Energy and Macronutrient Composition of Banked Donor Human Milk from a New Zealand Milk Bank

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Abstract

Background: Donor human milk (DHM) is the recommended alternative to commercial infant formula when an infant's mother's own milk is not available (Arslanoglu et al., 2013; Daniels et al., 2017; Meek & Noble, 2022). DHM use has been associated with reductions in the incidence and severity of prematurity-related morbidities, such as necrotizing enterocolitis (Miller et al., 2018). Although DHM is crucial for supporting the health of preterm infants, its macronutrient composition is known to vary significantly, posing challenges for fortification and clinical use (Perrin et al., 2020).

Objective: Our aim was to understand the energy and macronutrient composition of DHM in New Zealand and contribute to the paucity of information which currently exists in the New Zealand context. Additionally, our study aimed to identify donor characteristics associated with DHM macronutrient composition.

Methods: A secondary data analysis of 696 single-donor pools from 149 donors was conducted to describe the energy and macronutrient composition of DHM which had been donated to New Zealand's first human milk bank in the Christchurch Women's Hospital from July 2022 till July 2024. Single linear regression was performed in SPSS to explore the association between DHM macronutrients and the following variables: lactation stage, gestational age of the infant when the milk was expressed (i.e. preterm or term), donor age, and donor ethnicity.

Results: DHM contained on average 74.9 kcal/100 mL energy; 4.0 g/100 mL fat; 1.2 g/100 mL true protein; and 8.1 g/100 mL total carbohydrate, consistent with published reference values for DHM. Mean true protein fell below the recommended intake for preterm infants, and mean energy was on the lower end of the recommended range. Mean fat and true protein varied considerably, with differences of 37.6-fold and 32.7-fold respectively. Gestational age and lactation stage were the strongest predictors of DHM macronutrient composition. There was also some suggestion of small differences due to donor age and ethnicity.

Conclusion: There is a large variation in macronutrient composition between batches of DHM donated to the Christchurch Women's Hospital Milk Bank, with some batches lacking adequate true protein and energy needed for optimal growth and development of vulnerable preterm infants. Our study highlights the need to explore the growth and health outcomes of the recipient infants and if this DHM variability has significant clinical implications.

Keywords: breastmilk; preterm; term; protein; fat; carbohydrate

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Abbreviations

AAP	American Academy of Pediatrics
AGA	Appropriate for gestational age
ALA	Alpha-linoleic acid
AND	Academy of Nutrition and Dietetics
BMI	Body mass index
BPD	Bronchopulmonary dysplasia
CI	Confidence interval
CWHMB	Christchurch Women's Hospital Milk Bank
DHA	Docosahexaenoic acid
DHM	Donor human milk
EPA	Eicosapentaenoic acid
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
FDA	Food and Drug Administration
FT mid-IR	Fourier-Transform Mid-Infrared
GA	Gestational age
HMA	Human milk analyser
HMO	Human milk oligosaccharides
HoP	Holder pasteurisation
IgA	Immunoglobulin A
IQ	Intelligence quotient
Mid-IR	Mid-infrared
MOM	Mother's own milk
MUFA	Monounsaturated fatty acid
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
NPN	Non-protein nitrogen
OR	Odds ratio
PUFA	Polyunsaturated fatty acid
ROP	Retinopathy of prematurity
SD	Standard deviation
SGA	Small for gestational age
sIgA	Secretory Immunoglobulin A
T2DM	Type 2 diabetes mellitus
UNICEF	United Nations International Children's Emergency Fund
USA	United States of America
WHO	World Health Organization

Chapter 1. Introduction

Preterm birth is a significant health issue in New Zealand. The latest data from the New Zealand Ministry of Health (2023) reveal that in 2023, nearly 5,000 babies in New Zealand were born preterm, accounting for an average of 7.9% of total births nationwide, and this trend has been slowly ticking upwards since 2015. Prematurity is characterised by physiological immaturity, especially of the gastrointestinal, respiratory, immune, and renal systems, and the skin barrier (Deffrennes et al., 2024; Erickson et al., 2021; Henderickx et al., 2021; Oranges et al., 2015; van Duuren et al., 2024); therefore, preterm infants often require specialised hospital care. There is robust evidence that demonstrates a human milk diet, compared to commercial infant formula, significantly reduces the incidence and severity of several prematurity-related morbidities, including necrotising enterocolitis (Miller et al., 2018; Xu et al., 2020), bronchopulmonary dysplasia (Hair et al., 2016; Patel et al., 2017), and retinopathy of prematurity (Speigler et al., 2016; Goyal et al., 2024).

Human milk is the optimal nutrition for human infants, but mothers of hospitalised preterm infants may have challenges providing enough milk due to physical separation from their infant, stress, fatigue, inadequate resources, or inadequate milk supply (Hill et al., 2005; Ikonen et al., 2018; Niela-Vilén et al., 2015). When a mother's own milk (MOM) is not available, donor human milk (DHM) is recommended as the best alternative by the World Health Organization (WHO) (2019), the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) (Arslanoglu et al., 2013), and the American Academy of Pediatrics (AAP) (Daniels et al., 2017; Meek & Noble, 2022). DHM is milk from lactating mothers, surplus to the needs of their own infant, which is gifted for use in infants who are not their own. Before the introduction of infant formula, the sharing of breastmilk between women was a common practice dating back centuries (Moro, 2018). The establishment of formal human milk banks has facilitated milk donation and now plays a crucial role in supporting the health and development of hospitalised preterm infants. The first milk bank in New Zealand opened in the Christchurch Women’s Hospital in 2014 and provides pasteurised DHM to preterm infants in the Neonatal Intensive Care Unit (NICU) for at least the first week after birth or until the mother has established her own supply (Te Whatu Ora, 2024).

DHM has benefits for preterm infants due to non-nutritive elements, and because it encourages an exclusive human milk diet after the infant is discharged from the hospital (Wilson et al., 2018).

However, DHM macronutrient and energy composition is known to vary significantly, which poses challenges for fortification and clinical use (Perrin et al., 2020). This variability may be attributed to factors including inter-individual donor characteristics like age, ethnicity, and body mass index (BMI); lactation stage; or the infant's gestational age. While these variables have been extensively documented in the context of MOM, less has been reported on DHM. The fact that DHM is typically surplus milk rather than a 24-hour collection makes direct comparisons with MOM studies challenging. Additional processes specifically related to DHM, including milk collection and handling practices such as expression methods, freezing, pooling, and pasteurisation, may also influence composition

Only one study to date has reported on DHM macronutrient composition in New Zealand (Lamb et al., 2021). The authors found that preterm milk contained, on average, more fat, energy, protein, and carbohydrates than mature term milk, and that there was significant variability across donations. Although the study, limited by the small sample size, their findings were comparable to similar research conducted internationally, which also found that the macronutrients of DHM vary significantly (John et al., 2019; Kreissl et al., 2016; Leghi et al., 2020; Mills et al., 2019). There were also limitations to these studies, including small sample sizes and inconsistent analysis methods, indicating that more research is required, especially in the New Zealand context.

1.1 Aims and objectives

Aim

Examine the variation in energy content and macronutrient composition (fat, protein, and carbohydrate) of DHM donated to the Christchurch Women's Hospital Milk Bank between July 2022–July 2024.

Objectives

I. Describe the energy and macronutrient composition (fat, protein, and carbohydrate) of DHM donated to the Christchurch Women's Hospital Milk Bank from July 2022–July 2024.
II. Explore the association between donor characteristics (age and ethnicity) and the energy and macronutrient composition of DHM.
III. Explore how lactation stage and gestational age of the infant (preterm versus term) affects the energy content and macronutrient composition of DHM.

1.2 Thesis structure

This thesis is structured into four main chapters along with additional appendices. Chapter 1 introduces the research, outlining the study's scope, rationale, and background while also presenting the research aims, objectives, and the researchers' contributions. Chapter 2 provides a comprehensive review of the existing literature, focusing on 3 key areas: the benefits of human milk for preterm infants, the macronutrient composition of DHM, and the external and biological factors which may influence its composition. This chapter also identifies the gap in the current literature, which the study aims to address. Chapter 3 contains the research manuscript, which includes the abstract, introduction, methods, results, discussion, and conclusion. This manuscript was written with the intention of publication. Chapter 4 concludes the thesis by summarising the key findings and offering recommendations based on the study's results. It also reflects on the strengths and limitations of the research.

1.3 Researchers' contributions

Table 1.1

Researchers' Contributions

Researchers	Contribution
Stephanie Cox *Masters Researcher*	Primary researcher/writer, including literature review, statistical analysis, and ethics application
Dr Ying Jin *Primary Supervisor*	Conceptualisation and design of research, thesis guidance and assistance, reviewing manuscript
Professor Lisa Te Morenga *Secondary Supervisor*	Thesis guidance and assistance, reviewing manuscript
Schol Obery *Clinical Nurse Specialist and Milk Bank Manager – Neonatal Department, Waitaha, Canterbury*	Provision of data (third party not involved in the secondary data analysis)

Chapter 2. Literature Review

The purpose of this literature review is to highlight the benefits of human milk as the preferred nutrition for preterm infants, examine the existing literature on the macronutrient composition of donor human milk (DHM), discuss the external and biological factors that may influence its composition, and identify gaps in knowledge of DHM composition to inform future research. PubMed, Scopus, Cochrane Library and CINAHL were used to find peer-reviewed journal articles published in English, between 2015 and 2025. The final search strategy can be found in Appendix A.

2.1 Benefits of human milk as nutrition for infants

Multiple advantages of a human milk diet compared to commercial infant formula are described in the literature. Human milk produced by a lactating mother supplies all the necessary energy and nutrients for infants in their first 6 months (Perrella et al., 2021). It is easily digested and contains bioactive factors that support immune development and nutrient absorption. Key components like immunoglobulin A (IgA), white blood cells, lysozyme, lactoferrin, and oligosaccharides prevent bacteria from attaching to mucosal surfaces, protecting against respiratory and gastrointestinal infections (Agostoni et al., 2009; Ballard & Morrow, 2013; Carr et al., 2021). Additionally, growth factors and various hormones support the development and maturation of the gastrointestinal tract (Ballard & Morrow, 2013; World Health Organisation, 2009). Furthermore, bile-salt-stimulated lipase promotes fat digestion in the small intestine, enhancing feed tolerance (Ballard & Morrow, 2013; Hamosh, 1996; Lindquist & Hernell, 2010).

Consuming human milk is linked to a lower risk of infants developing chronic disease. There is robust international evidence to suggest that a human milk diet decreases the risk of obesity in childhood (5–10 years) (Qiao et al, 2020; Yan et al. 2014); and decreases the risk of developing type 2 diabetes mellitus (T2DM) in adolescence (10–19 years) (Horta et al., 2015), and in adulthood (20–71 years) (Horta & de Lima, 2019). Human milk contributes to these outcomes due to its bioactive components; for example, the hormone leptin helps regulate appetite and control excess weight gain, and the hormone adiponectin contributes to insulin sensitivity (Ballard & Morrow, 2013).

A human milk diet may influence children's intelligence (IQ). Studies have shown that children who were fed human milk, compared to commercial infant formula, displayed higher cognitive function and neurodevelopment (Lapidaire et al., 2022; Peña-Ruiz et al., 2023). However, a commentary article by

the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee highlight possible confounding factors such as maternal socioeconomic status and maternal IQ, which could influence the results of the children (Agostoni et al., 2009); although, the consensus supports a positive correlation between human milk feeding and enhancing IQ. Table 2.1 presents an overview of the benefits of human milk, as reported in the literature.

Table 2.1

Short- and Long-Term Benefits of a Human Milk Diet for Infants Compared with an Infant Formula Diet

Benefit	Reference	Comments
Decreased risk of gastrointestinal and respiratory infection in infancy	Quigley et al. (2024)	Meta-analysis of 12 trials, (*n* = 2,296)
	Frank et al. (2019)	International longitudinal birth cohort (*n* = 12,527)
	Davisse-Paturet et al. (2020)	Longitudinal birth cohort in France (*n* = 1,603)
Decreased risk of atopic conditions (e.g., eczema, asthma)	Dogaru et al. (2014)	Systematic review and meta-analysis
	Lodge et al. (2015)	Systematic review and meta-analysis
Lower incidence of diarrheal infection in infancy	Quigley et al. (2016)	Large cohort study (*n* = 15,809) from the United Kingdom
	Davisse-Paturet et al. (2020)	Longitudinal birth cohort in France (*n* = 12,527)
Lower incidence of acute otitis media	Bowatte et al. (2015)	Systematic review and meta-analysis
	Al-Nawaiseh et al. (2022)	Retrospective case-control study in Jordan (*n* = 196)
	Davisse-Paturet et al. (2020)	Longitudinal birth cohort in France (*n* = 12,527)
Reduced risk of childhood leukaemia	Amitay and Keinan-Boker (2015)	Systematic review and meta-analysis
Reduced risk of inflammatory bowel disease	Xu et al. (2017)	Systematic review and meta-analysis
	Agrawal et al. (2021)	Systematic review and meta-analysis
Decreased risk of T2DM in adolescence	Horta and de Lima (2019)	Systematic review and meta-analysis

Benefit	Reference	Comments
	Horta et al. (2015)	Systematic review and meta-analysis
	Agostoni et al. (2009)	A commentary by the ESPGHAN Committee
Decreased risk of obesity in childhood	Lee et al. (2019)	Large cohort study (*n* = 38,049) from Korea
	Horta et al. (2015)	Systematic review and meta-analysis
	Qiao et al. (2020)	Meta-analysis
	Yan et al. (2014)	Meta-analysis
Increased IQ in childhood	Amiel Castro et al. (2021)	Longitudinal birth cohort in England (*n* = 11,096)
	Lapidaire et al. (2022)	Prospective follow-up of a randomised control trial from England
	Peña-Ruiz et al. (2023)	Secondary data analysis of a longitudinal survey representative of the Mexican population (*n* = 35,000)

2.2 Human milk feeding recommendations for term infants

The World Health Organization (WHO), the United Nations International Children's Emergency Fund (UNICEF), and the American Academy of Pediatrics (AAP) recommend that human milk be the sole nutrition for term infants during the first 6 months of life due to the benefits discussed in 2.1 (Kramer & Kakuma, 2012; Meek & Noble, 2022). The recommendation aligns with that of the New Zealand Ministry of Health (2021). If human milk is unavailable, commercial infant formula is advised as the only suitable substitute (Ministry of Health, 2021). If exclusive breastfeeding is not possible, feeding some human milk alongside commercial infant formula is recommended as it will contribute to improved immunity and decreased risk of long-term health complications (Sankar et al., 2015).

2.3 Definition of preterm and feeding recommendations for preterm infants

Preterm infants are those born before 37 weeks of gestation, measured from the first day of the mother's last normal menstrual period (Khandre et al., 2022; World Health Organisation, 2009). A full-term pregnancy lasts between 37 and 42 completed weeks gestation, so preterm infants are born earlier than expected (American College of Obstetricians and Gynaecologists, 2013). Preterm infants are subcategorised based on the number of weeks of gestation: a) "extremely preterm" when the

infant is born less than 28 weeks gestation; b) “very preterm” when the infant is born between 28 to less than 32 weeks gestation and; “moderate to late preterm” when the infant is born between 32 to 37 weeks gestation (Khandre et al., 2022; Kramer et al., 2012). Prematurity is characterised by physiological immaturity, especially of the gastrointestinal, respiratory, immune, and renal systems, and the skin barrier (Humberg et al., 2020). Because of this, preterm infants often require specialised care in a neonatal intensive care unit (NICU) to help support their growth and development (McDonald et al., 2020). A human milk diet is associated with significant reductions in the incidence and severity of several prematurity-related morbidities, as shown in table 2.2. When a mother’s own milk (MOM) is not available, DHM is recommended as the best alternative by the WHO (2019), the ESPGHAN (Arslanoglu et al., 2013) and the AAP (Meek & Noble, 2022). The recommendations state that DHM should be used as a bridge to support the infant until the mother is able to establish her own supply.

Table 2.2

Impact of Human Milk Compared with Infant Formula on Morbidities Associated with Prematurity

Category	Morbidity	Protective effect of human milk
Gastrointestinal	Feeding intolerance attributed to immaturity of the gastrointestinal system (Fanaro, 2013; Indrio et al., 2022; Ortigoza, 2022)	Feeding intolerance occurred less often (Assad et al., 2016)
	Necrotising enterocolitis (NEC) (Gephart et al., 2012; Lamireau et al., 2023; Neu & Walker, 2011; Su et al., 2023)	A human milk diet significantly reduces NEC risk, protective effect increases with an exclusive HM diet and higher doses of human milk (Assad et al., 2016; Chowning et al., 2016; Hair et al., 2016; Miller et al., 2018; Quigley et al., 2024; Spiegler et al., 2016)
Respiratory	Bronchopulmonary dysplasia (BPD) (García-Muñoz Rodrigo et al., 2017; Stoll et al., 2010; Xu et al., 2023)	A human milk diet is associated with reduced risk of BPD, increased dose and duration of HM linked to greater protective effect (Hair et al., 2016; Patel et al., 2017; Spiegler et al., 2016; Verd et al., 2023; Xu et al., 2020)
Ophthalmologic	Retinopathy of prematurity (ROP) (Arya et al., 2025; Blazon et al., 2024; Sabri et al., 2022)	A human milk diet associated with decreased incidence of ROP (Bharwani et al., 2016; Goyal et al., 2024; Hair et al., 2016; Muneer et al., 2018; Spiegler et al., 2016)

2.4 Challenges of providing MOM in the NICU

Mothers of preterm infants can face significant challenges which contribute to difficulties feeding their infant with their milk. An immature suck-swallow-breathe coordination and weak sucking ability impairs effective oral feeding for preterm infants (Kamity et al., 2021; Lau, 2015). Additionally, maternal factors linked to preterm birth, including type 1 diabetes, obesity, multiple pregnancy (e.g. twin or triplet pregnancy), and pregnancy-induced hypertension may delay the onset of lactogenesis II (initiation of copious milk secretion), meaning the mother may struggle to produce enough milk for the infant. (Hartmann & Cregan, 2001; Hill et al., 2005; Ikonen et al., 2018). Moreover, when preterm infants require care in the NICU, the separation of mother and infant before lactogenesis II occurs, prevents early breastfeeding establishment, making continued breastfeeding more difficult (Levene et al., 2025; Meier et al., 2017; Parker et al., 2021). These factors may contribute to insufficient human milk intake in preterm infants, increasing the risk of morbidities associated with not consuming human milk.

2.5 Growth and nutritional requirements for preterm infants

Adequate growth for preterm infants is generally defined as 15–20 g/kg of body weight per day (Fenton et al., 2018). To maintain this growth, the ESPGHAN recommend the same energy and macronutrient intake as if the infant were still in utero (detailed in table 2.3) (Arslanoglu et al., 2019; Embleton et al., 2023). Whilst human milk is adequate for full term infants, it does not meet the energy and macronutrient needs of preterm infants (Belfort et al., 2019). This issue is further compounded during periods of illness or stress, common in preterm infants, where additional nutrients are crucial for proper recovery and growth (Hay, 1996; Ong & Belfort, 2021).

Table 2.3

Recommended Intakes of Energy and Macronutrients for Preterm Infants as per the ESPGHAN guidelines (Embleton et al., 2023)

Nutrient	Per kg/day
Energy	115–140 kcal
Fat	4.8–8.6 g
Protein	3.5–4.0 g
Carbohydrate	11.0–15.0 g

Note. ESPGHAN: European Society for Paediatric Gastroenterology, Hepatology and Nutrition

2.5.1 Need for fortification of human milk for preterm infants

Human milk alone may not provide the necessary energy and nutrients for optimal growth and development for preterm infants; therefore, it should be fortified to meet these needs. Commercial human milk fortifiers are supplements that are added to expressed human milk to enhance its energy, protein, mineral, and vitamin content (Ong & Belfort, 2021). A 2020 meta-analysis by Brown et al. (*n* = 1,456) assessed the effects of multi-nutrient fortified human milk versus unfortified human milk on preterm infant growth and showed that fortification led to a modest increase in the in-hospital growth rates of infants. Brown et al. report that the trials had small sample sizes and weak methodology; however, their findings were consistent with those by Salas et al. (2023) in a blind randomised trial (*n* = 150), and by Gu et al. (2021) which reviewed 16 articles, suggesting a consistent effect. These findings are particularly important in neonatal care, as inadequate growth for preterm infants is linked to poorer developmental outcomes (Morniroli et al., 2023).

The most common method used to fortify human milk is standard fortification. Standard fortification involves adding a fixed amount of fortifier per 100 mL of human milk, aiming to meet the recommended nutrient requirements of preterm infants (Arslanoglu et al., 2019). A drawback of standard fortification is that it assumes each mother's milk contains the reference nutrient concentrations and does not account for biological and external factors that may influence the energy and macronutrient content of an individual's milk. Consequently, mothers whose milk has lower than expected concentrations of nutrients may not meet the needs of preterm infants, even when fortified (Arslanoglu et al., 2019; Rochow et al., 2015). Adjustable and target methods are individualised approaches to human milk fortification. The adjustable fortification method involves tailoring the protein and energy content of milk based on the individual infant’s metabolic response, which is guided by blood urea nitrogen concentrations. The targeted fortification method analyses each milk sample and fortifies macronutrients individually to achieve each infant’s needs (Arslanoglu et al., 2019). A 2020 Cochrane review concluded that individualised fortification was generally associated with improved growth velocities in weight, length, and head circumference in preterm infants when compared to standard fortification, based on low- to moderate-certainty evidence (Fabrizio et al., 2020). Presently, there are no nationally standardised protocols for fortifying human milk in New Zealand, and practices vary between milk banks.

2.6 Macronutrient analysis of human milk

A standard device used to quantify the macronutrient components of human milk is the Miris Human Milk Analyser (HMA), developed in Sweden. The Miris HMA is widely used in clinical and research settings and is a reliable and practical method for analysing human milk, particularly protein and fat (Borràs-Novell et al., 2020; Groh-Wargo et al., 2016; Perrin et al., 2019). The Miris HMA requires a small sample (3 mL) of homogenised milk, heated to 40°C, and uses mid-infrared (mid-IR) spectroscopy to provide results in 60 seconds (Miris, 2025, July 8). Mid-IR measures macronutrients by analysing how the molecular bonds within a sample absorb specific wavelengths of infrared light (Skolik et al., 2018). The infrared data are translated into macronutrient concentrations using calibration models based on established reference methods: the Rose-Gottlieb method for fat, the Kjeldahl method for protein (measuring total nitrogen), and the oven-drying method for total solids. Total carbohydrate is calculated indirectly by subtracting the sum of fat, protein, and ash from the total solids. Energy content is calculated using the Atwater conversion factors (kcal/100 mL). Crude protein is calculated by multiplying the total nitrogen content by a standard conversion factor of 6.38. Crude protein includes all nitrogen-containing compounds in the milk, including non-protein nitrogen (NPN) such as urea, nucleic acids, and free amino acids. True protein is estimated by subtracting the estimated non-protein nitrogen (NPN) (~20%).

Clinically, true protein is more relevant than crude protein, as it more accurately reflects the bioavailable amino acid content (Belfort et al., 2024). Overestimating true protein due incorrect estimations of NPN, or a higher NPN content in a particular sample, may lead to under-fortification when DHM is used for preterm infants, which could lead to suboptimal growth and development. Additionally, it is important to consider crude or true protein when comparing protein values across different studies, where either may have been reported. There are also limitations to mid-IR based on carbohydrate measurement. Reported values depend on whether the instrument is calibrated to total carbohydrate (which includes lactose and human milk oligosaccharides (HMO)) or lactose alone (Belfort et al., 2024). Because HMOs are not digestible for energy, calibration to total carbohydrate may overestimate the available energy content of human milk (Belfort et al., 2024). Even though mid-IR carbohydrate analysis has a weaker correlation with reference methods, it does meet the Food and Drug Administration (FDA) standards of ± 15% (Belfort et al., 2024). In contrast, fat analysis by mid-IR

is considered reliable, provided that proper homogenisation and sample handling protocols are followed (Belfort et al., 2024).

2.7 Background to DHM and milk banking

Before the advent of commercialised infant formula, the sharing of breastmilk between women when a mother's milk was unavailable was common, with historical records indicating the practice dated back to classical antiquity (200 BC) (Moro, 2018; Stevens et al., 2009; Thorley, 2008). Informal arrangements of milk sharing included wet nursing and cross-feeding. Wet nursing refers to the practice in which a woman breastfeeds another's child, typically when the biological mother cannot or chooses not to breastfeed, often with a prior agreement or mutual obligation (Stevens et al., 2009; Thorley, 2008). Cross-feeding is an informal act of support where a lactating mother breastfeeds a child who is not her own without expectation of compensation (Thorley, 2008).

The formalisation of milk banks began in Vienna in 1909 (Jones, 2003) and rose in popularity around 1950 when artificial feeding with formula was recognised to have limitations compared to human milk (Unger & O'Connor, 2024). Advances in technology and hygiene led to the establishment of formal milk banks, providing a more reliable and safe system for distributing human milk to those who needed it most (Jones, 2003; Unger & O'Connor, 2024). Over time, the increased recognition of the health benefits of human milk, as outlined in 2.1, has driven the demand for these services, and more than 60 countries now have established milk banks (Israel-Ballard et al., 2024). Despite this, there is no global standard to guide the operational procedures of human milk banking (Israel-Ballard et al., 2024).

2.7.1 Milk banking practices and informal milk sharing in New Zealand

Five active non-profit milk banks are currently operating in New Zealand: in Christchurch, Wellington, Palmerston North, and Whangarei (Te Whatu Ora, 2024, July 8). The first started in Christchurch Women's Hospital, which supplies pasteurised DHM to preterm and other ill infants in the NICU (who have higher nutritional and immunity needs) whilst the mothers build their milk production (Te Whatu Ora, 2024, July 8). These formal milk banking practices in New Zealand are guided by the Operational Guidelines for Milk Banks in Australia and New Zealand (Human Milk Regulation Working Group, 2025, June 4). In addition to formal milk banking, informal human milk donation between women also occurs in New Zealand. Informal milk donation involves peer-to-peer milk sharing and is

usually facilitated by social media or word of mouth (Te Whatu Ora, 2024, July 8). A mixed-methods, national survey by Harris et al. (2024) explored the experiences of parents (*n* = 496) and health professionals (*n* = 232) with both informal and formal milk donation in New Zealand. They found that informal milk donation was common in hospitals and communities and was largely supported by both healthcare professionals and parents.

2.7.2 Pasteurisation of banked human milk

Pasteurisation is a critical process used by human milk banks to eliminate viral and bacterial pathogens, ensuring the microbiological safety of milk for the vulnerable preterm infant recipients. Holder pasteurisation (HoP) is the most commonly used method in non-profit settings and is the recommended method in most international human milk bank guidelines, including New Zealand (Arslanoglu et al., 2023; Human Milk Regulation Working Group, 2025; National Institute for Health and Clinical Excellence, 2010[1]; Unger & O'Connor, 2024; Updegrove et al., 2020; Weaver et al., 2019). HoP, which was developed specifically for human milk, involves heating milk to 62.5℃ for 30 minutes to minimise nutrient loss while still eliminating pathogens (Moro et al., 2019). While alternative methods, such as vat pasteurisation and retort sterilisation (table 2.4), are used in some commercial (for-profit) milk bank settings, this literature review focuses on HoP, as it is the standard method employed by non-profit human milk banks, including the current study. Table 2.4 provides an overview of key pasteurisation methods and their implications for milk quality. The specific effects of HoP on the macronutrient composition of DHM are explored in Section 2.9.5.

[1] Reviewed July 2018; no changes made to recommendations.

Table 2.4

Summary of Pasteurisation Methods Used for Human Milk

Method	Protocol	Used in	Impact on milk
Holder pasteurisation (HoP)	Milk is heated to 62.5℃ for 30 minutes in small batches in a water bath, followed by rapid cooling (Moro et al., 2019)	Recommended method in most international human milk bank guidelines	Kills most pathogens; preserves function of many bioactive components (e.g., lysozyme and sIgA) (Lima et al., 2017)
Vat pasteurisation	Milk is heated in a large industrial vat to 63℃ for 30 minutes, following by rapid cooling (Kim et al., 2023)	Dairy industry; some larger-scale human milk banks in the United States of America	Similar to HoP as vat pasteurisation follows similar protocols
Retort sterilisation	Milk in commercially sealed containers is heated to 121℃ for 5 minutes under pressure, typically at 1-1.4 bar above atmospheric pressure (Jimenez et al., 2024)	Commonly used in the canning industry; commercial (for-profit) milk banks (e.g., Prolacta Milk Bank, Medolac Milk Bank) Product is shelf-stable for up to 2 years	Eliminates all detectable microorganisms, including spores (Jimenez et al., 2024) Greater loss of proteins and bioactive components compared to vat pasteurisation (Conboy-Stephenson et al., 2024; Kim et al., 2023; Meredith-Dennis et al., 2018)

Note. sIgA = Secretory immunoglobulin A

2.8 What is already known about the composition of DHM

Human milk is a dynamic and complex fluid uniquely tailored to meet the nutritional and developmental needs of infants. Although the composition of MOM has been extensively studied and documented in the literature, the following section will focus on what is currently known about the macronutrient composition of DHM. This is important because unlike MOM, DHM is not produced in response to the needs of a specific infant and may vary significantly in composition. Understanding the macronutrient profile of DHM may help inform more precise fortification strategies and may improve preterm infant growth outcomes.

2.8.1 Energy content and macronutrient composition

Human milk is composed of approximately 87% water and 12.4 g/L of solid macronutrient components, including about 3.8% (3.5–4.0 g/L) fat, 1% (0.8–1.0 g/L) protein, and 7% (6.0–7.0 g/mL) carbohydrates (Kim & Yi, 2020; Kleinman & Greer, 2013). These macronutrients play vital roles in infant growth and development. Fats, including essential fatty acids and triglycerides, provide a large portion of an infant's energy intake, are crucial for brain and visual development, and support neural

tissue formation (Martin et al., 2016). Proteins, including whey, casein, and immunoglobulins, support growth and immune function, and also play a role in digestion and nutrient absorption (Lönnerdal, 2003; Martin et al., 2016). Carbohydrates, predominantly lactose, serve as the primary energy source, and prebiotic HMOs support gut health and influence the infant's microbiome (Belfort et al., 2024; Walsh et al., 2020). Although the composition of DHM varies significantly, especially after processing, pooling and due to interindividual influences, general patterns in its macronutrient content have been reported in the literature. Reference values for DHM macronutrients from the Academy of Nutrition and Dietetics, as cited by Perrin et al. (2020), are presented in table 2.5. These reference values likely reflect milk with varied characteristics; however, the specific details of how these values were derived (e.g., regarding processing, pooling, and donor characteristics) are not fully described. Section 2.9 explores how such factors can influence the macronutrient composition of DHM.

Table 2.5

Reference Values for the Macronutrient Composition of DHM (Academy of Nutrition and Dietetics, as cited in Perrin et al., 2020)

Nutrient	Value per 100 mL
Energy	65 kcal
Fat	3.2 g
Protein	1.2 g
Carbohydrate	7.8 g

Recent international literature reports that the energy content of DHM varies considerably, with mean values between 55.0 ± 8.27 and 76.0 ± 3.0 kcal/100 mL (Barbarska et al., 2017; Donovan et al., 2017; Kim et al., 2020; Lamb et al., 2021; Meredith-Dennis et al., 2018; Piemontese et al., 2019; Quitadamo et al., 2021; Walter et al., 2023). Importantly, several of these studies (Barbarska et al., 2017; Donovan et al., 2017; Meredith-Dennis et al., 2018; Piemontese et al., 2019; Quitadamo et al., 2021) reported mean energy values falling below the American of Nutrition and Dietetics reference for DHM (65.0 kcal/100 mL) (Perrin et al., 2020). Even similar studies reported varying results. For example, Piemontese et al. (2019) reported mean energy content 7.7% higher than that of Quitadamo et al. (2021), despite both studies analysing multi-donor pools of DHM from Italy, using the same analysis method and with comparable pool sizes and mean gestational age of donors. While this discrepancy may not be clinically significant, it highlights the potential influence of confounders such as lactation

stage or timing and method of breast expression. Studies from Australasia analysing single-donor pools with similar sample characteristics and methodologies to Piemontese et al. (2019) and Quitadamo et al. (2021) reported mean energy values that were 21.1% and 29.1% higher, respectively, than those reported by Quitadamo et al. (2021) (Lamb et al., 2021; Walter et al., 2023). Overall, these findings highlight the wide variability in the energy content of DHM and the potential influence of pooling, gestational age of the infant, lactation stage, and geographical location.

Across the broader literature on MOM, fat is consistently identified as the most variable macronutrient in human milk (Ballard & Morrow, 2013; Belfort et al., 2024). This variability is also evident in DHM, particularly in raw single-donor pools, which have shown a wide range, from 1.1–7.4 g/100 mL (*n* = 179; Barbarska et al., 2017), likely reflecting interindividual donor factors such as stage of lactation, individual physiology, and how the sample was collected (full versus partial breast expression). Walter et al. (2023) similarly reported a wide range of fat content in 95 single-donor pooled samples from Australia (1.46–9.39 g/100 mL); however, the authors highlight the likely influence of handling processes which may unevenly distribute fat content and contribute to the observed wide range. This is discussed further in section 2.9.5. In contrast, Meredith-Dennis et al. (2018) observed narrower fat ranges in multi-donor pooled DHM: 2.3–3.3 g/100 mL for retort sterilised, 3.9–4.6 g/100 mL for vat pasteurised, and 2.8–4.6 g/100 mL for HoP; however, the small sample sizes (*n* = 3) limit statistical power and generalisability. Mean concentrations across the literature typically fall between 2.55 ± 0.85–4.1 ± 0.2 g/100 mL, although differences in processing methods, donor characteristics, and measurement methods, as presented in table 2.7, limits the ability to draw reliable conclusions (Adhisivam et al., 2019; Barbarska et al., 2017; Donovan et al., 2017; John et al., 2019; Kim et al., 2020; Lamb et al., 2021; Meredith-Dennis et al., 2018; Moukarzel et al., 2017; Perrin et al., 2020; Quitadamo et al., 2021; Walter et al., 2023).

Reported protein content of DHM showed relatively consistent mean values across studies, despite differences in pooling practices, lactation stage, and analytical methods. Mean true protein values ranged from 0.7 ± 0.2–1.4 ± 0.3 g/100 mL (Adhisivam et al., 2019; Barbarska et al., 2017; Lamb et al., 2021; Quitadamo et al., 2021; Walter et al., 2023), and mean total protein values ranged from 0.8 ± 0.0–1.7 ± 0.2 g/100 mL (Adhisivam et al., 2019; Barbarska et al., 2017; Donovan et al., 2017; John et al., 2019; Meredith-Dennis et al., 2018; Perrin et al., 2017; Piemontese et al., 2019; Quitadamo et al., 2021; Walter et al., 2023). Studies using mid-IR analysis of single-donor, mature DHM reported

similar true protein values: 0.80 ± 0.10 g/100 mL (Lamb et al., 2021) and 0.93 ± 0.18 g/100 mL (Walter et al., 2023). Comparable values were also observed in multi-donor pools, with Quitadamo et al. (2021) reporting 0.89 ± 0.20 g/100 mL, and Piemontese et al. (2019) reporting 0.86 ± 0.20 g/100 mL. Interestingly, even where methodologies varied (such as pasteurised versus raw, or analysis technique), mean total protein values remained within a relatively narrow range, for example, 1.4 ± 0.2 g/100 mL in HoP DHM using Fourier-Transform Mid-Infrared (FT mid-IR) spectroscopy (Donovan et al., 2017) and 1.5 ± 0.20 g/100 mL (Perrin et al., 2017) in raw DHM using bicinchoninic acid assay. Together, these findings suggest that protein content in DHM is less variable than fat or energy, and is generally consistent between donors and with different processing conditions, when measured using validated techniques.

Carbohydrate concentrations similarly reflected relatively stable values across studies. Mean carbohydrate values ranged from 5.9 ± 0.7–8.25 ± 0.22 g/100 mL (Adhisivam et al., 2019; Barbarska et al., 2017; Donovan et al., 2017; Kim et al., 2020; Lamb et al., 2021; Meredith-Dennis et al., 2018; Walter et al., 2023). Studies analysing single-donor pooled, mature DHM reported similar total carbohydrate values: 8.2 ± 0.20 g/100 mL (Lamb et al., 2021), and 8.25 ± 0.22 g/100 mL (Walter et al., 2023). Kim et al. (2020) also reported similar mean total carbohydrate from mature DHM (mean lactation stage was 80 days postpartum): 8.2 ± 0.4 g/100 mL, although pooling method was not specified. Lower values were reported for HoP milk from early lactation stage (< 4 weeks postpartum): 5.9 ± 0.7 (multi-donor pool; Adhisivam et al., 2019), and 6.8 ± 0.20 (pooling not specified; preterm; Donovan et al. 2017), and raw milk from a single-donor pool: 7.4 ± 0.3 (Barbarska et al., 2017). Findings from Meredith-Dennis et al. (2018) showed that carbohydrate values remained stable across pooled DHM regardless of the pasteurisation method used (7.0–7.2 g/100 mL for vat and retort; 7.2–7.3 g/100 mL for HoP). Across the studies, there were differences in measurement techniques. For example, Donovan et al. (2017) and Meredith-Dennis et al. (2018) used FT mid-IR spectroscopy, a lab-based method that indirectly estimates carbohydrate content by measuring fat and protein and subtracting them from total solids. In contrast, Adhisivam et al. (2019), Barbarska et al. (2017), Kim et al. (2020), Lamb et al. (2021), and Walter et al. (2023) used mid-IR spectroscopy (via Miris HMA), which also indirectly estimates carbohydrates but has been shown to overestimate values compared to FT mid-IR (Perrin et al., 2019). Differences in carbohydrate measurements from different instruments have been shown to be statistically significant ($p < 0.0001$) (Perrin et al., 2019). Together,

these findings suggest that while minor variability exists, likely due to methodological differences, the carbohydrate content in DHM is relatively stable. To facilitate comparison across the literature, tables 2.6, 2.7, 2.8 and 2.9 summarises reported energy, fat, protein, and carbohydrate values in DHM respectively from selected studies.

Table 2.6

Reported Energy Composition of DHM Across Selected Studies

Study & Country	Pasteurisation method	Pooling	Sample size	Gestational age of infant	Lactation stage	Analysis method	Mean (±SD) and/or range (g/100 mL)
Quitadamo et al. (2021) Italy	HoP	Multi-donor pool (3 donors)	*n* = 100	Mean GA = 37.59 weeks	Donation commenced within 1 month postpartum (66.7%), 1-3 months postpartum (27.6%), 3+ months postpartum (5.7%)	Mid-IR via Miris HMA	55.0 ± 8.3
Meredith-Dennis et al. (2018) USA	Retort	Multi-donor pool (200 donors)	*n* = 3	Mixed, term and preterm	Mixed, early and mature	FT Mid-IR	58.8 ± 5.7 52.4 – 62.9
Piemontese et al. (2019) Italy	HoP	Multi-donor pool (3.5 ± 1.7 donors)	*n* = 460	Mean GA = 38.51 ± 2.73 weeks	Mature	Mid-IR via Miris HMA	59.4 ± 7.8
Barbarska et al. (2017) Poland	Raw	Single-donor pool	*n* = 179 (45 donors)	24.4% from preterm	Not specified	Mid-IR via Miris HMA	61.7 ± 6.50 46.0 – 86.0
Meredith-Dennis et al. (2018) USA	HoP	Multi-donor pool (2 donors)	*n* = 3	11% from preterm	< 1 month for preterm; < 2 months for term	FT Mid-IR	64.6 ± 8.5 58.1 – 74.0
Donovan et al. (2017) USA	HoP	Not specified	*n* = 21 Term	Not specified	Collected during first year postpartum	FT Mid-IR	64.9
Kim et al. (2020) South Korea	HoP	Not specified	*n* = 54 (26 donors)	Term	Mean 80 days, median 40.5 days postpartum	Mid-IR via Miris HMA	65.2 ± 7.0
Lamb et al. (2021) New Zealand	HoP	Single-donor pool	*n* = 50	Term	Mature	Mid-IR via Miris HMA	68.0 ± 9.0
Meredith-Dennis et al. (2018) USA	Vat	Multi-donor pool (250 donors)	*n* = 3	Mixed, term and preterm	Mixed, early and mature	FT Mid-IR	69.0 ± 1.4 67.6 – 70.3
Donovan et al. (2017) USA	HoP	Not specified	*n* = 16 Preterm	Not specified	Collected during first 4	FT Mid-IR	69.3

Study & Country	Pasteurisation method	Pooling	Sample size	Gestational age of infant	Lactation stage	Analysis method	Mean (±SD) and/or range (g/100 mL)
					weeks postpartum		
Walter et al. (2023) Australia	HoP	Single-donor pool	*n* = 95	Mixed, term (*n* = 71) and preterm (*n* = 24)	Mean duration lactation 110.5 days, median 91.0 days, minimum 7 days, maximum 345 days	Mid-IR via Miris HMA	73.7 ± 9.3 50.7 – 125.0 Difference between term and preterm not statistically significant
Lamb et al. (2021) New Zealand	HoP	Single-donor pool	*n* = 11	Preterm	Mature	Mid-IR via Miris HMA	76.0 ± 3.0

Note. Although the primary aims of Adhisivam et al. (2019), Donovan et al. (2017), Kim et al. (2020), Piemontese et a. (2019) and Quitadamo et al. (2021) were to address the effects of HoP on DHM, these studies also reported macronutrient composition in DHM samples, providing relevant data for inclusion in this review.
Preterm defined as milk expressed <37 weeks gestation
Term defined as milk expressed ≥37 weeks gestation
USA = United States of America
SD = Standard deviation
HMA = Human milk analyser

Table 2.7

Reported Fat Composition of DHM Across Selected Studies

Study & Country	Pasteurisation method	Pooling	Sample size	Gestational age of infant	Lactation stage	Analysis method	Mean (±SD) and/or range (g/100 mL)
Quitadamo et al. (2021) Italy	HoP	Multi-donor pool (3 donors)	*n* = 100	Mean GA = 37.59 weeks	Donation commenced within 1 month postpartum (66.7%), 1-3 months postpartum (27.6%), 3+ months postpartum (5.7%)	Mid-IR via Miris HMA	2.6 ± 0.9
Adhisivam et al. (2019) India	HoP	Multi-donor pool (90 donors)	*n* = 30	Mean GA 36.5 weeks	Transitional milk (5–10 days postpartum)	Mid-IR via Miris HMA	2.7 ± 0.5
Piemontese et al. (2019) Italy	HoP	Multi-donor pool (3.5 ± 1.7 donors)	*n* = 460	Mean GA = 38.51 ± 2.73 weeks	Mature	Mid-IR via Miris HMA	2.8 ± 0.8
Moukarzel et al. (2017) Canada	HoP	Multi-donor pool	*n* = 45	Term	Not specified	Creamatocrit	2.8 ± 1.0 1.1 – 4.8
Meredith-Dennis et al. (2018) USA	Retort	Multi-donor pool (200 donors)	*n* = 3	Not specified	Not specified	FT Mid-IR	3.0 ± 0.6 2.3 – 3.3
Kim et al. (2020) South Korea	HoP	Not specified	*n* = 54 (26 donors)	Term	Mean 80 days, median 40.5 days postpartum	Mid-IR via Miris HMA	3.1 ± 0.8
Barbarska et al. (2017) Poland	Raw	Single-donor pool	*n* = 179 (45 donors)	24.4% from preterm	Not specified	Mid-IR via Miris HMA	3.1 ± 0.8 1.1 – 7.4
Meredith-Dennis et al. (2018) USA	HoP	Multi-donor pool (2 donors)	*n* = 3	11% from preterm	< 1 month for preterm; < 2 months for term	FT Mid-IR	3.4 ± 0.9 2.8 – 4.6
Lamb et al. (2021) New Zealand	HoP	Single-donor pool	*n* = 50	Term	Mature	Mid-IR via Miris HMA	3.5 ± 0.9
Perrin et al. (2016) USA	Raw	Multi-donor pool (51 donors)	*n* = 33	Term	Mean 4.8 ± 3.3 months, median 4.0 months postpartum	Nuclear Magnetic Resonance (NMR) spectroscopy	3.5 ± 1.7
Donovan et al. (2017) USA	HoP	Not specified	*n* = 21	Term	Collected during first year postpartum	FT Mid-IR	3.7 ± 0.4

Study & Country	Pasteurisation method	Pooling	Sample size	Gestational age of infant	Lactation stage	Analysis method	Mean (±SD) and/or range (g/100 mL)
Donovan et al. (2017) USA	HoP	Not specified	*n* = 16	Preterm	Collected during first 4 weeks postpartum	FT Mid-IR	3.9 ± 0.6
Walter et al. (2023) Australia	HoP	Single-donor pool	*n* = 95	Mixed, term (*n* = 71) and preterm (*n* = 24)	Mean duration lactation 110.5 days, median 91.0 days, minimum 7 days, maximum 345 days	Mid-IR via Miris HMA	3.9 ± 1.0 1.5 – 9.4 Mean term = 3.7 g/100 ml; mean preterm = 4.3 g/100 ml (*p* = 0.014)
Lamb et al. (2021) New Zealand	HoP	Single-donor pool	*n* = 11	Preterm	Mature	Mid-IR via Miris HMA	4.0 ± 0.3
Meredith-Dennis et al. (2018) USA	Vat	Multi-donor pool (250 donors)	*n* = 3	Not specified	Not specified	FT Mid-IR	4.1 ± 0.2 3.9 – 4.6
John et al. (2019) USA	HoP	Single-donor pool	*n* = 1111 (443 donors)	13.1% from preterm	33.4% transition milk; 61.0% mature milk; 5% mature milk	FT Mid-IR	2.7 – 5.9

Note. Although the primary aims of Adhisivam et al. (2019), Donovan et al. (2017), Kim et al. (2020), Piemontese et a. (2019) and Quitadamo et al. (2021) were to address the effects of HoP on DHM, these studies also reported macronutrient composition in DHM samples, providing relevant data for inclusion in this review.
Preterm defined as milk expressed <37 weeks gestation
Term defined as milk expressed ≥37 weeks gestation
USA = United States of America
SD = Standard deviation
HMA = Human milk analyser

Table 2.8

Reported Protein Composition of DHM Across Selected Studies

Study & Country	Pasteurisation method	Pooling	Sample size	Gestational age of infant	Lactation stage	Analysis method	Mean (±SD) and/or range (g/100 mL)
Barbarska et al. (2017) Poland	Raw	Single-donor pool	*n* = 179 (45 donors)	24.4% from preterm	Not specified	True protein Mid-IR via Miris HMA	0.7 ± 0.2 0.3 – 1.2
Lamb et al. (2021) New Zealand	HoP	Single-donor pool	*n* = 50	Term	Mature	True protein Mid-IR via Miris HMA	0.8 ± 0.1
Walter et al. (2023) Australia	HoP	Single-donor pool	*n* = 95	Mixed, term (*n* = 71) and preterm (*n* = 24)	Mean duration lactation 110.5 days, median 91.0 days, minimum 7 days, maximum 345 days	True protein Mid-IR via Miris HMA	0.9 ± 0.2 0.6 – 1.6 Difference between term and preterm not statistically significant
Lamb et al. (2021) New Zealand	HoP	Single-donor pool	*n* = 11	Preterm	Mature	True protein Mid-IR via Miris HMA	1.1 ± 0.2
Adhisivam et al. (2019) India	HoP	Multi-donor pool (90 donors)	*n* = 30	Mean GA 36.5 weeks	Transitional milk (5-10 days postpartum)	True protein Mid-IR via Miris HMA	1.4 ± 0.3
Meredith-Dennis et al. (2018) USA	Retort	Multi-donor pool (200 donors)	*n* = 3	Not specified	Not specified	Total protein FT Mid-IR	0.8 ± 0.0 0.7 – 0.8
Meredith-Dennis et al. (2018) USA	Vat	Multi-donor pool (250 donors)	*n* = 3	Not specified	Not specified	Total protein FT Mid-IR	0.8 ± 0.0 0.8 – 0.8
Barbarska et al. (2017) Poland	Raw	Single-donor pool	*n* = 179 (45 donors)	24.4% from preterm	Not specified	Total protein Mid-IR via Miris HMA	0.8 ± 0.2 0.4 – 1.5
Meredith-Dennis et al. (2018) USA	HoP	Multi-donor pool (2 donors)	*n* = 3 11% from preterm	11% from preterm	< 1 month for preterm; < 2 months for term	Total protein FT Mid-IR	1.0 ± 0.1 0.9 – 1.1
Donovan et al. (2017) USA	HoP	Not specified	*n* = 21	Term	Collected during first year postpartum	Total protein FT Mid-IR	1.1 ± 0.1
Walter et al. (2023) Australia	HoP	Single-donor pool	*n* = 95	Mixed, term (*n* = 71) and preterm (*n* = 24)	Mean duration lactation 110.5 days, median 91.0 days, minimum 7 days, maximum 345 days	Total protein Mid-IR via Miris HMA	1.2 ± 0.2 0.7 – 2.0 Difference between term and preterm not statistically significant

Study & Country	Pasteurisation method	Pooling	Sample size	Gestational age of infant	Lactation stage	Analysis method	Mean (±SD) and/or range (g/100 mL)
Donovan et al. (2017) USA	HoP	Not specified	*n* = 16	Preterm	Collected during first 4 weeks postpartum	Total protein FT Mid-IR	1.4 ± 0.2
Perrin et al. (2016) USA	Raw	Multi-donor pool (51 donors)	*n* = 33	Term	Mean 4.8 ± 3.3 months, median 4.0 months postpartum	Total protein Bicinchoninic acid assay	1.5 ± 0.2
Adhisivam et al. (2019) India	HoP	Multi-donor pool (90 donors)	*n* = 30	Mean GA 36.5 weeks	Transitional milk (5-10 days postpartum)	Total protein Mid-IR via Miris HMA	1.7 ± 0.2
John et al. (2019) USA	HoP	Single-donor pool	*n* = 1111 (443 donors)	13.1% from preterm	33.4% transition milk; 61.0% mature milk; 5% mature milk	Total protein FT Mid-IR	0.8 – 2.2
Kim et al. (2020) South Korea	HoP	Not specified	*n* = 54 (26 donors)	Term	Mean 80 days, median 40.5 days postpartum	Protein type not specified Mid-IR via Miris HMA	1.0 ± 0.2

Note. Although the primary aims of Adhisivam et al. (2019), Donovan et al. (2017), Kim et al. (2020), Piemontese et al. (2019) and Quitadamo et al. (2021) were to address the effects of HoP on DHM, these studies also reported macronutrient composition in DHM samples, providing relevant data for inclusion in this review.
True protein refers to the actual amount of bioavailable protein, whereas total protein includes non-protein nitrogen, potentially overestimating actual protein content.
Preterm defined as milk expressed <37 weeks gestation
Term defined as milk expressed ≥37 weeks gestation
USA = United States of America
SD = Standard deviation
HMA = Human milk analyser

Table 2.9

Reported Carbohydrate Composition of DHM Across Selected Studies

Study & Country	Pasteurisation method	Pooling	Sample size	Gestational age of infant	Lactation stage	Analysis method	Mean (±SD) and/or range (g/100 mL)
Adhisivam et al. (2019) India	HoP	Multi-donor pool (90 donors)	*n* = 30	Mean GA 36.5 weeks	Transitional milk (5-10 days postpartum)	Mid-IR via Miris HMA	5.9 ± 0.7
Donovan et al. (2017) USA	HoP	Not specified	*n* = 21	Term	Collected during first year postpartum	FT Mid-IR	6.7 ± 0.2
Donovan et al. (2017) USA	HoP	Not specified	*n* = 16	Preterm	Collected during first 4 weeks postpartum	FT Mid-IR	6.8 ± 0.2
Meredith-Dennis et al. (2018) USA	Retort	Multi-donor pool (200 donors)	*n* = 3	Not specified	Not specified	FT Mid-IR	7.0 – 7.2
Meredith-Dennis et al. (2018) USA	Vat	Multi-donor pool (250 donors)	*n* = 3	Not specified	Not specified	FT Mid-IR	7.0 – 7.2
Meredith-Dennis et al. (2018) USA	HoP	Multi-donor pool (2 donors)	*n* = 3	11% from preterm	< 1 month for preterm; < 2 months for term	FT Mid-IR	7.2 – 7.3
Barbarska et al. (2017) Poland	Raw	Single-donor pool	*n* = 179 (45 donors)	24.4% from preterm	Not specified	Mid-IR via Miris HMA	7.4 ± 0.3 6.3 – 7.9
Lamb et al. (2021) New Zealand	HoP	Single-donor pool	*n* = 50	Term	Mature	Mid-IR via Miris HMA	7.9 ± 0.3
Lamb et al. (2021) New Zealand	HoP	Single-donor pool	*n* = 11	Preterm	Mature	Mid-IR via Miris HMA	8.2 ± 0.2
Walter et al. (2023) Australia	HoP	Single-donor pool	*n* = 95	Mixed, term (*n* = 71) and preterm (*n* = 24)	Mean duration lactation 110.5 days, median 91.0 days, minimum 7 days, maximum 345 days	Mid-IR via Miris HMA	8.3 ± 0.2 7.0 – 9.8 Difference between term and preterm not statistically significant
Kim et al. (2020) South Korea	HoP	Not specified	*n* = 54 (26 donors)	Term	Mean 80 days, median 40.5 days postpartum	Mid-IR via Miris HMA	8.2 ± 0.4

Note. Although the primary aims of Adhisivam et al. (2019), Donovan et al. (2017), Kim et al. (2020), Piemontese et al. (2019) and Quitadamo et al. (2021) were to address the effects of HoP on DHM, these studies also reported macronutrient composition in DHM samples, providing relevant data for inclusion in this review.
Preterm defined as milk expressed <37 weeks gestation
Term defined as milk expressed ≥37 weeks gestation
USA = United States of America
SD = Standard deviation
HMA = Human milk analyser

2.9 Variation in macronutrient composition of DHM

Research examining the macronutrient composition of DHM has revealed significant variability due to the differences in biological and external factors. This section will examine the effects of donor and infant characteristics, lactation stage, circadian rhythm, and collection and processing on the energy, fat, protein, and carbohydrate composition of DHM.

2.9.1 Influence of donor characteristics (ethnicity, parity, body mass index, diet, and age)

Donor characteristics, such as ethnicity, parity (the number of previous births), body mass index (BMI), diet, and age have been explored as potential contributors to the variability in human milk composition. Current evidence on the influence of donor ethnicity on the macronutrient composition of DHM is limited. An observational study by Butts et al. (2018) conducted in New Zealand compared samples of MOM from mothers of Asian, Māori, Pacific Island, and European descent. Whilst no significant ethnic differences were found in macronutrient content, differences in fatty acid profiles were observed between the ethnic groups; however, these showed a slight positive correlation with the respective donor dietary fatty acid intake. Due to limited evidence, the extent to which ethnic differences influence the macronutrient profile of DHM specifically, and the clinical implications of this, remains unclear.

The influence of parity on the composition of DHM is not fully understood. Bachour et al. (2011) report that the lipid concentration in mature milk increased with parity, based on a sample of MOM from 64 lactating women, suggesting a possible physiological adaptation over successive pregnancies, but only up to the third pregnancy. Conversely, Léké et al. (2019) (also studying MOM) report women who have only given birth once had milk with a higher fat concentration (n = 88). Mangel et al. (2017), examining colostrum from 67 mothers, observed a positive correlation between carbohydrate as well as fat content with higher parity. Burianova et al. (2019) reported similar findings from 1,558 MOM samples from 192 lactating women. The variations in the lactation stages studied (colostrum compared to mature milk) limit the comparability of findings across these studies. Furthermore, none of these studies focused on DHM, which is often pooled and processed, potentially altering or masking influences of parity. Further research is required to increase understanding about how parity influence milk composition and whether these effects are reflected in DHM.

Emerging evidence suggests that donor BMI may influence the macronutrient composition of DHM, particularly its protein and fatty acid content. Research on MOM indicates that the total protein and lipid content is significantly, and positively correlated with increased donor BMI (Borràs-Novell et al., 2023; Burianova et al., 2019; Chang et al., 2015; Dritsakou et al., 2017; Lithoxopoulou, Gkampeta, et al., 2025; Mangel et al., 2017). A higher BMI has also been associated with increased concentrations of saturated fats and omega-6 fatty acids, and decreased concentrations of alpha-linoleic acid (ALA), docosahexaenoic acid (DHA), and monounsaturated fatty acids (MUFA) in MOM (de la Garza Puentes et al., 2019; Tekin-Guler et al., 2023). To date, the study by Bzikowska-Jura et al. (2021) appears to be the only one that has investigated the association between donor BMI, and the composition of DHM, although their findings did not indicate any significant correlation. Furthermore, Bzikowska-Jura et al. (2021) argue that BMI is not a direct indicator of adiposity, as it does not differentiate between fat mass and lean body mass; therefore, may limit its validity as a measure for assessing the influence of BMI on human milk protein and lipid profiles.

Whilst donor diet does not significantly influence the total fat content of human milk, it has been shown to affect the fatty acid composition. Several studies have shown that a higher dietary intake of polyunsaturated fatty acids (PUFAs), particularly omega-3 fatty acids such as ALA, DHA, and eicosapentaenoic acid (EPA), may lead to increased concentrations of these fatty acids in MOM (Armand et al., 2018; Aumeistere et al., 2019; Kim et al., 2017; Liu et al., 2019). No significant correlations have been observed between maternal diet and MOM protein or carbohydrate content (Aumeistere et al., 2019; Butts et al., 2018; Bzikowska-Jura et al., 2021; Kim et al., 2017).

Maternal age has not been consistently identified as a factor influencing the macronutrient composition of human milk. Only one study was identified (Mills et al., 2019) (*n* = 2,966; United Kingdom), that directly examined the relationship between donor age and the macronutrient composition of DHM. The authors found no significant association. In contrast, research on MOM suggests that donor age may influence milk composition, particularly in the early postpartum period. Argov-Argaman et al. (2017) (*n* = 34; Israel) and Dritsakou et al. (2017) (*n* = 120; Greece) found that older mothers produced transitional milk with a higher fat content. Additionally, Dritsakou et al. (2017) also reported a higher fat content in mature milk from older mothers. Argov-Argaman et al. (2017) classified older mothers as those aged ≥ 37 years; however, Dritsakou et al. (2017) did not specify exact age groups. Moreover, Hochman et al. (2024) (*n* = 99; Brazil) found that mothers between the

ages of 20−34 years produced colostrum with a higher fat content than mothers < 20 years or > 34 years, and while Borràs-Novell et al. (2023) (*n* = 277; Spain) found no associations with fat, they did observe slightly higher true protein in older mothers (≥ 35 years) during the first 4 weeks postpartum. Conversely, Fernández-Tuñas et al. (2025) (*n* = 52; Spain) found no associations between maternal age (≥ 35 years vs. < 35 years) and milk macronutrients at any point during lactation. The authors of these studies note that discrepancies may be due to confounders related to advanced age, such as elevated serum cholesterol or obstetric complications (Borràs-Novell et al., 2023; Dritsakou et al., 2017), or differences in study methodologies (Hochman et al., 2024). Additionally, many of the studies had small sample sizes, making it difficult to detect true effects, and inconsistent age classifications which limits the ability for comparison. Overall, the influence of age on the macronutrient composition of human milk, particularly in donor milk bank settings, remains unclear. In summary, donor characteristics can lead to interindividual variability in the macronutrient composition of human milk, which may have implications for infant nutrition and developmental outcomes.

2.9.2 Influence of infant characteristics (gestational age and sex)

Infant characteristics, such as gestational age (term versus preterm), birth weight, and sex have been explored as potential contributors to the variability in human milk composition. The macronutrient composition of human milk varies based on the gestational age of the mother's infant at birth. Preterm (< 37 weeks) milk generally has higher protein and fat compared to term milk (≥ 37 weeks). Research examining DHM has found similar patterns. For example, Tanaka et al. (2023) and Holritz et al. (2024) found a 31.6% and 5.1% increase in the protein content of preterm compared to term milk, respectively, and Quitadamo et al. (2018) found that donors who gave birth at < 29 weeks gestation had milk with a higher protein content compared to those who delivered at term. Conversely, Walter et al. (2023) did not find a statistically significant difference between the protein content in preterm versus term milk, attributing differences seen were due to lactation stage, rather than gestational age. Across the literature, fat was also reported to be significantly higher from preterm samples, with Holritz et al. (2024) and Walter et al. (2023) reporting a 5.1% and 23.8% increase in preterm versus term samples, respectively; however, Quitadamo et al. (2018) did not find a significant correlation between fat and a gestational age of < 29 weeks. Carbohydrate content in DHM was not found to be significantly influenced by gestational age by Walter et al. (2023) or Quitadamo et al. (2018).

Research by Mills et al. (2019), revealed that in their unadjusted analyses, donor milk from preterm births had a significantly higher mean protein content compared to term donor milk ($p < 0.001$). However, after adjusting for factors, such as lactation stage, gestational age was no longer a significant predictor, but the authors did find that donors who had given birth to preterm infants who were also born small for gestational age (SGA) had milk with higher energy, fat, and protein compared to donors who gave birth to an infant that was appropriate for gestational age (AGA), even after adjusting for other variables. They also found that carbohydrate content was significantly lower in the preterm SGA group compared with the term AGA group, even after controlling for confounding variables. Overall, in Mills' et al. (2019) study, gestational age alone did not significantly predict macronutrient content, and the authors suggested that stage of lactation or SGA status may be a more important determinant for protein concentrations in DHM.

The findings from the discussed studies are presented in table 2.10 and highlight the variability in macronutrient composition of DHM by gestational age. Importantly, many of the studies reported mean energy, fat, and carbohydrate values in preterm DHM samples which fall below the reference ranges recommended by the Academy of Nutrition and Dietetics (AND) for preterm infants (2.1 g/100 mL protein; 2.4 g/100 mL fat; 7.5 g/100 mL carbohydrate; 77 kcal/100 mL energy), as cited in Perrin et al. (2020). Given the elevated nutritional needs of preterm infants, such discrepancies bring attention to the necessity of individualised fortification approaches to ensure DHM meets the growth and developmental needs of the recipient preterm infants.

If human milk composition varies by infant sex, DHM from mothers nursing female infants may not meet the nutritional needs of male recipients, and vice versa. No studies were identified that examined sex-based differences in the macronutrients of DHM. There has been research on the sex-based differences in MOM, but the evidence is mixed. Fischer Fumeaux et al. (2019) conducted a robust longitudinal study ($n = 500$) and found a statistically significant increase in fat for male infants, especially among those preterm, though *r*- and exact *p*-values were not reported. In contrast, a study by Suwaydi et al. (2024), also longitudinal and with a large sample size ($n = 501$), found no sex-based differences in fat content with a mean difference of –0.09 (95% Confidence interval (CI): -0.23, 0.18; $p = 0.82$), highlighting the inconsistent results across studies. A cross-sectional study by Quinn (2013) ($n = 103$) similarly found no sex-based differences. Fischer Fumeaux et al. (2019) analysed milk from full breast expression, whereas Suwaydi et al. (2024) only collected 'fore' milk samples and Quinn

(2013) used mid-feed samples. Observational studies by Hahn et al. (2016) and Powe et al. (2010) analysed samples from full breast expression. Hahn et al. (2016) reported lower carbohydrate (Odds Ratio (OR) = 0.56, p = 0.012) and lower energy (OR = 0.00, p = 0.017) in milk for female infants, and Powe et al. (2010) reported milk for male infants had higher energy (p < 0.001), although the small sample size for Powe et al. (2010) (n = 25) limits generalisability. Lower quality studies have also examined sex-based differences in human milk but lack methodological rigor (Alhindi et al., 2019; Dafaallah et al., 2018; Lithoxopoulou, Karastogiannidou, et al., 2025). Future studies should optimally control for key confounders (e.g., lactation stage), and differentiate between DHM and MOM to determine whether infant sex meaningfully impacts DHM milk composition.

Table 2.10

Comparison of Energy and Macronutrient Content in Preterm versus Term DHM

Study & country	Study size		Macronutrient	Preterm mean (±SD) (1 d.p.) g/100 mL	Term mean (±SD) (1 d.p.) g/100 mL	Statistical significance
	Preterm	Term				
Tanaka et al. (2023) Japan	*n* = 41	*n* = 93	Total protein	1.2 †	1.0 †	*p* < 0.001
Mills et al. (2019) United Kingdom	*n* = 284	*n* = 887	True protein	1.0 ± 0.3 §	0.8 ± 0.2 §	*p* < 0.001
			Fat	3.3 ± 0.9	2.9 ± 1.9	*p* < 0.001
			Carbohydrate	7.1 ± 0.4	7.1 ± 0.5	*p* < 0.05
			Energy	63.5 ± 7.5	59.0 ± 8.3	*p* < 0.001
Quitadamo et al. (2018) Italy	*n* = 10 (4.4% < 32 weeks)	*n* = 80	Protein	1.7*	Not reported	*p* = 0.014
			Fat	4.0*	Not reported	*p* = 0.4202
			Carbohydrate	6.5*	Not reported	*p* = 0.3443
			Energy	67.0*	Not reported	*p* = 0.014
Holritz et al. (2024) Denmark	*n = 46* (20 donors)	*n* = 285 (45 donors)	Protein †	1.4 ± 0.3	1.2 ± 0.2	*p* < 0.001
			Fat	4.0 ± 0.5	3.8 ± 0.8	*p* = 0.002
			Energy	75.9 ± 4.8	72.3 ± 7.4	*p* < 0.001
Walter et al. (2023) Australia	*n* = 24	*n* = 71	True protein	1.0 §	0.9 §	*p* = 0.591
			Total protein	1.3 †	1.1 †	*p* = 0.528
			Fat	4.7	3.7	*p* = 0.014
			Carbohydrate	8.3	8.2	*p* = 0.399

Note. The values from Mills et al. (2019) come from unadjusted t-tests. Adjusted analyses found that gestational age alone did not significantly affect protein and fat content. Carbohydrate showed no consistent difference. True protein refers to the actual amount of bioavailable protein, whereas total protein includes non-protein nitrogen, potentially overestimating actual protein content.
Preterm defined as milk expressed <37 weeks gestation
Term defined as milk expressed ≥37 weeks gestation
SD = standard deviation
* Values are for milk from < 28 weeks gestation; the remainder of the preterm values are from <37 weeks
† Type of protein not specified

2.9.3 Influence of lactation stage

Lactation progresses through 3 main stages: colostrum, transitional, and mature milk. Colostrum is the first fluid produced, typically secreted around the third day postpartum. It is a rich source of immune-protective and growth factors (e.g., secretory IgA, lactoferrin, and epidermal growth factor). Transitional milk is produced after colostrum, lasting approximately 1 week and is higher in volume. By approximately 2 weeks postpartum, mature milk is established, providing the infant's long-term nutritional needs (Shah et al., 2022). Studies have consistently observed longitudinal variation in the composition of MOM over these different stages of lactation. Protein concentrations have been observed to be the highest in colostrum and decrease as lactation progresses (Grote et al., 2016; Kreissl et al., 2016; Lithoxopoulou, Gkampeta, et al., 2025); whereas fat and carbohydrate concentrations are lowest in colostrum and increase throughout transitional milk (Dritsakou et al., 2017; Lithoxopoulou, Gkampeta, et al., 2025; Sever et al., 2015). Similar patterns have been reported in DHM (Holritz et al., 2024; John et al., 2019; Mills et al., 2019; Muts et al., 2025; Saarela et al., 2005; Walter et al., 2023).

Most studies evaluating DHM indicate that protein concentrations decline as lactation progresses. A longitudinal study by Saarela et al. (2005) (n = 253) reported a gradual decrease in protein content from 1.98 g/100 mL in milk from 1 week postpartum to 1.14 g/100 mL in milk from 6 months postpartum ($p < 0.001$). Similar findings were observed in cross-sectional studies by Walter et al. (2023) (n = 200), and John et al. (2019) (n = 1,111). A more recent longitudinal study by Muts et al. (2025) (n = 820) also found a decline in protein concentration; however, this decline extended over 8 months before stabilising. In contrast, carbohydrate concentrations remained stable throughout lactation (Muts et al., 2025; Saarela et al., 2005; Walter et al., 2023). Evidence on the variability of fat appears mixed, with increases observed after 8 months in the study by Muts et al. (2025), although Walter et al. (2023) found no significant change by stage of lactation. It is important to note that the studies with a cross-sectional design may mask the interindividual variations in milk composition, potentially influencing the interpretation of nutrient patterns across lactation. Additionally, these studies only focused on DHM produced within the first 6–12 months of lactation, resulting in the composition of milk beyond this period being less well understood. There is limited documented information about the quantities of milk being donated to milk banks from each stage of lactation (dos

Santos et al., 2024); however, if the majority of donations are mature milk, the lower protein content may not meet the higher protein needs of preterm infant recipients.

The current Operational Guidelines for Milk Banks in Australia and New Zealand (Human Milk Regulation Working Group, 2025, June 4) recommend accepting donations only from women up to 12 months postpartum, with a preference for those who are less than 6 months postpartum. This guideline is consistent with several international milk banks (Italian Association of Donated Human Milk Banks, 2016; National Health Serivce, 2025, June 26; Oschsner Health, 2025, June 6). The guideline is in place as milk expressed after 12 months postpartum was thought to be of less nutritional value; however, this claim has been refuted. Czosnykowska-Łukacka et al. (2018), analysing MOM, observed a positive correlation with both true protein ($p < 0.05$) and fat ($p < 0.05$) during lactation up to the 48th month. Additionally, mean carbohydrate concentrations remained similar between the groups of 1–12 and 12–18 months lactation before significantly decreasing in the 18–24 months group and then stabilising. Additional longitudinal studies by Muts et al. (2025) (n = 820) and Perrin et al. (2017) (n = 131), which reported total protein content, observed similar patterns to those of Czosnykowska-Łukacka et al. (2018) and suggest that restricting milk donation to the first 6–12 months of lactation may be unnecessary.

2.9.4 Circadian variation

Circadian variation refers to the natural changes in the composition of human milk over 24 hours. Although not all constituents of human milk show circadian patterns, growing evidence suggests that specific nutrients vary throughout the day and night. Most research on circadian variation in human milk composition has focused on MOM, and only one identified study specifically examined DHM (Bzikowska-Jura et al., 2021). This study observed a significant positive correlation between afternoon milk expressions and higher fat content in DHM (r = 0.289, p = 0.044). Their findings are consistent with the existing literature examining MOM (Andreas et al., 2015; Jackson et al., 1988; Kent et al., 2006; Kociszewska-Najman et al., 2012; Lubetzky et al., 2006). No circadian variation has been identified for carbohydrates (Khan et al., 2013; Mitoulas et al., 2002; Paulaviciene et al., 2020), nor total protein (Khan et al., 2013; Mitoulas et al., 2002). However, some studies have identified a circadian rhythm in individual amino acids, specifically tryptophan (Cubero et al., 2005; Sanchez et al., 2013). Overall, these findings highlight the potential for a mismatch between the timing of milk

expression and the timing of recipient infant feeding, which may have implications for the development of the recipient infant's circadian clock.

It is important to note that many of the cited studies had relatively small sample sizes (often fewer than 100 participants), which may limit the generalisability and statistical power of the findings. Additionally, the studies reviewed vary considerably in methodology, including the stage of lactation, gestational age of the infant (term versus preterm), and the manner of collection (e.g., partial or complete breast expression). Fat content increases as the breast empties, meaning that if milk expression is stopped prematurely, it may result in samples with lower fat concentrations (Bowornkitiwong et al., 2023; Daly et al., 1993; Unger & O'Connor, 2024). These inconsistencies complicate direct comparisons across studies and may contribute to the observed variability in macronutrients. Furthermore, some studies focused specifically on milk from the early stages of lactation (Lubetzky et al., 2006; Paulaviciene et al., 2020), whilst others included samples from a broader lactation window of up to 12 months (Jackson et al., 1988; Mitoulas et al., 2002). Further research is warranted to establish consistent patterns across larger, more diverse populations, as well as DHM samples specifically. The influence of factors such as the stage of lactation and gestational and postpartum age at the time of milk expression should also be considered, as these variables are known to impact the macronutrient composition of human milk.

2.9.5 Influence of collection, storage, and processing

The nutritional composition of DHM may be influenced by a wide variety of factors relating to the processing and handling of the milk. These include milk expression, freezing for storage, milk handling during processing, pooling, and Holder pasteurisation (HoP). Differences in milk expression practices by individual donors can significantly influence the macronutrient profile of DHM, particularly its fat content. The literature often describes 'foremilk' (the milk released at the beginning of the feed) as being lower in fat than 'hindmilk' (the milk released towards the end of a feed) (Andreas et al., 2015; Pu et al., 2023). Whilst 'hindmilk' is accepted to have a higher fat content, the exact point at which 'foremilk' becomes 'hindmilk' has not been clearly established, and Bowornkitiwong et al. (2023) challenge the binary distinctions between these terms. Studies suggest that milk fat content changes incrementally during a feeding session, with fat concentration increasing as the breast is progressively emptied, rather than there being 2 distinct "types" of milk (Bowornkitiwong et al., 2023; Daly et al., 1993; Unger & O'Connor, 2024). This is thought to be due to the gradual release of fat

globules from the mammary gland as the breast becomes less full (Hassiotou et al., 2013; Mizuno et al., 2009). Empirical evidence linking incomplete or inconsistent expression methods to altered fat concentrations in DHM is currently limited; however, it is plausible that incomplete breast expression may result in a higher quantity of milk which has a lower fat content than that from full breast expression. This highlights a need for further research and the potential value of standardised expression protocols to ensure nutritional consistency in DHM.

Milk banking guidelines recommend freezing DHM at -20°C for storage (Unger & O'Connor, 2024). However, freezing may lead to fat hydrolysis (breakdown of fat), and subsequently a reduction in fat content (Zhang et al., 2022). Some studies have reported a decrease in fat following freezing (Kim et al., 2020; Kim et al., 2019; Păduraru et al., 2018), while others found no significant effect (Ahrabi et al., 2016; de Waard et al., 2018; Zhang et al., 2022). Variability in findings may reflect differences in milk type (DHM versus MOM), thawing and warming methods, fat measurement techniques, or fat adherence to container walls (Stinson et al., 2024). Overall, while results are inconsistent, freezing may pose a risk for fat loss in DHM.

Measurement accuracy of human milk macronutrients, particularly fat and lactose, is significantly influenced by homogenisation (Fusch et al., 2015). Homogenisation emulsifies milk, breaking down fat molecules into smaller particles and dispersing them evenly throughout the batch (Reyes et al., 2022). Even after homogenisation, fat globules can begin to reaggregate if analysis is delayed, potentially distorting results (Fusch et al., 2015). Even when homogenisation is performed, handling procedures can still introduce variability. For example, Walter et al. (2023), in their analysis of 95 single-donor pooled DHM samples, discussed that 1 batch had a significantly higher level of fat than what is normal for human milk and suggested that this was likely due to processing methods rather than the milk itself. The authors note that it is typical for the fat in human milk to rapidly separate and rise. Although batches were stirred, the 20-minute aliquoting process, which involved taking milk from the bottom of a jug, likely allowed the milk to separate, resulting in a disproportionately high fat content in the final samples. Similarly, Czank et al. (2009) demonstrated that low-speed centrifugation followed by resuspension could reduce fat variability in DHM pools by 7.4-fold. Transparent reporting of homogenisation, sample aliquoting, and analysis timing as well as standardised protocols for milk sample preparation is crucial to ensure reproducibility and comparability of results across studies.

Pooling milk from multiple donors aims to reduce variability in nutrient content (John et al., 2019; PATH, 2013; Tabasso et al., 2023). However, a simulation by John et al. (2019) found that although variability in pools decreased when the number of randomly selected donors increased, the fat content of DHM still varied significantly. This finding is supported by a systematic review of 14 studies conducted by Perrin et al. (2020), which found substantial variability, particularly in fat content with 2- to 4-fold differences, even when pooling occurred, including the data from John et al. (2019). Similarly, Young et al. (2019) found that true protein content was significantly more variable in milk pools made from 1–2 donors ($p = 0.014$) compared to pools made from 3 or more donors. Even so, pool size did not significantly affect the variability of total energy, fat, and carbohydrate content (Young et al., 2019). Together, these findings suggest that pooling can reduce some nutrient variability but does not eliminate it entirely, particularly for fat.

Pasteurisation is a critical step in the processing of DHM. HoP effectively reduces harmful pathogens, and while some bioactive components are diminished (Koenig et al., 2005; Oliveira et al., 2020; Van Gysel et al., 2012; Vass et al., 2020), many bioactive components remain, which have benefits to the preterm infant, such as reducing NEC and BPD (Avila-Alvarez et al., 2025; Bishop et al., 2010; Oliveira et al., 2020). Several studies have investigated the effects of HoP on the macronutrient content of DHM, with inconsistent findings across nutrients and studies. Carbohydrate content appears largely unaffected, with most studies reporting no significant change (Adhisivam et al., 2019; Davis et al., 2025; Kim et al., 2020; Lamb et al., 2021; Pitino et al., 2019; Quitadamo et al., 2021), although Piemontese et al. (2019) report a slight reduction in lactose (1.1%; $p < 0.0001$). In contrast, fat content tends to decrease following HoP, with reported reductions ranging from 5.5–25% (Adhisivam et al., 2019; Kim et al., 2020; Piemontese et al., 2019; Quitadamo et al., 2021). Protein concentrations also show a general decline post-HoP, with reductions ranging from 2.3–16.7% (Adhisivam et al., 2019; Kim et al., 2020; Piemontese et al., 2019; Vieira et al., 2011). However, some studies found no significant impact on total nitrogen or protein (Davis et al., 2025; Lamb et al., 2021; Pitino et al., 2019; Quitadamo et al., 2021) suggesting that confounders such as methodological differences may have influenced results. Overall, while carbohydrate appears relatively stable, fat and protein are more variable after HoP treatment. Table 2.11 presents a summary of the collection methods and processing of DHM and their effect on its macronutrient profile.

Table 2.11

Collection Methods and Processing of DHM and their Effect on its Macronutrient Profile

Processing/collection method	Carbohydrate	Fat	Protein
Milk expression duration	Higher lactose at the start of the feed and decreases towards the end of the feed (Andreas et al., 2015)	Decrease in fat content may be due to incomplete expression (Andreas et al., 2015; Bowornkitiwong et al., 2023; Pu et al., 2023)	No significant change identified across the duration of a feed (Pu et al., 2023)
Freezing (-20°C)	Significant decrease in HoP milk after 20 weeks (Kim et al., 2020)	Significant decrease in HoP after 20 weeks (Kim et al., 2020)	Significant decrease in HoP after 20 weeks† (Kim et al., 2020)
	No effect in raw milk (de Waard et al., 2018; Kim et al., 2019; Zhang et al., 2022)	Significant decrease in preterm colostrum (Păduraru et al., 2018)	No effect in raw milk (Ahrabi et al., 2016†; de Waard et al., 2018†; Kim et al., 2019; Zhang et al., 2022†)
		No effect in raw milk (Ahrabi et al., 2016; de Waard et al., 2018; Kim et al., 2019; Zhang et al., 2022)	
Holder Pasteurisation (HoP)	1.1%* decreased in lactose (Piemontese et al., 2019)	25% decrease (Adhisivam et al., 2019)	16.7% decrease† (Kim et al., 2020)
	No significant effect (Adhisivam et al., 2019; Davis et al., 2025; Kim et al., 2020; Lamb et al., 2021; Pitino et al., 2019; Quitadamo et al., 2021; Vieira et al., 2011)	16.2% decrease (Kim et al., 2020)	12.5% decrease § (Adhisivam et al., 2019)
		14.9% decrease (Quitadamo et al., 2021)	8.9% decrease (Quitadamo et al., 2021)
		5.5% decrease (Piemontese et al., 2019*; Vierira et al., 2011)	3.9% decrease (Vierira et al., 2011)
		No significant effect (Davis et al., 2025; Lamb et al., 2021; Pitino et al., 2019)	2.3%* decrease (Piemontese et al., 2019)
			No significant effect (Lamb et al., 2021§; Pitino et al., 2019†; Davis et al., 2025)

Note. True protein refers to the actual amount of bioavailable protein, whereas total protein includes non-protein nitrogen, potentially overestimating actual protein content.
Holder Pasteurisation is the recommended pasteurisation method in most international human milk banking guidelines and involves heating milk to 62.5℃ for 30 minutes, followed by rapid cooling.
* Percentage change calculated from reported means
† Total protein
‡ Total nitrogen
§ True protein

2.10 Summary and identification of research gap

Human milk is widely recognised as the optimal nutrition for preterm infants as it provides important immunological factors which decrease the risk of prematurity-related morbidities such as necrotising enterocolitis. However, research examining the macronutrient composition of DHM has highlighted not only a significant variability in energy, fat, protein, and carbohydrate, but mean values for energy and fat often falling below clinical reference values. This variability may impact growth and health outcomes for preterm and critically ill infants. While the composition of MOM has been extensively studied, less attention has been given to DHM. Furthermore, there is a paucity of research on DHM in the New Zealand context. To date, only one study in New Zealand has examined the macronutrient composition of DHM (Lamb et al., 2021). This study focused on the effects of pasteurisation and did not explore other potential influencing variables. Furthermore, their small sample size (n = 63) makes it challenging to fully understand the unique characteristics and nutritional profile of DHM in New Zealand or compare it with international research. The present study aims to address these gaps by providing data on the macronutrient and energy content of DHM in New Zealand, with a larger sample size, and focus on how lactation stage, gestational age (preterm versus term), donor age, and donor ethnicity influence milk composition. By expanding the evidence base in New Zealand, this research will contribute to valuable insights into the quality of DHM and the potential to improve nutritional outcomes for preterm infants.

Chapter 3. Research Study Manuscript

3.1 Abstract

Background: Human milk is the optimal nutrition for feeding preterm infants. When a mother's own milk (MOM) is not available, pasteurised donor human milk (DHM) is recommended as the next best option (Daniels et al., 2017; Meek & Noble, 2022) as it reduces the incidence and severity of prematurity-related morbidities, such as necrotising enterocolitis (Miller et al., 2018). The macronutrient composition of DHM is known to vary significantly, with energy and fat often falling below clinical reference values (Perrin et al., 2020). Unlike MOM, DHM undergoes processing and handling, which can affect its nutritional content. Additionally, inter-individual differences between donors, lactation stage, and gestational age of the infant may also impact macronutrient content (Muts et al., 2025; Perrin et al., 2020; Walter et al., 2023). Limited research has been done on the macronutrient composition of DHM in New Zealand, highlighting a need for further investigation.

Objective: Our aim was to describe the energy and macronutrient content of DHM which had been donated to the Christchurch Women's Hospital Milk Bank in New Zealand, and explore the relationship between macronutrient composition and the following variables: lactation stage, gestational age of the infant when the milk was expressed (i.e. preterm or term), donor age, and donor ethnicity.

Methods: The macronutrient and energy content of 696 single-donor pools from unique 149 donors were analysed with the Miris Human Milk Analyser. Mean, median and box and whisker plots were used to describe the data set and single linear regression for each macronutrient was conducted to evaluate the influence of lactation stage, gestational age, donor age and donor ethnicity.

Results: DHM contained, on average, 74.9 kcal/100 mL energy; 4.0 g/100 mL fat; 1.2 g/100 mL true protein; and 8.1 g/100 mL carbohydrate, consistent with published reference values for DHM. Mean true protein fell below the recommended intake for preterm infants (2.2—2.5 g/100 mL) and mean energy was on the lower end of the recommended range (72—88 kcal/100 mL). Fat and true protein varied considerably, with differences of 37.6-fold and 32.7-fold respectively. Gestational age and lactation stage were the strongest predictors of DHM true protein content. True protein decreased by 1.4% per week as lactation progressed and milk expressed preterm had 1.5% more true protein

compared to milk expressed at term. There was also some suggestion of differences due to donor age and ethnicity.

Conclusion: Our findings revealed that the true protein and energy concentrations in DHM in New Zealand may not be adequate to meet the needs of preterm infants, potentially leading to growth restriction and poorer health outcomes. The high variation in fat content is likely related to DHM processing and handling, rather than physiological differences. The findings underscore the need for point-of-care analysis and targeted fortification in New Zealand milk banks to ensure proper infant nutrition.

Keywords: breastmilk; preterm; term; protein; fat; carbohydrate

3.2 Introduction

It is well established that human milk provides the optimal nutrition for infants (Nagel et al., 2023). It can contain all the essential nutrients required during the first six months of life, including key macro- and micronutrients necessary for growth and development (some nutrients in human milk are dependent on the mother being replete, for example vitamin D) (Perrella et al., 2021). For vulnerable infants, particularly those born preterm (<37 weeks gestation), there is robust evidence that human milk, compared to commercial infant formula, significantly reduces the incidence and severity of several prematurity-related morbidities, including necrotising enterocolitis (Miller et al., 2018; Quigley et al., 2024), bronchopulmonary dysplasia (Hair et al., 2016; Patel et al., 2017; Xu et al., 2020), and retinopathy of prematurity (Goyal et al., 2024; Spiegler et al., 2016).

When a mother's own milk is not available, donor human milk (DHM) is recommended by the World Health Organisation (WHO) (2019), the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) (Arslanoglu et al., 2013), and the American Academy of Pediatrics (AAP) (Daniels et al., 2017; Meek & Noble, 2022). The formalisation of human milk banks has provided a safe and reliable system for distributing DHM to those who need it most (Jones, 2003; Unger & O'Connor, 2024). As of 2024, over 700 human milk banks exist worldwide (Israel-Ballard et al., 2024). In New Zealand, there are currently five active non-profit milk banks (Te Whatu Ora, 2024, July 8), the first started in Christchurch Women's Hospital and supplies pasteurised DHM to hospitalised preterm and other ill infants, whilst their mothers establish their own milk supply (Te Whatu Ora, 2024, July 8).

Formal milk banking practices in New Zealand are guided by the Operational Guidelines for Milk Banks in Australia and New Zealand (Human Milk Regulation Working Group, 2025, June 4).

Research examining the macronutrient composition of DHM shows there is substantial variability in energy, fat, protein, and carbohydrate composition, but also that mean values for energy and fat often fall below clinical reference values expected for human milk (Perrin et al., 2020). Unlike a mother's own milk, DHM undergoes additional processes, including freezing, pasteurisation, and handling (e.g., mixing and transferring milk between containers), all of which can influence its nutritional composition. Furthermore, factors such as circadian rhythm, lactation stage, and differences in breast expression can also contribute to variations in macronutrient composition in individual donations (Perrin et al., 2020).

To mitigate this variability, DHM from one or more donors is often pooled (John et al., 2019; Tabasso et al., 2023). For example, North American milk banks typically pool the milk from 2—5 individual donors (Human Milk Banking Association of North America, 2020, June 4), whereas in New Zealand, multiple batches of milk from a single donor are pooled (Human Milk Regulation Working Group, 2025, June 4). Despite these practices, variability remains a concern (John et al., 2019; Perrin et al., 2020; Young et al., 2019). Furthermore, there is limited data on the nutritional composition of DHM in the New Zealand context. Currently, one study (Lamb et al., 2021) has examined the macronutrient composition of DHM in New Zealand, with a focus on the effects of Holder Pasteurisation (HoP). The study had a small sample size *(n* = 63) and did not consider other variables that could influence milk composition, such as lactation stage (Holritz et al., 2024; John et al., 2019; Mills et al., 2019; Muts et al., 2025). This makes it difficult to fully understand the unique characteristics and nutritional profile of DHM in New Zealand, conduct meaningful comparisons with international research, or make clinical recommendations.

The present study aimed to examine the macronutrient and energy content of DHM and explore the relationship between energy and macronutrient composition and the following variables: lactation stage, gestational age of the infant (i.e. preterm or term), donor age, and donor ethnicity. The findings will provide data specific to the New Zealand population. Additionally, this study will highlight the importance of macronutrient analysis in milk banks with the overall goal to improve the nutritional quality of DHM and provide better outcomes for preterm infants.

3.3 Methods

3.3.1 Study design and ethical approval

This research was a retrospective secondary analysis of data collected from all registered donors who donated milk to the Christchurch Women's Hospital Milk Bank (CWHMB) between July 2022 and July 2024. The project was approved by the Massey University Human Ethics Committee: Ohu Matatika 1, Application OM1 24/58 (Appendix B). Before donating milk to the milk bank, all donors provided signed consent for a sample of their milk to be used for research (Appendix C). Because the present study was a secondary data analysis, no identifiable data were available to the researchers.

3.3.2 Donor eligibility and milk collection

According to the Operational Guidelines for Milk Banks in Australia and New Zealand (Human Milk Regulation Working Group, 2025, June 4), all donors completed a health and serological screening to ensure they met the inclusion criteria. These criteria included being healthy, less than 12 months postpartum, exclusively breast milk feeding their infant, and having milk surplus to their own infant's requirements. Details of donor inclusion and exclusion criteria, as well as the full screening process, can be found in the Operational Guidelines for Milk Banks in Australia and New Zealand (Human Milk Regulation Working Group, 2025, June 4). According to the CWHMB protocols (Appendix D), donors were instructed to express and freeze their milk at home until they had accumulated one litre. Milk was generally only accepted by the milk bank if it had been frozen within three months of the date it was expressed. The milk bank provided sterile, food-grade plastic collection bottles, and donors labelled each bottle with the expression date. Donor date of birth, ethnicity, and infants birth date were recorded by the milk bank manager and stored in a secure database. The characteristics provided were the donors' date of birth and ethnicity, and the infant's birth date.

3.3.3 Human milk analysis

Ten millilitre aliquots of HoP DHM, pooled from a single donor, were analysed by the milk bank staff for energy and macronutrient content using the Miris Human Milk Analyser (HMA) (Miris, Uppsala, Sweden) according to the manufacturer's instructions (Miris, 2025, July 8). The Miris HMA is widely used in clinical and research settings as it is a reliable method for analysing human milk composition, specifically protein and fat content (Borràs-Novell et al., 2023; Groh-Wargo et al., 2016; Perrin et al., 2019). The Miris HMA quantitatively measures the concentration of fat, crude protein, and total carbohydrate (g/100 mL) using mid-infrared transmission spectroscopy (Mid-IR). The infrared data are

translated into macronutrient concentrations using calibration models based on established reference methods: The Rose-Gottlieb method for fat, the Kjeldahl methods for protein (measuring total nitrogen), and the oven-drying method for total solids. Total carbohydrate is calculated indirectly by subtracting the sum of fat, protein, and ash from the total solids. True protein is calculated by built-in software, based on 80% of crude protein. Energy content is calculated using Atwater conversion factors (kcal/100 mL). Based on the manufacturer's technical report, the measurement ranges are 0.6–6 g/100 mL for fat, 0.6–2.4 g/100 mL for true protein, and 6.6–8.7 g/100 mL for carbohydrate (Miris, 2025, July 8). True protein was reported in this study as it more accurately reflects the nutritionally available amino acid content of the milk (Belfort et al., 2024) and therefore is more clinically relevant. Detailed description of the DHM processing and handling is provided in Appendix E.

3.3.4 Statistical analysis

Lactation stage was calculated as the difference between the infant's birth date and the latest expression date from a donation. Gestational age category was determined based on the infant's gestational age at the time the milk was expressed: preterm (<37 weeks) and term (≥37 weeks). Donor age was calculated from the donor's birth date and the latest expression date from a donation. Donors were then categorised into two groups: older donors (35 years and older), and younger donors (under 35 years). The age threshold of 35 was selected based on existing literature, which identified advanced maternal age as being 35 years and older, and that this may be associated with different physiological factors relevant to pregnancy and lactation (Kortekaas et al., 2020). When a donor reported more than one ethnicity, we assigned a single prioritised ethnicity value based on the New Zealand Ministry of Health ethnicity data protocols (Ministry of Health, 2017), with prioritisation in order of Māori, Pacific, European, Asian, and Others. Mature milk is defined as milk expressed after about 2 weeks postpartum, and colostrum is defined as milk expressed prior to 2 weeks postpartum. The milk bank categorised milk as either mature or colostrum prior to providing us with the data.

Statistical analysis was performed using SPSS version 20 (SPSS Inc. Chicago, Illinois, U.S.). Descriptive statistics were calculated for energy, fat, true protein, and carbohydrate, including both mean and median, and box and whisker plots provided a visual representation of the data. To assess the effect of lactation stage, preterm versus term status, donor age and donor ethnicity on the energy

and macronutrient composition of DHM, single variable linear regression analyses were conducted with energy, fat, true protein and carbohydrate as the dependent variables. Results were reported as mean differences, 95% confidence intervals (CI) and *p*-values. Multivariate adjusted models were not performed to avoid 'table 2 fallacy' (Westreich & Greenland, 2013), in which inclusion of multiple coefficients may lead to overfitting and incorrect conclusions. 13 samples were excluded from the analysis as values were biologically implausible, indicating obvious data entry errors. A sensitivity analysis was performed to examine the effect of the extreme carbohydrate outliers by performing the single linear regression with extreme outliers excluded.

3.4 Results

3.4.1 Donor and donation characteristics

The dataset included a total of 696 HoP DHM samples from 149 unique donors, with an average of 4.7 donations per donor. Table 3.1 presents the characteristics of both the milk samples and the donors who provided the samples. Individual donations to the milk bank included milk which had been expressed over periods ranging from one to 30 days. Donors ranged from 20 to 45 years old, and the mean age at first donation was 31.6±4.9 years. Most donors (75%) were younger than 35 years. More than half (55.6%) of donors identified as European and 15.4% as either Māori or Pacific. Most donations were term mature milk (62.6%), followed by preterm mature (35.3%). Preterm and term colostrum samples represented just 1.7% and 0.7% of all donations, respectively. Most milk was donated during the early lactation stage (mean 12±12.3 weeks postpartum), with 469 donations expressed before 12 weeks postpartum, and 225 donations expressed at 12 weeks postpartum or later. Donations ranged from birth to 55 weeks postpartum (Figure 3.1). Data on the lactation stage were missing for 2 cases.

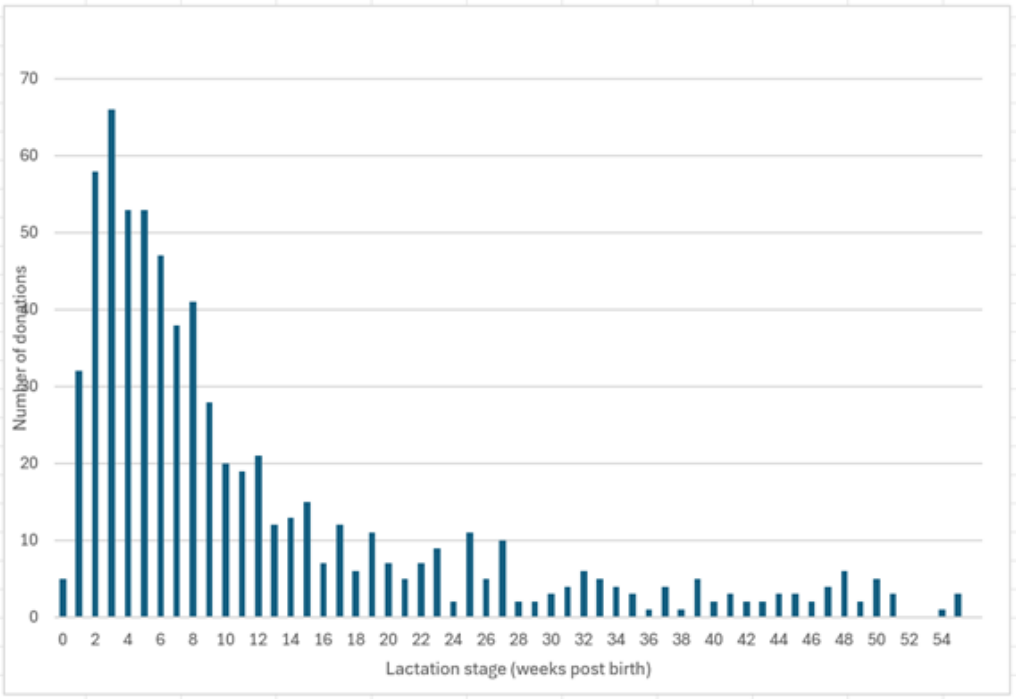
Table 3.1

Summary of donor and donation characteristics (n = 696)

	Descriptive statistic
Characteristics of donors	*n* = 149
Age at first donation, mean (SD)	31.6 (4.9)
Age group at first donation, *n* (%)	
<35 years	113 (75.3%)
≥35 years	37 (24.7%)
Lactation stage at first donation, mean (SD) weeks	12.6 (13.2)
Ethnicity*, *n* (%)	
European	84 (56.4%)
Māori	16 (10.7%)
Pacific	7 (4.7%)
Asian	28 (18.8%)
Others	14 (9.4%)
Characteristics of donations	*n* = 696
Age of donor, mean (SD) years	31.3 (5.3)
Age of group of donor, *n* (%)	
<35 years	481 (69.1%)
≥35 years	251 (30.9%)
Stage of lactation, mean (SD) weeks**	12 (12.3)
Preterm mature milk, *n* (%)	246 (35.3%)
Term mature milk, *n* (%)	433 (62.2%)
Preterm colostrum, *n* (%)	12 (1.7%)
Term colostrum, *n* (%)	5 (0.7%)

SD = standard deviation
Preterm mature milk defined as milk expressed <37 weeks gestation and after about 2 weeks postpartum
Term mature milk defined as milk expressed ≥37 weeks gestation and after about 2 weeks postpartum
Preterm colostrum defined as milk expressed in the first 1—2 weeks postpartum, but <37 weeks gestation
Term colostrum defined as milk expressed in the first 1—2 weeks postpartum, but ≥37 weeks gestation
*Ethnicity was prioritised in the following order: Māori, Pacific, Asian, Others, European
** Data for lactation stage was missing for 2 cases resulting in a sample size of *n*=694

Figure 3.1
Distribution of the number of donations across lactation (n = 694)



Note. Most milk donations were collected during early lactation (mean 12±12.3 weeks postpartum), with 469 donations expressed before 12 weeks and 225 from 12+ weeks postpartum. Donations ranged up to 55 weeks postpartum. Donations included milk expressed over periods ranging from one to 30 days. A single milk collection was assigned and date based on the last expression date.

3.4.2 Macronutrient content of DHM

The macronutrient and energy composition of HoP DHM samples from 149 unique donors is presented in Table 3.2 and Figure 3.2 shows the distributions of each. Both measures of central tendency were reported to provide a comprehensive summary of the data. Coefficients of variation were highest for fat (57.5%), followed by true protein (33.3%), energy (29.9%), and carbohydrate (7.4%).

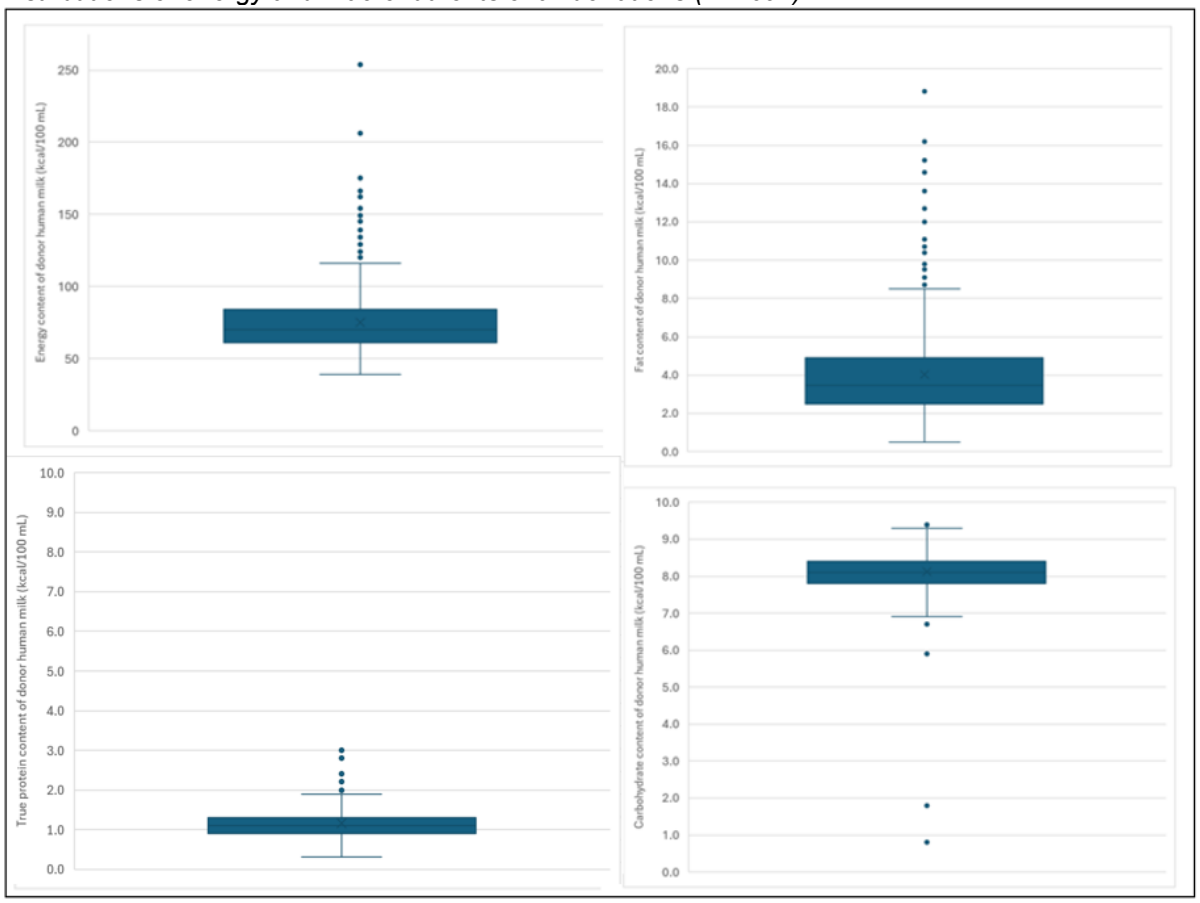
Table 3.2
Macronutrient composition of donations

	Cohort size	Mean (SD)	Median (25^{th} –75^{th} centiles)	Minimum – Maximum	Reference Value*
Energy (kcal/100 mL)	688	74.9 (22.4)	70 (61–84)	39–254	65
Fat (g/100 mL)	694	4.0 (2.3)	3.5 (2.5–4.9)	0.5–18.8	3.2
True protein (g/100 mL)	690	1.2 (0.4)	1.1 (0.9–1.3)	0.3–3.1	1.2
Carbohydrate (g/100 mL)	690	8.1 (0.6)	8.1 (7.8–8.4)	5.9–9.4	7.8

Note. Difference in cohort size relates to some cases in the dataset missing values
SD = standard deviation

*Reference value for the macronutrient composition of DHM as per the Academy of Nutrition and Dietetics (as cited in Perrin et al., 2020

Figure 3.2
Distributions of energy and macronutrients of all donations (n = 694)



3.4.3 Differences in DHM energy and macronutrient composition by donor characteristics, lactation stage and gestational age category

Donor ethnicity was associated with differing concentrations of some macronutrients. On average, DHM from donors in the 'others' ethnic group had significantly less energy (11.8%; $p = 0.040$) and fat (21.7%; $p = 0.048$) compared to DHM from European donors. DHM from Pacific and Māori donors had significantly more true protein (27.3%; $p < 0.001$ and 7.4% respectively; $p = 0.043$) than that from European donors. DHM from Asian donors had significantly more carbohydrate (2.1%; $p = 0.003$) and true protein (7.0%; $p = 0.028$), whilst milk from donors in the 'others' group had significantly less carbohydrate (5.1%; $p < 0.001$), compared to European donors (Table 3.3 and Figure 3.3).

Energy, true protein, and carbohydrate concentration of DHM were not associated with donor age. However, fat concentration was associated with donor age, with an increase in fat of 1.5% ($p = 0.013$)

per year of age (Table 3.3). There were no differences in energy and macronutrients, including fat, of DHM between older (≥35 years) and younger (<35 years) donors (Table 3.3 and Figure 3.4).

True protein was significantly negatively associated with lactation stage, decreasing by 1.4% per week as lactation progressed (p <0.001). DHM from early lactation (<4 weeks postpartum) contained 52.9% (p <0.001) more true protein, and DHM from mid-lactation (4–28 weeks postpartum) contained 15.2% (p <0.001) more true protein than DHM expressed later in lactation (>28 weeks postpartum). Energy, fat, and carbohydrate were not significantly impacted by lactation stage (Table 3.4).

Mean DHM expressed preterm was significantly higher in energy (6.1%; p = 0.005), fat (8.3%; p = 0.038), true protein (1.5%; p <0.001) and carbohydrate (1.4%; p = 0.014) than DHM expressed at term (Table 3.5 and Figure 3.5). Colostrum samples were excluded from the regression analysis due to insufficient sample sizes (n = 12 preterm colostrum; n = 5 term colostrum), which limited the reliability of estimates.

The sensitivity analysis revealed differences in the statistical significance of carbohydrate and donor ethnicity, and carbohydrate and lactation stage, but overall, the effect sizes were small and did not appear to have made a meaningful impact on the mean effect size. Results from the sensitivity analysis can be found in Appendix F.

Table 3.3

Macronutrient composition of donations by donor characteristics (696 samples from 149 donors)

	Energy, kcal/100 mL		Fat, g/100 mL		True protein, g/100 mL		Carbohydrate, g/100 mL	
	Mean difference (95% CI)	*p*-value	Mean difference (95% CI)	*p*-value	Mean difference (95% CI)	*p*-value	Mean difference (95% CI)	*p*-value
Donor age								
Per year	0.305 (-0.013, 0.623)	0.060	**0.041 (0.009, 0.074)**	**0.013**	-0.000 (-0.006, 0.005)	0.899	0.000 (-0.009, 0.008)	0.935
≥ 35 years compared to <35 years	-1.987 (-1.658, 5.632)	0.285	0.332 (-0.040, 0.705)	0.080	0.004 (-0.060, 0.068)	0.905	-0.038 (-0.136, 0.059)	0.440
Ethnicity								
European	Reference		Reference		Reference		Reference	
Māori	-2.651 (-7.542, 2.240)	0.288	-0.212 (-0.717, 0.293)	0.410	**0.087 (0.003, 0.172)**	**0.043**	-0.049 (-0.179, 0.081)	0.457
Pacific	0.082 (-8.242, 8.406)	0.985	-0.244 (-1.078, 0.590)	0.566	**0.377 (0.238, 0.516)**	**<0.001**	0.193 (-0.002, 0.407)	0.078
Asian	2.576 (-1.725, 6.878)	0.240	0.166 (-0.276, 0.607)	0.462	**0.083 (-0.009, 0.156)**	**0.028**	**0.172 (0.058, 0.286)**	**0.003**
Others	**-8.841 (-17.297, -0.385)**	**0.040**	**-0.881 (-1.754, -0.007)**	**0.048**	-0.075 (-0.221, 0.070)	0.311	**-0.411 (-0.636, -0.186)**	**<0.001**

Note. Bold indicates a statistically significant difference of $p < 0.05$.

CI = Confidence interval

Table 3.4

Macronutrient composition of donations by lactation stage (694 samples from 149 donors)

	Energy, kcal/100 mL		Fat, g/100 mL		True protein, g/100 mL		Carbohydrate, g/100 mL	
	Mean difference (95% CI)	*p*-value	Mean difference (95% CI)	*p*-value	Mean difference (95% CI)	*p*-value	Mean difference (95% CI)	*p*-value
Lactation stage								
Per week	-0.091 (-0.228, 0.04)	0.197	-0.001 (-0.015, 0.013)	0.936	**-0.013 (-0.015, -0.011)**	**<0.001**	-0.003 (-0.007, 0.000)	0.072
< 4 weeks	3.656 (-2.317, 9.629)	0.230	0.010 (-0.601, 0.622)	0.973	**0.648 (0.564, 0.732)**	**<0.001**	0.082 (-0.067, 0.256)	0.252
4–28 weeks	-1.691 (-7.126, 3.743)	0.541	-0.379 (-0.934, 0.176)	0.181	**0.148 (0.071, 0.224)**	**<0.001**	0.060 (-0.087, 0.207)	0.422
> 28 weeks	Reference		Reference		Reference		Reference	

Note. Bold indicates a statistically significant difference of $p < 0.05$.

CI = Confidence interval

Table 3.5

Macronutrient composition of preterm versus term DHM (696 samples from 149 donors)

	Energy, kcal/100 mL		Fat, g/100 mL		True protein, g/100 mL		Carbohydrate, g/100 mL	
	Mean difference (95% CI)	*p*-value	Mean difference (95% CI)	*p*-value	Mean difference (95% CI)	*p*-value	Mean difference (95% CI)	*p*-value
Term† versus preterm‡ mature	**-5.112 (-8.656, -1.567)**	**0.005**	**-0.386 (-0.750, -0.021)**	**0.038**	**-0.375 (-0.428, -0.322)**	**<0.001**	**-0.119 (-0.214, -0.024)**	**0.014**

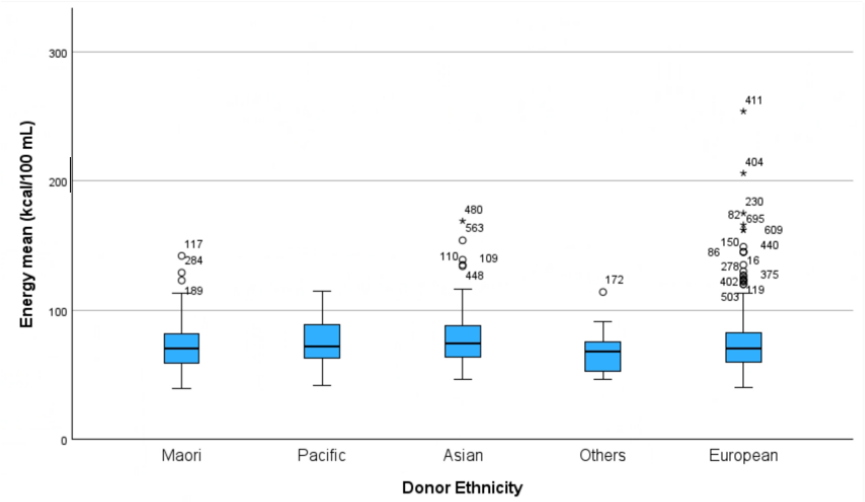
Note. Bold indicates a statistically significant difference of $p < 0.05$.

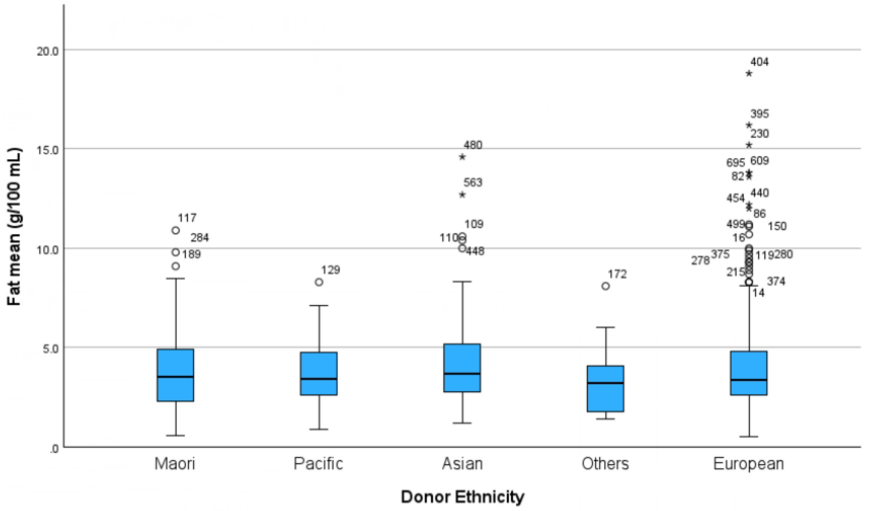
CI = Confidence interval

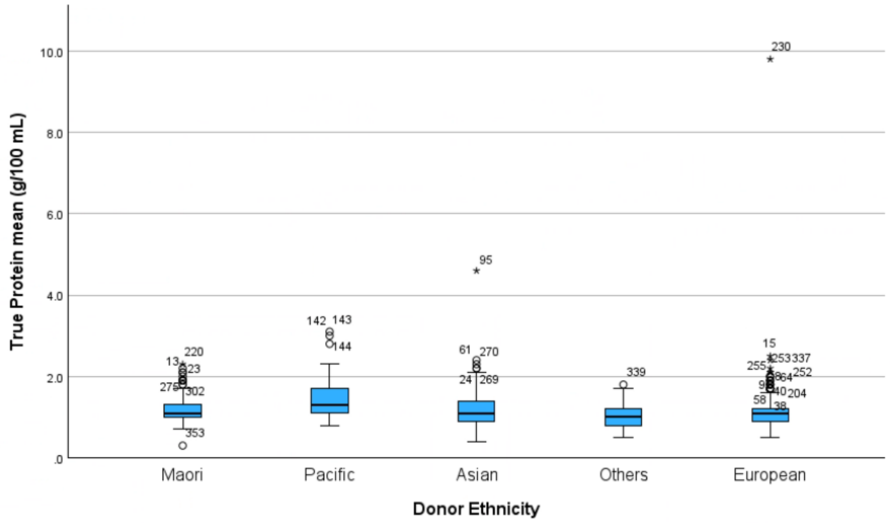
† Term mature milk defined as milk expressed ≥37 weeks gestation and after about 2 weeks postpartum

‡ Preterm mature milk defined as milk expressed <37 weeks gestation and after about 2 weeks postpartum

Figure 3.3
Distributions of energy and macronutrients according to donor ethnicity







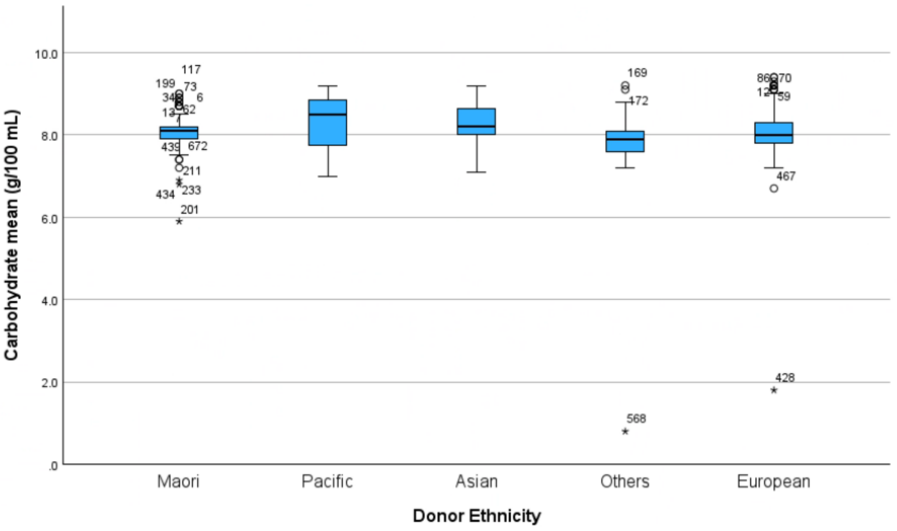


Figure 3.4
Scatter plot of fat mean (g/100 mL) by donor age (years)

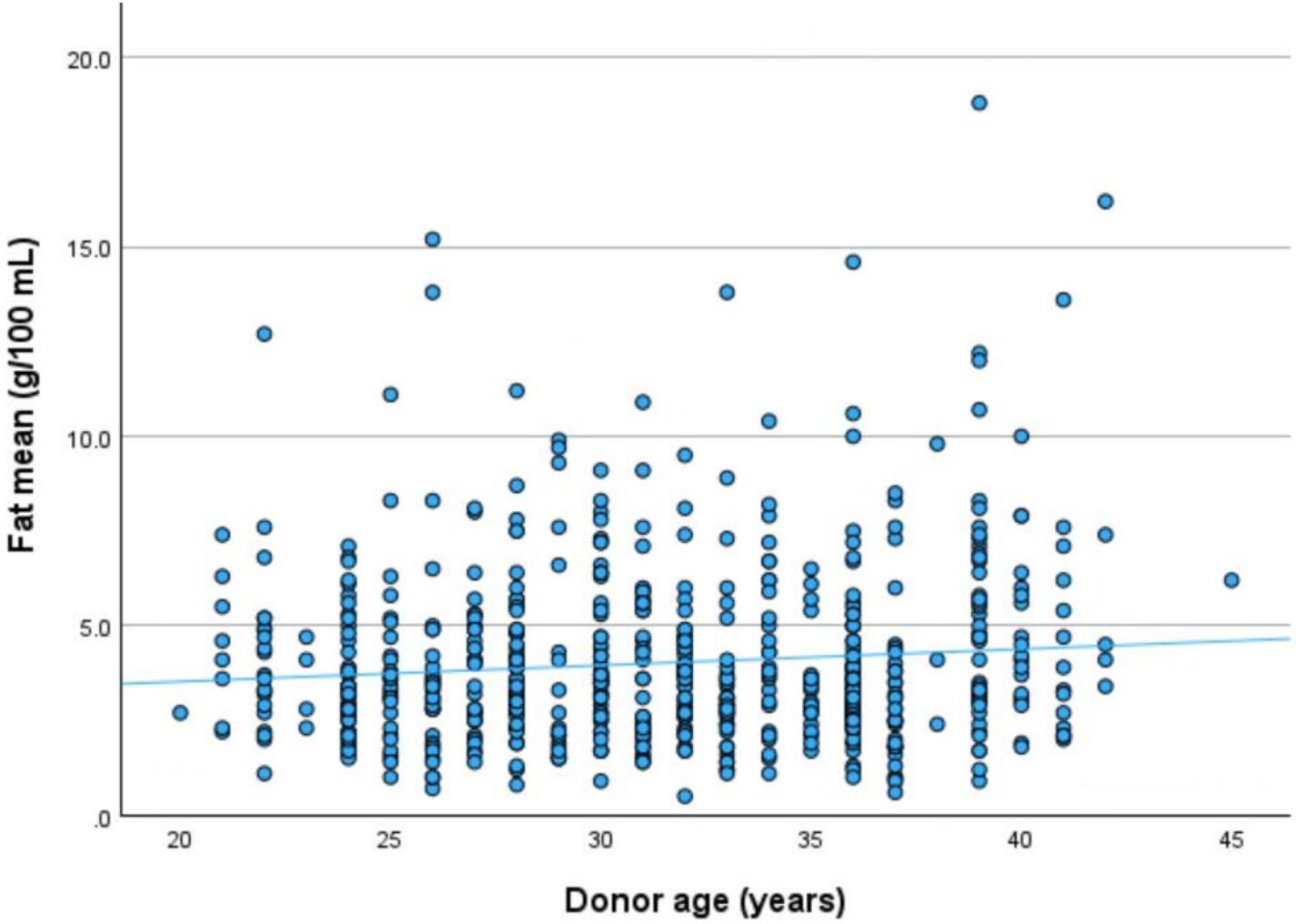
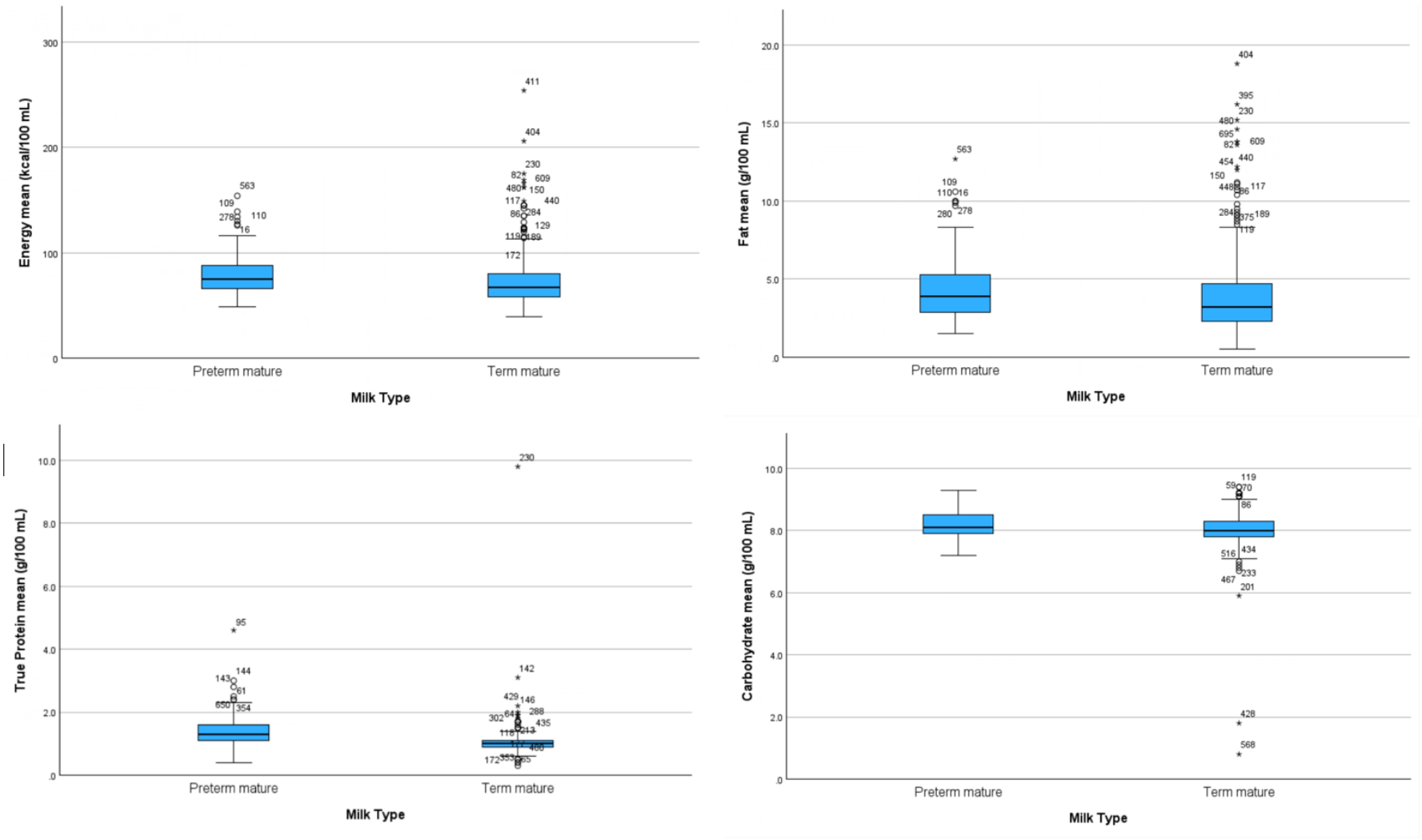


Figure 3.5
Distributions of energy and macronutrients according to milk type (preterm versus term)



3.5 Discussion

The present study reports the energy and macronutrient composition of donor human milk (DHM) from the largest known milk-bank data collection in New Zealand. The size of the dataset allowed us to examine the extent to which donor factors influence DHM composition and whether DHM meets the needs of preterm infants. Our findings revealed differences in energy and all macronutrients between milk expressed by mothers who had a preterm infant versus those who had a term infant. We also showed that lactation stage was a strong predictor of true protein content. There was some indication of differences in milk composition by ethnicity, with significantly higher mean true protein content in milk collections from our small sample of donors of Māori, Pacific, and Asian ethnicity, when compared with European donors. There was also a small, but clinically insignificant, increase in fat for each year older the donor was. The differences by age and ethnicity were relatively small and should be explored further to fully understand the effects of ethnicity and age on the macronutrient composition of DHM.

3.5.1 Energy and macronutrient composition of DHM

Our findings on the mean energy, fat, true protein, and carbohydrate composition of DHM are comparable to the only other New Zealand analysis of DHM by Lamb et al. (2021), and a recent Australian study on DHM by Walter et al. (2023) (Table 3.6). As with our study, Lamb et al. and Walter et al. analysed single-donor pooled HoP milk using mid-infrared (mid-IR) spectroscopy via the Miris HMA. However, both reported on a substantially smaller number of milk donations (Lamb et al. *n* = 63; Walter et al. *n* = 95) than our analysis (*n* = 696 donations from 149 unique donors). Between our study and international research, there were many differences in methods (e.g., pasteurisation, donor pooling practices and analysis techniques) which makes it difficult to make direct comparisons. Our findings for energy, protein, and carbohydrate were consistent with the Academy of Nutrition and Dietetics reference values for DHM (as cited in Perrin et al., 2020) (Table 3.7), providing credibility and suggesting our results are representative of DHM in general. Mean fat from our study was 20% higher than the Academy of Nutrition and Dietetics reference value, raising questions regarding the variation in DHM fat content or inconsistencies in analytical methods.

We found a high level of variation in fat composition between DHM collections in our study (37.6-fold). A large variation in fat content of human milk is consistent with international studies reporting on

fat content in both MOM and DHM; however, our range was substantially larger in magnitude. For example, Walter et al. (2023) observed a 6.3-fold variation between the lowest and highest fat content observed in their samples, John et al. (2019) observed a 2.2-fold variation, and Barbarska et al. (2017) observed a 6.7-fold variation. One determinant of the fat content in human milk is the point within a feed at which it is expressed. As the breast empties, fat content increases, with milk from the end of a feed (hind-milk) containing two to three times more fat than the milk expressed at the start (fore-milk) (Saarela et al., 2005). No guidance regarding milk expression is given to milk donors contributing to the CWHMB, so milk expressed after a donor has fed her own infant could have a higher fat content due to the predominance of hind-milk. Fat content can also be influenced by how the milk is handled, rather than from physiological differences. For instance, if the milk isn't properly homogenised before analysis, fat can separate and rise to the top, leaving milk at the bottom of a sample with a lower fat concentration (Czank et al., 2009; Fusch et al., 2015). These factors may have contributed to the large range in the fat content of individual samples observed in our analysis.

The wide range in fat content may also explain the large variation in our calculated energy values (39–254 kcal/100 mL) which were much greater than energy values observed by Walter et al. (2023) (50.7–125 kcal/100 mL), and most international studies of DHM composition, for example, 67.6–70.3 kcal/100 mL for vat pasteurised, term milk analysed by Meredith-Dennis et al. (2018) or 46.0–86.0 kcal/100 mL for raw, preterm milk analysed by Barbarska et al (2017). The mean energy content from our study was 3.9% higher than the Academy of Nutrition and Dietetics clinical reference value for DHM of 65 kcal/100 mL (Table 3.7). However, using the mean energy value from our findings, an infant consuming exclusively unfortified DHM would receive 119 kcal/kg/day (based on an intake of 160 mL/kg/day), which is on the lower end of the ESPGHAN guidelines recommendations of 115–140 kcal/kg/day (Embleton et al., 2023). Inadequate energy intake may result in extrauterine growth restriction (Perrin et al., 2023).

Our estimates of true protein content ranged from 0.3–3.1 g/100 mL, which was consistent with the findings of a recent systematic review of DHM macronutrient content by Perrin et al. (2020). In this review the mean protein concentrations from 10 studies ranged from 0.8–3.2 g/100 mL. Our observed true protein mean was also the same as the Academy of Nutrition and Dietetics reference value; although it is not specified if they reported true or total protein. In our study, the variation in carbohydrate content in our analysis was relatively small, with only a 1.6-fold difference between

minimum and maximum values. The indirect calculation of carbohydrate by the Miris HMA has been shown to overestimate values compared to other methods, and carbohydrate measured by mid-IR via HMA has been considered unreliable (Fusch et al., 2015; Perrin et al., 2019). However, our findings are consistent with existing literature on the analysis of DHM using the Miris HMA, suggesting that, despite its potential inaccuracies, our results are still comparable to other research on DHM.

Table 3.6

Comparison of published Australasian energy and macronutrient values for DHM with findings from the present study

Study and country	Gestational age of infant	Energy (kcal/100 mL)	Fat (g/100 mL)	True protein (g/100 mL)	Carbohydrate (g/100 mL)
Present study New Zealand *n* = 696	Mixed, term (*n* = 438) and preterm (*n* = 258)	68.0 ± 9.0	4.0 ± 2.3	1.2 ± 0.4	8.1 ± 0.6
Lamb et al. (2021) New Zealand *n* = 63	Mixed, preterm (*n* = 50) and preterm (*n* = 11)	68.0 ± 9.0	3.4 ± 1.0	0.9 ± 0.2	8.0 ± 0.2
Walter et al. (2023) Australia *n* = 95	Mixed, term (*n* = 71) and preterm (*n* = 24)	73.7 ± 9.3	3.9 ± 1.0	1.2 ± 0.2	8.3 ± 0.2

Note. All studies analysed Holder pasteurised (HoP) DHM from single-donor pools using Mid-IR spectroscopy via the Miris Human Milk Analyser.
True protein refers to the actual amount of bioavailable protein, whereas total protein includes non-protein nitrogen, potentially overestimating actual protein content.
Preterm defined as milk expressed <37 weeks gestation
Term defined as milk expressed ≥37 weeks gestation

Table 3.7

Comparison of DHM energy and macronutrient composition reference values with findings from the present study

Study and country	Energy (kcal/100 mL)	Fat (g/100 mL)	Protein (g/100 mL)	Carbohydrate (g/100 mL)
Present study	68.0 ± 9.0	4.0 ± 2.3	1.2 ± 0.4	8.1 ± 0.6
Academy of Nutrition and Dietetics*	65	3.2	1.2	7.8

Note. Values for the present study and from Miris are for true protein. It is not specified whether values from AND are true, or total protein. True protein refers to the actual amount of bioavailable protein, whereas total protein includes non-protein nitrogen, potentially overestimating actual protein content.
*From the Academy of Nutrition and Dietetics' Infant and Pediatric Feedings (cited in Perrin et al., 2020)

3.5.2 Influence of donor characteristics (ethnicity and age)

This study is the first to explore the relationship between donor ethnicity and the macronutrient composition of DHM in New Zealand. Previous work by Butts et al. (2018) examined this relationship in MOM but found no association between different ethnic groups in New Zealand. In contrast, our

study revealed some differences in the macronutrient composition of milk according to the ethnicity of the donor. Milk from donors of Māori, Pacific, and Asian ethnicity had a higher mean true protein when compared with European donors. Although this finding was statistically significant, most differences were very small and unlikely to make a meaningful clinical impact. Furthermore, despite Māori, Pacific, and Asian groups having more true protein, all ethnicities had milk which was below the recommended intake /100mL for preterm infants as recommended by the ESPGHAN guidelines.

Our study found no association between donor age and the energy, true protein, or carbohydrate concentrations in DHM, which is consistent with the only other study on DHM that we are aware of (Mills et al., 2019). However, our results did differ in that we observed a 1.5% increase in fat for every year older the donor was at the time of expression. This equates to 25% more fat in milk from the oldest donor (45 years) compared to the youngest donor (20 years). However, milk from both the oldest and the youngest donor still fall into the recommended fat intake as per the ESPGHAN guidelines and therefore there is a minimal nutritional significance and no practical implications for milk banks.

3.5.3 Influence of lactation stage

Previous research has consistently observed protein content being at its highest in early lactation and declining as milk moves from colostrum to mature (Grote et al. 2016; Kreissl et al. 2016; Lithoxopoulou, Gkampeta et al. 2025). Our results were consistent with this pattern, showing DHM from early lactation (<4 weeks postpartum) containing 52.9% more true protein, and DHM from mid-lactation (4–28 weeks postpartum) containing 15.2% more true protein compared to DHM expressed later in lactation (28–55 weeks). True protein was also shown to significantly decrease by 1.4% per week as lactation progressed. When expressed in terms of infant intake, a 1500g infant receiving 240 mL of DHM per day (160 mL/kg/day) would receive approximately 3.7 g of true protein from milk from early lactation, 2.5g from milk from mid-lactation, and only 2.2 g from milk from later in lactation. All of these values fall below the ESPGHAN guideline recommendations of 5.3–6 g/day (3.5–4 g/kg/day) for preterm infants, highlighting that, even in early lactation when true protein content is at its highest, DHM alone cannot meet preterm infants' protein requirements. The ESPGHAN guidelines do not specify whether their protein recommendations are for true or total protein (which includes both true and non-protein nitrogen). If their recommendations are based on total protein, this might explain the higher values they suggest. However, even with this consideration, our findings indicate that true

protein concentrations would still fall below their recommended intake and therefore fortification of true protein should be considered in clinical settings.

Lactoferrin makes up about 20% of true protein content in human milk (Kulesza-Brończyk et al., 2023). Lactoferrin has antimicrobial properties and also plays a key role in immune function (Legrand, 2016). As protein content is higher in milk collected in early lactation it is likely that it is also higher in lactoferrin (Kulesza-Brończyk et al., 2023), and therefore, be particularly beneficial for preterm or high-risk infants who are vulnerable to infection. A large proportion of the milk being donated to the CWHMB comes from the first 12 weeks postpartum, and therefore is likely to contain higher lactoferrin concentrations, making it particularly helpful for these at-risk infants. Prioritising milk from earlier in lactation for vulnerable preterm infants, could not only help meet protein requirements but also provide more of the beneficial lactoferrin. Further research is needed to explore the role of lactoferrin in DHM and to better define the optimal lactoferrin concentrations for these vulnerable populations.

Our findings concur with existing research on DHM, which indicates lactation stage is not a predictor of carbohydrate concentration (Muts et al., 2025; Saarela et al., 2005). Regarding lactation stage and fat content of DHM, Muts et al. (2025) observed an increase after 8 months, but Walter et al. (2023) saw no change. Similarly to Walter et al. (2023), we did not observe an association between fat and lactation stage. However, this may be due to the distribution of our dataset, as most of our donations were from the first 12 weeks postpartum (n = 496) compared to after 12 weeks (n = 115). This uneven distribution may have biased our regression model toward early-lactation trends, reducing sensitivity to potential changes in carbohydrate later in lactation.

3.5.4 Influence of gestational age category (preterm versus term)

Preterm milk contained significantly more energy, fat, true protein, and carbohydrate than term mature milk, as expected and consistent with other similar studies on DHM (Holritz et al., 2024; Mills et al., 2019; Tanaka et al., 2023; Walter et al., 2023). The higher protein content in preterm milk may reflect the increased nutritional demands of a preterm infant, needed to support rapid growth and organ development (Gates et al., 2023; Ziegler et al., 1976). Walter et al. (2023) included lactation stage in their mixed-model analysis and found that protein content was influenced mainly by lactation stage rather than gestational age. In contrast, Mills et al. (2019) reported that protein content remained significantly associated with gestational age even after controlling for lactation stage. Although we did

not adjust our regression analysis to account for lactation stage in our analysis, we categorised preterm milk as milk expressed before 37 weeks gestation, whilst term milk referred to milk expressed at 37 weeks or later, even if from the same donor. Therefore, it is reasonable to believe that some of the observed differences in our study may have reflected time postpartum (and therefore lactation stage), rather than purely the effect of gestational age. We also did not consider the degree of prematurity, although Mills et al. (2019) found that the level of prematurity did not significantly alter their results. Overall, while there was a difference between preterm and term milk, both contained mean concentrations of energy, fat, true protein, and carbohydrates within the recommended nutrient range for preterm infants according to the ESPGHAN guidelines (based on an intake of 160 mL/kg/day).

3.6 Strengths and limitations

Our study had three main strengths. Firstly, we included a diverse range of variables, some of which have not been previously explored in a New Zealand DHM population (donor age and ethnicity, and lactation stage). Although we may not be able to make definitive conclusions about how some of these variables impact DHM energy and macronutrient composition, our research sets the groundwork for future, more targeted studies. Secondly, true protein was reported as opposed to total protein, reflecting only the bioavailable amino acids and providing a more accurate estimation of the nutritional content available to the recipient infant. Finally, our analysis provided a comprehensive overview of DHM energy and macronutrient composition as we included data from all DHM batches donated to the CWHMB over two years (n = 696). The only other study to date on New Zealand DHM by Lamb et al. (2021) analysed a much smaller sample size (n = 63).

Whilst this study is of clinical interest for nutrition precision and personalised nutritional guidance for infant feeding, as it reports the known macronutrient composition of DHM, it presents some limitations. Firstly, other variables that may have influenced the macronutrient composition, such as donor body mass index, diet quality, circadian rhythm and whether the donation was ‘fore’ or ‘hind’ milk (i.e., whether it came from the start or end of a given expression), were not explored or accounted for, as this information was not available to us. Secondly, the regression model used for statistical analysis did not account for individual donor variability as a random effect, which could have led to an underestimation of between-donor variability.

3.7 Conclusion

Our findings contribute to the growing understanding of the macronutrient composition of DHM in New Zealand. The most concerning result was that the average true protein and energy concentrations of the DHM may not be high enough to meet the nutritional needs of preterm infants. This could increase the risk of extra-uterine growth restriction and poorer health outcomes. We also found extreme variability in fat content, which was likely due to differences in milk handling and processing or expression practices, rather than actual physiological differences, although more research is needed to confirm this. Overall, the large variability between batches, and the low true protein and energy concentrations highlight the importance of point-of-care milk analysis and targeted fortification in New Zealand milk banks to ensure that infants receive adequate nutrition.

Chapter 4. Discussion

The purpose of this study was to understand the energy and macronutrient composition of donor human milk (DHM) from a New Zealand milk bank. Additionally, the study aimed to identify factors which influence DHM macronutrient content. A secondary data analysis was conducted to describe the energy and macronutrient composition of DHM which had been donated to New Zealand's first human milk bank in the Christchurch Women's Hospital (CWHMB), and then single linear regression analysis was performed to explore the association between DHM macronutrients and the following variables: lactation stage, gestational age of the infant when the milk was expressed (i.e. preterm or term), donor age, and donor ethnicity. Existing research examining the macronutrient composition has highlighted not only a significant variability in fat and protein but that mean values often fall below preterm infant needs (Perrin et al., 2020). This variability and inconsistency may impact growth and health outcomes for preterm and critically ill infants. Chapter four will discuss how well our study achieved it's aims and objectives, consider the research impact and practical applications, discuss the study's strengths and limitations, and finally, recommendations for future research are outlined.

4.1 Achievements of aims and objectives

The aim of this research project was to understand the energy and macronutrient composition of DHM from the CWHMB and explore the biological and external factors which may influence its composition. The study was a secondary data analysis using descriptive statistics and single linear regression analysis. To achieve the research aim, three objectives were created.

The first objective was to describe the energy and macronutrient composition (fat, protein, and carbohydrate) of DHM. This objective was achieved by calculating the mean and median of each macronutrient and using box and whisker plots to provide a visual representation of the data. Samples of every single donation made to the CWHMB from July 2022 until July 2024 were analysed prior to the study by the CWHMB staff using the Miris human milk analyser (HMA), a validated method for measuring the macronutrient composition of human milk. The Miris HMA is also a very common milk analysis method, enabling meaningful comparison with other published DHM studies. Our mean energy, fat, true protein, and carbohydrate concentrations were relatively similar to the American Academy of Nutrition and Dietetics reference values for DHM (cited in Perrin et al., 2020), which suggests that our results are representative of DHM in general and provides validity to our study.

However, our range of fat was substantially larger than reports in existing literature, suggesting that DHM processing or expression practices outside of our control may have influenced the results.

The second objective was to explore the association between donor characteristics (age and ethnicity) and the energy and macronutrient composition of DHM. To achieve this objective, single variable linear regression analyses were conducted with energy, fat, true protein, and carbohydrates as the dependent variables and donor age and donor ethnicity as the independent variables. Results were reported as mean difference, 95% confidence intervals (CI), and *p*-values. Although we found some statistically significant difference according to ethnicity, our ethnic groups were small and uneven in size (Māori, $n = 16$; Pacific, $n = 7$; Asian, $n = 28$; Others, $n = 14$; European, $n = 84$). This meant statistical power was reduced and therefore limited our ability to make generalisable interpretations regarding differences in DHM between ethnicities.

The third objective was to explore how lactation stage and gestational age of the infant affect the energy and macronutrient composition of DHM. Like the second objective, single variable linear regression analyses were conducted. In line with existing research, we found lactation stage to be a strong predictor of true protein content. Unlike a similar Australian study (Walter et al., 2023), we did not observe an association between fat and lactation stage; however, our dataset had an uneven distribution, with more DHM batched from the first 12 weeks of lactation, and this may have biased our regression model, reducing sensitivity to possible associations later in lactation. Preterm mature milk had higher energy and macronutrient content that term milk, likely reflecting the greater nutritional needs of preterm infants, though some differences may be attributed to lactation stage rather than gestational age alone. Overall, our study achieved the third objective and was able to demonstrate that both lactation stage and gestational age of the infant at the time the milk was expressed are determinants of the energy and macronutrient composition of DHM from the CWHMB.

4.2 Research impact

At the time of writing, this study is the largest in Australasia to report the energy and macronutrient composition of banked DHM. The findings highlight the substantial variability of the energy and fat content of DHM from single-donor pools. Lactation stage was a strong predictor of true protein content and gestational age was a strong predictor of energy and all macronutrients, all of which agree with existing literature. Our study raises awareness that, on average, DHM from the CWHMB

has true protein concentrations below the recommended intake for preterm infants, and this finding may also extend to other New Zealand and international milk banks. Overall, the findings from this study support the use point-of-care analysis and prompts milk banks to consider individualised approaches to DHM fortification to ensure the preterm infant recipients receive the adequate nutrients.

4.3 Strengths

Our research project had two main strengths. Firstly, the secondary data analysis design allowed us to utilise a large dataset which would otherwise not have been feasible to collect within the scope of this student research project. By having access to all data collected from the CWHMB, we were able to examine associations that may not have been detectable in a small sample and assured the study design was appropriate for the research question. Moreover, our large data set allowed us to provide evidence on DHM specific to the New Zealand context, which is important for informing local clinical practice. The second strength was that our dataset reported true protein values rather than total protein. Total protein measures include non-protein nitrogen, whereas true protein reflects only the bioavailable amino acids, providing a more accurate estimate of the infant's nutritional intake and, therefore, is more clinically relevant (Belfort et al., 2024). Specifying the protein type allows for clear interpretation of findings without assumptions about bioavailability and allows results to be more easily compared with other literature. Many existing studies on DHM macronutrients report total protein. While these results provide a general overview of macronutrient content, they don't accurately reflect the recipients' intake, which is an important and clinically relevant consideration (de Halleux & Rigo, 2013; Donovan et al., 2017; John et al., 2019; Meredith-Dennis et al., 2018; Moukarzel et al., 2017; Perrin et al., 2017).

4.4 Limitations

This research project had several limitations. Firstly, the project was designed as a secondary data analysis, and therefore, the researchers had limited control over the variables that were collected. The data was collected for clinical purposes, rather than research, and may not have been as strictly controlled as would be expected in an observational trial. Some assumptions had to be made to account for this. For example, it was assumed that if a given macronutrient value appeared to be in error, the other macronutrients in that sample were unaffected. We also assumed that all milk bank staff were adequately trained in handling and processing milk samples, and that each analysis was

conducted consistently. The CWHMB uses the Operational Guidelines for Milk Banks in Australia and New Zealand (Human Milk Regulation Working Group, 2025, June 4) to guide their procedures; however, standardised DHM analysis protocols in New Zealand don't exist. Secondly, the regression model used in the statistical analysis did not account for individual donor variability as a random effect, potentially leading to an underestimation of between-donor variability. This was due to the scope of the research project and the ability of the student researcher. Finally, although milk donated to the CWHMB was representative of the ethnic groups present in New Zealand, it is not known if these proportions also reflect the ethnic groups of the infants receiving the DHM, which could be relevant if ethnicity is a significant predictor of DHM energy and macronutrient composition.

4.5 Recommendations for future research

- Other variables which may possibly influence DHM macronutrient composition such as parity, donor BMI, donor diet, and infant birthweight should be explored to provide a more comprehensive understanding of the factors which influence DHM macronutrients.
- If multiple batches of DHM are provided from the same donor, individual donor variability should be accounted for as a random effect in statistical analysis. Adding a random effect will allow the statistical model to account for correlations from the same donor and therefore estimate overall effects more accurately.
- Sufficiently large and evenly sized subgroups should be analysed before making conclusions about the effects of donor ethnicity on DHM macronutrient composition.
- We found extreme variability in fat content, which was likely due to differences in milk handling and processing or expression practices, rather than actual physiological differences. Detailed descriptions of DHM collection, processing and handling should always be considered in analyses and included to provide transparency to the reader.

5. References

Adhisivam, B., Vishnu Bhat, B., Rao, K., Kingsley, S. M., Plakkal, N., & Palanivel, C. (2019). Effect of Holder pasteurization on macronutrients and immunoglobulin profile of pooled donor human milk. *The Journal of Maternal-Fetal & Neonatal Medicine*, *32*(18), 3016-3019. https://doi.org/10.1080/14767058.2018.1455089

Agostoni, C., Braegger, C., Decsi, T., Kolacek, S., Koletzko, B., Michaelsen, K. F., Mihatsch, W., Moreno, L. A., Puntis, J., Shamir, R., Szajewska, H., Turck, D., & van Goudoever, J. B. (2009). Breast-feeding: A commentary by the ESPGHAN committee on nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, *49*(1), 112-125. https://doi.org/https://doi.org/10.1097/MPG.0b013e31819f1e05

Agrawal, M., Sabino, J., Frias-Gomes, C., Hillenbrand, C. M., Soudant, C., Axelrad, J. E., Shah, S. C., Ribeiro-Mourão, F., Lambin, T., Peter, I., Colombel, J. F., Narula, N., & Torres, J. (2021). Early life exposures and the risk of inflammatory bowel disease: Systematic review and meta-analyses. *EClinicalMedicine*, *36*, 100884. https://doi.org/10.1016/j.eclinm.2021.100884

Ahrabi, A. F., Handa, D., Codipilly, C. N., Shah, S., Williams, J. E., McGuire, M. A., Potak, D., Aharon, G. G., & Schanler, R. J. (2016). Effects of extended freezer storage on the integrity of human milk. *The Journal of Pediatrics*, *177*, 140-143. https://doi.org/https://doi.org/10.1016/j.jpeds.2016.06.024

Al-Nawaiseh, F. K., Al-Jaghbir, M. T., Al-Assaf, M. S., Al-Nawaiseh, H. K., & Alzoubi, M. M. (2022). Breastfeeding initiation and duration and acute otitis media among children less than two years of age in Jordan: Results from a case–control study. *BMC Pediatrics*, *22*(1), 370. https://doi.org/10.1186/s12887-022-03427-7

Alhindi, M. A., Musallam, H. M., & Elhefian, E. A. (2019). Characteristics of human breast milk based on infant's gender. *Journal or Basic and Applied Research in Biomedicine*, *5*(1), 8-10. https://doi.org/10.51152/jbarbiomed.v5i1.21

American College of Obstetricians and Gynaecologists. (2013). ACOG Committee opinion no. 579: Definition of term pregnancy. *Obstetrics and Gynecology*, *122*(5), 1139-1140. https://doi.org/10.1097/01.AOG.0000437385.88715.4a

Amiel Castro, R., Glover, V., Ehlert, U., & O'Connor, T. G. (2021). Breastfeeding, prenatal depression and children's IQ and behaviour: A test of a moderation model. *BMC Pregnancy and Childbirth*, *21*(1), 62. https://doi.org/10.1186/s12884-020-03520-8

Amitay, E. L., & Keinan-Boker, L. (2015). Breastfeeding and childhood leukemia incidence: A meta-analysis and systematic review. *JAMA Pediatrics*, *169*(6), e151025-e151025. https://doi.org/10.1001/jamapediatrics.2015.1025

Andreas, N. J., Kampmann, B., & Mehring Le-Doare, K. (2015). Human breast milk: A review on its composition and bioactivity. *Early Human Development*, *91*(11), 629-635. https://doi.org/https://doi.org/10.1016/j.earlhumdev.2015.08.013

Argov-Argaman, N., Mandel, D., Lubetzky, R., Hausman Kedem, M., Cohen, B. C., Berkovitz, Z., & Reifen, R. (2017). Human milk fatty acids composition is affected by maternal age. *The Journal of Maternal-Fetal & Neonatal Medicine*, *30*(1), 34-37. https://doi.org/10.3109/14767058.2016.1140142

Armand, M., Bernard, J. Y., Forhan, A., Heude, B., Charles, M.-A., Annesi-Maesano, I., Bernard, J. Y., Botton, J., Charles, M.-A., Dargent-Molina, P., de Lauzon-Guillain, B., Ducimetière, P., De Agostini, M., Foliguet, B., Forhan, A., Fritel, X., Germa, A., Goua, V., Hankard, R., . . . Thiebaugeorges, O. (2018). Maternal nutritional determinants of colostrum fatty acids in the

EDEN mother-child cohort. *Clinical Nutrition*, *37*(6), 2127-2136. https://doi.org/10.1016/j.clnu.2017.10.007

Arslanoglu, S., Boquien, C.-Y., King, C., Lamireau, D., Tonetto, P., Barnett, D., Bertino, E., Gaya, A., Gebauer, C., Grovslien, A., Moro, G. E., Weaver, G., Wesolowska, A. M., & Picaud, J.-C. (2019). Fortification of human milk for preterm infants: Update and recommendations of the European Milk Bank Association (EMBA) working group on human milk fortification. *Frontiers in Pediatrics*, *7*. https://doi.org/10.3389/fped.2019.00076

Arslanoglu, S., Corpeleijn, W., Moro, G. E., Braegger, C., Campoy, C., Colomb, V., Decsi, T., Domellöf, M., Fewtrell, M., Hojsak, I., Mihatsch, W., Mølgaard, C., Shamir, R., Turck, D., & van Goudoever, J. B. (2013). Donor human milk for preterm infants. *Journal of Pediatric Gastroenterology and Nutrition*, *57*(4), 535-542. https://doi.org/https://doi.org/10.1097/MPG.0b013e3182a3af0a

Arslanoglu, S., Moro, G. E., Tonetto, P., De Nisi, G., Ambruzzi, A. M., Biasini, A., Profeti, C., Gagliardi, L., Salvatori, G., & Bertino, E. (2023). Recommendations for the establishment and operation of a donor human milk bank. *Nutrition Reviews*, *81*(Suppl 1), 1-28. https://doi.org/10.1093/nutrit/nuad012

Arya, S., Saini, V., S, M. B., Kuniabdullah, S., & Irfan Khan, S. (2025). Evaluation of the risk factors for retinopathy of prematurity in preterm infants admitted to the neonatal intensive care unit (NICU). *Cureus*, *17*(1), e77163. https://doi.org/10.7759/cureus.77163

Assad, M., Elliott, M. J., & Abraham, J. H. (2016). Decreased cost and improved feeding tolerance in VLBW infants fed an exclusive human milk diet. *Journal of Perinatology*, *36*(3), 216-220. https://doi.org/10.1038/jp.2015.168

Aumeistere, L., Ciproviča, I., Zavadska, D., Andersons, J., Volkovs, V., & Ceļmalniece, K. (2019). Impact of maternal diet on human milk composition among lactating women in Latvia. *Medicina*, *55*(5), 173. https://doi.org/10.3390/medicina55050173

Avila-Alvarez, A., Fernandez-Gonzalez, S. M., Sucasas-Alonso, A., & Ansede, A. S. (2025). Initiation of enteral feeding with mother's own milk or donor human milk in very preterm infants: Impact on bronchopulmonary dysplasia and other prematurity-related morbidities. *Nutrients*, *17*(3), 508. https://doi.org/doi:10.3390/nu17030508

Bachour, P., Yafawi, R., Jaber, F., Choueiri, E., & Abdel-Razzak, Z. (2011). Effects of smoking, mother's age, body mass index, and parity number on lipid, protein, and secretory immunoglobulin A concentrations of human milk. *Breastfeeding Medicine*, *7*, 179-188. https://doi.org/10.1089/bfm.2011.0038

Ballard, O., & Morrow, A. L. (2013). Human milk composition: Nutrients and bioactive factors. *Pediatric Clinics of North America*, *60*(1), 49-74. https://doi.org/10.1016/j.pcl.2012.10.002

Barbarska, O., Zielińska, M., Pawlus, B., & Wesołowska, A. (2017). Characteristics of the regional human milk bank in Poland - Donors, recipients and nutritional value of human milk. *Roczniki Państwowego Zakładu Higieny*, *68*(4), 395-400.

Belfort, M. B., Edwards, E. M., Greenberg, L. T., Parker, M. G., Ehret, D. Y., & Horbar, J. D. (2019). Diet, weight gain, and head growth in hospitalized US very preterm infants: A 10-year observational study. *The American Journal of Clinical Nutrition*, *109*(5), 1373-1379. https://doi.org/https://doi.org/10.1093/ajcn/nqz008

Belfort, M. B., Stellwagen, L., North, K., Unger, S., O'Connor, D. L., & Perrin, M. T. (2024). Deciphering macronutrient information about human milk. *Journal of Perinatology*, *44*(9), 1377-1381. https://doi.org/10.1038/s41372-024-02029-8

Bharwani, S. K., Green, B. F., Pezzullo, J. C., Bharwani, S. S., Bharwani, S. S., & Dhanireddy, R. (2016). Systematic review and meta-analysis of human milk intake and retinopathy of prematurity: A significant update. *Journal of Perinatology*, *36*(11), 913-920. https://doi.org/10.1038/jp.2016.98

Bishop, C. E., Vasquez, M. M., Petershack, J. A., & Blanco, C. L. (2010). Pasteurized donor human milk for VLBW infants: The effect on necrotizing enterocolitis and related factors. *Journal of Neonatal-Perinatal Medicine*, *3*(2), 87-93. https://doi.org/10.3233/npm-2010-0099

Blazon, M. N., Rezar-Dreindl, S., Wassermann, L., Neumayer, T., Berger, A., & Stifter, E. (2024). Retinopathy of prematurity: Incidence, risk factors, and treatment outcomes in a tertiary care center. *Journal of Clinical Medicine*, *13*(22), 6926.

Borràs-Novell, C., Herranz Barbero, A., Aldecoa-Bilbao, V., Feixas Orellana, G., Balcells Esponera, C., Sánchez Ortiz, E., García-Algar, O., & Iglesias Platas, I. (2020). Infrared analyzers for the measurement of breastmilk macronutrient content in the clinical setting. *Expert Review of Molecular Diagnostics*, *20*(9), 867-887. https://doi.org/10.1080/14737159.2020.1816465

Borràs-Novell, C., Herranz Barbero, A., Balcells Esponera, C., López-Abad, M., Aldecoa Bilbao, V., Izquierdo Renau, M., & Iglesias Platas, I. (2023). Influence of maternal and perinatal factors on macronutrient content of very preterm human milk during the first weeks after birth. *Journal of Perinatology*, *43*(1), 52-59. https://doi.org/10.1038/s41372-022-01475-6

Bowatte, G., Tham, R., Allen, K. J., Tan, D. J., Lau, M., Dai, X., & Lodge, C. J. (2015). Breastfeeding and childhood acute otitis media: A systematic review and meta-analysis. *Acta Paediatrica*, *104*(467), 85-95. https://doi.org/10.1111/apa.13151

Bowornkitiwong, W., Komoltri, C., & Ngerncham, S. (2023). The relationship between creamatocrit and cumulative percentage of total milk volume: A cross-sectional study in mothers of very preterm infants in Bangkok, Thailand. *International Breastfeeding Journal*, *18*(1), 63. https://doi.org/10.1186/s13006-023-00599-5

Brown, J. V., Lin, L., Embleton, N. D., Harding, J. E., & McGuire, W. (2020). Multi-nutrient fortification of human milk for preterm infants. *Cochrane Database of Systematic Reviews*(6). https://doi.org/10.1002/14651858.CD000343.pub4

Burianova, I., Bronsky, J., Pavlikova, M., Janota, J., & Maly, J. (2019). Maternal body mass index, parity and smoking are associated with human milk macronutrient content after preterm delivery. *Early Human Development*, *137*, 104832. https://doi.org/https://doi.org/10.1016/j.earlhumdev.2019.104832

Butts, C. A., Hedderley, D. I., Herath, T. D., Paturi, G., Glyn-Jones, S., Wiens, F., Stahl, B., & Gopal, P. (2018). Human milk composition and dietary intakes of breastfeeding women of different ethnicity from the Manawatu-Wanganui region of New Zealand. *Nutrients*, *10*(9). https://doi.org/10.3390/nu10091231

Bzikowska-Jura, A., Machaj, N., Sobieraj, P., Barbarska, O., Olędzka, G., & Wesolowska, A. (2021). Do maternal factors and milk expression patterns affect the composition of donor human milk? *Nutrients*, *13*(7). https://doi.org/10.3390/nu13072425

Carr, L. E., Virmani, M. D., Rosa, F., Munblit, D., Matazel, K. S., Elolimy, A. A., & Yeruva, L. (2021). Role of human milk bioactives on infants' gut and immune health. *Frontiers in Immunology*, *12*, 604080. https://doi.org/10.3389/fimmu.2021.604080

Chang, N., Jung, J. A., Kim, H., Jo, A., Kang, S., Lee, S. W., Yi, H., Kim, J., Yim, J. G., & Jung, B. M. (2015). Macronutrient composition of human milk from Korean mothers of full term infants born at 37-42 gestational weeks. *Nutrition Research and Practice*, *9*(4), 433-438. https://doi.org/10.4162/nrp.2015.9.4.433

Chowning, R., Radmacher, P., Lewis, S., Serke, L., Pettit, N., & Adamkin, D. H. (2016). A retrospective analysis of the effect of human milk on prevention of necrotizing enterocolitis and postnatal growth. *Journal of Perinatology*, *36*(3), 221-224. https://doi.org/10.1038/jp.2015.179

Conboy-Stephenson, R., Ross, R. P., Kelly, A. L., & Stanton, C. (2024). Donor human milk: The influence of processing technologies on its nutritional and microbial composition. *Frontiers in Nutrition*, *Volume 11 - 2024*. https://doi.org/10.3389/fnut.2024.1468886

Cubero, J., Valero, V., Sanchez, J., Rivero, M., Parvez, H., Rodriguez, A. B., & Barriga, C. (2005). The circadian rhythm of tryptophan in breast milk affects the rhythms of 6-sulfatoxymelatonin and sleep in newborn. *Neuroendocrinology Letters*, *26*.

Czank, C., Simmer, K., & Hartmann, P. E. (2009). A method for standardizing the fat content of human milk for use in the neonatal intensive care unit. *International Breastfeeding Journal*, *4*, 3. https://doi.org/10.1186/1746-4358-4-3

Czosnykowska-Łukacka, M., Królak-Olejnik, B., & Orczyk-Pawiłowicz, M. (2018). Breast milk macronutrient components in prolonged lactation. *Nutrients*, *10*(12). https://doi.org/10.3390/nu10121893

Dafaallah, S., Amin, M., Mohammed, A., Kabbara, E., & Abbas, T. (2018). Biochemical differences in human breast milk contents accroding to infant's gender. *Anatomy Physiology & Biochemistry International Journal*, *5*(3), 555661. https://doi.org/10.19080/APBIJ.2018.05.555661

Daly, S. E., Di Rosso, A., Owens, R. A., & Hartmann, P. E. (1993). Degree of breast emptying explains changes in the fat content, but not fatty acid composition, of human milk. *Experimental Physiology*, *78*(6), 741-755. https://doi.org/10.1113/expphysiol.1993.sp003722

Daniels, S., Corkins, M., de Ferranti, S., Golden, N. H., Kim, J. H., Magge, S. N., Schwarzenberg, S. J., Meek, J. Y., Johnston, M. G., O'Connor, M. E., Stellwagen, L. M., Thomas, J. P., Ware, J. L., Schanler, R. J., Watterberg, K. L., Aucott, S. W., Benitz, W. E., Cummings, J. J., Eichenwald, E. C., . . . Stewart, D. L. (2017). Donor human milk for the high-risk infant: Preparation, safety, and usage Osptions in the United States. *Pediatrics*, *139*(1). https://doi.org/10.1542/peds.2016-3440

Davis, A., Lee, S. W., Hampel, D., Shahab-Ferdows, S., Allen, L. H., Bode, L., Mansen, K., Israel-Ballard, K., & Perrin, M. T. (2025). The impact of Holder pasteurization on macronutrients, vitamins, minerals and bioactive factors in human milk processed in a milk bank setting. *Current Developments in Nutrition*, *9*(8), 107490. https://doi.org/https://doi.org/10.1016/j.cdnut.2025.107490

Davisse-Paturet, C., Adel-Patient, K., Forhan, A., Lioret, S., Annesi-Maesano, I., Heude, B., Charles, M. A., & de Lauzon-Guillain, B. (2020). Breastfeeding initiation or duration and longitudinal patterns of infections up to 2 years and skin rash and respiratory symptoms up to 8 years in the EDEN mother-child cohort. *Maternal & Child Nutrition*, *16*(3), e12935. https://doi.org/https://doi.org/10.1111/mcn.12935Digital

de Halleux, V., & Rigo, J. (2013). Variability in human milk composition: Benefit of individualized fortification in very-low-birth-weight infants. *The American Journal of Clinical Nutrition*, *98*(2), 529S-535S. https://doi.org/https://doi.org/10.3945/ajcn.112.042689

de la Garza Puentes, A., Martí Alemany, A., Chisaguano, A. M., Montes Goyanes, R., Castellote, A. I., Torres-Espínola, F. J., García-Valdés, L., Escudero-Marín, M., Segura, M. T., Campoy, C., & López-Sabater, M. C. (2019). The effect of maternal obesity on breast milk fatty acids and its association with infant growth and cognition—The PREOBE follow-up. *Nutrients*, *11*(9), 2154. https://doi.org/doi:10.3390/nu11092154

de Waard, M., Mank, E., van Dijk, K., Schoonderwoerd, A., & van Goudoever, J. B. (2018). Holder-pasteurized human donor milk: How long can it be preserved? *Journal of Pediatric Gastroenterology and Nutrition*, *66*(3), 479-483. https://doi.org/10.1097/mpg.0000000000001782

Deffrennes, S., Rayyan, M., Fidlers, T., van den Heuvel, L., Levtchenko, E., & Arcolino, F. O. (2024). Impact of preterm birth on kidney health and development. *Front Med (Lausanne)*, *11*, 1363097. https://doi.org/10.3389/fmed.2024.1363097

Dogaru, C. M., Nyffenegger, D., Pescatore, A. M., Spycher, B. D., & Kuehni, C. E. (2014). Breastfeeding and childhood asthma: Systematic review and meta-analysis. *American Journal of Epidemiology*, *179*(10), 1153-1167. https://doi.org/10.1093/aje/kwu072

Donovan, R., Kelly, S. G., Prazad, P., Talaty, P. N., Lefaiver, C., Hastings, M. L., & Everly, D. N. (2017). The effects of human milk fortification on nutrients and milk properties. *Journal of Perinatology*, *37*(1), 42-48. https://doi.org/10.1038/jp.2016.166

dos Santos, B. G., Shenker, N., Weaver, G., & Perrin, M. T. (2024). Donation barriers, enablers, patterns and predictors of milk bank donors in the United States and United Kingdom. *Maternal & Child Nutrition*, *20*(3), e13652. https://doi.org/https://doi.org/10.1111/mcn.13652

Dritsakou, K., Liosis, G., Valsami, G., Polychronopoulos, E., & Skouroliakou, M. (2017). The impact of maternal- and neonatal-associated factors on human milk's macronutrients and energy. *The Journal of Maternal-Fetal & Neonatal Medicine*, *30*(11), 1302-1308. https://doi.org/10.1080/14767058.2016.1212329

Embleton, N. D., Jennifer Moltu, S., Lapillonne, A., van den Akker, C. H. P., Carnielli, V. P., Fusch, C., Gerasimidis, K., van Goudoever, J. B., Haiden, N., Iacobelli, S., Johnson, M. J., Meyer, S., Mihatsch, W., de Pipaon, M. S., Rigo, J., Zachariassen, G., Bronsky, J., Indrio, F., Köglmeier, J., . . . Domellöf, M. (2023). Enteral nutrition in preterm infants (2022): A position paper from the ESPGHAN committee on nutrition and invited experts. *Journal of Pediatric Gastroenterology and Nutrition*, *76*(2), 248-268. https://doi.org/https://doi.org/10.1097/MPG.0000000000003642

Erickson, G., Dobson, N. R., & Hunt, C. E. (2021). Immature control of breathing and apnea of prematurity: The known and unknown. *Journal of Perinatology*, *41*(9), 2111-2123. https://doi.org/10.1038/s41372-021-01010-z

Fabrizio, V., Trzaski, J. M., Brownell, E. A., Esposito, P., Lainwala, S., Lussier, M. M., & Hagadorn, J. I. (2020). Individualized versus standard diet fortification for growth and development in preterm infants receiving human milk. *Cochrane Database of Systematic Reviews*, *11*(11), Cd013465. https://doi.org/10.1002/14651858.CD013465.pub2

Fanaro, S. (2013). Feeding intolerance in the preterm infant. *Early Human Development*, *89*(2), S13-S20. https://doi.org/https://doi.org/10.1016/j.earlhumdev.2013.07.013

Fenton, T. R., Anderson, D., Groh-Wargo, S., Hoyos, A., Ehrenkranz, R. A., & Senterre, T. (2018). An attempt to standardize the calculation of growth velocity of preterm infants; Evaluation of practical bedside methods. *The Journal of Pediatrics*, *196*, 77-83. https://doi.org/10.1016/j.jpeds.2017.10.005

Fernández-Tuñas, M. d.-C., Torres-López, L., Vidal-Martínez, S., García-Couceiro, N., Pérez-Muñuzuri, A., & Couce, M. L. (2025). Impact of perinatal factors on breast milk composition and volume in preterm infants. *Scientific Reports*, *15*(1), 19876. https://doi.org/10.1038/s41598-025-04740-8

Fischer Fumeaux, C. J., Garcia-Rodenas, C. L., De Castro, C. A., Courtet-Compondu, M.-C., Thakkar, S. K., Beauport, L., Tolsa, J.-F., & Affolter, M. (2019). Longitudinal analysis of

macronutrient composition in preterm and term human milk: A prospective cohort study. *Nutrients*, *11*(7), 1525. https://doi.org/doi:10.3390/nu11071525

Frank, N. M., Lynch, K. F., Uusitalo, U., Yang, J., Lönnrot, M., Virtanen, S. M., Hyöty, H., Norris, J. M., Rewers, M., Bautista, K., Baxter, J., Felipe-Morales, D., Driscoll, K., Frohnert, B. I., Gallant, M., Gesualdo, P., Hoffman, M., Karban, R., Liu, E., . . . Triplett, E. (2019). The relationship between breastfeeding and reported respiratory and gastrointestinal infection rates in young children. *BMC Pediatrics*, *19*(1), 339. https://doi.org/10.1186/s12887-019-1693-2

Fusch, G., Rochow, N., Choi, A., Fusch, S., Poeschl, S., Ubah, A. O., Lee, S.-Y., Raja, P., & Fusch, C. (2015). Rapid measurement of macronutrients in breast milk: How reliable are infrared milk analyzers? *Clinical Nutrition*, *34*(3), 465-476. https://doi.org/https://doi.org/10.1016/j.clnu.2014.05.005

García-Muñoz Rodrigo, F., Losada Martínez, A., Elorza Fernández, M. D., Moreno Hernando, J., Figueras Aloy, J., & Vento Torres, M. (2017). The burden of respiratory disease in very-low-birth-weight infants: Changes in perinatal care and outcomes in a decade in Spain. *Neonatology*, *112*(1), 30-39. https://doi.org/10.1159/000455966

Gates, A., Hair, A. B., Salas, A. A., Thompson, A. B., & Stansfield, B. K. (2023). Nutrient composition of donor human milk and comparisons to preterm human milk. *The Journal of Nutrition*, *153*(9), 2622-2630. https://doi.org/https://doi.org/10.1016/j.tjnut.2023.07.012

Gephart, S. M., McGrath, J. M., Effken, J. A., & Halpern, M. D. (2012). Necrotizing enterocolitis risk: State of the science. *Advances in Neonatal Care*, *12*(2), 77-87. https://doi.org/10.1097/ANC.0b013e31824cee94

Goyal, K., Arya, S., & Guliani, B. P. (2024). Effect of breast milk feeding on retinopathy of prematurity in neonates less than 1800 grams: A cohort. *Journal of Neonatology*, *38*(1), 52-59. https://doi.org/10.1177/09732179231220208

Groh-Wargo, S., Valentic, J., Khaira, S., Super, D. M., & Collin, M. (2016). Human milk analysis using mid-infrared spectroscopy. *Nutrition in Clinical Practice*, *31*(2), 266-272. https://doi.org/https://doi.org/10.1177/0884533615596508

Grote, V., Verduci, E., Scaglioni, S., Vecchi, F., Contarini, G., Giovannini, M., Koletzko, B., & Agostoni, C. (2016). Breast milk composition and infant nutrient intakes during the first 12 months of life. *European Journal of Clinical Nutrition*, *70*(2), 250-256. https://doi.org/10.1038/ejcn.2015.162

Gu, X., Shi, X., Zhang, L., Zhou, Y., Cai, Y., Jiang, W., & Zhou, Q. (2021). Evidence summary of human milk fortifier in preterm infants. *Translational Pediatrics*, *10*(11), 3058-3067. https://doi.org/10.21037/tp-21-476

Hahn, W., Song, J., Song, S., & Kang, N. (2016). Do gender and birth height of infant affect calorie of human milk? An association study between human milk macronutrient and various birth factors. *The Journal of Maternal-Fetal & Neonatal Medicine*, *30*(13), 1608-1612. https://doi.org/10.1080/14767058.2016.1219989

Hair, A. B., Peluso, A. M., Hawthorne, K. M., Perez, J., Smith, D. P., Khan, J. Y., O'Donnell, A., Powers, R. J., Lee, M. L., & Abrams, S. A. (2016). Beyond necrotizing enterocolitis prevention: Improving outcomes with an exclusive human milk–based diet. *Breastfeeding Medicine*, *11*(2), 70-74. https://doi.org/10.1089/bfm.2015.0134

Hamosh, M. (1996). Digestion in the newborn. *Clinics in Perinatology*, *23*(2), 191-209.

Harris, S., Bloomfield, F. H., & Muelbert, M. (2024). Formal and informal human milk donation in New Zealand: A mixed-method national survey. *International Breastfeeding Journal*, *19*(1), 61. https://doi.org/10.1186/s13006-024-00667-4

Hartmann, P., & Cregan, M. (2001). Lactogenesis and the effects of insulin-dependent diabetes mellitus and prematurity. *The Journal of Nutrition*, *131*(11), 3016S-3020S. https://doi.org/https://doi.org/10.1093/jn/131.11.3016S

Hassiotou, F., Hepworth, A. R., Williams, T. M., Twigger, A. J., Perrella, S., Lai, C. T., Filgueira, L., Geddes, D. T., & Hartmann, P. E. (2013). Breastmilk cell and fat contents respond similarly to removal of breastmilk by the infant. *PloS one*, *8*(11), e78232. https://doi.org/10.1371/journal.pone.0078232

Hay, W. W., Jr. (1996). Assessing the effect of disease on nutrition of the preterm infant. *Clinical Biochemistry*, *29*(5), 399-417. https://doi.org/10.1016/0009-9120(96)00062-8

Henderickx, J. G. E., Zwittink, R. D., Renes, I. B., van Lingen, R. A., van Zoeren-Grobben, D. J. L. J. G., Boeren, S., van Elburg, R. M., Knol, J., & Belzer, C. (2021). Maturation of the preterm gastrointestinal tract can be defined by host and microbial markers for digestion and barrier defense. *Scientific Reports*, *11*(1), 12808. https://doi.org/10.1038/s41598-021-92222-y

Hill, P. D., Aldag, J. C., Chatterton, R. T., & Zinaman, M. (2005). Comparison of milk output between mothers of preterm and term infants: The first 6 weeks after birth. *Journal of Human Lactation*, *21*(1).

Hochman, V. G. A., Nascimento, R., da Silva, C. B. M., Quinderé, P. N. A., Melo, R. X., Velarde, L. G. C., Bueno, A. C., & Vieira, A. A. (2024). Relationship between maternal age and macronutrient content of colostrum. *Journal of Human Lactation*, *40*(2), 286-295. https://doi.org/10.1177/08903344241233500

Holritz, L., Olin, A. B., Knudsen, B. R., Svendsen, W. E., & Dimaki, M. (2024). Exploring trends of macronutrients in Danish human donor Milk. *Clinical Nutrition Open Science*, *58*, 54-68. https://doi.org/10.1016/j.nutos.2024.09.009

Horta, B. L., & de Lima, N. P. (2019). Breastfeeding and type 2 diabetes: Systematic review and meta-analysis. *Current Diabetes Reports*, *19*(1), 1. https://doi.org/10.1007/s11892-019-1121-x

Horta, B. L., Loret de Mola, C., & Victora, C. G. (2015). Long-term consequences of breastfeeding on cholesterol, obesity, systolic blood pressure and type 2 diabetes: A systematic review and meta-analysis. *Acta Paediatrica*, *104*(S467), 30-37. https://doi.org/https://doi.org/10.1111/apa.13133

Human Milk Regulation Working Group. (2025, June 4). *Operational guidelines for milk banks in Australia and New Zealand*. Australian Government, Department of Health, Disability and Ageing. https://www.health.gov.au/resources/publications/operational-guidelines-for-milk-banks-in-australia-and-new-zealand?language=en

Humberg, A., Fortmann, I., Siller, B., Kopp, M. V., Herting, E., Göpel, W., & Härtel, C. (2020). Preterm birth and sustained inflammation: Consequences for the neonate. *Seminars Immunopathology*, *42*(4), 451-468. https://doi.org/10.1007/s00281-020-00803-2

Ikonen, R., Paavilainen, E., Helminen, M., & Kaunonen, M. (2018). Preterm infants' mothers' initiation and frequency of breast milk expression and exclusive use of mother's breast milk in neonatal intensive care units. *Journal of Clinical Nursing*, *27*(3-4), e551-e558. https://doi.org/https://doi.org/10.1111/jocn.14093

Indrio, F., Neu, J., Pettoello-Mantovani, M., Marchese, F., Martini, S., Salatto, A., & Aceti, A. (2022). Development of the gastrointestinal tract in newborns as a challenge for an appropriate nutrition: A narrative review. *Nutrients*, *14*(7). https://doi.org/10.3390/nu14071405

Israel-Ballard, K., LaRose, E., & Mansen, K. (2024). The global status of human milk banking. *Maternal & Child Nutrition*, *20 Suppl 4*(Suppl 4), e13592. https://doi.org/10.1111/mcn.13592

Italian Association of Donated Human Milk Banks. (2016). *Breast Milk*. http://www.aiblud.com/donor/

Jackson, D. A., Imong, A. M., Silprasert, A., Ruckphaopunt, S., Woolridge, M. W., Baum, J. D., & Amatayakul, M. (1988). Circadian variation in fat concentration of breast-milk in a rural northern Thai population. *British Journal of Nutrition*, *59*, 349-363.

Jimenez, P. S., Bangar, S. P., Suffern, M., & Whiteside, W. S. (2024). Understanding retort processing: A review. *Food Science & Nutrition*, *12*(3), 1545-1563. https://doi.org/10.1002/fsn3.3912

John, A., Sun, R., Maillart, L., Schaefer, A., Hamilton Spence, E., & Perrin, M. T. (2019). Macronutrient variability in human milk from donors to a milk bank: Implications for feeding preterm infants. *PloS one*, *14*(1), e0210610. https://doi.org/10.1371/journal.pone.0210610

Jones, F. (2003). History of North American donor milk banking: One hundred years of progress. *Journal of Human Lactation*, *19*(3), 313-318. https://doi.org/10.1177/0890334403255857

Kamity, R., Kapavarapu, P. K., & Chandel, A. (2021). Feeding problems and long-term outcomes in preterm infants. A systematic approach to evaluation and management. *Children (Basel)*, *8*(12). https://doi.org/10.3390/children8121158

Kent, J., Mitoulas, L., Cregan, M., Geddes, D., Doherty, D., & Hartmann, P. (2006). Volume and frequency of breastfeeds and fat content of breastmilk throughout the day. *Pediatrics*, *117*(3), e387-395. https://doi.org/10.1542/peds.2005-1417

Khan, S., Hepworth, A. R., Prime, D. K., Lai, C. T., Trengove, N. J., & Hartmann, P. E. (2013). Variation in fat, lactose, and protein composition in breast milk over 24 hours: Associations with infant feeding patterns. *Journal of Human Lactation*, *29*(1), 81-89. https://doi.org/10.1177/0890334412448841

Khandre, V., Potdar, J., & Keerti, A. (2022). Preterm birth: An overview. *Cureus*, *14*(12), e33006. https://doi.org/10.7759/cureus.33006

Kim, B. J., Koh, J., Liang, N., Yang, J., Ozturk, G., Barile, D., & Dallas, D. C. (2023). Effect of vat pasteurization, ultra-high temperature sterilization, retort sterilization and homogenization on soluble proteins in donor human milk detected via proteomics. *LWT*, *182*, 114842. https://doi.org/https://doi.org/10.1016/j.lwt.2023.114842

Kim, H., Kang, S., Jung, B.-M., Yi, H., Jung, J. A., & Chang, N. (2017). Breast milk fatty acid composition and fatty acid intake of lactating mothers in South Korea. *British Journal of Nutrition*, *117*(4), 556-561. https://doi.org/10.1017/S0007114517000253

Kim, J., Lee, N. M., Yi, D. Y., Chung, S.-H., & Choi, Y.-S. (2020). The effect of Holder pasteurization and storage on macronutrients in donor human milk. *Perinatology*, *31*(1), 26-31. https://doi.org/10.14734/PN.2020.31.1.26

Kim, M. H., Shim, K. S., Yi, D. Y., Lim, I. S., Chae, S. A., Yun, S. W., Lee, N. M., Kim, S. Y., & Kim, S. (2019). Macronutrient analysis of human milk according to storage and processing in Korean mother. *Pediatric Gastroenterology, Hepatology & Nutrition*, *22*(3), 262-269. https://doi.org/10.5223/pghn.2019.22.3.262

Kim, S. Y., & Yi, D. Y. (2020). Components of human breast milk: From macronutrient to microbiome and microRNA. *Clinical and Experimental Pediatrics*, *63*(8), 301-309. https://doi.org/10.3345/cep.2020.00059

Kleinman, R. E., & Greer, F. R. (2013). *Pediatric Nutrition*. American Academy of Pediatrics.

Kociszewska-Najman, B., Borek-Dzieciol, B., Szpotanska-Sikorska, M., Wilkos, E., Pietrzak, B., & Wielgos, M. (2012). The creamatocrit, fat and energy concentration in human milk produced

by mothers of preterm and term infants. *The Journal of Maternal-Fetal & Neonatal Medicine*, *25*(9), 1599-1602. https://doi.org/10.3109/14767058.2011.648239

Koenig, A., de Albuquerque Diniz, E. M., Barbosa, S. F., & Vaz, F. A. (2005). Immunologic factors in human milk: The effects of gestational age and pasteurization. *Journal of Human Lactation*, *21*(4), 439-443. https://doi.org/10.1177/0890334405280652

Kortekaas, J. C., Kazemier, B. M., Keulen, J. K. J., Bruinsma, A., Mol, B. W., Vandenbussche, F., Van Dillen, J., & De Miranda, E. (2020). Risk of adverse pregnancy outcomes of late- and postterm pregnancies in advanced maternal age: A national cohort study. *Acta Obstetricia et Gynecologica Scandinavica*, *99*(8), 1022-1030. https://doi.org/10.1111/aogs.13828

Kramer, M. S., & Kakuma, R. (2012). Optimal duration of exclusive breastfeeding. *Cochrane Database of Systematic Reviews*, *2012*(8), Cd003517. https://doi.org/10.1002/14651858.CD003517.pub2

Kramer, M. S., Papageorghiou, A., Culhane, J., Bhutta, Z., Goldenberg, R. L., Gravett, M., Iams, J. D., Conde-Agudelo, A., Waller, S., Barros, F., Knight, H., & Villar, J. (2012). Challenges in defining and classifying the preterm birth syndrome. *American Journal of Obstetrics & Gynecology*, *206*(2), 108-112. https://doi.org/10.1016/j.ajog.2011.10.864

Kreissl, A., Zwiauer, V., Repa, A., Binder, C., Thanhaeuser, M., Jilma, B., Berger, A., & Haiden, N. (2016). Human milk analyser shows that the lactation period affects protein levels in preterm breastmilk. *Acta Paediatrica*, *105*(6), 635-640. https://doi.org/https://doi.org/10.1111/apa.13348

Kulesza-Brończyk, B., Bień, A., Sobieraj, P., Orczyk-Pawiłowicz, M., Lis-Kuberka, J., Czosnykowska-Łukacka, M., & Bzikowska-Jura, A. (2023). Factors affecting total protein and lactoferrin in human milk. *Scientific Reports*, *13*(1), 22434. https://doi.org/10.1038/s41598-023-50124-1

Lamb, R. L., Haszard, J. J., Little, H. M. J., Franks, A. F., & Meeks, M. G. (2021). Macronutrient composition of donated human milk in a New Zealand population. *Journal of Human Lactation*, *37*(1), 114-121. https://doi.org/10.1177/0890334420963666

Lamireau, N., Greiner, E., & Hascoët, J.-M. (2023). Risk factors associated with necrotizing enterocolitis in preterm infants: A case–control study. *Archives de Pédiatrie*, *30*(7), 477-482. https://doi.org/https://doi.org/10.1016/j.arcped.2023.07.003

Lapidaire, W., Lucas, A., Clayden, J. D., Clark, C., & Fewtrell, M. S. (2022). Human milk feeding and cognitive outcome in preterm infants: The role of infection and NEC reduction. *Pediatric Research*, *91*(5), 1207-1214. https://doi.org/10.1038/s41390-021-01367-z

Lau, C. (2015). Development of suck and swallow mechanisms in infants. *Annals of Nutrition and Metabolism*, *66 Suppl 5*(0 5), 7-14. https://doi.org/10.1159/000381361

Lee, J. W., Lee, M., Lee, J., Kim, Y. J., Ha, E., & Kim, H. S. (2019). The protective effect of exclusive breastfeeding on overweight/obesity in children with high birth weight. *Journal of Korean Medical Science*, *34*(10). https://doi.org/10.3346/jkms.2019.34.e85

Leghi, G. E., Middleton, P. F., Netting, M. J., Wlodek, M. E., Geddes, D. T., & Muhlhausler, B. S. (2020). A systematic review of collection and analysis of human milk for macronutrient composition. *The Journal of Nutrition*, *150*(6), 1652-1670. https://doi.org/https://doi.org/10.1093/jn/nxaa059

Legrand, D. (2016). Overview of lactoferrin as a natural immune modulator. *The Journal of Pediatrics*, *173*, S10-S15. https://doi.org/https://doi.org/10.1016/j.jpeds.2016.02.071

Léké, A., Grognet, S., Deforceville, M., Goudjil, S., Chazal, C., Kongolo, G., Dzon, B. E., & Biendo, M. (2019). Macronutrient composition in human milk from mothers of preterm and term neonates

is highly variable during the lactation period. *Clinical Nutrition Experimental*, *26*, 59-72. https://doi.org/https://doi.org/10.1016/j.yclnex.2019.03.004

Levene, I., O'Brien, F., Fewtrell, M., & Quigley, M. A. (2025). The relationship of early expressed milk quantity and later full breastmilk feeding after very preterm birth: A cohort study. *Maternal & Child Nutrition*, *21*(1), e13719. https://doi.org/https://doi.org/10.1111/mcn.13719

Lima, H. K., Wagner-Gillespie, M., Perrin, M. T., & Fogleman, A. D. (2017). Bacteria and bioactivity in Holder pasteurized and shelf-stable human milk products. *Current Developments in Nutrition*, *1*(8), e001438. https://doi.org/10.3945/cdn.117.001438

Lindquist, S., & Hernell, O. (2010). Lipid digestion and absorption in early life: An update. *Current Opinion in Clinical Nutrition and Metabolic Care*, *13*(3), 314-320. https://doi.org/10.1097/MCO.0b013e328337bbf0

Lithoxopoulou, M., Gkampeta, A., Rallis, D., Tzafilkou, K., Drogouti, E., & Tsakalidis, C. (2025). Determination of macronutrient profile and energy composition in human milk via mid-infrared spectrometer analysis: An experimental study. *Journal of Parenteral and Enteral Nutrition*, *49*(4), 517-527. https://doi.org/https://doi.org/10.1002/jpen.2741

Lithoxopoulou, M., Karastogiannidou, C., Karagkiozi, A., Zafeiriadou, I. E., Pilati, E., Diamanti, E., Kalogiannis, S., & Vassilopoulou, E. (2025). From mother-fetus dyad to mother-milk-infant triad: Sex differences in macronutrient composition of breast milk. *Nutrients*, *17*(9), 1422. https://doi.org/10.3390/nu17091422

Liu, Y., Liu, X., & Wang, L. (2019). The investigation of fatty acid composition of breast milk and its relationship with dietary fatty acid intake in 5 regions of China. *Medicine*, *98*(24), e15855. https://doi.org/10.1097/md.0000000000015855

Lodge, C. J., Tan, D. J., Lau, M. X. Z., Dai, X., Tham, R., Lowe, A. J., Bowatte, G., Allen, K. J., & Dharmage, S. C. (2015). Breastfeeding and asthma and allergies: A systematic review and meta-analysis. *Acta Paediatrica*, *104*(S467), 38-53. https://doi.org/https://doi.org/10.1111/apa.13132

Lönnerdal, B. (2003). Nutritional and physiologic significance of human milk proteins. *The American Journal of Clinical Nutrition*, *77*(6), 1537S-1543S. https://doi.org/https://doi.org/10.1093/ajcn/77.6.1537S

Lubetzky, R., Littner, Y., Mimouni, F. B., Dollberg, S., & Mandel, D. (2006). Circadian variations in fat content of expressed breast milk from mothers of preterm infants. *Journal of the American College of Nutrition*, *25*(2), 151-154. https://doi.org/10.1080/07315724.2006.10719526

Mangel, L., Mimouni, F. B., Feinstein-Goren, N., Lubetzky, R., Mandel, D., & Marom, R. (2017). The effect of maternal habitus on macronutrient content of human milk colostrum. *Journal of Perinatology*, *37*(7), 818-821. https://doi.org/10.1038/jp.2017.51

Martin, C. R., Ling, P. R., & Blackburn, G. L. (2016). Review of infant feeding: Key features of breast milk and infant formula. *Nutrients*, *8*(5), 279. https://doi.org/10.3390/nu8050279

McDonald, F. B., Dempsey, E. M., & O'Halloran, K. D. (2020). The impact of preterm adversity on cardiorespiratory function. *Experimental Physiology*, *105*(1), 17-43. https://doi.org/https://doi.org/10.1113/EP087490

Meek, J. Y., & Noble, L. (2022). Policy statement: Breastfeeding and the use of human milk. *American Academy of Pediatrics*, *150*(1). https://doi.org/10.1542/peds.2022-057988

Meier, P. P., Johnson, T. J., Patel, A. L., & Rossman, B. (2017). Evidence-based methods that promote human milk feeding of preterm infants: An expert review. *Clinics in Perinatology*, *44*(1), 1-22. https://doi.org/https://doi.org/10.1016/j.clp.2016.11.005

Meredith-Dennis, L., Xu, G., Goonatilleke, E., Lebrilla, C. B., Underwood, M. A., & Smilowitz, J. T. (2018). Composition and variation of macronutrients, immune proteins, and human milk oligosaccharides in human milk from nonprofit and commercial milk banks. *Journal of Human Lactation*, *34*(1), 120-129. https://doi.org/10.1177/0890334417710635

Miller, J., Tonkin, E., Damarell, R. A., McPhee, A. J., Suganuma, M., Suganuma, H., Middleton, P. F., Makrides, M., & Collins, C. T. (2018). A systematic review and meta-analysis of human milk feeding and morbidity in very low birth weight infants. *Nutrients*, *10*(6), 707. https://doi.org/10.3390/nu10060707

Mills, L., Coulter, L., Savage, E., & Modi, N. (2019). Macronutrient content of donor milk from a regional human milk bank: variation with donor mother-infant characteristics. *British Journal of Nutrition*, *122*, 1155-1167. https://doi.org/doi:10.1017/S0007114519002228

Ministry of Health. (2017). *HISO 10001:2017 Ethnicity Data Protocols*. Wellington: Ministry of Health Retrieved from https://www.tewhatuora.govt.nz/assets/Our-health-system/Digital-health/Health-information-standards/HISO-10001-2017-Ethnicity-Data-Protocols.pdf

Ministry of Health. (2021). *Healthy eating guidelines for New Zealand babies and toddlers (0-2 years old)*. Wellington: Ministry of Health Retrieved from https://www.health.govt.nz/system/files/2021-09/healthy-eating-guidelines-for-new-zealand-babies-and-toddlers-nov21-v3.pdf

Ministry of Health. (2023). *Health and Independence Report*. Wellington: Minsitry of Health

Miris. (2025, July 8). *Human milk analysis and target fortification*. https://www.mirissolutions.com/

Mitoulas, L. R., Kent, J. C., Cox, D. B., Owens, R. A., Sherriff, J. L., & Hartmann, P. E. (2002). Variation in fat, lactose and protein in human milk over 24h and throughout the first year of lactation. *British Journal of Nutrition*, *88*, 29-37. https://doi.org/10.1079/BJN2002579

Mizuno, K., Nishida, Y., Taki, M., Murase, M., Mukai, Y., Itabashi, K., Debari, K., & Iiyama, A. (2009). Is increased fat content of hindmilk due to the size or the number of milk fat globules? *International Breastfeeding Journal*, *4*(1), 7. https://doi.org/10.1186/1746-4358-4-7

Morniroli, D., Tiraferri, V., Maiocco, G., De Rose, D. U., Cresi, F., Coscia, A., Mosca, F., & Giannì, M. L. (2023). Beyond survival: The lasting effects of premature birth. *Frontiers in Pediatrics*, *11*, 1213243. https://doi.org/10.3389/fped.2023.1213243

Moro, G. E. (2018). History of milk banking: From origin to present time. *Breastfeeding Medicine*, *13*(S1), S-16-S-17. https://doi.org/10.1089/bfm.2018.29077.gem

Moro, G. E., Billeaud, C., Rachel, B., Calvo, J., Cavallarin, L., Christen, L., Escuder-Vieco, D., Gaya, A., Lembo, D., Wesolowska, A., Arslanoglu, S., Barnett, D., Bertino, E., Boquien, C. Y., Gebauer, C., Grovslien, A., Weaver, G. A., & Picaud, J. C. (2019). Processing of donor human milk: Update and recommendations from the European Milk Bank Association (EMBA). *Frontiers in Pediatrics*, *7*, 49. https://doi.org/10.3389/fped.2019.00049

Moukarzel, S., Soberanes, L., Dyer, R. A., Albersheim, S., Elango, R., & Innis, S. M. (2017). Relationships among different water-soluble choline compounds differ between human preterm and donor milk. *Nutrients*, *9*(4), 369. https://doi.org/doi:10.3390/nu9040369

Muneer, A., Bari, A., Naveed, S., & Ali, A. S. (2018). Is human milk feeding protective for retinopathy of prematurity? *Pakistan Journal of Medical Sciences*, *34*(6), 1534-1538. https://doi.org/10.12669/pjms.346.15799

Muts, J., Lukowski, J. I. A., Twisk, J. W. R., Schoonderwoerd, A., van Goudoever, J. B., van Keulen, B. J., & Van Den Akker, C. H. P. (2025). Macronutrient concentrations in human milk beyond

the first half year of lactation: A cohort study. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, *110*(3), 248-252. https://doi.org/10.1136/archdischild-2024-327319

Nagel, E. M., Elgersma, K. M., Gallagher, T. T., Johnson, K. E., Demerath, E., & Gale, C. A. (2023). Importance of human milk for infants in the clinical setting: Updates and mechanistic links. *Nutrition in Clinical Practice*, *38*(S2), S39-S55. https://doi.org/https://doi.org/10.1002/ncp.11037

National Health Serivce. (2025, June 26). *Human Milk Bank*. https://bwc.nhs.uk/human-milk-bank/

National Institute for Health and Clinical Excellence. (2010). *Donor breast milk banks: The operation of donor bank services*. https://www.nice.org.uk/guidance/cg93/evidence/full-guideline-243964189

Neu, J., & Walker, W. A. (2011). Necrotizing enterocolitis. *New England Journal of Medicine*, *364*(3), 255-264. https://doi.org/10.1056/NEJMra1005408

Niela-Vilén, H., Axelin, A., Melender, H. L., & Salanterä, S. (2015). Aiming to be a breastfeeding mother in a neonatal intensive care unit and at home: a thematic analysis of peer-support group discussion in social media. *Maternal & Child Nutrition*, *11*(4), 712-726. https://doi.org/10.1111/mcn.12108

Oliveira, M. N. S., Rodrigues, A. M., Faria, A. M. C., Pereira, S. C. L., & Maioli, T. U. (2020). Effects of Holder pasteurization on immune composition of human milk. *Breastfeeding medicine: The Official Journal of the Academy of Breastfeeding Medicine*, *15*(12), 803-808. https://doi.org/10.1089/bfm.2020.0124

Ong, M. L., & Belfort, M. B. (2021). Preterm infant nutrition and growth with a human milk diet. *Seminars in Perinatology*, *45*(2), 151383. https://doi.org/https://doi.org/10.1016/j.semperi.2020.151383

Oranges, T., Dini, V., & Romanelli, M. (2015). Skin physiology of the neonate and infant: Clinical implications. *Advances in Wound Care*, *4*(10), 587-595. https://doi.org/10.1089/wound.2015.0642

Ortigoza, E. B. (2022). Feeding intolerance. *Early Human Development*, *171*, 105601. https://doi.org/10.1016/j.earlhumdev.2022.105601

Oschsner Health. (2025, June 6). *Mothers' Milk Bank of Louisiana at Ochsner*. https://www.ochsner.org/services/mothers-milk-bank-at-ochsner-baptist/

Păduraru, L., Zonda, G. I., Avasiloaiei, A.-L., Moscalu, M., Dimitriu, D. C., & Stamatin, M. (2018). Influence of refrigeration or freezing on human milk macronutrients and energy content in early lactation: Results from a tertiary centre survey. *Paediatrics & Child Health*, *24*(4), 250-257. https://doi.org/10.1093/pch/pxy164

Parker, L. A., Sullivan, S., Cacho, N., Engelmann, C., Krueger, C., & Mueller, M. (2021). Indicators of secretory activation in mothers of preterm very low birth weight infants. *Journal of Human Lactation*, *37*(3), 581-592. https://doi.org/10.1177/0890334420980424

Patel, A. L., Johnson, T. J., Robin, B., Bigger, H. R., Buchanan, A., Christian, E., Nandhan, V., Shroff, A., Schoeny, M., Engstrom, J. L., & Meier, P. P. (2017). Influence of own mother's milk on bronchopulmonary dysplasia and costs. *Archives of Disease in Childhood: Fetal and Neonatal Edition*, *102*(3), F256-f261. https://doi.org/10.1136/archdischild-2016-310898

PATH. (2013). *Strengthening Human Milk Banking: A Global Implementation Framework (Version 1.1)*. Bill & Melinda Gates Foundation. https://media.path.org/documents/MCHN_strengthen_hmb_frame_Jan2016.pdf

Paulaviciene, I. J., Liubsys, A., Molyte, A., Eidukaite, A., & Usonis, V. (2020). Circadian changes in the composition of human milk macronutrients depending on pregnancy duration: A cross-sectional study. *International Breastfeeding Journal*, *15*(1), 49. https://doi.org/10.1186/s13006-020-00291-y

Peña-Ruiz, L. S., Unar-Munguía, M., Colchero, M. A., Alarid-Escudero, F., & Pérez-Escamilla, R. (2023). Breastfeeding is associated with the intelligence of school-age children in Mexico. *Maternal & Child Nutrition*, *19*(4), e13534. https://doi.org/https://doi.org/10.1111/mcn.13534

Perrella, S., Gridneva, Z., Lai, C. T., Stinson, L., George, A., Bilston-John, S., & Geddes, D. (2021). Human milk composition promotes optimal infant growth, development and health. *Seminars in Perinatology*, *45*(2), 151380. https://doi.org/https://doi.org/10.1016/j.semperi.2020.151380

Perrin, M. T., Belfort, M. B., Hagadorn, J. I., McGrath, J. M., Taylor, S. N., Tosi, L. M., & Brownell, E. A. (2020). The nutritional composition and energy content of donor human milk: A systematic review. *Advances in Nutrition*, *11*(4), 960-970. https://doi.org/https://doi.org/10.1093/advances/nmaa014

Perrin, M. T., Festival, J., Starks, S., Mondeaux, L., Brownell, E. A., & Vickers, A. (2019). Accuracy and reliability of infrared analyzers for measuring human milk macronutrients in a milk bank setting. *Current Developments in Nutrition*, *3*(11), nzz116. https://doi.org/https://doi.org/10.1093/cdn/nzz116

Perrin, M. T., Fogleman, A. D., Newburg, D. S., & Allen, J. C. (2017). A longitudinal study of human milk composition in the second year postpartum: Implications for human milk banking. *Maternal & Child Nutrition*, *13*(1). https://doi.org/10.1111/mcn.12239

Perrin, T., Pradat, P., Larcade, J., Masclef-Imbert, M., Pastor-Diez, B., & Picaud, J. C. (2023). Postnatal growth and body composition in extremely low birth weight infants fed with individually adjusted fortified human milk: A cohort study. *European Journal of Pediatrics*, *182*(3), 1143-1154. https://doi.org/10.1007/s00431-022-04775-3

Piemontese, P., Mallardi, D., Liotto, N., Tabasso, C., Menis, C., Perrone, M., Roggero, P., & Mosca, F. (2019). Macronutrient content of pooled donor human milk before and after Holder pasteurization. *BMC Pediatrics*, *19*(1), 58. https://doi.org/10.1186/s12887-019-1427-5

Pitino, M. A., Unger, S., Doyen, A., Pouliot, Y., Aufreiter, S., Stone, D., Kiss, A., & O'Connor, D. L. (2019). High hydrostatic pressure processing better preserves the nutrient and bioactive compound composition of human donor milk. *The Journal of Nutrition*, *149*(3), 497-504. https://doi.org/10.1093/jn/nxy302

Powe, C. E., Knott, C. D., & Conklin-Brittain, N. (2010). Infant sex predicts breast milk energy content. *American Journal of Human Biology*, *22*(1), 50-54. https://doi.org/https://doi.org/10.1002/ajhb.20941

Pu, Z., Sun, Z., Liu, J., Zhang, J., Cheng, M., Zhang, L., & Zhou, P. (2023). Differences in the content of human milk oligosaccharides between foremilk and hind milk. *Food Bioengineering*, *2*(2), 105-113. https://doi.org/https://doi.org/10.1002/fbe2.12049

Qiao, J., Dai, L. J., Zhang, Q., & Ouyang, Y. Q. (2020). A meta-analysis of the association between breastfeeding and early childhood obesity. *Journal of Pediatric Nursing*, *53*, 57-66. https://doi.org/10.1016/j.pedn.2020.04.024

Quigley, M., Embleton, N. D., Meader, N., & McGuire, W. (2024). Donor human milk for preventing necrotising enterocolitis in very preterm or very low-birthweight infants. *Cochrane Database of Systematic Reviews*, *9*(9), CD002971. https://doi.org/10.1002/14651858.CD002971.pub6

Quigley, M. A., Carson, C., Sacker, A., & Kelly, Y. (2016). Exclusive breastfeeding duration and infant infection. *European Journal of Clinical Nutrition*, *70*(12), 1420-1427. https://doi.org/10.1038/ejcn.2016.135

Quinn, E. A. (2013). No evidence for sex biases in milk macronutrients, energy, or breastfeeding frequency in a sample of Filipino mothers. *American Journal of Physical Anthropology*, *152*(2), 209-216. https://doi.org/https://doi.org/10.1002/ajpa.22346

Quitadamo, P. A., Palumbo, G., Gatta, A., Cianti, L., Copetti, M., Gentile, M. A., & Cristalli, P. (2018). How do characteristics of donors and their children influence volume and composition of banked milk? *Journal of Pediatric and Neonatal Individualized Medicine*, *7*(1), e070121. https://doi.org/10.7363/070121

Quitadamo, P. A., Sorrentino, L., Palumbo, G., Cianti, L., Copetti, M., Gentile, M. A., Cristalli, P., & Mantovani, M. P. (2021). Effect of Holder pasteurization on macronutrients and energy content of pooled donor human milk. *Journal of Pediatric and Neonatal Individualized Medicine*, *10*(2), e100202. https://doi.org/10.7363/100202

Reyes, S. M., Patra, B., & Elliott, M. J. (2022). The impact of homogenization on donor human milk and human milk-based fortifiers and implications for preterm infant health. *Current Developments in Nutrition*, *6*(1), nzab147. https://doi.org/10.1093/cdn/nzab147

Rochow, N., Landau-Crangle, E., & Fusch, C. (2015). Challenges in breast milk fortification for preterm infants. *Current Opinion in Clinical Nutrition and Metabolic Care*, *18*(3), 276-284. https://doi.org/10.1097/MCO.0000000000000167

Saarela, T., Kokkonen, J., & Koivisto, M. (2005). Macronutrient and energy contents of human milk fractions during the first six months of lactation. *Acta Paediatrica*, *94*(9), 1176-1181. https://doi.org/10.1111/j.1651-2227.2005.tb02070.x

Sabri, K., Ells, A. L., Lee, E. Y., Dutta, S., & Vinekar, A. (2022). Retinopathy of prematurity: A global perspective and recent developments. *Pediatrics*, *150*(3). https://doi.org/10.1542/peds.2021-053924

Salas, A. A., Gunawan, E., Nguyen, K., Reeves, A., Argent, V., Finck, A., & Carlo, W. A. (2023). Early human milk fortification in infants born extremely preterm: A randomized trial. *Pediatrics*, *152*(3). https://doi.org/10.1542/peds.2023-061603

Sanchez, L. C., Cubero, J., Sanchesz, J., Franco, L., Rodriguez, A. B., Rivero, M., & Barriga, C. (2013). Evolution of the circadian profile of human milk amino acids during breastfeeding. *Journal of Applied Biomedicine*, *11*, 59-70. https://doi.org/10.2478/v10136-012-0020-0

Sankar, M. J., Sinha, B., Chowdhury, R., Bhandari, N., Taneja, S., Martines, J. C., & Bahl, R. (2015). Optimal breastfeeding practices and infant and child mortality: A systematic review and meta-analysis. *Acta Paediatrica*, *104*(S467), 3-13. https://doi.org/https://doi.org/10.1111/apa.13147

Sever, O., Mandel, D., Mimouni, F. B., Marom, R., Cohen, S., & Lubetzky, R. (2015). Macronutrients in human milk: Colostrum lactose but not fat or protein predicts mature human milk content. *Childhood Obesity and Nutrition*, *7*(3), 162-165. https://doi.org/10.1177/1941406415577676

Shah, R., Sabir, S., & Alhawaj, A. F. (2022). Physiology, Breast Milk. In *Stat Pearls [Internet]*. StatPearls Publishing.

Skolik, P., McAinsh, M. R., & Martin, F. L. (2018). Chapter two - Biospectroscopy for plant and crop science. In J. Lopes & C. Sousa (Eds.), *Comprehensive Analytical Chemistry* (Vol. 80, pp. 15-49). Elsevier. https://doi.org/https://doi.org/10.1016/bs.coac.2018.03.001

Spiegler, J., Preuß, M., Gebauer, C., Bendiks, M., Herting, E., & Göpel, W. (2016). Does breastmilk influence the development of bronchopulmonary dysplasia? *Journal of Pediatrics*, *169*, 76-80.e74. https://doi.org/10.1016/j.jpeds.2015.10.080

Stevens, E., Patrick, T., & Pickler, R. (2009). A history of infant feeding. *The Journal of perinatal education*, *18*, 32-39. https://doi.org/10.1624/105812409X426314

Stinson, L. F., George, A., Gridneva, Z., Jin, X., Lai, C. T., & Geddes, D. T. (2024). Effects of different thawing and warming processes on human milk composition. *The Journal of Nutrition*, *154*(2), 314-324. https://doi.org/10.1016/j.tjnut.2023.11.027

Stoll, B. J., Hansen, N. I., Bell, E. F., Shankaran, S., Laptook, A. R., Walsh, M. C., Hale, E. C., Newman, N. S., Schibler, K., Carlo, W. A., Kennedy, K. A., Poindexter, B. B., Finer, N. N., Ehrenkranz, R. A., Duara, S., Sánchez, P. J., O'Shea, T. M., Goldberg, R. N., Van Meurs, K. P., . . . Higgins, R. D. (2010). Neonatal outcomes of extremely preterm infants from the NICHD neonatal research network. *Pediatrics*, *126*(3), 443-456. https://doi.org/10.1542/peds.2009-2959

Su, Y., Xu, R.-H., Guo, L.-Y., Chen, X.-Q., Han, W.-X., Ma, J.-J., Liang, J.-J., Hao, L., & Ren, C.-J. (2023). Risk factors for necrotizing enterocolitis in neonates: A meta-analysis. *Frontiers in Pediatrics*, *Volume 10 - 2022*. https://doi.org/10.3389/fped.2022.1079894

Suwaydi, M. A., Lai, C. T., Warden, A. H., Perrella, S. L., McEachran, J. L., Wlodek, M. E., Geddes, D. T., & Gridneva, Z. (2024). Investigation of relationships between intakes of human milk total lipids and metabolic hormones and infant sex and body composition. *Nutrients*, *16*(16), 2739. https://doi.org/10.3390/nu16162739

Tabasso, C., Piemontese, P., Pesenti, N., Perrone, M., Menis, C., Liotto, N., Amato, O., Orsi, A., Mallardi, D., Mosca, F., & Roggero, P. (2023). Pooling strategies to modify macronutrient content of pasteurized donor human milk. *Breastfeeding Medicine*, *18*(5), 370-376. https://doi.org/10.1089/bfm.2023.0043

Tanaka, M., Date, M., Miura, K., Ito, M., Mizuno, N., & Mizuno, K. (2023). Protein and immune component content of donor human milk in Japan: Variation with gestational and postpartum age. *Nutrients*, *15*(10), 2278. https://doi.org/doi:10.3390/nu15102278

Te Whatu Ora. (2024, July 8). *Donated breast milk*. https://www.cdhb.health.nz/health-services/human-milkbank/

Tekin-Guler, T., Koc, N., Kara-Uzun, A., & Fisunoglu, M. (2023). The association of pre-pregnancy obesity and breast milk fatty acids composition and the relationship of postpartum maternal diet, breast milk fatty acids and infant growth. *Breastfeeding Medicine*, *18*(6), 475-482. https://doi.org/10.1089/bfm.2023.0002

Thorley, V. (2008). Sharing breastmilk: Wet nursing, cross feeding, and milk donations. *Breastfeeding Review*, *16*(1), 25-29.

Unger, S. L., & O'Connor, D. L. (2024). Review of current best practices for human milk banking. *Maternal & Child Nutrition*, *20 Suppl 4*(Suppl 4), e13657. https://doi.org/10.1111/mcn.13657

Updegrove, K., Festival, J., Hackney, R., Jones, F., Kelly, S., Sakamoto, P., & Vickers, A. (2020). *HMBANA standards for donor human milk banking: An overview (Version 1.0)*. Human Milk Banking Association of North America. https://www.hmbana.org/file_download/inline/95a0362a-c9f4-4f15-b9ab-cf8cf7b7b866

van Duuren, I. C., van Hengel, O. R. J., Penders, J., Duijts, L., Smits, H. H., & Tramper-Stranders, G. A. (2024). The developing immune system in preterm born infants: From contributor to potential solution for respiratory tract infections and wheezing. *Allergy*, *79*(11), 2924-2942. https://doi.org/https://doi.org/10.1111/all.16342

Van Gysel, M., Cossey, V., Fieuws, S., & Schuermans, A. (2012). Impact of pasteurization on the antibacterial properties of human milk. *European Journal of Pediatrics*, *171*(8), 1231-1237. https://doi.org/10.1007/s00431-012-1750-4

Vass, R. A., Bell, E. F., Colaizy, T. T., Schmelzel, M. L., Johnson, K. J., Walker, J. R., Ertl, T., & Roghair, R. D. (2020). Hormone levels in preterm and donor human milk before and after Holder pasteurization. *Pediatric Research*, *88*(4), 612-617. https://doi.org/10.1038/s41390-020-0789-6

Verd, S., Porta, R., Ginovart, G., Avila-Alvarez, A., García-Muñoz Rodrigo, F., Izquierdo Renau, M., & Ventura, P. S. (2023). Human milk feeding is associated with decreased incidence of moderate-severe bronchopulmonary dysplasia in extremely preterm infants. *Children*, *10*(7), 1267.

Vieira, A. A., Soares, F. V. M., Pimenta, H. P., Abranches, A. D., & Moreira, M. E. L. (2011). Analysis of the influence of pasteurization, freezing/thawing, and offer processes on human milk's macronutrient concentrations. *Early Human Development*, *87*(8), 577-580. https://doi.org/https://doi.org/10.1016/j.earlhumdev.2011.04.016

Walsh, C., Lane, J. A., van Sinderen, D., & Hickey, R. M. (2020). Human milk oligosaccharides: Shaping the infant gut microbiota and supporting health. *Journal of Functional Foods*, *72*, 104074. https://doi.org/10.1016/j.jff.2020.104074

Walter, L., Clifford, V., Sulfaro, C., Brown, R., Ribeiro, D., Welvaert, M., Shuttleworth, G., & Klein, L. D. (2023). Macronutrient content of pasteurised donor human milk: Variability between batches from single-donor pools at an Australian milk bank. *Journal of Paediatrics and Child Health*, *59*(12), 1281-1288. https://doi.org/10.1111/jpc.16497

Weaver, G., Bertino, E., Gebauer, C., Grovslien, A., Mileusnic-Milenovic, R., Arslanoglu, S., Barnett, D., Boquien, C. Y., Buffin, R., Gaya, A., Moro, G. E., Wesolowska, A., & Picaud, J. C. (2019). Recommendations for the establishment and operation of human milk banks in Europe: A consensus statement from the European Milk Bank Association (EMBA). *Frontiers in Pediatrics*, *7*, 53. https://doi.org/10.3389/fped.2019.00053

Westreich, D., & Greenland, S. (2013). The table 2 fallacy: presenting and interpreting confounder and modifier coefficients. *American Journal of Epidemiology*, *177*(4), 292-298. https://doi.org/10.1093/aje/kws412

Wilson, E., Edstedt Bonamy, A. K., Bonet, M., Toome, L., Rodrigues, C., Howell, E. A., Cuttini, M., & Zeitlin, J. (2018). Room for improvement in breast milk feeding after very preterm birth in Europe: Results from the EPICE cohort. *Matern Child Nutr*, *14*(1). https://doi.org/10.1111/mcn.12485

World Health Organisation. (2009). *Infant and young child feeding: Model chapter for textbooks for medical students and allied health professionals.* World Health Organisation. https://www.ncbi.nlm.nih.gov/books/NBK148970/

World Health Organisation. (2019). *Donor human milk for low birth weight infants*. https://www.who.int/tools/elena/interventions/donormilk-infants

Xu, L., Lochhead, P., Ko, Y., Claggett, B., Leong, R. W., & Ananthakrishnan, A. N. (2017). Systematic review with meta-analysis: Breastfeeding and the risk of Crohn's disease and ulcerative colitis. *Alimentary Pharmacology & Therapeutics*, *46*(9), 780-789. https://doi.org/https://doi.org/10.1111/apt.14291

Xu, Y.-P., Chen, Z., Dorazio, R. M., Bai, G.-N., Du, L.-Z., & Shi, L.-P. (2023). Risk factors for bronchopulmonary dysplasia infants with respiratory score greater than four: A multi-center, prospective, longitudinal cohort study in China. *Scientific Reports*, *13*(1), 17868. https://doi.org/10.1038/s41598-023-45216-x

Xu, Y., Yu, Z., Li, Q., Zhou, J., Yin, X., Ma, Y., Yin, Y., Jiang, S., Zhu, R., Wu, Y., Han, L., Gao, Y., Xue, M., Qiao, Y., Zhu, L., Tu, W., Wu, M., Wan, J., Wang, W., . . . Han, S. (2020). Dose-dependent effect of human milk on bronchopulmonary dysplasia in very low birth weight infants. *BMC Pediatrics*, *20*(1), 522. https://doi.org/10.1186/s12887-020-02394-1

Yan, J., Liu, L., Zhu, Y., Huang, G., & Wang, P. P. (2014). The association between breastfeeding and childhood obesity: A meta-analysis. *BMC Public Health*, *14*, 1267. https://doi.org/10.1186/1471-2458-14-1267

Young, B. E., Borman, L. L., Heinrich, R., Long, J., Pinney, S., Westcott, J., & Krebs, N. F. (2019). Effect of pooling practices and time postpartum of milk donations on the energy, macronutrient, and zinc concentrations of resultant donor human milk pools. *The Journal of Pediatrics*, *214*, 54-59. https://doi.org/10.1016/j.jpeds.2019.07.042

Zhang, L., Qu, J., Huppertz, T., Liu, J., Sun, Z., & Zhou, P. (2022). Effects of different freeze-thaw processes on the bioactivity and digestibility of human milk. *Lebensmittel-Wissenschaft & Technologie*, *156*, 113025. https://doi.org/https://doi.org/10.1016/j.lwt.2021.113025

Ziegler, E. E., O'Donnell, A. M., Nelson, S. E., & Fomon, S. J. (1976). Body composition of the reference fetus. *Growth*, *40*(4), 329-341.

6. Appendices

Appendix A: Literature review search strategy

To be included in the review, studies needed to assess the macronutrient composition of donor human milk (DHM). Studies were included if they were published between the period of 2015 — 2025; published in English; were peer-reviewed journal articles that were quantitative and reported human milk macronutrient composition; included healthy mothers with varying milk production levels and of all age groups and only used pump or manual expression of milk as the collection methods, as opposed to drip milk (which is not acceptable to be donated). To identify relevant articles, databases were searched between January 2025 and March 2025. The databases were Google Scholar, Scopus, PubMed, Cochrane Library and Discover. The author developed the search strategies and refined them through discussion with the research team. The following search terms were used:

- “donor human milk” OR “donor breast milk” OR “milk bank” OR “human donor milk” OR “breast milk” OR “lactation” AND
- “milk composition” OR “nutrient content” OR “macronutrient”

The resulting articles from the search were exported to EndNote (version 21), and duplicates were removed.

Appendix B: Ethics approval

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

17/01/2025

Dear: Dr Ying Jin

Re: Ethics Application - OM1 24/58 - Macronutrient composition of donor human milk over postpartum periods in the first human milk bank in New Zealand.

Thank you for the above application that was considered by the Massey University Human Ethics Committee:

Ohu Matatika 1 at their meeting held on **Tuesday, 12 November 2024**

On behalf of the Committee I am pleased to advise you that the ethics of your application are approved.

Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Professor Tracy Riley,
Acting Chair, Research Ethics Chair's Committee

Appendix C: Christchurch Women's Hospital Consent Form

Te Whatu Ora
Health New Zealand
Waitaha Canterbury

Human Milk for Our Babies

(Place patient label here or complete details)

NAME:

GENDER: DOB: AGE: NHI:

ADDRESS:

Mother Wishing to Donate Human Milk: Consent

DONOR MILK CONSENT

As a mother exclusively breastfeeding/breastmilk feeding her baby I consent to donate my surplus milk to the Human Milk Bank.	☐ Yes	☐ No
I have read the information leaflet about donating milk to the Human Milk Bank	☐ Yes	☐ No
I have understood the process for collecting, storing and the transportation of my milk to the Human Milk Bank.	☐ Yes	☐ No
I have read and signed the health and lifestyle questionnaire.	☐ Yes	☐ No
To the best of my knowledge, there is no reason why I should not donate my milk.	☐ Yes	☐ No
I understand that once donated, the donated milk cannot be returned to me.	☐ Yes	☐ No
I consent to my breast milk being used for research and training purposes.	☐ Yes	☐ No
I understand I will need to be screened for the following blood infections prior to donating my breast milk: Human Immunodeficiency Virus 1 & 2 (HIV) Hepatitis B & C Human T Cell Lymphotropic Virus 1 & 2 (HTLV) Syphilis (SEIA)	☐ Yes	☐ No
I understand that the results of my blood tests will be communicated to me by milk bank staff. Blood results can be accessed by GP.	☐ Yes	☐ No
I consent to the information collected in relation to my milk donation being shared with CDHB staff as appropriate.	☐ Yes	☐ No
I understand that I will not receive any personal information relating to the recipients of my milk, including their identities.	☐ Yes	☐ No

Mother's name

Mother's signature _____ Date

STAFF USE ONLY

Statement of health care professional with an appropriate knowledge of the human milk bank policies.

I have discussed the process with the mother and explained the following:

- The benefits of human milk for the sick and preterm baby
- Information about donating human milk
- How to collect and store the milk
- Reasons for temporarily stopping donation
- The screening process

Name of Health Care Professional

Job Title

Signature of Health Care Professional _____ Date

C230052

Ref 2401159 Authorised by: Clinical Director Neonatal Services - Te Whatu Ora Waitaha Canterbury Page 1 of 1 Sept 2022

Appendix D: Christchurch Women's Hospital Milk Bank Protocols and Donor Information

Canterbury District Health Board
Te Poari Hauora ō Waitaha

Human Milk for Our Babies
NEONATAL SERVICES

Human Milk for Our Babies

INSTRUCTIONS AND FREQUENTLY ASKED QUESTIONS
FOR HUMAN MILK DONORS

Introduction

Breastmilk is the optimal food for babies and is particularly important for premature and critically ill babies. These babies have higher very specific nutritional and immunity needs. Mothers may be unable to provide milk for their baby for a number of reasons including maternal illness, prescription medications or low milk supply. We have a facility for accepting donor human milk to help in these situations.

Mothers who volunteer to donate to the human donor milk bank are usually producing more milk than their baby needs.

If you are interested in becoming a human milk donor we require you to complete a lifestyle questionnaire and to undertake blood tests. Factors that may exclude you from becoming a donor include smoking, drinking alcohol regularly, taking certain medications or recreational drugs.

What is the Human Donor Milk Bank?

This is a facility that accepts donated human milk from registered screened donors which is then pasteurised and stored frozen until required.

How can I become a donor?

If you are still interested in becoming a human milk donor after reading this pamphlet you will guided through the process by NICU/Milk Bank staff. You can also complete the lifestyle questionnaire and consent form on the Human Milk Bank website and return these to the Human Donor Milk Bank.

The Human Donor Milk Bank appreciates both the support from the donor mothers, volunteers and those who contribute financially to maintain this facility.

Information for mothers who are expressing and donating milk

Thank you for the gift of your breastmilk to the Human Donor Milk Bank.

If your baby is in the Neonatal Unit, you may be using one of our hospital grade electric breast pumps. If you are at home and using your own breast pump, the milk bank staff will assess the suitability of the pump for expressing.

As a donor you will receive the following:

- Lifestyle questionnaire
- Consent to donate form
- Expressing equipment – if required and available
- Sterile collection bottles
- Expressing labels for the collection bottles
- Human Milk Bank donor card
- Plastic bags and ties
- Amounts tag

It is important to keep everything as clean as possible to avoid contaminating the milk for donation. Before you start expressing, please ensure you read the general hygiene guidelines.

Milk production depends on milk removal or 'supply and demand'. There is no absolute right time to express – express when it is convenient for you and ensure that you express often enough to encourage production. One option for expressing is when your baby only feeds on one side — just express the other side after the feed. You will find what works for you.

Contacting the Human Donor Milk Bank

If you have any questions or concerns, please contact the Human Donor Milk Bank.

Phone: (03) 364 4344
Email: milkbanknicu@cdhb.health.nz
Website: google/CDHB Human Donor Milk Bank.

The milk bank hours are on the back of this brochure. If urgent, ask to speak to the Neonatal Unit ACNM on (03) 364 4699, who will contact the Milk Bank Manager.

Frequently asked questions for human milk donors

Who can donate human milk?
Any mother who wishes to provide the gift of human milk for the Human Donor Milk Bank is invited to complete the lifestyle questionnaire and screening blood tests. Any milk provided is in excess of her own baby's needs, growth and development.

Why donate?
A key concern for premature babies is a severe gut disorder called NEC (Necrotising Enterocolitis). Human milk is associated with lowering the risk of NEC. Donating to a milk bank ensures screened and pasteurised donor milk is available for these premature and other vulnerable babies.

What steps are required to be a donor?
You will be requested to complete a lifestyle questionnaire. Following this you will be asked to have a blood test to screen for infections that can be transmitted through breastmilk.

What are the blood tests I will consent to?
The screening blood tests will include HIV 1 & 2, Hepatitis B and C, HTLV 1 & 2. HTLV 1 & 2 are risk factors for the onset of adult leukaemia and lymphoma. **The blood tests are done at no cost to you.**

How much do I need to donate?
Keep freezing your breastmilk until you have approximately 1 litre of milk to donate. Single donations will be considered for mothers who have a moderate to large supply of excess milk that has been stored frozen for up to 3 months.

What happens to my milk if it is not used?
There are a variety of reasons why your milk may not be suitable for use by the donor milk bank although acceptable for your own baby. We will contact you to discuss this if it is the case. Milk that has been donated but cannot be dispensed is offered to research groups and your permission is requested as part of the consent process.

What happens if I take medications, drink alcohol and/or smoke?
Most prescribed medications are compatible with breastfeeding your own baby but need to be discussed when donating milk for the milk bank (for instance, blood pressure medications, antibiotics, or even herbal medicines need to be discussed). We would also like to discuss if you have had recent vaccinations or travelled/lived overseas. We discourage drinking alcohol while you are expressing for the milk bank. We also support a smoke free policy. It is important that you provide accurate details regarding your prescribed and social drug use, alcohol intake and smoking habits.

What happens if I am unwell?
You should continue to breastfeed your baby as usual and express milk for comfort as necessary. You may use this expressed milk for your own baby. If you have a fever or are unwell we advise you not to donate your milk to the Human Donor Milk Bank.

If you feel unwell, place a 'U' on the donation bottle at that time so it can be identified in the future. Place an 'M' on the bottle if you take medications in the 24 hours before expressing for the Milk Bank.

Will you accept frozen milk?
We generally accept milk that has been frozen up to 3 months from the date it was expressed. We are also able to accept older milk under special circumstances. Please ask the Milk Bank for advice before discarding your milk. You must, however, meet the donor criteria.

What happens to the milk before it is given to the babies?
Initially you freeze your milk at home and it will remain there until you have used up all your bottles or accumulated at least 1 litre of milk to donate. On arrival at the bank your milk is given a unique number and remains frozen until required for pasteurisation. A sample is checked for a bacterial count and then is heat treated (pasteurised). A further check of the bacterial count may occur on random samples for quality control purposes.

Will I be paid?
No. All our donors are volunteers.

What equipment will I need?
The milk bank will supply you with a donor pack that will include bottles, labels, bags and tags and a donor card. It is assumed that you will already be using a breast pump.

Will I be able to meet the babies who are receiving my milk?
No. Your contact will be with the Milk Bank staff. We hope to organise yearly meetings where donors get to meet other donors and Milk Bank staff as a way of thanking you for your contribution.

Are my details confidential?
All information collected in relation to your donation will be shared with CDHB staff as appropriate. This information, except personal information about the recipients of your milk, will be placed on your general medical record and shared with your GP as appropriate. Your details will not appear on the pasteurised milk.

I am unable to supply milk but wish to support the milk bank
Donations to the Canterbury Neonatal Unit Trust Fund (Westpac Papanui Branch, 030854 0584185 00) who funded the establishment of the Milk Bank are appreciated from those who wish to contribute financially to maintain this facility. This may be one off or in the form of on going fundraising. Details can be found on our website.

Human milk storage information

This applies to mothers who are storing their expressed milk at home. Hand hygiene and cleaning of the electric pump parts are very important.

Storage guidelines

- Collect milk in the sterile bottles supplied by the Human Donor Milk Bank.
- Store milk from each expression in separate bottles.
- Leave a gap of 2 cm at the top of each bottle to allow for expansion when the milk freezes.
- Use the labels provided to label each bottle with the date of milk expression.
- Place the labelled bottles of milk in the plastic bag provided.
- Freeze expressed milk for the Human Donor Milk Bank as soon as possible after each expression — immediately is best.

If you do not have a freezer please ask for advice from the Human Donor Milk Bank staff.

How long can I store frozen expressed breastmilk?

- 2 weeks in the freezer section located inside a refrigerator
- 3 months in a separate door refrigerator/freezer
- 6 months in a deep freeze or chest freezer

Community pick up for donations

The Human Donor Milk Bank has a weekly community pick up service for your donations.

You can also drop off your donations directly to the Milk Bank (NICU reception, 4th floor, Christchurch Women's Hospital) providing the donation remains frozen and the drop off has been prearranged. Please bring your donor card.

General hygiene when expressing at home

Routine hand washing decreases the risk of contamination of pumps, pump parts, storage containers and expressed milk.

Hand hygiene

Before expressing milk or handling equipment:

1. Wash your hands by lathering with soap and hot water, or use an alcohol-based hand rub (these can be purchased from your supermarket).

   We encourage you to keep your finger nails short.
2. Dry hands with disposable paper towels. Use the towel to turn off tap.
3. Daily washing/showering is sufficient for breast hygiene purposes.

Pump hygiene

Keep the outside of the breast pump and tubing clean and wipe away any milk spots:

1. Ensure your hands are clean and dry.
2. Dismantle and wash the breast kit parts after each pumping session.
3. A separate bowl or container should be used to wash the milk collection kit to avoid contamination from the skin.
4. Rinse breast kit parts in cool water to remove residue, then wash all parts in hot soapy water (washing-up detergent) to clean. Use a designated toothbrush or bottle brush for removing any solidified milk.
5. Rinse in hot water to wash off detergent residues.
6. Shake off excess water.
7. For mothers staying in the neonatal unit who already use tablet disinfectant please continue to use that method.
8. For mothers at home place the milk collection kit between dry paper towels to air dry and place in a zip lock bag when dried. Alternatively, boil the parts for 5 minutes or use a microwave/steam sterilising unit.

The Neonatal Unit will provide additional labels for you on request.

Human Milk Bank, Christchurch Women's Hospital, Neonatal Unit, 2 Riccarton Ave, Christchurch
Google — CDHB Human Milk Bank | milkbanknicu@cdhb.health.nz | 03 364 4344

Acknowledgments
Canterbury Neonatal Unit Trust
Chair: Paul McEwan

References
Robyn Noble, DMLT,BAppSc(MedSc),IBCLC Guidelines for Establishment and Operation of HMBs in UK.
Frances Jones, Mary Rose Tully, Best Practice for Expressing, Storing and Handling of HM. 2nd Edition,2011.
We acknowledge the use of material from Mothers Milk Banks Australia.

Appendix E: Human Milk Analysis Procedure

Processing and analysis of DHM for the present study was conducted by the CWHMB staff; however, the primary researcher had the opportunity to visit the milk bank and learn how to perform the analyses to gain hands-on experience and earn a deeper understanding of how DHM is processed.

Containers of frozen, unpasteurized human milk, pooled from a single donor were defrosted overnight. Once defrosted, the milk was poured through a food-grade, double sieve into a large jug lined with a plastic insert and swirled to mix it. This milk was then divided into 250 mL lots for Holder Pasteurisation (HoP)[2]. From each 250 mL pasteurised batch, a 20 mL sample was transferred into a separate, smaller container and placed in a water bath and warmed to 40°C. Samples needed to be approximately 40°C before being placed into the Miris HMA, as per the manufacturer's protocol (Miris Human Milk Analyser User Manual, n.d.). Once the milk reached the correct temperature, each container was gently swirled to mix it. A 10 mL aliquot was then drawn using a sterile syringe and transferred into a new, clean, glass container. Immediately prior to analysis, the 10 mL aliquot was placed in the Miris Ultrasonic Processor (Miris, Uppsala, Sweden) to homogenise for 12 seconds (1.5 seconds per mL) as per the user manual (Miris Ultrasonic Processor User Manual, n.d.). The probe was wiped clean between aliquots, with the MIRIS cleaning solution to prevent cross-contamination. After homogenisation, approximately 4 mL of milk was drawn using the syringe, taking care to avoid air bubbles, and incrementally (about 1 mL at a time) injected into the Miris HMA inlet for analysis. Analyses were performed in triplicate, and the mean was used for statistical analyses. Calibration was conducted weekly using the Miris Calibration Control Kit to ensure reliability and accuracy.

[2] Holder Pasteurisation is the recommended pasteurisation method in most international human milk banking guidelines and involves heating milk to 62.5℃ for 30 minutes, followed by rapid cooling.

Appendix F: Sensitivity Analyses

Table 6.1
Macronutrient composition of donations by donor characteristics post sensitivity analysis (696 samples from 149 donors)

	Carbohydrate, g/100 mL	
	Mean difference (95% CI)	*p*-value
Donor age		
Per year	0.004 (-0.003, 0.011)	0.247
≥ 35 years compared to <35 years	-0.026 (-0.104, 0.052)	0.518
Ethnicity		
European	Reference	
Māori	-0.066 (-0.169, 0.038)	0.457
Pacific	**0.176 (0.005, 0.347)**	**0.043**
Asian	**0.156 (0.065, 0.247)**	**<0.001**
Others	-0.181 (-0.363, -0.001)	0.051

Note. Bold indicates a statistically significant difference of p <0.05.

Table 6.2
Macronutrient composition of donations by lactation stage post sensitivity analysis (694 samples from 149 donors)

	Carbohydrate, g/100 mL	
	Mean difference (95% CI)	*p*-value
Lactation stage		
Per week	**-0.004 (-0.007, 0.001)**	**0.010**
< 4 weeks	0.095 (-0.034, 0.223)	0.148
4–28 weeks	0.092 (-0.025, 0.209)	0.122
> 28 weeks	Reference	

Note. Bold indicates a statistically significant difference of p <0.05.

Table 6.3
Macronutrient composition of preterm versus term DHM post sensitivity analysis (696 samples from 149 donors)

	Carbohydrate, g/100 mL	
	Mean difference (95% CI)	*p*-value
Preterm† versus term‡ mature	**-0.087 (-0.162, -0.012)**	**0.023**

Note. Bold indicates a statistically significant difference of p <0.05.
Preterm mature milk defined as milk expressed <37 weeks gestation and after 2 weeks postpartum
Term mature milk defined as milk expressed ≥37 weeks gestation and after 2 weeks postpartum