falling below the minimum recommendation from week 3 (≈ 108 – 110 kcal/kg/day) and reaching about 100 kcal/kg/day by week 6, with wider variability at week 6. Across weeks 3–6, PBM infants consistently had higher mean energy intakes than PFM infants, with between-group differences of roughly 10–20 kcal/kg/day.

For cumulative energy deficit, both groups started with a substantial negative balance in week 1, with PFM infants showing a larger initial deficit (≈ -450 kcal/kg) than PBM infants (≈ -250 to -280 kcal/kg). Over time, the PBM group's deficit improved steadily, approaching zero by weeks 5–6, whereas the PFM group remained in negative balance throughout and showed a decline by week 6. Although PBM infants had lower energy intake in week 1, they achieved and maintained intakes closer to or above ESPGHAN recommendations earlier resulting in a numerically smaller cumulative energy deficit in the PBM group by week.

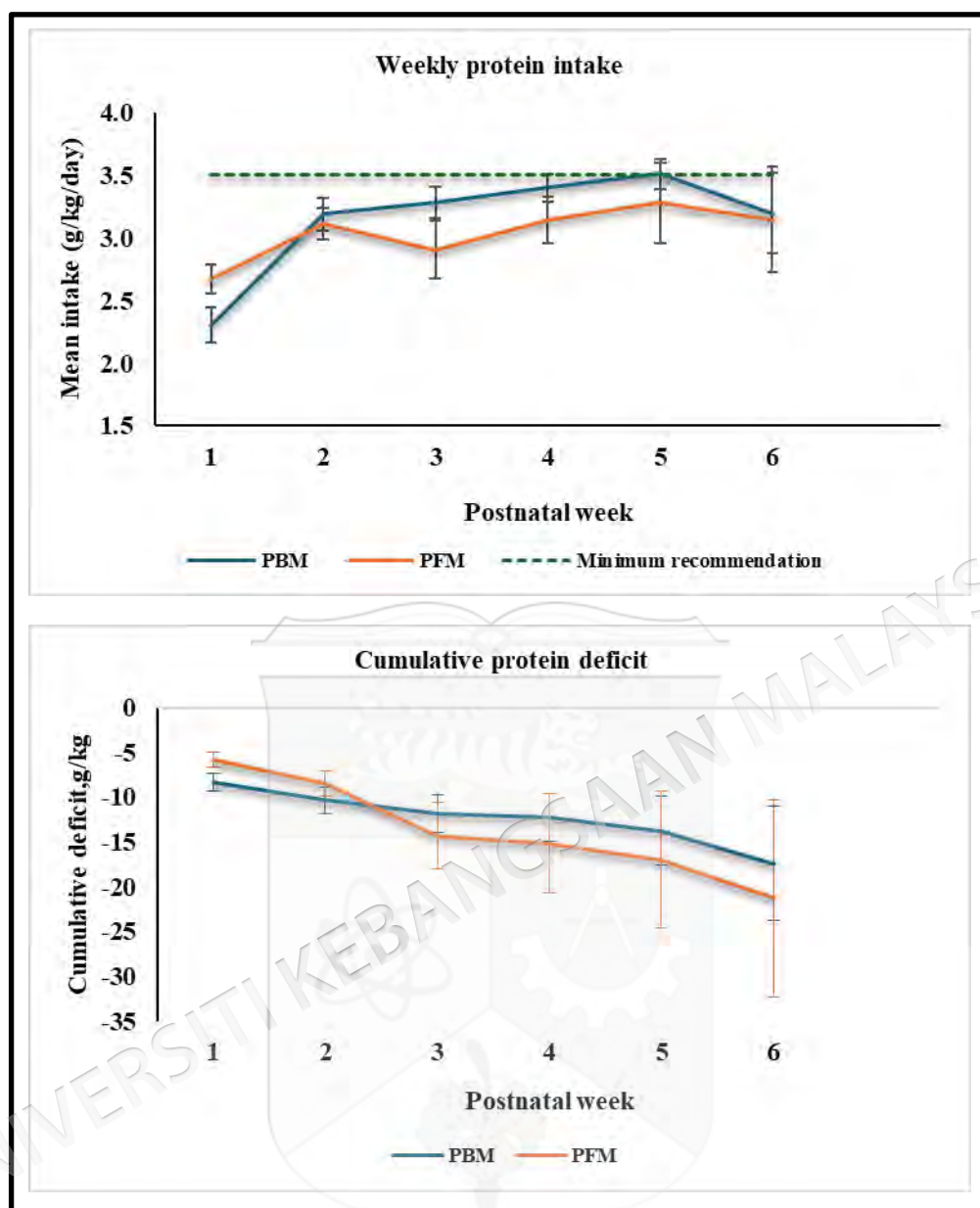


Figure 4.3 Mean weekly protein intake and cumulative protein deficits between two feeding groups (error bars represent the standard error (SE))

Figure 4.3 presents mean weekly protein intake and cumulative protein deficit for PBM and PFM across postnatal weeks 1 to 6. For weekly protein intake, both groups increased from week 1 to week 2. At week 1, the PBM group recorded approximately 2.3 g/kg/day while the PFM group recorded approximately 2.7 g/kg/day. By week 2, both groups reached approximately 3.0-3.1 g/kg/day.

From week 2 onwards, the PBM group recorded numerically higher mean protein intake compared to the PFM group during weeks 2–5, with similar values at week 6 (Figure 4.3). The PBM group demonstrated a progressive increase from week 2 (~3.0 g/kg/day) through week 5 (~3.5 g/kg/day), before declining to ~3.2 g/kg/day at week 6. The PFM group remained below the minimum recommendation throughout weeks 2–6, with intake ranging between 2.9–3.3 g/kg/day and exhibiting a declining trend from week 3 onwards.

For cumulative protein deficit, both groups began with deficits of approximately -7 to -8 g/kg at week 1. The cumulative deficit progressed negatively in both groups, reaching approximately -17 g/kg (PBM) versus -23 g/kg (PFM) by week 6. The PFM group exhibited a numerically greater cumulative deficit from week 3 onwards, although error bars showed substantial overlap between groups by week 6.

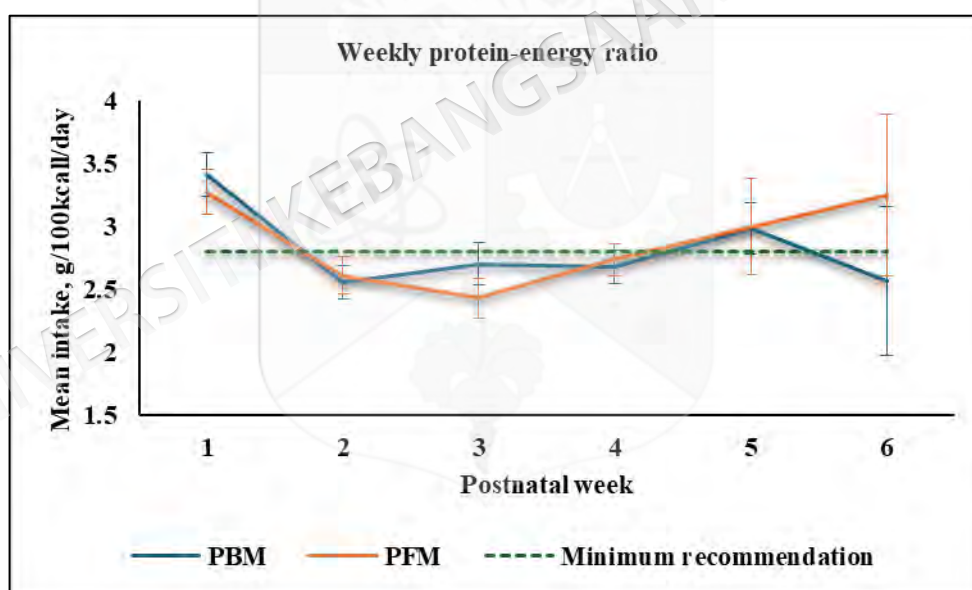


Figure 4.4 Mean weekly protein-energy ratio intake (PER) between two feeding groups (error bars represent the standard error (SE))

At week 1, both groups were within the recommended range at week 1. From weeks 1 to 2, both groups declined, reaching approximately 2.5–2.6 g/100 kcal/day by week 2 (below minimum recommendation). PER remained below the minimum recommendation from weeks 2 through 4 (range 2.4–2.7 g/100 kcal/day).

From week 5 onwards, both groups improved, reaching approximately 2.9–3.0 g/100 kcal/day at week 5 (approaching minimum recommendation) and approximately 3.2 g/100 kcal/day at week 6 (within range). PBM was slightly higher at week 1 (≈ 3.4 vs 3.2 g/100 kcal/day), but PFM reversed this at week 6 (≈ 3.2 vs PBM ≈ 2.6 g/100 kcal/day).

Weekly trends in fat, carbohydrate, and fluid intake are presented in Appendix F Figures 1-3. For all three macronutrients, intake increased from week 1 to week 2 and exceeded the minimum recommended levels from week 2 onwards in both groups. Weekly patterns were broadly similar between PBM and PFM infants, although greater variability was observed in the PFM group at later time points.

4.3 COMPARING GROWTH OUTCOMES OF PRETERM INFANTS BY FEEDING TYPE AT DISCHARGE, AND AT 1, 3, AND 6 MONTHS CORRECTED AGE

4.3.1 Comparison of Growth Outcomes at Discharge

Postnatal growth outcomes at discharge, and proportion of infants with postnatal growth failure (PGF) are summarized in Table 4.6 and compared between infants fed PBM and those fed PFM.

Table 4.6 Growth outcomes at discharge by feeding group

Variables	PBM (n=34)	PFM (n=42)	MD (95% CI)	p-value
Weight (g) at discharge, median (IQR, range)	2218 (1983 - 2423; 1800 - 3100)	2205 (1970 - 2365; 1360 - 3050)	-	0.98
Weight-for-age Z score at discharge, median (IQR, range)	-1.25 (-1.71 - -0.85; -3.09 - 0.66)	-1.43 (-1.9 - -0.79; -4.29 - -0.26)	-	0.81
Changes in weight -for-age Z score from birth to discharge, mean (SD)	-1.27 (0.35)	-1.13 (0.55)	-0.14 (-0.36 - 0.08)	0.21
Infants with changes of weight-for-age Z score of ≥ -1.28 from birth to discharge (Postnatal growth failure, PGF), n (%)	16 (47.1)	19 (45.2)	-	0.87
Head circumference at discharge (cm), mean (SD)	31.53 (1.42)	31.73 (1.40)	-0.20 (-0.86 - 0.46)	0.55
Head circumference-for-age Z score (HCZ) at discharge, mean (SD)	-0.86 (0.81)	-0.80 (0.86)	-0.06 (-0.45 - 0.33)	0.75

Values are presented as mean (SD) or median (IQR, range) as appropriate. Mean differences (MD) with 95% confidence intervals are shown for outcomes analyzed using independent samples t-tests. P values were obtained using independent samples t-tests for mean (SD) variables, Mann-Whitney U tests for median (IQR) variables, and Pearson chi-square or Fisher's exact tests for categorical variables.

Table 4.6 shows that discharge anthropometry were similar between groups. Median discharge weight was 2218 g in the PBM group and 2205 g in the PFM group ($p = 0.98$). Discharge Z scores also did not differ between groups, with comparable weight-for-age Z score (WAZ) ($p = 0.81$) and head circumference -for-age Z score ($p = 0.75$). PMA at discharge was similar in both groups (median 36 weeks in both; $p = 0.45$). Change in WAZ (Δ WAZ) indicated a greater decline in the PBM group (-1.27 ± 0.35) compared with the PFM group (-1.13 ± 0.55), although the difference was not statistically significant ($p = 0.21$). Postnatal growth failure at discharge occurred in 47.1% of PBM infants and 45.2% of PFM infants, with no difference between groups ($p = 0.87$).

4.3.2 Comparison of Post Discharge Growth Outcomes at 1, 3, and 6 Months CA

Throughout post-discharge follow-up, feeding group allocation remained fixed at the classifications established at NICU discharge (PBM $n = 34$; PFM $n = 42$) and was not revised based on post-discharge feeding transitions. The reduction in available data at 1, 3, and 6 months CA reflects attrition due to missed clinic appointments, transfer of care to other facilities, and non-attendance, rather than any reclassification of feeding group. Table 4.7 presents growth outcomes (mean (SD) for weight, length, and head circumference and the corresponding Z scores (WAZ, LAZ, HCZ) at 1, 3, and 6 months CA by feeding group.

Post-discharge nutritional intake was not measured in this study. As described in Section 3.10.3, feeding type at each outpatient visit was recorded categorically through unstructured caregiver interview by the attending dietitian, but quantitative nutrient intake assessment was not conducted under routine outpatient clinical conditions. Growth outcomes at 1, 3, and 6 months CA are therefore presented in the absence of corresponding post-discharge intake data. The analytical models examining post-discharge growth trajectories use NICU-period nutrient intake as the nutritional exposure variable rather than post-discharge intake, and the implications of this methodological limitation are discussed in Section 5.2.3 and in the study limitations in Chapter 5.

Table 4.6a Post-discharge feeding type at ± 3 months corrected age (n = 57)

Post-Discharge Feeding	n (%)
PBM group remained on breast milk	17 (29.8%)
PBM group transitioned to formula	10 (17.5%)
PFM group remained on formula	25 (43.9%)
PFM group transitioned to breast milk	5 (8.8%)
Total	57 (100%)

Of the 22 infants predominantly breast milk post-discharge, 16 (72.7%) received exclusive breast milk and 6 (27.3%) received mixed feeds. Of the 35 infants on predominantly formula post-discharge, 19 (54.3%) received exclusive formula and 16 (45.7%) received mixed feeds. Post-discharge feeding type was recorded by caregiver report at the first outpatient visit. Of 76 enrolled infants, 57 attended at least one post-discharge follow-up visit (PBM NICU group: 27 of 34, 79.4%; PFM NICU group: 30 of 42, 71.4%).

As shown in Table 4.6a, feeding transitions from NICU allocation were evident at the first post-discharge visit: 10 of 27 PBM-classified infants (37%) had transitioned to formula and 5 of 30 PFM-classified infants (16.7%) had transitioned to breast milk, confirming that NICU feeding group and post-discharge feeding type were not equivalent and should be treated as distinct variables in the analysis.

At each post-discharge clinic visit, caregivers were asked by the dietitian about the infant's current feeding pattern as part of routine dietetic assessment. Information collected included the primary milk source, feeding frequency, approximate amount consumed per feed where bottle-feeding was used, direct breastfeeding practices where applicable, use of mixed feeding, changes between milk types, and whether complementary foods had been introduced. However, no standardised dietary recall instrument, feeding diary, weighed-feed record, or validated research questionnaire was used. Because feeding after discharge was highly variable and included mixed feeding, changes between milk types, variable intake volume per feed, and direct breastfeeding sessions that could not be quantified reliably, these data were used descriptively rather than to calculate post-discharge nutrient intake.

Table 4.7a Growth outcomes at 1 month CA by feeding group

Outcome	PBM (mean ± SD)	PFM (mean ± SD)	p-value
Weight (kg)	4.52 ± 0.77	4.44 ± 0.94	0.72
WAZ	0.24 ± 1.20	0.08 ± 1.58	0.66
Length (cm)	54.05 ± 2.86	54.15 ± 4.43	0.92
LAZ	-0.10 ± 1.46	-0.02 ± 2.28	0.87
Head circumference (cm)	37.07 ± 1.76	37.74 ± 1.56	0.15
HCZ	0.14 ± 1.46	0.72 ± 1.26	0.12

Table 4.7b Growth outcomes at 3 months CA by feeding group

Outcome	PBM (mean ± SD)	PFM (mean ± SD)	p-value
Weight (kg)	5.88 ± 0.81	5.75 ± 0.92	0.56
WAZ	-0.38 ± 1.16	-0.63 ± 1.23	0.43
Length (cm)	59.99 ± 2.58	59.94 ± 3.31	0.95
LAZ	-0.31 ± 1.25	-0.40 ± 1.48	0.80
Head circumference (cm)	40.00 ± 1.19	39.70 ± 1.59	0.44
HCZ	-0.02 ± 1.07	-0.31 ± 1.17	0.36

Table 4.7c Growth outcomes at 6 months CA by feeding group

Outcome	PBM (mean ± SD)	PFM (mean ± SD)	p-value
Weight (kg)	7.20 ± 0.84	7.17 ± 1.19	0.91
WAZ	-0.55 ± 0.94	-0.71 ± 1.36	0.66
Length (cm)	65.54 ± 3.02	64.68 ± 3.96	0.43
LAZ	-0.54 ± 1.23	-1.01 ± 1.72	0.31
Head circumference (cm)	42.53 ± 1.08	42.40 ± 1.71	0.79
HCZ	-0.10 ± 0.62	-0.41 ± 1.32	0.34

Values are mean (SD). CA is corrected age. Sample sizes differ by age and indicator due to missing follow-up data (1 month: WAZ n=61, LAZ n=63, HCZ n=53; 3 months: WAZ n=57, LAZ n=60, HCZ n=51; 6 months: WAZ n=44, LAZ n=44, HCZ n=36).

Across follow-up at 1, 3, and 6 months CA, anthropometric measurements and corresponding z-scores were broadly similar between infants fed PBM and those fed PFM (Tables 4.7a–c). At each time point, mean body weight, length, and head circumference did not differ statistically between feeding groups (all $p > 0.05$). Likewise, WAZ, LAZ, and HCZ were comparable between PBM and PFM infants at all follow-up visits (all $p > 0.05$). From 1 to 6 months CA, mean body weight increased in both feeding groups, while mean z-scores declined progressively over time.

Next, Table 4.8 summarizes the number and percentage of infants classified as underweight ($WAZ < -2$), stunted ($LAZ < -2$), and low head circumference ($HCZ < -2$) at discharge and at 1, 3, and 6 months CA in the PBM and PFM groups. Nutritional status at discharge was included to characterise growth status at the end of NICU admission prior to post-discharge follow-up. Group differences for each nutritional status indicator were assessed separately at each time point.

Table 4.8 Prevalence of Undernutrition at discharge and at 1,3 and 6 months CA by Feeding Group

Age (CA)	Indicator	PBM, n (%)	PFM, n (%)	p-value
At Discharge	Underweight	3 (17.6)	6 (27.3)	0.48
	Stunted	3 (17.6)	4 (18.2)	0.48
	Low HC	1 (5.9)	4 (18.2)	0.94
1 month	Underweight	1 (3.4)	4 (12.5)	0.36
	Stunted	1 (3.3)	4 (12.1)	0.36
	Low HC	1 (3.6)	0 (0)	1.00
3 months	Underweight	2 (6.9)	3 (10.7)	0.67
	Stunted	3 (10.3)	3 (9.7)	1.00
	Low HC	1 (3.6)	3 (13)	0.32
6 months	Underweight	2 (9.5)	6 (26.1)	0.25
	Stunted	0 (0)	4 (17.4)	0.11
	Low HC	0 (0)	2 (9.5)	0.50

Values are presented as n (%). Nutritional status was classified according to WHO growth standards. Group differences were tested using Fisher's exact test (two-sided). Percentages were calculated based on available data within each feeding group at each time point. Sample sizes differ by age due to missing follow-up data: 1 month. Sample sizes differ by age and indicator due to missing follow-up data: 1 month: WAZ n = 61, LAZ n = 63, HCZ n = 53, 3 months: WAZ n = 57, LAZ n = 60, HCZ n = 51, 6 months: WAZ n = 44, LAZ n = 44, HCZ n = 36.

At 1, 3, and 6 months CA, the prevalence of underweight, stunting, and low head circumference (HC) did not differ significantly between PBM and PFM groups (p-values all > 0.05). Although numerically higher rates of underweight and stunting were observed in the PFM group at 6 months, these findings were based on small numbers and were not statistically significant.

Next, Table 4.9 summarizes mean monthly weight and length gain between 1–3 months and 3–6 months CA by feeding group. Growth velocity was calculated as the change in weight or length between 1–3 months and 3–6 months CA, divided by the interval duration.

Table 4.9 Comparison of post discharge growth velocity between feeding groups

Measurement interval (months CA)	Outcome	PBM	PFM	p value
1–3	Weight gain (kg/month)	0.75 ± 0.31	0.68 ± 0.34	0.41
	Length gain (cm/month)	3.21 ± 1.12	3.04 ± 2.48	0.76
3–6	Weight gain (kg/month)	0.44 ± 0.15	0.48 ± 0.23	0.46
	Length gain (cm/month)	1.99 ± 0.82	1.80 ± 1.35	0.60

Data are presented as mean ± SD and median (IQR; range). P-values were obtained using independent samples t-tests. Length of stay in the NICU is presented as median (IQR; range). Sample sizes for weight and length outcomes differ by interval: 1–3 months (weight: PBM n=28, PFM n=29; length: PBM n=27, PFM n=25), and 3–6 months (weight: PBM n=21, PFM n=23; length: PBM n=21, PFM n=21).

Table 4.9 summarizes post-discharge growth velocity between 1–3 and 3–6 months CA by feeding group. Growth velocity was higher during 1–3 months than during 3–6 months in both groups. No significant differences in weight or length gain were observed between PBM and PFM infants in either interval (all $p > 0.05$).

4.4 DESCRIBING BODY COMPOSITION AND BONE MINERALIZATION AT 6 MONTHS CA USING AVAILABLE DXA DATA

Table 4.10 DXA-derived Body Composition and Bone Mineralization at 6-9 Months CA

Variable (mean SD)	Overall (n=12)	PBM(n=6)	PFM (n=6)	Observed range (min-max)
Baseline measurements				
Age (months CA)	6.83 ± 1.03	6.33 ± 0.52	7.33 ± 1.21	6–9
Weight (kg)	7.50 ± 0.84	7.30 ± 0.54	7.70 ± 1.08	6.7 – 9.4
Length (cm)	67.19 ± 2.73	66.30 ± 3.25	68.08 ± 1.98	61.2 – 71.0
Bone Outcomes				
Bone area (cm ²)	675.51 ± 75.69	669.54 ± 89.74	681.47 ± 66.84	591.7–820.6
Bone mineral content (g)	169.23 ± 29.15	157.60 ± 17.61	180.86 ± 35.14	144.05–239.32
Bone mineral density (g/cm ²)	0.25 ± 0.024	0.24 ± 0.01	0.26 ± 0.03	0.22–0.31
Body Composition				
Lean body mass (g)	4887.08 ± 785.96	4539.92 ± 738.89	5234.25 ± 723.82	3921.3–6117.5
Fat mass (g)	3169.33 ± 668.00	3259.42 ± 724.63	3079.25 ± 661.16	2542.7–4544.7
Percentage body fat (%)	39.36 ± 6.68	41.75 ± 7.82	36.97 ± 4.84	30.5–53.7
Total body mass (g)	8056.42 ± 909.70	7799.33 ± 666.06	8313.52 ± 1037.80	7004–10108

Values are mean ± SD. Observed range represents minimum to maximum values among infants with DXA measurements.

Table 4.10 presents anthropometric measurements and DXA-derived bone and body composition outcomes for the 12 preterm infants scanned at 6 to 9 months CA. At the time of DXA assessment, infants had a mean weight of 7.50 ± 0.84 kg and a mean length of 67.19 ± 2.73 cm.

For bone outcomes, the mean bone area was 675.51 ± 75.69 cm², mean BMC was 169.23 ± 29.15 g, and mean BMD was 0.25 ± 0.02 g/cm². Body composition analysis showed a mean lean body mass of 4887.08 ± 785.96 g, fat mass of 3169.33 ± 668.00 g, and percentage body fat of $39.36 \pm 6.68\%$. The mean DXA-derived total body mass was 8056.42 ± 909.70 g.

Table 4.11 DXA-Derived Bone and Body Composition Outcomes Compared with Published Preterm Reference Values at 6–9 Months CA

Outcome	Current Study (n = 12)	Reference Values
Bone mineral content (g)	169.23 ± 29.15	125.5 ± 25.5^a 137.14 ± 22.46^b
Bone mineral density (g/cm ²)	0.25 ± 0.02	0.22 ± 0.02^b
Bone area (cm ²)	675.51 ± 75.69	486.5 ± 57^a
Lean body mass (g)	4887.08 ± 785.96	5034 ± 482^a 5750.13 ± 765.11^b
Percentage body fat (%)	39.36 ± 6.68	27.5 ± 6.0^a

Values are presented as mean $\pm$ SD. Reference values are derived from published preterm cohorts (^aCooke and Rawlings, 1999; ^b Quintal et al., 2014) and are shown for contextual comparison only. No formal statistical comparisons were performed due to differences in study population, age at assessment, DXA device and methodology, and sample size.

Table 4.11 compares DXA-derived bone mineral and body composition outcomes in the current study (n = 12), with published cohort values from Rawlings (1999) and Quintal et al. (2014).

Mean BMC and bone area in the present study were numerically higher than those reported by Rawlings (1999) and Quintal et al. (2014), while BMD values were broadly comparable. Quintal et al. did not report bone area, and BMD values were not available for comparison.

For body composition outcomes, lean body mass was within the range reported in previous cohorts, whereas percentage body fat appeared higher than values reported by Rawlings (1999). Quintal et al (2014) did not report percentage body fat. These comparisons are descriptive only and should be interpreted cautiously given differences in study population, age at assessment, DXA device and methodology, and sample size.

4.5 ASSOCIATION BETWEEN NICU FEEDING TYPE AND GROWTH OUTCOMES AT DISCHARGE, 1, 3 AND 6 MONTHS CA

Table 4.12 addresses whether NICU feeding type was associated with changes in anthropometric Z scores from birth to discharge, Δ WAZ, Δ LAZ, Δ H CZ, and the occurrence of postnatal growth failure (PGF), before and after adjustment for sex and GA at birth.



Table 4.12 Association between NICU Feeding Group and Changes in Anthropometric Z scores from Birth to Discharge

Outcome	PBM	PFM	Unadjusted effect (95% CI)	Unadjusted p	Adjusted effect β or OR (95% CI)	Adjusted p
Δ WAZ	-1.27 (0.35)	-1.13 (0.55)	-0.14 (-0.36 to 0.08)	0.21	-0.04 (-0.18 to 0.25)	0.73
Δ LAZ	-1.30 (1.00)	-1.10 (1.00)	-0.20 (-0.71 to 0.31)	0.43	-0.05 (-0.48 to 0.39)	0.83
Δ H CZ	-1.14 (0.94)	-0.91 (0.82)	-0.23 (-0.64 to 0.18)	0.28	0.10 (-0.32 to 0.51)	0.65
PGF, n (%)	16 (47.1)	19 (45.2)	OR 1.08 (0.43 to 2.67)	0.87	OR 1.38 (0.50 to 3.78)	0.53

Continuous outcomes are presented as mean (SD). Unadjusted effects are mean differences with 95% confidence intervals; p values are from univariable comparisons. Adjusted effects (β) for Δ WAZ, Δ LAZ, and Δ H CZ are from multivariable linear regression models for feeding group. PGF is presented as n (%); adjusted p value is from multivariable logistic regression with the same covariates. Adjusted OR is for PFM relative to PBM.

Unadjusted mean differences in Δ WAZ, Δ LAZ, and Δ HCH between PBM and PFM were small and not statistically significant (all $p > 0.05$). After adjustment, feeding group was not independently associated with Δ WAZ, Δ LAZ, or Δ HCH (all adjusted $p > 0.05$). Similarly, the odds of PGF did not differ by feeding group in the adjusted model (adjusted $p = 0.53$).

Next, following the comparison of growth changes by feeding group, univariable linear regression analyses were performed to examine crude associations between NICU feeding type and early nutrient intake (Week 1–3) with Δ WAZ from birth to discharge (Table 4.13).

Table 4.13 Univariable associations between NICU feeding group, early nutrient intake, and Δ WAZ

Predictor	β coefficient (B)	Std. Error	p value	95% CI
Feeding group (PFM vs PBM)	-0.02	0.13	0.91	-0.27 to 0.24
Protein intake, Week 1–3 (g/kg/day)	0.06	0.10	0.91	-0.19 to 0.22
Energy intake, Week 1–3 (kcal/kg/day)	-0.001	0.01	0.70	-0.01 to 0.01
Protein-to-energy ratio, Week 1–3	-0.02	0.09	0.84	-0.21 to 0.16

Outcome is Δ WAZ, analyzed using separate univariable linear regression models (one predictor per model). B values are unstandardized coefficients with 95% confidence intervals. Feeding group coded as PFM vs PBM (reference). Univariable analyses were restricted to infants with complete intake data ($n=66$).

In univariable linear regression analyses ($n = 66$), Δ WAZ from birth to discharge was not associated with feeding group, Week 1–3 protein intake, Week 1–3 energy intake, or the Week 1–3 protein-to-energy ratio (all $p > 0.05$).

Next, Table 4.14 evaluates whether feeding type and early nutrient intake during Weeks 1–3 were independently associated with Δ WAZ at discharge after adjustment for gestational age, sex, and birth weight z-score.

Table 4.14 Multivariable linear regression examining associations between NICU feeding group, nutrient intake, and Δ WAZ at discharge

Predictor	β coefficient	Std. Error	p value	95% CI
Feeding group (PFM vs PBM)	0.07	0.13	0.60	-0.34 to 0.20
Protein intake, Week 1–3 (g/kg/day)	-0.01	0.10	0.92	-0.22 to 0.20
Gestational age at birth (weeks)	0.04	0.03	0.21	-0.02 to 0.10
Sex (Female vs Male) *	0.09	0.13	0.49	-0.17 to 0.34
Birthweight z score (BWZ)	0.01	0.07	0.90	-0.13 to 0.15

As shown in Table 4.14, NICU feeding group was not independently associated with Δ WAZ from birth to discharge ($p = 0.60$). Protein intake during Weeks 1–3 was also not associated with Δ WAZ ($\beta = -0.01$, $p = 0.92$). GA at birth, infant sex, and birthweight Z score were not independently associated with Δ WAZ in the adjusted model ($p = 0.21$, $p = 0.49$, and $p = 0.90$, respectively).

Sensitivity models replacing protein intake with either energy intake or protein-to-energy ratio during Weeks 1–3 (Appendix G, Tables 1–2) similarly showed no association with Δ WAZ.

Next, Table 4.15 presents the linear mixed model examining associations between NICU feeding type, nutrient intake during Weeks 4–6, and repeated WAZ measurements at 1, 3, and 6 months CA.

Table 4.15 Linear mixed models examining associations between NICU feeding group, Week 4–6 nutrient intake, and WAZ from 1 to 6 months CA

Predictor	Estimate	Std. error	Df	P	95% CI
(Intercept)	-8.81	2.52	23.54	0.001	-14.01 to -3.60
Feeding group (PFM vs PBM)	1.00	0.41	23.93	0.03	0.15 to 1.85
Age 3 months (vs 1 month)	-0.49	0.14	43.38	0.001	-0.79 to -0.20
Age 6 months (vs 1 month)	-0.79	0.21	53.52	<0.001	-1.21 to -0.38
GA at birth (weeks)	-0.33	0.07	22.81	<0.001	-0.47 to -0.18
Birth weight z score (BWZ)	-0.81	0.20	23.51	<0.001	-1.21 to -0.40
Protein intake (wk4 -wk6)	-0.23	0.29	25.61	0.43	-0.83 to 0.36

As shown in Table 4.15, birth weight z-score (BWZ) was negatively associated with WAZ across post-discharge follow-up after adjustment (estimate = -0.81 , $p < 0.001$). GA at birth was also negatively associated with follow-up WAZ (estimate = -0.33 , $p < 0.001$). Compared with 1 month CA, WAZ was lower at 3 months CA and 6 months CA, indicating progressive decline over the follow-up period. Infants in the PFM group had higher WAZ across follow-up compared with PBM infants in this model (estimate = 1.00 , $p = 0.03$). Protein intake during Weeks 4–6 was not independently associated with WAZ across follow-up ($p = 0.43$).

Sensitivity analyses replacing protein intake with energy intake or PER ratio yielded similar findings, with no independent associations observed between nutrient intake and WAZ across follow-up (Appendix G, Tables 3–4). BWZ remained statistically associated in both models (energy: $p = 0.003$; PER: $p = 0.001$).

CHAPTER V

DISCUSSION

5.1 MACRONUTRIENT INTAKE DURING NICU ADMISSION

5.1.1 Baseline Clinical Differences and Implications for Nutritional Delivery

In this study, some baseline clinical characteristics differed between the predominantly fortified breast milk (PBM) and predominantly formula milk (PFM) groups. The PBM group had a lower median gestational age (GA) at birth (32 weeks, IQR 30–33, range 24–35) compared with the PFM group (33 weeks, IQR 31–34, range 28–35, $p=0.01$) (Table 4.1). Although the overall distribution of GA categories did not differ significantly between groups ($p=0.24$), the PBM group had a numerically higher proportion of very preterm infants born at 28–31 weeks gestation (41.2% vs 26.2%), while the PFM group had a greater proportion of moderate preterm infants born at 32–34 weeks gestation (71.4% vs 52.9%).

In addition to differences in GA, the PBM group had a higher proportion of very low birthweight (VLBW) infants. Thirteen of 34 PBM infants (38.2%) weighed 1000–1499 g compared with 6 of 42 PFM infants (14.3%). The overall distribution of birthweight categories differed at the borderline level of statistical significance ($p = 0.05$) (Table 4.1). These differences in birthweight distribution have important implications for early feeding tolerance and progression in the neonatal intensive care unit (NICU). In a prospective multicenter study among low birthweight neonates admitted to public hospital NICUs in Addis Ababa, Ethiopia, lower birthweight was independently associated with delayed attainment of full enteral feeding. Neonates in the VLBW range (1000–1499g) were 69% less likely to achieve full enteral feeding at any given time compared to heavier low birthweight counterparts (2000–2499g), after

adjustment for GA and other clinical covariates in multivariable Cox regression analysis (AHR = 0.31, 95% CI 0.142–0.67, $p = 0.003$) (Terefe et al., 2024). Accordingly, the greater representation of VLBW infants in the PBM group represents an additional biologically plausible contributor to slower feeding progression.

In addition, respiratory morbidity at birth also differed significantly between groups. The PBM group had a higher rate of resuscitation required at birth (58.8% vs 35.7%, $p=0.04$) and a higher incidence of respiratory distress syndrome (85.3% vs 61.9%, $p=0.02$) (Table 4.2). These findings are consistent with established evidence that infants born at 28–31 weeks GA have structurally and functionally immature lungs with reduced surfactant production, predisposing them to respiratory distress syndrome (RDS) compared with infants born at 32–34 weeks (Sweet et al. 2019).

Length of stay in the NICU was significantly longer in the PBM group (median 30 days, IQR 22–48, range 14–95) compared with the PFM group (median 21 days, IQR 17–34, range 14–85; $p = 0.01$) (Table 4.2). At first glance, this longer hospitalization might suggest greater medical complexity or slower clinical stabilization among PBM infants. However, discharge criteria in the unit protocol are based on reaching a weight of 1800 g, attaining approximately 35–36 weeks' GA, achieving full enteral feeding, and no longer requiring medical support. In the present cohort, postmenstrual age (PMA) at discharge was similar between groups (median 36 weeks GA in both PBM and PFM; $p = 0.45$), indicating that infants in both groups were discharged at a comparable level of physiological maturity.

Therefore, the longer hospitalization in the PBM group is likely explained by differences in GA at birth rather than delayed clinical progression. Infants born at lower GA require more postnatal days to reach the same discharge PMA compared with those born at higher GA, even if their clinical trajectory is similar. In this cohort, the PBM group had a median GA at birth of 32 weeks and was discharged at a median PMA of 36 weeks, corresponding to an expected median length of stay of approximately 28 days, closely aligned with the observed median of 30 days. In contrast, the PFM group had a median GA at birth of 33 weeks and was discharged at a median PMA of 36

weeks, corresponding to an expected median length of stay of 21 days, matching the observed value.

This pattern, whereby GA at birth largely determines length of stay when discharge occurs at a relatively fixed PMA, has been consistently documented in international cohorts. A European multicentre study of very preterm infants born at 22–31 weeks' gestation demonstrated that lower GA was associated with substantially longer hospitalization durations across regions (Maier et al. 2018). Similarly, a large U.S. national database study of preterm infants born at 23–36 weeks' GA between 2016 and 2020 reported a strong association between lower GA and prolonged hospitalization ($p < 0.001$) (Qattea et al. 2024). An international comparison across 11 countries within the iNeo collaboration also found that length of stay among extremely preterm infants decreased significantly with each additional week of GA at birth (El Rafei et al. 2021).

Taken together, the similar PMA at discharge despite differing lengths of stay suggests that discharge decisions were guided primarily by attainment of physiological stability rather than chronological age alone. This interpretation aligns with contemporary neonatal discharge practices, which emphasize readiness criteria such as thermoregulation, feeding competence, and cardiorespiratory stability, typically achieved between 35 and 37 weeks' PMA age in clinically stable preterm infants.

One notable attribute of the present cohort is the relatively low prevalence of major neonatal morbidities across both feeding groups with no significant differences noted. Necrotising enterocolitis (NEC) occurred in only 7.9% of infants overall, patent ductus arteriosus in only 2.6%, and intraventricular haemorrhage (IVH) in only 6.7% (Table 4.2). These rates are substantially lower than those reported in cohorts of similar or lower gestational ages from other settings. Abdul Hamid et al. 2021 documented IVH (in any grade from 1-4) in 72% of Malaysian preterm infants in the same NICU in an earlier period, which is much higher than the 12.1% observed in the present study's PBM group and a slightly higher rate of NEC (12%). In Southeast and East Asian cohorts of very preterm infants (<32 weeks' GA), NEC (stage ≥ 2) has been observed in approximately 4.7–8.1% of infants, and severe IVH (grade ≥ 3) in roughly 7.5–10.4% (Boo et al. 2021; Cao et al. 2021). However, comparisons across studies should be

interpreted cautiously. Differences in case definitions, grading thresholds, inclusion of suspected versus confirmed diagnoses, and documentation practices may substantially influence reported morbidity rates. In particular, the inclusion of IVH of any grade versus only severe grades, as well as differences in diagnostic criteria for NEC (suspected vs Bell stage ≥ 2), may partly explain discrepancies between studies, including the earlier local cohort. Besides, the present cohort had a more mature GA distribution compared with cohorts specifically targeting very or extremely preterm infants, with 63.2% of infants born at 32–34 weeks GA and only 3.9% born before 28 weeks gestation (Table 4.1). The risk of major morbidities such as NEC and IVH is inversely related to GA, with NEC incidence generally higher among the most premature infants and decreasing with increasing maturity, and IVH remaining a frequent complication of very preterm birth (Stoll et al. 2015). The relatively low burden of major neonatal complications observed in the present cohort is therefore consistent with its more mature gestational profile

It is also plausible that early clinical condition may have influenced feeding practices during admission. Infants with greater prematurity or respiratory instability may have required prolonged parenteral support or cautious advancement of enteral feeds, which could in turn affect the predominant milk source received during hospitalization. As such, differences in baseline clinical status, including GA and early respiratory morbidity, may have contributed to the observed distribution of PBM and PFM exposure rather than reflecting feeding choice alone.

5.1.2 Feeding Practice Differences Between the Feeding Groups

Both groups started enteral feeds early, PBM infants progressed through volume milestones more slowly than PFM infants (Table 4.3). Although the median day of first enteral feed was the same (day 1), the distribution differed ($p = 0.01$), which suggests that more PBM infants started later within the observed range (Table 4.3). A similar pattern was observed for the day reaching 120 mL/kg/day ($p = 0.01$), again with similar medians but a wider spread in PBM (Table 4.3). The difference became more apparent at 150 mL/kg/day, where PBM reached this target slightly later than PFM (median day 6 vs day 5; $p = 0.04$) (Table 4.3).

The slower advancement observed in the PBM group may partly reflect underlying differences in gestational maturity. The physiological basis for the effect of GA on feeding progression lies in gastrointestinal maturation. Preterm infants born at lower GA exhibit immature intestinal motility, delayed gastric emptying, reduced digestive enzyme activity, and impaired coordination of gastrointestinal function compared with more mature infants, all of which may contribute to feeding intolerance and slower advancement of enteral nutrition (Neu & Walker 2011). Consistent with this, a retrospective Ethiopian cohort study of VLBW neonates reported that GA independently predicted time to full enteral feeding, with infants born at ≥ 33 weeks being five times more likely to achieve full feeds earlier compared with those born at 28–32 weeks (AHR = 5.0; 95% CI: 2.29–11.13) (Getahun et al. 2024). These findings suggest that even modest differences in gestational maturity, such as those observed between the PBM and PFM groups in the present study, may translate into clinically meaningful differences in feeding progression.

This pattern is consistent with how feeding advancement is commonly managed in preterm care, where progression is adjusted according to infant clinical stability. Recommendation from European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) for enteral nutrition in preterm infants describe variability in advancement practices across units and support individualisation based on clinical condition (Embleton et al. 2023). In addition, practical factors related to milk availability may have contributed. Establishing and maintaining adequate milk supply after preterm birth is frequently challenging, particularly among mothers of VLBW infants, and early milk volume is known to vary substantially (Hoban et al. 2018; Meier et al. 2004). In contrast, formula feeds are immediately available and allow standardized volume provision, which may facilitate more predictable advancement in some clinical contexts. Taken together, differences in gestational maturity, clinical stability, and feeding logistics may collectively explain the observed distributional differences in feeding milestones between PBM and PFM infants.

The most distinct differences between groups were feeding at discharge. Exclusive breast milk at discharge was expectedly far more common in PBM than PFM (76.5% vs 16.7%; $p < 0.001$) (Table 4.3). At the same time, we found that 7 infants in

the PFM group were discharged on exclusive breast milk (Table 4.3). However, this finding does not conflict with the group allocation definition because classification was based on predominant milk source during admission, whereas discharge feeding reflects later lactation establishment and readiness for full oral milk feeds. A shift from predominantly formula intake earlier in admission to exclusive breast milk at discharge is plausible in preterm infants, as breastfeeding establishment can improve over time with continued expression support and feeding progression. Breastfeeding status at discharge has been linked to in-hospital breastfeeding support and feeding practices in very preterm and high-risk preterm cohorts (Casey et al. 2018; Maastrup et al. 2014; Zachariassen et al. 2010). The practice environment at Hospital Canselor Tuanku Muhriz, which aligns with Baby-Friendly Hospital Initiative standards promoting breastfeeding continuation (WHO, 2018, Abdul Hamid et al. 2021), may have supported this transition. Importantly, discharge feeding reflects feeding status at a single time point and does not necessarily represent cumulative nutritional exposure during the NICU stay. Because direct breast feeding volumes (feeding at breast) were not quantified in this study, the exact proportion of milk intake derived from breast milk in the later phase of admission cannot be confirmed. Therefore, while exclusive breast milk at discharge suggests successful lactation establishment, it should not be interpreted as equivalent to exclusive breast milk exposure throughout hospitalization.

From an analytical perspective, this distinction is important. Group allocation based on predominant intake during admission captures early nutritional exposure, which may be more relevant to early growth trajectories, whereas discharge feeding status reflects feeding competence and lactation success at physiological maturity. The presence of some PFM-classified infants discharged on exclusive breast milk therefore reflects dynamic feeding progression rather than misclassification.

All PBM infants received breast milk fortifier, with initiation at a median day of life 9 (Table 4.3). ESPGHAN recommends introducing multicomponent fortifier once enteral feeds reach roughly 40 to 100 mL/kg/day, which for many infants is reached within the first week, depending on stability and local advancement practice (Embleton et al. 2023). Using that volume as the reference point, a median start at day 9 (ranging from day 9-14) suggests fortification was commonly introduced after feeds

were well established, rather than at the earliest point permitted by the guideline. When compared with recent trials and cohorts, this cohort timing appears intermediate. In Chinnappan et al. 2021, fortification began at a median of about 7 to 8 days in infants already tolerating at least 100 mL/kg/day with most feeds as expressed breast milk. In contrast, several cohorts and trials that use higher volume thresholds, such as initiation around 100 mL/kg/day or 120 to 150 mL/kg/day, often start fortification in the second postnatal week or later (Mandhari et al. 2023; Sanchez-Holgado et al. 2024). Based on this day 9 practice sits within the range reported in recent studies, but it is later than the earliest ESPGHAN volume window and earlier than protocols that wait for higher volumes.

This fortification timing matters because an unfortified period during the first week can contribute to lower protein density in breast milk based feeding, even when total fluid volumes rise quickly. In this cohort, PBM infants reached 150 mL/kg/day later than PFM (Table 4.3), and fortification started at a median day 9 (Table 4.3), so part of week 1 and early week 2 intake for many PBM infants may have occurred before consistent fortifier use. This provides a feeding pathway explanation for why early macronutrient targets can be difficult to meet in breast milk based feeding without early and consistent fortification, while also keeping interpretation cautious because this study did not quantify nutrient intake specifically before and after the day fortifier was introduced.

5.1.3 Energy, Protein, and Protein-Energy Ratio During Weeks 1–3

In this study, across weeks 1–3, mean energy intake did not differ significantly between PBM and PFM groups (101.81 vs 102.38 kcal/kg/day, $p=0.87$), nor did mean protein intake (2.78 vs 2.87 g/kg/day, $p=0.51$) (Table 4.4a). The protein-energy ratio was also comparable between groups (2.92 vs 2.89 g/100 kcal, $p=0.86$). Similarly, fat intake (4.94 vs 5.24 g/kg/day, $p=0.21$), carbohydrate intake (10.77 vs 11.02 g/kg/day, $p=0.20$), and fluid intake (158.87 vs 166.73 mL/kg/day, $p=0.19$) showed no significant differences between groups.

This equivalence in macronutrient delivery is notable given that the two groups received fundamentally different milk sources during this period. As shown in Table 4.3, 82.4% of PBM energy intake came from breast milk compared with only 20.9% in the PFM group, while conversely, 74.0% of PFM energy came from formula versus only 13.7% in the PBM group. Breast milk and formula differ substantially in baseline nutrient composition, with unfortified breast milk containing approximately 0.9–1.0 g protein per 100 mL and 65–70 kcal per 100 mL, while preterm formula typically provides 1.8–2.0 g protein per 100 mL and 75–80 kcal per 100 mL (Embleton et al. 2023).

The most plausible explanation for the observed equivalence lies in the fortification practice applied to the PBM group. All PBM infants received breast milk fortifier during admission, with fortification initiated at a median of day 9 of life (Table 4.3). Standard fortification with PreNAN® BMF in this unit increases the nutrient density of breast milk from 66 kcal and 1.27 g protein per 100 mL to 83.4 kcal and 2.71 g protein per 100 mL from week 2 onward. This represents an increase of approximately 17.4 kcal and 1.44 g protein per 100 mL following fortification. At full feeding volumes of 150–180 mL/kg/day, fortified breast milk would therefore provide approximately 125–150 kcal/kg/day and 4.1–4.9 g/kg/day of protein, substantially increasing the nutrient density relative to unfortified breast milk and closer to the nutrient density of preterm formula. Therefore, the similarities in weeks 1–3 macronutrient delivery between PBM and PFM groups most likely reflects the combined effect of fortification in the PBM group raising breast milk nutrient density toward formula levels, while both groups progressed through similar trajectories of volume advancement during the early postnatal period.

When compared against ESPGHAN 2022 recommendations for preterm infant nutrition, both PBM and PFM groups remained substantially below guideline ranges for energy and protein throughout weeks 1–3 (Table 4.4b). The PBM group achieved mean energy intake of 101.81 kcal/kg/day approximately 13.2 kcal/kg/day below the ESPGHAN minimum recommendation of 115 kcal/kg/day, representing approximately 89% of the minimum target. Mean protein intake was 2.78 g/kg/day approximately 0.72 g/kg/day below the minimum recommendation of 3.5 g/kg/day, representing

approximately 79% of the minimum target (Embleton et al. 2023). The PFM group showed similarly large shortfalls: 102.38 kcal/kg/day energy (89% of minimum) and 2.87 g/kg/day protein (82% of minimum).

The protein-energy ratio in both groups (2.92 vs 2.89 g/100 kcal, $p=0.86$) fell below the ESPGHAN-recommended minimum of 3.2 g/100 kcal for preterm infants (Embleton et al. 2023) (Table 4.4a). A protein-energy ratio below 3.2 g/100 kcal indicates that protein delivery is proportionally lower than energy delivery relative to recommended ratios, which may limit lean tissue accretion even when absolute energy intake is adequate. In contrast, fat intake in both groups (4.94–5.24 g/kg/day) was within the ESPGHAN recommended range of 4.8–8.1 g/kg/day, and fluid intake (158.87–166.73 mL/kg/day) was within the 150–180 mL/kg/day range (Embleton et al. 2023) (Table 4.4b). Carbohydrate intake in the PBM group (10.77 g/kg/day) was marginally below the recommended minimum of 11 g/kg/day, though ESPGHAN notes that carbohydrate recommendations assume energy and protein targets are being met (Embleton et al., 2023).

This shared shortfall appears to reflect early clinical and feeding-related constraints rather than differences in milk type. Firstly, ESPGHAN explicitly acknowledges that recommended intake ranges are intended for stable, growing preterm infants and may not be immediately achievable during periods of acute illness or feeding establishment (Embleton et al. 2023). In the present cohort, both groups included substantial proportions of infants with significant respiratory morbidity 85.3% RDS in the PBM group and 61.9% in the PFM group (Table 4.2) and both groups were actively transitioning from parenteral to enteral nutrition during weeks 1–3. Approximately 50% of PBM infants and 42.9% of PFM infants received PN during admission (Table 4.3).

Secondly, the timing of fortification initiation in the PBM group (median day 9) means that a significant proportion of week 1, and potentially part of week 2, involved delivery of unfortified or sub-optimally fortified breast milk. Even if fortification commenced at day 9 and was continued thereafter, the inclusion of 8–9 days of

predominantly unfortified feeds in the weeks 1–3 average would pull the mean intake downward.

Thirdly, both groups showed a short delay in reaching full enteral feeds. PBM infants reached 120 mL/kg/day on median day 5 and 150 mL/kg/day on day 6, while PFM infants reached both volumes on day 5. Nutrient intake was lowest in Week 1 (energy: PBM 70.09 vs PFM 84.95 kcal/kg/day; protein: PBM 2.30 vs PFM 2.67 g/kg/day), increased markedly by Week 2, and remained relatively stable through Weeks 3–5 before a slight decline in Week 6. Thus, the lower mean intake across Weeks 1–3 largely reflects the transition phase in Week 1 rather than sustained inadequate delivery during the subsequent weeks.

Fourthly, respiratory morbidity may have directly affected feeding tolerance and advancement rate in both groups. Infants with respiratory distress syndrome often require fluid restriction during the acute phase of illness to minimize pulmonary oedema and optimize respiratory function (Demirel et al. 2016). In the present cohort, 85.3% of PBM infants and 61.9% of PFM infants had RDS (Table 4.2), and 58.8% of PBM infants and 35.7% of PFM infants required resuscitation at birth, indicating significant early respiratory compromise. Fluid restriction in the context of RDS management would necessarily limit the volume of milk that can be delivered, which in turn constrains total nutrient intake even when milk of adequate nutrient density is available.

In addition to weekly average intakes, cumulative nutrient deficits provide a measure of the total shortfall from ESPGHAN targets accumulated over the first three weeks of life. In this study, cumulative energy deficits were –187.26 kcal/kg in the PBM group and –147.65 kcal/kg in the PFM group ($p=0.18$), while cumulative protein deficits were –9.86 g/kg in PBM and –8.06 g/kg in PFM ($p=0.34$). Neither difference reached statistical significance, indicating that the two feeding groups accumulated similar total deficits relative to ESPGHAN targets despite different milk sources.

When daily energy intake averages 101.81–102.38 kcal/kg/day against a minimum target of 115 kcal/kg/day, the daily deficit is approximately 12.6–13.2 kcal/kg/day. Over 21 days (3 weeks), this would accumulate to approximately 265–277

kcal/kg if the deficit were sustained at this level throughout. The observed cumulative deficits of -187.26 and -147.65 kcal/kg are somewhat smaller than this theoretical maximum, which likely reflects the fact that intake improved progressively over the three-week period as volumes were established and fortification was initiated.

Similarly, the protein deficits of -9.86 and -8.06 g/kg/day arise from daily protein intakes averaging 2.78 – 2.87 g/kg/day against a minimum target of 3.5 g/kg/day, creating a daily deficit of approximately 0.63 – 0.72 g/kg/day. Over 21 days, this would accumulate to approximately 13.2 – 15.1 g/kg/day if sustained throughout, and the observed cumulative deficits are again somewhat smaller, consistent with progressive improvement in protein delivery as fortification was established in PBM and as full-volume formula feeds were achieved in PFM.

Abdul Hamid et al. (2021) reported cumulative energy and protein deficits across the first four postnatal weeks in both a Malaysian NICU and a UK unit, even though feeding protocols and fortifier use differed between centres. This is consistent with the pattern in the present cohort, where early intakes remained below ESPGHAN targets during the first weeks of admission, so early deficits appear to be a common feature of routine NICU care rather than a problem unique to this sample.

Importantly, Abdul Hamid et al. (2021) compared outcomes between neonatal units, not between feeding groups within one unit, so their findings cannot be used to infer PBM versus PFM effects directly. In our study, cumulative deficits were not statistically different between PBM and PFM during weeks 1–3, which supports the interpretation that feeding type alone did not determine overall nutritional adequacy during early admission.

Although their Malaysian cohort was more immature and clinically complex than ours, both studies achieved early energy delivery in the ~ 100 kcal/kg/day range during the initial weeks. This consistency suggests that energy provision within this range reflects established clinical practice within the unit, applied across varying GA profiles. Although ESPGHAN provides recommended energy targets for preterm infants (Embleton et al. 2023), achieving higher intakes during the early postnatal

period may be constrained by physiological tolerance, feeding progression, and clinical stability.

Differences in achieved nutrient intake may therefore be better understood in the context of fortification strategy rather than gestational maturity alone. This distinction becomes clearer when comparing the present data with studies that implemented structured or individualized fortification protocols.

Rochow et al. (2021) implemented an individualized target fortification protocol in 183 very preterm infants (≤ 32 weeks' gestation) in Germany, comparing outcomes with a standard fortification control group. In that study, protein intake averaged 3.6 ± 0.3 g/kg/day in the standard fortification arm and 4.5 ± 0.2 g/kg/day in the individualized fortification group during the first four weeks of life, with corresponding energy intakes of approximately 122 ± 8 kcal/kg/day and 141 ± 8 kcal/kg/day, respectively. Thus, even Rochow's standard fortification group exceeded the present cohort's protein intake by approximately 0.7–0.8 g/kg/day and energy intake by around 20 kcal/kg/day.

Several methodological differences likely account for this contrast. Rochow's protocol incorporated real-time measurement of breast milk macronutrient content using mid-infrared spectroscopy, with systematic titration of fortifier dosing to achieve predefined nutrient targets for each infant. Such individualized milk analysis is not routinely available in most neonatal units, including the present setting, where fortification follows a standard protocol without adjustment based on measured milk composition. Additionally, Rochow's cohort was exclusively human milk-based, demonstrating what is achievable when fortification is optimized within a breast milk framework, rather than comparing human milk and formula feeding strategies.

Notably, Rochow's cohort included infants born at or before 32 weeks' GA, who would typically be expected to have greater feeding intolerance. The achievement of higher protein and energy intakes in that population suggests that fortification strategy, particularly individualized nutrient titration, can substantially influence intake adequacy independent of gestational maturity. The within-study difference of 0.9

g/kg/day in protein intake between Rochow's standard and individualized groups further illustrates the magnitude of improvement achievable through systematic optimization.

Taken together, this comparison indicates that protein intakes of 3.6–4.5 g/kg/day and energy intakes of 122–141 kcal/kg/day are attainable during the first postnatal weeks when fortification is actively individualized. In contrast, the present cohort's intakes likely reflect outcomes achievable under standard fortification protocols without routine milk analysis.

Fu et al. (2026) analyzed macronutrient content in breast milk samples from 366 very preterm infants (<1500 g birthweight and <33 weeks' GA) in a US multicenter cohort and modeled the proportion of feeds that would achieve specific protein targets under standard versus enhanced fortification strategies at feeding volumes of 150–180 mL/kg/day. Under standard fortification (22 kcal/oz), 75% of samples achieved protein delivery of at least 3.5 g/kg/day (the ESPGHAN minimum), but only 11% achieved 4.0 g/kg/day and none reached 4.5 g/kg/day. In contrast, enhanced fortification (targeting 26 kcal/oz) enabled 100% of samples to achieve ≥ 3.5 g/kg/day and ≥ 4.0 g/kg/day, with 56% reaching 4.5 g/kg/day.

These findings are relevant when interpreting the present cohort's protein delivery during Weeks 1–3. The mean protein intake of 2.78–2.87 g/kg/day falls below the ESPGHAN minimum of 3.5 g/kg/day and below the range modeled as achievable under standard fortification at full feeding volumes in Fu's analysis. This suggests that protein delivery in the present cohort likely reflects both the use of standard fortification protocols and the inclusion of early postnatal days during which feeding volumes were still being established and fortification may not yet have been fully implemented.

However, important differences limit direct comparison. Fu's study modeled modelled theoretical protein delivery based on measured milk composition at established feeding volumes of 150–180 mL/kg/day, whereas the present study measured actual daily nutrient intake across variable feeding volumes, including periods of advancement. Furthermore, Fu's cohort was exclusively human milk-fed,

and the analysis evaluated fortification strategy rather than breast milk versus formula feeding.

Taken together with Rochow et al. (2021), these data indicate that protein intakes of 3.5–4.5 g/kg/day and energy intakes of 120–140 kcal/kg/day are achievable during early postnatal weeks when fortification is systematically optimized. The lower intakes observed in the present cohort are therefore more consistent with standard fortification practice and early feeding progression rather than inherent limitations of breast milk itself.

5.1.4 Energy, Protein, and Protein-Energy Ratio During Week 4-6 intake

By Weeks 4–6, the nutritional intake trajectory appeared to diverge between feeding groups, although interpretation is limited by substantial attrition in the PFM cohort. During this period, the PBM group (n = 20 remaining) achieved a mean energy intake of 119.90 kcal/kg/day and protein intake of 3.39 g/kg/day, whereas the PFM group (n = 11 remaining) achieved 108.06 kcal/kg/day and 3.05 g/kg/day, respectively (Table 4.5a). Although neither difference reached statistical significance (p = 0.09 for energy; p = 0.17 for protein), the PBM group met the ESPGHAN minimum energy target of 115 kcal/kg/day, while the PFM group remained below this threshold (Table 4.5b).

However, these findings must be interpreted in the context of discharge patterns. Median length of stay was significantly shorter in the PFM group (21 days) compared with the PBM group (30 days; p = 0.01), meaning that by Weeks 4–6, most PFM infants had already been discharged. The remaining 11 PFM infants therefore represent a selected subgroup requiring prolonged hospitalization, potentially due to feeding difficulties, slower growth, or ongoing medical concerns. As such, the lower intakes observed in the PFM group during this period likely reflect selection effects rather than intrinsic differences related to formula feeding.

In contrast, the PBM group's improved energy delivery during Weeks 4–6 is consistent with full establishment of feeding volumes and sustained fortification. By this stage, fortification had been implemented for several weeks and volumes were likely stabilized at 150–180 mL/kg/day. Despite improvement, protein intake remained

marginally below the ESPGHAN minimum of 3.5 g/kg/day, suggesting that standard fortification may not fully meet protein targets even at established feeding volumes. This observation aligns with findings from Fu et al (2026), where standard fortification frequently fell short of higher protein thresholds despite adequate volumes.

Cumulative deficits by Week 6 were numerically larger in the PFM group, particularly for energy; however, given the small sample size and potential selection bias, these differences should be interpreted cautiously. Importantly, the protein–energy ratio remained below the ESPGHAN minimum in both groups during Weeks 4–6 (2.73 g/100 kcal in PBM vs 2.87 g/100 kcal in PFM; $p = 0.52$), indicating that proportional protein delivery lagged behind energy provision even as absolute intakes improved. This pattern suggests that standard fortification strategies, whether through breast milk fortifier addition or provision of standard preterm formula, may not fully achieve the protein-to-energy balance recommended for optimal lean tissue accretion (Embleton et al. 2023). Overall, the Weeks 4–6 findings indicate progressive improvement in intake among PBM infants as fortification became fully established, while differences between groups are substantially confounded by discharge timing and cohort attrition.

5.2 GROWTH AT HOSPITAL DISCHARGE AND FOLLOW UP

5.2.1 Lack of Independent Predictors of Δ WAZ at Discharge

In univariable linear regression analyses (Table 4.13, $N = 66$), change in weight z-score (Δ WAZ) from birth to discharge was not associated with feeding group ($p = 0.91$), Week 1–3 protein intake ($p = 0.91$), Week 1–3 energy intake ($p = 0.70$), or the Week 1–3 protein-to-energy ratio ($p = 0.84$). In the adjusted multivariable model (Table 4.14), no predictor reached statistical significance, including feeding group ($\beta = 0.07$, $p = 0.60$), protein intake ($\beta = -0.01$, $p = 0.92$), GA at birth ($\beta = 0.04$, $p = 0.21$), sex ($\beta = 0.09$, $p = 0.49$), and BWZ ($\beta = 0.01$, $p = 0.90$). Sensitivity models replacing protein with energy intake or protein-to-energy ratio showed similarly null results. The absence of independent predictors meeting the statistical threshold in the NICU model requires careful interpretation.

The null findings in the NICU phase are most likely explained by a combination of methodological and biological factors. First, limited variation in nutritional exposure between groups may have reduced the ability to detect intake-growth associations. As discussed in Section 5.1.3, both groups were managed under the same standardised NICU protocol and achieved closely similar mean energy and protein intakes during Weeks 1–3. Where true differences in nutritional delivery between groups are small, the modest sample size limits the ability to detect corresponding differences in ΔWAZ .

Second, nutrient intake was summarised as a mean across the first three weeks of life. This approach was necessary because daily intake varied substantially during enteral advancement, feeding interruptions, and parenteral nutrition tapering, particularly among infants with differing clinical stability. Averaging intake across this period provided a more stable estimate of overall early nutritional exposure rather than reflecting short-term fluctuations related to acute illness. However, early postnatal nutrition may exert time-dependent effects. Evidence from controlled trials indicates that protein intake during the first seven to ten days of life may have a particularly strong influence on early somatic growth. van de Lagemaat et al. (2024) reported that each 1 g/kg/day increase in protein intake during the first week of life was associated with a 0.34 higher weight z-score at term-equivalent age. Therefore, although the three-week average was appropriate for capturing overall nutritional exposure in this observational cohort, it may have diluted associations operating within a narrower biological window.

Thirdly, the early postnatal course in preterm infants is characterised by physiological weight loss, fluid redistribution, and illness-related growth suppression, all of which independently influence ΔWAZ in ways not captured by nutritional variables alone (McNelis et al. 2025). Okalany et al (2024), in a retrospective cohort of infants weighing less than 1500 g in a Ugandan referral unit, identified respiratory distress syndrome (adjusted relative risk 1.30, 95% CI 1.01–1.67), duration of bubble CPAP use (aRR 1.35, 95% CI 1.10–1.66), and prolonged hospitalisation (aRR 1.85, 95% CI 1.31–2.61) as independent predictors of postnatal growth failure at discharge after adjustment, while nutritional intake variables were not independently significant.

This is consistent with the present study, where clinical and biological heterogeneity within the cohort likely obscured any nutritional signal in the NICU phase models.

Additionally, the precision with which nutritional intake was measured may have contributed to the findings. In the present study, intake from breast milk was estimated from reference values rather than directly assayed from individual samples, and direct breastfeeding volumes were not quantifiable. Belfort et al. (2025), in an observational study of very preterm infants across 12 middle-income countries including Malaysia, similarly noted that diet classification at discharge could not differentiate maternal milk from donor milk, nor disaggregate mixed diets into volume proportions, limiting dose-response analysis. In their cohort, infants on formula only had only a marginally less negative ΔWAZ than those on mixed diets (adjusted difference 0.15Z Z scores, 95% CI 0.10 to 0.20) despite the different sample size, indicating that feeding type is a relatively modest determinant of in-hospital weight trajectory compared with GA, birth size, and morbidity.

The finding that postnatal growth failure (PGF) was prevalent and comparable between groups at discharge (47.1% PBM vs 45.2% PFM, $p = 0.87$) is consistent with this pattern. Fenton et al. (2020) argued that the high prevalence of $\Delta WAZ \leq -1.28$ observed in preterm cohorts partly reflects the inevitable physiological weight loss in the early postnatal period and the mathematical properties of Z score change from a non-zero starting position, rather than exclusively representing nutritional inadequacy. Fenton et al. (2024) further proposed that growth trajectories in preterm infants should be evaluated in the context of parallel progression relative to reference curves rather than by threshold-based definitions alone, and that a range of growth patterns may be appropriate depending on individual clinical context. Accordingly, the similar PGF rates in both feeding groups in the present study are may therefore more consistent with a shared pattern of physiological z-score decline in a moderately preterm cohort than with a feeding-related difference in growth adequacy.

A formal consideration of statistical power is necessary when interpreting null findings in observational studies of this size. With a final sample of 76 infants, the study was designed to detect large between-group differences in macronutrient delivery.

However, the within-group variability in actual intake, the narrow absolute difference between groups in achieved nutrient delivery (mean energy difference 0.57 kcal/kg/day and mean protein difference 0.09 g/kg/day during Weeks 1 to 3), and the multiple clinical covariates competing for explanatory variance in a modest sample all substantially reduce the power to detect a nutrient-growth association at the level of individual regression coefficients. The study was not powered to detect the small to moderate effect sizes that characterise nutrient-growth associations in observational cohorts, which have been estimated at $r = 0.20$ to $r = 0.35$ in comparable settings (Abdul Hamid et al. 2021; Collins et al. 2010). Null findings in this context therefore represent absence of statistical evidence rather than definitive evidence of the absence of a true nutritional effect and should not be interpreted as confirming that protein delivery has no influence on growth in this population.

The null findings are nonetheless clinically informative and consistent with a body of observational evidence suggesting that under standardised NICU feeding protocols, differences in achieved nutrient delivery between breast milk-fed and formula-fed infants are substantially attenuated by fortification (Arslanoglu et al. 2019; Embleton et al. 2023). The present data suggest that when standard fortification is applied consistently, the two feeding substrates produce comparable macronutrient delivery during early admission, and that this broadly similar nutrient delivery was accompanied by comparable discharge growth. This is directly relevant to clinical practice in Malaysian NICUs where the choice between PBM and PFM is not freely made but is determined by maternal milk supply, clinical instability, and fortifier availability (Abdul Hamid et al. 2021; Cheah et al. 2021): the findings suggest that neither substrate confers a clear growth advantage under current standardised care conditions when nutrient delivery is broadly equivalent. What the data indicate clearly is that both groups achieved only approximately 79% to 82% of the ESPGHAN minimum protein target during the first three weeks of life. This shared protein shortfall appears more closely related to transition-phase feeding constraints and delayed achievement of fortified/full enteral intake than to feeding substrate alone (Cormack et al. 2016; Embleton et al. 2023). Therefore, the null association between nutrient intake and ΔWAZ in the present study should be interpreted cautiously. It suggests that improving the adequacy of early protein delivery, rather than focusing only on whether

infants received PBM or PFM, may be the more relevant target for future quality improvement initiatives at this centre.

Similarly, rates of underweight, stunting, and low head circumference at 1, 3, and 6 months CA did not differ significantly between PBM and PFM groups at any time point. This pattern is consistent with the interpretation that baseline biological characteristics and early clinical course exerted a greater influence on subsequent nutritional status than milk type alone within the context of standardised NICU feeding practices (Belfort et al. 2025; McNelis et al. 2025). The significant negative associations between birthweight z-score, GA, and post-discharge WAZ trajectory (Table 4.15) further support this interpretation: factors determined before and at birth, rather than NICU feeding type, were the strongest predictors of the post-discharge growth pattern (Fenton et al. 2024; McNelis et al. 2025). Taken together, the null findings across discharge and post-discharge growth analyses reinforce the importance of individualised post-discharge nutritional follow-up for infants at the extremes of birth size and gestational maturity, irrespective of their in-hospital feeding substrate, and highlight the need for future adequately powered studies to evaluate the effect of optimised protein delivery on growth outcomes in this population.

5.2.2 Birth Weight Z-Score and Gestational Age as Predictors of Post-Discharge WAZ Trajectory

In the linear mixed model for WAZ from 1 to 6 months CA (Table 4.15), BWZ was negatively associated with WAZ across the post-discharge follow-up period (estimate = -0.81 , 95% CI -1.21 to -0.40 , $p < 0.001$). GA at birth was similarly negatively associated with WAZ (estimate = -0.33 , 95% CI -0.47 to -0.18 , $p < 0.001$). The association with BWZ remained in sensitivity models replacing protein intake with energy intake ($p = 0.003$) and protein-to-energy ratio ($p = 0.001$). These findings indicate that, after adjustment for feeding group and nutrient intake, infants with higher BWZ and greater gestational maturity had lower WAZ across follow-up. Because this direction is counterintuitive, it requires cautious interpretation.

One possible explanation is regression toward the mean on the z-score scale. Infants who begin at a higher BWZ may show a greater apparent decline as their later measurements move closer to the reference population mean, whereas infants who begin lower may show less downward movement or relative catch-up. This does not necessarily mean that infants with higher BWZ or greater gestational maturity had poorer nutritional status. Rather, the negative coefficients may partly reflect the effect of starting position, the transition between preterm and post-discharge growth references, and adjustment for correlated perinatal variables.

This interpretation is consistent with recent work cautioning that z-score-based growth metrics are sensitive to the infant's starting position and may misclassify growth patterns when used without clinical context (McNelis et al. 2025; Fenton et al. 2024). Fenton et al. (2024) further emphasised that preterm growth assessment should consider trajectory over time rather than relying only on threshold-based z-score cut-offs. Therefore, the negative BWZ coefficient in the present model should not be interpreted as evidence that infants with higher BWZ were clinically growing poorly, nor should it be reversed to imply that infants with lower BWZ were clearly the highest-risk group.

The negative association between GA and post-discharge WAZ should be interpreted with the same caution. Greater gestational maturity may be associated with differences in birth size, clinical stability, length of stay, and timing of transition to outpatient growth references. Therefore, the GA coefficient may reflect both biological maturity and model-related effects rather than a simple causal association between higher GA and poorer post-discharge growth.

Comparison with regional follow-up studies provides useful context. Rohsiswatmo et al. (2024) reported early post-discharge WAZ decline followed by later recovery in an Indonesian preterm cohort, while Lan et al. (2022) reported that mean WAZ improved from birth to 3–6 years in a Shanghai cohort. However, Lan et al. (2022) also found that early growth restriction in head circumference and height was associated with later adverse physical growth outcomes. These findings suggest that early post-discharge decline may not represent permanent growth restriction, but it

remains clinically relevant because some early growth deficits may persist or relate to later outcomes.

Overall, BWZ and GA were associated with post-discharge WAZ trajectory in the adjusted model, but the negative direction means they should not be interpreted as straightforward risk markers in isolation.

5.2.3 Longitudinal Decline in WAZ from 1 to 6 Months Corrected Age

WAZ declined progressively across the post-discharge follow-up period in both feeding groups. In the LMM (Table 4.15), WAZ at 3 months CA was significantly lower than at 1 month CA (estimate = -0.49 , 95% CI -0.79 to -0.20 , $p = 0.001$), and WAZ at 6 months CA was lower still (estimate = -0.79 , 95% CI -1.21 to -0.38 , $p < 0.001$). Descriptively, mean WAZ was -0.38 in the PBM group and -0.63 in the PFM group at 3 months CA, declining to -0.55 and -0.71 , respectively, at 6 months CA. Growth velocities were comparable between groups at both 1–3 months CA (0.75 vs. 0.68 kg/month, $p = 0.41$) and 3–6 months CA (0.44 vs. 0.48 kg/month, $p = 0.46$), suggesting that the decline was broadly parallel across feeding groups, with no evidence of a differential post-discharge growth trajectory by NICU feeding type.

The continuing decline in WAZ after discharge may reflect several interrelated factors. The transition from NICU to home feeding represents a period of nutritional vulnerability. During NICU admission, feeds are prescribed and advanced under clinical supervision, with fortification used where indicated. After discharge, infants may transition to unfortified breast milk, standard formula, mixed feeding, or other feeding patterns, with nutrient density and intake volume less tightly controlled than during admission. This transition occurs during a period when catch-up growth may be expected, potentially widening the gap between nutritional requirements and intake achieved after discharge (Japakasetr et al. 2016; McNelis et al. 2025).

The post-discharge period may also involve changes in tissue accretion that are not fully reflected by WAZ alone. Ramel et al. (2011) reported that preterm infants at term CA had significantly lower fat-free mass than term-born peers, but this difference was no longer statistically evident by 3–4 months CA, suggesting early lean mass catch-

up. The same study also showed that illness burden remained associated with fat-free mass beyond the effect of inpatient nutrition. This supports the interpretation that post-discharge growth is influenced by both nutrition and biological recovery from neonatal morbidity.

Regional studies provide useful context for the present findings. Japakasetr et al. (2016), in a Thai NICU setting, reported that infants receiving conventional post-discharge nutrition showed persistent growth deficits, whereas structured post-discharge nutritional support was associated with improved growth outcomes. Rohsiswatmo et al. (2024) reported that WAZ in an Indonesian preterm cohort declined to a nadir at approximately 3 months CA before recovering gradually. In contrast, the present cohort continued to show WAZ decline up to 6 months CA. This suggests that catch-up growth had not yet become apparent within the observation window of the present study. Because follow-up ended at 6 months CA, it cannot be determined whether recovery would have occurred later in infancy. The continuing decline should therefore be interpreted as evidence of persistent early post-discharge growth vulnerability, rather than as proof of irreversible growth failure.

Lan et al. (2022) similarly showed that early growth patterns may have longer-term relevance. In their Shanghai cohort, mean weight z-scores improved by 3–6 years of age, but early head circumference and height growth restriction were associated with adverse physical outcomes at school age. This supports the clinical importance of monitoring growth beyond discharge, particularly during the first 6 months CA.

Although the present study did not quantify post-discharge nutrient intake, unmeasured variability in post-discharge feeding may have contributed to the observed WAZ decline. By 1, 3, and 6 months CA, many infants had transitioned away from their NICU feeding classification. Maternal lactation continuity, formula type, use of post-discharge formula, feeding volume, timing of complementary feeding, and access to dietetic support were not measured in sufficient detail to calculate post-discharge nutrient intake. Therefore, the WAZ decline cannot be attributed directly to specific post-discharge feeding practices. It should instead be interpreted as a pattern of post-discharge growth vulnerability requiring closer nutritional surveillance.

Given the progressive WAZ decline and the absence of sustained catch-up growth within the 6-month observation window, these findings support consideration of structured post-discharge nutritional follow-up. This may include assessment of whether enriched formula, continued fortification, or intensified dietetic monitoring is appropriate for selected infants. Regular growth monitoring should also be incorporated into the post-NICU care pathway to identify infants with persistent growth faltering and guide timely nutritional review.

5.2.4 Feeding Group Effect in the Linear Mixed Model

In the LMM for WAZ from 1 to 6 months CA (Table 4.15), PFM infants had higher WAZ compared with PBM infants after adjustment for GA, BWZ, age, and Week 4–6 protein intake (estimate = 1.00, 95% CI 0.15 to 1.85, $p = 0.03$). This contrasts with the null feeding group effect at discharge in the regression model (Table 4.14; $\beta = 0.07$, $p = 0.60$) and requires careful interpretation.

Several explanations are possible for this apparent post-discharge difference between feeding groups. First, it may reflect residual confounding by baseline clinical severity. In this observational cohort, the feeding groups differed in gestational age, clinical morbidity, and length of stay. Although the LMM adjusted for GA and BWZ, residual confounding by unmeasured or incompletely captured variables, such as cumulative nutrient deficit, duration of parenteral nutrition, illness severity, feeding tolerance, infection burden, and discharge readiness, cannot be excluded. Second, sociodemographic factors not available in this study may also have influenced post-discharge growth. Maternal education, household resources, access to post-discharge nutrition products, and follow-up support may differ between families and may influence growth independently of NICU feeding type.

The feeding group variable in this model reflects predominant NICU milk exposure, not the infant's actual feeding at the time WAZ was measured after discharge. By 1, 3, and 6 months CA, infants may have transitioned to different feeding regimens, including exclusive breast milk, exclusive formula, mixed feeding, or other feeding patterns. Because post-discharge nutrient intake was not quantified, the feeding group coefficient should not be interpreted as a direct effect of ongoing formula or breast milk

exposure after discharge. Rather, it may represent a composite of NICU feeding classification, baseline clinical differences, and unmeasured post-discharge factors.

The negative association of GA with WAZ in the same model also supports cautious interpretation. More mature GA was associated with lower WAZ across follow-up after adjustment, which is counterintuitive and may reflect regression toward the mean, reference-chart transition, residual confounding, or model instability. This is important because the PBM group had lower median GA at birth than the PFM group. Partial adjustment for GA may not fully capture differences in baseline growth position and clinical course across a wide GA range.

The feeding group coefficient was also not the main focus of the sensitivity analyses. The sensitivity models replaced the nutrient intake variable, but the robustness of the feeding group effect across alternative covariate structures was not fully tested. Therefore, the statistically higher WAZ observed among PFM infants in the LMM should be interpreted as an adjusted association rather than evidence of nutritional superiority of formula feeding.

These findings are consistent with the broader interpretation that post-discharge growth is shaped by multiple biological, clinical, and social factors rather than NICU feeding type alone. Lan et al. (2022) showed that early growth restriction can have longer-term physical growth implications, but also that later growth trajectories are influenced by multiple factors beyond early feeding exposure. In the present study, the available data do not support a causal claim that PFM improves post-discharge WAZ compared with PBM.

Therefore, given the observational design, baseline group differences, unmeasured post-discharge intake, and possible residual confounding, the higher post-discharge WAZ observed in PFM infants should not be interpreted as evidence of formula superiority over fortified breast milk. Future studies with individual-level post-discharge dietary assessment, sociodemographic data, and larger sample sizes would be needed to determine whether milk type has an independent effect on post-discharge WAZ trajectory in this population.

5.3 BODY COMPOSITION AND BONE MINERALIZATION AT 6–9 MONTHS CORRECTED AGE

DXA-derived bone and body composition data were available for 12 preterm infants scanned at a mean corrected age of 6.83 ± 1.03 months (range 6–9 months CA) (Table 4.10). Of these 12 infants, 6 had received predominantly breast milk and 6 had received predominantly formula during their NICU stay. The PBM infants scanned were younger at assessment (mean 6.33 ± 0.52 months) compared with PFM infants (mean 7.33 ± 1.21 months), though this one-month difference in scan timing was not statistically tested given the small sample size. Mean body weight at scan was 7.50 ± 0.84 kg (range 6.7–9.4 kg) and mean length was 67.19 ± 2.73 cm (range 61.2–71.0 cm).

5.3.1 Cohort Characteristics and Measurement Methods

Mean bone mineral content (BMC) was 169.23 ± 29.15 g, with bone mineral density (BMD) of 0.25 ± 0.02 g/cm² and bone area of 675.51 ± 75.69 cm² (Table 4.10). Body composition analysis showed mean lean body mass of 4887.08 ± 785.96 g, fat mass of 3169.33 ± 668.00 g, and percentage body fat of $39.36 \pm 6.68\%$. The observed range for percentage body fat (30.5–53.7%) was wide, reflecting substantial inter-individual variation in body composition at this age. Descriptively, PBM infants scanned had numerically lower BMC (157.60 ± 17.61 vs 180.86 ± 35.14 g) and BMD (0.24 ± 0.01 vs 0.26 ± 0.03 g/cm²) compared with PFM infants and also had lower lean body mass (4539.92 ± 738.89 vs 5234.25 ± 723.82 g) but higher percentage body fat (41.75 ± 7.82 vs $36.97 \pm 4.84\%$). However, these numerical differences cannot be interpreted as statistically significant or clinically meaningful given the sample size of only 6 infants per group, the lack of adjustment for body size or scan age, and the overlapping standard deviations.

In the absence of a concurrent term-born control group scanned under identical conditions, the primary value of these data lies in comparison with published reference cohorts to contextualize whether the observed values fall within expected ranges for preterm infants at this age. Three published studies provide reference data: Rawlings et al. (1999), Quintal et al. (2014), and Rohana et al. (2007), though each differs substantially from the present cohort in study era, geographic setting, GA distribution,

feeding practices, scan timing, and DXA platform. These differences must be considered systematically as they are likely to account for observed numerical discrepancies.

Rawlings et al. (1999) enrolled 17 preterm infants born at mean 28 weeks GA (range 24–33 weeks) in the United Kingdom during the 1990s and scanned them at exactly 6 months CA using a Hologic QDR 2000 with pencil-beam technology and infant whole-body software version 5.56. All infants were formula-fed post-discharge. Mean body weight at scan was approximately 6.98 kg. The authors explicitly acknowledged that their older DXA system, which uses pencil-beam technology, may underestimate bone mineral content in small and poorly mineralized infants due to limitations in point-by-point scanning and greater susceptibility to movement artifacts (Rawlings et al. 1999). The study measured bone mineral content (BMC, total grams of mineral in the skeleton), bone area (cm²), and percentage body fat but did not report bone mineral density (BMD, mineral per unit area).

Quintal et al. (2014) enrolled 20 preterm infants born at mean 31.1 ± 2.3 weeks gestation in Brazil between 2006 and 2008 and scanned them at exactly 6 months corrected age using Hologic Discovery A with fan-beam technology and software version 12.3.3, a newer platform providing greater measurement precision than the Rawlings system. All infants were exclusively breast milk-fed throughout admission and post-discharge to 6 months CA. Only 4 of the 7 very low birth weight infants in this cohort received breast milk fortifier during admission, meaning that the majority received unfortified breast milk throughout (Quintal et al. 2014). Mean body weight at scan was 7.29 ± 0.80 kg. They measured BMC (137.14 ± 22.46 g), BMD (0.22 ± 0.02 g/cm²), bone area, lean body mass (5750.13 ± 765.11 g), and percentage body fat, and compared their preterm cohort with a concurrent term-born reference group scanned at the same CA.

Rohana et al. (2007) enrolled 41 VLBW infants (median 31 weeks GA) in Malaysia and scanned them at a mean CA of 37.5 ± 0.6 weeks a near-term timepoint, not 6 months CA. Scans were performed using a Norland pencil-beam absorptiometer, a different manufacturer's platform entirely from the Hologic systems used in the other

studies. Bone mineral content was expressed as grams per kilogram body weight rather than absolute grams, and body composition was not reported. Given the substantially different scan timing (term-equivalent age vs 6 months), different DXA platform (Norland vs Hologic), and different units of measurement (g/kg vs absolute grams), direct numerical comparison between Rohana and colleagues and the present study is not appropriate. However, their finding that infants with complicated neonatal courses defined by prolonged ventilation, sepsis, or NEC had significantly lower BMC than uncomplicated peers (18.2 ± 3.0 vs 29.6 ± 12.2 g/kg, $p=0.05$) is contextually relevant, as the present cohort included infants with high rates of respiratory distress syndrome (85.3% in PBM, 61.9% in PFM) and resuscitation requirements (58.8% in PBM, 35.7% in PFM) (Rohana et al. 2007).

The present study used a Hologic Discovery DXA scanner with fan-beam technology (comparable to Quintal et al. 2014) and scanned infants at 6–9 months CA with a mean of 6.83 ± 1.03 months. All infants were immobilized using a Baby Wrap vacuum cushion during scanning to minimize movement artifacts. Mean body weight at scan was 7.50 ± 0.84 kg heavier than both the Rawlings cohort (6.98 kg) and marginally heavier than the Quintal cohort (7.29 kg).

5.3.2 Bone Mineral Content and Bone Mineral Density: Apparent Differences and Their Explanations

The most straightforward explanation for higher BMC and bone area is larger body size. Rawlings et al. (1999) demonstrated in their cohort that BMC is proportionally related to bone area ($r^2=0.98$, $p<0.0001$) and is significantly influenced by concurrent body weight, such that larger infants are expected to have proportionally greater absolute BMC. Infants in the present study had a mean weight of 7.50 ± 0.84 kg at the time of scanning, compared with approximately 6.98 kg in Rawlings and 7.29 ± 0.80 kg in Quintal. More importantly, the scan window in the present study spanned 6–9 months CA rather than the fixed 6-month timepoint used in both reference studies. Of the 12 infants scanned in the present cohort, 6 were scanned at 6 months, 3 at 7 months, 2 at 8 months, and 1 at 9 months. Infants scanned at older ages were correspondingly heavier and had greater skeletal size, which alone would produce higher absolute bone measures. The wide within-cohort range of BMC (144.05–239.32 g) and bone area

(591.7–820.6 cm²) reflects this heterogeneity in scan timing, with infants scanned at 9 months having substantially higher values than those scanned at 6 months. Therefore, the higher mean BMC and bone area in the present cohort relative to the reference cohorts likely reflects a combination of slightly greater mean body weight and the inclusion of older infants scanned at 7–9 months CA.

The substantial difference in bone area between the present study (675.51 cm²) and Rawlings (486.5 cm²) a 189 cm² or 39% discrepancy requires closer examination, as body size differences alone cannot fully account for such a large gap. Rawlings et al. (1999) used Hologic QDR 2000 with pencil-beam technology, an older system that scans in a point-by-point manner with a narrow X-ray beam. This technology is slower, more susceptible to movement artifacts, and less precise in defining bone edges compared with fan-beam technology. The authors explicitly acknowledged that their system may underestimate bone mineral content in small and poorly mineralized infants, and pencil-beam systems are known to produce systematically lower bone area measurements compared with fan-beam systems in the same subjects due to differences in edge detection algorithms (Rawlings et al. 1999).

In contrast, both Quintal et al. (2014) and the present study used Hologic Discovery systems with fan-beam technology, which scan in a sweeping motion with a wider X-ray field, providing faster scan times and greater measurement precision, particularly in small subjects. Fan-beam systems use more sophisticated edge detection algorithms that define bone boundaries more accurately, typically resulting in larger measured bone area compared with pencil-beam systems for the same skeletal size. Therefore, the 189 cm² difference in bone area between the present study and Rawlings is most plausibly attributable to the combination of: (1) pencil-beam versus fan-beam technology, (2) older software with less precise edge detection in the Rawlings system, and (3) slightly larger body size and older scan age in the present cohort. The fact that the present cohort's bone area (675.51 cm²) is not directly compared with Quintal's bone area (not reported in their publication) prevents assessment of whether fan-beam platforms alone account for the discrepancy or whether body size differences also contribute.

Rawlings et al. (1999) was conducted in the 1990s in the United Kingdom, a period during which nutritional targets and fortification practices were less aggressive than current ESPGHAN 2022 standards. All infants in that cohort were formula-fed post-discharge, but the protein and mineral content of 1990s-era formulas (typically 1.8–2.0 g protein per 100 mL and 40–50 mg calcium per 100 mL) was lower than contemporary preterm formulas (typically 2.0–2.2 g protein per 100 mL and 70–90 mg calcium per 100 mL) (Embleton et al. 2023). Quintal et al. (2014) enrolled infants between 2006 and 2008 in Brazil, during which only 4 of 7 very low birth weight infants received breast milk fortifier during admission, meaning that the majority received unfortified breast milk (approximately 1.0 g protein per 100 mL and 30 mg calcium per 100 mL) throughout the NICU period.

5.3.3 The Role of Nutrition in Bone Mineralization

The present cohort differed substantially in nutritional practices: all 6 PBM infants among the 12 scanned received breast milk fortifier during admission (initiated at median day 9), and all 6 PFM infants received preterm formula (typically 2.0 g protein per 100 mL and 80–90 mg calcium per 100 mL), both of which provide higher mineral content than unfortified breast milk. More systematic mineral delivery during the NICU period, consistent with contemporary ESPGHAN guidelines (Embleton et al. 2023) may have contributed in part to greater bone mineral accrual by 6–9 months CA. However, we observed in Section 5.1.3 that both PBM and PFM groups in the present cohort fell substantially short of ESPGHAN energy and protein targets during weeks 1–3 (achieving only 89% of minimum energy and 79–82% of minimum protein targets), which would be expected to compromise mineral delivery during that critical period.

Mean bone mineral density in the present cohort (0.25 ± 0.02 g/cm²) was numerically higher than the Quintal value of 0.22 ± 0.02 g/cm², though this 0.03 g/cm² difference represents only a 14% increase and falls within the range of inter-individual variation (Table 4.11). Rawlings et al. (1999) did not report BMD, preventing comparison. BMD is calculated as BMC divided by bone area (g/cm²), meaning that it is less sensitive to absolute body size than BMC alone, though still influenced by skeletal dimensions. The finding that BMD in the present cohort was comparable to or

slightly higher than Quintal's cohort despite both using similar fan-beam Hologic systems suggests that the present infants had adequate bone mineralization relative to skeletal size at 6–9 months CA.

Importantly, none of the 12 infants in the present cohort had BMD values below 0.22 g/cm^2 (range $0.22\text{--}0.31 \text{ g/cm}^2$, Table 4.10), and all values fell within or above the reference range reported by Quintal et al. (2014). Metabolic bone disease of prematurity is typically diagnosed when BMC or BMD falls more than 2 standard deviations below age- and sex-matched reference means, often accompanied by radiographic evidence of osteopenia, elevated alkaline phosphatase ($>900 \text{ IU/L}$), or fractures (Quintal et al. 2014; Embleton et al. 2023). None of the 12 infants in the present cohort met these diagnostic criteria, and there were no clinical reports of fractures or radiographic osteopenia during the follow-up period. Therefore, while the small sample size precludes definitive conclusions, the available data do not suggest that metabolic bone disease of prematurity was present in this cohort at 6–9 months CA.

Quintal et al. (2014) demonstrated that preterm infants, despite having significantly lower BMC and BMD than term-born peers at 40 weeks post-conceptual age ($p<0.001$ for both), achieved comparable values by 6 months CA (BMC $p=0.054$, BMD $p=0.62$), indicating the capacity for accelerated catch-up mineralization during the first 6 months of life. That the present cohort's BMD values ($0.25 \pm 0.02 \text{ g/cm}^2$) met or exceeded the Quintal preterm reference values ($0.22 \pm 0.02 \text{ g/cm}^2$) at a similar timepoint is consistent with this pattern of catch-up mineralization, though this observation must be interpreted with caution given the small sample size and the methodological differences between studies.

5.3.4 Lower lean mass and higher fat mass compared with reference cohorts

Mean lean body mass in the present cohort ($4887.08 \pm 785.96 \text{ g}$) was substantially lower than the $5750.13 \pm 765.11 \text{ g}$ reported by Quintal et al. (2014) at 6 months CA a difference of 863 g representing a 15% reduction in lean mass (Table 4.11). This difference is noticeable given that the present cohort had similar or slightly greater mean body weight (7.50 kg vs 7.29 kg). The present cohort's lean mass was also numerically lower than the $5034 \pm 482 \text{ g}$ reported by Rawlings et al. (1999), though again observed

in the context of greater mean body weight in the present cohort (7.50 kg vs 6.98 kg). The lower lean mass despite similar or greater total body weight means that the present cohort had a higher proportion of body mass composed of fat tissue rather than muscle, organ, or skeletal mass.

Percentage body fat in the present cohort ($39.36 \pm 6.68\%$) was considerably higher than the approximately $27.5 \pm 6.0\%$ reported by Rawlings et al. (1999), representing an 11.9 percentage point increase (Table 4.11). Neither Quintal et al. (2014) nor Rohana et al. (2007) reported percentage body fat, preventing further comparison. As a contextual comparison, de Fluiter et al. (2020) reported that healthy term-born infants at 6 months had mean fat mass percentage of 24.1% when measured by air displacement plethysmography and 25.0% when measured by DXA with a vacuum cushion. These values were lower than the mean fat mass percentage of 39.36% observed in the present preterm cohort at 6–9 months corrected age.

Before interpreting this difference as a true adipose-predominant body composition profile in the present cohort, DXA measurement methodology must be considered. De Fluiter et al. (2020) showed that DXA performed without a vacuum cushion overestimated fat mass percentage in 6-month-old infants. Mean fat mass percentage was 36.6% using DXA without a cushion, compared with 24.1% using air displacement plethysmography and 25.0% using DXA with a vacuum cushion. Thus, DXA without a cushion produced values 12.5 percentage points higher than air displacement plethysmography and 11.6 percentage points higher than DXA with a cushion.

In the present study, all 12 infants were scanned using a Baby Wrap vacuum cushion designed to reduce movement during DXA scanning. This method is consistent with the approach that de Fluiter et al. (2020) found to produce fat mass percentage values comparable to air displacement plethysmography. The mean fat mass percentage of 39.36% in the present cohort is therefore less likely to be explained solely by movement artefact. However, interpretation remains cautious because the present DXA sample was small, consisted of preterm infants rather than healthy term-born infants, and was assessed at 6–9 months CA rather than exactly 6 months.

The pattern of lower lean mass relative to body weight, combined with proportionally higher fat mass, is consistent with the adipose-predominant body composition pattern observed in preterm infants during post-discharge catch-up growth. Several mechanisms may account for this finding.

The present cohort's protein delivery during the NICU period, while broadly adequate by contemporary standards, was substantially below ESPGHAN targets during the first three postnatal weeks. During week 1, the PBM group received only 2.30 ± 0.80 g/kg/day protein 66% of the 3.5 g/kg/day minimum target (as documented in Section 5.1.3). Across weeks 1–3, protein intake averaged 2.78–2.87 g/kg/day in both groups. Early protein deficits during critical periods of lean tissue accrual may have lasting effects on body composition months later. Calor et al. (2021) demonstrated in 58 very and extremely preterm infants that protein intake during the first four postnatal weeks was an independent predictor of bone mineral density at 6 months CA ($\beta=0.05$, $p=0.04$), and that fat intake in the same period was also an independent predictor ($\beta=0.04$, $p=0.04$), suggesting that early macronutrient delivery has lasting effects on body composition measurable months after discharge. Whether the early protein shortfalls documented in the present cohort contributed to the lower lean mass observed at 6–9 months cannot be determined definitively, but the temporal sequence and the evidence from Calor and colleagues make this a plausible contributing factor.

Preterm infants have been documented to accumulate fat tissue preferentially relative to lean tissue during the first year of life, particularly during periods of rapid weight gain following early postnatal growth faltering. This pattern has been described as a compensatory response to early growth restriction, in which adipose tissue accrues more rapidly than lean tissue when nutrition becomes adequate, resulting in a body composition profile that differs from term-born peers despite achieving similar absolute weight (Embleton et al. 2023). The present cohort experienced substantial cumulative energy and protein deficits during weeks 1–3 (-187.26 to -147.65 kcal/kg energy, -9.86 to -8.06 g/kg protein), which would be expected to result in early growth restriction. Subsequent catch-up growth during weeks 4–6 and post-discharge would then favor fat accumulation, consistent with the observed 39.36% body fat at 6–9 CA.

Additionally, the protein-to-energy ratio during the early postnatal period may affect later body composition. The present cohort achieved a protein-energy ratio of 2.92–2.89 g/100 kcal during weeks 1–3 (as documented in Section 5.1.3), below the ESPGHAN-recommended minimum of 3.2 g/100 kcal (Embleton et al. 2023). A protein-energy ratio below 3.2 g/100 kcal may favor fat deposition over lean tissue accrual even when absolute energy intake is adequate. Whether the persistently low protein-energy ratio throughout weeks 1–6 in the present cohort contributed to the adipose-predominant body composition observed at 6–9 months is plausible but cannot be confirmed without serial body composition measurements during the NICU period and post-discharge.

Toftlund et al. (2025) followed 192 very preterm children to 6 years of age and found that those fed breast milk post-discharge had significantly higher bone mineral density than formula-fed peers after adjustment for body size and other covariates ($\beta = -0.02$, 95% CI -0.03 to -0.01 , $p = 0.001$). Notably, fortification of breast milk post-discharge did not further improve BMD compared with unfortified breast milk in that study, suggesting that non-nutritive components of breast milk such as growth factors, hormones, or immune-active molecules rather than added minerals alone, may mediate a lasting skeletal benefit (Toftlund et al. 2025). This finding is contextually relevant to the present cohort given that 6 of the 12 infants scanned received predominantly breast milk during their NICU admission. However, Toftlund et al assessed outcomes at 6 years of age, and whether the skeletal benefit of breast milk is already detectable at 6–9 months CA the timepoint assessed in the present study is unknown.

In summary, DXA assessment at 6–9 months CA in this small subsample of preterm infants did not demonstrate evidence of metabolic bone disease and showed bone mineral density values comparable to published preterm reference cohorts using similar technology. The higher absolute bone mineral content and bone area observed relative to older reference studies are most plausibly explained by differences in body size, scan timing, and DXA platform rather than superior mineralization alone. However, the pattern of relatively lower lean mass and higher fat mass suggests an adipose-predominant body composition at this age, which may reflect early protein deficits, suboptimal protein-to-energy ratio during critical postnatal weeks, and the

known tendency for preferential fat accretion during catch-up growth in preterm infants. Given the small sample size, lack of serial body composition data, and absence of standardized post-discharge nutritional tracking, these findings should be interpreted cautiously but highlight the importance of optimizing early protein delivery and monitoring body composition beyond discharge.



CHAPTER VI

CONCLUSION

This prospective observational cohort study compared PBM and PFM feeding during NICU admission and examined their associations with growth outcomes at discharge and up to 6 months CA. By integrating week-by-week quantification of achieved macronutrient intake with longitudinal anthropometric follow-up and, for a subsample, DXA-derived body composition and bone mineralisation assessment, the study addressed four specific objectives aligned with major evidence gaps in Malaysian and Southeast Asian neonatal nutrition research.

During weeks 1 to 3 of life, both feeding groups achieved similar mean energy and protein intakes. Despite differences in milk source, nutrient delivery did not significantly differ between groups, suggesting that standardised feeding protocols and fortification practices contributed to comparable macronutrient provision. However, both groups remained below recommended protein and energy targets during early admission, indicating that achieving optimal early nutritional adequacy remains challenging in routine NICU practice regardless of milk substrate.

Baseline clinical differences were evident between groups. The PBM group had lower GA, a higher proportion of very low birth weight infants and greater respiratory morbidity. These differences are biologically consistent with slower advancement to full enteral feeds and prolonged hospitalisation. Importantly, discharge postmenstrual age was similar between groups, suggesting that differences in length of stay were primarily explained by gestational maturity at birth rather than differential clinical recovery or discharge readiness.

Milk source alone did not independently determine early growth trajectory in this cohort. Changes in weight-for-age z-score at discharge were similar between PBM and PFM groups, and no nutritional or clinical predictor reached significance in the NICU model. In the post-discharge period, BWZ and GA at birth were consistently associated with WAZ trajectory, with both remaining significant across all model specifications. Although PFM infants had a significantly higher WAZ across follow-up, this difference is most plausibly attributable to residual confounding by baseline clinical immaturity and illness severity rather than a causal nutritional effect, as growth velocities were comparable between groups throughout. The similarity in achieved nutrient delivery between feeding groups, attributable to the standardised fortification protocol at HCTM, limited the ability to attribute growth outcomes to feeding type per se and these findings highlight the dominant influence of intrauterine growth trajectory and gestational maturity over feeding exposure in determining post-discharge weight outcomes.

Overall, these findings suggest that nutrient adequacy and baseline biological factors are more influential determinants of early growth than milk category under standardised NICU conditions. These findings support the distinction between milk category and nutrient dose, suggesting that quantitative macronutrient delivery may be more predictive of growth outcomes than qualitative milk source alone. Ensuring adequate protein and energy delivery in the first weeks of life remains a central clinical priority, and these findings emphasise that optimising early nutritional strategy in biologically vulnerable infants is essential not only for short-term weight gain but also for shaping longer-term growth trajectory and body composition.

This study makes several novel contributions to the existing literature. It represents the first prospective cohort study from a Malaysian tertiary NICU to simultaneously quantify week-by-week achieved macronutrient intake, longitudinal growth trajectories from NICU admission through 6 months CA, and DXA-derived body composition and bone mineralisation beyond term-equivalent age. Prior Malaysian publications in this field were either limited to in-hospital outcomes, used prescribed rather than achieved intake values, or reported body composition only at term-equivalent age. The multidimensional outcome framework employed in this study,

encompassing nutrient intake, anthropometric growth, body composition, and bone mineralisation within the same cohort, provides a more complete evaluation of early nutritional strategy than has previously been available in this setting.

The DXA data address an important local evidence gap, as no previously published Malaysian study has reported DXA-derived body composition and bone mineralisation outcomes in preterm infants at 6 to 9 months CA. The observed percentage body fat of 39.4% appeared higher than values reported in published term-born cohorts, suggesting a relatively adipose-predominant body composition profile in this small preterm cohort. This provides preliminary local data for understanding post-discharge growth quality among preterm infants discharged from the NICU in this population.

Bone mineral density values were comparable to published preterm cohorts assessed using similar DXA-based approaches, suggesting no marked DXA-based evidence of impaired bone mineralisation in this cohort at 6 to 9 months CA. However, this should be interpreted cautiously because DXA alone does not establish the presence or absence of metabolic bone disease, and the DXA subsample was small. Together, these findings provide preliminary local descriptive data to guide future adequately powered studies on preterm body composition, bone mineralisation, and post-discharge nutrition in the Malaysian context.

6.1 CLINICAL IMPLICATIONS

The findings of this study carry implications at multiple levels, including the individual infant, the family, the healthcare system, and national planning. Presenting the conclusions at these four levels allows a more comprehensive synthesis of the study's significance and practical impact.

At the level of the individual preterm infant, one of the most clinically important findings is that BWZ and GA were consistently associated with post-discharge WAZ trajectory across model specifications. However, the negative direction of association in the adjusted model means that these variables should be interpreted cautiously. The finding may reflect regression toward the mean, transition between growth references,

residual confounding, or model instability. Clinically, birth size and gestational maturity should still be considered when planning post-discharge follow-up, but they should not be used alone to conclude that infants with lower BWZ were the highest-risk group in this cohort. Infants at the extremes of birth size or GA, and those showing poor early post-discharge growth, should be prioritised for structured dietetic review.

At 6 to 9 months CA, the mean percentage body fat in the present preterm cohort was 39.4%, compared with approximately 23% to 25% reported in healthy term-born infants at a similar age. This suggests a potentially adipose-predominant body composition profile among preterm infants in this cohort after NICU discharge. However, this finding should be interpreted cautiously because the DXA subsample was small and no long-term metabolic outcomes were assessed. Further follow-up is needed to determine whether this body composition pattern persists and whether it has later clinical relevance. These findings support the value of assessing growth quality during post-discharge follow-up, rather than relying on body weight alone. Nutritional monitoring after discharge should therefore consider whether weight gain reflects appropriate lean tissue accretion or disproportionate fat accumulation.

Neurodevelopmental outcomes were not assessed in this study. However, previous studies have reported associations between early protein deficits, postnatal growth faltering, and later cognitive or motor outcomes in preterm infants. The protein shortfalls documented in both feeding groups during Weeks 1 to 3, with mean intakes reaching only 79% to 82% of the ESPGHAN minimum protein target, may represent a potentially modifiable nutritional exposure relevant to later developmental follow-up. Future longitudinal assessment of this cohort at 18 to 24 months CA using standardised developmental tools could examine whether the early nutritional patterns observed in this study are associated with later cognitive or motor differences.

At the family level, the finding that fortified PBM and PFM were associated with broadly comparable macronutrient delivery and similar growth outcomes has implications for feeding counselling after discharge. Families of preterm infants may face difficulty sustaining mother's own milk provision after NICU discharge, particularly when breastfeeding or milk expression must continue alongside return-to-

work demands, perceived milk insufficiency, workplace constraints, and postpartum confinement practices in the Malaysian context (Ahmad et al. 2022; Foong et al. 2021).

The present findings suggest that, under a standardised NICU fortification protocol, growth outcomes among infants predominantly receiving breast milk were broadly comparable to those among infants predominantly receiving formula. In this cohort, BWZ and GA were statistically associated with post-discharge WAZ trajectory, but the negative direction of association in the adjusted model means these findings require cautious interpretation. Feeding counselling should therefore remain balanced, supporting breast milk provision where feasible while recognising that growth monitoring, nutrient adequacy, and individual clinical risk remain central after discharge.

This finding does not diminish the established immunological and morbidity-protective value of breast milk, which remains the preferred substrate for reasons beyond growth. However, it provides some local reassurance for families who are unable to sustain adequate breast milk supply after discharge, as formula transition was not associated with worse growth outcomes in this cohort. The post-discharge feeding transition data support this interpretation: 37.0% of PBM-classified infants who attended follow-up had transitioned to formula by the first post-discharge visit, and 19.3% of all follow-up infants were receiving mixed feeds. These findings suggest that feeding transitions after NICU discharge are common in this population and that family-level counselling should address such transitions directly.

The progressive WAZ decline observed through 6 months CA in both feeding groups also indicates that the post-discharge period is a window of nutritional vulnerability regardless of NICU feeding type. Families require clear guidance on feeding adequacy, appropriate use of fortification or formula where clinically indicated, and early warning signs of growth faltering. The present findings support structured dietetic counselling at each post-discharge clinic visit, with feeding review tailored to each infant's growth trajectory and clinical risk profile.

At the healthcare system level, the present study identifies several points relevant to neonatal nutrition protocols and post-discharge service planning. The protein shortfall of approximately 18 to 21 percentage points below the ESPGHAN minimum target during the first three weeks of admission in both feeding groups represents a clear target for service review. Potential strategies include earlier fortification once enteral feeds are established, fortification protocols with greater protein density, or adjustable fortification guided by blood urea nitrogen levels, as reported in the Thai randomised controlled trial by Khorana et al. (2024). In this cohort, standard fortification produced broadly comparable protein intakes between PBM and PFM groups, but mean protein intake remained below guideline targets. This suggests that the key constraint may relate more to the timing, dose, and consistency of nutrient delivery than to feeding substrate alone.

The progressive WAZ decline through 6 months CA in both groups points to a need for closer post-discharge nutritional surveillance. The present cohort continued to show WAZ decline up to 6 months CA, whereas Rohsiswatmo et al. (2024) reported a WAZ nadir at approximately 3 months CA followed by later recovery in an Indonesian preterm cohort. Because post-discharge nutrient intake was not quantified in the present study, the observed decline cannot be attributed directly to specific post-discharge feeding practices. However, the pattern supports the need for structured follow-up during the early post-discharge period.

The healthcare system implication is that post-discharge nutrition pathways should be strengthened for selected infants. These findings support consideration of structured post-discharge nutrition pathways, with assessment of whether enriched formula, continued fortification, or intensified dietetic monitoring is appropriate for selected infants. Regular growth monitoring after discharge may help identify infants with persistent growth faltering and guide timely nutritional review. Findings from Japakasetr et al. (2016) suggest that structured nutritional follow-up can improve growth outcomes in a Southeast Asian setting, although the present study did not test an enriched formula intervention directly.

The DXA feasibility data generated by this study are also useful for healthcare system planning. The completion of DXA scanning in preterm infants at 6 to 9 months CA using a feed-and-wrap technique without pharmacological sedation supports the feasibility of incorporating DXA into selected clinical follow-up or research pathways at this centre. However, because the DXA subsample was small, these findings should not be interpreted as justification for routine DXA monitoring of all preterm infants. BWZ and GA may still be useful clinical descriptors when planning post-discharge follow-up, but the negative direction of association in the adjusted model means they should not be used alone to classify infants as high risk.

Post-discharge dietetic resources should instead be directed using a broader clinical risk profile, including birth size, gestational maturity, illness history, feeding tolerance, discharge growth status, and early post-discharge growth pattern.

At the national level, this study contributes to the Malaysian evidence base for preterm nutrition practice. Current local guidance on preterm feeding recognises the importance of breast milk fortification and post-discharge nutrition, but much of the supporting literature is drawn from international settings with different feeding systems, fortifier access, and cultural contexts. The present study provides locally derived quantitative data on achieved nutrient delivery, growth trajectories, and exploratory DXA-derived body composition and bone outcomes in a Malaysian tertiary NICU cohort. This helps address a local data gap that has previously limited interpretation of Malaysian preterm feeding outcomes.

The finding that PBM and PFM were associated with broadly comparable growth outcomes under the current unit protocol, while both groups remained below ESPGHAN minimum protein targets during the early postnatal weeks, has implications for future national discussion on preterm nutrition practice. Areas for consideration include fortification protocols, minimum protein delivery targets, timing of fortifier initiation, and structured post-discharge nutritional follow-up. The present study can support this process by providing local estimates of protein delivery shortfalls, post-discharge growth patterns, and exploratory body composition outcomes to inform future

multicentre studies, audit activities, and quality improvement work across Malaysian NICUs.

At the population health level, the potentially adipose-predominant body composition profile observed in this cohort at 6 to 9 months CA suggests the need for longer-term follow-up of Malaysian preterm survivors. As neonatal survival improves and more preterm infants are discharged from NICU, post-discharge growth quality may become an increasingly important component of early-life nutritional care. However, the present findings should be interpreted cautiously because the DXA subsample was small and no later metabolic outcomes were assessed.

These findings support the need for structured post-discharge monitoring that extends beyond weight gain alone and includes parental feeding guidance, growth surveillance, and assessment of infants with persistent growth faltering or disproportionate adiposity. Future multicentre follow-up studies would be needed to determine whether early body composition patterns persist beyond infancy and whether they are associated with later metabolic outcomes. A national preterm growth and nutrition registry could help generate longitudinal Malaysian data on feeding exposure, growth trajectory, body composition, and longer-term outcomes, and could support future service planning and intervention studies.

6.2 STRENGTHS AND LIMITATIONS

6.2.1 Strengths

This study has several important strengths. The prospective observational cohort design allowed temporal alignment of nutritional exposure and subsequent growth outcomes, reducing ambiguity regarding directionality of associations. Prospective collection of nutritional and anthropometric data minimised recall bias and ensured systematic documentation. Detailed quantification of energy and macronutrient intake across defined postnatal periods (week 1, weeks 1 to 3, weeks 4 to 6) revealed temporal patterns. This granular time-segmented analysis enabled identification of critical windows of nutritional vulnerability, particularly during early transitional feeding, which are often obscured in studies reporting only cumulative or discharge-level intake.

Longitudinal follow-up of growth outcomes extended to 6 months CA, enabling assessment of trajectories beyond the typical discharge endpoint. Use of linear mixed-effects models accounted for repeated measures and adjusted for baseline GA, birthweight z-score, and Week 4–6 protein intake, thereby improving interpretation of growth trajectory findings within the limits of the observational design. This modelling approach allowed separation of within-infant longitudinal change from between-infant baseline differences, strengthening interpretation of growth trajectory findings.

Inclusion of DXA-derived body composition and bone mineralisation data provided objective assessment beyond anthropometry, addressing an important evidence gap in Southeast Asian preterm populations where such data remain limited. The integration of anthropometric, nutritional, and body composition outcomes within a single cohort strengthens the multidimensional interpretation of growth quality rather than weight gain alone. To date, published DXA data in Malaysian preterm populations have been extremely limited, with prior reports focusing primarily on measurements at term-equivalent age. The present study extends this evidence by providing follow-up body composition data beyond term age, representing the first contemporary longitudinal DXA assessment in this population. Conducting the study within a standardised, protocol-driven tertiary NICU reduced variability in feeding advancement practices, allowing clearer evaluation of nutrient delivery effects independent of major protocol differences.

6.2.2 Limitations

This study has several limitations that should be acknowledged. First, feeding allocation was not randomised. Feeding group reflected clinical factors, and while statistical models adjusted for these factors, residual confounding by unmeasured illness severity cannot be excluded. Additionally, as a single-centre study conducted within a practice-driven tertiary NICU, findings may not be fully generalisable to units with different fortification practices, discharge criteria, or post-discharge follow-up systems.

Second, the overall sample size was modest, limiting power to detect small but clinically relevant differences in growth velocity. The DXA subsample was small ($n = 12$), and scans were performed across a variable corrected age range (6 to 9

months), limiting precision of body composition comparisons. Although more than 30 parents initially consented, successful scans were reduced due to the requirement for natural sleep using the feed-and-wrap technique without pharmacological sedation. This pragmatic approach prioritised infant safety but limited completion rates and contributed to selection bias within the DXA cohort.

Third, there was attrition in follow-up measurements. Loss to follow-up may not have been random, as infants with feeding difficulties, slower growth, or socioeconomic barriers to clinic attendance may have been underrepresented in later assessments, potentially biasing post-discharge growth estimates.

Fourth, macronutrient intake from breast milk was estimated using reference values rather than direct milk analysis. Actual breast milk composition varies between mothers, introducing measurement error in calculated protein and energy intake. Additionally, direct breastfeeding during later admission was not consistently quantified, as intake volumes during direct feeding sessions could not be precisely measured as it was only recorded from nursing chart. This may have resulted in underestimation of total enteral intake among infants receiving mixed feeding and introduced additional measurement imprecision.

Finally, post-discharge nutritional intake was not systematically captured. Caregivers frequently modified feeding practices after discharge, including mixed direct breastfeeding and formula use, changes in formula type, and variable introduction of complementary feeding. Consequently, growth trajectories at 3 and 6 months CA reflect combined effects of NICU and post-discharge nutrition, and the independent contribution of early in-hospital intake cannot be fully isolated.

6.3 SUGGESTIONS FOR FUTURE STUDIES

Future work should address the methodological and biological gaps identified in this study. Larger prospective cohorts across multiple Malaysian neonatal centres are needed to improve statistical power, capture wider variation in feeding practice, and test whether findings from this single tertiary NICU are consistent across different care settings, fortification protocols, and post-discharge follow-up pathways.

Research on early nutritional delivery should focus on whether earlier, higher-protein, or individualised fortification can improve protein intake during the first weeks of NICU admission. Randomised or protocol-based evaluations within standard care pathways would provide stronger causal evidence on optimal protein and energy delivery. These studies should examine whether individualised fortification creates sufficient intake contrast to clarify feeding-type effects on growth, particularly when comparing predominantly fortified breast milk and predominantly formula-fed infants.

Body composition follow-up should extend beyond 6 months CA. Serial DXA assessment at term-equivalent age and at 6, 12, and 24 months CA would help identify when adipose-predominant growth patterns emerge, whether they persist, and whether early nutritional interventions affect later lean mass, fat mass, and bone mineral accrual. Larger DXA samples are needed to allow valid feeding-group comparisons and to build local descriptive data for Malaysian preterm infants.

Future studies should incorporate biological and developmental outcomes alongside anthropometry and DXA. Potential mediators such as insulin-like growth factor-1, leptin, adiponectin, and bone metabolism markers may help explain how early protein-energy balance relates to lean tissue accretion, adiposity, and skeletal mineralisation. Long-term follow-up into childhood should include cardiometabolic markers, adiposity distribution, bone outcomes, physical growth, and standardised neurodevelopmental assessment at 18 to 24 months CA and beyond. Combining these outcomes within one longitudinal cohort would provide a stronger basis for improving preterm nutrition care in Malaysia.

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APPENDIX A

ETHICAL APPROVAL



FAKULTI SAINS KESIHATAN - FACULTY OF HEALTH SCIENCES

Rujukan : UKM.FSK.PRA.600-5/2/1

Tarikh : 09 September 2024

YBhg. Dato' Dr. Azmi Baharudin
Pengarah
Hospital Canselor Tuanku Muhriz
Universiti Kebangsaan Malaysia

YBhg. Prof. Dato'.

PERMOHONAN MENJALANKAN PROJEK PENYELIDIKAN DI HOSPITAL CANSELOR TUANKU MUHRIZ

Dengan segala hormatnya perkara di atas adalah dirujuk.

2. Sukacita dimaklumkan bahawa Program Dietetik, Fakulti Sains Kesihatan, Universiti Kebangsaan Malaysia ingin memohon kebenaran bagi pihak pelajar untuk menjalankan projek penyelidikan di Hospital Canselor Tuanku Muhriz.

3. Perincian maklumat pelajar dan penyelidikan yang akan dijalankan adalah seperti berikut :

Nama Pelajar :	Badmus Suad Mazidatul-khayr
Penyelia :	Dr. Haslina binti Abdul Hamid
Tajuk Kajian :	"Association between breastmilk fortifications and growth, body composition and bone mineralization among preterm infants"
Tarikh :	2 September 2024 – 1 September 2026
Tempat :	Unit Neonatal (HCTM) dan Unit Laporan Perubatan.

4. Untuk pengetahuan pihak YBhg. Prof. Dato' proses pengumpulan data akan dilakukan melalui analisis rekod perubatan elektronik melalui sistem dan rekod perubatan (fizikal) di dalam unit neonatal dan jika perlu, rekod akan diakses melalui unit Laporan Perubatan. Tiada sebarang intervensi perubatan atau prosedur secara terus akan dilakukan kepada pesakit. Data yang akan di ambil adalah data demografi, antropometri, biokimia, klinikal dan rekod sejarah di wad. Kajian ini telah mendapat kelulusan etika daripada jawatankuasa Etika Penyelidikan Universiti Kebangsaan Malaysia (nombor rujukan JEP-2024-519)

5. Sebarang pertanyaan, pihak YBhg. Prof. Dato' boleh berhubung dengan saya sebagai penyelia pelajar di talian 019-4883607 atau emel haslina86@ukm.edu.my.

Segala pertimbangan dan kerjasama daripada pihak YBhg. Prof. Dato' amatlah dihargai dan didahului dengan ucapan terima kasih.

Sekian.



CERTIFIED TO ISO 9001:2015

CERT. NO. QMS 01100

PROGRAM DIETETIK, FAKULTI SAINS KESIHATAN
Universiti Kebangsaan Malaysia, Kampus Kuala Lumpur, Jalan Raja Muda Abdul Aziz,
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PENERAJU SAINS KESIHATAN

Yang benar,



DR. HASLINA BINTI ABDUL HAMID
Pensyarah dan Penyelia Utama
Program Dietetik
Fakulti Sains Kesihatan
Universiti Kebangsaan Malaysia

- s.k. - **Prof. Dr. Cheah Fook Choe**
Jabatan Pediatrik
Fakulti Perubatan
Universiti Kebangsaan Malaysia
- **Dr. Nor Aini Jamil @ A. Wahab**
Ketua
Program Dietetik
Fakulti Sains Kesihatan
Universiti Kebangsaan Malaysia
- **Prof. Madya Dr. Faizah Binti Mohd Zaki**
Jabatan Radiologi
Fakulti Perubatan
Universiti Kebangsaan Malaysia
- **Dr. Wan Nurulhuda binti Wan Md Zin**
Jabatan Pediatrik
Fakulti Perubatan
Universiti Kebangsaan Malaysia
- **Dr. Afifah binti Mohamed**
Program Pengimejan Diagnostik dan Radioterapi
Fakulti Sains Kesihatan
Universiti Kebangsaan Malaysia



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APPENDIX B

INFORMATION SHEET

PARTICIPANT INFORMATION SHEET

Research Title:

Associations between breast milk fortification and growth, body composition and bone mineralization among preterm infants

Introduction:

You are invited to participate in a research study. Before participating in this study, it is important that you take time to read and understand the information in this information sheet.

Purpose of study:

The purpose of this research is to know the effects of milk types used in feeding preterm infants in the Neonatal intensive care unit (NICU). The results of this study will be useful in suggesting the optimal way to feed preterm infants to improve growth and development before and after discharge from the NICU. This study will also provide information on growth (weight, height and head circumference), body composition and bone status of the infants before and after discharge from the NICU.

What will the study involve?

There will be no intervention on your infants' feeding during NICU or after discharge. Your infants' medical and growth data will be collected during admission. After that, follow-up appointments will be set to measure weight, height, head circumference at 1,3 and 6-months corrected age. At 6 months of age, a more specific measurement that involves using a scan machine will be used to see your infants' body composition and bone status. You will also be required to answer a few questions related to your infants' feeding at these appointment visits.

Benefits:

Participating in this study is unlikely to offer you a direct advantage. However, you will get detailed growth, body composition and bone status information of your infant which is not routinely provided in usual health appointments. Your participation could help us improve the feeding protocols in NICU and suggest more comprehensive health monitoring for preterm infants after discharge.

Risk:

In this study, a scan machine called DXA will be used to measure body composition and bone status. DXA scan involves a very low amount of radiation exposure (less than standard chest x-ray machine), however it is safe to use and should not pose any side effects.

Do you have to take part?

Participation in this study is voluntary. If you agree to take part, then you will be asked to sign an "Informed Consent Form". You will be given a copy of the form and this Information Sheet. Your treatment is not affected if you decide not to participate in this study. Your infant will be reviewed appropriately during her follow up based on the standard procedure.

Should you decide to participate, you can still withdraw from the study without penalty. The researcher may also remove you from the study for a variety of reasons. In this event, you will not be penalized or lose your rights as a patient.

Data & Confidentiality:

The data from this study will be made into a report which may be published. Access to only by the research team and the REC UKM. The data will be reported in a collective manner with no reference to an individual. Hence your infant's identity will be kept confidential.

Payment and Compensation:

You do not have to pay to take part in this study. However, you will be paid to participate in this study. You do have to pay the usual hospital charges.

This study is being conducted with a Universiti Kebangsaan Malaysia Research Grant (GGPM-2022-047). Each Participant will be given an honorarium of RM100 for the 3 outpatient visits.

Who can I ask about the study?

If you have any questions, you can direct them to the research team. You can also come to UKM for clarifications.

Dr Haslina binti Abdul Hamid: Principal investigator

Dietetics program

Universiti Kebangsaan Malaysia Kuala Lumpur Campus

Phone number: +60 19 488 3607

Email: haslina86@ukm.edu.my

Co investigators:

Prof Cheah Fook Choe

Faculty of medicine

Universiti Kebangsaan Malaysia Kuala Lumpur

Dr Nor Aini Binti Jamil

Dietetics program

Universiti Kebangsaan Malaysia Kuala Lumpur

Student: Badmus Suad Mazidatul-khayr

Dietetics program

Universiti Kebangsaan Malaysia Kuala Lumpur

Phone number: +60 11 682 49185



APPENDIX C

INFORMED CONSENT FORM

PARTICIPANT INFORMED CONSENT FORM

Research Title: Associations between breast milk fortification and growth, body composition and bone mineralization among preterm infants.

Researcher's Name:

I....., with an infant birth certificate number:

- have read the information in the patient information sheet including information regarding the risk in this study.
- have been given time to think about it and all my questions have been answered to my satisfaction.
- understands that I may freely choose to withdraw my infant from this study at any time without reason and without repercussion.
- understands that my anonymity will be ensured in the write-up.

I voluntarily agree to be part of the research study, to follow the study procedures and to provide necessary information to the doctor, nurses or other staff members as requested.

.....
Participant Signature

.....
Date

.....
Witness (if any)

.....
Signature

.....
IC Number

.....
Date

.....
Researcher

.....
Signature

.....
IC Number

.....
Date

APPENDIX D

APPLICATION TO WRITE THESIS IN ENGLISH LANGUAGE



FAKULTI SAINS KESIHATAN • FACULTY OF HEALTH SCIENCES

Reference No. : UKM.FSK.SWZ/600-4/6/1(P104813)

Date : 30th January 2024

Basmus Suad Mazidatul-Khayr
 12, Balogun Fabiyi Street Ojoo Ibadan
 Nihort Jericho Ibadan
 20013 Ibadan
 Oyo
 Nigeria

Dear Madam,

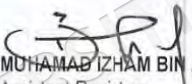
APPLICATION TO WRITE A THESIS IN ENGLISH

With all due respect allow me to refer to the above.

2. I am pleased to inform that the Faculty has agreed to approve your application to write a thesis in English.

Thank you.

Yours sincerely,


MUHAMAD IZHAM BIN AZMIL
 Assistant Registrar
 for Deputy Dean (Postgraduate)
 Faculty of Health Sciences

- c.c. - **Assoc. Prof. Dr. Mahadir Bin Ahmad**
 Head of Graduate Research Program
 Center For Community Health Studies (ReaCH)
 Faculty of Health Sciences
- **Dr. Haslina Binti Abdul Hamid**
 Main Supervisor
 Center For Community Health Studies (ReaCH)
 Faculty of Health Sciences

/ssr

DEPUTY DEAN OFFICE (POSTGRADUATE) FACULTY OF HEALTH SCIENCES

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Tarikh Kuatkuasa 01-09-2019



FAKULTI SAINS KESIHATAN
(Faculty of Health Sciences)

PERMOHONAN MENULIS TESIS DALAM BAHASA INGGERIS
(Application to Writing Thesis in English)

BAHAGIAN A: MAKLUMAT DIRI PELAJAR
Part A: Student's Detail

Nama: (Name)	BADMUS SUAD MAZIDATUL-KHAYR
No.Pendaftaran: (Registration Number)	P133181
Alamat Tetap: (Permanent Address)	The Academia, Southcity Apartment, Seri Kebangan
No. Telefon: (Telephone Number)	+60 11 682 49185
Email: (E-mail)	P133181@siswa.ukm.edu.my
Program: (Program)	Masters' Dietetics
Pusat Pengajian: (School)	Universiti Kebangsaan Malaysia / Faculty of Health Sciences
Bentuk Pendaftaran: (Type of registration)	Full Time
Tajuk Tesis (Bahasa Malaysia): (Title for Thesis in Bahasa Malaysia)	KESAN SUSU IBU TERHADAP PERTUMBUHAN, KOMPOSISI BADAN DAN MINERALISASI TULANG DI KALANGAN BAYI PRAMATANG
Tajuk Tesis (Bahasa Inggeris): (Title for Thesis in English)	EFFECTS OF BREAST MILK FORTIFICATION ON GROWTH, BODY COMPOSITION AND BONE MINERALIZATION AMONG PRETERM INFANTS
Sebab Memohon: (Reason to Changes)	International student
**Bagi pelajar Warganegara, lampirkan bersama surat Iantikan Penyelia Bersama Luar Negara (for Non-Malaysian Student, please attach the appointment letter of Non-Malaysian Co-Supervisor)	
Semester / Sesi Mula Pertukaran: (Semester / Session of Changes Application)	1 2023/2024

PEJABAT TIMBALAN DEKAN (SISWAZAH), FSK, UKM	
TARIKH:	17/1/24
SEGERA	
MAKLUMAN	
LULUS	☒
TIDAK DILULUSKAN	
CATATAN:	

UKM/FSK/SPS/09
Tarikh Kuatkuasa 01-09-2019

Tandatangan (Signature):



Tarikh (Date): 16th of January 2024

BAHAGIAN B: PERAKUAN PENYELIA UTAMA:

Part B: Approval by Main Supervisor

Permohonan ini *disokong/ tidak disokong:

Tandatangan: _____



Tarikh: 16th of January 2024

Cop Rasmi:

BAHAGIAN C: PENGESAHAN TIMBALAN DEKAN (SISWAZAH)

Part C: Approval by Deputy Dean (Post-graduate)

Permohonan *diluluskan/ tidak diluluskan:

Tandatangan: _____



Tarikh: 8/1/2024

Cop Rasmi:

PROF. Madya DR. MOHD 'IZZUDDIN HAIROL
Timbalan Dekan (Siswazah)
Fakulti Sains Kesihatan
Universiti Kebangsaan Malaysia

UNIVERSITI KEBANGSAAN MALAYSIA

APPENDIX E

CASE NOTE AND DATA COLLECTION SHEET

Seen by Dietitian -Follow-up visit

Female/Chinese

GA :

Chronological age : 7 months 28 days

Corrected age : 6 months 9 days

DOB:

Date of discharge from NICU : 17/4/25 (NICU stay : 22 days)

Consultation for : Follow-up for growth and nutrition monitoring

Nutritional assessment

Social history:

Came in with mother housewife (34years old), , Father's age 34 years old,lorry driver. Also follow up at KK Kuchai Lama. First baby, stay with mother at home.

Medical History

1. moderate prematurity at 33 weeks, LBW, AGA
2. anemia of prematurity
3. feeder and grower
4. infant with single umbilical artery. postnatal US KUB - normal, planned for outpatient US KUB on 26/5/25
5. Resolved Presumed sepsis I/V/O spontaneous prematurity
6. Resolved Neonatal jaundice secondary to prematurity
7. u/I Maternal HVS C+S yield yeast species

follow -up ophthalmology HPKK

Anthropometry data:

Birth weight:1.695

Length: 40

HC:30

Biochem: last 13/5/25

Date	Birth	Discharge	1month CA 26/6	2 months CA	3 months CA	5m CA	6m CA
Weight (kg)	1.695	2.12	4.5	5.08 (0 zscore)	5.9	6.7	7.1 (>-2)
Length (cm)	40		55	57 (0 zscore)	60	62.7	65.7 (0)
Head Circumference (cm)	30	31-	39	39.5 zscore)	40	42	42.5 (0)

For WHO growth chart using corrected age, baby is gaining appropriate weight, HC and length gain for age.

Diet history:

During NICU admission: preterm formula and EBM, but predominantly FM

Postdischarge feeding: Similac neosure for 1 month, claimed to be constipated so changed to Dupro step 1.

Current feeding: Dupro 6 scoop + 170ml, 4hourly, 6x/day

Started weaning in early October (5m+ CA), 2 scoops rice cereal (brown rice porridge) + 1 scoop milk, but still limit the feeding to only 3x a week as mother noticed a change in the stool texture – more drier.

BO : once a day

Nutritional Diagnosis: No diagnosis at this time.

Intervention:

- Mother was advised to continue milk feeding as usual.
- Can increase CF once a day instead of 3x/week, increasing to twice a day when tolerating well

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S			
1			Nutrition and Growth in Preterm Infants																			
2																						
3			Patient details:															Diagnosis	Yes/No	Grade/Severity		
4			Study number																	NEC (Grade)		
5			Date of birth																	IVH (grade)		
6			Sex																	PVL		
7			Time of birth																	Late onset sepsis		
8			Gestation age																	Chronic lung disease		
9			Birth weight																	ROP		
10																						
11																						
12																						
13			Time of start of daily nursing charts					0:00		Date of admission							15/08/25					
14			Time of birth in complete hours					11		Date of Discharge							7/9/25					
15																						
16			Duration of Day 1		23																	
17																						
18			glucose solutions							PN			Other fluids		Enteral feeds							
19			Working Body Weight	Actual Body Weight	COH / LT	Volume of 5% glucose	Volume of 10% glucose	Volume of 12.5% glucose	Volume of 15% glucose	Volume of 20% glucose	Volume of PN	What is the concentration of glucose in this PN?	Volume of Lipid	Any other fluid (total volume in the duration)	Volume of EBM	Volume of Fortified EBM	Volume of Formula milk	Volume of Other Formula	Name of formula (if other)	Myo		
20			kg	kg		ml	ml	ml	ml	ml				ml	ml	ml	ml	ml				
21		Day 1																				
22		Day 2																				
23		Day 3																				
24		Day 4																				
25		Day 5																				

APPENDIX F

ADDITIONAL FIGURES ON NUTRIENT INTAKES

Weekly Fat Intake

Appendix Figure F.1 presents mean weekly fat intake for PBM and PFM infants from weeks 1 to 6. Both groups showed an increase from week 1 to week 2, exceeding the minimum recommended intake from week 2 onwards. Fat intake remained relatively stable through week 5 in both groups. At week 6, the PFM group demonstrated a decline and increased variability compared to the PBM group.

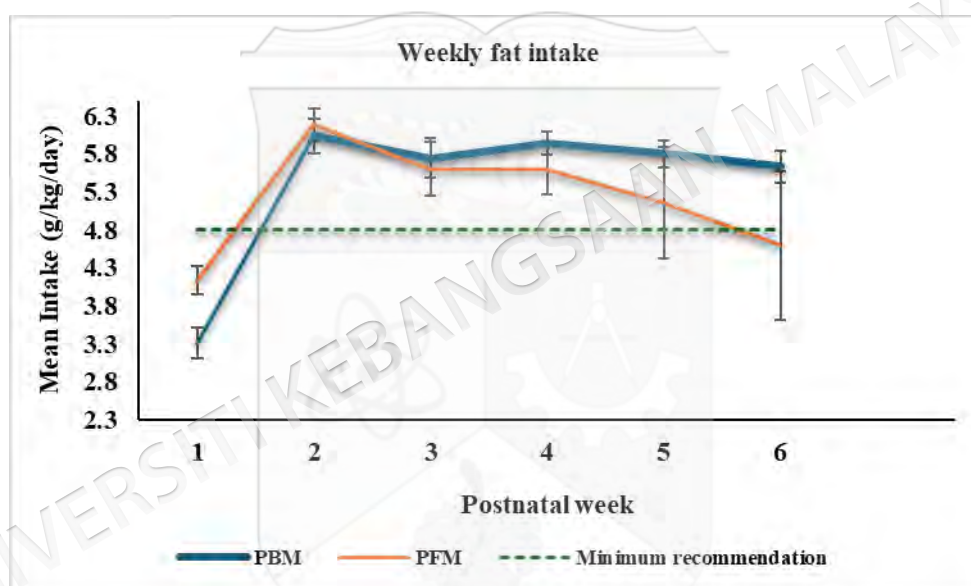


Figure F.1 Mean weekly fat intake between two feeding groups (error bars represent the standard error (SE))

Figure F.1 presents mean weekly fat intake for PBM and PFM across postnatal weeks 1 to 6. Both groups increased from week 1 to week 2. At week 1, the PBM group recorded approximately 3.3 g/kg/day while the PFM group recorded approximately 4.2 g/kg/day. By week 2, both groups reached approximately 5.9-6.1 g/kg/day.

From week 2 onwards, both groups exceeded the minimum target through week 5, with values ranging between 5.5-6.0 g/kg/day. At week 6, the PBM group maintained fat intake at approximately 5.6 g/kg/day, while the PFM group declined to approximately 4.8 g/kg/day. The PBM group showed gradual decline from weeks 3 to

6, while the PFM group demonstrated a more pronounced decrease between weeks 5 and 6. Error bars for the PFM group at week 6 were substantially larger compared to earlier weeks and to the PBM group at the same time point.

Weekly Carbohydrate Intake

Appendix Figure F.2 shows mean weekly carbohydrate intake across postnatal weeks 1 to 6. Both groups increased markedly from week 1 to week 2 and remained above the minimum recommended intake thereafter. PBM infants recorded numerically higher carbohydrate intake than PFM infants from weeks 2 to 6, with relatively stable trends in both groups.

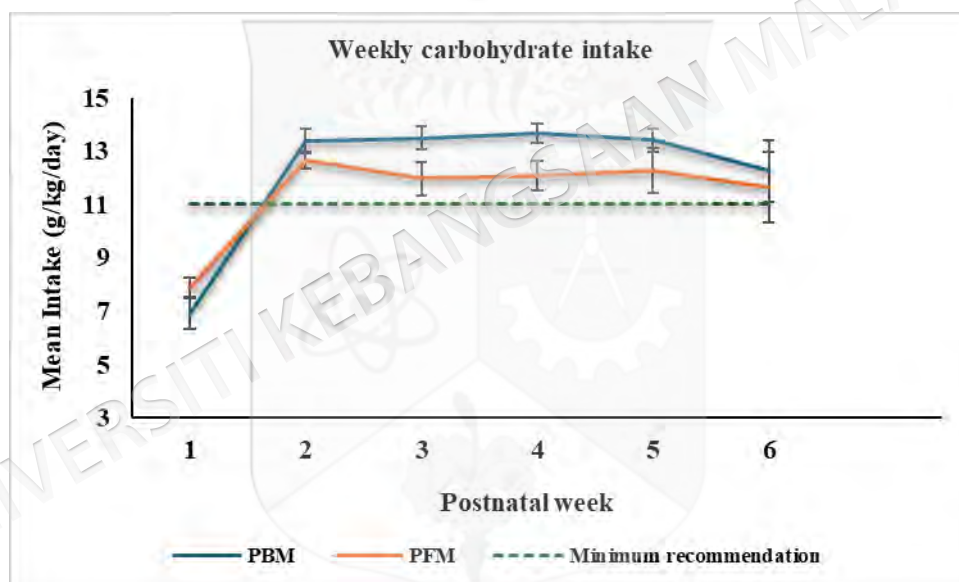


Figure F.2 Mean weekly carbohydrate intake between two feeding groups (error bars represent the standard error (SE))

Figure F.2 presents mean weekly carbohydrate intake for PBM and PFM across postnatal weeks 1 to 6.

Both groups increased from week 1 to week 2. At week 1, the PBM group recorded approximately 7.0 g/kg/day while the PFM group recorded approximately 8.0 g/kg/day. By week 2, the PBM group reached approximately 13.0 g/kg/day, and the PFM group reached approximately 12.5 g/kg/day.

From week 2 onwards, both groups exceeded the minimum target throughout the study period. The PBM group maintained values between 13.0-13.5 g/kg/day from weeks 3 to 6, while the PFM group ranged between 11.5-12.5 g/kg/day during the same period. The PBM group recorded consistently higher carbohydrate intake compared to the PFM group across weeks 2 to 6, with a difference of approximately 1.0-1.

Weekly Fluid Intake

Appendix Figure F.3 presents weekly fluid intake for PBM and PFM infants. Both groups exceeded the minimum recommended intake throughout the study period. Fluid intake increased from week 1 to week 2, followed by a gradual decline in PBM infants and greater variability in PFM infants during weeks 5 and 6.

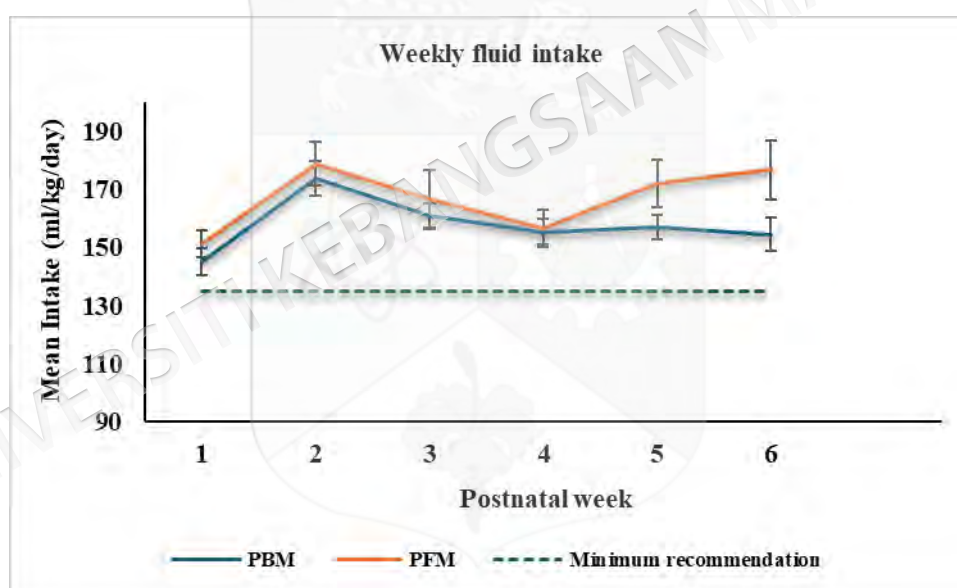


Figure F.3 Mean weekly fluid intake between two feeding groups (error bars represent the standard error (SE))

Figure F.3 presents mean weekly fluid intake for PBM and PFM across postnatal weeks 1 to 6. Both groups increased from week 1 to week 2. At week 1, both groups recorded approximately 145-150 ml/kg/day. By week 2, both groups reached approximately 170-175 ml/kg/day. Both groups exceeded this minimum threshold throughout the entire study period.

From week 2 onwards, both groups showed gradual decline from week 2 to week 4, reaching approximately 155-160 ml/kg/day. The PFM group recorded marginally higher fluid intake compared to the PBM group during weeks 2 to 6. From week 4 onwards, the PFM group showed an increasing trend, reaching approximately 177 ml/kg/day by week 6, while the PBM group maintained relatively stable intake around 155 ml/kg/day. Error bars for both groups increased during weeks 5 and 6, with the PFM group at week 6 showing the largest variability observed across the study period.



APPENDIX G

ADDITIONAL STATISTICAL ANALYSES

Appendix Table G.1 presents sensitivity model for energy and PER between week 1-6

Table G.1 Sensitivity model (Energy wk1–3)

Predictor	β coefficient	Std. Error	p value	95% CI
Feeding group (PFM vs PBM)	-0.06	0.13	0.64	-0.32 to 0.20
Energy intake, Week 1–3 (kcal/kg/day)	0.002	0.004	0.71	-0.01 to 0.01
Gestational age at birth (weeks)	0.04	0.03	0.20	-0.02 to 0.10
Sex (Female vs Male) *	0.08	0.13	0.57	-0.18 to 0.33
Birthweight z score (BWZ)	0.01	0.07	0.89	-0.13 to 0.15

Table G.2 Sensitivity model (PER wk1–3)

Predictor	β coefficient	Std. Error	p value	95% CI
Feeding group (PFM vs PBM)	-0.07	0.13	0.61	-0.33 to 0.19
PER, Week 1–3 (g/100 kcal/day)	-0.01	0.10	0.92	-0.20 to 0.18
Gestational age at birth (weeks)	0.04	0.03	0.23	-0.02 to 0.10
Sex (Female vs Male) *	0.09	0.13	0.49	-0.17 to 0.34
Birthweight z score (BWZ)	0.01	0.07	0.91	0.13 to 0.15

Values are unstandardized regression coefficients (β) from a multivariable linear regression model with Δ WAZ at discharge as the dependent variable (N = 66). The feeding group coefficient compares PFM relative to PBM. Protein intake represents the Week 1–3 mean (g/kg/day). *Sex coding: 1 = Male 2 = Female (so the coefficient reflects female relative to male).

In the sensitivity model using energy intake during Weeks 1–3, neither feeding group (PFM vs PBM) nor energy intake was associated with Δ WAZ at discharge. BWZ, GA at birth, and sex were also not associated with Δ WAZ in this model (all $p > 0.05$).

Similarly, in the sensitivity model using PER during Weeks 1–3, feeding group and PER were not associated with Δ WAZ at discharge. Birth weight z-score, GA, and sex likewise showed no association with Δ WAZ (all $p > 0.05$).

Table G.3 Sensitivity model 1: Energy intake during Weeks 4 -6

Predictor	Estimate	Std. error	Df	p-value	95% CI
(Intercept)	2.17	2.91	18.85	0.36	-3.39 to 8.80
Feeding group (PFM vs PBM)	0.06	0.37	18.92	0.88	-0.72 to 0.84
Age 3 months (vs 1 month)	-0.52	0.14	42.94	<0.001	-0.79 to -0.25
Age 6 months (vs 1 month)	-0.73	0.19	51.47	<0.001	-1.11 to -0.36
GA at birth (weeks)	-0.16	0.07	18.90	0.04	-0.31 to -0.01
Birth weight z score (BWZ)	-0.64	0.19	19.27	0.003	-1.03 to -0.26
Energy intake (week 4 - 6)	-0.02	0.01	20.34	0.26	-0.04 to 0.01

Table G./4 Sensitivity model 2: PER during Weeks 4- 6

Predictor	Estimate	Std. error	Df	p-value	95% CI
(Intercept)	6.67	3.74	14.03	0.10	-1.35 to 14.69
Feeding group (PFM vs PBM)	0.57	0.43	13.40	0.21	-0.36 to 1.50
Age 3 months (vs 1 month)	-0.23	0.17	31.23	0.18	-0.58 to 0.11
Age 6 months (vs 1 month)	-0.50	0.24	38.46	0.04	-0.98 to -0.02
GA at birth (weeks)	0.21	0.11	14.25	0.09	-0.45 to 0.04
Birth weight z score (BWZ)	-0.91	0.23	14.42	0.001	-1.40 to -0.42
PER (week 4 to 6)	0.27	0.37	15.65	0.47	-0.51 to 1.05

Linear mixed model with random intercept for infant were used to account for repeated measures at 1, 3, and 6 months CA. Separate models were fitted for protein (n = 29), energy (n = 25), and PER (n = 20) due to incomplete Week 4–6 intake data.

In the model using energy intake during Weeks 4–6, neither feeding group nor energy intake was associated with WAZ over follow-up (p = 0.88 and p = 0.26, respectively). WAZ was significantly lower at 3 months, and 6 months compared with 1 month (both p < 0.001). GA at birth was negatively associated with WAZ (p = 0.04), and BWZ was also negatively associated with WAZ (p = 0.003).

In the model using PER during Weeks 4–6, feeding group and PER were not associated with WAZ ($p = 0.21$ and $p = 0.47$, respectively). WAZ at 6 months was lower than at 1 month ($p = 0.04$), while the 3 month comparison was not statistically different ($p = 0.18$). BWZ remained significantly and negatively associated with WAZ ($p = 0.001$). GA did not reach statistical significance in this model ($p = 0.09$).

Overall, sensitivity analyses replacing protein intake with energy intake or PER during Weeks 4–6 showed no independent association between nutrient intake and WAZ trajectory after adjustment. BWZ was the only consistent predictor across models.



APPENDIX H

FENTON CALCULATOR

DO NOT ENTER DAYS OF AGE AS IT CAUSES CALCULATOR TO INCORRECTLY ROUND UP OR DOWN. IF YOU HAVE DAYS AND WEEKS OF AGE, AND YOU WISH TO USE THAT PRECISION, USE THE ACUTAL AGE CALCULATOR
****SGA is defined AT BIRTH ONLY as < 10th percentile for weight, LGA is > 90th percentile for weight**

Data Entry Cells - Girls					Calculated Cells Girls							
ID	Gest Age or Postmenstrual Age 22-49 completed wks	Weight grams	Length cm	HC cm	z-scores			Percentiles				
					SGA, AGA or LGA by weight at birth	Weight Z	Length Z	Head circumference Z	Weight %	Length %	Head circumference %	
	22	643	30.3	19.5	LGA	1.7				96%		
	24	451	27.3	21.8	SGA	-1.9	-1.9	0.0	3%	3%	50%	
	49	2720	47.0	32.6	SGA	-4.7	-5.2	-4.8	0%	0%	0%	
	36	1952	42.3	29.9	SGA	-1.9	-1.9	-1.9	3%	3%	3%	

Source:
www.ucalgary.ca/fenton
 Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMJ Open* 2013;3(8):e006593.
 Paper is available free with open access from:
<http://www.biomedcentral.com/1471-2431/13/59>
 Calculator written with assistance of Timothy P Stevens, MD, MPH, University of Rochester, Rochester, NY.

* Data not available for length and head circumference of infants at 22 weeks gestation
 **SGA is defined as < 10th percentile for weight, LGA is > 90th percentile for weight

Procedure to calculate Z-scores and percentiles for your research database:
 1 Prepare your research data of age (in completed weeks of gestational age), weight (grams), Head circumference (centimeters), and/or Length
 2 Copy up to 500 girl infants' data into the white columns, with an id number
 3 This application will calculate girls exact Z-score and Percentile placement
 4 Infant age must be reported as weeks, i.e. even weeks since the last mens
 5 Copy and paste "Values" the resulting Z-scores and Percentiles into your

Version 6 - includes SD23 correction for extreme weight z-scores, to improve accuracy of extreme values. While assessment revealed minimal differences with the SD23 correction, we wanted to follow the WHO's lead to provide the most accurate extreme z-scores as possible [1]. Head circumference and length were normally distributed throughout these charts so no correction was needed.

DO NOT ENTER DAYS OF AGE AS IT CAUSES CALCULATOR TO INCORRECTLY ROUND UP OR DOWN. IF YOU HAVE DAYS AND WEEKS OF AGE, AND YOU WISH TO USE THAT PRECISION, USE THE ACUTAL AGE CALCULATOR
****SGA is defined AT BIRTH ONLY as < 10th percentile for weight, LGA is > 90th percentile for weight**

Data Entry Cells - Boys					Calculated Cells - Boys							
ID	Gest Age or Postmenstrual Age 22-49 completed wks	Weight grams	Length cm	HC cm	z-scores			Percentiles				
					SGA, AGA or LGA by weight at birth	Weight Z	Length Z	Head circumference Z	Weight %	Length %	Head circumference %	
	22	695	31.6	22.2	LGA	2.1				98%		
	24	473	27.8	19.8	SGA	-1.9	-1.9	-1.9	3%	3%	3%	
	36	2838	47.7	33.0	AGA	0.0	0.0	0.0	50%	49%	50%	
	49	2036	43.2	30.2	SGA	-6.5	-7.8	-7.5	0%	0%	0%	

Source:
www.ucalgary.ca/fenton
 Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMJ Open* 2013;3(8):e006593.
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APPENDIX I

NICHD CALCULATOR

NEONATAL RESEARCH NETWORK Login

HOME ABOUT NETWORK CENTERS STUDIES PUBLICATIONS TOOLS DATA REQUESTS OPPORTUNITIES LINKS MEMBER LOGIN

NICHD Neonatal Research Network Web-Based Adjusted Age Calculator

Date of Birth:	09/09/2024	Enter the Child's Birth Date (mm/dd/yyyy).
Developmental Test Date:	12/02/2024	Enter the Developmental Test Visit Date (mm/dd/yyyy).
Gestational Age Weeks:	34	Enter the Estimated Gestational Age at Birth (in Weeks) (18-40).
Gestational Age Days:	0	Enter the Estimated Gestational Age at Birth (in Days) (0-6).
	Calculate Clear	Click on the "Calculate" button.
Adjusted Age for Developmental Test Date:	1 months 12 days	
Chronological Age:	2 months 24 days	
Adjusted Age for Developmental Test Date:	1 months 12 days	
Chronological Age:	2 months 24 days	
18-Month Adjusted Age Date:	4/20/2026	

APPENDIX J

WHO ANTHROPOMETRY SOFTWARE AND GROWTH CHARTS

