# The nutrient composition of human milk between 7–10 months postpartum and its contribution to infant energy intake

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# Abstract

**Background:** Human milk (HM) is the optimal source of nutrition for infants, with exclusive breastfeeding recommended by the World Health Organization and New Zealand Ministry of Health for the first 6 months. Complementary foods are introduced thereafter, while breastfeeding continues into late infancy. HM composition beyond 6 months postpartum remains under-researched in New Zealand, with many studies classifying HM from two weeks postpartum as "mature milk," potentially overlooking changes later in lactation. Understanding how energy is derived from infant milk sources and complementary foods is crucial for accurate assessment of nutrient intakes and evidence-based infant feeding guidelines.

**Aim:** To determine the nutrient composition of human milk between 7–10 months postpartum and its contribution to infant energy intake.

**Methods:** The cross-sectional study included 625 parent-infant dyads from the multi-centred First Food New Zealand (FFNZ) study (Dunedin and Auckland, 2020–2022). This secondary analysis focuses on a subgroup of 121 mothers, with infants aged 7–10 months, who provided HM samples. Demographic and anthropometric measures, 24-hour dietary recalls, and HM intake volume data (via the dose-to-mother stable isotope technique) were collected. Macronutrients (fat, lactose, protein), vitamins (vitamin A, C, E, folate), minerals (Na, Mg, P, K, Ca), and trace elements (Fe, Cu, Se, Zn, I) were analysed using validated methods.

**Results:** Thirteen of 18 nutrients were within reported literature ranges. HM iodine (62.82 µg/L), iron (133.9 µg/L), selenium (10.21 µg/L), and vitamin C (2.39 mg/100 mL) concentrations were lower than reported literature values, whereas vitamin E (0.27 mg/100 mL) and magnesium (34.30 mg/L) were higher. No clear differences were observed across maternal or infant characteristics. Significant differences ($p<0.05$) in energy intake were observed across feeding groups. Breastfed and formula-fed infants differed in energy from infant milk sources (mean ± SD: 2068 ± 561 kJ/day vs 2251 ± 601 kJ/day) and total energy intake (3239 ± 525 kJ/day vs 3534 ± 609 kJ/day). Additionally, breastfed and mixed-fed infants differed in energy from complementary foods (1170 ± 741 kJ/day vs 1448 ± 728 kJ/day) and total energy intake (3239 ± 525 kJ/day vs 3538 ± 559 kJ/day). All significant differences remained after adjusting for infant age and sex.

**Conclusion:** HM nutrient concentrations at 7–10 months postpartum were broadly consistent with international literature, though lower iodine, iron, and selenium concentrations may place infants at risk of inadequate intake. Differences in energy intakes between feeding groups were also observed. Future work should integrate HM composition with measured HM intakes to clarify HM's nutrient contribution in late infancy and to refine infant feeding guidelines.

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## List of Abbreviations

| Abbreviation | Definition |
|---|---|
| 25OHD | 25-hydroxyvitamin D |
| AAS | Atomic absorption spectroscopy |
| AOAC | Association of Official Agricultural Chemists |
| BMI | Body mass index |
| CI | Confidence interval |
| DHA | Docosahexaenoic acid |
| DNA | Deoxyribonucleic acid |
| DTM | Dose-to-mother |
| EER | Estimated energy requirement |
| FAAS | Flame atomic absorption spectroscopy |
| FFNZ | First Foods New Zealand |
| HM | Human milk |
| HPLC | High performance liquid chromatography |
| ICP-AES | Inductively coupled plasma atomic emission spectroscopy |
| ICP-MS | Inductively coupled plasma mass spectrometry |
| ICP-OES | Inductively coupled plasma optical spectroscopy |
| IU | International units |
| LC-MS | Liquid chromatography tandem mass spectrometry |
| LC-UV | Liquid chromatograph with ultraviolet detection |
| MOH | Ministry of Health |
| NPN | Non protein nitrogen |
| NRV | Nutrient reference value |
| NZ | New Zealand |
| NZDEP | New Zealand Index of Deprivation |
| RDI | Recommended daily intake |
| SD | Standard deviation |
| T3 | Triiodothyronine |
| T4 | Thyroxine |
| UHPLC-MS | Ultra-high performance liquid chromatography tandem mass spectrometry |
| UHPLC-UV | Ultra-high performance liquid chromatography tandem ultraviolet detection |
| US | United States |
| WHO | World Health Organization |

## Glossary

| Term | Definition |
|---|---|
| Breastfed | An infant who consumes human milk and not commercial infant formula |
| Early infancy/first half of infancy | <6 months of age |
| Feeding group | A term used to describe which type of milk source(s) an infant receives either breastfed, formula-fed, or mixed-fed |
| Formula-fed | An infant who consumes commercial infant formula and not human milk |
| Infancy | The first year of life |
| Late infancy/second half of infancy | 6–12 months of age |
| Mixed-fed | An infant who consumes both human milk and commercial infant formula |

# Chapter 1 Introduction

## 1.1 Background

Human milk (HM) is considered the “gold standard” for early life nutrition, designed by evolution to support the growth of the nutritionally vulnerable, developing infant. Breastfeeding provides widely recognised health and nutritional benefits for both the infant and the mother and is therefore recommended by the World Health Organization (WHO) to be continued until 2 years or beyond (WHO & UNICEF, 2003). Established benefits include: protection against common childhood illnesses, future overweight/obesity, and diabetes in the infant, and protection against breast and ovarian carcinoma in the mother, with longer durations of breastfeeding offering greater protection (Chowdhury et al., 2015; Horta & de Lima, 2019; Horta et al., 2015; Ministry of Health, 2021; Victora et al., 2016; WHO, 2023). HM before 6 months of age particularly provides a nutritionally complete dietary source for infants as it contains sufficient proteins, fats, carbohydrates, fluid, vitamins, and minerals (Butts et al., 2018; Grote et al., 2016). HM also contains numerous bioactive compounds that cannot be reproduced in commercial infant formula, including antibodies, antioxidants, enzymes, hormones, and growth factors (Lonnerdal, 2014; Victora et al., 2016). Together with the nutritional constituents of HM, these components play crucial roles in supporting infant growth, development, and host defence as they possess antimicrobial, anti-inflammatory, and growth-promoting properties (Cacho & Lawrence, 2017; Reniker et al., 2023)

As a result of scientific evidence supporting breastfeeding, evidence-based infant feeding guidelines have been developed both globally and in New Zealand (NZ) for health professionals and parents/caregivers. In 2021, the NZ Ministry of Health (MOH) updated the infant feeding guidelines, “Healthy Eating Guidelines for NZ Babies and Toddlers (0 to 2 years old)”, which include statements on breast- and formula-feeding and complementary feeding (MOH, 2021). When an infant receives all their nutrients from HM (mother’s milk directly from the breast or expressed), it is defined as ‘exclusive breastfeeding’. Exclusive breastfeeding is recommended for the first 6 months of an infant’s life by the WHO and UNICEF (2003) and the MOH (2021). Infants who receive both HM (mother’s milk directly from the breast, expressed, or donated) and commercial infant formula are classified as ‘mixed-fed’. Commercial infant formula is the only alternative milk source recommended by the MOH (2021) until 12 months of age. In NZ, breastfeeding is initiated by 97.9% of mothers at <1 month postpartum, dropping to 73.3% at 7–7.9 months, and to 68.6% at 10–10.9 months (Brown et al., 2023). The decision to forgo or

discontinue breastfeeding may be due to pain, fatigue, the return to work, or supply issues (Brown et al., 2014; Douglas, 2022; Kozhimannil et al., 2014).

Alongside the breastfeeding recommendations, the MOH infant feeding guidelines include recommendations on introducing complementary food. Around 6 months of age, when infants show signs of readiness, it is recommended that complementary foods be introduced alongside HM (or commercial infant formula), a practice referred to as complementary feeding (MOH, 2021). This marks a notable shift in dietary patterns, as HM alone can no longer provide sufficient energy and nutrients for optimal infant growth and development (Fewtrell et al., 2017). The First Food New Zealand (FFNZ) study reported that 75.4% of NZ infants were introduced to complementary foods within the recommended timeframe, while 22.4% were introduced early (<5 months) and 2.3% late (≥7 months) (Brown et al., 2023). However, the FFNZ study also reported that NZ infants aged 7–10 months are not meeting the MOH's food group recommendations, with fewer than half of infants consuming grain foods, meat and protein-rich foods, and milk products daily (Brown et al., 2023). This contrasts with findings from the Growing Up in NZ study, which previously indicated high adherence to all food group recommendations at 9 months of age (de Castro et al., 2018).

HM is a complex biological system that adapts to meet the changing needs of infants across different stages of growth and development, with its macro- and micronutrient composition altering throughout lactation (Dror & Allen, 2018b; Yang et al., 2018). In addition to essential nutrients, HM contains bioactive components with important functions, including anti-inflammatory and antimicrobial properties (Christian et al., 2021; Reniker et al., 2023; Szyller et al., 2024). Importantly, HM macronutrients provide a source of energy for the developing infant, with carbohydrates and protein providing 16.7 kJ/g and fat offering a higher energy yield of 37.7 kJ/g (Kent, 2007; United States National Research Council, 1989). Fat not only contributes a high energy-density but also supports the absorption of fat-soluble vitamins and the development of adipose tissue, which is vital for thermoregulation (Kumar et al., 2017; Lidell, 2019). Protein is essential for tissue growth, maintenance, and repair, and also plays a role in immune function (Kent, 2007). HM supplies a complete amino acid profile, including all nine essential amino acids that cannot be synthesised endogenously (Kumar et al., 2017). Lactose is the predominant carbohydrate in HM, alongside smaller amounts of monosaccharides and HM oligosaccharides (Gridneva et al., 2019). Micronutrients in HM further support immune function, neurodevelopment, and metabolic regulation (Dror & Allen, 2018b; Shergill-Bonner, 2017). As a responsive system,

the composition of HM is shaped by both internal (maternal physiology) and external influences (diet, environment, and infection). To generate meaningful insights, research must recognise HM as a biologically dynamic system influenced by multiple interacting factors, thereby better informing approaches that support optimal maternal and infant health (Christian et al., 2021).

The nutrient composition of HM has been reported to be highly variable between individuals and within individuals, particularly between lactation stages (Czosnykowska-Lukacka et al., 2018; Gidrewicz & Fenton, 2014; Grote et al., 2016). For example, zinc concentrations have a well-established inverse relationship with time postpartum (Bilston-John et al., 2021; Casey et al., 1989; Domellof et al., 2004; Han et al., 2022; Han et al., 2011; Krebs et al., 1985; Krebs et al., 1995; Simmer et al., 1990), where studies have reported standard deviations of up to 1270 µg/L within a narrow range of time (Javad et al., 2018). In the second half of infancy (6–12 months) studies have not demonstrated relationships between minerals and trace elements in HM and maternal factors such as age (Javad et al., 2018; Prentice & Barclay, 1991; Rajalakshmi & Srikantia, 1980), parity (Prentice & Barclay, 1991; Rajalakshmi & Srikantia, 1980), supplementation or status (Domellof et al., 2004; Krebs et al., 1995; Shashiraj et al., 2006), socioeconomic status (Javad et al., 2018; Rajalakshmi & Srikantia, 1980), or occupation (Javad et al., 2018). Iodine is a notable exception, as maternal supplementation during lactation has been demonstrated to increase HM concentrations (Dror & Allen, 2018a) and improve maternal and infant iodine status in iodine-deficient populations (Jin et al., 2021). In NZ, this is of relevance given low soil iodine levels and current public health initiatives promoting maternal supplementation. Supplementation with selenium has also been reported to increase corresponding HM concentrations (Dorea, 2002; Falize et al., 2024; Keikha et al., 2021), although evidence largely relates to the first half of infancy (≤6 months), and routine supplementation of lactating mothers is not currently recommended in NZ.

There is limited NZ-specific research focused on the composition of HM and its contribution to infant energy intake, particularly in later infancy when complementary foods are introduced. In an NZ study in the Manawatu-Wanganui region (n=78), reported an association between polyunsaturated fatty acids, docosahexaenoic acid (DHA), arachidonic, and linoleic fatty acids, and magnesium mineral content of HM with ethnicity; however, samples were collected a 6–8 weeks postpartum (Butts et al., 2018). An NZ study by Lamb et al. (2021) examined the macronutrient composition of HM samples from 27 mothers, encompassing mature term, mature preterm, and transitional preterm HM. Lamb et al. (2021) reported that mature milk of term infants

contained 284.5 kJ/100 mL energy, 0.8 g/100 mL protein, 3.5 g/100 mL fat, and 7.9 g/100 mL carbohydrate (Lamb et al., 2021). Though both studies provide valuable information on HM composition in an NZ context, the former only focuses on a specific region, and the latter was only able to provide a small sample size, with neither focused on the 7–10 month period. There is limited research in NZ examining infant energy intake beyond 6months of age, specifically from HM and complementary foods, across different feeding groups (i.e. breastfed, formula-fed, and mixed-fed). Such research is needed to better understand the unique dietary intake of NZ infants.

Increasing the volume of research on HM composition and infant energy intake NZ-wide during late infancy is a key part of creating future infant feeding guidelines specific to the NZ population, for the benefit of future generations' development. As our physical and social environment progressively changes, the modern diet continues to evolve, with the potential to impact HM and infant energy intake. Therefore, up-to-date HM data is valuable for guiding NZ public health recommendations, adequately supporting the health and well-being of NZ families, and informing future research.

## 1.2 Purpose

HM is recognised by both the NZ MOH and the WHO as beneficial for maternal and infant health beyond the exclusive breastfeeding period (<6 months), with continued breastfeeding recommended alongside complementary feeding from 6 months to 2 years and beyond (MOH, 2021; WHO & UNICEF, 2003). While the macro- and micronutrient composition of HM during exclusive breastfeeding is well documented internationally, data for NZ is limited overall, and completely lacking during late infancy, once complementary feeding is established. Understanding factors influencing HM composition during this stage is important for identifying infants at risk of nutrient inadequacies and guiding strategies to optimise health outcomes.

There is limited evidence in late infancy comparing feeding groups in relation to energy intake from both infant milk sources and complementary foods, and almost no research specifically examining mixed-fed infants. Although recent NZ studies have begun to explore adherence to infant guidelines (Brown et al., 2023; de Castro et al., 2018) and infant feeding patterns (Brown, 2023), the nutrient composition of HM and the relative contribution of HM to total energy intake remains poorly characterised in NZ, particularly in the second half of infancy, where existing research is limited to individual nutrients (Han et al., 2022, Jin et al., 2020, 2022).

Therefore, the purpose of the current study was to characterise the nutrient composition of HM between 7–10 months postpartum in NZ, and to understand how its contribution to infant energy intake varies across feeding groups and how its nutrient composition varies in relation to maternal and infant characteristics. This information is needed to build a clearer picture of infant dietary intake in NZ to inform future infant feeding guidelines and policies.

## 1.3 Aim

To determine the nutrient composition of human milk between 7–10 months postpartum and its contribution to infant energy intake.

### 1.3.1 Objectives

1) To determine the nutrient (macronutrient, vitamin, mineral, and trace element) composition of human milk;
2) To describe the relationship between infant and maternal characteristics with the composition of human milk (i.e. minerals and trace elements);
3) To determine the energy intake from infant milk sources and complementary foods of infants who were breastfed, formula-fed, and mixed-fed.

## 1.4 Thesis Structure

This thesis comprises four chapters. **Chapter One** introduces the research, outlining the background, purpose, aims, objectives, and researcher contributions. **Chapter Two** reviews the literature, examining breastfeeding recommendations, estimation of HM intake, collection of HM samples, infant energy intakes, nutrient concentrations of HM, and factors influencing the nutrient composition of selected minerals (Na, Mg, P, K, Ca) and trace elements (Fe, Cu, Zn, Se, I), alongside the analytical methods employed to assess these nutrients. **Chapter Three** presents the study findings in the form of a manuscript prepared for publication. **Chapter Four** discusses the outcomes and implications of the research, highlighting its strengths and limitations, and provides final recommendations based on the research outcomes. The **Appendices** include summarised data on nutrient concentrations from the literature, providing a concise overview and point of reference, as well as additional figures that were not included in the manuscript.

## 1.5 Researchers Contributions

**Table 1.1** Summary of the researchers' contributions to the study

| Author | Contribution to Thesis |
|---|---|
| **Ariella Weinberg**<br>MSc Nutrition and Dietetic candidate | Primary author of the thesis. Completed the literature review, statistical analysis, and interpretation of results for this secondary analysis. |
| **Professor Cathryn Conlon**<br>Academic supervisor | Academic supervisor. Co-investigator of the FFNZ study, involved in the study design, collection of data, and guided the data analysis for this project. Provided ongoing feedback across all thesis components. Reviewed and approved thesis chapters and manuscript. |
| **Dr Lisa Daniels**<br>Academic supervisor | Academic supervisor. Designed the human milk components of the FFNZ study, collected the data, obtained funding for the composition analysis, supported data analysis, and provided guidance and feedback on all thesis components. Reviewed and approved thesis chapters and manuscript. |

# Chapter 2 Literature Review

## 2.0 Introduction

Breastfeeding is recognised by the New Zealand (NZ) Ministry of Health (MOH) as essential for maternal and infant health, even after the introduction of complementary foods. However, there is limited NZ research on the macro- and micronutrient composition of human milk (HM) and the relationship of minerals and trace elements with infant and maternal factors, with no studies specifically addressing the 7–10 month postpartum period. Research is also scarce on the relative contributions of HM and complementary foods to infant diets across different feeding types. This chapter reviews current literature on breastfeeding guidelines, HM collection and measurement methods in research, infant energy intakes, HM macronutrient, vitamin, mineral, and trace element composition, and infant and maternal factors influencing HM mineral and trace element concentrations, both in NZ and internationally. This literature review will examine results from 6 to 12 months postpartum, excluding exclusively breastfed infants.

Relevant literature was found by searching online electronic databases, including PubMed, Google Scholar, and Massey Discover, using a combination of the search terms below (Figure 2.1). Additional literature was discovered from the reference lists of relevant articles. Journal articles were retrieved between October 2024 and November 2025. Relevant literature was exported to EndNote 20. A summary of the literature review's findings on nutrient concentration for each investigated nutrient and infant energy intakes is included in the appendices (Appendix A–Appendix S).

Date searched: September 2025

Electronic Databases: PubMed, Google Scholar, Massey Discover

Search Terms:

- "Human milk" OR "breast milk" OR breastmilk OR "mothers milk" OR "maternal milk"
- Composition OR makeup OR "make up" OR constitution OR concentration
- Macronutrient OR carbohydrate OR protein OR fat OR lipid OR energy OR micronutrient OR vitamin OR mineral OR "trace element" OR sodium OR magnesium OR phosphorus OR potassium OR calcium OR iron OR copper OR zinc OR selenium OR iodine
- Factor OR characteristic
- Zealand OR Aotearoa OR NZ

**Figure 2.1** Search Strategy for Literature Review

## 2.1 Recommendations for Breastfeeding

The NZ MOH recommends that infants are exclusively breastfed until around 6 months of age, which aligns with the World Health Organization (WHO) recommendation that infants are exclusively breastfed for the first 6 months of life. Both organisations recommend that breastfeeding is continued until 2 years of age and beyond (MOH, 2021; WHO & UNICEF, 2003). The WHO states that from 6 to 12 months of age, it is appropriate for HM to provide half or more of an infant's energy requirements (WHO, 2023). To ensure adequate nutrition for infants, the MOH (2021) states that breastfeeding should continue alongside the commencement of complementary foods. These recommendations pertain to the observed nutritional and health benefits of breastfeeding for both infant and mother (Chowdhury et al., 2015; Horta & Victora, 2013; Sankar et al., 2015). Furthermore, HM composition changes in response to an infant's specific growth and development requirements (Horta & Victora, 2013), reinforcing HM as the gold standard for infant nutrition.

## 2.2 Estimating Human Milk Volume

Accurate methods for estimating HM volume are crucial for assessing infant nutrient intakes and developing nutrient intake recommendations for vulnerable growing infants. As described in the literature, a number of methods are used to estimate HM intake with varying degrees of accuracy, commonly: test-weighing, the 'one size fits all' approach, and the dose-to-mother (DTM) deuterium oxide technique.

The traditional ‘test weighing’ method involves the infant being weighed before and after every feed over a 24-hour period, assuming the change in weight indicates HM intake, as used by Dewey et al. (1984), Heinig et al. (1993), Jarjou et al. (2012), Mitoulas et al. (2002), and Neville et al. (1991). Nevertheless, systematic error can arise in test-weighing due to insensible water loss across long feeds (Heinig et al., 1993). Hendrikson et al. (1985) estimated insensible water losses at 0.03 mL/kg/minute for infants in the first year, whereas Heinig et al. (1993) used their own experimental measurements of insensible water loss (0.048 g/kg/minute); however, this estimation remains unvalidated (Kinoshita et al., 2024). Even so, test-weighing has been reported to overestimate or underestimate HM intake by up to 40%; therefore, it is now considered an imprecise and impractical method that is not appropriate for use in clinical practice (Savenije & Brand, 2006).

Some studies, such as Lim et al. (2018), apply a single estimate for all infants of a given age, adopting a ‘one size fits all’ approach. However, this method implies there is no variation in HM intakes, despite evidence that variance exists (da Costa et al., 2010, as cited in Haszard et al., 2024). Consequently, when studies use a ‘one size fits all’ approach to compare nutrient intakes between breastfed and formula-fed infants, the estimates for formula-fed infants are likely to be more accurate than those for breastfed infants, introducing considerable error in the curation of nutrient intakes for the former group (Haszard et al., 2024).

The deuterium oxide DTM technique, first described by Coward et al. (1982), has been shown to accurately and reliably measure HM intake (Bandara et al., 2015; da Costa et al., 2010; Daniels et al., 2019; Lopez-Teros et al., 2017). Deuterium oxide ($^2H_2O$) is a stable isotope of hydrogen ($^2H$) which is orally administered, metabolised synonymous to water, and excreted in bodily fluids, including saliva and HM. Analysis of salvia samples by Fourier Transform Infrared spectroscopy enables the assessment of HM intake (International Atomic Energy Agency, 2010). Matsiko et al. (2020) reported that saliva yields significantly higher estimates due to slightly greater isotope enrichment in saliva and a poorer model fit for urine samples. Thus, Matsiko et al. (2020) determined that saliva sampling from infants is more precise and practical than urine sampling for estimating HM intake.

Currently, there is limited data on HM intake in infants aged 6–12 months globally. Mean reported intake volumes range between 403 mL/day and 875 mL/day (Dewey et al., 1984; Jarjou et al., 2012; Krebs et al., 1994; Neville et al., 1991). Jarjou et al. (2012) employed the deuterium oxide DTM technique to measure intakes of Gambian infants at 12 months of age, resulting in an

average (mean ± SD) intake of 763 ± 213 mL/day. Jarjou et al. (2012), using the deuterium oxide DTM technique, reported an average (mean ± SD) intake of 763 ± 213 mL/day in Gambian infants at 12 months of age. While considerable variation was reported between infants (IQR: 607–898 mL), intake within the same infant was consistent, with no differences between sexes. In contrast, a review by da Costa et al. (2010), which analysed data from 12 countries across five continents using the same method, reported that variability in intake increased later in infancy and that male infants consumed more HM than females. However, their review included young children aged 0–24 months and did not specifically focus on the second half of infancy (6–12 months).

The current nutrient reference values (NRVs) in NZ for infants 7–12 months are based on an assumed average HM intake of 600 mL/day for this age group, as per the results of Dewey et al. (1984), which is a 'one size fits all' approach. Yet, several limitations exist. The study by Dewey et al. (1984) is outdated, with a small sample of mother-infant dyads studied for HM intake (n=8 at 6 months decreasing to n=3 at 12 months postpartum), and relies on test weighing, which has been shown to be less accurate compared to the deuterium oxide DTM technique (Savenije & Brand, 2006). Consequently, the reliance on the value from the study by Dewey et al. (1984) has likely contributed to inaccuracies in the derivation of many NRVs for infants aged 7 to 12 months.

## 2.3 Collection of Human Milk Samples

Accurate and appropriate analysis of the nutrient composition of HM requires representative sampling, as its nutrient composition varies throughout the course of a feed (Leghi et al., 2020). The gold standard of milk sampling is sub-sampling and pooling of HM from a full breast expression at each feed across a 24-hour period (Kent et al., 2006; Nommsen et al., 1991). This method may impose a significant burden on mothers, making it impractical to carry out. A systematic review by Leghi et al. (2020) analysed the effect of sampling method on macronutrient composition. Protein and fat concentrations remained similar when studies analysed HM collected over a full day or combined pre- and post-feed samples (either once in the morning or across the whole day). However, studies that used only post-feed HM or a single full expression in the morning reported higher protein and fat concentrations (Leghi et al., 2020). Lactose concentration was similar in studies that took a full expression at one time or across 24 hours for pre- or post-feed samples. Nevertheless, fat concentrations were the most affected by the sampling method, as they can increase up to threefold within a single feed from a single breast. Leghi et al. (2020) reported that fat concentrations were higher in post-feed samples or a full expression at one time point than in pre-feed samples. Additionally, fat content increases progressively throughout the

day (Kent et al., 2006). The systematic review by Leghi et al. (2020) concluded that both the most common sampling methods of a full 24-hour expression and a single full expression provide the most representative samples of a feed. Given the methodological challenges of 24-hour sampling, a single full expression is considered the most practical option. Standardising factors such as the time interval since the last feed can further improve analytical accuracy (Leghi et al., 2020). Although Leghi et al. (2020) did not focus on HM at 6–12 months postpartum, the sampling measures they recommend can be applied in studies in the second half of infancy for comparability and accuracy.

## 2.4 Infant Energy Intakes

By 6 months, HM can no longer solely meet infants' increasing energy and nutrient requirements (Fewtrell et al., 2017; WHO et al., 2004). The MOH (2021) recommends that parents offer iron-rich foods, vegetables, and fruit as first foods to infants from 6 months of age and to continue to offer these foods daily alongside HM (or commercial infant formula). The MOH (2021) states that by 12 months of age, most children should be eating the same foods as the family, which should include nutrient-dense foods. Even so, de Castro et al. (2018) reported that 50% of NZ infants at 9 months consumed high sugar, fat, and/or salt foods, which can inappropriately increase energy intake and displace important nutrient intakes (e.g., iron).

Mean daily energy intakes of infants 6–12 months of age range between 577 kJ/day and 970 kJ/day (Ahn & Jeong, 1998; Dewey et al., 1984; Heinig et al., 1993; Lim et al., 2018). Lim et al. (2018) reported that breastfed infants' average (mean ± SD) daily energy intakes were 2728 ± 469 kJ/day at 6 months, 2427 ± 511 kJ/day at 9 months, and 2996 ± 782 kJ/day at 12 months. Additionally, in the same study, formula-fed infants had average (mean ± SD) intakes of 2653 ± 762 kJ/day at 6 months, 2874 ± 766 kJ/day at 9 months, and 3176 ± 904 kJ/day at 12 months. Lim et al. (2018) reported that at 9 months, breastfed infants consumed significantly less energy than formula-fed infants, whereas Heinig et al. (1993) reported that breastfed infants had lower energy intakes at 6, 9, and 12 months. However, Lim et al. (2018) did not measure the volume intake of directly breastfed infants and instead assumed an intake of 780 mL/day at 6 months and 600 mL/day at 9 and 12 months (i.e. "one size fits all approach"). Additionally, Heinig et al. (1993) used test-weighing to measure HM intake. Therefore, energy intake calculations and the results of the two aforementioned studies may be less representative than those that could be obtained using more precise methods, such as the deuterium oxide DTM technique.

### 2.4.1 Energy from Milk Feeding

Mean daily energy intakes from infant milk sources (HM or commercial infant formula) of infants 6–12 months of age range between 176 kJ/day and 641 kJ/day (Dewey et al., 1984; Heinig et al., 1993; Mitoulas et al., 2002; Owino et al., 2007). Heinig et al. (1993) and Mitoulas et al. (2002) both reported a decrease in mean energy intake from infant milk sources with time from 6 to 12 months postpartum. Heinig et al. (1993), who used test weighing, reported a decrease in average (mean ± SD) energy intake from 2167 ± 481 kJ/d at 6 months to 1272 ± 720 kJ/d at 12 months in breastfed infants, and from 2682 ± 682 kJ/d at 6 months to 2080 ± 812 kJ/d at 12 months in formula-fed infants. A decrease was also reported in the average (mean ± SD) percentage of energy received from infant milk sources with increasing infant age, from 85% ± 15% to 38% ± 22% in breastfed infants and from 84% ± 13% to 51% ± 17% in formula-fed infants, from 6 to 12 months of age (Heinig et al., 1993). The same trend is reported by Dewey et al. (1984), with an average (mean ± SD) decrease from 93% ± 7% at 7 months to 64% ± 0.5% at 12 months. However, Dewey et al. (1984) included only eight infants at 7 months, decreasing to only two infants at 12 months; thus, their findings are unlikely to be representative of all infants. On average, across all feeding groups, NZ infants aged 7 months obtain a substantial proportion of their total dietary energy from infant milk sources, with 50% derived from HM and 28% from commercial infant formula (Leong et al., 2018).

### 2.4.2 Energy from Complementary Foods

Mean daily energy intakes from complementary foods of infants 6–12 months of age range between 93 kJ/day and 521 kJ/day (Campos et al., 2010; Heinig et al., 1993; Hernandez et al., 2011; Owino et al., 2007). Campos et al. (2010) reported average (mean ± SD) daily energy intakes from complementary food of 1272 ± 837 kJ/day at 7–9 months and 2054 ± 967 kJ/day at 10–12 months in rural breastfed Guatemalan infants, while at the same timepoints Hernandez et al. (2011) reported average (mean ± SD) intakes of 1356 ± 828 kJ/day and 2192 ± 1067 kJ/day, respectively, in urban breastfed Guatemalan infants. Heinig et al. (1993) reported average (mean ± SD) daily energy intakes from food increased from 389 ± 439 kJ/day, to 1142 ± 799 kJ/day, to 2180 ± 929 kJ/day at 6, 9, and 12 months, respectively, in breastfed infants. Heinig et al. (1993) reported the same increase in energy within formula-fed infants.

Overall, few studies have reported values for both infant milk source energy intake and complementary food energy intake in infants aged 6–12 months, particularly using the gold-standard method for measuring HM intake (deuterium oxide DTM technique). This gap makes it

challenging to accurately determine the relative contributions of these sources to total energy intake during this developmental period. Furthermore, data on the energy intakes of mixed-fed infants are particularly scarce, leaving a major gap in understanding for this entire feeding group.

## 2.5 Human Milk Nutrient Composition

This literature review examines selected nutrient components of HM, specifically energy, macronutrients, trace elements, and some vitamins. These nutrients were chosen based on their feasibility for reliable analysis within NZ laboratories and within funding constraints. In addition, this section considers the concentrations used to inform current NZ nutrient reference values (NRVs) and explores factors that influence HM nutrient concentrations.

### 2.5.1 Energy

HM provides the infant with energy to grow and synthesise tissue, to maintain metabolic activities, physiological functions, heat generation, and muscular activity. The current NZ estimated energy requirements (EER) for infants at 6 months are 2500 kJ/day (girls) and 2700 kJ/day (boys), which rise to 3200 kJ/day (girls) and 3500 kJ/day (boys) at 12 months (Australian National Health and Medical Research Council et al., 2006). These requirements, based on data from studies that measure EER using doubly labelled water, are adjusted for additional growth requirements, with an extra 90 kJ/day for infants aged 7–12 months. Even with the introduction of complementary foods, HM remains the primary source of energy and all macronutrients at 6–12 months of age, in both developing and developed countries (Campos et al., 2010; Mitoulas et al., 2002). An infant's growth is a sensitive indicator of whether energy requirements are being met. At 6 months of age, infants use approximately 6% of their energy for growth, decreasing to 3% at 12 months of age (WHO et al., 2001). Infants who are formula-fed have, on average, higher total energy expenditure (TEE) than breastfed infants, and therefore higher energy requirements, with an average of +7% at 6 months, +6% at 9 months, and +3% at 12 months of age (Butte et al., 1990; Butte et al., 2000; Davies et al., 1990; Jiang et al., 1998, as cited in Australian National Health and Medical Research Council et al., 2006).

Mean HM energy concentrations in the literature range between 243 kJ/100 mL and 297 kJ/100 mL at 6–12 months postpartum globally (Chang et al., 2015; Kim et al., 2017; Kumari-Maurya et al., 2025; Lauber & Reinhardt, 1979; Mitoulas et al., 2002; Nakamori et al., 2009; Nommsen et al., 1991; Yamawaki et al., 2005). An Australian longitudinal study by Mitoulas et al. (2002) reported stable HM energy concentrations at 9 and 12 months postpartum with average values (mean ± SD) of 281 ± 9.0 kJ/100 mL and 279 ± 14.0 kJ/100 mL, respectively. Nommsen et al.

(1991) reported stable average (mean ± SD) HM concentrations of energy at 6–12 months postpartum between 292 ± 28.0 kJ/100 mL and 297 ± 31.0 kJ/100 mL among mothers in the United States (US). However, many studies aggregate data between multiple months (Kim et al., 2017; Kumari-Maurya et al., 2025; Nakamori et al., 2009; Yamawaki et al., 2005), making it difficult to understand the energy concentrations in HM on a month-by-month basis.

The analytical methodology used to directly measure or indirectly calculate energy concentrations in HM influences the reported results. Nommsen et al. (1991) and Mitoulas et al. (2002) determine energy concentrations as described by Garza et al. (1985), using an indirect calculation method by multiplying lactose, protein, and fat by 16.5, 23.6, and 38.7 kJ/g, respectively. Nommsen et al. (1991) also used the gold standard of energy estimation, bomb calorimetry, in a subgroup, which generated similar results to those described by Garza et al. (1985). In contrast, Nakamori et al. (2009) and Yamawaki et al. (2005) determine energy concentration according to the Atwater general factor system, multiplying values of 16.7, 16.7, and 37.7 kJ/g for lactose, protein, and fat concentrations, respectively. Studies that calculate energy concentration according to Garza et al. (1985) tend to report higher values. Methodological variability in the literature complicates direct comparisons of energy concentration and, consequently, the determination of energy intake from HM of infants aged 6–12 months.

### 2.5.2 Fat

Fat constitutes the primary energy-yielding component of HM, of which 97–98% is made up of triglycerides (Campos et al., 2010; Picciano, 2001). HM energy density is strongly correlated with fat concentration (Nommsen et al., 1991). Fat is an efficient source of energy deposition for developing infants, with an energy cost of only 1–4% to store, compared to an energy cost of 25% for glucose (Flatt, 1995). For infants aged 7–12 months of age, the NRV was calculated using an estimated average daily intake of HM for this age group (600 mL/day), multiplied by an average HM fat concentration (4 g/100 mL), and supplemented with median intake data from complementary foods, sourced from American dietary studies (Australian National Health and Medical Research Council et al., 2006). However, caution is warranted when interpreting these values, as the HM data originate from 2002 and the complementary food data are based on studies conducted in 1994–96, which may no longer reflect current dietary patterns or compositions.

An important fatty acid in HM for developing infants is docosahexaenoic acid (DHA), an omega-3 long-chain polyunsaturated fatty acid. It is essential for brain growth and functional

development, playing a crucial role in maintaining normal neural functions (Horrocks & Yeo, 1999). The nervous system is rich in DHA, and HM-derived DHA is readily incorporated into developing infants' brains. This is evidenced by breastfed infants exhibiting a higher proportion of DHA in their brain cortex and erythrocytes compared to formula-fed infants (Makrides et al., 1994)

Fat is recognised as the most variable nutrient in HM across all stages of lactation, exhibiting substantial intra-individual variation (Dror & Allen, 2018b; Mitoulas et al., 2002; Picciano, 2001). Despite this variability, Dewey et al. (1984) reported that the average fat concentration remains relatively stable during the 7–12 months postpartum period. In contrast, Neville et al. (1986) documented a significant increase in fat concentration between 6 and 12 months postpartum. As both aforementioned studies were conducted in the US within a similar time frame, these factors are unlikely to explain the discrepancies in findings.

Studies have reported average (mean ± SD) HM fat concentrations to be between 2.46 ± 1.26 g/100 mL and 5.16 ± 2.73 g/100 mL at 6–12 months postpartum (Ayah et al., 2007; Chang et al., 2015; Dewey et al., 1984; Dijkhuizen et al., 2004; Kim et al., 2017; Kumari-Maurya et al., 2025; Liyanage et al., 2008; Mitoulas et al., 2002; Nagra, 1989; Nakamori et al., 2009; Yamawaki et al., 2005). Studies differ in both HM sample collection methods and analytical methods. HM is known to transition to have higher fat content across the duration of a feed (Kent et al., 2006), although studies worldwide employ different sampling methods. Chang et al. (2015), Dewey et al. (1984), and Kim et al. (2017) collected a full expression, whereas Kumari-Maurya et al. (2025) sampled 25 mL mid-feed. Mitoulas et al. (2002) and Nagra (1989) collected samples before and after a feed (≤1 mL and 20 mL, respectively). Nakamori et al. (2009) sampled 20 mL in the morning, not directly associated with a feed, and Yamawaki et al. (2005) collected 50 mL at an intermediate time during feeding. When studies do not employ standardised protocols, such as consistent sample volumes and timings, it becomes difficult to make meaningful comparisons, even when uniform approaches would have been straightforward to implement.

The literature describes a range of methods for analysing HM fat. Older studies have commonly employed the creamatocrit method (Ayah et al., 2007; Dijkhuizen et al., 2004; Liyanage et al., 2008; Lucas et al., 1978), the gravimetric Rosë-Gottlieb method (Nakamori et al., 2009; Yamawaki et al., 2005), or colorimetric methods (Dewey et al., 1984; Mitoulas et al., 2002), whereas more recent studies typically use an HM analyser (HMA), which relies on mid-infrared transmission spectroscopy (Chang et al., 2015; Kumari-Maurya et al., 2025). Among these, the Rosë-Gottlieb method is generally regarded as the gold standard for HM fat analysis (Giuffrida et al., 2019).

Comparisons between methods have yielded inconsistent findings: Giuffrida et al. (2019) reported that the HMA underestimated fat relative to the Rosë-Gottlieb analysis, whereas Zhu et al. (2017) reported an overestimation. The creamatocrit method has been described as highly reproducible (Tie et al., 2021) and provides values that correlate strongly with Rosë-Gottlieb measurements (Hundrieser et al., 1984). However, the creamatocrit method generally reports higher fat and energy concentrations than an HMA, which consistently provides lower values despite significant correlations with creamatocrit results (Andreassa et al., 2024; O'Neill et al., 2013). While correlations exist between techniques, differences in absolute concentrations highlight methodological variability, which complicates comparisons of HM fat concentrations across studies. However, researchers may favour using an HMA because it is fast and simple to use and has practical clinical applications (Kwan et al., 2020; O'Neill et al., 2013).

### 2.5.3 Protein

The protein fraction of HM is comprised of digestible true protein, providing all essential amino acids (van Sadelhoff et al., 2021), alongside non-protein nitrogen, which accounts for 20–25% of the total nitrogen (Picciano, 2001).

HM protein concentration beyond 6 months is lower when compared to the earlier postpartum period (<6 months) (Lonnerdal et al., 2017; Rajalakshmi & Srikantia, 1980), with reported mean concentrations in the literature between 0.80 g/100 mL and 1.55 g/100 mL globally (Chang et al., 2015; Dewey et al., 1984; Gridneva et al., 2018; Kim et al., 2017; Kugananthan et al., 2017; Kumari-Maurya et al., 2025; Mitoulas et al., 2002; Nagra, 1989; Nakamori et al., 2009; Nommsen et al., 1991; Yamawaki et al., 2005). Chang et al. (2015) reported higher average (mean ± SD) HM protein concentrations of 1.30 ± 0.20 g/100 mL at 6–7 months and 1.20 ± 0.20 g/100 mL at 7–8 months in Korean mothers. These elevated values likely reflect the inclusion of non-protein nitrogen (NPN) in the analysis, whereas most studies report protein concentration based on true protein alone. Similarly, Dewey et al. (1984), Kim et al. (2017), and Nommsen et al. (1991) reported higher average (mean ± SD) concentrations ranging from 1.24 ± 0.22 g/100 mL to 1.55 ± 0.48 g/100 mL, although it was not specified whether NPN was included. Nommsen et al. (1991) also noted that their use of bovine serum albumin as the standard in the Modified Lowry Assay may have led to slightly elevated protein values due to differences in amino acid composition.

Longitudinal studies by Mitoulas et al. (2002) and Nagra (1989) reported relatively stable protein concentrations in HM between 6 and 12 months postpartum, with average values (mean ± SD) between 0.98 ± 0.02 g/100 mL and 1.01 ± 0.6 g/100 mL (Nagra, 1989) and between 0.8 ± 0.04

g/100 mL and 0.83 ± 0.06 g/100 mL (Mitoulas et al., 2002). Similarly, Kim et al. (2017) found no differences between samples at 6 and 7–11 months. Although these studies demonstrate the stability of protein concentrations, Kugananthan et al. (2017) reported a drop at 9 months, with concentrations rising again at 12 months postpartum. At 9 months postpartum, Kugananthan et al. (2017) included more mothers from the cross-sectional cohort compared to other months, which may explain this finding. Similarly, Nommsen et al. (1991) observed a decrease in protein concentration at the 6- and 9-month time points, followed by a rise again at 12 months postpartum. Additionally, a negative correlation was established between HM output volume and protein concentration at both 6 and 9 months postpartum.

There are multiple methods used for protein analysis described in the literature, including the Kjeldahl method (Nakamori et al., 2009; Yamawaki et al., 2005), the Bradford assay (Gridneva et al., 2018; Kugananthan et al., 2017; Mitoulas et al., 2002), a modified Lowry assay (Nommsen et al., 1991), or the use of an HMA with mid-infrared spectroscopy (Chang et al., 2015; Kumari-Maurya et al., 2025). The Modified Lowry assay (Peterson, 1977) measures total protein, including non-protein nitrogen, which can lead to an overestimation of true protein. While NPN may have biological roles (Gowen et al., 2025), it does not reflect nutritive protein content. Therefore, for consistency and nutritional relevance, true protein should be measured (Belfort et al., 2024). Studies that have used the modified Lowry assay (Dewey et al., 1984; Nommsen et al., 1991), which did not indicate exclusion of NPN, reported unusually high protein values at 6–12 months postpartum, suggesting that the method is less representative of true nutritive protein. If such methods are used, NPN should be taken into account to ensure accurate and comparable estimates of protein content. Nakamori et al. (2009) and Yamawaki et al. (2005), who use the Kjeldahl method, applied a conversion factor of 6.38, which is typically used for dairy milk to convert from total nitrogen to true protein. However, Lonnerdal (2003) argued that since NPN accounts for a higher portion in HM (around 20–25% of total nitrogen), it is more appropriate to subtract the NPN fraction from total nitrogen and apply a factor of 6.25; otherwise, true protein may be overestimated.

The Kjeldahl method (Barbano et al., 1990; Licon, 2022) is widely regarded as the reference for HM protein analysis, with results aligned with those from direct amino acid analysis, the most accurate yet costly and time-consuming approach (Giuffrida et al., 2019; Hambraeus et al., 1976; Lonnerdal, 2003). More recently, the use of an HMA has been adopted as a rapid alternative. Although HMA protein concentrations are correlated with Kjeldahl concentrations, multiple studies

show consistent underestimation when compared with both the Kjeldahl method (Giuffrida et al., 2019; Menjo et al., 2009; Zhu et al., 2017) and the Dumas method, which is analogous to the Kjeldahl method (Thajer et al., 2019). In contrast, in a study of donor human milk across three milk bank settings (n=25) that compared results against the Kjeldahl reference method, Perrin et al. (2019) reported that the HMA was sufficient for protein analysis. In comparison with a colorimetric method, Silvestre et al. (2014) reported that the HMA underestimated protein concentrations relative to the Bradford assay. In contrast, Casadio et al. (2010) reported a small but significant difference between the two methods yet concluded that the HMA provides an efficient method for evaluating protein content. Overall, these findings suggest that protein concentrations measured by an HMA may be underestimated compared to more traditional methods of protein analysis and should be interpreted with caution. However, similar to fat, researchers may favour using an HMA as it is fast and simple to use and has practical clinical application (Kwan et al., 2020; O'Neill et al., 2013).

### 2.5.4 Lactose

Lactose is the primary carbohydrate in HM (Gridneva et al., 2019) and one of the most stable constituents of HM across lactation (Chang et al., 2015; Picciano, 2001). Multiple longitudinal studies have shown no significant variation in lactose concentration across 6–12 months postpartum (Chang et al., 2015; Gridneva et al., 2019; Mitoulas et al., 2002; Wack et al., 1997). Unlike many other nutrients in HM, lactose concentrations show minimal variation between different mothers (Chang et al., 2015). Instead, the primary source of variation is within the same mother over time (Picciano, 2001). Reported mean concentrations for HM lactose at 6–12 months postpartum range between 5.88 g/100 mL and 7.82 g/100 mL (Chang et al., 2015; Dewey et al., 1984; Gridneva et al., 2019; Kugananthan et al., 2017; Kumari-Maurya et al., 2025; Lauber & Reinhardt, 1979; Mitoulas et al., 2002; Nagra, 1989; Nommsen et al., 1991; Wack et al., 1997; Yamawaki et al., 2005).

There have been multiple different analytical methods used for lactose, including enzymatic methods (Dewey et al., 1984; Gridneva et al., 2019; Kugananthan et al., 2017; Mitoulas et al., 2002; Nommsen et al., 1991; Yamawaki et al., 2005), infrared analysis using an HMA (Chang et al., 2015; Kumari-Maurya et al., 2025), colorimetry (Lauber & Reinhardt, 1979), and high-performance liquid chromatography (Wack et al., 1997). A systematic review by Leghi et al. (2020) reported that analytical methods had a greater impact on lactose concentrations in HM than the specific sample collection methods (e.g., time of day). The literature generally agrees that mid-

infrared HMAs are unsuitable for lactose analysis, as they cannot distinguish lactose from HMOs, which make up 15-25% of HM carbohydrates, leading to overestimation (Casadio et al., 2010; McCune et al., 2023; Perrin et al., 2019; Smilowitz et al., 2014). Comparisons of HPLC with HMAs have shown inconsistent results: Menjo et al. (2009) reported a significant positive correlation, but accuracy only at a lactose concentration of 6–7 g/100 mL, whereas McCune et al. (2023) found no correlation between an HMA and HPLC (AOAC method 984.22) or with an enzymatic assay (AOAC method 2006.06) (see also Perrin et al., 2019). Some types of enzymatic assays may be inflated by HMOs if their enzymes cleave lactose moieties (Casadio et al., 2010; McCune et al., 2023). This may account for the higher lactose concentrations reported by Dewey et al. (1984) and Nommsen et al. (1991), which together reported mean concentrations ranging between 7.35 g/100 mL and 7.82 g/100 mL using enzymatic hydrolysis according to Dahlqvist (1964). However, both the HPLC (AOAC method 984.22) and the specific enzymatic assay (AOAC method 2006.06) methods described by McCune et al. (2023) are considered reliable, unaffected by HMOs, and strongly correlated; therefore, they are the most suitable for research.

### 2.5.5 Analysis of Minerals

Atomic absorption spectrometry (AAS) techniques have been widely used for elemental analysis of milk, using flame atomic absorption spectrometry (FAAS) and electrothermal atomization atomic absorption spectrophotometry (ET-AAS) (Caroli, 2006). Flame atomic absorption spectrometry (FAAS) offers a relatively fast, simple, and cost-effective approach (Caroli, 2006). However, its limitations include the need for large sample volumes and high detection limits, making it inappropriate for elements present in very low concentrations (Bolann et al., 2007; Caroli, 2006). FAAS remains appropriate for the analysis of elements such as calcium, potassium, magnesium, sodium, copper, iron, and zinc (Caroli, 2006; Hampel et al., 2018). Graphite furnace atomic absorption spectrometry (GFAAS) is a form of ET-AAS that offers good detection power and requires only small sample volumes. However, issues tend to arise with the method, including the buildup of carbonaceous residue, foaming, and the splattering of samples during the drying stage. Consequently, several techniques have been developed over the years to address these issues (Caroli, 2006).

Inductively coupled plasma-atomic emission spectrometry (ICP-AES), also referred to as inductively coupled plasma-optical emission spectrometry (ICP-OES) (Sabzkoohi et al., 2023), enables simultaneous multi-element analysis within a relatively short timeframe; however, like FAAS, it is limited by high detection thresholds (Bolann et al., 2007; Caroli, 2006). In contrast,

inductively coupled plasma mass spectrometry (ICP-MS) is currently regarded as the best method for mineral and trace element analysis, as it offers both multi-element capability and high sensitivity, with low detection limits (Bolann et al., 2007; Caroli, 2006; Dubascoux et al., 2018). This allows for the quantification of a broad range of minerals in HM. The primary disadvantages of ICP-MS are its high cost and the requirement for skilled personnel, which may limit its application in certain research contexts (Bolann et al., 2007; Caroli, 2006; Dubascoux et al., 2018).

Ultimately, while ICP-MS is the most desirable method due to its high sensitivity, low detection limits, and multielement capability, this does not imply that other methods are inadequate. FAAS and ICP-AES/OES remain valuable depending on the context; the choice of method should be guided by the nutrients of interest, expected concentration ranges, available resources, and laboratory expertise (Bolann et al., 2007).

For the purposes of this literature review, future references to AAS include FAAS, GFAAS, or instances where a study reports only AAS. This is to compare temporal trends in analytical methods as they are all based on the principle of atomic absorption spectroscopy, rather than implying that these techniques are synonymous.

The measurement of minerals and trace elements in HM at 6–12 months postpartum has evolved over the past several decades. Early studies primarily relied on AAS, particularly for analysis of magnesium, iron, copper, zinc, calcium, potassium, sodium, and selenium. In some cases, a validated semi-automated micro-method was used for calcium and phosphorus analysis (Jarjou et al., 2012; Prentice & Barclay, 1991). Over the past two decades, ICP-based techniques (ICP-AES/OES and ICP-MS) have become increasingly used. ICP-MS has been employed in the most up-to-date studies of magnesium, iron, copper, and zinc (Han et al., 2022; Javad et al., 2018; Kim et al., 2017; Nakamori et al., 2009; Yamawaki et al., 2005), while potassium and sodium analyses have also transitioned entirely to ICP-AES/OES or ICP-MS (Javad et al., 2018; Kim et al., 2017; Wack et al., 1997; Yamawaki et al., 2005). Selenium analysis has similarly evolved from AAS (Al-Awadi & Srikumar, 2001; Li et al., 1999) to ICP-AES in 2005 and ICP-MS in 2020 (Jin et al., 2020; Yamawaki et al., 2005). Iodine analysis in recent years has been dominated by ICP-MS (Andersson et al., 2010; Henjum et al., 2016; Jin et al., 2022), which has been deemed the most appropriate method (Hampel et al., 2018), although earlier, an adaptation of the Sandell-Kolthoff method (Dunn et al., 1993) was used in 2009 (Wang et al., 2009). This progression reflects a clear trend in HM research towards using analytical methods with multielement capability, such

as ICP-AES/OES and ICP-MS, as well as methods that offer high sensitivity and low detection limits, such as ICP-MS.

### 2.5.6 Zinc

Sufficient zinc intake in infancy is crucial as this mineral plays a vital role in biological processes such as cell growth and differentiation, and is required for hundreds of enzymes that regulate metabolism (Darnton-Hill, 2013; Vallee & Auld, 1990). Zinc is a component of enzymes that maintain the structural integrity of proteins (Australian National Health and Medical Research Council et al., 2006), such as its structural role in deoxyribonucleic acid (DNA) transcription proteins (Georgieff, 2007). This mineral also plays a role in immunity, with zinc deficiency leading to increased morbidity and mortality from respiratory infections and diarrhoea in infants (Black & Fischer Walker, 2012). Zinc is involved in length-related outcomes, with zinc deficiency in infancy resulting in stunting (Dror & Allen, 2018b). Umeta et al. (2003) reported that low zinc concentrations in the HM of Ethiopian mothers at 5–11 months postpartum contributed to the stunting of infants. However, a systematic review by Reyes et al. (2024) suggested that it is zinc intake that may be important for length-related outcomes in the second half of infancy as opposed to HM zinc concentration. Umeta et al. (2003) employed a cross-sectional study design, and therefore, the longitudinal effect of low zinc concentrations on infant length was not investigated.

The NRV for zinc at 7–12 months of age was calculated by estimating absorbable zinc requirements to replace endogenous losses, assuming an absorption of 30% (Australian National Health and Medical Research Council et al., 2006). This estimation was based on adult data, considering growth needs only, without taking into account HM concentrations, whereas the NRV for younger infants aged 0–6 months considers HM. This demonstrates a gap in the literature, where the NRV could be more accurately estimated using up-to-date HM intake and composition, as well as complementary food intake data for infants aged 7–12 months.

Zinc has been identified as a nutrient at risk of deficiency for breastfed infants past 6 months (Krebs, 2007). As time progresses, the space between zinc HM intake and requirements narrows. Consequently, at 7 months of age, zinc HM intake is nearly equal to the estimated requirements for net absorption. Therefore, to meet zinc requirements from HM alone, absorption would need to be close to 100% (Krebs & Hambidge, 1986).

The zinc concentrations reported in the literature at 6–12 months postpartum range between a mean of 340 µg/L and 2300 µg/L, with many studies exhibiting large standard deviations, up to

1270 µg/L (Casey et al., 1989; Dewey et al., 1984; Domellof et al., 2004; Han et al., 2022; Javad et al., 2018; Kim et al., 2017; Lauber & Reinhardt, 1979; Nagra, 1989; Nakamori et al., 2009; Rajalakshmi & Srikantia, 1980; Ren et al., 2024; Rios-Leyvraz & Yao, 2023; Simmer et al., 1990; Vaughan et al., 1979; Yamawaki et al., 2005). A meta-analysis by Rios-Leyvraz and Yao (2023) reported a mean (95% CI) zinc concentration of 1180 µg/L (1050, 1320) for 6–12 months postpartum, from an average of 56 studies. A longitudinal study by Han et al. (2022) reported a mean (95% CI) concentration of 340 µg/L (295, 392) at 12 months postpartum in the HM of NZ mothers (n=38), placing NZ mothers on the lower end of values reported globally, even at this later stage of lactation.

*Factors influencing HM zinc concentrations*

There is well-established evidence that HM zinc concentrations decrease throughout the 6–12 months postpartum period (Bilston-John et al., 2021; Casey et al., 1989; Domellof et al., 2004; Han et al., 2022; Han et al., 2011; Krebs et al., 1985; Krebs et al., 1995; Simmer et al., 1990). Karra et al. (1986) reported a significant average (mean ± SD) decrease of 26% ± 0.4% between 7 and 12 months postpartum. A longitudinal American study by Casey et al. (1989) reported average (mean ± SD) zinc concentrations as follows: 1098 ± 602 µg/L at 6 months, 922 ± 458 µg/L at 7 months, 745 ± 209 µg/L at 8 months, 772 ± 392 µg/L at 9 months, 621 ± 347 µg/L at10months, 530 ± 327 µg/L at 11 months, and 543 ± 301 µg/L at 12 months postpartum, demonstrating this decrease with time postpartum. Domellof et al. (2004) suggested that the decrease with time is due to breastfeeding weaning (gradually replacing HM with complementary foods) occurring during this period of lactation. Conversely, Casey et al. (1989) reported that the decrease was due to time postpartum alone, and not specifically related to the volume decrease from breastfeeding weaning. Unlike the aforementioned studies, Nagra (1989) reported that zinc concentrations remained stable between 6 and 12 months postpartum in Pakistani mothers, with mean concentrations ranging from 800 µg/L to 1000 µg/L. While Nagra (1989) reported a decline in zinc concentrations earlier in lactation (<6 months), the small sample size (n=15–20) may have limited the study's ability to detect the continued decrease commonly reported in other studies.

The key factors that influence zinc concentrations are the stage of lactation and interindividual variability (Krebs et al., 1995). There have been no relationships reported between HM zinc concentrations and maternal age (Javad et al., 2018; Rajalakshmi & Srikantia, 1980), nutritional status, parity (Rajalakshmi & Srikantia, 1980), zinc supplementation or status (Domellof et al.,

2004; Krebs et al., 1995), socioeconomic status (Javad et al., 2018; Rajalakshmi & Srikantia, 1980), occupation, or smoking by family members (Javad et al., 2018)

Overall, zinc concentrations in HM are well-researched in diverse geographical locations. The frequently-reported decline across the 6–12 month postpartum period appears to be largely independent of maternal or infant factors, with increasing time postpartum (and thus infant age) being the primary determinant of concentration changes.

### 2.5.7 Copper

Copper is the third most abundant trace element in the human body and is an essential cofactor in multiple metalloenzymes. It is also involved in many physiological functions, such as erythropoiesis, thermal regulation, immune and cardiac function, skeletal mineralisation, and connective tissue synthesis (Solomons, 1985). Copper deficiency in infancy has been associated with bone disease and anaemia (Levy et al., 1985; Solomons, 1985).

The copper NRV for infants aged 7–12 months was calculated by combining the average HM concentration at this stage of lactation (≤0.20 mg/L) with an average HM intake volume of 600 mL/day and American data for median intake from complementary foods (0.10 mg/day) (Australian National Health and Medical Research Council et al., 2006). As the NZ NRVs were formed in 2006, more up-to-date HM copper concentrations, from NZ mothers, would form a more appropriate infant copper NRV.

The mean concentrations of copper in the literature range between 119 µg/L and 300 µg/L at 6–12 months postpartum (Casey et al., 1989; Dewey et al., 1984; Domellof et al., 2004; Javad et al., 2018; Kim et al., 2017; Lauber & Reinhardt, 1979; Nagra, 1989; Nakamori et al., 2009; Rajalakshmi & Srikantia, 1980; Ren et al., 2024; Simmer et al., 1990; Vaughan et al., 1979; Yamawaki et al., 2005). In Japan, Yamawaki et al. (2005) note a large standard deviation for copper of 50 ug/L at 6–12 months postpartum. Similar or greater variability has been reported elsewhere; in Korea, Kim et al. (2017) reported a standard deviation of 96.1 µg/L at 7–11 months postpartum, and in Sweden, Domellof et al. (2004) reported a standard deviation of 220 µg/L at 9 months postpartum.

*Factors Influencing HM Copper Concentrations*

HM copper concentrations are reported to be lower in the second 6 months of life, decreasing between 1 and 12 months postpartum (Bilston-John et al., 2021; Ren et al., 2024). The literature

reveals a range of copper concentration trends from 6 to 12 months postpartum. When specifically assessed by month postpartum (between 6 and 12 months), no changes in copper concentrations have been reported by some (Casey et al., 1989; Nagra, 1989). However, other studies have reported decreasing concentrations over the same period. In NZ mothers, a steady decrease in HM copper concentrations from 6 to 12 months postpartum was reported (Han et al., 2022). Similarly, Kim et al. (2017) reported this pattern among mothers in Korea, and Javad et al. (2018) among mothers in Iran, with significant decreases in copper concentrations between mothers at 6 and 7–11 months, and at 6–7 and 12 months postpartum, respectively. These differing trends are unlikely to be explained by geographical variation alone, as studies from diverse regions (e.g., NZ, Iran, Korea) reported similar temporal patterns. Javad et al. (2018) reported no correlation between HM copper concentrations and maternal socioeconomic status, age, occupation, or smoking by family members during the 1–12 month postpartum period. Although these studies did not solely focus on the 6–12 months postpartum period, the results are still applicable to this age range.

However, geographical location may influence the absolute concentrations of copper in HM. For example, Domellof et al. (2004) reported that Honduran mothers had significantly higher HM copper concentrations than Swedish mothers at 9 months postpartum. In the Honduran subsample, a positive correlation was observed between HM copper concentrations and complementary food intake, whereas no correlation was found in the Swedish subsample. Additionally, Domellof et al. (2004) reported no correlation between copper HM concentrations and maternal copper status in neither Honduran nor Swedish mothers. These results suggest that differences in infant feeding practices, such as type and/or amount of complementary food provided, may influence HM copper concentrations more than maternal copper status. This highlights the importance of considering dietary and cultural contexts when comparing HM copper concentrations across populations.

While copper concentrations in HM have been reported across many countries in the second half of infancy, recent evidence is limited. Up-to-date concentrations are needed to inform NRV calculations and ensure they accurately reflect modern dietary and physiological contexts. As the copper NRV does not incorporate NZ HM data, it may not be fully generalisable to NZ infants. International trends in copper concentrations may not reflect population differences; however, there remains a possibility that actual concentrations differ, highlighting the need for further research in NZ.

### 2.5.8 Calcium

Calcium is the most abundant mineral in the body, playing key roles in neuromuscular and cardiac function, as well as in the development and maintenance of the skeleton (Australian National Health and Medical Research Council et al., 2006). Calcium is stored in teeth and bones, with the highest rate of calcium accretion occurring in the first year of life. Umeta et al. (2003) reported that low concentrations of HM calcium contributed to the stunting of infants aged 5–11 months. At 12 months of age, HM calcium concentration has been reported to be a positive predictor of infant weight (second to HM intake) in a sample of Gambian mother-infant dyads (Jarjou et al., 2012). However, a systematic review by Reyes et al. (2024) suggested that calcium intake (rather than calcium HM concentration) is more important as a predictor of infant length in the second half of infancy, particularly in settings where maternal and infant undernutrition are common.

The current calcium NRV for infants aged 7–12 months was determined using an estimate of HM calcium at this age (210 mg/L), an average intake of 600 mL/d, and an estimated intake from complementary food (140 mg/d) (Australian National Health and Medical Research Council et al., 2006). However, this calculation used data from American studies over 25 years old, indicating the need for up-to-date NZ data to establish more meaningful NRVs to be set for this age group. Calcium in HM is highly bioavailable, with approximately 60–70% of the calcium being absorbed (Abrams, 2006). Even with the introduction of complementary foods, HM remains the leading source of calcium in the diets of breastfed infants at 6–12 months of age, as reported in two studies of Guatemalan infants (Campos et al., 2010; Hernandez et al., 2011).

A meta-analysis by Rios-Leyvraz and Yao (2023) determined the average (mean 95% CI) calcium concentration of HM at 6–12 months postpartum to be 214 mg/L (163, 266). Reported mean concentrations in the literature range between 152 and 489 mg/L (Dewey et al., 1984; Jarjou et al., 2012; Javad et al., 2018; Kim et al., 2017; Kumari-Maurya et al., 2025; Nagra, 1989; Prentice & Barclay, 1991; Rios-Leyvraz & Yao, 2023; Vaughan et al., 1979; Yamawaki et al., 2005), with the exception of an Ethiopian study by Umeta et al. (2003) who reported a markedly low concentration of 14.0 ± 0.4 mg/L.

*Factors Influencing HM Calcium Concentrations*

Many studies use a cross-sectional study design when investigating HM calcium concentration. However, when a longitudinal approach is used, results within 6–12 months postpartum are often pooled, making it difficult to adequately analyse trends in calcium concentrations month by month. For example, Bilston-John et al. (2021) and Han et al. (2022) reported a decrease in calcium

concentrations from 1 month to 12 months postpartum, while Jarjou et al. (2012) reported a decrease between 3 and 12 months postpartum. However, without a focus on the 6–12 months postpartum period, the same trend cannot be assumed for this time frame.

A longitudinal study by Nagra (1989) reported average values (mean ± SD) ranging from 313 ± 13.0 mg/L to 321 ± 12.0 mg/L throughout the 6–12 month postpartum period, with no definitive trend. Likewise, Dewey et al. (1984) reported that concentrations were maintained at roughly the same level between 7 and 12 months postpartum for mothers not purposefully decreasing breastfeeding, whereas Vaughan et al. (1979) reported stable concentrations from 9 months postpartum. Conversely, Karra et al. (1986) found that calcium concentrations were significantly lower at 12 months than at 7 months postpartum, with an average (mean ± SD) decrease of 9.0% ± 0.5% between the two time points.

Bilston-John et al. (2021) and Kim et al. (2017) indicated that maternal calcium supplementation does not influence HM calcium concentrations. There have been no significant relationships found between HM calcium concentrations and maternal age (Javad et al., 2018; Prentice & Barclay, 1991), parity, nutrition status (Prentice & Barclay, 1991), occupation, socioeconomic status, or smoking by family members (Javad et al., 2018). Conversely, from 7 months postpartum, Dewey et al. (1984) identified breastfeeding weaning as a factor that decreases HM calcium concentrations. Therefore, according to the current literature, it can be inferred that, aside from breastfeeding weaning, neither maternal nor infant factors significantly impact calcium concentrations.

Although calcium has a moderately well-established body of evidence in the second half of infancy, there is a complete absence of NZ-specific data. Current, local calcium concentrations are required to inform NRV calculations and ensure they accurately reflect the nutrients received by infants in NZ.

#### 2.5.9 Iron

Iron is a key component of enzymes involved in redox reactions and proteins, including haemoglobin, myoglobin, and cytochromes (Australian National Health and Medical Research Council et al., 2006). As a component of haemoglobin, iron facilitates the transport of oxygen to tissues. Additionally, iron is a structural component of enzymes, which are involved in various metabolic processes, including neurotransmitter synthesis and function, phagocyte antimicrobial activity, and the production of DNA, collagen, and bile acids (WHO & Food and Agricultural

Organization of the United Nations, 2002). Although there is well-established mechanistic evidence on the role of iron in brain development, evidence on the impact of iron deficiency or iron supplementation throughout all months of infancy on cognition remains limited (McCann et al., 2020). Iron deficiency and iron deficiency anaemia, which are common in infants aged 6–24 months, can result in poor weight gain and a higher incidence of respiratory and intestinal infections (Castillo-Duran & Cassorla, 1999; Saloojee & Pettifor, 2001).

Iron is a nutrient with a high risk of deficiency in breastfed infants after 6 months of age (Krebs, 2007). An infant's iron stores are depleted by 6 months, and HM is no longer a sufficient sole source of iron to support the infant's requirements; thus, the infant becomes critically dependent on dietary iron from complementary foods (Eussen et al., 2015). This is reflected by the iron NRV increasing from 0–6 months to 7–12 months postpartum. Nonetheless, the NRVs for the 7–12-month age group did not account for HM iron concentrations. Instead, the iron NRVs were set through modelling components of iron requirements, estimating absorption requirements, and applying a 10% upper limit for iron absorption (Australian National Health and Medical Research Council et al., 2006).

Although HM iron concentrations are low, bioavailability is high; infants absorb ~49% of HM iron compared with 10% from cow's milk (Saarinen et al., 1977). Reported mean HM iron concentrations at 6–12 months postpartum range between 180 µg/L and 850 µg/L (Dewey et al., 1984; Domellof et al., 2004; Javad et al., 2018; Kim et al., 2017; Lauber & Reinhardt, 1979; Nakamori et al., 2009; Ren et al., 2024; Shashiraj et al., 2006; Vaughan et al., 1979; Yamawaki et al., 2005). Extreme values include a study by Dewey et al. (1984), which reported an average (mean ± SD) concentration of 180 ± 100 µg/L at 7–11 months postpartum, and Yamawaki et al. (2005), which reported a mean of 850 µg/L with a large standard deviation of 660 µg/L at 6–12 months postpartum. The high value reported by Yamawaki et al. (2005) is unlikely to be attributed to the analysis method, as Nakamori et al. (2009) also used ICP-AES yet reported lower average values (mean ± SD) of 400 ± 140 µg/L at 6–8 months postpartum and 460 ± 150 µg/L at 9–12 months postpartum. As the former study is the only one in the literature that examines iron concentrations in a sample of Japanese mothers, additional research is needed to determine whether this finding reflects ethnic and/or geographical differences among mothers.

*Factors Influencing HM Iron Concentrations*

Studies investigating iron concentration trends between 6 and 12 months postpartum have concluded that concentrations remain stable (Bilston-John et al., 2021; Dewey et al., 1984; Han

et al., 2022). There has been no correlation reported between maternal iron status and HM iron concentrations (Domellof et al., 2004; Shashiraj et al., 2006). Domellof et al. (2004) reported a positive correlation between complementary food energy intake and HM iron concentrations in a sample of Honduran mothers at 9 months postpartum, suggesting a relationship between breastfeeding weaning and HM iron concentration; however, this relationship was not reported in the Swedish cohort of the same study. Javad et al. (2018) reported no significant relationships between HM iron concentrations and maternal age, occupation, socioeconomic status, or smoking by family members. Notably, Dewey et al. (1984) identified significant inter-mother variation in HM iron concentrations during the 7–11 months postpartum period, suggesting individuality as the primary source of variability. Therefore, the current literature suggests there are no known maternal or infant factors that influence HM iron concentrations at 6–12 months postpartum.

Overall, iron appears to be a nutrient at risk of deficiency in infants during the second half of infancy. Despite its low concentrations in HM, including iron in future NRV calculations would be important to illustrate its limited contribution. Globally, reported iron concentrations vary widely. Since neither maternal nor infant factors appear to affect calcium concentrations, future research should explore the reasons for the wide range of reported concentrations, which could inform interventions in populations at risk of deficiency.

#### 2.5.10 Magnesium

Approximately 50–60% of the body's magnesium lies within bone, 30–40% in muscles and soft tissues, and 1% in extracellular fluid. Magnesium acts as a cofactor for numerous enzymes, playing key roles in energy metabolism, RNA and DNA synthesis, maintaining the electrical potential of cell membranes and nervous tissue, and protein synthesis (WHO & Food and Agricultural Organization of the United Nations, 2002). Acute magnesium deficiency and magnesium toxicity are rare, with deficiency typically occurring only in cases of protein-energy malnutrition (Lonnerdal, 1995).

For infants aged 7–12 months, the NRV was calculated based on an estimate of magnesium HM concentration (34 mg/L) and an average HM intake volume (600 mL/day). The concentration of 34 mg/L used by the Australian National Health and Medical Research Council et al. (2006) to establish the NRV represents an average derived from 10 studies reviewed by Atkinson et al. (1995) at 6 months postpartum. Mean magnesium concentrations reported in the literature range between 22.0 mg/L and 33.6 mg/L (Dewey et al., 1984; Javad et al., 2018; Kim et al., 2017; Nagra,

1989; Rajalakshmi & Srikantia, 1980; Vaughan et al., 1979; Yamawaki et al., 2005). The majority of studies reviewed by Atkinson et al. (1995) were conducted before 1992 and focused on mothers in the US. This highlights the need to reassess the magnesium NRVs for NZ infants aged 7–12 months using updated evidence and more representative populations.

*Factors Influencing HM Magnesium Concentrations*

Multiple studies have reported that magnesium HM concentrations remain relatively stable throughout 6 to 12 months postpartum (Bilston-John et al., 2021; Han et al., 2022; Nagra, 1989; Rajalakshmi & Srikantia, 1980). Similarly, Vaughan et al. (1979) reported no consistent trends in magnesium concentrations over the 1–31 months postpartum period. Conversely, Karra et al. (1986) reported an average (mean ± SD) decrease of 9.0% ± 2.2% from 7 to 12 months postpartum, though the change was not statistically significant. Neville et al. (1991) reported a significant difference in trends between breastfeeding weaning and non-weaning mothers, where the magnesium concentrations of the former increased with time post-partum, whereas those of the latter decreased over time postpartum (from 6 to 18 months). However, Neville et al. (1986) only included a small sample of 11 mothers; therefore, the findings may not accurately reflect the true effect of time postpartum. The only study that has reported magnesium concentrations month-by-month between 6 and 12 months postpartum is by Nagra (1989), which examined mothers in Pakistan. Nagra (1989) reported stable average (mean ± SD) concentrations between 22.0 ± 5.0 mg/L and 28.0 ± 4.0 mg/L. These values are on the lower end of those reported in the literature. The limited detail provided in Nagra's study makes it challenging to determine reasons for these lower values, aside from potential population or ethnic differences.

In the second half of infancy, there have been no relationships found between HM magnesium concentrations and maternal age (Javad et al., 2018; Rajalakshmi & Srikantia, 1980), nutritional status, parity (Rajalakshmi & Srikantia, 1980), socioeconomic status, occupation, or smoking by family members (Javad et al., 2018).

In summary, HM magnesium concentrations demonstrate little variation between 6 and 12 months postpartum, and current evidence does not indicate any influence of infant or maternal factors. Given the outdated and non-representative HM data underpinning the current magnesium NRV (for 7–12 months of age), research conducted in a population representative of NZ would provide a more robust and relevant basis for developing NZ-specific NRVs.

### 2.5.11 Sodium and Potassium

Sodium and potassium are essential electrolytes involved in cellular homeostasis, as well as in the nervous and muscular systems. Their roles include regulating heart and muscle function, maintaining osmotic pressure, serving as cofactors for enzymes, contributing to pH balance, and participating in oxidation-reduction reactions (Pohl et al., 2013). Sodium ions are the primary cations in human extracellular fluid, while potassium ions are the major cations of the human intracellular fluid, constantly moving to maintain electrolyte and water balance (Australian National Health and Medical Research Council et al., 2006). Sodium stimulates cell proliferation and protein synthesis, thereby increasing cell mass and acting as a growth factor (Haycock, 1993). Insufficient sodium intake during early life can impair growth and potentially disrupt other physiological functions (Araya et al., 2023).

The sodium NRV for infants aged 7–12 months was extrapolated using data from the 0–6 month NRV (a concentration of 160 mg/L and an average intake of 780 mL/day) with adjustments for relative energy requirements and consideration of metabolic body weights (Australian National Health and Medical Research Council et al., 2006). It is unclear whether this was due to limited data on HM sodium concentrations beyond 6 months or other reasons. However, as complementary feeding occurs throughout 7 to 12 months of age, utilising data from a stage where HM is the sole nutrient source may be inappropriate.

The potassium NRV for infants aged 7–12 months was calculated using an average HM intake of 600 mL/day, an HM potassium concentration of 500 mg/L, and an average intake from complementary foods (440 mg/day) at this age. However, the potassium concentration used is derived predominantly from data collected at 1–6 months postpartum, with limited representation of values beyond 6 months (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2004).

Sodium and potassium concentrations in HM are often reported together in studies. At 6–12 months postpartum mean sodium HM concentrations reported in the literature range between 76.0 mg/L and 252 mg/L at (Dewey et al., 1984; Javad et al., 2018; Kim et al., 2017; Nagra, 1989; Wack et al., 1997; Yamawaki et al., 2005), while reported mean potassium concentrations range between 330 mg/L and 472 mg/L at 6–12 months postpartum (Dewey et al., 1984; Kim et al., 2017; Nagra, 1989; Wack et al., 1997; Yamawaki et al., 2005). A meta-analysis by the US National Academies of Sciences Engineering and Medicine (2019) reported a mean sodium concentration of 110 mg/L and a mean potassium concentration of 435 mg/L at 7–12 months postpartum.

However, as they only included studies from the US, the findings may not reflect global concentrations. A longitudinal study by Nagra (1989) reported the noticeably highest values among those included in this literature review, with average (mean ± SD) values ranging from 235 ± 10.0 mg/L to 252 ± 3.0 mg/L across 6–12 months postpartum in Pakistani mothers**.** In contrast, longitudinal study by Wack et al. (1997), conducted over the same postpartum period, reported lower average (mean ± SD) values ranging from 122 ± 123 mg/L to 130 ± 142 mg/L.

*Factors Influencing HM Sodium and Potassium Concentrations*

Han et al. (2022), Nagra (1989), and Wack et al. (1997) reported that concentrations of both sodium and potassium remained stable throughout 6–12 months postpartum. Conversely, Bilston-John et al. (2021) reported that sodium concentrations increased in months 9 and 12 postpartum. Moreover, Javad et al. (2018) reported HM sodium concentrations of mothers at 12 months postpartum were higher than those at 6–7 months postpartum. Resultingly, there is inconsistency in the reported trends of sodium concentrations at 6–12 months postpartum, making it difficult to draw firm conclusions about sodium's changes in HM over time.

Wack et al. (1997) reported that sodium concentrations varied more within individual mothers than between mothers, whereas potassium concentrations had a similar level of variation both within and between mothers. Furthermore, for sodium, Dewey et al. (1984) reported that mothers producing <300 mL/day of HM had both higher variability and concentrations, demonstrating an inverse relationship with HM output and sodium concentrations.

Data on sodium and potassium concentrations in HM during the second half of infancy are lacking. Obtaining up-to-date local HM concentrations for these minerals would improve the relevance of NRVs for NZ infants receiving HM. Additionally, future research should investigate HM sodium and potassium concentrations longitudinally, given the mixed evidence for sodium and the overall limited knowledge of temporal changes in both minerals during the second half of infancy.

### 2.5.12 Selenium and Iodine

Selenium and iodine are critical for thyroid function, making adequate infant status essential (Kohrle et al., 2005; Zimmermann, 2009). Iodine is incorporated into thyroid hormones thyroxine (T4) and triiodothyronine (T3), which regulate biochemical reactions, enzymatic activities, and protein synthesis (Triggiani et al., 2009). Selenium, a component of iodothyronine deiodinases, facilitates the conversion of T4 to the active T3. These hormones play a crucial role in normal metabolic regulation, growth, and development. Additionally, selenium plays an antioxidant role

in reduction-oxidation reactions (Australian National Health and Medical Research Council et al., 2006).

The NRVs for infants aged 7–12 months for both selenium and iodine were extrapolated from HM concentrations and intake at 0–6 months of age on a metabolic weight basis (Australian National Health and Medical Research Council et al., 2006). The HM concentration used for the selenium NRV (15 µg/L) is based on NZ data, whereas the iodine value (115 µg/L) is not. This suggests a clear gap in knowledge of NZ HM selenium and iodine concentrations beyond 6 months postpartum. In NZ infants at 12 months of age, Daniels et al. (2023a) reported that HM was the greatest contributor of selenium (39%) to total intake when compared to categories of complementary food. However, Daniels et al. (2023a) did not directly measure HM selenium concentrations and rather assumed it to be 20.7 µg/L, which is higher than the values reported in this literature review, and was also limited by a 'one-size-fits-all' intake estimate of 600 mL/day. Additionally, Daniels et al. (2023a) reported that HM selenium intake was correlated with lower infant selenium plasma concentrations.

Few studies exist on selenium concentrations in HM at 6–12 months postpartum. Reported mean selenium concentrations in the literature range between 11.35 µg/L and 22.49 µg/L (Al-Awadi & Srikumar, 2001; Jin et al., 2020; Li et al., 1999; Ren et al., 2024; Shalom et al., 2024; Yamawaki et al., 2005). The lowest concentration was reported in a meta-analysis by Shalom et al. (2024), which investigated mothers with infants born at term at 6–12 months of age, while the highest concentration was reported in a meta-analysis by Ren et al. (2024), based on Chinese mothers at 8–12 months postpartum. However, the evidence base is limited. Shalom et al. (2024) included only three studies with a total of 17 samples, and Ren et al. (2024) drew upon just two studies. The only other NZ study reporting selenium concentration was conducted by Jin et al. (2020), which reported a median ($25^{th}$, $75^{th}$ percentile) concentration of 12 µg/L (11, 13).

For iodine, data at 6–12 months postpartum are also scarce, with reported concentrations ranging between 43.6 µg/L and 360 µg/L (Andersson et al., 2010; Henjum et al., 2016; Jin et al., 2022; Wang et al., 2009). A Nepalese study by Henjum et al. (2016) reported the highest average concentrations at 7–12 months postpartum, with medians [$25^{th}$, $75^{th}$ percentiles] between 215 µg/L [193, 400] and 360 µg/L [125, 480] (extracted from supplementary data), where 81% of mothers were classified as iodine sufficient. However, this population was specifically investigated for excessive iodine intake in infants, which likely accounts for the notably higher HM iodine concentrations compared with other studies. A study by Wang et al. (2009) reported a mean

(range) HM iodine concentration of 122 µg/L (16.5–291) among infants and mothers in China with adequate iodine status at 6–12 months postpartum. In contrast, in Switzerland, Andersson et al. (2010) reported a mean HM iodine concentration of 42.6 µg/L, with both infants and mothers below the WHO (2007) recommended urinary iodine sufficiency threshold of 100 µg/L at 12 months postpartum. In addition, Jin et al. (2022), in an observational longitudinal study conducted in Palmerston North, New Zealand, reported that while breastfed infants (n=33) at 12 months of age were iodine sufficient, the breastfeeding mothers (n=33) remained deficient, reporting a median [25$^{th}$, 75$^{th}$ percentile] HM iodine concentration of 35.0 µg/L [26.0, 54.0] at 12 months postpartum. These discrepancies likely reflect differences in national salt iodization programmes and the policies regarding iodine supplementation for lactating women across the study settings: Nepal (Henjum et al., 2016), NZ (Jin et al., 2022), Switzerland (Andersson et al., 2010), and Yongjing County in Gansu Province, China (Wang et al., 2009).

*Factors Influencing HM Selenium and Iodine Concentrations*

HM selenium concentrations are influenced by maternal dietary intake, with lower concentrations in geographical regions with selenium-poor soils (Kumpulainen, 1989; Roekens et al., 1985). Notably, NZ, particularly in the South Island, is a low selenium environment (Robinson, 1989; Thomson, 2004). Likewise, HM iodine concentrations vary geographically (Picciano, 2001). Although NZ soils are iodine poor (Thomson, 2004), mandatory iodized salt fortification in bread has improved the population's intake of iodine (Skeaff et al., 2013), though potentially still not to adequate levels (Edmonds et al., 2016). Additionally, mothers are recommended to take an iodine supplement of 150 µg from the start of pregnancy and whilst breastfeeding in NZ (MOH, 2021), whereas for lactating mothers from countries with successful national iodized salt programmes, such as Switzerland (Andersson et al., 2010), the WHO does not recommend iodine supplementation (WHO Secretariat et al., 2007). Maternal intake of either a single high dose or a daily iodine supplement during lactation has been shown to increase HM iodine concentrations (Dror & Allen, 2018a) and improve maternal and infant iodine status (Jin et al., 2021). While urinary iodine is commonly used as a biomarker of iodine status, recent evidence indicates that HM iodine concentrations closely reflect maternal iodine status and may therefore serve as a useful indicator of both maternal and infant iodine status (Liu et al., 2022).

Selenium concentrations may decrease with higher maternal parity (WHO, 1998), though this evidence from developing countries may not be applicable to NZ mothers. Given the low selenium and iodine levels in NZ soils, it is important that future literature explores how this influences HM

concentrations and subsequently infant growth and development. Three reviews by Dorea (2002), Falize et al. (2024), and Keikha et al. (2021) have found that, generally, selenium supplementation does increase HM selenium concentrations, though none of these reviews focused on the 6–12 months postpartum period.

Bilston-John et al. (2021) reported stability in iodine concentrations throughout 6–12 months postpartum. In contrast, for selenium, an NZ study by Han et al. (2022) reported increasing concentrations from 6 to 12 months postpartum, after a nadir at 6 months. Similarly, Bilston-John et al. (2021) reported an increase in selenium concentration between 9 and 12 months postpartum in Australian mothers.

Given the low selenium and iodine content of NZ soils, it is essential to characterise HM concentrations across all stages of lactation as both nutrients are critical for thyroid function and neurodevelopment. The scarcity of data beyond 6 months postpartum represents a significant research gap, limiting the ability to identify infants at risk of deficiency.

### 2.5.13 Phosphorus

Phosphorus is involved in numerous biological processes, including acid-base balance, bone mineralisation, cell signalling, and energy production. Additionally, it is a structural constituent of cell membranes and nucleic acids (Dror & Allen, 2018b). Phosphorus in HM has higher bioavailability than cow's milk and soy-based formula, with an absorption of 85 to 90% (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997) compared to 72% and 59%, respectively.

The phosphorus NRV was calculated using an estimate for phosphorus from HM at this age and an estimate of intake from complementary foods (Australian National Health and Medical Research Council et al., 2006). The concentration used of 124 mg/L is an average from two studies from the US, predating 1992, with a total of only 30 mothers (Atkinson et al., 1995; Greer et al., 1982; Prentice & Barclay, 1991). Using recent, NZ-specific data would better validate or update this value.

Throughout 6–12 months postpartum, four studies conducted across Korea (Kim et al., 2017), Pakistan (Nagra, 1989), Zaire (Prentice & Barclay, 1991), and Japan (Yamawaki et al., 2005) have reported average (mean ± SD) phosphorus concentrations between 117 ± 13.0 mg/L and 142 ± 36.9 mg/L.

*2.5.13.1 Factors Influencing HM Phosphorus Concentrations*

Han et al. (2022), Nagra (1989), and Prentice and Barclay (1991) reported phosphorus concentrations to remain stable throughout 6–12 months postpartum, whereas Bilston-John et al. (2021) reported phosphorus concentrations to increase in months 9 and 12 postpartum. When investigating maternal factors, Prentice and Barclay (1991) reported no influence of maternal age, nutritional status, or parity on phosphorus concentrations. This suggests that, as per current evidence, phosphorus concentrations are not influenced by any factors other than potential changes with time postpartum.

Although phosphorus is an important mineral, it remains relatively under-researched in HM during the second half of infancy. Further research is needed to establish the true physiological range of phosphorus during this period, both globally and in NZ.

### 2.5.14 Vitamin A and E

Fat-soluble vitamins are less frequently studied than macronutrients and minerals in HM through the 6–12 month period. Among these, vitamin A is the most commonly reported, typically analysed as retinol. Present in HM as retinyl esters in the fat fraction (Stoltzfus & Underwood, 1995), vitamin A is crucial for epithelial differentiation, immune function, growth, reproduction, and vision (Ross & Tan, 2016). With low vitamin A stores at birth, the infant relies on an adequate supply from HM and complementary foods for liver accretion and normal growth and development (Dror & Allen, 2018b). HM has been reported as the leading source of vitamin A in the diet of rural Guatemalan infants at 6–12 months of age (Campos et al., 2010). Breastfeeding protects against Vitamin A deficiency (Sommer et al., 1986; Tarwotjo et al., 1989), which is one of the most prevalent nutrient deficiencies throughout developing countries (McLaren, 1986).

Reported mean vitamin A concentrations in developing countries range between 12.6 µg/100 mL and 50.7 µg/100 mL (Ayah et al., 2007; Dijkhuizen et al., 2004; Kumari-Maurya et al., 2025; Liyanage et al., 2008; Rice et al., 1999; Roy et al., 1997). While studies from developing countries provide valuable HM data, their findings are likely not applicable to NZ mothers. Maternal nutrition during lactation strongly influences the vitamin A content of HM, and women in developing countries tend to consume less than half of the daily vitamin A intake of those in developed countries, resulting in lower vitamin A status (Lonnerdal, 1986; Newman, 1994). In developed countries, Kim et al. (2017) reported average (mean ± SD) concentrations from Korean mothers' of 32.1 ± 15.7 µg/100 mL at 7–11 months postpartum, and Kim et al. (1990) reported an average (mean ± SD) concentration of 58.7 ± 25.8 µg/100 mL in mothers in the US at 6–7 months

postpartum. As the studies by Kim et al. (1990) and Kim et al. (2017) were conducted in developed countries, their findings are likely more applicable to an NZ context.

The current vitamin A NRV for infants aged 7–12 months was calculated using an HM concentration of 310 µg/L, from a study by Canfield et al. (2003), alongside an average intake of 600 mL/day and a contribution of vitamin A from complementary food (Australian National Health and Medical Research Council et al., 2006). However, Canfield et al. (2003) reported concentrations over the whole 1–12 months postpartum period and did not specifically focus on the 6–12 months postpartum period. A meta-analysis by Dror and Allen (2018c) reported a positive correlation between retinol and fat in HM in mature milk, a finding also reported by Kim et al. (2017). Notably, the literature exhibits near unanimity in analytical approach, with retinol almost exclusively measured using high-performance liquid chromatography (HPLC).

There is limited composition data available on vitamin E in HM at 6–12 months postpartum. Comprising eight naturally occurring isomers, it is typically measured using alpha-tocopherol equivalents, the major form of vitamin E. This nutrient exhibits antioxidant activity, preventing the production of free radicals and protecting cell membranes from radical damage (Burton et al., 1983). For infants aged 7–12 months, the current vitamin E NRV was extrapolated from data of infants aged 0–6 months on a body weight basis, using a value of 4.90 mg/L for HM concentrations, which demonstrates again a gap in HM knowledge past 6 months postpartum (Australian National Health and Medical Research Council et al., 2006). Kim et al. (2017) reported an average (mean ± SD) alpha-tocopherol concentration of 0.23 ± 0.13 mg/100 mL at 7–11 months postpartum, as determined by HPLC. Kim et al. (2017) also reported that alpha-tocopherol was correlated with both vitamin A and fat in HM.

While vitamin A has been extensively studied, particularly in developing countries where such research is crucial for health outcomes, further work is needed in developed countries to provide a more complete understanding of its concentrations once complementary feeding is introduced. In contrast, data on HM vitamin E concentrations remain limited across all countries, underscoring the need for more research globally during the 6–12 month postpartum period.

#### 2.5.15 Folate and Vitamin C

Folate and vitamin C (ascorbic acid) are both water-soluble vitamins, which the body does not store and therefore need to be replenished daily by the diet. Folate is also known as vitamin B9 or formerly as folacin (WHO & Food and Agricultural Organization of the United Nations, 2004).

Folate is essential for amino acid metabolism, DNA synthesis, and cell division, with deficiency leading to megaloblastic anaemia (Carmel, 2005). Vitamin C acts as a cofactor in the synthesis of connective tissue proteins as well as aiding the absorption of iron (United States National Research Council, 1989).

The reported mean concentration of folate in HM varies across studies, ranging from 1.90 µg/100 mL to 5.68 µg/100 mL. Differences in reported values may reflect the various forms of folate quantified, including free folacin (Ek, 1983; Udipi et al., 1987), total folacin/folate (Ek, 1983; Su et al., 2022; Udipi et al., 1987), and folic acid (Sakurai et al., 2005). However, folic acid may not be an appropriate measure, as it is not the form naturally present in HM (Tamura & Picciano, 2006). There are also different analytical methods, such as a microbiological assay with Lacticaseibacillus casei (L. casei) (Ek, 1983; Udipi et al., 1987), HPLC with fluorimetric detection (Sakurai et al., 2005), and liquid chromatography-mass spectrometry (LC-MS) (Su et al., 2022). According to Hampel et al. (2018) and Tamura and Picciano (2006), the microbiological assay with L. casei was considered the most reliable method. However, Hampel et al. (2018) did not evaluate LC-MS, and the conclusion of Tamura and Picciano (2006) was based on the findings of Lim et al. (1998), which also did not include LC-MS, likely due to its limited use at the time of publication (Redeuil et al., 2017). In contrast, the European Food Safety Authority (2014) has reported that LC-MS provides the highest specificity and sensitivity for folate analysis, although this evidence primarily relates to plasma/serum, whole blood, tissue, and food samples, rather than HM specifically. In the context of the microbiological assay, free folacin/folate refers to the fraction directly utilisable by L. casei without enzymatic treatment, whereas total folacin/folate represents the sum of free folate plus polyglutamates released following conjugase treatment (Hoppner et al., 1972). Butterworth et al. (1969) reported that a comparable enzymatic cleavage occurs in humans, and therefore, it is essential to measure both free and total folate using this method. In contrast, Su et al. (2022) defined total folate as the combined concentration of five species of folate measured by LC-MS.

The only concentration for vitamin C identified in this literature review was reported by Sakurai et al. (2005), which measured an average (mean ± SD) concentration of 3.7 ± 1.3 mg/100 mL in HM at 6–12 months postpartum using HPLC analysis. The vitamin C NRV for infants aged 7–12 months was derived from a concentration of 3.0 mg/100 mL reported in studies of infants ≤6 months, adjusted for body weight. Campos et al. (2010) and Hernandez et al. (2011) reported HM to be the leading source of folate and vitamin C at 7–12 months postpartum in both urban and

rural Guatemalan infants. However, neither of the aforementioned studies directly measured HM intake and instead assumed that the energy from HM intake was equal to the energy requirement of the infant, minus the energy intake measured from complementary foods.

Overall, both water-soluble vitamins, particularly vitamin C, require further investigation of the concentrations in HM, specifically in the second half of infancy, as it remains difficult to establish true physiological ranges, especially within an NZ context. Future studies should also investigate different analytical methods for determining vitamin C concentrations during this period to determine the most accurate and reliable approach.

## 2.6 Summary

An analysis of key literature regarding HM nutrients and infant energy intake has revealed that HM remains an important source of nutrients alongside complementary foods in the second half of infancy. Some nutrients, such as zinc and iron, exhibit considerable variability in reported concentrations. A range of analytical methods has been used to assess HM composition. Modern analytical methods such as ICP-MS offer high sensitivity, rapid analysis, and low detection limits for minerals and trace elements. Evidence of the influence of infant and maternal factors on HM composition is limited and often inconsistent. However, there is consistent evidence indicating a positive association between time postpartum and zinc concentrations as well as between maternal supplementation and selenium and iodine levels. Formula-fed infants generally have higher reported energy intakes than breastfed infants, while little evidence exists on mixed-fed infants as a distinct feeding group. In NZ, there is a lack of data on HM composition beyond 6 months postpartum, and no NZ study has examined a broad range of nutrients in the second half of infancy. Addressing these gaps is essential to provide accurate population-specific data, inform infant feeding guidelines, and better understand the contribution of HM to infant nutrient intake.

# Chapter 3 Manuscript

## 3.0 Abstract

**Background:** Human milk (HM) is the optimal source of nutrition for infants, delivering health benefits to both infants and mothers. While exclusive breastfeeding is recommended by the World Health Organization and the New Zealand (NZ) Ministry of Health for the first 6 months, complementary foods are typically introduced at around 6 months, with breastfeeding often continuing into late infancy. HM composition during the complementary feeding period remains under-researched in NZ and globally, with most studies classifying HM expressed beyond two weeks postpartum as “mature milk,” which may obscure potential changes in nutrient content that may occur over the course of lactation. Understanding HM composition in relation to maternal and infant characteristics, and how energy is derived from infant milk sources and complementary foods across feeding practices, is essential to accurately assess nutrient intake and inform evidence-based feeding guidelines during this critical stage.

**Aim:** To determine the nutrient composition of human milk between 7–10 months postpartum and its contribution to infant energy intake.

**Methods:** The cross-sectional study included 625 parent-infant dyads from the multi-centred First Food New Zealand (FFNZ) study (Dunedin and Auckland, 2020–2022). This secondary analysis focuses on a subgroup of 121 mothers, with infants aged 7–10 months, who provided HM samples. Demographic and anthropometric measures, 24-hour dietary recalls, and HM intake volume data (via the dose-to-mother stable isotope technique) were collected. Macronutrients (fat, lactose, protein), vitamins (vitamin A, C, E, folate), minerals (Na, Mg, P, K, Ca), and trace elements (Fe, Cu, Se, Zn, I) were analysed using validated methods. Energy was calculated using Atwater factors. Mineral and trace elements concentrations were descriptively analysed in relation to maternal (age, BMI, ethnicity, parity, employment, deprivation, supplement use) and infant (age, ethnicity, sex, term status, BMI z-score, feeding group) characteristics.

**Results:** Thirteen of 18 nutrients were within reported literature ranges. Median HM iodine (62.82 µg/L), iron (133.9 µg/L), selenium (10.21 µg/L), and vitamin C (2.39 mg/100 mL) concentrations were lower than reported literature values, whereas vitamin E (0.27 mg/100 mL) and magnesium (34.30 mg/L) were higher. No clear differences were observed across maternal or infant characteristics. Significant differences ($p<0.05$) in energy intake were observed across feeding groups. Breastfed and formula-fed infants differed in energy intake from infant milk sources (mean ± SD: 2068 ± 561 kJ/day vs 2251 ± 601 kJ/day) and total energy intake (3239 ± 525 kJ/day vs 3534 ± 609 kJ/day). Additionally, breastfed and mixed-fed infants differed in energy intake from

complementary foods (1170 ± 741 kJ/day vs 1448 ± 729 kJ/day) and total energy intake (3239 ± 525 kJ/day vs 3538 ± 559 kJ/day). All significant differences remained after adjusting for infant age and sex.

**Conclusion:** These findings indicate that HM nutrient concentrations in NZ mothers between 7–10 months postpartum are broadly consistent with international literature, though lower iodine, iron, and selenium concentrations may place infants at risk of inadequate intake. Differences in energy intakes between feeding groups were also observed. Future work should integrate HM composition with measured intakes to determine nutrient adequacy. This will clarify HM's contribution in late infancy and ensure feeding guidelines adequately address potential nutrient gaps.

**Key words:** human milk, breast milk, infant, New Zealand

## 3.1 Introduction

Human milk (HM) is widely regarded as the “gold standard” for early life nutrition, an evolutionary refined fluid uniquely tailored to support the growth and development of the nutritionally vulnerable infant. Breastfeeding has well-established health and nutritional benefits for both the infant and the mother and is therefore recommended by the World Health Organization (WHO) to continue until 2 years of age or beyond (WHO & UNICEF, 2003). Infant benefits include protection against common childhood illnesses, future overweight/obesity, and diabetes. Maternal benefits include protection against breast and ovarian carcinoma in the mother, with longer durations of breastfeeding offering greater protection (Chowdhury et al., 2015; Horta & de Lima, 2019; Horta et al., 2015; MOH, 2021; Victora et al., 2016; WHO, 2023). HM provides a complete source of nutrition, containing macronutrients, fluid, vitamins, and minerals (Butts et al., 2018; Grote et al., 2016), as well as a range of bioactive components such as antibodies, enzymes, antioxidants, hormones, and growth factors which cannot be replicated by commercial infant formula (Lonnerdal, 2014; Victora et al., 2016). These bioactive substances contribute to immune protection, developmental programming, and infant growth and development (Reniker et al., 2023).

In response to scientific evidence supporting the benefits of breastfeeding, evidence-based infant feeding guidelines have been developed both internationally and in New Zealand (NZ) to guide health professionals and inform parents and caregivers. In 2021, the NZ Ministry of Health (MOH) released updated guidelines, which provide recommendations on breastfeeding, formula feeding, and the introduction of complementary foods (MOH, 2021). Exclusive breastfeeding, defined as an infant receiving only HM (either directly from the breast, expressed, or donated milk), is recommended for the first 6 months by the WHO and UNICEF (2003) and the MOH (2021). Commercial infant formula is recommended as the only suitable alternative to HM for infants under 12 months of age (MOH, 2021). In NZ, breastfeeding initiation is high, with 97.9% of mothers initiating breastfeeding before 1 month of age; however, rates decline to 73.3% by 7–7.9 months and 68.6% by 10–10.9 months (Brown et al., 2023). Common reasons for discontinuation of breastfeeding include maternal pain, fatigue, return to work, and concerns around milk supply (Brown et al., 2014; Douglas, 2022; Kozhimannil et al., 2014). Complementary foods are recommended from around 6 months of age, when infants exhibit signs of developmental readiness, as HM alone is no longer sufficient to meet infants’ evolving energy and nutrient requirements for optimal growth and development (MOH, 2021).

The composition of HM is dynamic and may be influenced by various factors throughout lactation. One of the most consistently observed influences is the duration of time postpartum, which has been associated with changes in the concentrations of several micronutrients, including calcium, copper, selenium, and zinc (Bilston-John et al., 2021; Domellof et al., 2004; Han et al., 2011; Kim et al., 2017). In contrast, there is relatively limited evidence regarding the role of maternal or infant characteristics in shaping the micronutrient profile of HM (Domellof et al., 2004; Javad et al., 2018; Rajalakshmi & Srikantia, 1980). So far, international studies have found no significant relationships between HM minerals and age, socioeconomic status, occupation, smoking by family members (Javad et al., 2018), as well as nutritional status, parity, or between urban and poor rural groups (Rajalakshmi & Srikantia, 1980).

There is currently limited NZ-specific research examining the nutrient composition of HM in the second half of infancy (6–12 months), particularly between 7 and 10 months postpartum, and its contribution to infant energy intake. One NZ study reported that mature term milk contained 284.5 kJ/100 mL energy, 0.8 g/100 mL protein, 3.5 g/100 mL fat, and 7.9 g/100 mL carbohydrate (Lamb et al., 2021), while another reported a mean zinc concentration of 340 µmol/L at 12 months postpartum (Han et al., 2022). However, the general classification of milk expressed beyond 14 days postpartum as “mature milk” (Kim & Yi, 2020) may obscure important changes that occur beyond 6 months postpartum, when complementary foods are typically introduced. Neither study provides a comprehensive profile of both macro- and micronutrient composition beyond 6 months postpartum. Understanding how HM composition changes over the course of lactation when complementary foods are introduced is crucial for evaluating infant nutritional intake and adequacy during this developmentally and nutritionally vulnerable period. Therefore, the purpose of this study is to determine the nutrient composition of human milk between 7–10 months postpartum and its contribution to infant energy intake.

## 3.2 Methods

### 3.2.1 Study Design

This cross-sectional study included maternal and infant data from the FFNZ observational study. The primary aim of the FFNZ study was to determine the iron status, growth, food and nutrient intakes, HM intake, eating and feeding behaviours, dental health, oral motor skills, and choking risk of NZ infants in general, including those using baby food pouches and those using the baby-led approach to complementary feeding (Taylor et al., 2021). This research reports a secondary outcome of the FFNZ study, investigating the nutrient composition of HM between 7 and 10

months postpartum and its contribution to infant energy intake. Detailed methods of the FFNZ study have already been published (Taylor et al., 2021); therefore, only methods applicable to this study have been included.

### 3.2.2 Participants and Recruitment

The FFNZ study recruited 625 caregiver-infant dyads, with 121 of these dyads included in the full HM subgroup. The inclusion criteria for parents were: residing in Auckland or Dunedin, could communicate in English, and were aged 16 years or older. To meet the eligibility criteria, infant participants were required to be 7 to 10 months of age at the time of participation and not be taking part in a nutrition intervention study that could influence their diet. There were no other exclusion criteria.

Recruitment for this study was accomplished through both advertising and word of mouth. Advertisements were placed on social media and in community hubs. FFNZ aimed to recruit a sample that was broadly representative of NZ infants with regard to ethnicity and socioeconomic status. To recruit a diverse range of participants, Māori and Pasifika community health organisations were engaged with, and suburbs with high proportions of Māori, Pasifika, and Asian populations were targeted. Mothers registered their interest through phone contact with researchers. Screening was conducted by means of a phone call questionnaire, and verbal consent was obtained once eligibility was confirmed. Written consent was obtained at the first visit prior to commencement of participation. Ethical approval was obtained from the Health and Disability Ethics Committees of New Zealand (19/STH/151). This study was registered with the Australian New Zealand Clinical Trials Registry (registration number: ACTRN12620000459921).

### 3.2.3 Data Collection

Mother-infant dyads included in the full HM subgroup had a total of five visits, either at home or in clinic, over a 2-week period. At the first visit, initiation of the deuterium oxide (stable isotope) ‘dose-to-mother’ (DTM) technique began, where the mother was given an oral dose of deuterium oxide after collecting baseline saliva samples from both the mother and infant. Subsequent post-dose saliva samples were collected from the infant and mother at 3 visits over the following 14 days to estimate daily infant HM intake (g/day). The first 3 visits consisted of questionnaires, 24-hour infant diet recalls, and infant and maternal anthropometric measurements. After completion of the two-week deuterium oxide DTM oxide assessment period (visits 1-3), a full HM expression

(complete emptying of the breast) sample from one breast was collected from the mother at the fourth and fifth visits.

*Demographic Data*

At the first visit, an online questionnaire was completed to gather demographic data that described the study participants and infant feeding practices. Demographic data were collected on: infant and maternal dates of birth (to determine age), whether born at term, delivery route (e.g., vaginal or caesarean), ethnicity (classified by NZ census categories), number of breastfeeding occasions, infant sex, parity, maternal education level, employment, health status, medication, physical activity, and supplement use. To streamline data, prioritised ethnicity was used to allocate participants to a single ethnicity if they identified with more than one. Area-level socioeconomic deprivation was approximated using participants' home addresses based on the NZDep2018 index of deprivation ordinal scale, ranging from 1 (least deprived) to 10 (most deprived) (Atkinson et al., 2019).

*Anthropometric Assessment*

Infant length and weight (nude) were measured by trained researchers using calibrated equipment and standard operating procedures (de Onis et al., 2004). Infant length was measured using a 99 cm length board (model SE210; Seca), and electronic scales were used to measure infant weight (models 334 and 354; Seca). Two measurements were taken, and a third measurement was taken if the difference exceeded the specific threshold (de Onis et al., 2004). For mothers whose infants' HM intake was assessed using the deuterium oxide DTM technique, maternal weight was measured in duplicate with an electronic scale (Tanita HD-351) at the first (day 0) and final (day 14) study visits, while mother's height was measured at the third study visit (day 7) using a portable stadiometer (Wedderburn Portable Height Rod WS-HRP). All other mothers self-reported maternal height and weight via a questionnaire. The infant's BMI z-score was determined using the WHO Child Growth Standards (WHO, 2006), and maternal BMI was calculated.

*Assessment of Infant Diet and Formula Intake*

Two multiple-pass 24-hour diet recalls were collected by trained researchers. The second diet recall was conducted to account for within-person variance in intake, which took place on a different day of the week, including weekends. The multiple-pass 24-hour diet recall followed the four stages used in the 2008/09 NZ Adult Nutrition Survey to assess intake from midnight to

midnight the day prior to the recall (MOH & University of Otago, 2011). Infant energy intakes from complementary food were calculated using a multiple-source method. The recalls included the intake of commercial infant formula. Trained research staff entered the 24-hour diet recall data into the nutrient analysis software: FoodWorks (version 10, Xyris Software), using the NZ Food Composition database FOODfiles™ 2018 version 01. Due to the software's lack of data on commercial infant formula and baby food products, the research team supplemented it with data on 104 commercial infant formula products and 385 baby food products available on the market at the time of the study.

*Breastfeeding Status*

The screening questionnaire assessed breastfeeding status using the question, "*Is baby still being breastfed?*", with the answer options of "*Yes*" or "*No*". In the analysis, feeding groups were defined as follows: breastfed infants received HM only and no commercial infant formula; formula-fed infants received commercial infant formula only and no HM; and mixed-fed infants received both HM and commercial infant formula. Infants were considered not to be breastfed in the analysis if they were not breastfed for any amount during participation.

*Maternal Dietary Supplement Use*

In the questionnaire, mothers who provided HM samples were asked, "*Have you taken any supplements while breastfeeding?*", with the response options of "*Yes*" or "*No*", followed by "*If yes, please state the brand (e.g., Elevit, Go Healthy etc.) and type (e.g., iron, iodine, vitamin B6, multivitamin etc.)"*.

*Human Milk Intake*

The deuterium oxide DTM technique (International Atomic Energy Agency, 2010) was used to assess HM intake in a sample of 157 infant-mother dyads over a 14-day period. On day 0 (once ensuring neither mother nor infant had consumed food or fluid 30 minutes prior), baseline saliva samples were collected from the mother and infant (described below in "Saliva Sampling Technique). The mother then received an accurately-measured oral dose of 30 g deuterium oxide ($^2H_2O$). Once consumed, the bottle was then refilled with 50 mL of bottled water, inverted, and consumed by the mother through a straw to ensure the full dose was consumed. Post-dose saliva samples were collected from the mother and infant on days 2–3, 7–8, and 13–14, following the same procedure described below in "Saliva Sampling Technique". All saliva samples were stored at -20ºC until analysis of deuterium enrichment by Fourier transform infrared spectrometry (International Atomic Energy Agency, 2010). HM volume was estimated using calculations and

methods described by the International Atomic Energy Agency (2010) and Liu et al. (2019). For infant-mother dyads who did not undergo HM intake measurement using the deuterium oxide DTM technique, infant HM intakes were estimated using the predictive equation developed by Haszard et al. (2024). The predictive equation used questionnaire data (infant age, maternal employment status, infant appetite changes, infant BMI, and maternal BMI, number of breastfeeds per day, and commercial infant formula consumption) and dietary assessment data (commercial infant formula and energy intake). These data were then used to calculate energy intake from HM, which was subsequently used to assess difference in energy intake between feeding groups.

*Saliva Sampling Technique*

One or two sterile cotton rolls were placed in the mother's mouth for approximately 2 minutes, or until they were sodden. The sodden cotton roll(s) were placed into a disposable 10 mL syringe, and saliva was extracted into a collection tube (at least 1 mL in both a sample and a spare tube). A researcher swabbed a cotton roll around the inside of the infant's mouth until it was sodden. Similarly, 0.5–1 mL of saliva was extracted into collection tubes (sample and spare).

*Human Milk Collection*

At the end of the 14-day deuterium oxide DTM assessment period, a full milk expression sample was collected from the mother. To standardise collection methods, mothers were asked not to breastfeed their baby or apply any creams or powders to the chosen collection breast for at least 2 hours before the sample was expressed. After washing their hands, mothers cleaned the nipple and surrounding area with distilled water using a gauze pad (to remove potential residue impacting accurate trace element analysis). Mothers expressed their sample with either a manual or electric breast pump until the breast was empty or 150 mL of milk had been collected into the provided bottle (maximum capacity of the collection bottle), in a dimly lit room (to prevent degradation of light-sensitive nutrients). Milk was required to be expressed before 12:00 pm to ensure uniformity among samples. Milk sample bottles were wrapped in tin foil (to prevent degradation of light-sensitive nutrients) and stored in the refrigerator until they were collected by researchers, aiming to do so within 24 hours. Breast pump parts that made contact with HM were cleaned with a cold rinse, a warm soap wash to remove HM, Milton's sterilisation step to kill off bacteria, fungi, and viruses and, lastly, acid wash baths (5% HCl and 10% $HNO_3$) to remove residual trace elements which could impact HM trace element analysis.

### 3.2.4 Analysis of Human Milk

To aliquot HM, samples were swirled in a laminar flow hood to ensure the HM was well mixed, with the lights turned off to protect light-sensitive nutrients. Using a 1 mL pipette, 6 x 1.5 mL tubes of HM were aliquoted for nutrient analysis (trace elements, minerals, macronutrients, fatty acids, and iodine). A further 2 mL was aliquoted for the analysis of vitamins, and 2 x 10 mL and 1 x 50 mL were reserved as spares, as described in **Table 3.1**. The tubes were then stored at -80 ºC until analysis.

**Table 3.1** Sampling tubes for individual human milk analysis

| Tube # | Nutrient | Tube size | Tube type |
|---|---|---|---|
| A. | Vitamins | 2 mL | Amber screw top tube |
| B. | Trace element - sample | 1.5 mL | Clear flip lid tube |
| C. | Trace element - spare | 1.5 mL | Clear flip lid tube |
| D. | Major elements | 1.5 mL | Clear flip lid tube |
| E. | Macronutrients | 1.5 mL | Clear flip lid tube |
| F. | Fatty acids | 1.5 mL | Clear flip lid tube |
| G. | Iodine | 1.5 mL | Clear flip lid tube |
| H. | Spare | 10 mL | Clear screw top tube |
| I. | Spare | 10 mL | Clear screw top tube |
| J. | Spare | 50 mL | Clear blue lid tube |

Funding constraints prevented individual-level analyses for certain nutrients, including macronutrients, vitamins, and fatty acids. Instead, these were analysed using pooled samples. For the pooled analysis, samples from 100 randomly selected participants (who provided sufficient sample for at least 35 mL to be pooled) were thawed. Exactly 35 mL from each individual HM sample was pipetted into a 3.5 L collection bottle, gently inverted, and split across smaller aliquots (50–100 mL) before analysis.

Results were converted from mass to volume using a factor of 1.03 g/mL to account for the density of HM (Institute of Medicine (US) Committee on Nutritional Status During Pregnancy and Lactation, 1991).

*Pooled Sample Nutrient Analyses*

Macronutrients were analysed by Hill Laboratories, Hamilton, NZ. Total fat content was determined using the Rosë-Gottlieb method (AOAC, 2023). Lactose was measured by gas-liquid chromatography (Jaynes & Asan, 1973). Nitrogen content was measured using the Kjeldahl method (Barbano et al., 1990; Licon, 2022). The protein concentration was calculated from total nitrogen using a factor of 6.25 (Lonnerdal, 2003). Energy concentration (kJ) was calculated using 16.7 kJ/g for protein, 37.7 kJ/g for fat, and 16.7 kJ/g for carbohydrates.

Vitamins were analysed by AssureQuality Ltd., Auckland, NZ. Folate was analysed with liquid chromatography in tandem with mass spectroscopy (LC-MS). Vitamin B12 was analysed using ultra-high performance liquid chromatography with ultraviolet detection (UHPLC-UV). Ascorbic acid (vitamin C) was analysed using liquid chromatography with ultraviolet detection (LC-UV). Vitamins A, D, E, and K were analysed using high-performance liquid chromatography (HPLC).

*Individual Nutrient Analyses*

Minerals (sodium, magnesium, manganese, phosphorus, potassium, and calcium) and trace elements (iron, copper, zinc, selenium, and iodine) were analysed by the Centre for Trace Element Analysis, Department of Chemistry, University of Otago, NZ, by inductively coupled plasma mass spectrophotometry (ICP-MS) as described by Daniels et al. (2019).

### 3.2.5 Statistical Analysis

SPSS (IBM) version 29 was used for statistical analysis, with a significance level set at $p \leq 0.05$. All variables were assessed for normality. Descriptive statistics were used to analyse the frequencies of participant characteristics and medians [$25^{th}$, $75^{th}$ percentile] of HM nutrient concentrations. Descriptive statistics were also used to examine the median [$25^{th}$, $75^{th}$ percentile] distribution of nutrients across maternal (age, BMI, ethnicity, parity, employment, household deprivation, supplement use) and infant (age, ethnicity, sex, preterm status, BMI z-score) characteristics. A descriptive approach was adopted, as a statistical model to rigorously test for significance was not developed for this study. This decision was made to avoid overinterpretation or the implication of significance without appropriate analytical justification. Pairwise comparisons of energy intake from infant milk sources (i.e. HM and commercial infant formula), complementary foods, and total intake across feeding groups (i.e. breastfed, formula-fed, and mixed-fed) were conducted using Mann-Whitney U tests.

## 3.3 Results

### 3.3.1 Participants

The flow of participants through the study is shown in **Figure 3.1**. The final number of infants included in the study was 625.

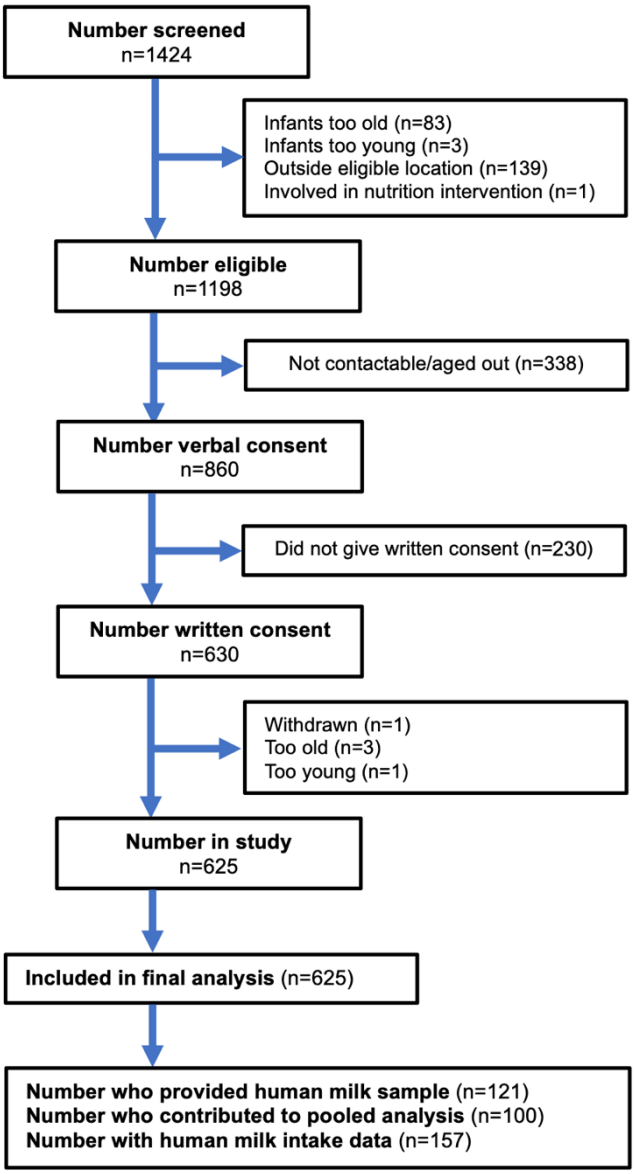



**Figure 3.1** First Foods New Zealand study screening, consent, and inclusion pathway

**Table 3.2** describes the characteristics of the entire FFNZ study participants (n=625) and the two subgroups used for analysis in this study. In total, 121 mothers provided a HM sample (hereafter called 'full HM subgroup'); due to funding constraints, a pooled analysis requiring 100 samples was undertaken for some nutrients (hereafter called 'pooled HM subgroup'). The average (mean

± SD) age of infants in the 'full HM subgroup' was 8.2 ± 0.8 months of age (range: 7.0–9.9 months) with a mean BMI z-score of 0.3. Infant and mother prioritised ethnicity distribution differed between the FFNZ cohort and the 'full HM subgroup', though ethnicity distribution remained similar for both subgroups. The mean age of the respondent (mothers) in the 'full HM subgroup' was 33 years (range: 21–44 years) with a mean BMI of 27.4 kg/m$^2$. Infant-mother dyads of high area-level socioeconomic deprivation (score 7–10) were the least represented (21.5%).

**Table 3.2** Demographic characteristics

| Characteristics | FFNZ cohort[a] | Full HM subgroup (individual analysis)[a] | Pooled HM subgroup (pooled analysis)[a] |
|---|---|---|---|
| | n = 625 | n = 121 | n = 100 |
| **Respondent** | | | |
| Age (years)[b], mean ± SD | 32.7 ± 4.9 | 33.3 ± 4.7 | 33.4 ± 4.6 |
| Respondent (mother) | 617 (98.7) | 121 (100.0) | 100 (100.0) |
| Anthropometry | | | |
| Weight (kg)[c], mean ± SD | 75.9 ± 18.1 | 75.0 ± 16.8 | 74.4 ± 16.6 |
| Height (cm)[d], mean ± SD | 165.2 ± 7.4 | 165.3 ± 6.2 | 165.2 ± 5.7 |
| BMI (kg/m$^2$)[e], mean ± SD | 27.8 ± 6.4 | 27.4 ± 5.8 | 27.3 ± 5.9 |
| Ethnicity[f] | | | |
| Māori | 85 (13.6) | 10 (8.3) | 8 (8.0) |
| Pacific | 32 (5.1) | 1 (0.8) | 1 (1.0) |
| European | 409 (65.4) | 101 (83.5) | 84 (84.0) |
| Asian | 84 (13.4) | 3 (2.5) | 2 (2.0) |
| Other | 15 (2.4) | 6 (5.0) | 5 (5.0) |
| Parity[g] | | | |
| One | 303 (48.6) | 42 (34.7) | 36 (36.0) |
| Two | 191 (30.6) | 50 (41.3) | 41 (41.0) |
| Three or more | 130 (20.8) | 29 (24.0) | 23 (23.0) |
| Employment | | | |
| Working | 207 (33.1) | 33 (27.3) | 26 (26.0) |
| Parental leave | 230 (36.8) | 57 (47.1) | 48 (48.0) |
| Unemployed | 188 (30.1) | 31 (25.6) | 26 (26.0) |
| Education | | | |
| School | 94 (15.0) | 10 (8.3) | 9 (9.0) |
| Polytech | 125 (20.0) | 36 (29.8) | 27 (27.0) |
| University | 405 (64.8) | 75 (62.0) | 64 (64.0) |
| Deprivation[h] | | | |
| 1–3 (low) | 180 (28.8) | 44 (36.4) | 37 (37.0) |
| 4–6 | 282 (45.1) | 51 (42.1) | 41 (41.0) |
| 7–10 (high) | 163 (26.1) | 26 (21.5) | 22 (22.0) |
| **Infant** | | | |
| Age (months), mean ± SD | 8.4 ± 0.8 | 8.2 ± 0.8 | 8.2 ± 0.8 |
| Ethnicity[f] | | | |
| Māori | 131 (21.0) | 21 (17.4) | 17 (17.0) |

| | | | |
|---|---|---|---|
| Pacific | 44 (7.0) | 1 (0.8) | 1 (1.0) |
| European | 344 (55.0) | 89 (73.6) | 74 (74.0) |
| Asian | 90 (14.4) | 5 (4.1) | 4 (4.0) |
| Other | 16 (2.6) | 5 (4.1) | 4 (4.0) |
| Sex (female) | 289 (46.2) | 56 (46.3) | 48 (48.0) |
| Preterm (yes)[i] | 46 (7.4) | 8 (6.6) | 7 (7.0) |
| Anthropometry | | | |
| Length[j], mean ± SD | 70.7 ± 3.1 | 70.1 ± 2.9 | 70.1 ± 3.0 |
| Weight[k], mean ± SD | 8.8 ± 1.1 | 8.7 ± 1.1 | 8.6 ± 1.1 |
| BMI z-score[l], mean ± SD | 0.3 ± 1.0 | 0.3 ± 1.0 | 0.3 ± 1.0 |
| Feeding group | | | |
| Breastfed | 300 (48.0) | 99 (81.8) | 85 (85) |
| Formula-fed | 220 (35.2) | 0 (0.0) | 0 (0.0) |
| Mixed-fed | 105 (16.8) | 22 (18.2) | 15 (15.0) |

[a] n (%), unless otherwise specified
[b] Missing data: FFNZ cohort n=2, full HM subgroup n=1
[c] Missing data: FFNZ cohort n=21, full HM subgroup n=1
[d] Missing data: FFNZ cohort n=18, full HM subgroup n=2
[e] Missing data: FFNZ cohort n=29, full HM subgroup n=3
[f] Prioritised ethnicity
[g] Missing data: FFNZ cohort n=1
[h] The New Zealand Deprivation Index 2018, ordinal scale ranges from one to ten. One represented areas with the least deprived scores, and ten represented areas with the most deprived scores, 1–2 (low), 4–6, or 7–10 (high)
[i] Born at 37 weeks' gestation or older
[j] Missing data: FFNZ cohort n=7, full HM subgroup n=2, pooled HM subgroup n=1
[k] Missing data: FFNZ cohort n=9, full HM subgroup n=1, pooled HM subgroup n=1
[l] Missing data: FFNZ cohort n=16, full HM subgroup n=3, pooled HM subgroup n=2

*Supplement Use by Mothers*

Of the 'full HM subgroup', 73 mothers (60.3%) reported currently taking a dietary supplement. For six mothers (5.0%), supplement data were missing. The reported frequency of use varied widely, ranging from twice daily to once a month, with some women describing their intake as "irregular." The most commonly reported supplements were multivitamins and/or multiminerals (n=33, 27.4%), with 16 (13.2%) of these containing varying doses of iodine (150–299 mcg), followed by iodine/potassium iodate (n=28, 23.1%). Overall, 44 (36.3%) mothers were taking iodine supplements; however, not all mothers specified the brand or dose, and frequency of intake varied widely, and therefore, it cannot be assumed that all achieved the MOH's recommendation of 150 µg per day. Iron (n=17, 14.1%), vitamin C (n=13, 10.7%), herbal (e.g., fenugreek, St John's wort, garlic and echinacea) (n=12, 9.9%), vitamin D (n=9, 7.4%), magnesium (n=9, 7.4%), probiotics (n=8, 6.6%), fish oil (n=5, 4.1%) and zinc (n=5, 4.1%) supplements were also reported. There were also 12 other less-frequently reported dietary supplements (e.g., calcium, vitamin A, and vitamin K). Among supplement users, 52.1% reported taking one, 15.1% two, 13.7% three, and 19.2% four or more.

### 3.3.2 Macronutrient, Vitamin, Mineral, and Trace Element Concentrations in Human Milk at 7–10 Months Postpartum

Average concentrations of HM macronutrients, vitamins, minerals, and trace elements, alongside reported ranges for each nutrient from previous literature, are presented in **Table 3.3**. As macronutrients (fat, protein, lactose) and vitamins (vitamin A, folate, vitamin C, vitamin E) could only be analysed from a pooled sample, results are presented as average composition estimates rather than statistical means. Of the 18 nutrients analysed, 13 were within previously reported literature ranges. Vitamin B12, D, K, and manganese were not detectable under their respective detection limits of <0.02 µg/100 g, <20 IU/100 g, <3.00 µg/100 g, and <0.05 µg/100 g. One participant's zinc concentration was below the detection limit of 0.15 mg/kg, and 14 participants' iron concentrations were below the detection limit of 0.07 mg/kg; these were recorded as the values of the respective lowest limits.

**Table 3.3** Average macronutrient, vitamin, mineral, and trace element concentrations in human milk at 7–10 months postpartum

| **Pooled analysis (n=100)** | | |
|---|---|---|
| ***Macronutrients*** | **Average**[ab] | **Literature range**[c] |
| Energy (kJ/100 mL) | 271.9 | 242.7 – 297.1 |
| Fat (g/100 mL) | 3.80 | 2.46 – 5.16 |
| Protein (g/100 mL) | 1.09 | 0.80 – 1.55 |
| Lactose (g/100 mL) | 6.59 | 5.88 – 7.82 |
| ***Vitamins*** | | |
| Vitamin A (µg/100 mL) | 42.23 | 10.31 – 58.71 |
| Folate (µg/100 mL) | 5.15 | 1.90 – 5.68 |
| Vitamin C (mg/100 mL) | 2.39 | 3.70[d] |
| Vitamin E (mg/100 mL) | 0.27 | 0.23[d] |
| **Individual analysis (n=121)** | | |
| ***Minerals*** | **Median** [25$^{th}$, 75$^{th}$][a] | **Literature range**[c] |
| Na (mg/L) | 91.57 [67.88, 114.3] | 76.00 – 252.0 |
| Mg (mg/L) | 34.30 [30.44, 39.60] | 22.00 – 33.60 |
| P (mg/L) | 131.9 [116.4, 148.3] | 117.0 – 142.2 |
| K (mg/L) | 439.8 [404.3, 492.9] | 330.0 – 472.0 |
| Ca (mg/L) | 238.1 [219.9, 259.8] | 14.00 – 488.9 |
| ***Trace elements*** | | |
| Fe (µg/L) | 133.9 [98.36, 192.6][e] | 180.0 – 850.0 |
| Cu (µg/L) | 146.3 [110.2, 209.1] | 119.0 – 300.0 |
| Se (µg/L) | 10.21 [8.99, 12.26] | 11.35 – 22.49 |
| Zn (µg/L) | 554.1 [374.9, 872.9][f] | 340.0 – 2300 |
| I (µg/L) | 62.82 [42.27, 99.09] | 35.00 – 360.0 |

[a] Values converted from mass to volume using a factor of 1.03 to account for the density of human milk

[b] Reported by the laboratory as an average of measured samples

[c] Literature values 6–12 months postpartum, excluding exclusively breastfed infants

[d] Reported as a single value as only one study provided a concentration for 6-12 months postpartum
[e] n=14 concentrations retained at the detection limit/minimum value of 72.1 µg/L (converted from 0.07 mg/kg)
[f] n=1 concentration retained at the detection limit/minimum value of 154.5 µg/L (converted from 0.15 mg/kg)

### 3.3.3 Comparisons of Selected Human Milk Minerals and Trace Elements Across Infant and Maternal Characteristics

To explore potential patterns in HM mineral and trace element composition, descriptive analyses were conducted according to maternal and infant characteristics. **Figure 3.2** presents comparisons between maternal and infant characteristics and sodium and phosphorus HM concentrations, **Figure 3.3** shows comparisons for potassium and calcium, and **Figure 3.4** presents comparisons for copper, zinc, and iodine. Comparisons between maternal and infant characteristics for magnesium, iron, and selenium were included in **Appendices T–V**. Nutrients were stratified by maternal factors, including age, BMI, ethnicity, parity, employment status, and NZ deprivation score, as well as by infant factors such as age, infant ethnicity, sex, term status, and BMI z-score. No marked differences in mineral and trace element concentrations were observed across the examined characteristics.

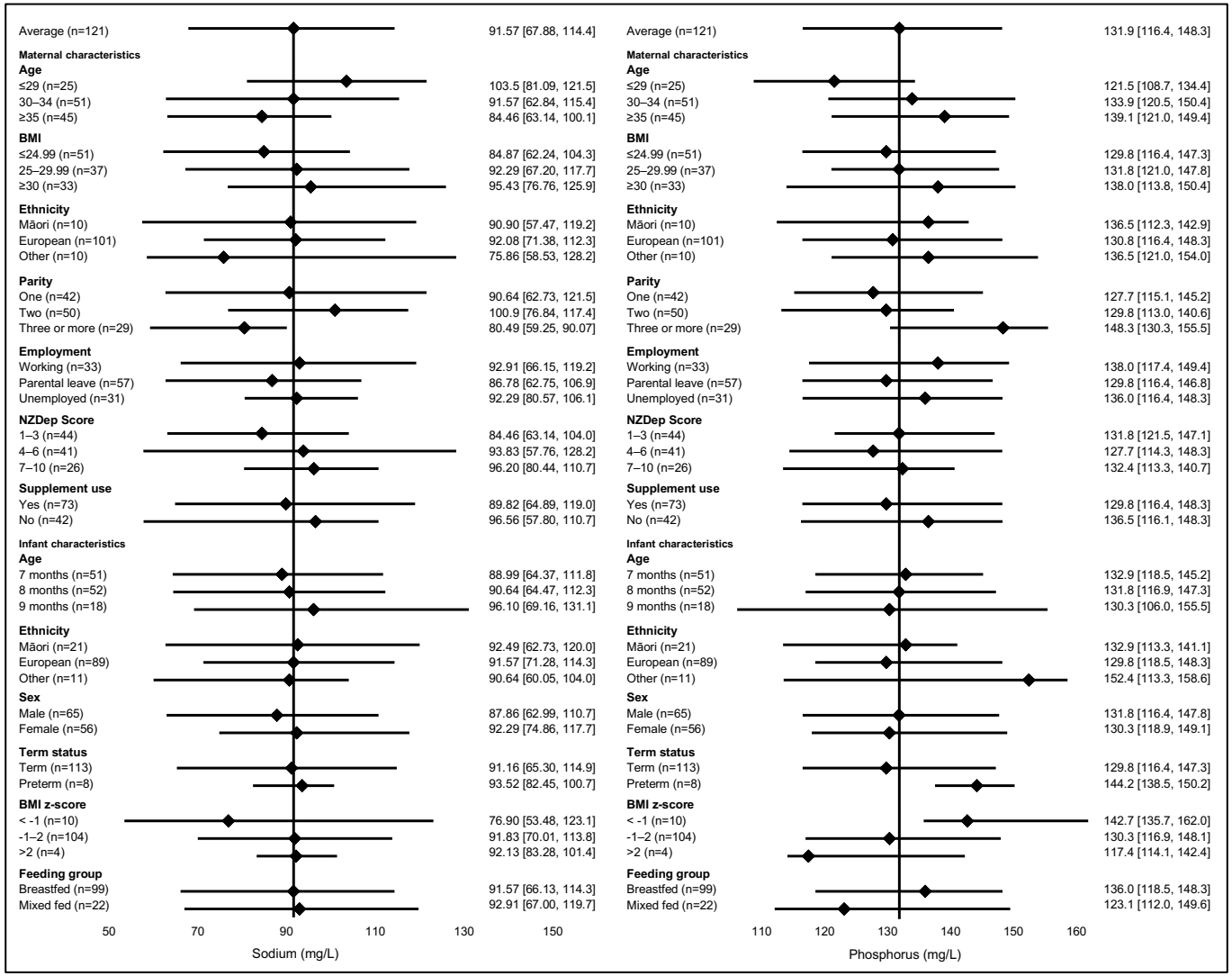



**Figure 3.2** Distribution of human milk sodium and phosphorus concentrations according to maternal and infant characteristics at 7–10 months postpartum[a]

Values are presented as median [25$^{th}$, 75$^{th}$ percentile]. Left: sodium, right: phosphorus

[a] 'Other' ethnicities includes Asian (maternal n=2; infant n=4), Pacific (maternal n=1; infant n=1) and other (maternal n=5; infant n=4)

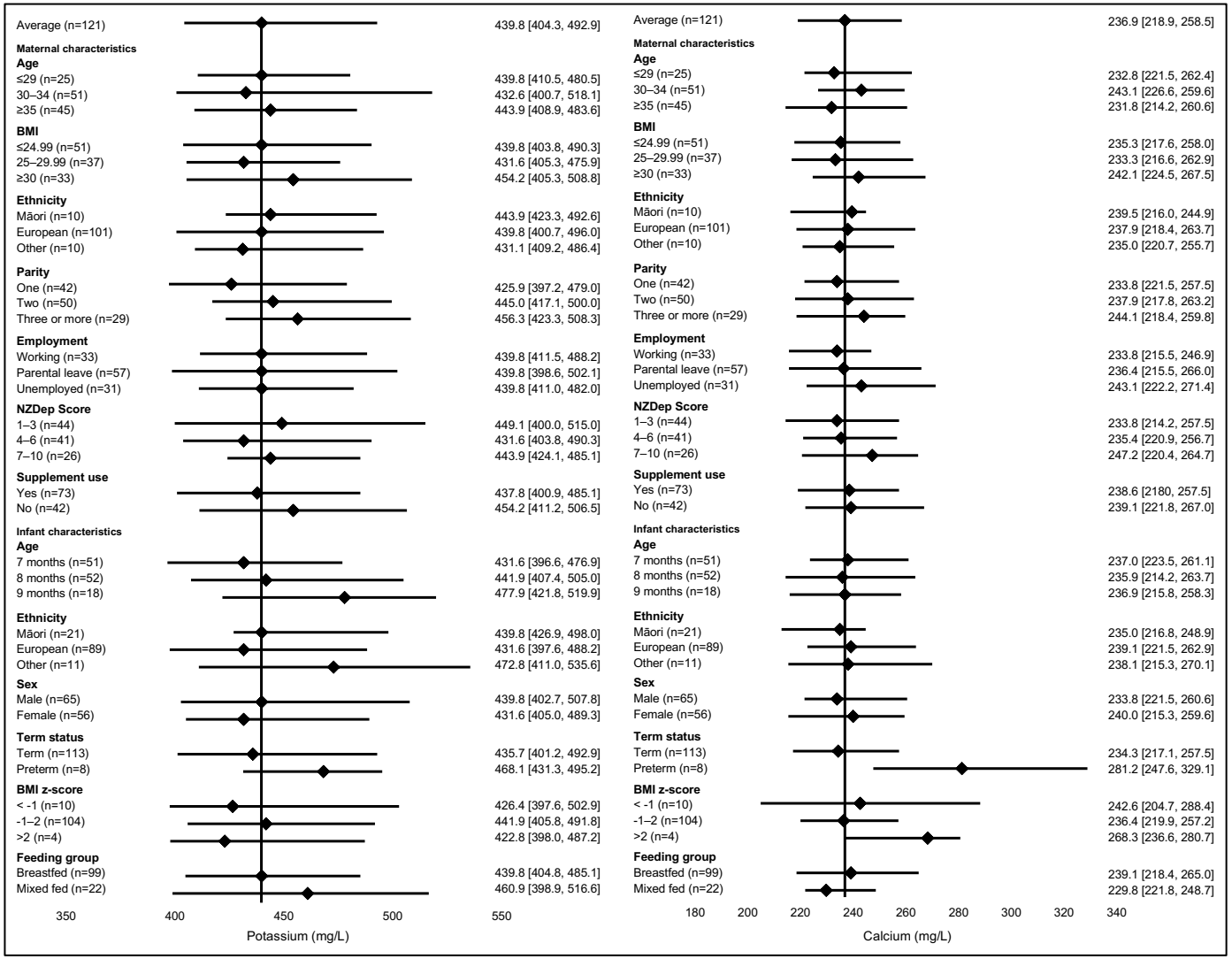



**Figure 3.3** Distribution of human milk potassium and calcium concentrations according to maternal and infant characteristics at 7–10 months postpartum[a]

Values are presented as median [25th, 75th percentile]. Left: potassium, right: calcium.

[a] 'Other' ethnicities includes Asian (maternal n=2; infant n=4), Pacific (maternal n=1; infant n=1) and other (maternal n=5; infant n=4)

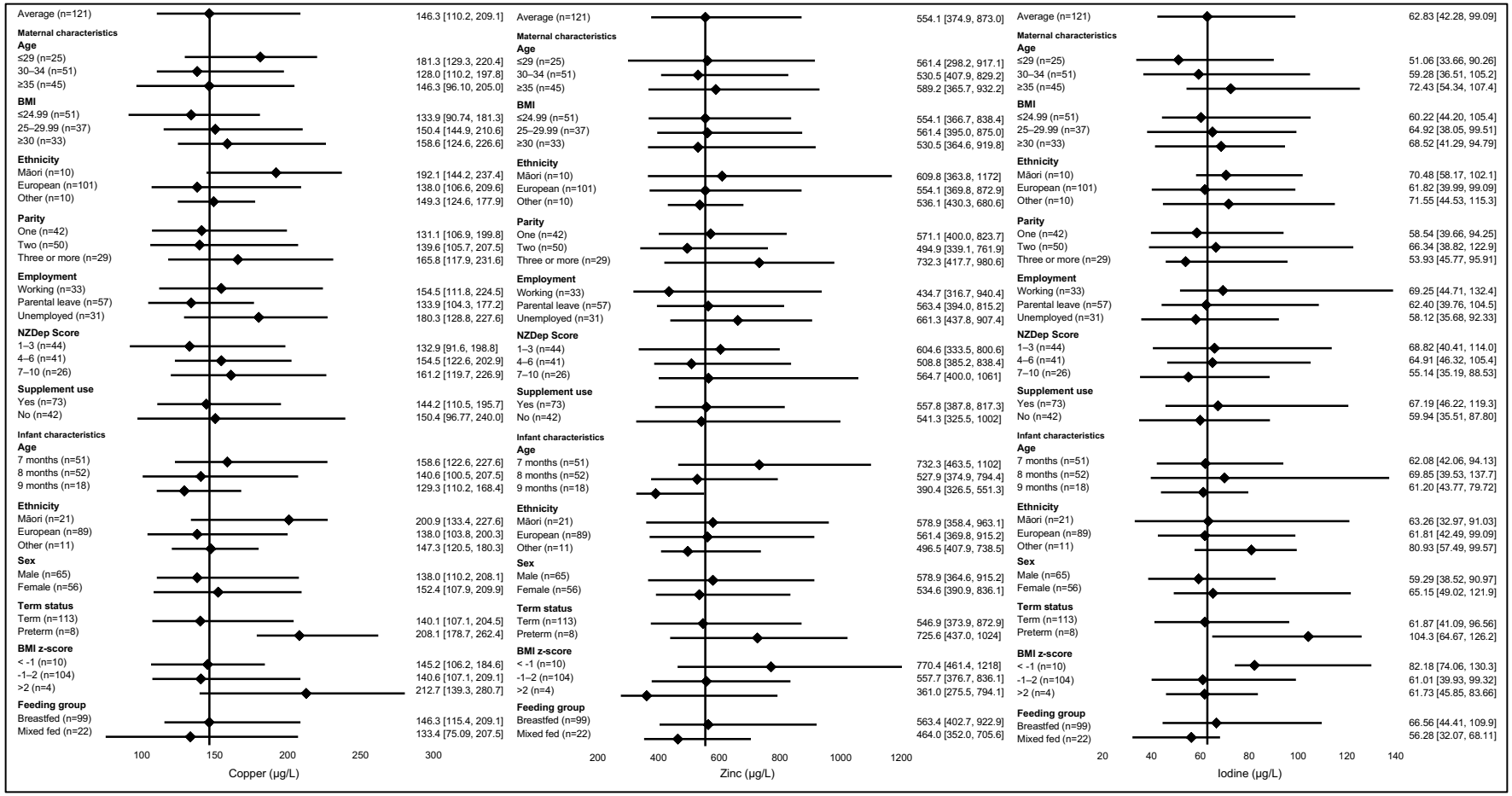



**Figure 3.4** Distribution of human milk copper, zinc, and iodine concentrations according to maternal and infant characteristics at 7–10 months postpartum[a]

Values are presented as median [25th, 75th percentile]. Left: copper, middle: zinc, right: iodine.

[a] 'Other' ethnicities includes Asian (maternal n=2; infant n=4), Pacific (maternal n=1; infant n=1) and other (maternal n=5; infant n=4)

### 3.3.4 Energy Intake from Milk and Complementary Food Sources, and Total Energy Intake of Infants Aged 7–10 Months

**Table 3.4** presents the mean ± SD daily energy intakes (kJ/day) from infant milk sources (HM and/or commercial infant formula), complementary food, and combined intake by feeding group (i.e. breastfed, formula-fed, mixed-fed). Unadjusted and adjusted pairwise mean differences between feeding groups with 95% confidence intervals are also reported. Outliers were excluded from the analysis to reduce skewness, including two extreme values (>4000 kJ/day) for complementary food and one (>7000 kJ/day) for total energy intake, as these values were substantially beyond the typical range observed in the sample. All infants were consuming complementary foods, as reported in diet recall data presented elsewhere (Brown, 2023). For infants whose HM intake was not directly measured using the deuterium oxide DTM technique, intake was estimated using predictive equations developed by Haszard et al. (2024). These equations were derived from HM intake data for the same cohort of 157 mother-infant dyads (aged 7–10 months) included in the current study, whose intake data had been measured by the deuterium oxide DTM technique. These were used to calculate energy intake from HM, as presented within energy intake from infant milk sources in **Table 3.4** and **Table 3.5**.

**Table 3.4** Daily mean energy intake (kJ/day) from infant milk sources, complementary food, and total energy intake by feeding group with unadjusted and adjusted mean differences

| | **Energy intake from infant milk sources (kJ/day)** | **Energy intake from complementary food (kJ/day)** | **Total energy intake (kJ/day)** |
|---|---|---|---|
| | | Mean ± SD | |
| **Breastfed (n=300)** | 2068 ± 561.2 | 1170 ± 741.1 | 3239 ± 524.5 |
| **Formula-fed (n=217)**[a] | 2251 ± 601.2 | 1283 ± 661.8 | 3534 ± 609.0 |
| **Mixed-fed (n=105)** | 2089 ± 637.2 | 1448 ± 728.8 | 3538 ± 559.0 |
| | | Mean difference (95% CI) | |
| **BF vs FF** | **-183.1 (55.75, 310.4)** | -113.0 (-41.15, 267.1) | **-295.1 (174.0, 416.3)** |
| **BF vs MF** | -21.71 (-134.5, 177.9) | **-277.9 (88.92, 466.9)** | **-298.7 (150.1, 447.3)** |
| **FF vs MF** | 161.4 (-3.63, 326.4) | -165.0 (-34.71, 364.7) | 3.53 (-153.5, 160.5) |
| | | Adjusted mean difference (95% CI)[b] | |
| **BF vs FF** | **-199.9 (83.17, 316.6)** | -81.84 (-52.24, 215.9) | **-280.8 (163.5, 398.2)** |
| **BF vs MF** | -63.23 (-80.25, 206.7) | **-205.4 (40.57, 370.2)** | **-267.6 (123.4, 411.9)** |
| **FF vs MF** | 136.7 (-14.57, 287.9) | -123.5 (-50.20, 297.2) | 13.21 (-138.8, 165.2) |

Abbreviations: BF: breastfed; FF: formula-fed; MF: mixed-fed (human milk and commercial infant formula)
Bolded values indicate significance ($p < 0.05$)
[a] Outliers removed (n=2) >4000 kJ/day from complementary food and outlier removed (n=1) >7000 kJ/day from total energy intake
[b] Adjusted for infant sex and age

In **Figure 3.4,** energy intake (mean ± SD) from infant milk sources and complementary foods by feeding group is presented. The mean (95% CI) HM intake of breastfed infants is 806 mL/d (780, 832) with breakdown by age: 895 mL/d (856, 933) at 7 months, 803 mL/d (765, 843) at 8 months, and 644 mL/d (591, 696) at 9 months of age. Full infant milk intake data are published elsewhere by Daniels et al., 2025 (under review, *European Journal of Nutrition*).

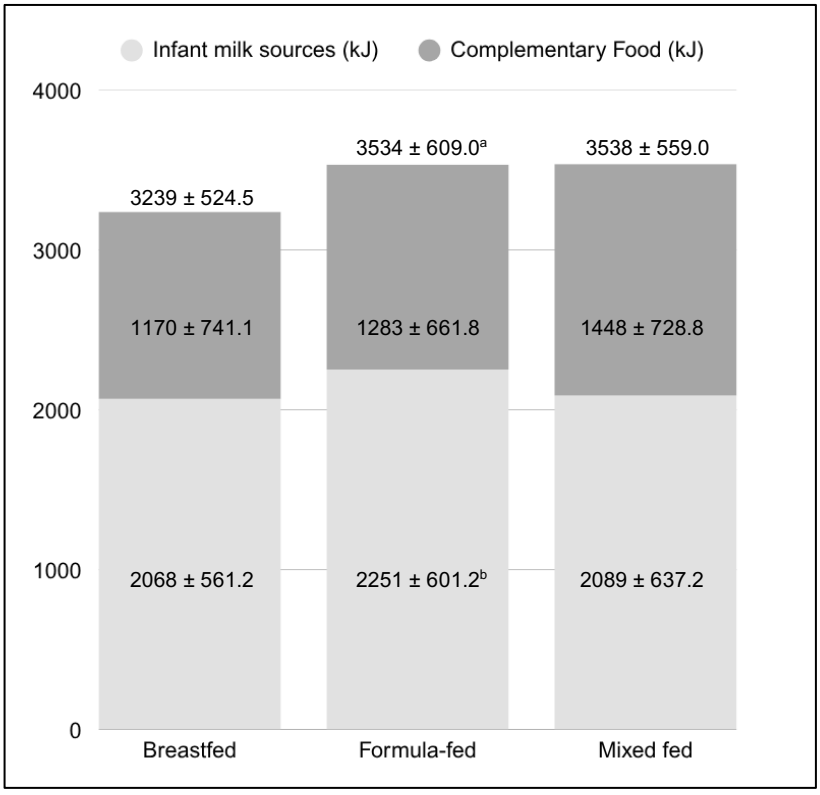



**Figure 3.4.** Mean ± SD daily energy intake from infant milk sources and complementary food by feeding group
[a] Outlier removed (n=1) >7000 kJ/day
[b] Outliers removed (n=2) >4000 kJ/day

In **Table 3.5,** a cross-sectional descriptive analysis of (mean ± SD) energy intakes from infant milk sources, complementary food, and total energy intake stratified by infant age and feeding group is presented. As expected during the transition into established complementary feeding, energy intake from infant milk sources declined, while energy intake from complementary foods increased. The mean total energy intakes exceed the recommendations at each month within all feeding groups.

**Table 3.5** Cross-sectional analyses of (mean ± SD) energy intake (kJ/day) from infant milk sources, complementary food, total energy intake, and estimated energy requirements (EER), stratified by infant age and feeding group

| | | **All groups** | | **Breastfed** | | **Formula-fed** | | **Mixed-fed** | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| | Energy EER (kJ) (male/female) | Energy total (kJ/day) | | | | | | | |
| **7 months (n=241, 120, 82, 38)**[a] | 2800/2500 | 3306 | 588 | 3124 | 432 | 3506 | 708 | 3442 | 561 |
| **8 months (n=231, 116, 73, 42)** | 3000/2700 | 3382 | 583 | 3268 | 530 | 3481 | 604 | 3522 | 631 |
| **9 months (n=153, 64, 64, 25)** | 3100/2800 | 3598 | 630 | 3401 | 619 | 3766 | 667 | 3673 | 395 |
| **Total (n=625, 300, 219, 105)** | | 3405 | 606 | 3239 | 524 | 3573 | 672 | 3529 | 559 |
| | | Energy from infant milk sources (kJ/day) | | | | | | | |
| **7 months (n=241, 120, 83, 38)** | | 2370 | 551 | 2293 | 493 | 2512 | 568 | 2304 | 636 |
| **8 months (n=231, 116, 73, 42)** | | 2105 | 551 | 2060 | 530 | 2137 | 537 | 2173 | 626 |
| **9 months (n=153, 64, 64, 25)** | | 1785 | 578 | 1650 | 500 | 1928 | 635 | 1766 | 549 |
| **Total (n=625, 300, 220, 105)** | | 2128 | 601 | 2066 | 561 | 2217 | 625 | 2123 | 642 |
| | | Energy from complementary food (kJ/day) | | | | | | | |
| **7 months (n=241, 120, 83, 38)** | | 936 | 538 | 829 | 478 | 969 | 493 | 1139 | 722 |
| **8 months (n=231, 116, 72, 42)**[b] | | 1245 | 637 | 1208 | 692 | 1244 | 545 | 1349 | 625 |
| **9 months (n=153, 64, 64, 25)** | | 1813 | 757 | 1751 | 854 | 1838 | 687 | 1907 | 679 |
| **Total (n=625, 300, 219, 105)** | | 1261 | 720 | 1172 | 740 | 1314 | 673 | 1406 | 730 |

n values are presented as total participants at each month, followed by the number of breastfed, formula-fed, and mixed-fed infants.

[a] Outlier removed (n=1) >7000 kJ/day

[b] Outliers removed (n=2) >4000 kJ/day

## 3.4 Discussion

This study aimed to determine the nutrient composition of HM between 7 and 10 months postpartum and its contribution to infant energy intake. Participants were predominantly of European ethnicity, from low- to medium socioeconomic deprivation backgrounds, with many holding tertiary qualifications. Overall, the concentrations of macronutrients, vitamins, minerals, and trace elements were broadly consistent with values previously reported in the literature, although the concentrations of iron and selenium were lower, and iodine concentrations were below those proposed to indicate adequate iodine status. No significant relationships were observed between mineral or trace element concentrations and maternal or infant characteristics. Importantly, this study is among the first to include mixed-fed infants when assessing energy intake in the second half of infancy, demonstrating that their total energy intake and intake from complementary foods differ from those who are breastfed but not those who are formula-fed. Across all feeding groups, the mean total energy intake exceeded the estimated energy requirements. These findings suggest that HM in NZ may be deficient in iodine, iron, and selenium, while infants, on average, may have excessive energy intakes, highlighting areas where further research is warranted.

### 3.4.1 The Macronutrient, Vitamin, Mineral, and Trace Element Composition of Human Milk at 7–10 Months Postpartum

In the NZ context, this study provides novel results on the macronutrient, vitamin, mineral, and trace element composition of HM between 7 and 10 months postpartum. While the results are broadly consistent with international literature, some differences were observed, which are discussed below.

The average fat concentration in this study (3.80 g/100 mL) is comparable to that reported for an Australian cohort by Mitoulas et al. (2002) (3.72 g/100 mL), despite differences in the analytical methods used. The present study employed the Rosë-Gottlieb method, whereas Mitoulas et al. (2002) used colorimetric spectrophotometry. Research by Nakamori et al. (2009) and Yamawaki et al. (2005) also employed the Rosë-Gottlieb method and reported moderately lower mean fat concentrations (3.08 g/100 mL and 3.17 g/100 mL, respectively). Yamawaki et al. (2005) collected 50 mL of HM at an intermediate time during suckling (Kojima et al., 2004), without standardising the time of collection, whereas Nakamori et al. (2009) collected 20 mL of HM in the morning, although they did not report whether this was in relation to a feed. Fat content is known to be higher at the end of a feed and throughout the day, which can introduce variability in reported

values (Kent et al., 2006; Leghi et al., 2020). To address this, samples in the present study were collected either until the breast was fully emptied or until 150 mL had been expressed, and before 12:00 pm, thereby ensuring a representative and uniform estimate of fat concentration. Therefore, the current study's results likely captured higher-fat HM compared to Nakamori et al. (2009) and Yamawaki et al. (2005), and a concentration that is more representative of HM as a whole. For reliable comparisons, future studies should adopt uniform standardised collection protocols, such as those employed in the present study, to minimise discrepancies caused by sampling differences and ensure that results are comparable.

Protein concentration in HM is known to be lower beyond 6 months postpartum when compared to earlier in lactation (Lonnerdal et al., 2017; Rajalakshmi & Srikantia, 1980). The average protein concentration in the present study (1.09 g/100 mL) was higher than the mean concentration reported by Nakamori et al. (2009) (0.99 g/100 mL) but comparable to that of Yamawaki et al. (2005) (1.04 g/100 mL), both of whom also used the Kjeldahl method to assess nitrogen content and excluded non-protein nitrogen (NPN) to determine true protein concentration. However, the two aforementioned studies used a factor of 6.38 to convert total nitrogen to true protein, whereas the current study used a factor of 6.25, which is more appropriate for HM (Lonnerdal, 2003). The higher factor of 6.38 is intended for calculation from dairy milk, which contains less NPN than HM (Lonnerdal, 2003). Interestingly, the average protein concentration of this study resembles those reported by Nommsen et al. (1991) at 6 and 9 months postpartum (1.14 g/100 mL and 1.16 g/100 mL, respectively), who noted that their use of bovine serum albumin as the standard in the Modified Lowry Assay may have resulted in slightly elevated protein values due to differences in amino acid composition, and who also did not report the exclusion of NPN. Given these methodological factors, it is likely that their genuine protein concentrations may have matched or been lower than observed in the current study, though the extent of the elevation was not reported on.

The average lactose concentration in the present study (6.59 g/100 mL) aligns with the findings of Gridneva et al. (2019) (6.53 g/100 mL at 9 months and 6.69 g/100 mL at 12 months) in Australian mothers and Yamawaki et al. (2005) (6.46 g/100 mL at 6–12 months) in Japanese mothers. However, it is slightly lower than the values reported by Chang et al. (2015), Dewey et al. (1984), Nommsen et al. (1991), and Wack et al. (1997), who all reported mean concentrations of 7.00 g/100 mL or higher. Although this study does not report lactose concentrations on a month-by-month basis, it is unlikely that these values vary substantially across this period, as lactose is

considered one of the most stable constituents of HM throughout lactation (Chang et al., 2015; Picciano, 2001).

The energy concentration observed in this study (271.9 kJ/100 mL) is comparable to values reported in the literature previously. It is slightly higher than those reported by Nakamori et al. (2009) (255.6 kJ/100 mL) and Yamawaki et al. (2005) (261.9 kJ/100 mL), both of whom indicated they also employed the Atwater factor system to calculate energy concentration. It is difficult to compare this study's findings with those that used energy calculations according to Garza et al. (1985), such as Mitoulas et al. (2002) and Nommsen et al. (1991), as the total coefficients used in Garza's method result in higher energy estimates than those in the Atwater system.

Of the four vitamins analysed above the detection limits, only vitamin A had a moderately established body of literature reporting concentrations in the second half of infancy, primarily in relation to fat concentrations. The median vitamin A concentration (42.23 µg/100 mL) falls within the range reported in the literature (10.31–58.71 µg/100 mL), with near-unanimous use of HPLC for analysis, consistent with the current study's methodology. For vitamin E, the only study that reported concentrations in the second half of infancy was Kim et al. (2017), which demonstrated a slightly lower concentration (0.23 mg/100 mL) at 7–11 months postpartum compared to the findings presented here (0.27 mg/100 mL). The limited data on vitamin E concentrations during this period constrain the ability to assess whether the concentration observed in this study falls within a typical range and whether it is comparable to concentrations worldwide. To the best of our knowledge, only Sakurai et al. (2005) have reported vitamin C concentrations in the second half of infancy, with a mean of 3.70 mg/100 mL, greater than the 2.39 mg/100 mL measured in the current study. Therefore, the present study's findings contribute to a broader understanding of the vitamin composition of HM during the establishment of complementary feeding.

The current study's mineral and trace element analyses revealed several nutrients to be outside of previously reported values. Magnesium concentrations (34.30 mg/L) were higher than literature values (22.00–33.60 mg/L). However, a study by Javad et al. (2018), which also employed ICP-MS for magnesium analysis, currently regarded as the best method for mineral and trace element analysis because of its multi-element capability, high sensitivity, and low detection limits (Bolann et al., 2007; Caroli, 2006; Dubascoux et al., 2018), reported comparable concentrations of 33.60 mg/L at 6–7 months and 32.00 mg/L at 12 months postpartum.

Notably, iron concentrations (133.9 µg/L) were lower than studies previously reported in the literature (180.0–850.0 µg/L). Previous studies, including those by Domellof et al. (2004) and Shashiraj et al. (2006), reported no association between maternal iron status and HM iron concentrations in the second half of infancy, suggesting maternal status is unlikely to explain the low concentrations found in this study. When examining analytical methods, the only other study reporting iron concentrations in the second half of infancy that also employed ICP-MS by Javad et al. (2018), reported notably higher concentrations of 420 µg/L at 6–7 months and 380 µg/L at 12 months postpartum. Given that infant iron stores are typically depleted by 6 months and dietary iron becomes critical (Eussen et al., 2015), these findings underscore the importance of introducing iron-rich foods between 7 and 10 months of age, supporting the need for the current MOH's (2021) "Health Eating Guidelines for NZ Babies and Toddlers (0–2 years old)" recommendation of offering iron-rich foods as first foods. The low HM iron concentrations observed in this study may help to explain some of the findings of McLean et al. (2024), where it was reported that only 77.8% of NZ infants aged 7–10 months were iron sufficient, with 9.1% iron-depleted, 10.4% experiencing early functional iron deficiency, and 2.8% with iron deficiency anaemia.

Similarly, the selenium concentration (10.21 µg/L) was below the ranges reported in the literature (11.35–22.49 µg/L). This may be partially explained by NZ's naturally low soil selenium content, particularly in the South Island (Robinson, 1989; Thomson, 2004), where all HM samples were collected. As the selenium content in food is dependent on soil selenium levels (Hartikainen, 2005), mothers living in NZ (particularly in the South Island) may have lower dietary intake. Given that selenium concentrations in HM are influenced by maternal intake (Kumpulainen, 1989; Roekens et al., 1985), this likely contributes to the low values observed in this study. In NZ, lactating mothers and their infants have been reported to have suboptimal selenium intake and status in the second half of infancy (Jin et al., 2020; McLachlan et al., 2004). Daniels et al. (2023a) reported that a substantial proportion of NZ infants at 12 months have selenium intakes below recommended levels, with HM identified as the primary contributor; however, selenium concentrations in HM were not quantified in their study and were instead assumed to be 20.6 µg/L, approximately half the concentration observed in the current study. A recent meta-analysis by Shalom et al. (2024) reported a mean (SD) selenium concentration of 11.35 µg/L (3.30) at 6–12 months postpartum. However, this estimate was only based on 17 individual samples from three studies, highlighting the scarcity of available data. The only other NZ study that has analysed HM selenium concentrations in late infancy reported a median concentration of 12 µg/L, slightly

higher than the values in the present study (Jin et al., 2020). However, their work was conducted in Palmerston North, in the North Island, whereas this study's samples were collected in Dunedin, in the South Island, a region known to have poorer selenium concentrations. Currently, the limited evidence in the literature makes it difficult to determine whether the concentrations in the current study fall within the true physiological range for this stage of lactation.

In NZ, the MOH (2021) recommends that women take a daily iodine supplement of 150 µg from the beginning of pregnancy through to the end of lactation, in response to the low iodine content of locally produced foods. Despite this recommendation, only around one-third of the mothers in this study reported using a supplement that provided sufficient iodine, and among these women, intake did not always align with recommended frequency. This suggests that, despite existing public health guidelines, both mothers and infants may remain at risk of inadequate iodine status. This study's finding that 36.3% of mothers were taking iodine supplements differs from that of Jin et al. (2022), who reported that only 6% of NZ lactating mothers at 12 months postpartum consumed iodine supplements. However, the 6% reported by Jin et al. (2022) refers specifically to an intake of at least 150 µg per day, in line with the NZ MOH's recommendations, whereas the present study's data did not provide sufficient detail to determine exact dosages or frequencies. Jin et al. (2022) also documented a lower median HM iodine concentration of 35.0 µg/L at 12 months postpartum, where breastfed infants met iodine sufficiency requirements, whereas their mothers did not. In the present study, the median HM iodine concentration (62.8 µg/L) is higher than the median concentration reported by Andersson et al. (2010) in Switzerland (43.6 µg/L), yet lower than the median concentration reported in the Yongjin Country, Gansu Province, China by Wang et al. (2009) (122 µg/L). These findings place this study's participants between populations with known deficiency and those with sufficiency. For infants under two years of age, HM iodine concentration is considered a reliable biomarker of iodine status in both the mother and infant (Liu et al., 2022), though no threshold value has been set by the WHO, such as that of urinary iodine concentration (WHO et al., 2007). One study has suggested that an HM iodine concentration of 75 µg/L may represent sufficient maternal intake (Azizi & Smyth, 2009), while another review has proposed an adequacy range of 100–200 µg/L (Andersson & Braegger, 2022). However, these recommendations do not clarify whether they remain applicable beyond 6 months postpartum. Nevertheless, if they are applicable, the findings of this study suggest that neither mothers nor their infants may be achieving an adequate iodine status. Further research is therefore needed to establish appropriate reference values for HM iodine concentrations beyond 6 months postpartum.

Establishing this understanding of HM nutrient composition in a NZ context is particularly important, as it helps future research to evaluate the nutrient intakes of breastfed infants during the introduction of complementary feeding and identify potential dietary deficiencies when complementary foods are first introduced alongside HM. The HM concentrations of this study, which lie outside of previously reported literature values, may reflect genuine differences in nutrient composition, or alternatively, they may highlight the limited attention given to research in this specific postpartum period. It is also possible that observed differences are unique to the NZ population, given the overall scarcity of local data, particularly within this postpartum period. To our knowledge, this dataset, which includes a detailed range of macronutrients, vitamins, minerals, and trace elements, is the first of its kind in NZ at 7–10 months postpartum.

### 3.4.2 Comparisons of Selected Human Milk Minerals and Trace Elements Across Infant and Maternal Characteristics

Overall, no substantial differences were observed in the concentrations of the minerals and trace elements in relation to maternal and infant characteristics. This likely reflects the relatively small sizes and uneven representation of certain subgroups in the analysis. While the present work employed a descriptive approach, it nonetheless offers valuable insights for future research, as evidence beyond 6 months postpartum remains scarce.

*Maternal Factors*

In the present study, there appeared to be a trend towards median HM sodium concentrations being lower with increasing maternal age, whereas phosphorus concentrations trended higher. At a similar lactation stage (7–12 months postpartum), Rajalakshmi and Srikantia (1980) reported no relationship between HM copper, magnesium, or zinc concentrations and maternal age or nutritional status, nor any differences between urban and rural groups. Similarly, Javad et al. (2018) reported no correlations between HM calcium, copper, iron, magnesium, potassium, sodium, or zinc and maternal socioeconomic status, age, occupation, or smoking by family members across 1–12 months postpartum. Earlier in lactation (<4 months), a study by Butte et al. (1987) also reported no relationship between maternal characteristics and HM minerals. In the present study, the absence of a clear relationship between maternal supplementation and HM minerals or trace element concentration in the analysis, even for nutrients known to be influenced by supplementation such as iodine (Dror & Allen, 2018a; Jin et al., 2021), was likely due to the heterogeneity in the types, doses, and frequencies of supplements taken among this study's participants.

*Infant Factors*

Zinc concentrations appeared to decline with increasing infant age and time postpartum, consistent with previous literature (Bilston-John et al., 2021; Casey et al., 1989; Domellof et al., 2004; Han et al., 2022; Han et al., 2011; Krebs et al., 1985; Krebs et al., 1995; Simmer et al., 1990). Therefore, this well-established trend may have confounded the results in the descriptive analysis of zinc in this study. Copper concentrations also appeared to decrease over time, aligning with Han et al. (2022), Kim et al. (2017), and Javad et al. (2018), while potassium concentrations appeared to increase slightly, contrasting with studies reporting stable concentrations (Han et al., 2022; Nagra, 1989; Wack et al., 1997). Within the narrow 7–10 months postpartum window, changes may be minimal.

Phosphorus and zinc concentrations appeared to be inversely related to infant BMI z-scores; however, interpretation is limited by the small number of infants with extreme z-scores. Reyes et al. (2024) noted that total HM micronutrient intake may be a better predictor of infant growth than HM nutrient concentrations themselves.

Calcium, copper, iodine, and phosphorus concentrations appeared to be higher in preterm infants; however, the small preterm subgroup (n=8) and overlapping distribution with term infants limit the strength of these conclusions. Data on the degree of prematurity were also unavailable. Compared with earlier in lactation (<6 months postpartum), Atinmo and Omololu (1982) reported higher copper in mature HM of preterm mothers, while Sabatier et al. (2019) observed higher copper concentrations in term milk, and Aquilio et al. (1996) and Mendelson et al. (1982) reported no differences**.** For calcium, Gidrewicz and Fenton (2014) reported higher concentrations in term HM at 2–4 weeks, shifting to higher in preterm at 5–6 weeks, with no differences by 10–12 weeks. In contrast, phosphate concentrations were lower in preterm HM across 2–12 weeks, differing from the higher values observed in the present study. Future research is warranted to clarify the impact of prematurity on HM nutrient composition in the second half of infancy.

### 3.4.3 Energy Intake from Infant Milk Sources and Complementary Foods of Infants who were Breastfed, Formula-fed, and Mixed-fed

The current study has demonstrated that breastfed infants had significantly lower total energy intake compared to those who were formula-fed or mixed-fed. Formula-fed infants derived more energy from infant milk sources than breastfed infants, while mixed-fed infants obtained more from complementary foods. These findings are consistent with those of Heinig et al. (1993), who

reported that at 6, 9, and 12 months postpartum, formula-fed infants had higher energy intake from infant milk sources than breastfed infants. Furthermore, Heinig et al. (1993) noted that at 6 and 9 months of age, formula-fed female infants consumed significantly more energy than their breastfed counterparts. In the present study, however, adjusting for infant age and sex did not alter the observed differences. Lim et al. (2018) similarly reported that total energy intake was significantly higher in formula-fed and mixed-fed infants compared to those who were breastfed, supporting the present study's results.

Differences in total energy intake between feeding groups may be partly explained by variation in energy expenditure and growth. Formula-fed infants generally have higher total energy expenditure (TEE) than breastfed infants, as demonstrated by four studies showing TEE to be approximately 7% higher at 6 months, 6% higher at 9 months, and 3% higher at 12 months of age (Butte et al., 1990; Butte et al., 2000; Davies et al., 1990; Jiang et al., 1998, as cited in Australian National Health and Medical Research Council et al., 2006). Correspondingly, formula-fed infants tend to gain weight more rapidly than breastfed infants, which has been characterised by greater accrual of fat-free mass, rather than adipose tissue (Bell et al., 2017; Dewey, 1998; Gale et al., 2012). While rapid weight gain during infancy has been linked to an increased risk of childhood obesity (Woo Baidal et al., 2016; Zheng et al., 2018), evidence suggests that feeding practices associated with infant formula use, such as high protein formula content, adding cereals to bottles, putting infants to bed with a bottle, and overfeeding, may play a stronger role in this risk than rapid weight gain itself (Appleton et al., 2018). In the current study, mixed-fed infants exhibited total energy intakes comparable to those of formula-fed infants. This raises the question of whether their TEE and growth mirror those of formula-fed infants or represent a pattern between those of breastfed and formula-fed infants.

Compared to the Australian and NZ estimated energy requirements (EERs), the mean total energy intakes observed in this study exceed the recommendations at each month across all feeding groups. This suggests that, on average, infants in NZ may be consuming excessive total energy during this transitional feeding period. Given the rising prevalence of obesity among NZ young children aged 6–27 months, further investigation is required to assess whether infant energy intakes are higher than required at this age (Daniels et al., 2023b).

The only study in the second half of infancy that has analysed mixed-fed infants is by Lim et al. (2018), which reported a mean total energy intake of 2707 kJ at 6 months, 2858 kJ at 9 months, and 3326 kJ at 12 months. In comparison, the present study reported a slightly higher mean total

energy intake among mixed-fed infants (3517 kJ/day), 7–10 months postpartum. While Lim et al. (2018) assumed HM intakes of 780 mL at 6 months and 600 mL at both 9 and 12 months to calculate energy intake, the current study employed the deuterium oxide DTM technique and accurate intake prediction calculations by Haszard et al. (2024), enabling a more precise measurement of energy intake from HM. Methodological differences in dietary assessment may account for variations in these findings. Lim et al. (2018) estimated dietary intake using a randomly selected day from a three-day food diary or a single 24-hour recall, whereas the present study used an average of two 24-hour multiple-pass recalls. However, since Lim et al. (2018) reported only total energy intake without distinguishing between contributions from infant milk sources and complementary foods, it is unclear whether the observed differences compared to the present study are attributable to variation in milk intake, complementary food intake, or both.

In relation to energy intake from complementary foods, the findings of this study are consistent with those of Heinig et al. (1993), who also reported no significant differences in energy intake between breastfed and formula-fed infants. The present study has yielded similar energy intakes from complementary foods in breastfed and formula-fed infants, consistent with several other studies conducted in the second half of infancy (Campos et al., 2010; Heinig et al., 1993; Hernandez et al., 2011). Despite the continued introduction and establishment of complementary feeding between 7 and 10 months of age, the results of this study demonstrate that HM continues to provide a substantial proportion (63.2%) of the total energy intake of breastfed infants. This finding supports the current MOH (2021) infant feeding guidelines, which recommend continued breastfeeding for up to two years or beyond. Formula-fed and mixed-fed infants also derived a substantial proportion of their total energy intake from infant milk sources at 62.0% and 60.2%, respectively, emphasising the critical role of infant milk sources in an infant's diet during this stage, irrespective of feeding type. This aligns with the findings of Heinig et al. (1993), who reported that at 9 months of age, breastfed infants obtained a mean (SD) of 64.0% (22.0%) and formula-fed infants a mean (SD) of 64.0% (14.0%) of their total energy intake from infant milk sources. The current study's findings further highlight the continued substantial nutritional contribution of infant milk sources as infants transition from exclusive milk feeding to more diverse dietary patterns.

### 3.4.4 Strengths and Limitations

A major strength of this study was the group of 121 lactating mothers who provided HM samples, enabling a robust nutrient analysis. Importantly, this study reports a range of macronutrients, vitamins, minerals, and trace elements, whereas many international studies are limited to only a

few nutrients. This is the first NZ study to comprehensively profile HM nutrient concentrations at 7–10 months postpartum, a critical period for the introduction and establishment of complementary feeding, providing valuable baseline data for research and clinical practice. Another strength was the use of the deuterium oxide DTM technique in combination with validated prediction equations to estimate HM intake, thereby allowing for the accurate measurement of infant energy intake from HM.

A key limitation was that maternal dietary intake was not assessed, which limited the investigation of its relationship with HM composition and introduced potential unmeasured confounding. This is an area that future NZ research could address. Individual-level samples were unable to be analysed for concentrations of macronutrients and vitamins due to funding constraints, thereby preventing comparisons with maternal and infant factors. In addition, there was a disproportionate representation of European mothers in the HM subgroup, restricting analysis of ethnic differences in HM composition and reducing generalisability to the wider NZ population. Analytical constraints also limited interpretation, as vitamin B12, vitamin D, and manganese values, together with some individual-level zinc and iron values, were below the detection threshold.

### 3.8. Conclusion

These findings indicate that HM nutrient concentrations in NZ mothers between 7 and 10 months postpartum are broadly consistent with international literature, though lower iodine, iron, and selenium concentrations may place infants at risk of inadequate intake. Future work should integrate HM composition with measured intakes to determine nutrient adequacy. This will clarify HM's contribution in late infancy and ensure feeding guidelines adequately address potential nutrient gaps.

# Chapter 4 Conclusion and Recommendations

## 4.1 Achievement of Aims and Objectives

This study aimed to determine the nutrient composition of human milk (HM) between 7 and 10 months postpartum and its contribution to infant energy intake. All study objectives were achieved.

The first objective of this thesis was to determine the nutrient (macronutrient, vitamin, mineral, and trace element) composition of HM. This thesis was able to successfully report concentrations for energy, macronutrients (fat, lactose, protein), vitamins (vitamin A, C, E, folate), minerals (Na, Mg, P, K, Ca), and trace elements (Fe, Cu, Se, Zn, I) from the HM of New Zealand (NZ) mothers between 7 and 10 months postpartum. HM nutrient concentrations were largely consistent with concentrations reported internationally for the second half of infancy. However, iron and selenium concentrations were lower than reported globally, and iodine concentrations were below the concentration suggested to indicate adequate maternal and infant status. This represents a novel finding, as no previous study in NZ has examined such a broad range of HM nutrients beyond 6 months postpartum. Clinically, these findings suggest that infants in the second half of infancy, who receive HM as their primary source of nutrition may be at risk of inadequate intake of iodine, iron, and selenium; however, further studies assessing actual nutrient intake from HM and complementary foods are needed to determine the true risk of deficiency.

The second objective of this thesis was to describe the relationship between infant and maternal characteristics with the composition of selected minerals and trace elements of HM. No significant relationships were observed between HM minerals and trace elements and infant characteristics (age, ethnicity, sex, term status, BMI z-score) or maternal characteristics (age, BMI, ethnicity, parity, employment, household deprivation). The descriptive nature of this analysis provided a valuable foundation for future research, as the literature pool on HM composition beyond 6 months postpartum remains scarce.

The third objective of this thesis was to determine the energy intake from infant milk sources and complementary foods of infants who were breastfed, formula-fed, and mixed-fed. The analysis of infant energy intake revealed significant differences between feeding groups (i.e. breastfed, formula-fed, and mixed-fed) using Mann-Whitney U tests for pairwise comparisons, and remained significant after adjusting for infant age and sex. Breastfed infants obtained less energy from infant milk sources (i.e. HM or commercial infant formula) than formula-fed infants and consumed less energy from complementary foods than mixed-fed infants. Overall, breastfed infants had lower

total energy intake compared with both formula-fed and mixed-fed infants. Across all infants, infant milk sources were the primary contributor to total energy intake, and total energy intake exceeded the EERs.

Addressing these objectives provides a comprehensive understanding of HM nutrient composition during late infancy and its role in meeting infant energy requirements. This thesis has highlighted that iodine, iron, and selenium concentrations may be insufficient in HM in NZ, emphasising the need to assess the nutrient intakes of NZ infants aged 7 and 10 months using local and up-to-date HM concentrations. This thesis has also identified differences in energy intake between infants receiving HM and those receiving infant formula. In addition, it has identified mixed-fed infants as a distinct feeding group, potentially requiring further research to compare their intakes and energy expenditure with those of breastfed and formula-fed infants.

### 4.2 Research Impact

To date, no studies have reported a broad range of nutrient concentrations in HM from NZ mothers between 7 and 10 months postpartum, and only limited international data are available for certain nutrients during this period. This research, therefore, provides novel insights into the macronutrient, vitamin, mineral, and trace element composition of HM in NZ once complementary feeding has been established. This study's findings indicate that HM from NZ mothers is likely to be insufficient in iodine, iron, and selenium. Further studies are needed to explore the underlying causes of these lower nutrient concentrations and to consider infant intakes in the context of both HM and complementary foods, in order to determine whether NZ infants are meeting their requirements. Such evidence will be important for shaping future infant feeding guidelines tailored to the nutrient intakes of NZ infants and for informing dietary advice, including supplementation if necessary. This study has also identified that infants aged 7–10 months may be at risk of excessive energy intake, regardless of feeding group. These findings are important for future research aiming to optimise infant feeding practices and prevent adverse outcomes, such as childhood obesity, by supporting strategies that ensure energy intake is balanced during late infancy.

Although this study focused on HM from NZ mothers, it contributes to the global literature on HM composition beyond the period of exclusive breastfeeding, where most existing research is concentrated. This knowledge is crucial for future research to accurately assess dietary intake in late infancy, particularly as the present findings confirm that HM remains the primary source of energy for breastfed infants, despite the introduction of complementary foods. The results

underscore the need for health promotion strategies that address key nutrients of concern, alongside strengthening existing initiatives, such as iodine supplementation for lactating mothers, to ensure they remain effective and widely adopted. These findings can be used by health professionals to support infant nutrition and to guide the care of both infants and lactating mothers.

### 4.3 Strengths

This is the first NZ study to provide a comprehensive profile of the nutrients in HM past the exclusive breastfeeding period. Given the limited national data on HM composition at any stage of lactation, this study makes a valuable contribution to understanding the nutritional profile of HM within the unique geographical and demographic context of NZ. Prior research in NZ has been limited to reporting on a single nutrient in HM during the second half of infancy (Han et al., 2022; Jin et al., 2020; Jin et al., 2022). Developing a clearer picture of HM composition in the NZ population can inform future research and support more contextually relevant clinical and public health decisions. This may include determining whether routine supplementation should be recommended for either the breastfeeding mother or breastfed infants directly in the second half of infancy, or whether infant feeding guidelines should be updated to address potential nutrient deficiencies (e.g., selenium supplementation for lactating mothers to improve HM concentrations).

Another strength is the focus on the 7–10 postpartum period. While there exists a growing body of research on lactation beyond 6 months postpartum, the specific window when complementary feeding is introduced and gradually established remains relatively underexplored. Investigating this stage offers valuable insights into how HM composition may adapt in response to the evolving nutritional needs of the infant, as reliance on HM as the main source of nutrition decreases. This focus contributes to a more comprehensive understanding of HM during an important, yet often overlooked, transitional period in infant feeding.

Additionally, this study included an analysis of mixed-fed infants (i.e. those consuming HM and commercial infant formula) during the second half of infancy. Previous research has typically focused on comparisons between breastfed and formula-fed infants, thereby excluding this distinct feeding group, with the exception of one study by Lim et al. (2018). Our findings indicate that the energy intakes of mixed-fed infants differ from those of breastfed infants and are similar to, though not synonymous with, those of formula-fed infants, supporting the recognition of mixed-fed infants as a distinct and established feeding group.

Finally, this study applied standardised techniques for HM sample collection. As nutrient composition can be influenced by factors such as time of day, stage of the feed, and light exposure, standardisation was essential to ensure uniformity among samples (Leghi et al., 2020; Lima et al., 2020). Samples were collected before 12:00 pm, with no feeding of the infant from the collection breast for at least two hours before collection. A full expression was obtained, and samples were collected in a dimly lit room before being wrapped in tin foil. Breast pump components that came into contact with HM were acid-washed, and no creams or powders were applied to the collection breast for at least two hours beforehand to minimise trace element contamination. These procedures minimised variability, degradation of light-sensitive vitamins, and potential trace element contamination, allowing for uniformity among samples and a true representation of HM composition among NZ mothers. In doing so, this study provides accurate evidence that strengthens the knowledge base on maternal and infant health in NZ.

### 4.4 Limitations

Limitations in this study should be recognised. First, this study did not collect maternal dietary intake by recall or record, and therefore, the potential impact on HM nutrient composition could not be assessed. Although maternal supplement use was recorded and subsequently analysed, information on dosage and frequency was limited. Together, these gaps may have introduced confounding when examining factors influencing HM composition. This would also have given in-depth insight into any cultural dietary patterns and how they impact nutrient concentrations.

Second, the subgroup of mothers who provided milk samples was not ethnically representative of the NZ population. European mothers were overrepresented, while Māori, Pacific, and Asian mothers were underrepresented. Consequently, this limited the ability to conduct meaningful comparisons of selected mineral and trace element concentrations between ethnic groups. This was particularly evident for Pacific participants, with only one mother-infant dyad included in the sample, preventing any robust analysis for this group. The number of Māori mothers who provided HM samples was limited and not aligned with national demographics. Given the scarcity of data on HM composition from Māori and Pacific mothers, especially during later lactation stages (7–10 months postpartum), targeted research is needed to ensure that findings are representative of these populations and to inform culturally appropriate guidance on infant nutrition. This underrepresentation restricts the generalisability of findings across ethnicities and highlights the need for more inclusive and representative sampling in future HM research in NZ.

Third, collecting data on macronutrient and vitamin concentrations in HM was not feasible at the individual level, primarily due to funding constraints. As a result, it was not possible to examine how maternal or infant factors may influence these nutrient concentrations. Additionally, some nutrient concentrations (i.e. vitamin B12, vitamin D, and manganese) analysed on an individual level fell below their respective analytical detection limits. For a few participants, the concentrations of iron (n=14) and zinc (n=1) fell below the detection limit and were recorded as the value of the lowest limit, which may have slightly overestimated the true concentrations of these nutrients. Despite using inductively coupled plasma mass spectroscopy, which is the preferred method for mineral and trace element analysis, these undetectable concentrations remain a limitation.

Finally, for this thesis, there was a lack of integration between HM nutrient composition data and information on HM volumes consumed. Without combining these two, it was not possible to estimate infants' total nutrient intakes from HM. Although the primary FFNZ study collected data on the volume of HM consumed by infants, incorporating these analyses was beyond the scope of the objectives of this thesis. Future work should link these data, using the reported nutrient concentrations, to provide a more comprehensive picture of infant nutrient intakes from HM.

### 4.5 Recommendations and Future Directions for Research

- Further research is warranted to investigate the influence of maternal dietary intake on the nutrient composition of HM during the later stages of lactation, particularly beyond 6 months postpartum. Dietary assessment methods should be tailored to the target population to ensure a balance between accuracy, feasibility, and participant burden.
- There is a critical need for research focusing on the HM composition of Māori and Pacific mothers. Current evidence is limited, with this study being the first in NZ to examine nutrient composition at 7–10 months postpartum. The only other analysis by ethnicity in NZ, conducted by Butts et al. (2018), assessed samples collected much earlier in lactation (6–8 weeks postpartum). To address longstanding health inequities and the underrepresentation of Māori and Pacific populations in this field, future research must prioritise inclusive sampling and ethnic-specific analyses to generate relevant and equitable nutritional insights. This also requires research methods that foster trust and cultural safety, such as community-led or co-designed approaches, to ensure Māori and Pacific mothers feel respected, supported, and comfortable contributing HM samples.

- Future research should aim to recruit larger sample sizes to investigate the relationship between HM nutrient concentrations and maternal and infant characteristics across a range of HM nutrients. Ensuring sufficiently large and evenly sized subgroups would allow for more robust statistical comparisons, for example, between infants born preterm and at term, across ethnicities, and by BMI z-scores.
- Further work should also investigate the contribution of HM concentrations to infant nutrient intake, in conjunction with dietary data. This would provide a more comprehensive understanding of nutrient intakes among NZ infants and help identify potential deficiencies and appropriate strategies to address them. While the primary FFNZ study has collected these dietary data, analysis was beyond the scope of this thesis and will be undertaken at a later stage.

In summary, this research provides an in-depth discussion around the nutrient composition of HM 7–10 months postpartum and its contribution to infant energy intake. Its findings present an opportunity to further investigate nutrient intakes of infants aged 7–10 months in relation to their intake of HM. The findings of this thesis have the potential to enhance infant feeding guidelines in NZ and lead to enhanced infant nutrition status.

# References


Abrams, S. A. (2006). Building bones in babies: Can and should we exceed the human milk-fed infant's rate of bone calcium accretion? *Nutrition Reviews*, *64*(11), 487-494. https://doi.org/10.1111/j.1753-4887.2006.tb00181.x

Ahn, H. S., & Jeong, J. Y. (1998). Ecological studies of maternal-infant nutrition and feeding in urban low income areas: III. Infant's nutrient intakes and growth patterns. *Korean Journal of Community Nutrition*, *3*(2), 174-189. https://www.kjcn.or.kr/journal/view.php?number=319

Al-Awadi, F. M., & Srikumar, T. S. (2001). Determination of selenium concentration and its chemical forms in the milk of Kuwaiti and non-Kuwaiti lactating mothers. *The Journal of Trace Elements in Experimental Medicine*, *14*(1), 57-67. https://doi.org/10.1002/1520-670X(2001)14:1<57::AID-JTRA1008>3.0.CO;2-K

Andersson, M., Aeberli, I., Wust, N., Piacenza, A. M., Bucher, T., Henschen, I., Haldimann, M., & Zimmermann, M. B. (2010). The Swiss iodized salt program provides adequate iodine for school children and pregnant women, but weaning infants not receiving iodine-containing complementary foods as well as their mothers are iodine deficient. *The Journal of Clinical Endocrinology & Metabolism*, *95*(12), 5217-5224. https://doi.org/10.1210/jc.2010-0975

Andersson, M., & Braegger, C. P. (2022). The role of iodine for thyroid function in lactating women and infants. *Endocrine Reviews*, *43*(3), 469-506. https://doi.org/10.1210/endrev/bnab029

Andreassa, N. P., Suano-Souza, F. I., & Sarni, R. O. S. (2024). Fat content and energy calculation in pasteurized human milk: Comparison between infrared analysis and creamatocrit method. *Breastfeeding Medicine*, *19*(11), 863-869. https://doi.org/10.1089/bfm.2024.0249

AOAC. (2023). 33.2.25 AOAC official method 905.02 fat in milk: Roese-Gottlieb method. In *Official methods of analysis of AOAC international* (22nd ed.). https://doi.org/10.1093/9780197610145.003.3059

Appleton, J., Russell, C. G., Laws, R., Fowler, C., Campbell, K., & Denney-Wilson, E. (2018). Infant formula feeding practices associated with rapid weight gain: A systematic review. *Maternal & Child Nutrition*, *14*(3), e12602. https://doi.org/10.1111/mcn.12602

Aquilio, E., Spagnoli, R., Seri, S., Bottone, G., & Spennati, G. (1996). Trace element content in human milk during lactation of preterm newborns. *Biological Trace Element Research*, *51*(1), 63-70. https://doi.org/10.1007/BF02790148

Araya, B. R., Ziegler, A. A., Grobe, C. C., Grobe, J. L., & Segar, J. L. (2023). Sodium and growth in preterm infants: A review. *Newborn*, *2*(2), 142-147. https://doi.org/10.5005/jp-journals-11002-0060

Atinmo, T., & Omololu, A. (1982). Trace element content of breastmilk from mothers of preterm infants in Nigeria. *Early Human Development*, *6*(3), 309-313. https://doi.org/10.1016/0378-3782(82)90125-6

Atkinson, J., Salmond, C., & Crampton, P. (2019). *NZDep2018 index of deprivation: Interim research report, December 2019*. https://www.otago.ac.nz/__data/assets/pdf_file/0025/327481/nzdep2018-index-of-deprivation-research-report-interim-dec-2019-730394.pdf

Atkinson, S. A., Alston-Mills, B. P., Lonnerdal, B., & Neville, M. C. (1995). Major minerals and ionic constituents of human and bovine milk. In R. G. Jensen (Ed.), *Handbook of Milk Composition* (pp. 593-619). Academic Press. https://doi.org/10.1016/B978-012384430-9/50026-3

Australian National Health and Medical Research Council, Australian Government Department of Health and Ageing, & New Zealand Ministry of Health. (2006). *Nutrient reference values for Australia and New Zealand*. Australian National Health and Medical Research Council. https://www.nhmrc.gov.au/sites/default/files/images/nutrient-refererence-dietary-intakes.pdf

Ayah, R. A., Mwaniki, D. L., Magnussen, P., Tedstone, A. E., Marshall, T., Alusala, D., Luoba, A., Kaestel, P., Michaelsen, K. F., & Friis, H. (2007). The effects of maternal and infant vitamin A supplementation on vitamin A status: A randomised trial in Kenya. *The British Journal of Nutrition*, *98*(2), 422-430. https://doi.org/10.1017/S0007114507705019

Azizi, F., & Smyth, P. (2009). Breastfeeding and maternal and infant iodine nutrition. *Clinical Endocrinology*, *70*(5), 803-809. https://doi.org/10.1111/j.1365-2265.2008.03442.x

Bandara, T., Hettiarachchi, M., Liyanage, C., Amarasena, S., & Wong, W. W. (2015). The deuterium oxide-to-the-mother method documents adequate breast-milk intake among Sri Lankan infants. *The Journal of Nutrition*, *145*(6), 1325-1329. https://doi.org/10.3945/jn.115.211771

Barbano, D. M., Clark, J. L., Dunham, C. E., & Fleming, R. J. (1990). Kjeldahl method for determination of total nitrogen content of milk: Collaborative study. *Journal of Association of Official Analytical Chemists*, *73*(6), 849-859. https://doi.org/10.1093/jaoac/73.6.849

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

Bell, K. A., Wagner, C. L., Feldman, H. A., Shypailo, R. J., & Belfort, M. B. (2017). Associations of infant feeding with trajectories of body composition and growth. *American Journal of Clinical Nutrition*, *106*(2), 491-498. https://doi.org/10.3945/ajcn.116.151126

Bilston-John, S. H., Narayanan, A., Lai, C. T., Rea, A., Joseph, J., & Geddes, D. T. (2021). Macro- and trace-element intake from human milk in australian infants: Inadequacy with respect to national recommendations. *Nutrients*, *13*(10), 3548. https://doi.org/10.3390/nu13103548

Black, R. E., & Fischer Walker, C. (2012). Role of zinc in child health and survival. *Nestle Nutrition Institute workshop series*, *70*, 37-42. https://doi.org/10.1159/000337393

Bolann, B. J., Rahil-Khazen, R., Henriksen, H., Isrenn, R., & Ulvik, R. J. (2007). Evaluation of methods for trace-element determination with emphasis on their usability in the clinical routine laboratory. *The Scandinavian Journal of Clinical and Laboratory Investigation*, *67*(4), 353-366. https://doi.org/10.1080/00365510601095281

Brown, C. R., Dodds, L., Legge, A., Bryanton, J., & Semenic, S. (2014). Factors influencing the reasons why mothers stop breastfeeding. *Canadian Journal of Public Health*, *105*(3), e179-185. https://doi.org/10.17269/cjph.105.4244

Brown, K. J. (2023). *Feeding and dietary practices of New Zealand infants: An observational study* [Unpublished doctoral dissertation]. Massey University. Auckland.

Brown, K. J., Beck, K. L., von Hurst, P., Heath, A. L., Taylor, R., Haszard, J., Daniels, L., Te Morenga, L., McArthur, J., Paul, R., Jones, E., Katiforis, I., Rowan, M., Casale, M., McLean, N., Cox, A., Fleming, E., Bruckner, B., Jupiterwala, R., . . . Conlon, C. (2023). Adherence to infant feeding guidelines in the first foods New Zealand study. *Nutrients*, *15*(21), 4650. https://doi.org/10.3390/nu15214650

Burton, G. W., Joyce, A., & Ingold, K. U. (1983). Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? *Archives of Biochemistry and Biophysics*, *221*(1), 281-290. https://doi.org/10.1016/0003-9861(83)90145-5

Butte, N. F., Garza, C., Smith, E. O., Wills, C., & Nichols, B. L. (1987). Macro- and trace-mineral intakes of exclusively breast-fed infants. *The American Journal of Clinical Nutrition*, *45*(1), 42-48. https://doi.org/10.1093/ajcn/45.1.42

Butterworth, C. E., Baugh, C. M., & Krumdiek, C. (1969). Availability to man of folate ingested in polyglutamate form. *The American Journal of Clinical Nutrition*, *22*(5), 670. https://ajcn.nutrition.org/

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), 1231. https://doi.org/10.3390/nu10091231

Cacho, N. T., & Lawrence, R. M. (2017). Innate immunity and breast milk. *Frontiers in Immunology*, *8*, 584. https://doi.org/10.3389/fimmu.2017.00584

Campos, R., Hernandez, L., Soto-Mendez, M. J., Vossenaar, M., & Solomons, N. W. (2010). Contribution of complementary food nutrients to estimated total nutrient intakes for rural Guatemalan infants in the second semester of life. *Asia Pacific Journal of Clinical Nutrition*, *19*(4), 481-490. https://www.ncbi.nlm.nih.gov/pubmed/21147708

Canfield, L. M., Clandinin, M. T., Davies, D. P., Fernandez, M. C., Jackson, J., Hawkes, J., Goldman, W. J., Pramuk, K., Reyes, H., Sablan, B., Sonobe, T., & Bo, X. (2003).

Multinational study of major breast milk carotenoids of healthy mothers. *European Journal of Nutrition*, *42*(3), 133-141. https://doi.org/10.1007/s00394-003-0403-9

Carmel, R. (2005). *Modern nutrition in health and disease* (11th ed.). Lippincott Williams & Wilkins.

Caroli, S. (2006). *The determination of chemical elements in food: Applications for atomic and mass spectrometry*. John Wiley & Sons. https://doi.org/10.1002/9780470141007

Casadio, Y. S., Williams, T. M., Lai, C. T., Olsson, S. E., Hepworth, A. R., & Hartmann, P. E. (2010). Evaluation of a mid-infrared analyzer for the determination of the macronutrient composition of human milk. *Journal of Human Lactation*, *26*(4), 376-383. https://doi.org/10.1177/0890334410376948

Casey, C. E., Neville, M. C., & Hambidge, K. M. (1989). Studies in human lactation: Secretion of zinc, copper, and manganese in human milk. *The American Journal of Clinical Nutrition*, *49*(5), 773-785. https://doi.org/10.1093/ajcn/49.5.773

Castillo-Duran, C., & Cassorla, F. (1999). Trace minerals in human growth and development. *Journal of Pediatric Endocrinology & Metabolism*, *12*(5), 589-601. https://doi.org/10.1515/JPEM.1999.12.5.589

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

Chowdhury, R., Sinha, B., Sankar, M. J., Taneja, S., Bhandari, N., Rollins, N., Bahl, R., & Martines, J. (2015). Breastfeeding and maternal health outcomes: A systematic review and meta-analysis. *Acta Paediatrica*, *104*(467), 96-113. https://doi.org/10.1111/apa.13102

Christian, P., Smith, E. R., Lee, S. E., Vargas, A. J., Bremer, A. A., & Raiten, D. J. (2021). The need to study human milk as a biological system. *The American Journal of Clinical Nutrition*, *113*(5), 1063-1072. https://doi.org/10.1093/ajcn/nqab075

Coward, W. A., Cole, T. J., Sawyer, M. B., & Prentice, A. M. (1982). Breast-milk intake measurement in mixed-fed infants by administration of deuterium oxide to their mothers. *Human Nutrition: Clinical Nutrition*, *36C*(2), 141-148. https://pubmed.ncbi.nlm.nih.gov/6286540/

Czosnykowska-Lukacka, M., Krolak-Olejnik, B., & Orczyk-Pawilowicz, M. (2018). Breast Milk Macronutrient Components in Prolonged Lactation. *Nutrients*, *10*(12). https://doi.org/10.3390/nu10121893

da Costa, T. H., Haisma, H., Wells, J. C., Mander, A. P., Whitehead, R. G., & Bluck, L. J. (2010). How much human milk do infants consume? Data from 12 countries using a standardized stable isotope methodology. *The Journal of Nutrition*, *140*(12), 2227-2232. https://doi.org/10.3945/jn.110.123489

Dahlqvist, A. (1964). Method for assay of intestinal disaccharidases. *Analytical Biochemistry*, *7*, 18-25. https://doi.org/10.1016/0003-2697(64)90115-0

Daniels, L., Heath, A.-L. M., Taylor, R. W., Bruckner, B., Diana, A., Zinzan-Dickie, I., McLean, N. H., Cox, A. M., Jones, E. A., Katiforis, I., Brown, K. J., Rowan, M., Casale, M., Jupiterwala, R., Harper, M., Conlon, C. A., Beck, K. L., Morenga, L. T., von Hurst, P. R., & Haszard, J. J. (2025). Human milk and total milk intakes of mixed fed infants: A cross-sectional study of infants aged 7-10 months [Manuscript under review].

Daniels, L., Gibson, R. S., Diana, A., Haszard, J. J., Rahmannia, S., Luftimas, D. E., Hampel, D., Shahab-Ferdows, S., Reid, M., Melo, L., Lamers, Y., Allen, L. H., & Houghton, L. A. (2019). Micronutrient intakes of lactating mothers and their association with breast milk concentrations and micronutrient adequacy of exclusively breastfed Indonesian infants. *The American Journal of Clinical Nutrition*, *110*(2), 391-400. https://doi.org/10.1093/ajcn/nqz047

Daniels, L., Haszard, J. J., Gibson, R. S., Taylor, R. W., Fleming, E. A., Miller, J. C., Thomson, C. D., & Heath, A. M. (2023). Selenium intakes and plasma selenium of New Zealand toddlers: Secondary analysis of a randomised controlled trial. *The British Journal of Nutrition*, *129*(7), 1193-1201. https://doi.org/10.1017/S0007114522002379

Daniels, L., Haszard, J. J., Taylor, R. W., & Taylor, B. J. (2023). Prevalence of low and high BMI during the first 3 years of life: using New Zealand national electronic health data. *Pediatric Obesity*, *18*(5), e13013. https://doi.org/10.1111/ijpo.13013

Darnton-Hill, I. (2013). *Zinc supplementation and growth in children*. World Health Organization. https://www.who.int/tools/elena/bbc/zinc-stunting.

de Castro, T. G., Gerritsen, S., Wall, C., Grant, C., Teixeira, J. A., Marchioni, D. M., Pillai, A., & Morton, S. (2018). *Infant feeding in New Zealand: Adherence to food and nutrition guidelines among the growing up in New Zealand cohort*. Ministry of Social Development. https://www.msd.govt.nz/documents/about-msd-and-our-work/publications-resources/research/infant-feeding/infant-feeding-in-new-zealand.pdf

de Onis, M., Onyango, A. W., Van den Broeck, J., Chumlea, W. C., & Martorell, R. (2004). Measurement and standardization protocols for anthropometry used in the construction of a new international growth reference. *Food and Nutrition Bulletin*, *25*(1 Suppl), S27-36. https://doi.org/10.1177/15648265040251S104

Dewey, K. G. (1998). Growth characteristics of breast-fed compared to formula-fed infants. *Biology of the Neonate*, *74*(2), 94-105. https://doi.org/10.1159/000014016

Dewey, K. G., Finley, D. A., & Lonnerdal, B. (1984). Breast milk volume and composition during late lactation (7-20 months). *Journal of Pediatric Gastroenterology and Nutrition*, *3*(5), 713-720. https://doi.org/10.1097/00005176-198411000-00014

Dijkhuizen, M. A., Wieringa, F. T., West, C. E., & Muhilal. (2004). Zinc plus beta-carotene supplementation of pregnant women is superior to beta-carotene supplementation alone

in improving vitamin A status in both mothers and infants. *The American Journal of Clinical Nutrition*, *80*(5), 1299-1307. https://doi.org/10.1093/ajcn/80.5.1299

Domellof, M., Lonnerdal, B., Dewey, K. G., Cohen, R. J., & Hernell, O. (2004). Iron, zinc, and copper concentrations in breast milk are independent of maternal mineral status. *The American Journal of Clinical Nutrition*, *79*(1), 111-115. https://doi.org/10.1093/ajcn/79.1.111

Dorea, J. G. (2002). Selenium and breast-feeding. *The British Journal of Nutrition*, *88*(5), 443-461. https://doi.org/10.1079/BJN2002692

Douglas, P. (2022). Re-thinking lactation-related nipple pain and damage. *Women's Health (London, England)*, *18*, 17455057221087865. https://doi.org/10.1177/17455057221087865

Dror, D. K., & Allen, L. H. (2018a). Iodine in human milk: A systematic review. *Advances in Nutrition*, *9*(suppl_1), 347S-357S. https://doi.org/10.1093/advances/nmy020

Dror, D. K., & Allen, L. H. (2018b). Overview of nutrients in human milk. *Advances in Nutrition*, *9*(suppl_1), 278S-294S. https://doi.org/10.1093/advances/nmy022

Dror, D. K., & Allen, L. H. (2018c). Retinol-to-fat ratio and retinol concentration in human milk show somilar time trends and associations with maternal factors at the population level: A systematic review and meta-analysis. *Advances in Nutrition*, *9*(suppl_1), 332S-346S. https://doi.org/10.1093/advances/nmy021

Dubascoux, S., Andrey, D., Vigo, M., Kastenmayer, P., & Poitevin, E. (2018). Validation of a dilute and shoot method for quantification of 12 elements by inductively coupled plasma tandem mass spectrometry in human milk and in cow milk preparations. *Journal of Trace Elements in Medicine and Biology*, *49*, 19-26. https://doi.org/10.1016/j.jtemb.2018.04.023

Dunn, J. T., Crutchfield, H. E., Gutekunst, R., & Dunn, A. D. (1993). Two simple methods for measuring iodine in urine. *Thyroid*, *3*(2), 119-123. https://doi.org/10.1089/thy.1993.3.119

Edmonds, J. C., McLean, R. M., Williams, S. M., & Skeaff, S. A. (2016). Urinary iodine concentration of New Zealand adults improves with mandatory fortification of bread with iodised salt but not to predicted levels. *European Journal of Nutrition*, *55*(3), 1201-1212. https://doi.org/10.1007/s00394-015-0933-y

Ek, J. (1983). Plasma, red cell, and breast milk folacin concentrations in lactating women. *The American Journal of Clinical Nutrition*, *38*(6), 929-935. https://doi.org/10.1093/ajcn/38.6.929

European Food Safety Authority. (2014). Scientific Opinion on Dietary Reference Values for folate. *EFSA Journal*, *12*(11). https://doi.org/10.2903/j.efsa.2014.3893

Eussen, S., Alles, M., Uijterschout, L., Brus, F., & van der Horst-Graat, J. (2015). Iron intake and status of children aged 6-36 months in Europe: A systematic review. *Annals of Nutrition & Metabolism*, *66*(2-3), 80-92. https://doi.org/10.1159/000371357

Falize, C., Savage, M., Jeanes, Y. M., & Dyall, S. C. (2024). Evaluating the relationship between the nutrient intake of lactating women and their breast milk nutritional profile: A systematic review and narrative synthesis. *The British Journal of Nutrition*, *131*(7), 1196-1224. https://doi.org/10.1017/S0007114523002775

Fewtrell, M., Bronsky, J., Campoy, C., Domellof, M., Embleton, N., Fidler Mis, N., Hojsak, I., Hulst, J. M., Indrio, F., Lapillonne, A., & Molgaard, C. (2017). Complementary feeding: A position paper by the European society for paediatric gastroenterology, hepatology, and nutrition (ESPGHAN) committee on nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, *64*(1), 119-132. https://doi.org/10.1097/MPG.0000000000001454

Flatt, J. P. (1995). Use and storage of carbohydrate and fat. *The American Journal of Clinical Nutrition*, *61*(4 Suppl), 952S-959S. https://doi.org/10.1093/ajcn/61.4.952S

Gale, C., Logan, K. M., Santhakumaran, S., Parkinson, J. R., Hyde, M. J., & Modi, N. (2012). Effect of breastfeeding compared with formula feeding on infant body composition: A systematic review and meta-analysis. *American Journal of Clinical Nutrition*, *95*(3), 656-669. https://doi.org/10.3945/ajcn.111.027284

Garza, C., Butte, N., & Dewey, K. (1985). Determination of the energy content of human milk. In R. Jensen & M. Neville (Eds.), *Human lactation: Methodologies* (pp. 121-126). Plenum Press.

Georgieff, M. K. (2007). Nutrition and the developing brain: Nutrient priorities and measurement. *The American Journal of Clinical Nutrition*, *85*(2), 614S-620S. https://doi.org/10.1093/ajcn/85.2.614S

Gidrewicz, D. A., & Fenton, T. R. (2014). A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. *BMC Pediatrics*, *14*, 216. https://doi.org/10.1186/1471-2431-14-216

Giuffrida, F., Austin, S., Cuany, D., Sanchez-Bridge, B., Longet, K., Bertschy, E., Sauser, J., Thakkar, S. K., Lee, L. Y., & Affolter, M. (2019). Comparison of macronutrient content in human milk measured by mid-infrared human milk analyzer and reference methods. *Journal of Perinatology*, *39*(3), 497-503. https://doi.org/10.1038/s41372-018-0291-8

Gowen, N., Gai, N., O'Mahony, J. A., O'Regan, J., & Goulding, D. A. (2025). Non-protein nitrogen in dairy ingredients: A closer look at its contribution in infant nutritional product formulation. *International Dairy Journal*, *164*, 106201. https://doi.org/10.1016/j.idairyj.2025.106201

Greer, F. R., Tsang, R. C., Levin, R. S., Searcy, J. E., Wu, R., & Steichen, J. J. (1982). Increasing serum calcium and magnesium concentrations in breast-fed infants: Longitudinal studies of minerals in human milk and in sera of nursing mothers and their

infants. *The Journal of Pediatrics*, *100*(1), 59-64. https://doi.org/10.1016/s0022-3476(82)80235-7

Gridneva, Z., Rea, A., Tie, W. J., Lai, C. T., Kugananthan, S., Ward, L. C., Murray, K., Hartmann, P. E., & Geddes, D. T. (2019). Carbohydrates in human milk and body composition of term infants during the first 12 months of lactation. *Nutrients*, *11*(7), 1472. https://doi.org/10.3390/nu11071472

Gridneva, Z., Tie, W. J., Rea, A., Lai, C. T., Ward, L. C., Murray, K., Hartmann, P. E., & Geddes, D. T. (2018). Human milk casein and whey protein and infant body composition over the first 12 months of lactation. *Nutrients*, *10*(9), 1332. https://doi.org/10.3390/nu10091332

Grote, V., Verduci, E., Scaglioni, S., Vecchi, F., Contarini, G., Giovannini, M., Koletzko, B., Agostoni, C., & European Childhood Obesity, P. (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

Hambraeus, L., Forsum, E., Abrahamsson, L., & Lonnerdal, B. (1976). Automatic total nitrogen analysis in nutritional evaluations using a block digestor. *Analytical Biochemistry*, *72*(1-2), 78-85. https://doi.org/10.1016/0003-2697(76)90508-x

Hampel, D., Dror, D. K., & Allen, L. H. (2018). Micronutrients in human milk: Analytical methods. *Advances in Nutrition*, *9*(suppl_1), 313S-331S. https://doi.org/10.1093/advances/nmy017

Han, S. M., Devaraj, S., Derraik, J. G. B., Vickers, M. H., Huang, F., Dubascoux, S., Godfrey, K. M., Chan, S. Y., Pang, W. W., Thakkar, S. K., Cutfield, W. S., & NiPPeR Study Group. (2022). A nutritional supplement containing zinc during preconception and pregnancy increases human milk zinc concentrations. *Frontiers in Nutrition*, *9*, 1034828. https://doi.org/10.3389/fnut.2022.1034828

Han, Y. H., Yon, M., Han, H. S., Johnston, K. E., Tamura, T., & Hyun, T. (2011). Zinc status and growth of Korean infants fed human milk, casein-based, or soy-based formula: Three-year longitudinal study. *Nutrition Research and Practice*, *5*(1), 46-51. https://doi.org/10.4162/nrp.2011.5.1.46

Hartikainen, H. (2005). Biogeochemistry of selenium and its impact on food chain quality and human health. *Journal of Trace Elements in Medicine and Biology*, *18*(4), 309-318. https://doi.org/10.1016/j.jtemb.2005.02.009

Haszard, J. J., Heath, A.-L. M., Taylor, R. W., Bruckner, B., Katiforis, I., McLean, N. H., Cox, A. M., Brown, K. J., Casale, M., Jupiterwala, R., Diana, A., Beck, K. L., Conlon, C. A., von Hurst, P. R., & Daniels, L. (2024). Equations to estimate human milk intake in infants aged 7 to 10 months: Prediction models from a cross-sectional study. *The American Journal of Clinical Nutrition*, *120*(1), 102-110. https://doi.org/10.1016/j.ajcnut.2024.04.009

Haycock, G. B. (1993). The influence of sodium on growth in infancy. *Pediatric Nephrology*, *7*(6), 871-875. https://doi.org/10.1007/BF01213376

Heinig, M. J., Nommsen, L. A., Peerson, J. M., Lonnerdal, B., & Dewey, K. G. (1993). Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: The DARLING study. *The American Journal of Clinical Nutrition*, *58*(2), 152-161. https://doi.org/10.1093/ajcn/58.2.152

Hendrikson, E. C., Seacat, J. M., & Neville, M. C. (1985). Insensible weight loss in children under one year of age. *Acta Paediatrica*, *74*(5), 678-680. https://doi.org/10.1111/j.1651-2227.1985.tb10012.x

Henjum, S., Kjellevold, M., Ulak, M., Chandyo, R. K., Shrestha, P. S., Froyland, L., Strydom, E. E., Dhansay, M. A., & Strand, T. A. (2016). Iodine concentration in breastmilk and urine among lactating women of Bhaktapur, Nepal. *Nutrients*, *8*(5), 255. https://doi.org/10.3390/nu8050255

Hernandez, L., Campos, R., Enneman, A., Soto-Mendez, M. J., Vossenaar, M., & Solomons, N. W. (2011). Contribution of complementary food nutrients to estimated total nutrient intakes for urban Guatemalan infants in the second semester of life. *Asia Pacific Journal of Clinical Nutrition*, *20*(4), 572-583. https://www.ncbi.nlm.nih.gov/pubmed/22094843

Hoppner, K., Lampi, B., & Perrin, D. E. (1972). The free and total folate activity in foods available on the Canadian market. *Canadian Institute of Food Science and Technology Journal*, *5*(2), 60-66. https://doi.org/10.1016/S0315-5463(72)74089-4

Horrocks, L. A., & Yeo, Y. K. (1999). Health benefits of docosahexaenoic acid (DHA). *Pharmacological Research*, *40*(3), 211-225. https://doi.org/10.1006/phrs.1999.0495

Horta, B. L., & de Lima, N. P. (2019). Breastfeeding and Type 2 Diabetes: Systematic Review and Meta-Analysis. *Curr Diab Rep*, *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*(467), 30-37. https://doi.org/10.1111/apa.13133

Horta, B. L., & Victora, C. G. (2013). *Long-term effects of breastfeeding: A systematic review*. World Health Organization Press https://iris.who.int/handle/10665/79198

Hundrieser, M. S. K. E., Clark, R. M., Jensen, R. G., & Ferris, A. M. (1984). A comparison of methods for determination of total lipids in human milk. *Nutrition Research 4*(1), 21-26. https://doi.org/10.1016/S0271-5317(84)80129-3

Institute of Medicine (US) Committee on Nutritional Status During Pregnancy and Lactation. (1991). *Nutrition During Lactation*. National Academies Press. https://doi.org/10.17226/1577

Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. (1997). *Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride*. National Academies Press (US). https://doi.org/10.17226/5776

Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. (2004). *Dietary reference intakes for water, potassium, sodium, chloride, and sulfate*. National Academies Press (US). https://doi.org/10.17226/10925

International Atomic Energy Agency. (2010). *Stable isotope technique to assess intake of human milk in breastfed infants* ((IAEA Human Health Series No. 7) https://www-pub.iaea.org/MTCD/Publications/PDF/Pub1429_web.pdf

Jarjou, L. M., Goldberg, G. R., Coward, W. A., & Prentice, A. (2012). Calcium intake of rural Gambian infants: A quantitative study of the relative contributions of breast milk and complementary foods at 3 and 12 months of age. *European Journal of Clinical Nutrition 66*(6), 673-677. https://doi.org/10.1038/ejcn.2012.7

Javad, M., Vahidinia, A., Samiee, F., Elaridi, J., Leili, M., Faradmal, J., & Rahmani, A. (2018). Analysis of aluminum, minerals and trace elements in the milk samples from lactating mothers in Hamadan, Iran. *Journal of Trace Elements in Medicine and Biology*, *50*, 8-15. https://doi.org/10.1016/j.jtemb.2018.05.016

Jaynes, H. O., & Asan, T. (1973). Determination of lactose in milk by gas chromotography. *Journal of Food Protection*, *36*(6), 333-336. https://doi.org/10.4315/0022-2747-36.6.333

Jin, Y., Coad, J., Pond, R., Kim, N., & Brough, L. (2020). Selenium intake and status of postpartum women and postnatal depression during the first year after childbirth in New Zealand - Mother and Infant Nutrition Investigation (MINI) study. *Journal of Trace Elements in Medicine and Biology*, *61*, 126503. https://doi.org/10.1016/j.jtemb.2020.126503

Jin, Y., Coad, J., Skeaff, S. A., Zhou, S. J., & Brough, L. (2022). Iodine status of postpartum women and their infants aged 3, 6 and 12 months: Mother and Infant Nutrition Investigation (MINI). *The British Journal of Nutrition*, *127*(4), 570-579. https://doi.org/10.1017/S000711452100129X

Jin, Y., Coad, J., Zhou, S. J., Skeaff, S., Benn, C., & Brough, L. (2021). Use of iodine supplements by breastfeeding mothers Is associated with better maternal and infant iodine status. *Biological Trace Element Research*, *199*(8), 2893-2903. https://doi.org/10.1007/s12011-020-02438-8

Karra, M. V., Udipi, S. A., Kirksey, A., & Roepke, J. L. (1986). Changes in specific nutrients in breast milk during extended lactation. *The American Journal of Clinical Nutrition*, *43*(4), 495-503. https://doi.org/10.1093/ajcn/43.4.495

Keikha, M., Shayan-Moghadam, R., Bahreynian, M., & Kelishadi, R. (2021). Nutritional supplements and mother's milk composition: A systematic review of interventional studies. *International Breastfeeding Journal 16*(1), 1. https://doi.org/10.1186/s13006-020-00354-0

Kent, J. C., Mitoulas, L. R., Cregan, M. D., Ramsay, D. T., Doherty, D. A., & Hartmann, P. E. (2006). Volume and frequency of breastfeedings and fat content of breast milk

throughout the day. *Pediatrics*, *117*(3), e387-395. https://doi.org/10.1542/peds.2005-1417

Kent, M. (2007). *The oxford dictionary of sports science & medicine* (3rd ed.). Oxford University Press.

Kim, H., Jung, B. M., Lee, B. N., Kim, Y. J., Jung, J. A., & Chang, N. (2017). Retinol, alpha-tocopherol, and selected minerals in breast milk of lactating women with full-term infants in South Korea. *Nutrition Research and Practice*, *11*(1), 64-69. https://doi.org/10.4162/nrp.2017.11.1.64

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

Kim, Y., English, C., Reich, P., Gerber, L. E., & Simpson, K. L. (1990). Vitamin A and carotenoids in human milk. *Journal of Agricultural and Food Chemistry*, *38*(10), 1930–1933. https://doi.org/10.1021/jf00100a011

Kinoshita, M., White, M. J., & Doolan, A. (2024). Clinical assessment of breastfeeding in preterm infants. *European Journal of Clinical Nutrition*, *78*(10), 825-829. https://doi.org/10.1038/s41430-024-01471-3

Kohrle, J., Jakob, F., Contempre, B., & Dumont, J. E. (2005). Selenium, the thyroid, and the endocrine system. *Endocrine Reviews*, *26*(7), 944-984. https://doi.org/10.1210/er.2001-0034

Kojima, T., Asoh, M., Yamawaki, N., Kanno, T., Hasegawa, H., & Yonekubo, A. (2004). Vitamin K concentrations in the maternal milk of Japanese women. *Acta Paediatrica*, *93*(4), 457-463. https://doi.org/10.1080/08035250410023692

Kozhimannil, K. B., Jou, J., Attanasio, L. B., Joarnt, L. K., & McGovern, P. (2014). Medically complex pregnancies and early breastfeeding behaviors: A retrospective analysis. *PLoS One*, *9*(8), e104820. https://doi.org/10.1371/journal.pone.0104820

Krebs, N. F. (2007). Food choices to meet nutritional needs of breast-fed infants and toddlers on mixed diets. *The Journal of Nutrition*, *137*(2), 511S-517S. https://doi.org/10.1093/jn/137.2.511S

Krebs, N. F., & Hambidge, K. M. (1986). Zinc requirements and zinc intakes of breast-fed infants. *The American Journal of Clinical Nutrition*, *43*(2), 288-292. https://doi.org/10.1093/ajcn/43.2.288

Krebs, N. F., Hambidge, K. M., Jacobs, M. A., & Rasbach, J. O. (1985). The effects of a dietary zinc supplement during lactation on longitudinal changes in maternal zinc status and milk zinc concentrations. *The American Journal of Clinical Nutrition*, *41*(3), 560-570. https://doi.org/10.1093/ajcn/41.3.560

Krebs, N. F., Reidinger, C. J., Hartley, S., Robertson, A. D., & Hambidge, K. M. (1995). Zinc supplementation during lactation: Effects on maternal status and milk zinc concentrations. *The American Journal of Clinical Nutrition*, *61*(5), 1030-1036. https://doi.org/10.1093/ajcn/61.4.1030

Krebs, N. F., Reidinger, C. J., Robertson, A. D., & Hambidge, K. M. (1994). Growth and intakes of energy and zinc in infants fed human milk. *The Journal of Pediatrics*, *124*(1), 32-39. https://doi.org/10.1016/s0022-3476(94)70251-9

Kugananthan, S., Gridneva, Z., Lai, C. T., Hepworth, A. R., Mark, P. J., Kakulas, F., & Geddes, D. T. (2017). Associations between maternal body composition and appetite hormones and macronutrients in human milk. *Nutrients*, *9*(3), 252. https://doi.org/10.3390/nu9030252

Kumar, V., Shukla, A. K., Sharma, P., Choudhury, B., Singh, P., & Kumar, S. (2017). Role of Macronutrient in Health. *World Journal of Pharmaceutical Research*, *6*(3), 373-381. https://doi.org/10.20959/wjpr20173-7955

Kumari-Maurya, S., Annapure, U. S., & Gupta, S. (2025). Nutrient composition of human milk of Indian mothers: Relation with maternal and infant anthropometry. *Journal of Food Science and Technology*, *62*(2), 273-282. https://doi.org/10.1007/s13197-024-06025-w

Kumpulainen, J. (1989). Selenium: Requirement and supplementation. *Acta Paediatrica*, *351*, 114-117. https://doi.org/10.1111/j.1651-2227.1989.tb11221.x

Kwan, C., Fusch, G., Rochow, N., Fusch, C., & collaborators, M. S. (2020). Milk analysis using milk analyzers in a standardized setting (MAMAS) study: A multicentre quality initiative. *Clin Nutr*, *39*(7), 2121-2128. https://doi.org/10.1016/j.clnu.2019.08.028

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

Lauber, E., & Reinhardt, M. (1979). Studies on the quality of breast milk during 23 months of lactation in a rural community of the Ivory Coast. *The American Journal of Clinical Nutrition*, *32*(5), 1159-1173. https://doi.org/10.1093/ajcn/32.5.1159

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/10.1093/jn/nxaa059

Leong, C., Haszard, J. J., Lawley, B., Otal, A., Taylor, R. W., Szymlek-Gay, E. A., Fleming, E. A., Daniels, L., Fangupo, L. J., Tannock, G. W., & Heath, A. M. (2018). Mediation analysis as a means of identifying dietary components that differentially affect the fecal microbiota of infants weaned by modified baby-led and traditional approaches. *Applied and Environmental Microbiology Journal*, *84*(18), e00914-00918. https://doi.org/10.1128/AEM.00914-18

Levy, Y., Zeharia, A., Grunebaum, M., Nitzan, M., & Steinherz, R. (1985). Copper deficiency in infants fed cow milk. *The Journal of Pediatrics*, *106*(5), 786-788. https://doi.org/10.1016/s0022-3476(85)80356-5

Li, F., Rossipal, E., & Irgolic, K. J. (1999). Determination of selenium in human milk by hydride cold-trapping atomic absorption spectrometry and calculation of daily selenium intake. *Journal of Agricultural and Food Chemistry*, *47*(8), 3265-3268. https://doi.org/10.1021/jf990268d

Licon, C. (2022). Proximate and other chemical analyses. In P. L. H. McSweeney & J. P. McNamara (Eds.), *Encyclopedia of dairy sciences* (3rd ed., Vol. 2). Academic Press. https://doi.org/10.1016/B978-0-12-374407-4.00517-3

Lidell, M. E. (2019). Brown adipose tissue in human infants. *Handbook of Experimental Pharmacology*, *251*, 107-123. https://doi.org/10.1007/164_2018_118

Lim, H.-S., Mackey, A. D., Tamura, T., Wong, S. C., & Picciano, M. F. (1998). Measurable human milk folate is increased by treatment with α-amylase and protease in addition to folate conjugase. *Food Chemistry*, *63*(3), 401-407. https://doi.org/10.1016/S0308-8146(98)00054-5

Lim, S. X., Toh, J. Y., van Lee, L., Han, W. M., Shek, L. P., Tan, K. H., Yap, F., Godfrey, K. M., Chong, Y. S., & Chong, M. F. (2018). Food sources of energy and macronutrient intakes among infants from 6 to 12 months of age: The growing up in Singapore towards healthy outcomes (GUSTO) study. *International Journal of Environmental Research and Public Health*, *15*(3), 488. https://doi.org/10.3390/ijerph15030488

Lima, H. K., Vogel, K., Hampel, D., Wagner-Gillespie, M., & Fogleman, A. D. (2020). The associations between light exposure during pumping and holder pasteurization and the macronutrient and vitamin concentrations in human milk. *Journal of Human Lactation*, *36*(2), 254-263. https://doi.org/10.1177/0890334420906828

Liu, S., Sharp, A., Villanueva, E., & Ma, Z. F. (2022). Breast milk iodine concentration (BMIC) as a biomarker of iodine status in lactating women and children <2 years of age: A systematic review. *Nutrients*, *14*(9), 1691. https://doi.org/10.3390/nu14091691

Liu, Z., Diana, A., Slater, C., Preston, T., Gibson, R. S., Houghton, L., & Duffull, S. B. (2019). Development of a nonlinear hierarchical model to describe the disposition of deuterium in mother-infant pairs to assess exclusive breastfeeding practice. *Journal of Pharmacokinetics and Pharmacodynamics*, *46*(1), 1-13. https://doi.org/10.1007/s10928-018-9613-x

Liyanage, C., Hettiarachchi, M., Mangalajeewa, P., & Malawipathirana, S. (2008). Adequacy of vitamin A and fat in the breast milk of lactating women in south Sri Lanka. *Public Health Nutrition*, *11*(7), 747-750. https://doi.org/10.1017/S1368980008001857

Lonnerdal, B. (1986). Effects of maternal dietary intake on human milk composition. *The Journal of Nutrition*, *116*(4), 499-513. https://doi.org/10.1093/jn/116.4.499

Lonnerdal, B. (1995). Magnesium nutrition of infants. *Magnesium Research*, *8*(1), 99-105. https://www.ncbi.nlm.nih.gov/pubmed/7669512

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

Lonnerdal, B. (2014). Infant formula and infant nutrition: Bioactive proteins of human milk and implications for composition of infant formulas. *The American Journal of Clinical Nutrition*, *99*(3), 712S-717S. https://doi.org/10.3945/ajcn.113.071993

Lonnerdal, B., Erdmann, P., Thakkar, S. K., Sauser, J., & Destaillats, F. (2017). Longitudinal evolution of true protein, amino acids and bioactive proteins in breast milk: A developmental perspective. *The Journal of Nutritional Biochemistry*, *41*, 1-11. https://doi.org/10.1016/j.jnutbio.2016.06.001

Lopez-Teros, V., Limon-Miro, A. T., Astiazaran-Garcia, H., Tanumihardjo, S. A., Tortoledo-Ortiz, O., & Valencia, M. E. (2017). 'Dose-to-mother' deuterium oxide dilution technique: An accurate strategy to measure vitamin A intake in breastfed infants. *Nutrients*, *9*(2), 169. https://doi.org/10.3390/nu9020169

Lucas, A., Gibbs, J. A., Lyster, R. L., & Baum, J. D. (1978). Creamatocrit: Simple clinical technique for estimating fat concentration and energy value of human milk. *The British Medical Journal*, *1*(6119), 1018-1020. https://doi.org/10.1136/bmj.1.6119.1018

Makrides, M., Neumann, M. A., Byard, R. W., Simmer, K., & Gibson, R. A. (1994). Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. *The American Journal of Clinical Nutrition*, *60*(2), 189-194. https://doi.org/10.1093/ajcn/60.2.189

Matsiko, E., Hulshof, P. J. M., van der Velde, L., Kenkhuis, M. F., Tuyisenge, L., & Melse-Boonstra, A. (2020). Comparing saliva and urine samples for measuring breast milk intake with the (2)H oxide dose-to-mother technique among children 2-4 months old. *The British Journal of Nutrition*, *123*(2), 232-240. https://doi.org/10.1017/S0007114519002642

McCann, S., Perapoch Amado, M., & Moore, S. E. (2020). The role of iron in brain development: A systematic review. *Nutrients*, *12*(7), 2001. https://doi.org/10.3390/nu12072001

McCune, S., Khwajazada, S., Yerabandi, N., Bode, L., Belfort, M., Todd, D., & Perrin, M. T. (2023). The influence of oligosaccharides when measuring lactose and total carbohydrates in human milk and comparison of methods. *The Journal of Nutrition*, *153*(7), 2117-2124. https://doi.org/10.1016/j.tjnut.2023.05.004

McLachlan, S. K., Thomson, C. D., Ferguson, E. L., & McKenzie, J. E. (2004). Dietary and biochemical selenium status of urban 6- to 24-month-old South Island New Zealand children and their postpartum mothers. *The Journal of Nutrition*, *134*(12), 3290-3295. https://doi.org/10.1093/jn/134.12.3290

McLaren, D. S. (1986). Global occurrence of vitamin A deficiency. In J. C. Bauernfeind (Ed.), *Vitamin A deficiency and its control* (pp. 1-18). Academic Press.

McLean, N. H., Haszard, J. J., Daniels, L., Taylor, R. W., Wheeler, B. J., Conlon, C. A., Beck, K. L., von Hurst, P. R., Te Morenga, L. A., McArthur, J., Paul, R., Katiforis, I., Brown, K. J., Gash, M. C., Rowan, M. M., Casale, M., Cox, A. M., Jones, E. A., Jupiterwala, R. M., . . . Heath, A. M. (2024). Baby food pouches, baby-led weaning, and iron status in New Zealand infants: An observational study. *Nutrients*, *16*(10), 1494. https://doi.org/10.3390/nu16101494

Mendelson, R. A., Anderson, G. H., & Bryan, M. H. (1982). Zinc, copper and iron content of milk from mothers of preterm and full-term infants. *Early Human Development*, *6*(2), 145-151. https://doi.org/10.1016/0378-3782(82)90101-3

Menjo, A., Mizuno, K., Murase, M., Nishida, Y., Taki, M., Itabashi, K., Shimono, T., & Namba, K. (2009). Bedside analysis of human milk for adjustable nutrition strategy. *Acta Paediatrica*, *98*(2), 380-384. https://doi.org/10.1111/j.1651-2227.2008.01042.x

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

Ministry of Health, & University of Otago. (2011). *Methodology report for the 2008/09 New Zealand adult nutrition survery* https://www.health.govt.nz/system/files/2011-10/methodology-report.pdf

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 24 h and throughout the first year of lactation. *The British Journal of Nutrition*, *88*(1), 29-37. https://doi.org/10.1079/BJNBJN2002579

Nagra, S. A. (1989). Longitudinal study in biochemical composition of human milk during first year of lactation. *Journal of Tropical Pediatrics*, *35*(3), 126-128. https://doi.org/10.1093/tropej/35.3.126

Nakamori, M., Ninh, N. X., Isomura, H., Yoshiike, N., Hien, V. T., Nhug, B. T., Nhien, N. V., Nakano, T., Khan, N. C., & Yamamoto, S. (2009). Nutritional status of lactating mothers and their breast milk concentration of iron, zinc and copper in rural Vietnam. *Journal of Nutritional Science and Vitaminology*, *55*(4), 338-345. https://doi.org/10.3177/jnsv.55.338

National Academies of Sciences Engineering and Medicine. (2019). *Dietary reference intakes for sodium and potassium* (V. A. Stallings, M. Harrison, & M. Oria, Eds.). The National Academies Press (US). https://doi.org/10.17226/25353

Neville, M. C., Allen, J. C., Archer, P. C., Casey, C. E., Seacat, J., Keller, R. P., Lutes, V., Rasbach, J., & Neifert, M. (1991). Studies in human lactation: Milk volume and nutrient composition during weaning and lactogenesis. *The American Journal of Clinical Nutrition*, *54*(1), 81-92. https://doi.org/10.1093/ajcn/54.1.81

Neville, M. C., Casey, C. E., Keller, R. P., & Archer, P. (1986). Changes in milk composition after six months of lactation: The effects of duration of lactation and gradual weaning. In M. Hamosh & A. S. Goldman (Eds.), *Human lactation 2: Maternal and environmental factors* (pp. 144-154). Springer. https://doi.org/10.1007/978-1-4615-7207-7

Neville, M. C., Keller, R., Seacat, J., Lutes, V., Neifert, M., Casey, C., Allen, J., & Archer, P. (1988). Studies in human lactation: Milk volumes in lactating women during the onset of lactation and full lactation. *The American Journal of Clinical Nutrition*, *48*(6), 1375-1386. https://doi.org/10.1093/ajcn/48.6.1375

Newman, V. (1994). Vitamin A and breast-feeding: A comparison of data from developed and developing countries. *Food and Nutrition Bulletin*, *15*(2), 1-16. https://doi.org/10.1177/156482659401500201

Nommsen, L. A., Lovelady, C. A., Heinig, M. J., Lonnerdal, B., & Dewey, K. G. (1991). Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: The DARLING study. *The American Journal of Clinical Nutrition*, *53*(2), 457-465. https://doi.org/10.1093/ajcn/53.2.457

O'Neill, E. F., Radmacher, P. G., Sparks, B., & Adamkin, D. H. (2013). Creamatocrit analysis of human milk overestimates fat and energy content when compared to a human milk analyzer using mid-infrared spectroscopy. *Journal of Pediatric Gastroenterology and Nutrition*, *56*(5), 569-572. https://doi.org/10.1097/MPG.0b013e31828390e4

Owino, V. O., Kasonka, L. M., Sinkala, M. M., Wells, J. K., Eaton, S., Darch, T., Coward, A., Tomkins, A. M., & Filteau, S. M. (2007). Fortified complementary foods with or without alpha-amylase treatment increase hemoglobin but do not reduce breast milk intake of 9-mo-old Zambian infants. *The American Journal of Clinical Nutrition*, *86*(4), 1094-1103. https://doi.org/10.1093/ajcn/86.4.1094

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/10.1093/cdn/nzz116

Peterson, G. L. (1977). A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Analytical Biochemistry*, *83*(2), 346-356. https://doi.org/10.1016/0003-2697(77)90043-4

Picciano, M. F. (2001). Nutrient composition of human milk. *Pediatric Clinics of North America*, *48*(1), 53-67. https://doi.org/10.1016/s0031-3955(05)70285-6

Pohl, H. R., Wheeler, J. S., & Murray, H. E. (2013). Sodium and potassium in health and disease. *Metal Ions in Life Sciences*, *13*, 29-47. https://doi.org/10.1007/978-94-007-7500-8_2

Prentice, A., & Barclay, D. V. (1991). Breast-milk calcium and phosphorus concentrations of mothers in rural Zaire. *European Journal of Clinical Nutrition 45*(12), 611-617. https://www.ncbi.nlm.nih.gov/pubmed/1810721

Rajalakshmi, K., & Srikantia, S. G. (1980). Copper, zinc, and magnesium content of breast milk of Indian women. *The American Journal of Clinical Nutrition*, *33*(3), 664-669. https://doi.org/10.1093/ajcn/33.3.664

Redeuil, K., Bénet, S., Affolter, M., Thakkar, S. K., & Campos-Giménez, E. (2017). A novel methodology for the quantification of B-vitamers in breast milk. *Journal of Analytical & Bioanalytical Techniques*, *8*(2), 1000352. https://doi.org/10.4172/2155-9872.1000352

Ren, Q., Li, K., Li, J., Pan, J., Liu, Y., Chen, Y., Xu, Y., & Xie, Q. (2024). Longitudinal changes in human milk minerals and vitamins in the Chinese population: A scoping review. *Nutrients*, *16*(11), 1710. https://doi.org/10.3390/nu16111710

Reniker, L. N., Frazer, L. C., & Good, M. (2023). Key biologically active components of breast milk and their beneficial effects. *Seminars in Pediatric Surgery*, *32*(3), 151306. https://doi.org/10.1016/j.sempedsurg.2023.151306

Reyes, S. M., Brockway, M. M., McDermid, J. M., Chan, D., Granger, M., Refvik, R., Sidhu, K. K., Musse, S., Monnin, C., Lotoski, L., Geddes, D. T., Jehan, F., Kolsteren, P., Allen, L. H., Hampel, D., Eriksen, K. G., Rodriguez, N., & Azad, M. B. (2024). Human Milk Micronutrients and Child Growth and Body Composition in the First 2 years: A Systematic Review. *Advances in Nutrition*, *15*(1), 100082. https://doi.org/10.1016/j.advnut.2023.06.005

Rice, A. L., Stoltzfus, R. J., de Francisco, A., Chakraborty, J., Kjolhede, C. L., & Wahed, M. A. (1999). Maternal vitamin A or beta-carotene supplementation in lactating Bangladeshi women benefits mothers and infants but does not prevent subclinical deficiency. *The Journal of Nutrition*, *129*(2), 356-365. https://doi.org/10.1093/jn/129.2.356

Rios-Leyvraz, M., & Yao, Q. (2023). Calcium, zinc, and vitamin D in breast milk: A systematic review and meta-analysis. *International Breastfeeding Journal 18*(1), 27. https://doi.org/10.1186/s13006-023-00564-2

Robinson, M. F. (1989). Selenium in human nutrition in New Zealand. *Nutrition Reviews*, *47*(4), 99-107. https://doi.org/10.1111/j.1753-4887.1989.tb02807.x

Roekens, E., Deelstra, H., & Robberecht, H. (1985). Trace elements in human milk, selenium a case study. *The Science of the Total Environment*, *42*(1-2), 91-108. https://doi.org/10.1016/0048-9697(85)90010-5

Ross, A. C., & Tan, L. (2016). Vitamin A deficiencies and excess. In R. M. Kliegman, B. F. Stanton, J. W. Geme, & N. F. Schor (Eds.), *Nelson Textbook of Pediatrics* (pp. 317-321). Elsevier.

Roy, S. K., Islam, A., Molla, A., Akramuzzaman, S. M., Jahan, F., & Fuchs, G. (1997). Impact of a single megadose of vitamin A at delivery on breastmilk of mothers and morbidity of their infants. *European Journal of Clinical Nutrition 51*(5), 302-307. https://doi.org/10.1038/sj.ejcn.1600398

Saarinen, U. M., Siimes, M. A., & Dallman, P. R. (1977). Iron absorption in infants: high bioavailability of breast milk iron as indicated by the extrinsic tag method of iron absorption and by the concentration of serum ferritin. *The Journal of Pediatrics*, *91*(1), 36-39. https://doi.org/10.1016/s0022-3476(77)80439-3

Sabatier, M., Garcia-Rodenas, C. L., Castro, C. A., Kastenmayer, P., Vigo, M., Dubascoux, S., Andrey, D., Nicolas, M., Payot, J. R., Bordier, V., Thakkar, S. K., Beauport, L., Tolsa, J. F., Fumeaux, C. J. F., & Affolter, M. (2019). Longitudinal changes of mineral concentrations in preterm and term human milk from lactating Swiss women. *Nutrients*, *11*(8), 1855. https://doi.org/10.3390/nu11081855

Sabzkoohi, H. A., Dodier, V., & Kolliopoulos, G. (2023). A validated analytical method to measure metals dissolved in deep eutectic solvents. *RSC Advances*, *13*(22), 14887-14898. https://doi.org/10.1039/d3ra02372a

Sakurai, T., Furukawa, M., Asoh, M., Kanno, T., Kojima, T., & Yonekubo, A. (2005). Fat-soluble and water-soluble vitamin contents of breast milk from Japanese women. *Journal of Nutritional Science and Vitaminology 51*(4), 239-247. https://doi.org/10.3177/jnsv.51.239

Saloojee, H., & Pettifor, J. M. (2001). Iron deficiency and impaired child development. *The British Medical Journal*, *323*(7326), 1377-1378. https://doi.org/10.1136/bmj.323.7326.1377

Sankar, M. J., Sinha, B., Chowdhury, R., Bhandari, N., Taneja, S., Martines, J., & 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

Savenije, O. E., & Brand, P. L. (2006). Accuracy and precision of test weighing to assess milk intake in newborn infants. *Archives of Disease in Childhood. Fetal and Neonatal Edition*, *91*(5), F330-332. https://doi.org/10.1136/adc.2005.091876

Shalom, O. I., Lubetzky, R., Mimouni, F. B., & Mandel, D. (2024). Selenium concentrations in expressed human milk: A systematic review and meta-analysis. *Journal of Perinatology*, *44*(11), 1607-1610. https://doi.org/10.1038/s41372-024-02057-4

Shashiraj, Faridi, M. M., Singh, O., & Rusia, U. (2006). Mother's iron status, breastmilk iron and lactoferrin--are they related? *European Journal of Clinical Nutrition*, *60*(7), 903-908. https://doi.org/10.1038/sj.ejcn.1602398

Shergill-Bonner, R. (2017). Micronutrients. *Paediatrics and Child Health*, *27*(8), 357-362. https://doi.org/10.1016/j.paed.2017.04.002

Silvestre, D., Fraga, M., Gormaz, M., Torres, E., & Vento, M. (2014). Comparison of mid-infrared transmission spectroscopy with biochemical methods for the determination of macronutrients in human milk. *Maternal & Child Nutrition*, *10*(3), 373-382. https://doi.org/10.1111/j.1740-8709.2012.00431.x

Simmer, K., Ahmed, S., Carlsson, L., & Thompson, R. P. (1990). Breast milk zinc and copper concentrations in Bangladesh. *The British Journal of Nutrition*, *63*(1), 91-96. https://doi.org/10.1079/bjn19900094

Skeaff, S. A., McLean, R., Mann, J., & Williams, S. (2013). *The impact of mandatory fortification of bread with iodine*. Ministry for Primary Industries. https://www.mpi.govt.nz/dmsdocument/4097-the-impact-of-mandatory-fortification-of-bread-with-iodine

Smilowitz, J. T., Gho, D. S., Mirmiran, M., German, J. B., & Underwood, M. A. (2014). Rapid measurement of human milk macronutrients in the neonatal intensive care unit: Accuracy and precision of fourier transform mid-infrared spectroscopy. *Journal of Human Lactation*, *30*(2), 180-189. https://doi.org/10.1177/0890334413517941

Solomons, N. W. (1985). Biochemical, metabolic, and clinical role of copper in human nutrition. *Journal of the American College of Nutrition*, *4*(1), 83-105. https://doi.org/10.1080/07315724.1985.10720069

Sommer, A., Tarwotjo, I., Djunaedi, E., West, K. P., Jr., Loeden, A. A., Tilden, R., & Mele, L. (1986). Impact of vitamin A supplementation on childhood mortality. A randomised controlled community trial. *Lancet*, *1*(8491), 1169-1173. https://doi.org/10.1016/s0140-6736(86)91157-8

Stoltzfus, R. J., & Underwood, B. A. (1995). Breast-milk vitamin A as an indicator of the vitamin A status of women and infants. *Bulletin of the World Health Organization*, *73*(5), 703-711. https://www.ncbi.nlm.nih.gov/pubmed/8846497

Su, Y., Mao, Y., Tian, F., Cai, X., Chen, R., Li, N., Qian, C., Li, X., Zhao, Y., & Wang, Y. (2022). Profile of folate in breast milk from Chinese women over 1-400 days postpartum. *Nutrients*, *14*(14), 2962. https://doi.org/10.3390/nu14142962

Szyller, H., Antosz, K., Batko, J., Mytych, A., Dziedziak, M., Wrzesniewska, M., Braksator, J., & Pytrus, T. (2024). Bioactive components of human milk and their impact on child's health and development, literature review. *Nutrients*, *16*(10), 1487. https://doi.org/10.3390/nu16101487

Tamura, T., & Picciano, M. F. (2006). Folate determination in human milk. *Journal of Nutritional Science and Vitaminology 52*(2), 161. https://doi.org/10.3177/jnsv.52.161

Tarwotjo, I., West, K., Mele, L., Nur, S., Nendrawati, H., Kraushaar, D., & Tilden, R. L. (1989). Determinants of community-based coverage: Periodic vitamin A supplementation. *American Journal of Public Health*, *79*(7), 847-849. https://doi.org/10.2105/ajph.79.7.847

Taylor, R. W., Conlon, C. A., Beck, K. L., von Hurst, P. R., Te Morenga, L. A., Daniels, L., Haszard, J. J., Meldrum, A. M., McLean, N. H., Cox, A. M., Tukuafu, L., Casale, M., Brown, K. J., Jones, E. A., Katiforis, I., Rowan, M., McArthur, J., Fleming, E. A., Wheeler, B. J., . . . Heath, A. M. (2021). Nutritional implications of baby-led weaning and baby food pouches as novel methods of infant feeding: Protocol for an observational study. *JMIR Research Protocols*, *10*(4), e29048. https://doi.org/10.2196/29048

Thajer, A., Fusch, G., Binder, C., Berger, A., & Fusch, C. (2019). Human milk analyser underestimated protein content of unfortified and fortified samples compared to elemental analysis. *Acta Paediatrica*, *108*(12), 2298-2300. https://doi.org/10.1111/apa.14982

Thomson, C. D. (2004). Selenium and iodine intakes and status in New Zealand and Australia. *The British Journal of Nutrition*, *91*(5), 661-672. https://doi.org/10.1079/BJN20041110

Tie, W. J., Kent, J. C., Tat Lai, C., Rea, A., Hepworth, A. R., Murray, K., & Geddes, D. T. (2021). Reproducibility of the creamatocrit technique for the measurement of fat content in human milk. *Food Chemistry*, *356*, 129708. https://doi.org/10.1016/j.foodchem.2021.129708

Triggiani, V., Tafaro, E., Giagulli, V. A., Sabba, C., Resta, F., Licchelli, B., & Guastamacchia, E. (2009). Role of iodine, selenium and other micronutrients in thyroid function and disorders. *Endocrine, Metabolic & Immune Disorders Drug Targets*, *9*(3), 277-294. https://doi.org/10.2174/187153009789044392

Udipi, S. A., Kirksey, A., & Roepke, J. L. (1987). Diurnal variations in folacin levels of human milk: Use of a single sample to represent folacin concentration in milk during a 24-h period. *The American Journal of Clinical Nutrition*, *45*(4), 770-779. https://doi.org/10.1093/ajcn/45.4.770

Umeta, M., West, C. E., Verhoef, H., Haidar, J., & Hautvast, J. G. (2003). Factors associated with stunting in infants aged 5-11 months in the Dodota-Sire District, rural Ethiopia. *The Journal of Nutrition*, *133*(4), 1064-1069. https://doi.org/10.1093/jn/133.4.1064

United States National Research Council. (1989). *Diet and health: Implications for reducing chronic disease risk*. National Research Council Committee on Diet and Health. https://doi.org/10.17226/1222

Vallee, B. L., & Auld, D. S. (1990). Zinc coordination, function, and structure of zinc enzymes and other proteins. *Biochemistry*, *29*(24), 5647-5659. https://doi.org/10.1021/bi00476a001

van Sadelhoff, J. H. J., Siziba, L. P., Buchenauer, L., Mank, M., Wiertsema, S. P., Hogenkamp, A., Stahl, B., Garssen, J., Rothenbacher, D., & Genuneit, J. (2021). Free and total amino acids in human milk in relation to maternal and infant characteristics and infant health outcomes: The ulm SPATZ health study. *Nutrients*, *13*(6), 2009. https://doi.org/10.3390/nu13062009

Vaughan, L. A., Weber, C. W., & Kemberling, S. R. (1979). Longitudinal changes in the mineral content of human milk. *The American Journal of Clinical Nutrition*, *32*(11), 2301-2306. https://doi.org/10.1093/ajcn/32.11.2301

Victora, C. G., Bahl, R., Barros, A. J., Franca, G. V., Horton, S., Krasevec, J., Murch, S., Sankar, M. J., Walker, N., Rollins, N. C., & Lancet Breastfeeding Series, G. (2016). Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. *Lancet*, *387*(10017), 475-490. https://doi.org/10.1016/S0140-6736(15)01024-7

Wack, R. P., Lien, E. L., Taft, D., & Roscelli, J. D. (1997). Electrolyte composition of human breast milk beyond the early postpartum period. *Nutrition*, *13*(9), 774-777. https://doi.org/10.1016/s0899-9007(97)00187-1

Wang, Y., Zhang, Z., Ge, P., Wang, Y., & Wang, S. (2009). Iodine status and thyroid function of pregnant, lactating women and infants (0-1 yr) residing in areas with an effective universal salt iodization program. *Asia Pacific Journal of Clinical Nutrition*, *18*(1), 34-40. https://www.ncbi.nlm.nih.gov/pubmed/19329393

Woo Baidal, J. A., Locks, L. M., Cheng, E. R., Blake-Lamb, T. L., Perkins, M. E., & Taveras, E. M. (2016). Risk factors for childhood obesity in the first 1,000 days: A systematic review. *American Journal of Preventive Medicine*, *50*(6), 761-779. https://doi.org/10.1016/j.amepre.2015.11.012

World Health Organization. (1998). *Complementary feeding of young children in developing countries: a review of current scientific knowledge*. https://iris.who.int/handle/10665/65932

World Health Organization. (2006). WHO child growth standards based on length/height, weight and age. *Acta Paediatrica*, *450*, 76-85. https://doi.org/10.1111/j.1651-2227.2006.tb02378.x

World Health Organization. (2023). *Infant and young child feeding*. Retrieved October 14, 2024, from https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding

World Health Organization, & Food and Agricultural Organization of the United Nations. (2002). *Human vitamin and mineral requirements* https://iris.who.int/bitstream/handle/10665/42716/9241546123.pdf?sequence=1

World Health Organization, & Food and Agricultural Organization of the United Nations. (2004). *Vitamin and mineral requirements in human nutrition* https://www.who.int/publications/i/item/9241546123

World Health Organization, Food and Agricultural Organization of the United Nations, & United Nations University. (2001). *Human energy requirements* https://openknowledge.fao.org/server/api/core/bitstreams/65875dc7-f8c5-4a70-b0e1-f429793860ae/content

World Health Organization, Food and Agricultural Organization of the United Nations, & United Nations University. (2004). Energy requirements of infants from birth to 12 months. In *Human energy requirements: Report of a joint FAO/WHO/UNU expert consultation*. https://www.fao.org/4/y5686e/y5686e00.htm#Contents

World Health Organization, & UNICEF. (2003). *Global strategy for infant and young child feeding* https://iris.who.int/bitstream/handle/10665/42590/9241562218.pdf?sequence=1

World Health Organization, UNICEF, & ICCIDD. (2007). *Assessment of iodine deficiency disorders and monitoring their elimination* (3rd ed.) https://apps.who.int/iris/bitstream/handle/10665/43781/9789241595827_eng.pdf

World Health Organization Secretariat, Andersson, M., de Benoist, B., Delange, F., & Zupan, J. (2007). Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: Conclusions and recommendations of the technical consultation. *Public Health Nutrition*, *10*(12A), 1606-1611. https://doi.org/10.1017/S1368980007361004

Yamawaki, N., Yamada, M., Kan-no, T., Kojima, T., Kaneko, T., & Yonekubo, A. (2005). Macronutrient, mineral and trace element composition of breast milk from Japanese women. *Journal of Trace Elements in Medicine and Biology*, *19*(2-3), 171-181. https://doi.org/10.1016/j.jtemb.2005.05.001

Yang, T., Zhang, L., Bao, W., & Rong, S. (2018). Nutritional composition of breast milk in Chinese women: A systematic review. *Asia Pacific Journal of Clinical Nutrition*, *27*(3), 491-502. https://doi.org/10.6133/apjcn.042017.13

Zheng, M., Lamb, K. E., Grimes, C., Laws, R., Bolton, K., Ong, K. K., & Campbell, K. (2018). Rapid weight gain during infancy and subsequent adiposity: A systematic review and meta-analysis of evidence. *Obesity Reviews*, *19*(3), 321-332. https://doi.org/10.1111/obr.12632

Zhu, M., Yang, Z., Ren, Y., Duan, Y., Gao, H., Liu, B., Ye, W., Wang, J., & Yin, S. (2017). Comparison of macronutrient contents in human milk measured using mid-infrared human milk analyser in a field study vs. chemical reference methods. *Maternal & Child Nutrition*, *13*(1), e12248. https://doi.org/10.1111/mcn.12248

Zimmermann, M. B. (2009). Iodine deficiency. *Endocrine Reviews*, *30*(4), 376-408. https://doi.org/10.1210/er.2009-0011

# Appendices

**Appendix A.** Literature values of estimated HM intake at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | mL/d, mean ± SD |
|---|---|---|---|
| Dewey et al., (1984) | US | 7 | 875.0 ± 142.0 |
| | | 8 | 834.0 ± 99.00 |
| | | 9 | 774.0 ± 180.0 |
| | | 10 | 691.0 ± 233.0 |
| | | 11 | 516.0 ± 215.0 |
| | | 12 | 759.0 ± 28.00 |
| Jarjou et al (2012) | Gambia | 12 | 763.0 ± 213.0 |
| Krebs et al. (1994) | US | 7 | 659.2 ± 154.5[a] |
| Neville et al. (1988) | US | 7 | 721.0 ± 154.0 |
| | | 8 | 622.0 ± 210.0 |
| | | 9 | 618.0 ± 220.0 |
| | | 10 | 551.0 ± 234.0 |
| | | 11 | 554.0 ± 240.0 |
| | | 12 | 403.0 ± 250.0 |

[a] Converted from g/day

**Appendix B.** Literature values of infant estimated energy intake at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Feeding type[a] | Milk intake (kJ/day)[b] | Food intake (kJ/day)[b] | Total intake (kJ/day)[b] |
|---|---|---|---|---|---|---|
| Ahn and Jeong (1998) | Korea | 7–9 | BF | — | — | 2331±402.0[c] |
| | | | FF | | | 2682±408.0[c] |
| Campos et al. (2010) | Guatemala | 7–9 | BF | — | 1272±836.8[c] | — |
| | | 10–12 | | | 2054±966.5[c] | |
| Dewey et al. (1984) | US | 7 | BF | 2372±382.4[cd] | — | 2552±364.0[c] |
| | | 8 | | 2276±409.6[cd] | | 2648±334.7[c] |
| | | 9 | | 2100±489.5[cd] | | 2531±485.3[c] |
| | | 10 | | 1882±594.1[cd] | | 2812±728.0[c] |
| | | 11 | | 1435±665.3[cd] | | 2761±518.8[c] |
| | | 12 | | 2079±64.60[cd] | | 3247±96.23[c] |
| Heinig et al. (1993) | US | 6 | BF | 2170±480.0[e] | 390.0±440.0[e] | 2560±420.0[e] |
| | | 9 | | 1840±600.0[e] | 1140±800.0[e] | 2980±680.0[e] |
| | | 12 | | 1270±720.0[e] | 2180±930.0[e] | 3450±660.0[e] |
| | | 6 | FF | 2680±680.0[e] | 490±410.0[e] | 3160±570.0[e] |
| | | 9 | | 2290±610.0[e] | 1270±560.0[e] | 3570±700.0[e] |
| | | 12 | | 2080±810.0[e] | 1980±870.0[e] | 4060±960.0[e] |
| Hernandez et al. (2011) | Guatemala | 7–9 | BF | — | 1356±828.4[c] | — |
| | | 10–12 | | | 1292±1067[c] | |
| Lim et al. (2018) | Singapore | 6 | BF | — | — | 2728±468.6[c] |
| | | | MF | | | 2707±410.0[c] |
| | | | FF | | | 2653±761.5[c] |
| | | 9 | BF | | | 2427±510.5[c] |
| | | | MF | | | 2858±573.2[c] |
| | | | FF | | | 2874±756.7[c] |
| | | 12 | BF | | | 2996±782.4[c] |
| | | | MF | | | 3326±686.2[c] |
| | | | FF | | | 3176±903.7[c] |
| Mitoulas et al. (2002) | Australia | 6 | BF | 1040±75.00 | — | — |
| | | 9 | | 995.0±132.0 | | |
| | | 12 | | 738.0±141.0 | | |
| Owino et al. (2007) | Zambia | 9 | BF | 1833 (1586, 2075)[cf] | 1477 (1251, 1703)[cf] | — |

[a] Abbreviations: BF = breastfed; FF = formula-fed; MF = mixed-fed
[b] Values are reported as mean ± SD unless otherwise specified
[c] Values converted from kcal
[d] Milk intake calculated from percentage kJ intake from milk and total kJ intake
[e] Converted from MJ
[f] Reported as mean (95% CI)

**Appendix C.** Literature values of HM energy concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Concentration (kJ/100 mL), mean ± SD |
|---|---|---|---|
| Chang et al. (2015) | Korea | 7–8 | 259.0 ± 54.80 |
| Kim et al. (2017) | Korea | 7–11 | 258.0 ± 48.10 |
| Kumari-Maurya et al. (2025) | India | 7–12 | 242.7 ± 47.70 |
| Lauber and Reinhardt (1979) | Ivory Coast | 6 | 297.1 ± 33.90 |
| | | 12 | 256.9 ± 24.30 |
| Mitoulas et al. (2002) | Australia | 6 | 258.0 ± 9.00 |
| | | 9 | 281.2 ± 9.00 |
| | | 12 | 279.0 ± 14.00 |
| Nakamori et al. (2009) | Vietnam | 6–12 | 255.6 ± 49.00 |
| Nommsen et al. (1991) | US | 6 | 295.8 ± 38.50 |
| | | 9 | 296.7 ± 31.00 |
| | | 12 | 295.4 ± 46.00 |
| Yamawaki et al. (2005) | Japan | 6–12 | 261.9 ± 47.30 |

**Appendix D.** Literature values of HM lactose concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Concentration (g/100 mL), mean ± SD |
|---|---|---|---|
| Chang et al. (2015) | Korea | 6–7 | 7.10 ± 0.40 |
| | | 7–8 | 7.10 ± 0.40 |
| Dewey et al. (1984) | US | 7–11 | 7.57 ± 0.36[a] |
| | | | 7.82 ± 0.36[b] |
| Gridneva et al. (2019) | Australia | 9 | 6.53 ± 0.53 |
| | | 12 | 6.69 ± 0.40 |
| Kim et al. (2017) | Korea | 7–11 | 6.99 ± 0.34 |
| Kugananthan et al. (2017) | Australia | 9 | 6.84 ± 0.88 |
| | | 12 | 6.97 ± 0.91 |
| Kumari-Maurya et al. (2025) | India | 7–12 | 6.30 ± 0.30 |
| Lauber and Reinhardt (1979) | Ivory Coast | 6 | 7.15 ± 0.72 |
| | | 12 | 7.00 ± 0.97 |
| Mitoulas et al. (2002) | Australia | 6 | 6.25 ± 1.70 |
| | | 9 | 6.28 ± 0.15 |
| | | 12 | 6.14 ± 2.90 |
| Nagra (1989) | Pakistan | 6 | 6.92 ± 0.17[c] |
| | | 7 | 6.80 ± 0.25[c] |
| | | 8 | 6.87 ± 0.27[c] |
| | | 9 | 6.91 ± 0.07[c] |
| | | 10 | 6.87 ± 0.17[c] |
| | | 11 | 5.88 ± 0.12[c] |
| | | 12 | 6.81 ± 0.21[c] |
| Nommsen et al. (1991) | US | 6 | 7.44 ± 0.19 |
| | | 9 | 7.35 ± 0.29 |
| | | 12 | 7.40 ± 0.27 |
| Wack et al. (1997) | US | 6–8 | 7.10 ± 0.40 |
| | | 8–10 | 7.00 ± 0.40 |
| | | 10–12 | 7.10 ± 0.40 |
| Yamawaki et al. (2005) | Japan | 6–12 | 6.46 ± 0.61 |

[a] Full lactation (≥500ml expressed per day)
[b] Volume unknown but nursing ≥4 times per day
[c] Converted from mg/dl

**Appendix E.** Literature values of HM fat concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Concentration (g/100 mL), mean ± SD[a] |
|---|---|---|---|
| Ayah et al. (2007) | Kenya | 6 | 3.01 (27.40, 32.90)[b] |
| Chang et al. (2015) | Korea | 7–8 | 3.20 ± 1.60 |
| Dewey et al. (1984) | US | 7–11 | 3.45 ± 1.53[c] |
| | | | 5.16 ± 2.73[d] |
| Dijkhuizen et al. (2004) | Indonesia | 6 | 3.38 ± 1.40 |
| Kim et al. (2017) | Korea | 7–11 | 3.20 ± 1.35 |
| Kumari-Maurya et al. (2025) | India | 7–12 | 3.00 ± 1.50 |
| Liyanage et al. (2008) | Sri Lanka | 7 | 2.46 ± 1.26 |
| Mitoulas et al. (2002) | Australia | 6 | 3.72 ± 0.14 |
| Nagra (1989) | Pakistan | 6 | 3.51 ± 0.02[e] |
| | | 7 | 3.58 ± 0.34[e] |
| | | 8 | 3.69 ± 0.27[e] |
| | | 9 | 3.72 ± 0.24[e] |
| | | 10 | 3.64 ± 0.33[e] |
| | | 11 | 3.65 ± 0.29[e] |
| | | 12 | 3.67 ± 0.30[e] |
| Nakamori et al. (2009) | Vietnam | 6–12 | 3.08 ± 1.26 |
| Yamawaki et al. (2005) | Japan | 6–12 | 3.17 ± 1.36 |

[a] Unless otherwise specified.
[b] Reported as mean (95% CI)
[c] Full lactation (≥500ml expressed per day)
[d] Volume unknown but nursing ≥4 times per day
[e] Converted from mg/dl

**Appendix F**. Literature values of HM protein concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Concentration (g/100 mL), mean ± SD |
|---|---|---|---|
| Chang et al. (2015) | Korea | 6–7 | 1.30 ± 0.20 |
| | | 7–8 | 1.20 ± 0.20 |
| Dewey et al. (1984) | US | 7–11 | 1.24 ± 0.22[a] |
| | | | 1.55 ± 0.48[b] |
| Gridneva et al. (2018) | Australia | 9 | 1.00 ± 0.11 |
| | | 12 | 1.07 ± 0.28 |
| Kim et al. (2017) | Korea | 7–11 | 1.24 ± 0.20 |
| Kugananthan et al. (2017) | Australia | 9 | 1.08 ± 0.46 |
| | | 12 | 1.28 ± 6.74 |
| Kumari-Maurya et al. (2025) | India | 7–12 | 0.70 ± 0.30 |
| Mitoulas et al. (2002) | Australia | 6 | 0.80 ± 0.04 |
| | | 9 | 0.83 ± 0.05 |
| | | 12 | 0.83 ± 0.06 |
| Nagra (1989) | Pakistan | 6 | 1.01 ± 0.06[c] |
| | | 7 | 0.99 ± 1.02[c] |
| | | 8 | 0.98 ± 0.02[c] |
| | | 9 | 0.99 ± 0.01[c] |
| | | 10 | 0.99 ± 0.00[c] |
| | | 11 | 1.01 ± 0.05[c] |
| | | 12 | 1.00 ± 0.02[c] |
| Nakamori et al. (2009) | Vietnam | 6–12 | 1.05 ± 0.17 |
| Nommsen et al. (1991) | US | 6 | 1.14 ± 0.15 |
| | | 9 | 1.16 ± 0.18 |
| | | 12 | 1.24 ± 0.15 |
| Yamawaki et al. (2005) | Japan | 6–12 | 0.99 ± 0.12 |

[a] Full lactation (≥500ml expressed per day)
[b] Volume unknown but nursing ≥4 times per day
[c] Converted from mg/dl

**Appendix G**. Literature values of HM zinc concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Zinc (µg/L), mean ± SD[a] |
|---|---|---|---|
| Casey et al. (1989) | US | 6 | 1098 ± 601.5[b] |
| | | 7 | 921.9 ± 457.7[b] |
| | | 8 | 745.3 ± 209.2[b] |
| | | 9 | 771.5 ± 392.3[b] |
| | | 10 | 621.1 ± 346.5[b] |
| | | 11 | 529.6 ± 326.9[b] |
| | | 12 | 542.7 ± 300.8[b] |
| Dewey et al. (1984) | US | 7–11 | 420.0 ± 220.0[c] |
| | | | 710.0 ± 510.0[d] |
| Domellof et al. (2004) | Sweden | 9 | 460.0 ± 260.0 |
| | Honduras | | 700.0 ± 180.0 |
| Han et al. (2022) | New Zealand | 12 | 340.0 (392.0, 295.0) |
| Javad et al. (2018) | Iran | 6–7 | 1410 ± 1270 |
| | | 12 | 970.0 ± 860.0 |
| Kim et al. (2017) | Korea | 7–11 | 745.7 ± 368.3 |
| Krebs et al. (1995) | US | 6 | 1092 ± 667.0[b] |
| | | 7 | 850.0 ± 510.0[b] |
| | | 8 | 870.0 ± 582.0[b] |
| | | 9 | 778.0 ± 510.0[b] |
| Lauber and Reinhardt (1979) | Ivory Coast | 6 | 2300 ± 900.0 |
| | | 12 | 1600 ± 900.0 |
| Nagra (1989) | Pakistan | 6 | 900.0 ± 100.0[e] |
| | | 7 | 1000.0 ± 100.0[e] |
| | | 8 | 800.0 ± 200.0[e] |
| | | 9 | 800.0 ± 200.0[e] |
| | | 10 | 1000 ± 300.0[e] |
| | | 11 | 1000 ± 100.0[e] |
| | | 12 | 900.0 ± 300.0[e] |
| Nakamori et al. (2009) | Vietnam | 6–8 | 590.0 (480.0, 850.0)[f] |
| | | 9–12 | 370.0 (200.0, 730.0)[f] |
| Rajalakshmi and Srikantia (1980) | India | 7–12 | 1120 ± 52.00 |
| Ren et al. (2024)[g] | China | 8–12 | 690.0 (550.0, 840.0)[f] |
| Rios-Leyvraz and Yao (2023)[h] | *Multiple* | 6–12 | 1180 (1050, 1320)[f] |
| Simmer et al. (1990) | Bangladesh | 6 | 928.4 ± 405.4[b] |
| | | 9 | 725.7 ± 614.6[b] |
| | | 12 | 542.7 ± 274.6[b] |
| Vaughan et al. (1979) | US | 7–9 | 750.0 ± 110.0[h] |
| | | 10–12 | 630.0 ± 90.00[h] |
| Yamawaki et al. (2005) | Japan | 6–12 | 650.0 ± 430.0 |

[a] Unless otherwise specified.
[b] Converted from µmol/L
[c] Full lactation (≥500ml expressed per day)
[d] Volume unknown but nursing ≥4 times per day
[e] Converted from mg/dl
[f] Reported as mean (95% CI)

[g] Meta-analysis
[h] Converted from µg/ml

**Appendix H**. Literature values of HM copper concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Copper (µg/L), mean ± SD[a] |
|---|---|---|---|
| Dewey et al. (1984) | US | 7–11 | 170.0 ± 50.00[b] |
| | | | 170.0 ± 70.00[c] |
| Domellof et al. (2004) | Sweden | 9 | 120.0 ± 220.0 |
| | Honduras | | 160.0 ± 210.0 |
| Javad et al. (2018) | Iran | 6–7 | 370.0 ± 210.0 |
| | | 12 | 270.0 ± 140.0 |
| Lauber and Reinhardt (1979) | Ivory Coast | 6 | 140.0 ± 70.00 |
| | | 12 | 130.0 ± 40.00 |
| Nagra (1989) | Pakistan | 6 | 290.0 ± 27.00[d] |
| | | 7 | 272.0 ± 20.00[d] |
| | | 8 | 281.0 ± 32.00[d] |
| | | 9 | 268.0 ± 30.00[d] |
| | | 10 | 275.0 ± 18.00[d] |
| | | 11 | 263.0 ± 25.00[d] |
| | | 12 | 277.0 ± 24.00[d] |
| Nakamori et al. (2009) | Vietnam | 6–8 | 190.0 ± 50.00 |
| | | 9–12 | 180.0 ± 50.00 |
| | | 12 | 130.0 ± 40.00 |
| Rajalakshmi and Srikantia (1980) | India | 7–12 | 170.0 ± 9.00 |
| Ren et al. (2024)[e] | China | 8–12 | 210 (180, 240)[f] |
| Simmer et al. (1990) | Bangladesh | 6 | 188.7 ± 69.27 |
| | | 9 | 158.9 ± 129.0 |
| | | 12 | 119.5 ± 79.43 |
| Vaughan et al (1979) | US | 7–9 | 300.0 ± 30.00[g] |
| | | 10–12 | 240.0 ± 40.00[g] |

[a] Unless otherwise specified.
[b] Full lactation (≥500ml expressed per day)
[c] Volume unknown but nursing ≥4 times per day
[d] Converted from mg/dl
[e] Meta-analysis
[f] Reported as mean (95% CI)
[g] Converted from µg/ml

**Appendix I**. Literature values of HM calcium concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Calcium (mg/L), mean ± SD[a] |
|---|---|---|---|
| Dewey et al. (1984) | US | 7–11 | 236.0 ± 29.00[b] |
| | | | 208.0 ± 53.00[c] |
| Jarjou et al. (2012) | Gambia | 12 | 152.0 ± 24.00 |
| Javad et al. (2018) | Iran | 6–7 | 242.1 ± 50.58 |
| | | 12 | 259.8 ± 71.11 |
| Kim et al. (2017) | Korea | 7–11 | 267.7 ± 58.10 |
| Kumari-Maurya et al. (2025) | India | 7–12 | 488.9 ± 144.7 |
| Nagra (1989) | Pakistan | 6 | 321.0 ± 12.00[d] |
| | | 7 | 313.0 ± 16.00[d] |
| | | 8 | 318.0 ± 7.00[d] |
| | | 9 | 315.0 ± 12.00[d] |
| | | 10 | 320.0 ± 16.00[d] |
| | | 11 | 316.0 ± 11.00[d] |
| | | 12 | 314.0 ± 21.00[d] |
| Prentice and Barclay (1991) | Zaire | 6 | 220.0 ± 10.00 |
| | | 9 | 198.0 ± 11.00 |
| | | 12 | 191.0 ± 8.00 |
| Rios-Leyvraz and Yao (2023)[e] | *Multiple* | 6–12 | 214.0 (163.0, 266.0)[f] |
| Umeta et al. (2003) | Ethiopia | 5–11 | 14.03 ± 0.40 |
| Vaughan et al. (1979) | US | 7–9 | 175.0 ± 28.00 |
| | | 10–12 | 170.0 ± 25.00 |
| Yamawaki et al. (2005) | Japan | 6–12 | 260.0 ± 54.00 |

[a] Unless otherwise specified.
[b] Full lactation (≥500ml expressed per day)
[c] Volume unknown but nursing ≥4 times per day
[d] Converted from mg/dl
[e] Meta-analysis
[f] Reported as mean (95% CI)

**Appendix J**. Literature values of HM iron concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Iron (µg/L), mean ± SD[a] |
|---|---|---|---|
| Dewey et al. (1984) | US | 7–11 | 180.0 ± 100.0[b] |
| | | | 200.0 ± 120.0[c] |
| Domellof et al. (2004) | Sweden | 9 | 290.0 ± 220.0* |
| | Honduras | | 210.0 ± 250.0* |
| Javad et al. (2018) | Iran | 6–7 | 530.0 ± 420.0 |
| | | 12 | 420.0 ± 330.0 |
| Kim et al. (2017) | Korea | 7–11 | 457.2 ± 540.3 |
| Lauber and Reinhardt (1979) | Ivory Coast | 6 | 550.0 ± 100.0 |
| | | 12 | 540.0 ± 120.0 |
| Nakamori et al. (2009) | Vietnam | 6–8 | 400.0 ± 140.0 |
| | | 9–12 | 460.0 ± 150.0 |
| Ren et al. (2024)[d] | China | 8–12 | 340 (310, 380)[e] |
| Shashiraj et al. (2006) | India | 6 | 260.0[f] |
| | | | 270.0[g] |
| Vaughan et al. (1979) | US | 7–9 | 420.0 ± 60.00 |
| | | 10–12 | 380.0 ± 50.00 |
| Yamawaki et al. (2005) | Japan | 6–12 | 850.0 ± 660.0 |

[a] Unless otherwise specified
[b] Full lactation (≥500ml expressed per day)
[c] Volume unknown but nursing ≥4 times per day
[d] Meta-analysis
[e] Reported as mean (95% CI)
[f] Non-anaemic mothers
[g] Anaemic mothers

**Appendix K**. Literature values of HM magnesium concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Magnesium (mg/L), mean ± SD |
|---|---|---|---|
| Dewey et al. (1984) | US | 7–11 | 31.90 ± 4.80[a] |
| | | | 29.50 ± 5.70[b] |
| Javad et al. (2018) | Iran | 6–7 | 33.55 ± 6.56 |
| | | 12 | 32.00 ± 8.91 |
| Kim et al. (2017) | Korea | 7–11 | 32.00 ± 5.40 |
| Nagra (1989) | Pakistan | 6 | 24.00 ± 3.00[c] |
| | | 7 | 22.00 ± 5.00[c] |
| | | 8 | 25.00 ± 5.00[c] |
| | | 9 | 26.00 ± 3.00[c] |
| | | 10 | 22.00 ± 4.00[c] |
| | | 11 | 28.00 ± 4.00[c] |
| | | 12 | 23.00 ± 5.00[c] |
| Rajalakshmi and Srikantia (1980) | India | 7–12 | 30.77 ± 1.07 |
| Vaughan et al. (1979) | US | 7–9 | 26.00 ± 3.30 |
| | | 10–12 | 29.00 ± 4.70 |
| Yamawaki et al. (2005) | Japan | 6–12 | 33.00 ± 7.00 |

[a] Full lactation (≥500ml expressed per day)
[b] Volume unknown but nursing ≥4 times per day
[c] Converted from mg/dl

**Appendix L.** Literature values of HM sodium concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Sodium (mg/L), mean ± SD |
|---|---|---|---|
| Dewey et al. (1984) | US | 7–11 | 84.00 ± 42.00[a] |
| | | | 76.00 ± 36.00[b] |
| Javad et al. (2018) | Iran | 6–7 | 154.7 ± 92.58 |
| | | 12 | 196.0 ± 149.6 |
| Kim et al. (2017) | Korea | 7–11 | 108.0 ± 55.60 |
| Nagra (1989) | Pakistan | 6 | 246.0 ± 4.00[c] |
| | | 7 | 239.0 ± 11.00[c] |
| | | 8 | 235.0 ± 10.00[c] |
| | | 9 | 241.0 ± 8.00[c] |
| | | 10 | 252.0 ± 3.00[c] |
| | | 11 | 246.0 ± 6.00[c] |
| | | 12 | 244.0 ± 8.00[c] |
| National Academies of Sciences Engineering and Medicine (2019)[d] | *Multiple* | 7–12 | 110.0[e] |
| Wack et al. (1997) | US | 6–8 | 130.0 ± 142.0 |
| | | 8–10 | 124.0 ± 65.00 |
| | | 10–12 | 122.0 ± 123.0 |
| Yamawaki et al. (2005) | Japan | 6–12 | 116.0 ± 61.00 |

[a] Full lactation (≥500ml expressed per day)
[b] Volume unknown but nursing ≥4 times per day
[c] Converted from mg/dl
[d] Meta-analysis
[e] Reported as mean

**Appendix L**. Literature values of HM potassium concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Potassium (mg/L), mean ± SD |
|---|---|---|---|
| Dewey et al. (1984) | US | 7–11 | 389.0 ± 41.00[a] |
| | | | 352.0 ± 77.00[b] |
| Kim et al. (2017) | Korea | 7–11 | 363.4 ± 56.90 |
| Nagra (1989) | Pakistan | 6 | 346.0 ± 9.00[c] |
| | | 7 | 339.0 ± 10.00[c] |
| | | 8 | 333.0 ± 12.00[c] |
| | | 9 | 330.0 ± 13.00[c] |
| | | 10 | 338.0 ± 10.00[c] |
| | | 11 | 343.0 ± 8.00[c] |
| | | 12 | 345.0 ± 12.00[c] |
| National Academies of Sciences Engineering and Medicine (2019)[d] | *Multiple* | 7–12 | 435.0 |
| Wack et al. (1997) | US | 6–8 | 472.0 ± 63.00 |
| | | 8–10 | 470.0 ± 72.00 |
| | | 10–12 | 445.0 ± 53.00 |
| Yamawaki et al. (2005) | Japan | 6–12 | 432.0 ± 70.00 |

[a] Full lactation (≥500ml expressed per day)
[b] Volume unknown but nursing ≥4 times per day
[c] Converted from mg/dl
[d] Meta-analysis

**Appendix M**. Literature values of HM selenium concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Selenium (ug/L), mean ± SD[a] |
|---|---|---|---|
| Al-Awadi and Srikumar (2001) | Kuwait | 6–12 | 16.00 ± 0.80 |
| | Non-Kuwait | | 14.00 ± 0.30 |
| Jin et al. (2020) | New Zealand | 12 | 12.00 (11.00, 13.00)[b] |
| Li et al. (1999) | Austria | 8–10[c] | 13.50 ± 4.50 |
| Ren et al. (2024)[d] | China | 8–12 | 22.49 (20.27, 24.71)[e] |
| Shalom et al. (2024)[d] | *Multiple* | 6–12 | 15.00 ± 1.00[f] |
| | | | 11.35 ± 3.30[g] |
| Yamawaki et al. (2005) | Japan | 6–12 | 13.00 ± 4.00 |

[a] Unless otherwise specified.
[b] Reported as median (25$^{th}$, 75$^{th}$ percentile)
[c] Rounded from 7.47–9.77 months (224–293 days)
[d] Meta-analysis
[e] Reported as mean (95% CI)
[f] Infants born at term
[g] No class

**Appendix N**. Literature values of HM iodine concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Iodine (ug/L), median [25th, 75th percentile] |
|---|---|---|---|
| Andersson et al. (2010) | Switzerland | 12 | 43.57[ab] |
| Henjum et al. (2016) | Nepal | 7 | 264.0 [94.75, 470.0][c] |
| | | 8 | 245.0 [122.5, 347.5][c] |
| | | 9 | 260.0 [197.5, 322.5][c] |
| | | 10 | 360.0 [125.0, 480.0][c] |
| | | 11 | 300.0 [202.5, 392.5][c] |
| | | 12 | 215.0 [192.5, 400.0][c] |
| Jin et al. (2022) | New Zealand | 12 | 35.00 [26.00, 54.00] |
| Wang et al. (2009) | China | 6–12 | 122.0 (16.48–291.0)[d] |

[a] Reported as median
[b] Converted from µg/kg
[c] Extracted from supplementary data
[d] Reported as median (range)

**Appendix O**. Literature values of HM phosphorus concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Phosphorus (mg/L), mean ± SD |
|---|---|---|---|
| Kim et al. (2017) | Korea | 7–11 | 127.2 ± 31.20 |
| Nagra (1989) | Pakistan | 6 | 119.0 ± 13.00[a] |
| | | 7 | 120.0 ± 9.00[a] |
| | | 8 | 123.0 ± 5.00[a] |
| | | 9 | 117.0 ± 13.00[a] |
| | | 10 | 118.0 ± 16.00[a] |
| | | 11 | 117.0 ± 12.00[a] |
| | | 12 | 120.0 ± 14.00[a] |
| Prentice and Barclay (1991) | Zaire | 6 | 136.0 ± 4.00 |
| | | 9 | 138.0 ± 7.00 |
| | | 12 | 139.0 ± 5.00 |
| Yamawaki et al. (2005) | Japan | 6–12 | 130.0 ± 25.00 |

[a] Converted from mg/dl

**Appendix P.** Literature values of HM vitamin A concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Vitamin A (µg/100 mL), mean ± SD[a] |
|---|---|---|---|
| Ayah et al. (2007) | Kenya | 6 | 12.60 (11.75, 13.75)[bc] |
| Dijkhuizen et al. (2004) | Indonesia | 6 | 27.00[d] |
| Kim et al. (1990) | US | 6–7 | 58.71 ± 25.75[e] |
| Kim et al. (2017) | Korea | 7–11 | 32.12 ± 15.71 |
| Kumari-Maurya et al. (2025) | India | 7–12 | 38.10 ± 21.77 |
| Liyanage et al. (2008) | Sri Lanka | 7 | 10.31 (8.31, 13.18)[bc] |
| Rice et al. (1999) | Bangladesh | 6 | 24.92 ± 17.47[c] |
| | | 9 | 22.63 ± 12.60 |
| Roy et al. (1997) | Bangladesh | 6 | 20.91 (18.33, 23.78)[bc] |
| | | 9 | 31.22 (20.91, 41.82)[bc] |
| Stoltzfus and Underwood (1995) | Indonesia | 6 | 50.70 ± 27.79[c] |
| | | 8 | 44.69 ± 28.36[c] |

[a] Unless otherwise specified.
[b] Reported as mean (95% CI)
[c] Converted from µmol/L
[d] Converted from nmol/g fat
[e] Converted from µg/100 g using a factor of 1.03

**Appendix Q.** Literature values of HM vitamin E concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Vitamin E (mg/100 mL), mean ± SD |
|---|---|---|---|
| Kim et al. (2017) | Korea | 7–11 | 0.23 ± 0.13 |

**Appendix R.** Literature values of HM folate concentration at 6–12 months postpartum

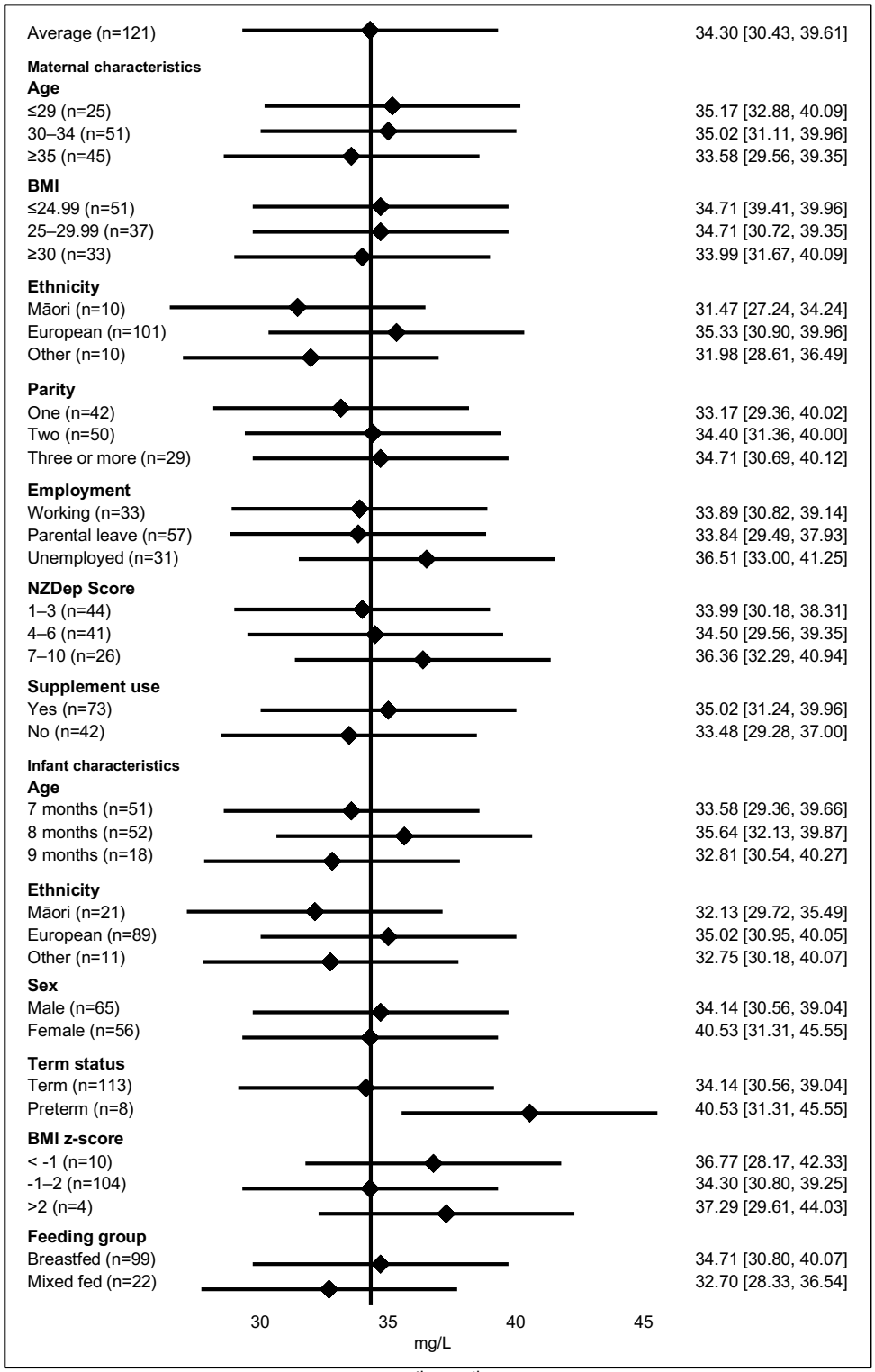
| Reference | Country | Time postpartum (months) | Folate (µg/100 mL) | Form |
|---|---|---|---|---|
| Ek (1983) | Norway | 9 | 4.04 ± 0.51[a] | Free folacin |
| | | | 3.13 ± 0.61[a] | Total folacin |
| Sakurai et al. (2005) | Japan | 6–12 | 5.30 ± 1.20 | Folic acid |
| Su et al. (2022) | China | 7–8[b] | 5.68 (4.48, 7.39)[c] | Total folate |
| Udipi et al. (1987) | US | 8 | 2.90 ± 0.50 – 5.60 ± 1.50[de] | Free folacin |
| | | 10 | 1.90 ± 0.40 – 4.50 ± 1.10[de] | |
| | | 8 | 3.50 ± 0.90 – 7.90 ± 3.70[de] | Total folate |
| | | 10 | 2.20 ± 0.40 – 7.70 ± 1.60[de] | |

[a] Converted from nmol/L
[b] Rounded from 6.67–8 months (121–180 days)
[c] Converted from ng/mL
[d] Converted from µg/L
[e] Samples were collected across the day from 0400-0600 to 2200-2400; ranges represent the minimum and maximum reported concentrations

**Appendix S.** Literature values of HM vitamin C concentration at 6–12 months postpartum

| Reference | Country | Time postpartum (months) | Vitamin C (mg/100 mL) |
|---|---|---|---|
| Sakurai et al. (2005) | Japan | 6–12 | 3.70 ± 1.30 |

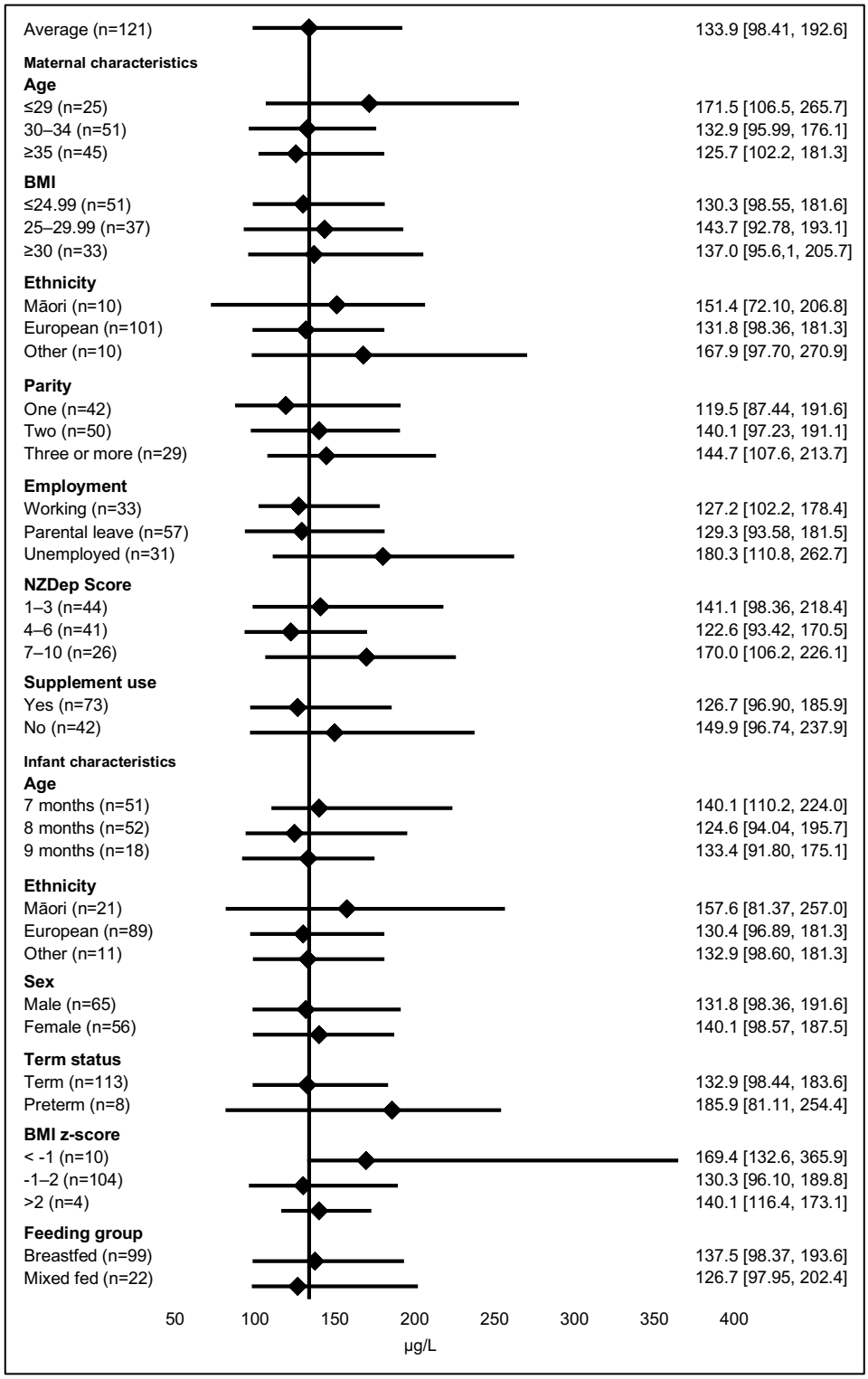
**Appendix T.** Distribution of HM magnesium concentrations according to maternal and infant characteristics at 7–10 months postpartum[a]




Values are presented as median [25th, 75th percentile].

[a] 'Other' ethnicities includes Asian (maternal n=2; infant n=4), Pacific (maternal n=1; infant n=1) and other (maternal n=5; infant n=4)

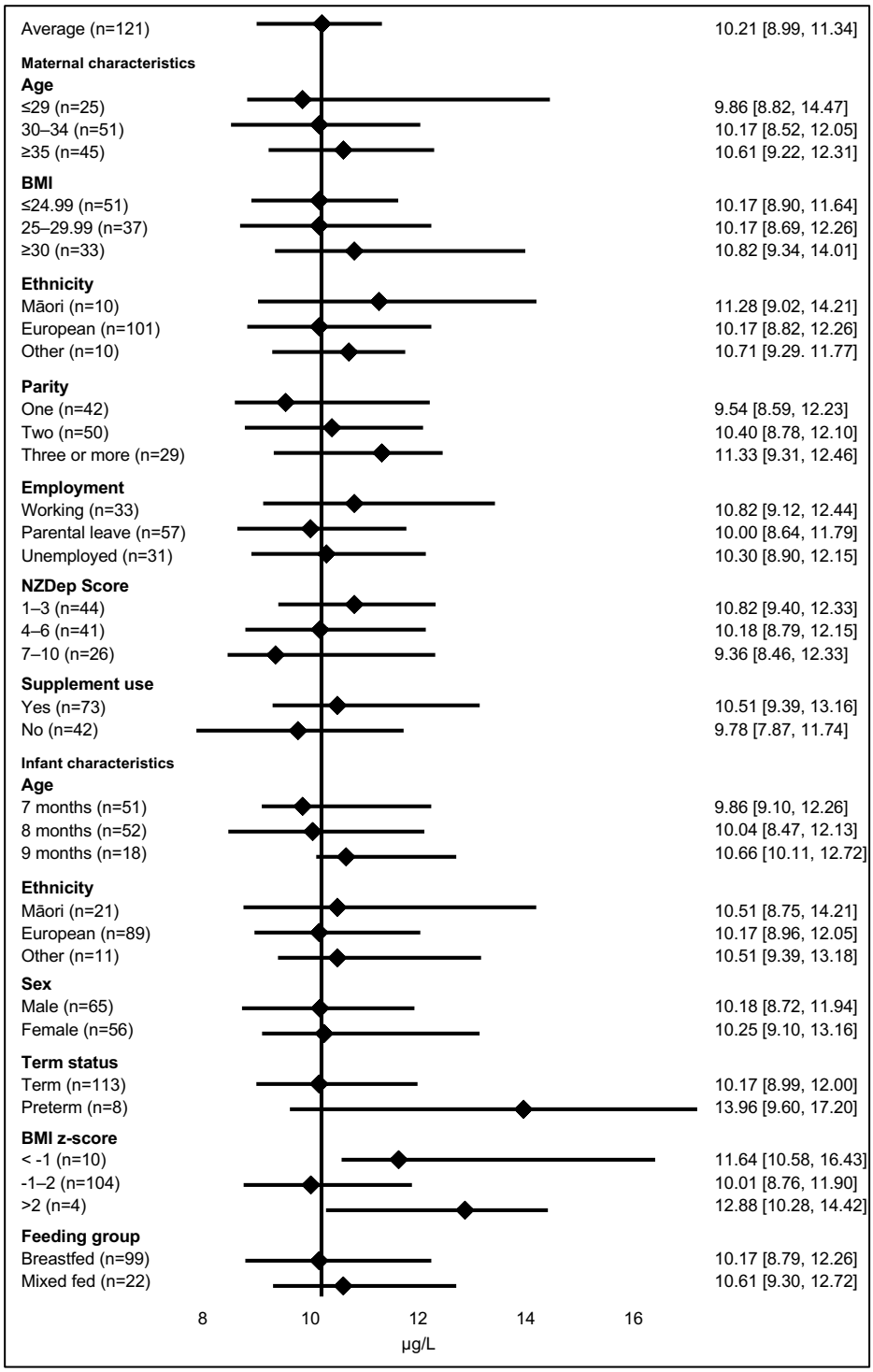
**Appendix U.** Distribution of HM iron concentrations according to maternal and infant characteristics at 7–10 months postpartum[a]




Values are presented as median [25th, 75th percentile]

[a] 'Other' ethnicities includes Asian (maternal n=2; infant n=4), Pacific (maternal n=1; infant n=1) and other (maternal n=5; infant n=4)

**Appendix V.** Distribution of HM selenium concentrations according to maternal and infant characteristics at 7–10 months postpartum[a]




Values are presented as median [25th, 75th percentile].

[a] 'Other' ethnicities includes Asian (maternal n=2; infant n=4), Pacific (maternal n=1; infant n=1) and other (maternal n=5; infant n=4)