

Maternal Perception vs Actual Breast Milk Supply: Is There a Difference? Protocol for the MABBS Observational Study

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Abstract

Background: It is well known that breastfeeding provides favourable health outcomes for both mother and baby. However, many mothers struggle to meet global breastfeeding recommendations, with Perceived Insufficient Milk Supply (PIMS) being a leading reason for early cessation. Currently, little is known about the relationship between PIMS, actual milk insufficiency, and human milk nutrient composition.

Objective: The main aims of the M?m? (mother) and Baby Breastfeeding Study (MABBS) are to estimate human milk volumes produced by a diverse sample of breastfeeding mothers at 3 months postpartum with differing perceptions of milk supply, and to investigate human milk composition in relation to milk volume.

Methods: In this observational study in Dunedin, Aotearoa New Zealand, a sample of 150 mother-infant dyads will be recruited. A diverse range of participants in terms of ethnicity and socio-economic status will be included. Human milk volume will be assessed using the 'dose-to-mother' stable isotope (deuterium oxide) technique. Mother participants will consume an accurate dose (30 g) of deuterium oxide after baseline saliva samples are collected from both mother and infant. Subsequent post-dose samples will be collected over 3 time-points to determine deuterium enrichment over a 14 day period using Fourier-Transform Infrared Spectrometry. Human milk macronutrient (energy, fat, carbohydrate, crude and true protein) and mineral and trace element (sodium, magnesium, phosphorus, potassium, calcium, iron, copper, zinc, selenium, iodine) composition of one full milk expression from one breast will be analyzed using the MIRIS Human Milk Analyzer and ICP-MS, respectively. Potential predictors and maternal perception of milk supply will be assessed via questionnaire. Infant body mass index (BMI) will be calculated from measures of weight and length at three different time points over four weeks, using standard techniques. These, alongside anthropometric measurements collected at the infant's Well Child Tamariki Ora visits, will be used to assess infant growth trajectory in the first six months of life. Regression models will be used to assess the associations between maternal perception of milk supply, human milk volumes, and composition.

Results: Recruitment for this study began in February 2025, and is anticipated to conclude in June 2026 with analysis expected to be completed by February 2027. As of the 25th of September 2025, 64 participants have been enrolled.

Conclusions: This research will provide new knowledge on whether maternal perception of milk supply aligns with actual human milk volume or nutrient composition. Such information will be extremely useful for health professionals working with breastfeeding mothers with milk supply concerns and for informing the design of breastfeeding support programmes and resources. Clinical Trial: Australian and New Zealand Clinical Trial Registry ACTRN12625000180415;

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Original Manuscript

Maternal Perception vs Actual Breast Milk Supply: Is There a Difference?

Protocol for the MABBS Observational Study

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Abstract

Background: It is well known that breastfeeding provides favourable health outcomes for both mother and baby. However, many mothers struggle to meet global breastfeeding recommendations, with Perceived Insufficient Milk Supply (PIMS) being a leading reason for early cessation. Currently, little is known about the relationship between PIMS, actual milk insufficiency, and human milk nutrient composition.

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supply, human milk volumes, and composition.

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Discussion: This research will provide new knowledge on whether maternal perception of milk supply aligns with actual human milk volume or nutrient composition. Such information will be extremely useful for health professionals working with breastfeeding mothers with milk supply concerns and for informing the design of breastfeeding support programmes and resources.

Trial Registration: Australian and New Zealand Clinical Trial Registry ACTRN12625000180415; <https://www.anzctr.org.au/ACTRN12625000180415.aspx>

Keywords: human milk; breastfeeding; milk supply; lactation; stable isotope; infants; perceived insufficient milk supply; human milk composition

Introduction

While national and international advisory groups recommend exclusive breastfeeding for the first six months of life and continued breastfeeding for up to two years of age and beyond (1,2), many mothers in New Zealand and globally are not meeting these recommendations. In New Zealand, 77% of infants are exclusively breastfed at discharge from maternity services (3), however this drops to under 50% by four months of age and less than 10% by six months of age (4). These percentages are lower than exclusive breastfeeding rates at six months in the USA (24.9% in 2019) (5), but higher than recent estimates in Australia (less than 1% in 2021) (6). It is evident that work is needed to improve exclusive breastfeeding rates for the first six months in New Zealand and other high income countries, especially considering the global goal of 60% of infants being exclusively breastfed to 6

months by 2030 set by the World Health Assembly (7).

There are many reasons why mothers may not be meeting recommended breastfeeding targets, such as pain, tiredness, inconvenience, or return to work or education (8,9). However, Perceived Insufficient Milk Supply (PIMS), a mother's perception that her production of milk is not adequate to satisfy her infant's needs, is reported to be the leading cause of early cessation of breastfeeding (10–12). Gatti et al. (10) concluded that of women who discontinue breastfeeding early, 35% describe PIMS as the primary reason. It is also a known barrier to continuing breastfeeding in New Zealand (13,14).

However, a major gap in the literature exists in that while PIMS is very common and leads to a large proportion of early breastfeeding cessation, how perception of insufficient milk supply relates to actual physiological milk volume is uncertain. Similarly, there also appears to be no research investigating the perception of high milk volume (oversupply) in this context. Only one study appears to have investigated the relationship between PIMS and human milk volume (15), using the 24-h test-weighing method in first time mothers only. The study found that at two weeks postpartum, there was no significant association between perceived and actual insufficient milk supply. No other research has examined older infants, particularly around the age of 3 months, immediately before breastfeeding rates decrease in New Zealand (4). This is perhaps due in part to the fact that accurately measuring human milk intake is complex. Standard approaches often assume a fixed volume of intake across infants of a specific age. For example, a commonly used “one size fits all” approach estimates individual intake at 780 mL/day for infants aged 0 to 6 months (16). However, we know such estimates mask considerable variation observed between mother-infant pairs, with intakes ranging from 400 to 1400 mL/day in infants at 2-5 months postpartum (17). Such standard approaches clearly undermine the ability to assess true intake in breastfed infants. Other approaches

include 24-h test-weighing, where the mother weighs the infant directly before and after all breastfeeds over a 24-h period. Test-weighing, while more individualized than the “one size fits all” approach, may impact usual feeding patterns and interfere with the physiological and behavioural aspects of lactation (18,19).

Measuring pumped (expressed) milk volume has also been used to estimate human milk production (18–21). However, this is not considered an accurate indicator of infant intake largely due to differences between maternal production capacity and infant need (22). Pumping output is also highly variable, influenced by factors such as device efficiency and maternal pumping technique, and individual responsiveness to pumping (24,25). As a result, expressed volume often reflects the effectiveness of the tools and methods used rather than the mother’s underlying physiological milk production. Another common method of determining human milk intake is the “minutes per feed” approach, which assumes that a set amount of milk is transferred to the baby per minute of breastfeeding. However, ultrasound imagery has previously found no relationship between the length of the breastfeed and the amount of milk transferred (26), suggesting considerable inaccuracy with this method. A promising approach to estimating human milk intake is the use of predictive modelling (27), although, to date, only limited data are available and the method is yet to be validated.

Finally, the dose-to-mother (DTM) stable isotope technique (19,28,29) is considered the gold standard method for estimating human milk volume. This method can estimate human milk intake over a 14-day period by giving the mother an accurate oral dose of deuterium oxide (a stable isotope, also known as ‘heavy water’) and then tracing the transfer of deuterium oxide from the mother to the infant through mother and infant saliva or urine samples. This method has been used to estimate human milk intake of infants all over the world, most commonly in early infancy (18,30–34) but also

in older infants aged 7-10 months in New Zealand (27). While it is more expensive than other methods, it is relatively non-invasive and provides an accurate estimate of human milk intake (29). To date, no research has used the gold standard dose-to-mother technique to investigate the relationship between perceived insufficient milk supply and actual milk intake in infants.

Furthermore, it is not known how differences in nutritional composition influence the perception of milk supply or volume. Determining human milk adequacy in terms of both volume (quantity) and composition (quality) will provide valuable information and has been highlighted as a known gap in the literature (35). For mothers with lower milk volume, it may be important to know whether the nutritional composition of the milk, particularly total energy, is different from a mother who has higher milk volume. Furthermore, determining the nutrient composition of human milk in New Zealand is particularly important because of unique local nutritional issues. In particular, iodine and selenium are low in the New Zealand food supply due to poor soil content (36) and are critical for infant development as both support thyroid hormone production and regulation, and therefore brain maturation (37,38).

The Māmā and Baby Breastfeeding Study (MABBS) will describe human milk volumes produced by a diverse sample of lactating mothers of 3-month-old infants with differing perceptions of milk supply. We will compare the lactating mothers' perceptions of milk supply with the volume of milk they produce as well as the nutrient composition of that milk.

The primary objective is to estimate the human milk intakes of fully breastfed infants aged 3 months, using the gold standard dose-to-mother deuterium oxide (D_2O) technique. The secondary objectives are, at 3-months postpartum, to: (1) estimate how human milk volumes differ between lactating mothers with differing perceptions of milk supply, (2) explore the characteristics that may predict

human milk volumes produced by a diverse range of lactating mothers, (3) explore the relationship between human milk volumes produced and infant growth as a potential means of assessing human milk adequacy, (4) investigate the association between human milk composition and human milk volumes, and (5) assess how demographic characteristics might modify the relationship between perception vs actual milk supply of lactating mothers.

Methods

Study design

The MABBS study is an observational, cross-sectional study of human milk intakes in infants aged 2.0 to <4 months in Dunedin, New Zealand. Participants will be included in the study if they are: a lactating mother who is 16 years of age or older; are living in Dunedin, New Zealand; have an infant aged 2.0 – <4 months at the time of their first visit; are fully breastfeeding (i.e. their infant is receiving human milk only) for two weeks prior to recruitment; and are intending to continue fully breastfeeding for the duration of the study (4 weeks). We will exclude participants who cannot provide informed consent or complete study procedures in English, are tandem breastfeeding (simultaneously breastfeeding another child) or breastfeeding multiples (twins or higher order multiples). To our knowledge, the dose-to-mother technique has not been specifically validated for use in twins or tandem-fed children, therefore the study design was based on estimating human milk intake in singleton dyads.

The study has been approved by the University of Otago Human Ethics Committee (Health) Te Pae Matatika Tangata (Haurora) Ōtākou Whakaihū Waka (H24/0225), and written informed consent will be obtained at the first appointment. The study is registered with the Australian New Zealand Clinical Trials Registry (registration number: ACTRN12625000180415).

Participants and Recruitment

In total, 150 mother-infant-dyads will be recruited. We initially planned to target recruitment to mothers with infants aged between 2 to 3 months, so that they would participate within the study age range of 3.0 and <4 months. This time point was selected because the prevalence of breastfeeding drops dramatically around 4 months of age in New Zealand (39), and this would allow us to estimate human milk intake just before this to understand factors that may be driving the decline around 4 months. While some literature suggests that PIMS is most common early in the breastfeeding journey (8,40), research in New Zealand shows that PIMS is a primary reason for early breastfeeding cessation around 4-5 months (14). Furthermore, around 3-4 months, many PIMS-related factors may be contributing to this decline, including changes in feeding behaviours such as an increased rate of cluster feeding (close intervals between breastfeeds); and maternal changes such as breasts feeling softer and less milk leakage as the body becomes more efficient at producing milk. This timepoint also occurs before the majority of mothers return to work in NZ (41), and before the recommended age for introducing complementary foods, both of which events which influence infant feeding. However, when recruitment commenced, we found that many mothers had already begun mixed feeding before 3 months of age, potentially due to perceived insufficient milk supply. As many of these women were from ethnic groups other than New Zealand European, the one-month age band (3.0 to <4 months of age) appeared to be limiting the ethnic diversity of our sample. Thus, from the 29th of August 2025, we revised our minimum age of inclusion to 2.0 months rather than 3.0 months of age, resulting in a final inclusion period of the infant aged between 2.0 and <4 months, and therefore the recruitment age range expanded to 1.5 to 2 months of age.

Recruitment will occur through advertisement on social media, word of mouth, and referral through health professionals (e.g., midwives, Well Child Tamariki Ora providers, lactation consultants) where

possible: and by study posters in parent spaces and community groups/spaces as needed. We aim to recruit a diverse range of participants in terms of ethnicity and socio-economic status, including approximately 20% Māori (indigenous people of NZ, to reflect local statistics of the region in which data collection is occurring (42)). Some targeted recruitment (identifying and approaching groups to promote the study) may be required to achieve an ethnically diverse sample. This will occur through utilizing networks within the maternity and healthcare sector as well as with Māori research colleagues.

Interested individuals will complete an online eligibility questionnaire administered through REDCap (43), which includes access to the information sheet about the study and the consent form to review. Once eligibility is confirmed, the potential participant will be sent an email offering them the opportunity to ask any questions and attaching the information sheet and consent form. A follow-up phone call or text will be set around three days later to confirm they have read the information, answer any questions about the study, obtain verbal consent, and schedule their appointments.

Data collection

Overview

Participation in the study will involve five contacts over four weeks following recruitment as is depicted in **Table 1**. Data collection will be conducted in the participant's home or in the research clinic (Department of Human Nutrition, University of Otago, Dunedin, NZ) depending on the participant's preference. The first visit (visit 1) will involve obtaining written consent, completion of the Main Questionnaire, and obtaining anthropometric measurements (infant weight and length and maternal weight and height) and baseline saliva samples from both the mother and the infant, as well as the administration of a 30g dose of stable isotope (deuterium oxide) to the mother. The mother

will be asked to take their own (mother only) “at home” saliva sample 3-4 hours after the dose is administered (to be retrieved at visit 2). Post-dose saliva samples will be taken by researchers at the second visit (visit 2) occurring 2-3 days after the administration of the isotope, as well as at the third visit (visit 3), 6-7 days after the isotope, and at the fourth visit (visit 4), 13-14 days after isotope administration. Infant anthropometric measurements (weight and length) will be repeated at the fourth visit (visit 4). After the fourth visit (visit 4), the mother will be asked to express a full milk expression from a single breast. The final visit (visit 5) will be conducted by an International Board-Certified Lactation Consultant (IBCLC) who will take the final anthropometric measurements from the infant, conduct an observed breastfeed with a one-off single test-weighing (pre and post feed weights), conduct an oral assessment of the infant, and ask the mother to complete a questionnaire about Dysphoric Milk Ejection Reflex (D-MER (44).

Demographic Data

Self-reported maternal and mother-reported child ethnicity will be collected as part of the eligibility questionnaire, using standard New Zealand census questions (45). Area-based household socioeconomic deprivation (NZDep2023) will be determined using the participant’s postal address (46). NZDep2023 combines 9 variables from the 2023 New Zealand national census to determine a socioeconomic deprivation score for each Statistical Area (a geographic area defined by Statistics New Zealand containing ~100-200 people). The deprivation score estimates the relative material and social deprivation for the area where the participant lives, where decile 1 represents areas with the least deprivation and decile 10 represents the area with the most deprivation. Maternal education, maternal work status, childcare use, and the number of children and adults living in the household will be collected in the Main Questionnaire administered in the first visit.

Maternal Perception of Milk Supply

Perception of milk supply will be assessed in the Main Questionnaire using two approaches. First, using the Perceived Infant Breastfeeding Satiety Subscale (PIBSS) of the Hill and Humenick (H&H) Lactation Scale (47) in the Main Questionnaire, which is a validated scale designed to determine perceived insufficient milk supply. The PIBSS subscale is a five-question subscale with responses recorded using a 7-point Likert scale (1 – strongly disagree, to 7 – strongly agree). Higher scores represent higher perceived milk supply. Second, a single question developed by the research team will be administered to assess maternal perception of milk supply: “How do you *currently* feel about the amount of breast milk you make for your baby/pēpi? (*think about over the past week*)?” with answers from a 5-point Likert scale (1 – not enough milk, 2 – sometimes not enough milk, 3 – enough milk, 4 – sometimes too much milk, 5 – too much milk). This continuous variable will be dichotomised to create a variable for comparing low (insufficient) perceived milk supply and high (oversupply) perceived milk supply, combining 1 – not enough milk and 2 – sometimes not enough milk to define low (insufficient) and 4 – sometimes too much milk and 5 – too much milk to define high (oversupply), to 3 – sufficient supply.

Dose-to-Mother Stable Isotope Technique

Human Milk Volume

Human milk volume will be determined using the gold standard stable isotope dose-to-mother technique (19,28,29). Baseline saliva samples (~ 2 mL) will be collected from the mother and infant participants at the first visit. Participants will be asked to ensure that neither they nor (themselves and their baby) have not consumed any food or drink for 30 minutes prior to the saliva sample collection (to prevent contamination of the sample with any food or drink residue). After ensuring appropriate sanitization, using gloved hands the researcher will place a dental cotton roll (Premium dental cotton rolls, Henry Schein, New Zealand) into the mother’s mouth to soak up saliva, without the cotton roll being touched by the mother’s hands. Once sodden, the cotton roll will be placed into

a 10 mL syringe and squeezed into a labelled tube (1.5 mL, screw top) until at least 1 mL saliva is extracted. This will be repeated to fill a second tube as a spare, in case of any measurement errors or spills during analysis. For the infant's saliva sample, the same process will be followed, however the researcher will hold one cotton roll and move it around the lower left and right sides of the infant's mouth and under the tongue until sodden. Filled sample and spare tubes will be placed into separate 'mother' and 'baby' labelled plastic zip lock bags and into a cooler box with an ice pack before being transferred into a -20°C freezer, until analysis.

After the baseline samples have been taken, an accurately measured dose of deuterium oxide (D_2O ; Aldrich, 99.8 atom % D) will be administered to the mother participant as a drink of water through a straw. The dose amount will be 30 g measured to the nearest 0.01 g (i.e. doses will range from a minimum of 30.00 g to a maximum of 30.09 g). To ensure the full dose is consumed by the participant, the dose bottle will be filled twice with 50 mL of regular drinking water, inverted, and consumed by the mother through the same straw.

The elimination of D_2O from the mother and transfer to the infant will be traced over the 14-day collection through the analysis of deuterium enrichment of the saliva samples. Post-dose (D_2O) saliva samples will be collected using the same methods described above from the mother and infant at 3 time points: 2-3 days post-dose, 6-7 days post-dose, and 13-14 days post-dose. The reduced sampling protocol from the original IAEA protocol will be used (19), and has shown high sensitivity and specificity (29). Infant morbidity will be assessed in visit 3 and 4, with a one-off question of "has baby been unwell in the last week?" to determine whether any illness occurred over the dose-to-mother period.

Any participants expressing breast milk will be asked to weigh and record the amount of milk

expressed and indicate whether this was fed back to the infant, either fully or partially, during the 14-day dose-to-mother period. An expression log and electronic kitchen scale (Salter, model 1023) will be provided to the participant for recording purposes. Participants will be asked not to feed the infant any expressed milk that had been stored prior to the consumption of the D₂O as this cannot be tracked, and therefore, infants will only consume expressed milk from the 14-day dose-to-mother period. Specific expression storage bags will be provided to participants to ensure accurate tracking of milk expressed during the dose-to-mother period and to minimize any confusion with milk expressed and stored outside of this time.

Maternal Fat Mass and Fat Free Mass

Maternal body composition will be measured through the dose-to-mother technique, providing calculated fat mass and fat-free mass (19). Participants will be asked to collect their own saliva sample 4 hours (or at least between 3 – 4 hours) after administration of the stable isotope using the same methods used by the researcher at their visit 1. They will be provided with equipment and instructions and asked to store the sample in their refrigerator until it is collected by a researcher at the second visit.

Human Milk Sample Collection

At the end of the dose-to-mother assessment period, a subsample of a complete human milk sample (full expression) will be collected from the participant in their home. At visit 4, participants will either be provided with a sterile breast pump (manual or electric) and collection bottle or given the option of using their personal breast pump, depending on preference. For those using their own pump, a microwave steam sterilizer bag (Medela, Switzerland or New Beginnings, Australia) will be provided so that the participant can sterilize the pump parts prior to expression, to minimize contamination. Participants will be asked to, prior to expression, clean their hands and the breast area

with de-mineralized water and a cleaning gauze as provided, in order to remove any trace elements from creams that may have been applied to the hands or nipple area (e.g., barrier creams, which commonly contain zinc). Participants will be asked to express a full milk sample from one breast, in the morning (before 12 pm), at least 2 hours after the last feeding or pumping session on that breast to standardize the collection period (48). Participants will be asked to gently invert their full milk sample three times immediately after collection and then to pour a 12 mL subsample into a collection container provided by the study that has been labelled with the participant ID, and to indicate on the container the volume collected. The 12 mL amount was chosen to balance analytical needs (this is the volume required for the planned analyses) with sensitivity to mothers who may have concerns about their milk supply (their infant can be fed the remainder of the expressed milk).

Breastfeeding Observation

A one-off breastfeeding will be observed in the final visit (visit 5) by an International Board-Certified Lactation Consultant (IBCLC). The IBCLC will use the Bristol Breastfeeding Assessment Tool (BBAT) (49) to assess adequate latch and transfer of milk by the infant. This tool has four main elements, scoring a breastfeeding on positioning, sucking, swallowing, and attachment. A score of 0 (poor feeding elements) to 8 (good feeding elements) will be recorded by the IBCLC.

Test-weighing

During the breastfeeding observation by the IBCLC, a single test-weighing of human milk transfer will be taken using an electronic baby scale (Seca model 334). Single test-weighing, the process of weighing the infant before and after a single breastfeed, is often used clinically by health professionals working with breastfeeding dyads to assess adequate milk transfer. The volume of milk transferred in the single test-weighing will be compared to the human milk volume estimated by the dose-to-mother technique, to investigate whether a one-off test-weighing is indicative of actual

human milk volume over a two-week period.

Anthropometry

Infant weight and length will be measured using an electronic baby scale (Seca model 334) and a 99 cm measuring mat (Seca model SE210). Infants will be weighed naked with only a dry nappy of known weight. Maternal weight (minimal clothing, no shoes) will be measured using an electronic scale (Tanita model HD-351) and height will be measured using a portable stadiometer (Wedderburn model WMHM200P or Seca model 213), depending on whether the visit is at the participants home or in the clinic. Measurements will be conducted by trained research staff in duplicate, following the World Health Organization (WHO) anthropometric protocol (50). A third measurement will be taken if the difference between the first and second measurement exceeds the maximum allowable difference (0.1 kg and 0.5 kg for infant and maternal weight, respectively, and 0.7 cm for length and height).

At visit 5 (the final visit), photographs will be taken of anthropometric measurements collected during Well Child Tamariki Ora visits. These will be entered into REDCap. Well Child Tamariki Ora visits are free health assessments that are offered to all NZ families and conducted between 6 weeks and 5 years of age (51). These anthropometric data will be used alongside those directly measured in the study to assess infant growth trajectory over time, which will be compared to the WHO Child Growth Standards (52). Infant weight and length data will be used to calculate Body Mass Index (BMI) and determine BMI-for-age z-score using sex specific reference data from the WHO Child Growth Standards (53).

Perception of Infant and Maternal Sleep

Infant sleep will be assessed using questions from a New Zealand sleep study (54), modified to

include questions about infant sleep location, and changes to infant sleep patterns. Participants will be asked a question about their own sleep quality and sleep quantity, developed by the research team, in the Main Questionnaire to assess maternal sleep perception.

Potential Predictors of Human Milk Intake

Human milk intake is complex and can be influenced by a range of different factors. A selection of potential maternal and infant factors that may influence human milk intakes has been identified through literature searches and advice from breastfeeding experts. These are summarized in **Table 2**.

Breastfeeding Self-Efficacy

Maternal breastfeeding self-efficacy will be assessed using the Breastfeeding Self-Efficacy Scale-Short Form (BSES-SF) (55) in the Main Questionnaire. This is a validated 14-item questionnaire with a 5-point Likert scale (1 – not confident to 5 – very confident) to determine the participant's confidence in their ability to breastfeed. Total scores range from 14 to 70, with higher scores indicating higher level of breastfeeding self-confidence.

Health History

Maternal and infant health history will be collected in the Main Questionnaire. Questions about birth and breastfeeding experience, breastfeeding support and practices, infant gestational age, parity, current medications or medical conditions, use of supplements, maternal dietary pattern and appetite, breast surgery, and return of menses will be included to determine their potential impact on human milk intake. The Main Questionnaire will also include questions about breastfeeding practices, such as the average number of breastfeeds in 24-hours, whether they are breastfeeding on demand or on a schedule, how much the infant spills, and questions on previous breastfeeding experience. Maternal stress will be assessed using the Global Measure of Perceived Stress Questionnaire (56).

Questions about nipple or breast trauma will be asked at visit 1 and repeated at subsequent visits (visits 2-4) to determine whether any changes occurred during the study period. Breastfeeding pain and maternal experience with Dysphoric Milk Ejection Reflex (D-MER) will be assessed at visit 5 (final) with the International Board-Certified Lactation Consultant (IBCLC). Breastfeeding pain will be the mother's self-reported usual pain during a breastfeed, on a scale of 0 (no pain) to 10 (worst pain). If pain is reported as >0 , follow-up questions will be asked about the type of pain, timing, frequency, and its impact on breastfeeding. This will be assessed separately to the questions around nipple or breast trauma as those focus on damage and injury, rather than general pain. Presence of D-MER will be assessed using the validated D-MER questionnaire (44). Those who are experiencing symptoms of pain or D-MER will be offered further support by the IBCLC based on clinical judgement.

Nicotine and Cannabis Use

Questions about nicotine use will be assessed in the Main Questionnaire. Due to the rising rates of e-cigarette ("vape") use in New Zealand (57) and internationally (58), as well as evidence that use could be negatively associated with breastfeeding duration (59), questions on cigarette and e-cigarettes use will both be included. The number of cigarettes per day, as well as the use, nicotine level, and flavour of e-cigarettes will be assessed via questionnaire.

Cannabis use will be assessed in the Main Questionnaire. As cannabis is illegal in New Zealand, care will be taken when asking these questions to ensure that confidentiality is maintained and participants feel safe responding. Participants will be reminded that all responses are confidential and no identifying information will be kept alongside their responses. The questions about cannabis will be optional, allowing participants to leave this section blank if they feel more comfortable. Cannabis

use will be assessed via questions used previously (60), modified to include questions about use after birth, along with use during pregnancy.

Household Temperature and Humidity

To explore the impact of potential heat stress on infant milk intake, household temperature and humidity will be measured using a digital hydrometer (Niome) with a temperature accuracy of $\pm 1^{\circ}\text{C}$ and humidity accuracy of $\pm 5\%$. For participants who choose to have their visits in the home that the infant is most commonly in, the digital hydrometer will be placed in the main room of the home where the visit takes place. The household temperature and humidity will be recorded after 15-20 minutes, which gives the hydrometer enough time to obtain an accurate reading of the room.

Laboratory analyses

Human milk volume

The deuterium enrichment in saliva samples will be determined by Fourier-Transform Infrared Spectrometry (Thermo Fisher Nicolet Summit, Thermo Fisher Scientific) with a liquid accessory attachment: SPECAC Pearl and CaF_2 100 μm pathlength top and bottom window plates. Enrichment will be determined using a modified method described by the International Atomic Energy Agency (19). Previously frozen saliva samples will be thawed at room temperature for 30 minutes, inverted four times and centrifuged at 1000 g for 10 minutes. A trained researcher will pipette 45 μL of sample (baseline or post-dose) onto the cell window, close the top cell window and measure the absorption spectrum based on settings described in the IAEA (19). Each sample will be measured in duplicate, unless the difference between the two sample measurements is >3 mg/kg, in which case a third or fourth measure will be taken until two measures are <3 mg/kg apart. The mean of the two closest measurements (within 3 mg/kg) will be the calculated D_2O at each timepoint. Following the

determination of D₂O enrichment, the calculation of breast milk volume, infant non-milk water intake, and classification of exclusive breastfeeding will be determined using a validated method (28,29). The mother's measured height and weight and the infant's measured length and weight (collection described above) will be used in the calculation of human milk volume.

Human milk composition

Macronutrients

Human milk samples will be collected from the participant on the same day of expression. Samples will be carefully mixed and aliquoted into a 10 mL collection tube and frozen within 48 hours of collection, at -20°C. Frozen samples will be defrosted at room temperature and analyzed within 2 hours. Once samples are defrosted, they will be placed into a heating bath where they will sit for approximately 20 minutes to warm to 40°C. When the samples have reached 40°C, they will be mixed by inversion and homogenized using an ultrasonic processor (Miris Ultrasonic Processor, Sweden). Macronutrient composition will be analyzed using a Human Milk Analyzer (HMA, Miris, Sweden). Three mL of sample will be slowly syringed into the HMA and repeated in triplicate (three analyses per sample) to ensure accuracy of the reading.

The HMA uses a combination of mid-infrared (mid-IF) transmission spectroscopy principles to measure concentrations of fat, carbohydrate (lactose and oligosaccharides), and both crude and true protein in human milk, as well as providing calculated values for total solids and energy. The HMA software uses internal calibration based on chemical standard methods validated for analysis of human milk. The Röse-Gottlieb method is used for total fat (61), and Kjeldahl (62) for crude protein. True protein is crude protein multiplied by 0.8, to correct for non-protein-nitrogen (NPN). The total carbohydrate content is calculated by the total solids minus fat, protein, and ash (63). Total solids are measured by drying oven (64).

Minerals and trace elements

Minerals and trace elements will be determined following methodology used previously (17). Specifically, minerals (sodium, magnesium, phosphorus, potassium, and calcium) and trace elements (iron, copper, zinc, selenium, and iodine) will be analyzed in the Centre for Trace Element Analysis, Department of Chemistry, University of Otago, New Zealand, by inductively coupled plasma mass spectrophotometry (Agilent 7900; Agilent Technologies). Briefly, 10 mL of quartz sub boiling 14 N nitric acid and 1 mL of 30% hydrogen peroxide will be added to approximately 1 g of the human milk sample. Samples will be microwave digested at 200°C for 15 min and dried down to approximately 1 mL. The final sample will be diluted to 25 mL with 2% aqueous nitric acid. The precision and accuracy will be checked with the use of in-house pooled samples and multielement reference standards (SRM1846, infant formula) from the National Institute of Standards and Technology.

Data management

Upon enrolment, participants will be assigned a unique identifying code (participant ID). To preserve confidentiality, during data collection all data will be recorded against the participant ID. The key that connects the participant ID and participant name and address will be kept secure, and although it will need to be accessible to research staff so that they can attend home visits (name and address) and collect data (participant ID), research staff will only have access to health information when it is essential for specific study-related tasks.

Responses to online questionnaires will be linked to participant ID numbers. Participant IDs will also be written on biological sample tubes and recording sheets. All study data will be stored on the secure REDCap database (43). In the unlikely event that data are unable to be entered directly into

the REDCap database, they will be entered on a hard copy datasheet and later transferred into REDCap. All paper copies will be stored in a locked office.

Data will be collected following rigorous protocols and cleaned as per standard data entry procedures. Quality control visits (observations of research staff conducting visits) will be conducted by senior research staff to ensure staff follow data collection protocols consistently. Data quality checks will be run on all entered data to check for accuracy, consistency, and completeness. The results database will be stored on research team members' computers, all of which are password protected.

Statistical analysis

Sample size

We anticipate a dropout rate, including incomplete data, of 20% based on recruitment and completion data from a similar recent study (65). Therefore, we aim to recruit 150 mother-infant dyads, which is expected to result in around 120 with complete data. With 120 participants, mean human milk intake can be estimated to a 95% confidence level of ± 0.18 SD. If the distribution of intakes is similar to previous data, this corresponds to around ± 27 mL/d (i.e. 3% of an estimated intake of 787 mL/d at 3 months (17)). A final sample of 120 will provide a robust sample size to estimate the correlation between human milk intake and PIMS, as well as to estimate differences between groups (66).

Data analysis plan

Human milk intakes of infants at 3 months of age will be described. Regression models will be used to assess how maternal perception of human milk supply is associated with actual human milk

volumes produced by lactating participants at 3 months postpartum. Pre-determined potential predictors (**Table 1**) of human milk intakes will be described using appropriate regression modelling. Perception of milk supply will be collected as a continuous scale and dichotomized to create a variable for comparing low (insufficient) perceived milk supply and high (oversupply) perceived milk supply. Effect modifications of perception vs actual milk volume by demographic characteristic will be investigated descriptively by presenting stratified results. Data will be illustrated graphically where appropriate.

BMI and weight velocity between birth and up to 5 months will be calculated from the Well Child Tamariki Ora data collected. An initial investigation will be conducted into whether infant milk intakes, and infant growth over time (birth to up to 5 months) can be used to assess human milk inadequacy (actual low milk volume), using regression models.

Statistical analyses will be conducted under the guidance of a biostatistician and using Stata version 18 (StataCorp, TX) software.

Results

Recruitment for this study began in February 2025 and is currently underway. As of the 25th of September 2025, 64 participants have been enrolled. Recruitment is anticipated to conclude in June 2026, with analysis expected to be completed by February 2027.

Discussion

To the best of our knowledge, there is currently no published research using the dose-to-mother technique to investigate whether PIMS is related to actual milk insufficiency nor has the association with nutrient composition been investigated, a gap in the literature as highlighted by the 2023 Lancet

Series on Breastfeeding (35). While one study has investigated the relationship between perceived and actual milk insufficiency (15), it was restricted to first-time mothers and used test-weighing methods to estimate infant milk intake, both of which inherently limit the interpretation of the findings.

Exploring the relationship between maternal perception and actual milk quantity and quality could have significant implications for improving breastfeeding support. If PIMS is linked to specific physiological characteristics of human milk, such as lower volume or suboptimal nutrient concentrations, this could provide a biological basis for potentially individualized interventions to support the continuation of breastfeeding. Alternatively, if perception is not linked to measurable differences, this reinforces the need for better psychosocial support and education to sustain breastfeeding, or the development of tools that can be used to assess milk supply and reassure mothers that their supply is sufficient. As perception is a modifiable factor, identifying its relationship to human milk volume and composition offers a critical opportunity to intervene early, support breastfeeding continuation, and improve infant and maternal health outcomes.

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Conflicts of Interest

None declared.

Data Availability

The data used in the present study are not publicly available due to ethical restrictions related to the consent provided by participants. An ethically compliant dataset may be made available by the corresponding author upon reasonable request and upon approval by the University of Otago Human Ethics Committee (Health).

Authors' Contributions

BB contributed to study design, data collection, and wrote the first and subsequent drafts of the manuscript. RT, CP, JH, BT, ALH, and IK contributed to the study design and critical review of the manuscript. SB and KG contributed to data collection and critical review of the manuscript. LD (Primary Investigator of MABBS) was responsible for study conception, study design, data collection, drafting of the manuscript and primary responsibility of the final content.

Abbreviations

BMI: Body Mass Index

D-MER: Dysphoric Milk Ejection Reflex

DTM: Dose-to-mother

D₂O: Deuterium oxide

HMA: Human Milk Analyzer

IBCLC: International Board-Certified Lactation Consultant

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

PIMS: Perceived Insufficient Milk Supply

WHO: World Health Organization

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Table 1. Overview of participant contacts

Recruitment: birth to 2 months	Recruitment of potential participants
Screening: 6 weeks to 2 months	Eligibility questionnaire
Visit 1 (baseline): 2.0 to <4 months	Written consent Baseline saliva samples (mother and infant) Isotope administration (mother only) Anthropometry (mother and infant) Main Questionnaire NB: The mother is asked to collect a saliva sample (mother only) 3-4 hours after the dose is administered.
Visit 2: 2 – 3 days after baseline	Follow- up (post-dose) saliva samples (mother and infant) Collect post-dose saliva sample –that was self-collected by the mother after Visit 1 (mother only)
Visit 3: 6 – 7 days after baseline	Follow- up (post-dose) saliva samples (mother and infant)
Visit 4: 13 – 14 days after baseline	Final (post-dose) saliva samples (mother and infant) Anthropometry (infant only)
Human Milk Collection: ~ 3 days after visit 4	12 mL subsample of full milk expression from single breast collected
Visit 5: 2 weeks after visit 4	Anthropometry (infant only) Single test-weigh = weight before and after a breastfeed (infant) Breastfeeding assessment Oral assessment D-MER questionnaire



Table 2. Potential predictors (maternal and infant) of human milk intakes

Maternal factors that potentially predict human milk intakes	Explanation of potential impact or hypothesis	Reference(s)
Breastfeeding self-efficacy	Mothers who have greater self-efficacy may have infants with higher intake.	Khresheh & Ahmad 2018 (67)
Breast/chest trauma	Mothers who have experienced breast/chest trauma may have infants with lower intake.	History of breast surgery: Kraut et al. 2017 (68) Abscess: Haifeng et al. 2022 (69) Chemotherapy: Stopenski et al. 2017 (70) Radiotherapy: Leal et al. 2014 (71) Spinal cord injury: Holmgren et al. 2018 (69)
Cannabis	Mothers who use cannabis may have infants with lower intake.	Castro-Navarro et al. 2024 (72)
Dysphoric Milk Ejection Reflex (D-MER)	Mothers who experience D-MER (sudden, intense negative emotions that occur immediately before and during milk let-down) may have infants with lower intake.	Ureño et al. 2018 (73)
Early return of menses	Mothers who have had return of menses may have infants with lower intake.	Spencer et al. 2023 (74)
Education	Mothers who are more educated may have infants with higher intake.	Bandara et al. 2015 (75) Haszard et al. 2024 (27)
Employment (current)	Mothers who are in current employment may have infants with lower intake.	Haszard et al. 2024 (27)
Following specific diet	Mothers who are consuming specific dietary patterns (e.g., low fat, dairy-free, vegetarian) may have infants with differing intakes.	Lee et al. 2016 (74) Spencer et al. 2023 (74)
Galactagogues/anti-galactagogues	Mothers who are consuming galactagogues (a substance that is thought to increase milk volume) may have infants	Brodribb et al. 2018 (76) Johnson et al. 2020 (77)

	with higher intakes, and mothers who are consuming anti-galactagogues may have infants with lower intake.	
Hormonal birth control	Mothers who are taking hormonal birth control may have infants with lower intake.	Berens et al. 2015 (78)
Mother BMI	Mothers with higher BMI may have infants with lower intake.	Hilson et al. 2004 (79) Haszard et al. 2024 (27)
Mother fat mass	Mothers who have higher fat mass may have infants with lower intake.	Diana et al. 2018 (80)
Parity	Mothers who have previously given birth may have infants with lower intake.	Whitehead et al. 1978 (81)
Previous breastfeeding experience	Mothers with previous breastfeeding success may have infants with higher intake while those who did not have success previously may have infants with lower intake.	Huang et al. 2019 (82)
Maternal morbidities	Mothers who have medical conditions may have infants with lower intake.	Spencer et al. 2023 (74)
Maternal Sleep	Hypothesize that maternal sleep may impact infant milk intake.	Maternal Sleep
Maternal use of medications	Mothers on certain medications may have infants with differing intake.	Farah et al. 2021 (83)
Mental health	Mothers who experience challenges with mental health may have infants with lower intake.	Spencer et al. 2023 (74)
Pain while breastfeeding	Mothers who experience pain while breastfeeding may have infants with lower intake.	McGuire 2018 (84)
Smoking/nicotine	Mothers who use nicotine may have infants with lower intake.	Napierala et al. 2016 (85)

Stress	Mothers with higher levels of stress may have infants with lower intake.	Nagel et al. 2022 (86)
Infant factors that potentially predict human milk intakes	Explanation of potential impact or hypothesis	Reference(s)
Age	It is known that infant intake varies over the course of lactation.	Bandara et al. 2015 (75) da Costa et al. 2010 (27) Whitehead et al. 1978 (81) Brown et al. 1982 (87) Haszard et al. 2024 (27)
Body weight	Smaller body weight may have lower intake.	Bandara et al. 2015 (75) Daniels et al. 2019 (17) Agne-Djigo et al. 2013 (88) Haisma 2003 (30) Haszard et al. 2024 (27)
Demand vs. schedule feeding	Infants fed on demand rather than by schedule may have higher intake.	Dewey et al. 1986 (89) Haszard et al. 2024 (27)
Growth trajectory	Infants with greater growth velocity may have higher intake.	Olga et al. 2023 (32)
Household temperature and humidity	Higher household temperature and humidity may have higher intake.	Baby et al. 2023 (90)
Number of breast feeds (Average #/d)	Infants feeding more often may have higher intake.	Brown et al. 1982 (87) Haszard et al. 2024 (27)
Sex	Male infants may have higher intake than female infants.	da Costa et al. 2010 (27)
Spilling (Average quantity)	Hypothesize that intake may differ in infants who spill.	
Term/preterm/early term	Infants born early may have lower intake.	Lapillonne et al. 2019 (91) Altuntas et al. 2015 (92) Boies & Vaucher 2016 (93)

Supplementary Files

Multimedia Appendixes

HRC Peer-Review.

URL: <http://asset.jmir.pub/assets/f040cc86791412a74432b03f9c7cd23c.pdf>

Ethics Peer-Review 1.

URL: <http://asset.jmir.pub/assets/54e4416e352c01027833797b9e421bc2.pdf>

Ethics Peer-Review 2.

URL: <http://asset.jmir.pub/assets/8c9d8918d6ba4da40455dc36e6287105.pdf>