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The impact of human milk feeding modality (breast vs bottle) on infant growth during the first 6 months postnatal

By

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Abstract

Background: Exclusive breastfeeding is recommended for the first 6 months, but expressing, or pumping milk has become common in high-income settings. While feeding human milk has long been equated with breastfeeding, there is some evidence of differences in feeding responsiveness and other feeding behaviours differing by feeding modality, that is, directly breast-feeding, or mixed breast- and bottle-feeding expressed human milk, that may impact infant growth. There is paucity of research comparing infant growth by human milk feeding modality. Additionally, discrepancies in infant growth measuring practices by healthcare practitioners could further complicate interpretation of infant growth data.

Objectives: To assess infant growth and growth trajectories during the first 6 months by feeding modality (directly breast-fed, or mixed breast- and bottle-fed expressed human milk). Also, to explore usual infant measuring practices by healthcare practitioners, and to assess agreement between infant anthropometric measures and z-scores computed by healthcare practitioners and trained researchers.

Methods: This was an exploratory, repeated-measures observational study, conducted with predominantly human milk-feeding mother-infant dyads at 2, 4, and 6 months postpartum. Descriptive statistics were computed for both mothers' and infants' sociodemographic characteristics, infant anthropometric measures and z-scores, and healthcare practitioner measuring practices. Independent samples t-tests were used to compare anthropometric measures and z-scores by infant feeding modality, and paired samples t-test for anthropometric measures and z-scores by healthcare practitioners and trained researchers. Infant growth trajectories over time were compared by feeding modality using a repeated measures ANOVA adjusted for covariates.

Results: Most infants were both breast- and bottle-fed human milk (74%). Compared to breastfed-only infants, weight-for-age, weight-for-length, and BMI-for-age z-scores were all significantly lower among infants who were mixed breast- and bottle-fed expressed human milk at 2 months (all $p<0.05$). Study participants were commonly measured using a paper-pencil method for length, an infant scale for weight, and a tape measure for head circumference. When comparing infant anthropometrics by healthcare practitioner and researcher, practitioners recorded infants as lighter at 2 months and longer at 6 months, with higher length-for-age z-scores at both 4 and 6 months, and lower weight-for-length and BMI-for-age z-scores at 6 months. However, there was a strong agreement between infant anthropometric z-scores between healthcare practitioners and researchers.

Conclusion: In contrast to previous work, infants fed only at the breast had higher anthropometric z-scores at several timepoints in the first 6 months compared to infants mixed breast- and bottle-fed human milk. However, growth trajectories over the first 6 months did not differ by modality. Although there were some statistically significant differences in anthropometric measures between healthcare practitioners and researchers, these small differences were not clinically meaningful, and there was strong agreement between measurers.

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1.0 Introduction

Human milk is the recommended food for infants especially during the initial 6 months of life, as it provides numerous health benefits to both the mother and the infant (1,2). The World Health Organization (WHO) endorses breastfeeding initiation within the first hour after delivery, exclusive breastfeeding for the first 6 months of life, and continued breastfeeding for 2 years and beyond (1,3). However, expressing milk or pumping is becoming more common for a variety of reasons, including lactation challenges, the advancement in electronic breast pumps, mother's desire to share feeding responsibilities with other caregivers, stigma associated with breastfeeding in public, and/or wanting other caregivers to participate in bonding associated with feeding (4).

While feeding human milk has long been regarded as the same as breastfeeding, recent evidence suggests that the feeding modality (direct breastfeeding vs bottle feeding human milk) influences feeding behaviours, such as the responsiveness of feeding (5,6). Among human milk-fed infants, bottle-fed infants tend to gain more weight, and have higher body mass index (BMI) z-scores and weight gain velocities when compared with breastfed infants (7). Currently, there is limited research investigating the growth differences among infants fed directly at the breast and those bottle-fed human milk, and none based in Nova Scotia. Therefore, this study aims to fill this gap.

Since most infants undergo routine physical measurements at their primary health care centers, as an additional outcome, the agreement between anthropometric measures taken by primary care providers and those by trained research staff will also be compared to gain an understanding of the accuracy of routine measurements.

2.0 Terminology

The WHO defines exclusive breastfeeding as the consumption of human milk alone, not accompanied by additional food or drink, even water, for the first six months of life (3). However, exceptions include drops or syrups containing vitamins, minerals, supplements, and medicines, if needed (2). Although the WHO includes breastfeeding by a wet nurse and expressed milk from a donor in their definition of exclusive breastfeeding, this thesis will limit exclusive breastfeeding to the sole provision of mother's milk, either by breast or bottle (1,2,8,9).

There are many forms of human milk feeding, with two commonly practiced feeding modalities including breastfeeding and bottle-feeding expressed mother's milk (10). The term 'breastfeeding' is used throughout this thesis to refer to direct feeding of mother's milk at the breast (1,10). Although bottles are commonly used to feed human milk substitutes like infant formula (11), throughout this thesis, bottle-feeding referred to the practice of feeding human milk from a bottle. Human milk may be fed immediately after expression or after a period of storage (for instance, frozen or in a fridge, and later re-heated), and may be fed to the infant by the mother herself or by another caregiver.

While other researchers cited through this thesis may have used different terminology, for example 'breastfeeding with a bottle', 'feeding expressed milk' (12), to ensure clarity here, language from the literature was changed to reflect the definitions provided above.

The term human milk feeding was used as a general term to encompass both breastfeeding and bottle-feeding human milk, and was also used when it is unclear whether authors of cited papers are speaking to a certain feeding modality.

3.0 Literature Review

3.1 Benefits of human milk feeding

Human milk feeding is a key public health intervention, well-established to reduce the burden of morbidity and mortality for both mother and child (13). Health Canada recommends human milk feeding as it provides the infant with all required nutrients and is considered an adaptive source of food that regulates over time to meet changing demands of the infant (14,15). Human milk is a unique infant food as it also supplies specific immunological bioactives to the infant such as lactoferrin, immunoglobulin A, tumor growth factor, and lysozyme, which rapidly increase nutrient absorption, growth stimulation, and regulation of immune system (15). These immunomodulatory, anti-inflammatory, and anti-microbial bioactives reduce the risk of both short-term and long-term morbidity (16). Other specific benefits of human milk feeding to both the infant and mother are described in detail below.

3.1.1 Benefits to the infant

Exclusive human milk feeding protects the child against a myriad of illnesses and diseases like diarrhea, lower respiratory tract infections, constipation, risk of ear infections (acute otitis media), eczema (atopic dermatitis) and childhood overweight and obesity (13,17,18). Human milk feeding also promotes sensory and cognitive development (19). Hauck and colleagues analyzed numerous studies from 1966 to 2009 and found that those infants who were ever breastfed had a 45% reduction in Sudden Infant Death Syndrome (SIDS) risk, those who were breastfed for more than 2 months had a 62% reduction, while those who were exclusively breastfed for 6 months had a 73% reduction, when compared to formula-fed infants (20). Furthermore, a meta-analysis of 7 studies discovered that feeding human milk decreased the risk of type 2 diabetes in infants by 39% when compared to infants who were formula-fed (OR [95% CI]: 0.61 [0.44, 0.85]) (21). Continued human milk feeding

for longer than 12 months is associated with higher human capital among children and adolescents, including better performance on intelligence tests, which some researchers have hypothesized could be related to the long-chain poly-unsaturated fatty acid content of human milk, thus enhancing retinal and neural development (22–24).

Through the above-mentioned evidence, human milk feeding is of great benefit to the infant and there are also maternal health benefits.

3.1.2 Benefits to the mother

There are numerous benefits of human milk feeding for maternal health, including reduced maternal bleeding after delivery, facilitation of positive metabolic changes, reduction in stress, delayed ovulation (25), as well as a reduction in risk for various cancers (26). Increased, exclusive, or predominant human milk feeding is associated with longer periods of amenorrhea that helps in birth spacing (13,26). A meta-analysis including 400 individual studies noted a 28% lower risk of developing ovarian cancer in mothers who breastfed for a minimum 12 months, as compared to those who never breastfed (27). A collaborative data analysis from 47 studies conducted over 30 different countries discerned that the likelihood of developing breast cancer decreased by more than 4.3% (95% Cl 2.9-5.8%) for each year a mother breastfeeds her infant, accompanied by a reduction of 7% (5.0-9.0; $p<0.0001$) for each additional birth (28). Mothers who practiced human milk feeding for 7-12 months after their first delivery were 28% less likely to later develop cardiovascular disease (29), and 23% less likely to develop coronary artery disease (30). For each additional year of lactation, a 15% (1-27%) decrease in the risk of diabetes was observed among Nurses' Health Study (NHS) participants and 14% (7-21%) among NHS II participants (31).

Given the recognized benefits for both infant and mother, numerous public health organizations have established recommendations for human milk feeding, which will be outlined below.

3.2 Human milk feeding and other infant and young child feeding recommendations

Global infant and young child feeding (IYCF) recommendations include skin-to-skin contact immediately after birth, with initiation of breastfeeding within the first hour (8,32). Initial immediate contact between the mother and infant improves breastfeeding initiation rates (8). Initial immediate contact also provides other health benefits, including early neuro-behavioural self-regulation, and improved cardiorespiratory, glucose, and temperature regulation in infants (33,34). Initial immediate contact also enhances mother-infant bonding, accompanied with lower stress levels in mothers due to production of prolactin and oxytocin during breastfeeding (35,36). In agreement with the WHO, Health Canada recommends that infants be exclusively breastfed to six months, at which point nutritionally adequate, safe, iron-rich complementary foods should be introduced, with breastfeeding being continued for two years and beyond (37,38).

The IYCF practices are designed to optimize infant nutritional status, health, and survival, as well as benefit maternal health (39,40). Health Canada, the Canadian Pediatric Society, Dietitians of Canada, and the Breastfeeding Committee for Canada published two sets of IYCF recommendations: Nutrition for Healthy Term Infants (NHTI) for birth to 6 months (41), and NHTI for 6 months to 24 months (42). Human milk feeding remains the preferred mode of infant feeding in other situations including premature infants, mothers living with human immunodeficiency virus, malnourished infants and children, families experiencing complex emergencies, and adolescent mothers (38,43). In Canada, other

recommendations include provision of daily vitamin D supplements of 10 µg (400 IU) for breastfed infants (44).

Despite the well-established benefits of human milk feeding, rates remain suboptimal in Canada and around the world, which will be explored below.

3.3 Global rates of human milk feeding

The gap between human milk feeding recommendations and current breastfeeding rates has grown considerably, especially among socially and economically vulnerable women (45,46). Many factors and causes have contributed to low adherence to recommended human milk feeding guidelines, for instance lack of knowledge around breastfeeding, complications in the health system, social and cultural norms, physical breastfeeding challenges, practical breastfeeding challenges like maternal time pressure, and finally, time and cost associated with breastfeeding support (45,47). Other reasons that contribute to an unsupportive breastfeeding culture include lack of resources, insufficient support at workplace for continued breastfeeding, contrast between recommendations and beliefs of physicians, and absence of support from family members (48). Additionally, lack of availability of support services, inadequate maternal leave policies, social determinants of health, and cultural factors act as barriers to the prevalence of breastfeeding (7). However, it should be noted that professional lactation support and lactation training given by healthcare practitioners is reported to enhance breastfeeding interventions and initiation rates (49). Worldwide, only 48% of infants are breastfed within the first hour of their delivery, 65% are breastfed for the first 2 days after birth, and only 42% of the infants are exclusively breastfed (50).

Human milk feeding is more prevalent in low- and middle-income countries (LMIC) than high-income countries (HIC) (13). In 2019, United Nations International Children's Emergency Fund (UNICEF) reported that exclusive breastfeeding increased from 35% in

2005 to 42% in 2018 in a total of 80 LMIC across the world (51), and the most recent 2020 data indicated that 44% of infants are exclusively breastfed to 6 months (52). Although the above stated data represents the worldwide rates of exclusive breastfeeding, the country-specific statistics differ significantly. For instance, the highest rates of exclusive breastfeeding were observed in LMIC like Rwanda (86.9%), Sri Lanka (82%), Burundi (82.3%), the Solomon Islands (76.2%), and Vanuatu (72.6%) in the year 2018 (53). Contrastingly, lower exclusive breastfeeding rates were found in HIC like USA (24.9% in 2019) (54), UK (1% in 2010) (55), and Norway (11.1% in 2014) (56). Given the current rates, predictions indicated that even in LMIC the prevalence of exclusive breastfeeding will remain below the global targets of 50% by 2025, and 70% by 2030 (57). Global breastfeeding data varies from the Canadian context, as outlined below.

3.4 Prevalence of human milk feeding in Canada, and Nova Scotia

In Canada, 89% of mothers initiated breastfeeding soon after childbirth, but only 26% exclusively breastfed to the recommended 6 months in 2011-2012 (58). Although a high initiation rate of early breastfeeding is observed across Canada, with highest in British Columbia (93.1%) (59), rates in Nova Scotia remain below the Canadian average (58). Statistics Canada reported that 88% of women in Nova Scotia initiated breastfeeding, and only 22% exclusively breastfed their infants (58). The most common reasons for breastfeeding cessation in Canada are: not enough human milk production (26.1%), willingness of the baby to consume solid foods (18.9%), and self-weaning of the infant (13.1%) (58,60).

In a cohort study of 4533 mothers in the province of Nova Scotia, 64.1% mothers initiated early breastfeeding, and by 6 weeks more than 40% of women had ceased to exclusively breastfeed their infants (12). You need more than a sentence here….

3.5 Bottle feeding human milk

Expression, of breast milk, is defined as the practice of removal of milk from the breast by hand or pump (61). Mothers choose to provide expressed human milk to their infants for numerous reasons, including challenges with latching at the breast and nipple pain associated with attachment (4,62). Other reasons why women choose to pump milk include building an emergency supply (63), increasing their milk supply (4,64), relieving breastfeeding issues like mastitis and breast engorgement (65), oversupply or undersupply of human milk (63), mother's intent to return to work commitments (63), and embarrassment associated with breastfeeding in public (62,63). Although breastfeeding and provision of expressed human milk is often seen as equivalent, there is a higher risk of pathogenic contamination inherent to expressed milk given the container changes and necessary milk storage (66,67). Factors like milk volume, room temperature during milk expression, temperature changes in the refrigerator and freezer, and conditions of the external environmental guide the duration and temperature guidelines for safe and efficient human milk storage (68). The optimal storage conditions for expressed milk are described in **Table 3-1** (68–70).

Table 3-1. Human milk storage recommendations

Storage location and temperatures			
Type of Breast Milk	At room temperature (25°C)	Refrigerator (4°C)	Freezer (-18°C) or colder
Freshly Expressed or Pumped	Less than 4 hours	Up to 4 days	• Up to 3 months for best quality • Up until 6 months for high quality • Up to 12 months is acceptable

Previously frozen	Thaw to room temperature, and ideally use within 1-2 hours	Thaw and use within 24 hours	Never refreeze expressed breast milk after it has been thawed
Leftover from a Feeding (Unfinished milk in the bottle)	Use within 2 hours after the baby is finished feeding		

To determine the portion size of human milk for healthy infants, infant cues should be followed to decide the amount of milk required per feed (71). Since a mother's preference of feeding modality can determine the quantity of human milk fed to the infant (4), the following section elaborates on the differences between feeding at the breast and feeding human milk with a bottle.

3.6 Differences in the practice of breastfeeding and bottle-feeding

There is growing literature questioning the long-term health impacts of bottle-feeding, given the numerous differences in feeding patterns spurred by this feeding modality (72). For example, among exclusively human milk-fed infants aged 21 days to 5 months (N=234) in a cross-sectional study in Barcelona, Spain, feeding sessions were recorded by registered nurses, and researchers observed that sucking patterns differed significantly between those fed only at the breast compared to those fed exclusively with a bottle (73). Infants who were breastfed had a higher mean number of sucks per minute and the same number of pauses but of shorter duration, as compared to bottle-fed infants (73). Differences in the sucking patterns associated with breastfeeding and bottle-feeding can potentially impact the weight gain velocity and body mass index (BMI) of infants (7).

Recent data from the Infant Feeding Practices Study II (IFPS II) found that infants who were bottle-fed showcased more rapid weight-gain than those fed directly at the breast (74,75), owing to the differences in feeding practices (4). Infant controlled length and timing of the feed, multiple suction movements and pauses, longer duration of pauses, and less

consumption of milk volume during breastfeeding is thought to facilitate less rapid weight-gain of breastfed infants compared to those bottle-fed human milk (4,73). Bottle-fed infants are believed to have substandard growth when compared with breastfed infants, due to lower and more uneven distribution of energy and fat in pumped human milk (72). A cohort study conducted among 2553 mother-infant dyads investigated weight-gain velocity and BMI in breastfed, bottle-fed, and formula-fed infants, and found that exclusively breastfed infants receiving additional expressed human milk had higher BMI scores than directly breastfed infants (mean: +0.14 ± 1.00 vs -0.02 ± 1.06; aβ + 0.12; 95% CI: 0.01 to 0.23) (7). Bottle-fed infants were observed to have intermediate BMI z-scores, but higher weight-gain velocities as compared to infants fed exclusively at the breast, and lower velocities than infants fed formula (76).

There are also differences in responsiveness, infant cues, and mother-infant behaviours during bottle-feeding and feeding at the breast (77), which are explained in detail below.

3.6.1 Responsive feeding by human milk feeding modalities

Responsive feeding is defined through an interdisciplinary lens as a practice that encourages infants to self-regulate their food intake, in order to fulfill their physiological and developmental needs that bring about cognitive, emotional, social, and physical growth in the long run (78,79). Responsive feeding is the ideal way of feeding, as it nurtures reciprocity between the caregiver and infant, and facilitates development of ideal eating patterns (6,80,81). Conversely, non-responsive feeding styles, such as controlling or pressuring behaviours are considered suboptimal feeding practices, because of the disengaged nature of the caregiver, and failure to establish a healthy eating routine among children, which could

lead to adverse outcomes like eating in the absence of hunger and obesity (81,82). The extent of responsiveness may impact infant weight and length during the first year of life (83).

3.7 Anthropometric measurement of infants

Growth assessment is a relatively simple and low-cost means of assessing the health and nutritional status of a population (84). Anthropometry is a universally recognized, portable, economical, and non-intrusive technique of evaluating the distribution, dimensions, and configuration of the human body (85). Repeated longitudinal anthropometric measurements, or growth monitoring, allows for the timely identification, diagnosis, and treatment of nutritional deficiencies or developmental defects (38,86).

3.7.1 Origin of infant growth monitoring

To assess the growth and nutritional status of infants and young children, and develop new growth references, from 1998 to 2003, the WHO coordinated an international, community-based effort known as Multicenter Growth Reference Study (MGRS) (87). This initiative was formed to replace the already existing 1997 National Center for Health Statistics (NCHS) and the WHO growth reference that had been in use for the international population (88,89). In MGRS 8,500 children were sampled from six different countries (Brazil, Ghana, India, Norway, Oman, and the United States), with the aim to collect anthropometric data of infants from 1 week up until 12 months of age (87). Additionally, this approach was designed to provide a single international reference to best describe and analyze physical growth of children under 5 years of age, and to understand growth during optimal conditions (87,90). In April 2006, new growth recommendations and growth charts were created by the WHO through the MGRS initiative (91).

3.7.2 Growth monitoring in practice

The WHO recommends the use of growth charts for monitoring physical development in infants (92). In Canada, the WHO growth charts replaced the use of previous charts developed by the American Centers for Disease Control and Prevention (CDC) (93). Percentiles and z-scores are used to evaluate growth in the neonatal and pediatric populations, while percentiles are more commonly used at a community level (91,94). The z-scores are measured in standard deviations, where z-score value of 0 is the median or mean for a normal curve (95). These z-scores can then be used to define malnutrition by comparing individuals' z-scores to the normed standards (95). The z-scores and their respective growth indicators, and depiction of nutritional status are described in **Table 3-2**, as modified from (94,96–98):

Table 3-2. z-scores and associated nutritional complications

	Growth Indicators or Nutritional Conditions				
z-score	Length-or Height-for-age z-score (LAZ)	Weight-for-age z-score (WAZ)	Weight-for Length or Height for age z-score (WLZ)	BMI-for-age z-score (BAZ)	Head Circumference-for-age (HCA)
≥3	Very tall	Flag: Assess growth using WLZ or BAZ	Obese	Obese	Macrocephaly
≥2	Moderately tall	Overweight for age	Overweight	Overweight	Macrocephaly
≥1	Normal range	Normal range	Normal range	Normal range	Normal range
0	Normal range	Normal range	Normal range	Normal range	Normal range
<1	Normal range	Normal range	Approaching rapid weight loss	Approaching rapid weight loss	Normal range
<2	Moderately stunted	Moderately underweight	Moderately wasted	Wasted	Microcephaly
<3	Severely stunted	Severely underweight	Severely wasted	Severely wasted	Microcephaly

The use of z-scores allows for comparisons across ages and sexes, can quantify growth status at both extremes of the distribution, and can track children's growth over time (99).

3.7.3 Commonly used measurements in primary care setting

Growth monitoring in infants includes assessment of weight, length, and head circumference in relation to the growth of an international reference population or compared with standardized growth norms (94). Anthropometric assessments of infants are done using weighing and measuring equipment, most commonly with weight scales and measuring boards (100). Detailed utilization of commonly used anthropometric equipment is explained in **Table 3-3** below (100,101).

Table 3-3. Infant anthropometric equipment and their respective usage

Type of measurement	Equipment	Usage	Largest acceptable differences between repeated measurements
Weight	Electronic Scale	• The child can be weighed directly and accurately on an electronic scale. • Weight can also be measured through adult-assistance on a regular scale, through taring.	0.5 kg
	Hanging Scale	• A hanging spring scale that can measure up to 25 kg in 100 g increments.	
Length	UNICEF Model Height/Length Measuring Board	• A recommended instrument to measure recumbent length of infants. • It measures a maximum of 130 cm of height. This tool is 30 cm wide and weighs an estimated 10 kg.	1.0 cm
	Infant Recumbent Length Board	• It is a lightweight and durable instrument, which can measure the recumbent length up to 100 cm.	

Head Circumference	Flexible measuring tape	• This tape is positioned above eyebrows and ears to measure the distance around the circumference of the infant's head.	0.5 cm

Other than these recommended and commonly used tools, infant meters also called infantometers are used to measure a child's length up to 24 months of age (102,103). Some practitioners also use the 'paper and pencil' method, where the child is placed on an examining table and the distance between a mark at the child's head and foot is determined using a measuring tape (104). In case of an emergency where standard anthropometric equipment is not available, tools like the 'Broselow tape', a length-based resuscitation tape, can be used to estimate weight (103). Head circumference measurement is another method to monitor infant growth and development, and is a swift, non-invasive method in determining the head size (101). The occipital head circumference should be measured twice, using a non-elastic tape measure, and plotted on the growth chart accordingly (105). At times, health care practitioners also use paper measuring tapes to measure head circumference of an infant by making a small rip in the paper at the junction, and then releasing it from the head and reading it (106).

Anthropometric measures can be used to calculate BMI z-scores of an infant, in order to assess the relevance of weight to height, and evaluate for wasting, overweight, or obesity (107). BMI is calculated by dividing weight (in kilograms) by height (in meters) squared (108). BMI z-scores reflect age-dependent variation of weight and length during infancy and predict future adiposity in infants (109).

These measurements should be used according to time and procedural recommendations provided by numerous health organizations in Canada, as described in detail below.

3.7.4 Recommended growth assessments

According to the collaborative health statement by Dietitians of Canada, Canadian Pediatric Society, The College of Family Physicians of Canada, and Community Health Nurses of Canada, growth charts should be used for all full-term infants as per the WHO Child Growth Standards (birth to 5 years of age) (91). It is recommended that length or height and weight data, for infants, should be collected by health care providers in each of the following windows: between birth to 28 days, 6 to 8 weeks, 7 to 9 months, and at 2 years of age (110,111). Additionally, the American Academy of Pediatrics, Dietitians of Canada, Canadian Pediatric Society and Community Health Nurses of Canada recommend measuring the head circumference at least 8 times during the initial 2 years of life (91,112). Specific changes in these anthropometric measurements, for instance, height, weight, WAZ, and WLZ, calculated between 6 months to over one year is referred as growth velocity, and can be charted (91,113). Growth velocity charts sensitize small variations in growth status when compared to the regular size-attained charts and are also more beneficial in evaluating growth changes and investigating growth concerns in infants (114,115).

A study involving a collaboration of two broad studies [The Western Australian Pregnancy Cohort and three additional European cohort studies (European Childhood Obesity Trial, Norwegian Human Milk Study, and Prevention of Coeliac Disease)] was modelled to compare individual growth trajectories in infants (N=6708) breastfed for >3 months and <3 months (116). It was observed that a shorter duration of breastfeeding for <3 months was associated with accelerated growth (OR: 2.66; 95% CI: 1.48, 4.79) and rapid growth (OR: 1.96; 95% CI: 1.51, 2.55) rather than normative growth of the infants breastfed >3 months (116). Accelerated growth is defined as change in WAZ > 0.5 or 0.67 (117), and rapid growth is explained as change in weight or LAZ greater than +0.67 from birth to 24 months of age (118). Another study analyzing growth velocity of infants in South Africa, as

part of the PROMISE-EBF trial (N=641) observed that infants who were not breastfed at 12 weeks had a 0.37 (95% CI 0.07, 0.66) increase in BAZ, a higher weight velocity, and were more overweight and obese at 2 years (119).

Overall, growth velocity is a valuable approach that describes age-dependent changes in velocity that categorize human post-natal growth (120). For instance, a birth cohort with 256 healthy neonates analyzed their growth velocity over the period of 5 years, and observed that weight, length, and head circumference velocity curves showed the highest values directly after birth, decreased through the first 2 years of life, and then remained constant up to 5 years (121). Thus, changes in growth velocity and its graphical representation are essential for proper growth assessment of infants, and is a useful screening tool to assess short-term changes in the growth of infants (121,122).

Growth assessment of infants can vary across different settings and by individual healthcare providers, therefore, the following section describes the agreement between health setting and researcher conducted anthropometric measurements.

3.7.5 Agreement between health setting and researcher conducted measurements

Routinely collected height, weight, head circumference, and body mass measurements are used in clinical practice, research, and epidemiological studies, and provide a swift, rapid, and economical method of data collection (111,123). However, not all measurements are necessarily taken following standard practice. This section will explore the agreement between researcher- and health-care professional (HCP) collected infant anthropometric measurements.

A study comparing length and weight measurements of infants at 6, 12, and 24 months obtained from the longitudinal UK birth control (born in Bradford) and routine data by health visitors, observed a weaker agreement for height than weight (111). Routinely

collected data by health visitors underestimated infant's length at 6 months (-0.7 cm (-0.9 to -0.6)) and overestimated at 12 (0.7 cm (0.5 to 0.9)) and 24 months (0.5 cm (0.4 to 0.6)), and overestimated weight at all the three ages (-0.04 kg (-1.2 to 0.9) to (-0.04 (-0.7 to 0.6)) (111). Another cross-sectional study conducted on infants (n=129) in Northern Pennsylvania from July 2016 to May 2018 assessed the agreement in infant growth monitoring between Special Supplemental Nutrition Program for Women, Infants, and Children (community) and clinical (primary care providers) care settings (124). A statistically significant bias toward WLZ was observed to be greater in the community setting (0.66 ± 1.04) than those obtained at the clinical setting (0.48 ± 1.11, $p<0.001$) (124). However, the poor growth evaluation agreement reported between community and clinical providers was attributed to irregular appointments and the dynamic nature of growth of infants (125,126).

Length measurements appear to be particularly problematic (124). In a multicenter randomized controlled study involving 837 infants (birth to 6 months), only 30% of length measurements by clinicians were accurate (≤0.5 cm difference from standardised measurements) (127). Likewise, another study including 160 children (0-23 months) compared clinician- calculated infant length measures using paper-pencil method to the researcher- administered standardized technique and found that clinicians over-estimated length by 1.88 cm (128).

3.8 Effect of feeding modality on infant growth

Even if the same food, human milk, is provided, bottle-feeding is distinct from feeding at the breast in its potential to impact infant growth (129). A study conducted in the United States between May 2005 and June 2007 noted that infants fed only by bottle gained 89 g more weight per month ($p=0.02$) when compared to infants fed only at the breast (129). While obesity can predispose a child to a myriad of diseases like hypertension, diabetes, and

cardiovascular disease later in life, feeding patterns in the early stage of infant life play a crucial role in determining their growth rate and development (130). The differences between breastfeeding and bottle-feeding can occur due to the sucking speed of an infant, associated milk volume intake, and the level of responsiveness (81,83). Numerous studies have drawn an association between the feeding practices and feeding modalities employed during infancy, and their respective effects on an infant's growth (131), which will be explained in detail below.

3.8.1 Breastfeeding and infant growth

Provision of human milk is a biological norm, and MGRS (and hence growth charts) are based on children following optimal IYCF practices, including exclusive human milk feeding. Human milk fed infants tend to grow faster than infants fed human milk substitutes in the first 6 months of life and tend to grow slowly in the proceeding 6 months (130,132,133). A cross-sectional study conducted with 360 healthy, exclusively breastfed infants in Belem, Brazil from October 2006 to December 2008, found greater mean weight at 5th and 6th month of life suggesting greater growth of exclusively breast-fed infants when compared to infants receiving other forms of feeding (134).

3.8.2 Bottle-feeding and infant growth

Bottle-fed infants have a higher risk of rapid weight gain, compared to feeding directly at the breast (77). A study analyzing WAZ trajectory in bottle-fed infants from birth to 12 months observed a 'rapid increase' in growth trajectory if they consumed 30% of feeds from a bottle during the neonatal period, subsequently increasing to 100% by 6 months, whereas 'high-stable' growth was observed in infants who took 100% of feeds from bottles throughout the period of infancy (135). Infants falling in the category of 'rapid increase' and

'high-stable' growth trajectories depicted significantly higher WAZ ($p < 0.001$) when compared with 'gradual increase' and 'low-stable' groups (135). The drastic weight gain through bottle-feeding may be attributed to greater intake of milk during bottle-feeding as compared to breastfeeding (77). Faster speed of the feed, less infant control, and external cues (e.g. milk remaining in bottle) may contribute to greater food intake (136). I will explore the relationship between infant growth rates over the two predominant feeding modalities, breast- and bottle-feeding human milk in this thesis.

3.9 Research gap and potential significance

Exclusive human milk feeding is recommended for the first 6 months, which can be provided by breast or bottle. While differences in growth trajectories in relation to weight and BMI z-scores have been observed between breastfed and formula-fed infants (137), there is a paucity of evidence analyzing the growth of infants that are bottle-fed human milk (138). As such, this thesis will assess infant growth rates over the first 6 months in predominantly human milk-fed infants who are either fed at breast only, as compared to those fed with a mix of breast and bottle-feeds.

Additionally, the standard anthropometric procedures exercised by HCP in Nova Scotia will be studied. Growth monitoring is an important screening tool that health professionals regard as a requirement of pediatric services throughout the world (139). Despite the importance of accurate infant measurements, little is known about the accuracy of clinician-measured infant anthropometrics in Nova Scotia. As such, I will study the agreement between HCP- and trained research staff- collected anthropometric measurements during the first 6 months postnatal.

4.0 Methods

4.1 Research objectives

The objectives of this study were as follows:

4.1.1 Primary objectives

1) To describe the growth of infants at 2, 4, 6 months postnatal, including length, weight, head circumference, WAZ, LAZ, WLZ, and BAZ, by human milk feeding modality (directly at the breast only, versus mixed breast- and bottle-fed expressed human milk)
2) To assess growth rates (WAZ and growth velocity) over the first 6 months among predominantly human milk-fed infants in Nova Scotia, by human milk feeding modality.

4.1.2 Secondary objectives

1) To assess usual infant anthropometric measurement practices among various primary and public health care settings in Nova Scotia.
2) To assess agreement in infant anthropometric assessments conducted by HCP during routine visits to those assessed by researchers using specialized infant anthropometric equipment.

4.2 Study setting

The study was conducted in Nova Scotia, a Canadian province located on the eastern coast of the country, with its proximity to the Atlantic Ocean (140). Halifax is the capital city of Nova Scotia and forms a major part of the Halifax Regional Municipality (HRM) along with Dartmouth, former Halifax County, and other Bedford areas (141). Halifax being the 14th largest municipality in Canada, and the 13th largest metropolitan area, contributes a

population of 297,000 to the total population of 439,819 of HRM (142,143). Nova Scotia has one of the lowest rates of breastfeeding in Canada, with only 22% of infants exclusively breastfed for 6 months in the year 2018, as compared to 33% nationwide (144).

4.3 Study design

This thesis research was an exploratory, repeated-measures observational study. The overarching study was designed to compute and compare anthropometric measures (length, weight, head circumference) and z-scores (WAZ, LAZ, WLZ, BAZ) at 3 timepoints, 2, 4, and 6 months postnatal and to assess potential differences in infant growth by feeding modality (directly at the breast only, versus mixed breast- and bottle-fed expressed human milk). The study design is further explained in **Table 4-1** below.

Table 4-1. Summary of data collection at 2, 4, and 6 months

Recruitment	2, 4, 6 Months Postnatal
Online N=129 mother-infant dyads	Mothers were asked to retrospectively provide weight, length, and head circumference measurements of the infant from HCP. The online questionnaire included: Sociodemographics (2 months only) IYCF practices Feeding modality
In-person n=36 (at 2 months) n=29 (at 4 months) n=31 (at 6 months)	Researchers recorded duplicate measures of weight, length, and head circumference of the infant (within ± 48 hours of HCP measurement)

4.4 Participants

4.4.1 Sampling methods

Mother-infant dyads were recruited using convenience sampling. Participants were recruited during the perinatal period through social media delivered advertisements like regular and boosted posts, and posters and pamphlets distributed via doctor's offices, Le

Leche League meetings, local community, and day care centers. The study was also publicized at community locations like grocery stores, municipal centers, and malls. Interested mothers could contact the research team via email, text, or phone. The participants were screened based on the eligibility criteria (*see section 4.4.3*) and were asked to provide implied consent for the questionnaire, and written consent for in-person administered anthropometric measurements.

4.4.2 Sample size estimates

In 2021, there were 7,447 births in Nova Scotia (145). Assuming 6 months of data collection, with previous reports that 22% of infants are exclusively breastfed in Nova Scotia (58), the eligible population would include ~800 infants across the province. Appreciating challenges in research participation among this busy group, the higher burden of participation in a repeated measures survey, and the fact that not all breastfeeding caregivers may express human milk to feed with a bottle, we aimed to recruit 10-15% of the population, or between 80-120 caregiver-infant dyads. All participants were invited to participate in a sub-study to collect in-person, researcher-administered infant anthropometric measurements (either at home or on-campus). Since these participants must have resided in the HRM, and given the physical distancing preferred by many during COVID (data collected in winter 2021), we expected ~25% of participants to consent to participate to this in-person portion of the study (i.e. n=20-30).

4.4.3 Eligibility criteria

Mother-infant dyads were eligible to participate in the study if they met the following criteria:

4.4.3.1 Inclusion criteria for mothers

Mothers were eligible to participate if they:

- were 19 years or older
- currently lived in HRM, in Nova Scotia,
- had a healthy singleton baby who was younger than 6 weeks of age,
- at time of recruitment, were predominantly feeding human milk,
- planned on predominantly feeding human milk for the first 6 months of life (human milk fed at the breast or as expressed milk in a bottle), and
- planned on attending a standard schedule of infant follow-up visits with their primary health care provider (minimum at 2 months, 4 months, and 6 months of age).

4.4.3.2 Exclusion criteria for mothers

Mothers were not eligible to participate if:

- their baby was born preterm (earlier than 37 weeks' gestation),
- their baby had a condition that impacted feeding (e.g. cleft palate), or
- they planned to move in the next 6 months.

Apart from this, the only additional eligibility criteria to participate in the 'in-person component' of the study was to undergo researcher-administered anthropometric measurements, to enable data collection visits either in the MAMA lab or at the participant's home.

4.4.4 Remuneration

Participants were provided a remuneration of $20 for each online questionnaire that they completed. For those participants recruited in the in-person sub-study, an additional $10 were given to them for each researcher-administered anthropometric measurement set.

4.5 Data collection

Data for the study was collected using self-administered online questionnaires, and in the sub-study, in-person anthropometric measures. Questionnaires took approximately 10 to 15 minutes to complete, and were administered at 2, 4, and 6 months. No invasive data collection techniques were used to measure the growth of infants. To measure infant growth with restricted in-person contact, data collection timepoints were selected to follow the post-natal follow-up schedule in Nova Scotia to enable the use of measurements taken by the family's regular HCP. However, given previous reports of inconsistent HCP methods and lower accuracy of measurements (111,127), repeated measures were taken through the in-person sub-study to assess accuracy and reliability. In this subset of participants, researcher-administered infant anthropometrics were taken within 48 hours of the HCP's appointment, to assess the degree of agreement between researcher-administered anthropometrics and parent-reported anthropometrics taken by the infant's HCP.

Consent was outlined on the first page of the online questionnaires (Appendices B, C, D). After reading through the consent information and confirming eligibility through a series of self-reported questions, participants were instructed that implied consent was given by clicking the 'next page' button that brought them to the beginning of the questionnaire.

4.6 Research tools

4.6.1 Questionnaires

The initial 2-month timepoint online questionnaire collected information on demographics, socioeconomic status, usual infant feeding practices and feeding modalities, dietary intake (including introduction of complementary foods), and maternal dietary intake. A shorter questionnaire was also administered at 4 and 6 months to track changes in the household, in feeding, and in the feeding environment since the previous visit. Importantly, these questionnaires asked participants to record HCP-measured infant anthropometric measures, as well as the methods and equipment used by their HCP to measure their infant.

4.6.2 Anthropometric measurements

The length of infants was measured using an infant length measuring board (Seca 416), following standard techniques (100). Measurements were taken to the nearest 0.1 cm, with the largest acceptable difference of 1.0 cm between repeated measures (100). Weight measurement in infants was determined using an electronic infant scale (Seca 727), by placing them on the scale with the same amount of clothing as the HCP visit. Weight was measured to the nearest 0.01 kg (146), the largest acceptable difference in duplicate measurements being 0.5 kg (100). To determine the head circumference of infants, a non-stretchable measuring tape (Seca 201) was placed above the eyebrows and ears to measure distance around back of the infant's head (101). The maximum difference between repeated measures of the head circumference was 0.5 cm (100). Participants were asked to report in detail the methods employed by their HCP to measure weight, length, and head circumference.

These anthropometric measurements were used to compute the growth trajectories of infants (WAZ, LAZ, WLZ, BAZ) from birth to 6 months. The WHO Anthro Survey Analyser

Tool (https://worldhealthorg.shinyapps.io/anthro/) was used to compute z-score distributions for four of the anthropometric indexes: WAZ, LAZ, WLZ, and BAZ (147). Since HCA z-scores were not computed using the WHO Anthro Survey Analyser, only the head circumference measures were reported. This tool created files including z-scores with corresponding flags to identify questionable values, a prevalence file including z-score summary statistics (mean and standard deviation), and a survey report depicting graphical representations of prevalence estimates and z-score distributions (147).

4.7 Ethical considerations

This study underwent ethics review by the Mount Saint Vincent University Research Ethics Board (MSVU #2018-155), and the University of Prince Edward Island Research Ethics Board (#6008074). Implied consent was given at each timepoint for all online data collection, and written, informed consent was obtained prior to the first in-person anthropometric measures among participants in the sub-study.

4.8 Data analysis

4.8.1 Primary objectives: Assessing growth rate of infants over 6 months

IBM SPSS v. 26.0 for Mac OS (IBM Corp, 2018) was used, with a significance level of $p<0.05$. Descriptive statistics were computed for both the mother's and infant's sociodemographic characteristics, and infant anthropometric measurements (length, weight, head circumference) and anthropometric z-scores (WAZ, LAZ, WLZ, BAZ; computed via WHO Anthro Analyser) reported as mean ± SD for continuous variables and *n* (%) for categorical variables. To assess differences by feeding modality (breast only, or mixed breast- and bottle-fed expressed human milk), independent sample t-tests for continuous variables, and chi square analysis for categorical variables, were employed.

To test potential differences in growth trajectories (WAZ, LAZ, WLZ, BAZ) by feeding modality during the first 6 months of life, a repeated measures ANOVA was administered with feeding modality as the main effect, and time (infant age) as the repeated-measures variable (148); unadjusted and adjusted models were generated. Before analyses, statistical outliers (anthropometric measures and z-scores lying outside of the <-3SD and >+3SD range) and other implausible data were eliminated. The normality of data distributions (a pre-requisite of repeated measures ANOVAs) was confirmed through Shapiro-Wilks tests or visual assessments of box plots and Q-Q plots. Adjusted analyses were controlled for covariates, identified as potential confounders based on previous research with human milk feeding modalities and/or infant growth (149). These confounders included total annual income (148), maternal age (150), maternal educational attainment (151), maternal height (152), maternal pre-pregnancy BMI (153), maternal weight gain during pregnancy (154), infant birth weight (148,150), infant length at birth (155), ethnicity of the mother and the infant (149), and infant sex (149). Before inclusion in the adjusted model, each covariate of interest was assessed for marginal statistical association with feeding modality using a univariate analysis and retained if $p<0.25$ (156). Relevant covariates were then assessed for collinearity, with elimination of redundant covariates when correlations between covariates exceeded $r>0.7$ (157). The following covariates were included for each of the adjusted models: WAZ, included covariates were maternal age, annual household income, and maternal height; LAZ, included covariates were maternal height, annual household income, infant sex, and pregnancy weight gain; WLZ, included covariates were maternal age and infant sex; BAZ, included covariates were maternal age and annual household income.

Rapid weight gain, defined as a difference in infant WAZ >0.67 between birth and 6 months of age (158), was computed and reported as a percentage of infants meeting this definition by feeding modality (chi-square test).

4.8.2 Secondary objectives: Assessing usual infant measuring practices, and agreement between HCP and researcher measures

Descriptive statistics were computed and reported as mean ± SD by measurer, while HCP measuring practices were reported as *n* (%). Paired sample t-tests were used to assess if the measurements differed significantly by measurer (HCP and researcher).

Agreement between z-score indicators (WAZ, LAZ, WLZ, BAZ) of infants measured by both their HCP and trained researchers was examined using Bland-Altman analyses (159). Bland-Altman tests are used to compare two contrasting techniques by calculating and plotting the difference between a pair of measurements against the mean of each participant of the study (160), and the magnitude of differences between the mean of HCP- and researcher administered measures (124). The limits of agreement constructed, depicting mean and 95% prediction interval for the difference between HCP- and trained researcher-collected measures, were used to assess the level of agreement between the two measures.

5.0 Dissemination of findings

The findings of this research have the potential to grow the limited literature regarding the influence of human milk feeding modality on infant growth, which could inform future research, or impact public health messaging or interventions regarding human milk feeding. The obtained data will be used to prepare a manuscript for submission to a peer-reviewed journal (e.g. *Breastfeeding Medicine or Applied Physiology, Nutrition, and Metabolism*), and presentations at conferences (e.g. *Science Atlantic or the Canadian Nutrition Society*).

Further, a lay summary of the findings from this research will be made available to study participants and the public on the MAMA lab website.

6.0 Results

6.1 Participant characteristics

Participant characteristics, by feeding modality, are detailed in **Table 6-1**. Women participants had a mean ± SD age of 31 ± 4 years, and most of the mothers were married (94%) and had completed post-secondary education (98%). Significantly fewer women who fed only at the breast were White (79%), primiparous (23%) and had annual household incomes ≥$60,000 (76%) compared with women who fed a mix of breast- and bottle-fed expressed human milk (94%, 59% and 95%, respectively).

Table 6-1. Sociodemographic characteristics of participants[a]

Participant characteristics	**All** *n = 129*	**Breastfed only** *n = 34*	**Breast- and bottle-fed** *n = 95*	**p value**[b]
Mother				
Age, *years*	31.4 ± 4.0	31.3 ± 4.5	31.4 ± 3.8	0.904
Marital status				0.165
Married	95 (74%)	28 (82%)	67 (71%)	
Common law	29 (22%)	4 (12%)	25 (26%)	
Other[c]	5 (4%)	2 (6%)	3 (3%)	
Maternal race[d]				0.032*
White	117 (85%)	27 (79%)	90 (94%)	
Other[e]	21 (15%)	9 (26%)	12 (13%)	
Annual household income				0.003*
<CAD$60,000	12 (10%)	8 (24%)	4 (5%)	
≥CAD$60,000	108 (90%)	25 (76%)	83 (95%)	
Education				0.538
High school diploma	3 (2%)	0 (0%)	3 (3%)	
College diploma	29 (23%)	9 (27%)	20 (21%)	
Undergraduate degree	54 (42%)	14 (41%)	40 (42%)	
Graduate or professional degree	43 (33%)	11 (32%)	32 (34%)	
Area of residence				0.340
Rural area	28 (22%)	4 (12%)	24 (25%)	
Town	21 (16%)	5 (15%)	16 (17%)	
Medium city	15 (12%)	4 (12%)	11 (12%)	
Large city	65 (50%)	21 (62%)	44 (46%)	
Number of children	1.7 ± 0.8	2.1 ± 0.8	1.5 ± 0.7	<0.001**
Primiparous	64 (50%)	8 (23%)	56 (59%)	<0.001**
Infant				
Sex, *male*	65 (50%)	14 (41%)	51 (54%)	0.211
Infant race[d]				0.156
White	118 (83%)	29 (85%)	89 (94%)	
Other[f]	24 (17%)	10 (29%)	14 (15%)	

[a]data presented as mean ± SD or n (%). Columns may not add to 100% due to rounding
*[b]independent sample t-test or chi square test, comparing sociodemographic characteristics of participants by feeding modality, * p<0.05, **p<0.001*
[c]participants' report of their 'other' marital status included single (n=4;3%), and divorced/separated (n=1;1%)
[d]total % in columns surpass 100% since multiple answers could have been selected
[e]mother participants' 'other' racial background included South Asian (n=6;5%), First Nations/Metis (n=5;4%), Black (n=4;3%), Chinese (n=2;2%), Latin American (n=2;2%), and Acadian (n=2;2%)
[f]infant participants' 'other' racial background included Black (n=8;6%), South Asian (n=7;5%), First Nations/Metis (n=5;4%), Chinese (n=2;2%), Latin American (n=2;2%), and Acadian (n=1;1%)

6.2 Anthropometric measurements and z-scores by usual infant feeding modality

There were no significant differences in infant anthropometric measurements at birth, 2, 4, or 6 months (length, weight, and head circumference), by feeding modality (directly at the breast only, versus mixed breast- and bottle-fed expressed human milk; see **Table 6-2**).

Table 6-2. Infant anthropometric measurements by usual feeding modality

Anthropometry	Breastfed		Breast- and bottle-fed		p value[a]
	n	Mean ± SD	n	Mean ± SD	
Length, *cm*					
Birth	29[b]	51.5 ± 2.2	83[f]	51.1 ± 2.1	0.349
2 months	27[c]	59.2 ± 2.5	88[g]	59.1 ± 2.4	0.852
4 months	30[d]	65.2 ± 2.6	90[h]	64.8± 2.5	0.490
6 months	29[e]	68.6 ± 2.8	84[i]	68.3 ± 2.6	0.671
Weight, *kg*					
Birth	34	3.7 ± 0.5	95	3.5 ± 0.5	0.098
2 months	34	5.7 ± 0.8	95	5.5 ± 0.7	0.109
4 months	33[j]	7.2 ± 0.9	94[j]	6.8 ± 0.9	0.071
6 months	33[j]	8.00 ± 1.0	93[j]	7.8 ± 1.0	0.383
Head circumference, *cm*					
Birth	13[k]	35.1 ± 1.7	54[o]	35.0 ± 1.5	0.751
2 months	23[l]	39.1 ± 1.2	72[p]	39.4 ± 1.3	0.307
4 months	31[m]	41.7 ± 1.4	84[q]	41.8 ± 1.6	0.621
6 months	28[n]	43.5 ± 1.4	75[r]	43.6 ± 1.4	0.751

[a]independent sample t-test comparing infant anthropometric measures by feeding modality
[b]missing data: n=4 no recorded data, n=1 eliminated as statistical outlier
[c]missing data: n=4 no recorded data, n=3 eliminated as statistical outliers
[d]missing data: n=1 no recorded data, n=3 eliminated as statistical outliers
[e]missing data: n=2 no recorded data, n=2 eliminated as statistical outliers, n=1 eliminated as implausible
[f]missing data: n=4 no recorded data, n=8 eliminated as statistical outliers
[g]missing data: n=3 no recorded data, n=4 eliminated as statistical outliers
[h]missing data: n=2 no recorded data, n=3 eliminated as statistical outliers
[i]missing data: n=7 no recorded data, n=4 eliminated as statistical outliers
[j]missing data: n=1 no recorded data
[k]missing data: n=19 no recorded data, n=1 eliminated as statistical outlier, n=1 eliminated as implausible
[l]missing data: n=9 no recorded data, n=1 eliminated as statistical outlier, n=1 eliminated as implausible
[m]missing data: n=3 no recorded data
[n]missing data: n=5 no recorded data, n=1 eliminated as statistical outlier
[o]missing data: n=37 no recorded data, n=4 eliminated as statistical outliers
[p]missing data: n=23 no recorded data
[q]missing data: n=11 no recorded data
[r]missing data: n=18 no recorded data, n=2 eliminated as statistical outliers

Normed anthropometric data are shared as z-scores in **Table 6-3**. Compared to breastfed-only infants, WAZ were significantly lower among infants who were both breast- and bottle-fed

expressed human milk at 2 months (p=0.019) and 4 months (p=0.017). Similarly, at 2 months both WLZ and BAZ were significantly lower among breast- and bottle-fed expressed human milk infants. LAZ did not differ by feeding modality at any timepoints.

Table 6-3. Infant anthropometric z-scores by usual feeding modality

Participant z-scores	**Breastfed**		**Breast- and bottle-fed**		**p value**[a]
	n[b]	**Mean ± SD**	**n**[b]	**Mean ± SD**	
WAZ					
Birth	34	0.80 ± 1.00	95	0.45 ± 0.90	0.062
2 months	34	0.48 ± 1.01	95	0.02 ± 0.96	0.019*
4 months	33	0.47 ± 0.98	93	-0.03 ± 1.04	0.017*
6 months	33	0.37 ± 1.00	93	0.08 ± 0.96	0.145
LAZ					
Birth	29	1.14 ± 1.14	83	0.82 ± 1.07	0.179
2 months	27	0.79 ± 1.09	88	0.58 ± 1.09	0.397
4 months	30	0.96 ± 1.11	90	0.74 ± 1.05	0.328
6 months	29	0.85 ± 1.08	84	0.58 ± 1.07	0.243
WLZ					
Birth	29	-0.17 ± 1.49	83	-0.35 ± 1.23	0.532
2 months	27	0.28 ± 1.04	88	-0.59 ± 1.31	0.002*
4 months	30	-0.25 ± 1.39	90	-0.62 ± 1.11	0.145
6 months	29	-0.08 ± 1.25	84	-0.26 ± 1.08	0.465
BAZ					
Birth	29	0.27 ± 1.29	83	-0.04 ± 1.01	0.184
2 months	27	0.43 ± 0.87	88	-0.46 ± 1.17	<0.001**
4 months	30	-0.23 ± 1.34	90	-0.61 ± 1.09	0.126
6 months	29	-0.19 ± 1.26	84	-0.37 ± 1.09	0.482

BAZ, BMI-for-age z-scores; LAZ, Length- or height-for-age z-scores; WAZ, Weight-for-age z-scores; WLZ, Weight-for-length z-scores

[a]*independent sample t-test comparing infant anthropometric measures by the feeding modality, * p<0.05, **p<0.001*

[b]*missing data described in Table 6-2*

6.3 Differences in infant growth patterns by feeding modality

There were no significant differences in the overall infant growth trajectories from birth to 6 months by feeding modality (directly at the breast only, versus mixed breast- and bottle-fed expressed human milk; see **Figure 6-1**).

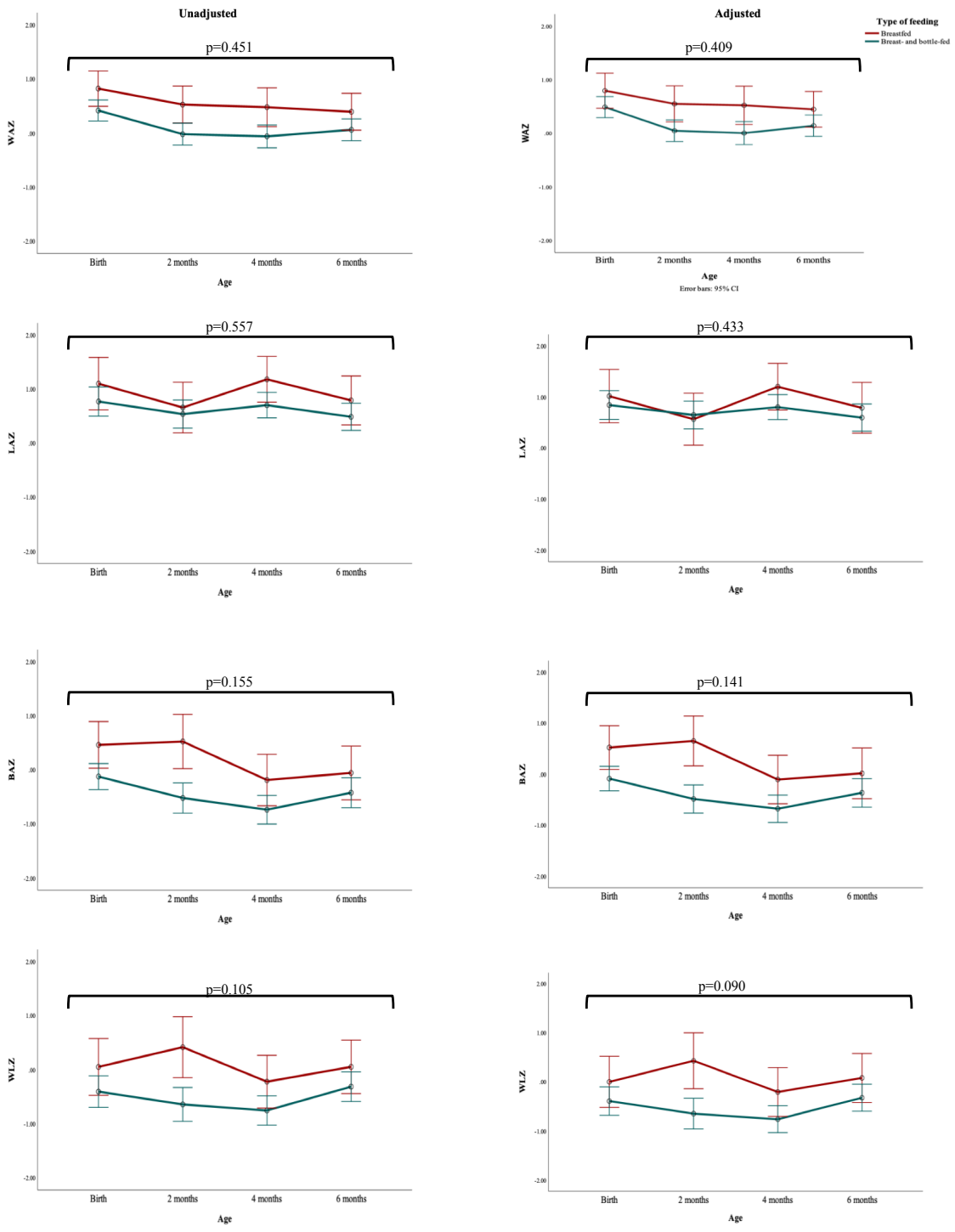

Figure 6-1. WAZ, LAZ, WLZ, and BAZ trajectory of infants (0-6 months), by feeding modality. Covariates in adjusted models are as follows: WAZ adjusted for maternal age, annual household income, and maternal height; LAZ adjusted for maternal height, annual household income, infant sex, and pregnancy weight gain; WLZ adjusted for maternal age and infant sex; BAZ adjusted for maternal age and annual household income.

6.4 Rapid weight gain by feeding modality

Overall, 30% of infants experienced rapid weight gain, defined as an increase in WAZ >0.67 between birth to 6 months of age, but there was no difference in the prevalence of rapid weight gain by feeding modality (12% breastfed versus 18% breast- and bottle-fed infants; p=0.415).

6.5 Infant anthropometric assessments: HCP versus researchers

Most HCP in this study were family doctors: 71% at 2 months; 68% at 4 months; 67% at 6 months (data not shown). Study participants were measured using three main tools/methods by their HCP: length by marking and measuring infants' respective lengths on a paper, weight using an infant scale, and head circumference using a tape measure (see **Table 6-4**).

Table 6-4. Infant measuring practices by the healthcare practitioner[a]

Measuring equipment	**Time period**		
	2 months	**4 months**	**6 months**
Length	*n = 123*	*n = 126*	*n = 120*
Infant length board	33 (26%)	34 (26%)	32 (25%)
Marking length on paper, then measuring it	71 (55%)	69 (53%)	67 (52%)
Tape measure beside the infant	19 (15%)	23 (18%)	21 (16%)
Weight	*n = 129*	*n = 126*	*n = 126*
Infant scale	127 (98%)	124 (96%)	125 (97%)
Parent holding infant on adult scale	2 (2%)	2 (2%)	1 (1%)
Head circumference	*n = 103*	*n = 118*	*n = 106*
Head circumference tape measure	92 (71%)	100 (77%)	89 (69%)
Regular tape measure	10 (8%)	16 (12%)	16 (12%)
String	1 (1%)	2 (2%)	1 (1%)

[a]*data presented as n (%). N=129, but some data missing due to no recorded observation.*

In comparing anthropometric measures at birth, 2, 4, and 6 months by HCP and researchers, we found that HCP recorded infants as significantly lighter at 2 months (5.77 ± 0.77 versus

5.83 ± 0.84; p=0.012) and significantly longer at 6 months (69.00 ± 2.66 versus 68.15 ± 2.11; p=0.003) (**Table 6-5**).

Table 6-5. Anthropometric measurements of infants by their healthcare practitioners at regularly scheduled appointments, and researchers (within ± 48 hours of healthcare practitioner appointment)

Anthropometry	**Healthcare practitioner**		**Researcher**		**p value**[a]
	n	**Mean ± SD**	**n**	**Mean ± SD**	
Length, *cm*					
Birth	112[b]	51.20 ± 2.15	-	-	-
2 months	115[c]	59.44 ± 2.22	34[f]	59.38 ± 1.79	0.841
4 months	120[d]	65.21 ± 2.64	28[g]	64.71 ± 2.09	0.075
6 months	113[e]	69.00 ± 2.66	31	68.15 ± 2.11	0.003*
Weight, *kg*					
Birth	129	3.57 ± 0.48	-	-	-
2 months	129	5.77 ± 0.77	34[h]	5.83 ± 0.84	0.012*
4 months	127[h]	7.12 ± 0.91	28[j]	7.15 ± 0.91	0.190
6 months	126[i]	8.13 ± 1.04	31	8.14 ± 1.05	0.573
Head circumference, *cm*					
Birth	67[k]	35.00 ± 1.50	-	-	-
2 months	95[l]	39.67 ± 1.28	32[o]	39.96 ± 1.40	0.130
4 months	115[m]	42.15 ± 1.40	29	42.23 ± 1.40	0.597
6 months	103[n]	43.73 ± 1.56	30[g]	43.88 ± 1.45	0.549

[a]*paired sample t-test comparing infant measures performed by healthcare practitioner and researchers*
[b]*missing: n=8 no recorded data, n=9 eliminated as statistical outliers*
[c]*missing: n=7 no recorded data, n=7 eliminated as statistical outliers*
[d]*missing: n=3 no recorded data, n=6 eliminated as statistical outliers*
[e]*missing: n=9 no recorded data, n=6 eliminated as statistical outliers, n=1 eliminated as implausible*
[f]*missing: n=1 no recorded data, n=1 eliminated as statistical outlier*
[g]*missing: n=1 eliminated as statistical outlier*
[h]*missing: n=2 no recorded data*
[i]*missing: n=3 no recorded data*
[j]*missing: n=1 no recorded data*
[k]*missing: n=56 no recorded data, n=5 eliminated as statistical outliers, n=1 eliminated as implausible*
[l]*missing: n=29 no recorded data, n=4 eliminated as statistical outliers, n=1 eliminated as implausible*
[m]*missing: n=14 no recorded data*
[n]*missing: n=23 no recorded data, n=3*
[o]*missing: n=1 no recorded data, n=3 eliminated as statistical outliers*

Normed z-score data, by measurer, are presented in **Table 6-6**. Significantly higher LAZ were computed from measurements by HCP compared to researchers at both 4 months (0.86 ± 1.03 versus 0.59 ± 0.76, respectively; p=0.046) and 6 months (0.89 ± 0.99 and 0.49 ± 0.74, respectively; p=0.002). At 6 months, WLZ and BAZ were both significantly lower among

HCP compared to researchers (WLZ: -0.02 ± 1.13 versus 0.25 ± 1.02, respectively; p=0.010) and (BAZ: -0.14 ± 1.13 versus 0.16 ± 1.03, respectively; p=0.006).

Table 6-6. Infant anthropometric z-scores using measurements from healthcare practitioners at regularly scheduled appointments, and researchers (within ± 48 hours of healthcare practitioner appointment)

Participant z-scores	**Healthcare practitioner**		**Researcher**		**p value**[a]
	n[b]	**Mean ± SD**	**n**[b]	**Mean ± SD**	
WAZ					
Birth	129	0.54 ± 0.93	-	-	-
2 months	129	0.46 ± 0.90	34	0.50 ± 0.97	0.142
4 months	126	0.34 ± 1.10	28	0.34 ± 0.93	0.966
6 months	126	0.40 ± 0.98	31	0.40 ± 0.99	0.902
LAZ					
Birth	112	0.90 ± 1.09	-	-	-
2 months	115	0.82 ± 0.98	34	0.74 ± 0.72	0.589
4 months	120	0.86 ± 1.03	28	0.59 ± 0.76	0.046*
6 months	113	0.89 ± 0.99	31	0.49 ± 0.74	0.002*
WLZ					
Birth	112	-0.30 ± 1.30	-	-	-
2 months	115	-0.27 ± 1.30	33	-0.17 ± 1.15	0.564
4 months	120	-0.27 ± 1.13	27	-0.08 ± 0.99	0.160
6 months	113	-0.02 ± 1.13	31	0.25 ± 1.02	0.010*
BAZ					
Birth	112	0.04 ± 1.09	-	-	-
2 months	115	-0.06 ± 1.11	33	0.02 ± 1.10	0.501
4 months	120	-0.26 ± 1.10	27	-0.07 ± 0.99	0.118
6 months	113	-0.14 ± 1.13	31	0.16 ± 1.03	0.006*

[a]*paired sample t-test comparing infant z-scores performed by healthcare practitioners and researchers*
[b]*missing data described in table 6-5*

In general, there was strong agreement between z-score indicators (WAZ, LAZ, WLZ, BAZ) at each time interval measured by HCP and researchers, with only a few observations falling outside of the 95% limits of agreement (see **Figure 6-2**).

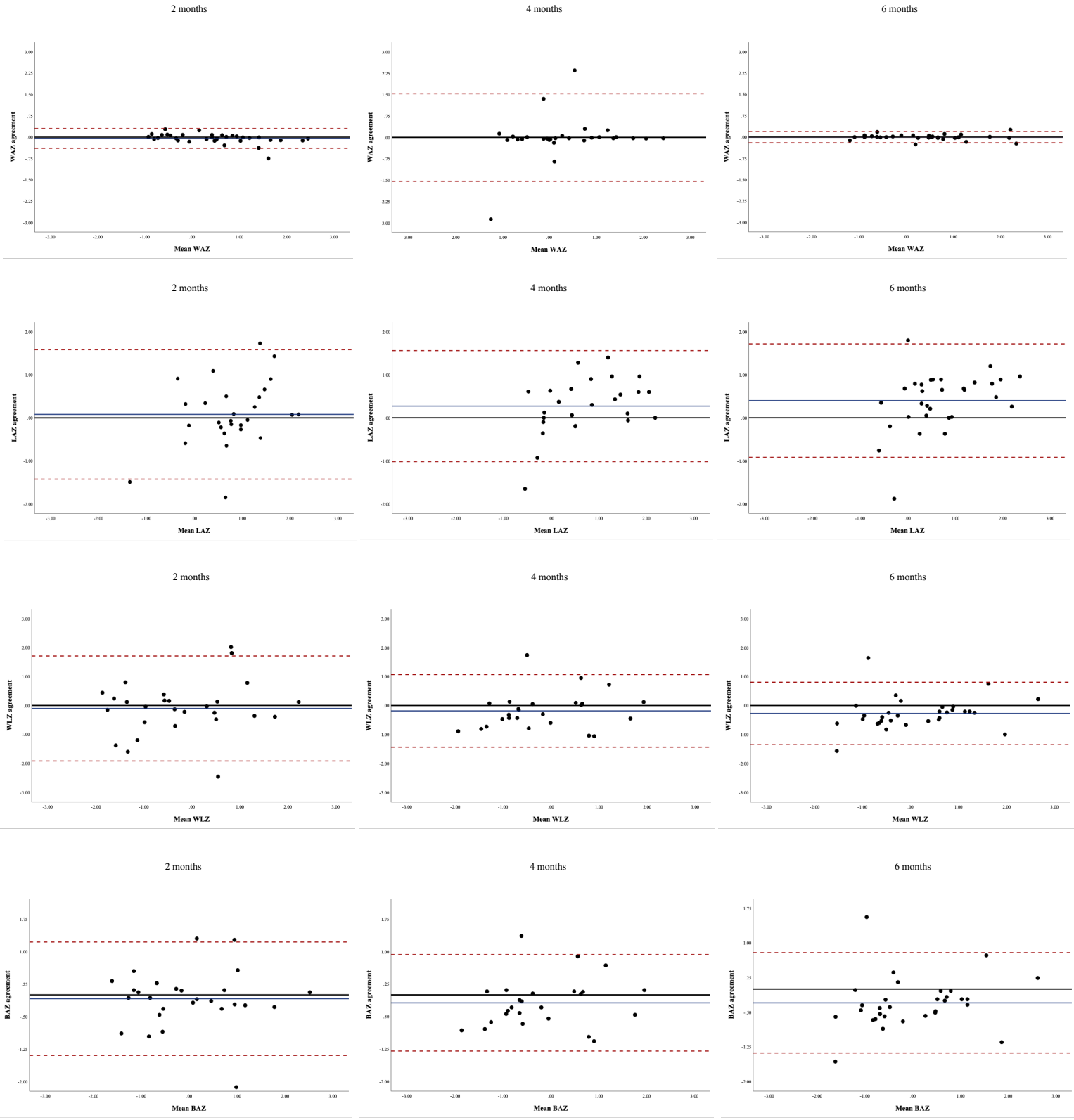

Figure 6-2 Bland-Altman analysis depicting agreement between z-score indicators of infants measured by both the HCP and trained researchers

7.0 Discussion

Despite numerous studies reporting growth differences between breast-fed and formula-fed infants (161,162), few have explored the impact of feeding modality on growth among infants fed only human milk. Through this exploratory, repeated measures observational study, I describe infant growth (length, weight, head circumference, WAZ, LAZ, WLZ, and BAZ) at birth, 2, 4, and 6 months; as well as infant growth trajectories (0 to 6 months) by human milk feeding modality (breast only, versus breast- and bottle-fed human milk). In addition, given that discrepancies in infant growth measurements across various settings have been previously reported (111,124), this research also documented usual anthropometric practices employed by Nova Scotian HCP, and assessed agreement in infant anthropometric assessments conducted by both HCP and researchers.

Among the total sample of 129 infants in the study, only 34 (26%) fed exclusively at the breast. This relatively small proportion may be evidence of a trend of increased expression and bottle-feeding of human milk, although it is important to note that most participants in this study were only feeding a small proportion of total daily feeds from a bottle (<10%), and hence were still predominantly feeding at the breast. Limited Canadian data are available on human milk expression, however, human milk feeding modality data were collected among 2553 mother-infant dyads in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort. Of the 61% of infants (n=1,686) in this cohort who were exclusively human milk fed at 3 months, 55% received some amount of expressed breastmilk from a bottle (7). Although the proportion of feeds at breast versus with a bottle were not described, this sizeable proportion of dyads engaging in bottle-feeding of human milk is notable.

A similar trend towards human milk expression has been observed in high-income countries other than Canada. For instance, a cohort study of 587 mothers residing in Perth, Australia reported that while 20% used an electronic pump at some point postpartum between 64% (4 weeks postpartum) to 67% (22 weeks postpartum) of mothers were manually pumping milk (163). Pumping was even more common in the United States: a recent study reported that 77% of mothers of singleton infants in Cincinnati, USA (n=346) pumped human milk (164). This presents a general trend in various high-income settings of mothers opting to pump their milk, which is relevant as this practice may be helping to drive the increase in breastfeeding rates observed in recent years (37,46,165).

7.1 Infant anthropometric measurements and z-scores by feeding modality

Although no differences in infant anthropometric measures (length, weight, or head circumference) were found by feeding modality at any time point, significant differences in WAZ (at 2 and 4 months), WLZ (at 2 months), and BAZ (at 2 months) were observed. Infant anthropometric z-scores are the recommended indicator in assessing infant growth over time (166), as they allow for comparison across a reference distribution (population-normed data), are standardized values that can be weighed across age, sex, and anthropometric measures, and can be easily analyzed (99). Additionally, z-scores are also able to quantify acute growth status at both ends of the distribution (>+3SD and <-3SD) (99). Given that, anthropometric z-scores are the more relevant indicator of infant growth, it is notable that differences were observed by feeding modality

Significant differences in multiple z-scores were seen at 2 months which aligns with previously described growth spurt in infants. As early as the 1970s, when Lampl *et al.* examined growth bursts in 31 Caucasian American infants, there has been an awareness that infant growth is episodic, with rapid bursts in length of 0.5 to 1.65 cm in a 24-hour period,

followed by between 2-28 days of stasis (167). Another study reported uneven spaced growth spurts centered at 2 months and 8 months (168). With this, noted differences in WAZ, WLZ, and BAZ at 2 months may be driven by feeding modality-related differences in the timing of these known growth spurts, however, this is just speculation based on results of our study.

Another notable finding of this study is that anthropometric z-scores of both breast- and bottle- fed infants were lower when compared with only breast-fed infants, which is inconsistent with other available literature. In a pilot study conducted with 37 infants (19 predominantly breastfed and 18 fed significant quantities of breastmilk through a bottle) compared infant growth and body composition, infants were similar in weight, head circumference, and WAZ by feeding modality, and reported no significant growth differences based on the human milk delivery method (169). In the IFPS II study (n=1291), human milk and infant formula-fed infants who consumed more than 30% of feeds from the bottle during the neonatal period depicted higher WAZ as compared to infants being fed less than 10% of feeds from the bottle (135). Another study conducted in South Africa recruited 641 infants to study growth between 3-24 months and observed higher BAZ (0.37 increment) in infants fed with a bottle as compared with breast-fed individuals (119). All these studies included infant formula-fed infants, and thus differences cannot be distinguished between the food (infant formula versus human milk) and feeding modality (breast versus bottle). However, no studies appear to have found higher anthropometric z-scores among only breast-fed infants. Although z-scores were significantly higher among breastfed-only infants in the current study, mean z-scores of both feeding modalities at all measured timepoints fell within ± 1SD of the median (where one would expect 68% of z scores to fall), hence growth of all infants was typical, making it challenging to understand the true impact of feeding modality on growth.

7.2 Infant growth trajectories by feeding modality

This study reports no significant differences in infant growth trajectories (WAZ, LAZ, WLZ, BAZ) from birth to 6 months, by infant feeding modality. This is similar to the above-mentioned pilot study of 37 infants, which reported no significant differences in the weight velocities of predominantly breastfed versus predominantly bottle-fed infants (p=0.12) (169). However, WAZ trajectory (weight gain velocity) from birth to 12 months in the CHILD birth cohort differed between infants in the two human milk feeding modality groups: Infants who were both breast- and bottle-fed human milk at 3 months had 14% higher odds of experiencing a higher weight gain velocity (change in WAZ from birth to 12 months; 0.14 (0.05-0.24) than exclusively beast-fed infants (7). This study differs in that feeding modality at 3 months is used to predict growth at 12 months. Alternatively, given the much larger sample size of the CHILD cohort, it may be that my study was underpowered to identify differences by feeding modality. Moving beyond human milk-fed infants, like bottle-feeding in the CHILD cohort, both CALINA and IFPS II studies observed higher WAZ trajectories among formula-fed infants (p=0.005, p<0.001; respectively) as compared with only breast-fed individuals (135,170).

7.3 Rapid weight gain in infants by feeding modality

Although, 30% of infants in this study depicted rapid weight gain, rates did not differ by feeding modality. Since children are optimally meant to grow on the same percentile trajectory throughout childhood (97), observing that approximately one third of children in this study experienced a growth increase of >0.67SD seems high, but actually aligns with other research. An Australian study of child growth among breast- only, formula-only, and mixed-fed infants (n=220) reported that 36% of infants experienced rapid weight gain (158).

Infants who were breast-fed for less than 4 months were three times more likely to experience rapid weight gain when compared with those who were breastfed for more than 4 months (158). However, authors of this study focussed more on food than modality differences, speculating that the high protein content of infant formula likely contributed to rapid weight gain in infancy (158). In addition, a longitudinal study of 129 infants residing in Chicago observed a higher prevalence of rapid weight gain among formula-fed (38%) and mixed-fed (28%) infants, when compared to those who were fed only at the breast (13%; p=0.002) (149). Although a different design that did not explicitly measure rapid weight gain, an intriguing observational study of 1899 American infants who were fed only human milk reported that weight gain was significantly lower among low-bottle users (<33% feeds from bottles; 729g weight gained/month) compared to high bottle users (>66% feeds from bottles; 780g weight gained/month; $p<0.05$) (129).

Although none of the above-mentioned studies collected data to explore mechanisms behind these differences in weight gain, it is possible that less responsive feeding, which is more common among bottle-feeding dyads (5), could be driving these differences. For instance, bottle-feeding tends to increase the pace of the feed, and caregivers often override infant cues with visual cues from the bottle, both of which could lead to overeating, and ultimately more weight gain (5).

7.4 Healthcare practitioner's infant measuring practices

The infant measuring practices most employed by HCP in Nova Scotia for length do not align with the recommended anthropometric measuring guidelines (100). Instead of using a length board, HCPs in Nova Scotia most commonly used paper and pencil method, in which children lie down on an examining table covered in paper, the HCP marks their length, and

then measures the distance between these marks using a measuring tape (104). This method has been previously shown to overestimate length compared to a standardized length board (104). Although HCPs overestimated infant length in the current study at 6 months ($p=0.003$), the difference of 69.00 ± 2.66 cm (HCP) versus 68.15 ± 2.11 cm (researcher) is not clinically meaningful, and as such, it does not appear that HCP necessarily need to change practice.

When considering head circumference measuring equipment, most HCPs used a tape measure, which is the recommended method to evaluate head size of an infant. Although recommendations call for a reusable cloth tape measure (100), paper tape measures are more commonly used in the United States due to lower slippage and stretch, and equally reliable results (106). Although participants were not specifically asked in the current study whether their HCP used paper or cloth/fabric tape measures, it may be that like peers in the United States, Nova Scotian HCP use paper tape measures for head circumference measures.

Even though HCP in this study used the standardized method of measuring infant head circumference, it was the anthropometric measurement with the highest number of missing, implausible, and statistical outlier observations. This could potentially be attributed to the measurement process being cumbersome, time-consuming, or requiring additional skilled training, or may be driven by a lack of knowledge regarding the importance of tracking head circumference growth. For instance, although the Canadian Paediatric Society recommends measuring head circumference between birth and 2 years, head circumference-for-age z-scores are not included in their cut-off guidelines for further assessment, referral, or intervention (91), so practitioners may not see clinical value in taking time to collect and record these measures. Findings from this study suggest that practitioner education or other

interventions may be needed, as head circumference measures are important in the diagnosis of microcephaly or megacephaly (depicting size of skull and brain volume), prognosis for hydrocephalus and intracranial expansion, and are vital for checking for developmental delays and tracking infant motor development (101,171).

7.5 Agreement in infant anthropometric measures and z-scores by HCP and researcher

Overall, there was a strong agreement in infant anthropometric measures and z-scores observed between HCP and researchers. Despite HCP not employing the recommended measuring equipment for length, there was strong agreement in infant z-scores (WAZ, LAZ, WLZ, BAZ) observed at every timepoint (2, 4, 6 months).

A cross-sectional study of agreement in infant growth assessments between community and clinical care settings in Northern Pennsylvania (n=129 infants) noted a high level of inconsistency and lack of agreement with WLZ, and strongest agreement for WAZ (124). A longitudinal UK birth cohort compared routine data collected by health visitors (n=836-1280) with research data (n=104-573) and found that health visitors underestimated length at 6 months (0.5 cm (-4.0 to -4.9)), and overestimated weight at all time points (6, 12, 24 months) (111). Conversely, strong agreement in all z-scores at all timepoints was seen in the current study. These strong results may be attributed to the setting, as most anthropometric measures took place with family doctors in Nova Scotia (71% at 2 months; 68% at 4 months; 67% at 6 months), rather than at community drop-in clinics. The time and one-on-one care afforded at these visits may have translated to higher quality measurements.

7.6 Strengths and limitations of the study

This study provides an in-depth analysis of infant growth (0 to 6 months) among predominantly human milk-fed infants, by feeding modality (only breast or mixed breast- and bottle-fed expressed human milk). Although numerous studies have compared infant growth between breastfed and formula-fed infants, this research is unique in assessing growth among infants consuming the same food by different modalities. A key strength of this study was the relatively large sample size of 129 mother-infant dyads, considering the low rate of human milk feeding in Atlantic Canada (only 30% exclusive breastfeeding in 2017-18) (165). Another strength is the repeated measures design with infant growth measured at 4 different time points, i.e. at birth, 2, 4, and 6 months. The low attrition rate of the participants, irrespective of the repeated-measures study design, is an additional strength of this study.

Limitations of the study include a potential for recall bias, as mothers were required to retrospectively recall the number of feeds of breastmilk per day, frequency of pumping in a typical day, and frequency of feeding pumped human milk to the infant. With this, there was potential for incorrect data recall (172). Retrospectively recalled data by mothers could be problematic when recollecting events in their infant's development (173), and in their own response of changing feeding practices, especially leading to an overestimate of the duration of exclusive breastfeeding (174). However, recall bias was limited by contacting participants before HCP appointments to remind them to write down anthropometric measures at the appointments, and by sending questionnaire reminders on the same day as their HCP visits. Another limitation of this study was that most participants were of high socioeconomic status. Although this aligns with the profile of breastfeeding mothers in Nova Scotia and Canada (165), this limits the generalizability of study findings to lower socioeconomic status

groups. Finally, although numerous potential confounding variables were included in the repeated measures ANOVA modelling, some potentially relevant covariates were not included in the data collection. These included mode of delivery (vaginal vs Cesarean-section) (175), area of residence (urban or rural) (176), and number of antenatal care visits (176).

7.7 Recommendations for future research

Given the finding of significantly different anthropometric z-scores by human milk feeding modality, future research should expand on this work, but with some modifications. In the present study, 26% of participants fed only at the breast, and the remainder of breast- and bottle-feeding dyads also fed mainly at the breast, with <10% of bottle-feeds on a typical day. In the future, effort should be given to recruiting more breastfeeding-only dyads, as well as more dyads who feed predominantly with a bottle. Recruiting more different dyads and reducing overlaps between groups may more clearly identify differences in growth. Similarly, although no differences in rapid weight gain by human milk feeding modality were observed, this is a common finding when comparing breast-fed and formula-fed infants (129,149,158), and there are mechanisms by which bottle-feeding could drive these differences. Again, recruiting a wider variety of human milk feeders could help to elucidate the role of feeding modality in rapid weight gain, and in turn obesity risk at later stage of life (124).

HCP in Nova Scotia did not collect head circumference measures as often as weight and length and had more implausible data for these measures than other anthropometry. It is possible that lack of knowledge and access to measuring equipment could be driving this, therefore, efforts should be made to ensure HCP across Nova Scotia appreciate the importance of tracking pediatric head circumference.

8.0 Conclusion

This novel research explores infant growth from birth to 6 months postnatal among predominately human milk fed infants, differing by feeding modality (directly at the breast only, versus mixed breast- and bottle-fed expressed human milk). There were no differences in anthropometric measures (weight, length, head circumferences) by modality, however, differences did exist in anthropometric z-scores, most notably at 2 months postpartum. Approximately one third of infants experienced rapid weight gain between 0-6 months, however, no differences were seen by feeding modality.

Healthcare practitioners used the gold standard growth measuring equipment and practices while recording weight and head circumference of infants. However, when measuring infant length, HCP tended to use the paper and pencil method (marking length on a paper and then measuring it) over the recommended length board. Irrespective of this deviation in selecting growth measuring equipment, strong agreement in all infant growth measures and z-scores was observed at each timepoint (2, 4, and 6 months), with only a few observations lying outside the 95% limit of agreement.

9.0 References

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10.0 Appendices

Appendix A: In-person questionnaire

Appendix B: Study poster

Appendix C: Study consent form

Appendix D: 2-month questionnaire

Appendix E: 4-month questionnaire

Appendix F: 6-month questionnaire

Appendix G: Ethical clearances from MSVU and UPEI

Appendix A: In-person questionnaire

REFINE: Anthropometrics Study In-Person Visit

Identification	
Subject ID: RA- _____	Visit: 1. 2 months 2. 4 months 3. 6 months
Date (DD/MM/YY): _____/_____/ _____	Time: _____:_____AM/PM
Anthropometric Measures	
1. What was infant wearing while weighed at primary care appointment?	1. Nude 2. Diaper only 3. Diaper and clothing 4. Other:
2. Infant weight (dressed as in primary care appointment)	1._____.____kg 2._____.____kg 3._____.____kg* 5. **Only repeat measurement a third time if >0.1kg difference*
3. How long since infant last ate before weighing (RA measurement)?	_____minutes
4. Approximately how long since infant last ate before weighing at primary care appointment?	_____minutes
5. Infant length	1._____.____cm 2._____.____cm 3._____.____cm* **Only repeat measurement a third time if >0.5cm difference.*
6. Infant head circumference	1._____.____cm 2._____.____cm 3._____.____cm* **Only repeat measurement a third time if >0.5cm difference.*

Milk Sample	
1. Was a milk sample collected at this visit?	1. Yes 2. No
2. Milk collection time.	_____:_____AM/PM
3. Approximate time since last meal before collection (maternal).	_____minutes
4. Breast side	1. Left 2. Right 3. Both
5. Milk volume	_____g
Remuneration	
Preferred email for e-transfer: _____	

ARE YOU BREASTFEEDING A NEWBORN?

You **may** be eligible to participate in a research study if your baby is **younger than 6 weeks of age** and you are **breastfeeding both directly at the breast and also pumping and feeding your milk in bottles.**
You must also **live in the Halifax area.**

We are interested in tracking breastfed babies growth to see if it differs by whether babies are mostly breast- or bottle-fed.
We are also interested in mom's body composition postpartum.
Researchers will ask you to come to the lab at three timepoints.
You will receive **$15 for each study session ($45 in total).**
In addition, you will get all of yours and your baby's measurements.

Contact researchers at the MAMA Lab for more information
www.mamalab.ca
mama.lab@msvu.ca
(902) 943-5652 (call or text)

REFINE Responsive Feeding of Infants with Expressed Milk
Mount Saint Vincent University
MAMA Lab
University of Prince Edward Island
researchNS Advancing Innovation

This study has received ethics approval from the Mount Saint Vincent University Research Ethics Board (#XXXX-XXX) and the Ethics Board of the University of Prince Edward Island (#XXXXXXX).

Excellence • Innovation • Discovery

CONSENT FORM
REFINE: Exploring the growth of infants breast- and bottle-fed human milk

Introduction
We invite you to take part in the research study called 'Exploring the growth of infants breast- and bottle-fed human milk'. This form gives information about the study. Before you decide if you want to participate, it is important that you understand the purpose of the study, what you will be asked to do, and the risks and benefits. We will explain all information before asking for your consent to participate. A member of the research team will be available to answer any questions. You may decide not to participate. You may also withdraw from the study at any time without any problems. Your participation is entirely voluntary.

Who is conducting the study?
Dr. Kyly Whitfield, Principal Investigator, Assistant Professor
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School of Nursing (Health Sciences), Queen's University
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This study is funded by Research Nova Scotia (formerly Nova Scotia Health Research Foundation). The researchers have no conflicts of interest to report.

What is the study about?
Mother's milk is the best food for babies, but how babies eat mother's milk is changing as pumping becomes more popular. Health professionals have long thought that feeding a bottle of pumped breastmilk was the same as breastfeeding. Yet, there is research showing that *how* baby eats mother's milk is important, and may have long-term impacts, for instance on baby's ability to understand if she/he is hungry or full.

We plan to invite 50 mother-infant pairs in the Halifax Regional Municipality to participate in this study. We will follow babies eating mother's milk from both the breast and a bottle from 6 weeks to 6 months. We aim to better understand how infants grow during this period of time.

Who can participate?
You may be eligible to participate if you:

- are 19 years or older,
- currently live in the Halifax Regional Municipality in Nova Scotia,
- have a healthy singleton baby who is younger than 6 weeks of age who is fedmother's milk directly from the breast and from a bottle,
- plan to exclusively feed your baby mother's milk up to 6 months.

You are not eligible to participate if:

- your baby was born preterm (earlier than 37 weeks gestation),
- your baby has a condition that impacts feeding (e.g. cleft palate), or
- you plan to move in the next 6 months.

Taking part in this study is completely voluntary. You do not need to answer any questions that you don't feel comfortable answering. You may choose not to take part or may leave the study at any time. You do not have to give a reason for your decision.

What will participation in this study look like?
Questionnaires
The study will begin when your baby is 6 weeks old and finish when your baby is 6 months old. After you sign this consent form, we will ask you to complete a questionnaire. It will ask questions about yourself (such as your age, ethnicity, diet), and about your baby (such as details of the delivery, and how you usually feed your baby). We will then ask you to fill out a shorter questionnaire at 4 and 6 months. Each of these questionnaires is likely to take about 5-10 minutes to fill out.

Growth and body composition
We will measure your baby's growth: weight, length, and head circumference (~7 minutes). We will also measure your body composition using a BodPod (~15 minutes, 3 minutes inside BodPod). The BodPod is a device that uses changing air pressure to calculate fat and lean body mass. Ideally you will come to our lab with your baby so that we can conduct all measurements. The other option is for a research team member to come to your home to measure your baby's growth only.

Milk samples
During these visits we will also ask you to give a milk sample. This is optional, and we will ask you to provide separate consent at the end of this consent form. You can use the hospital grade pump

in our research centre. The other option is for you to use your own pump if you're more comfortable with it. These milk samples will be identified only with your Study ID. The samples will be stored in our -80°C freezer for future analysis for nutrient content and other compounds such as

bioactives and hormones.

Participation in this study is entirely voluntary. It will not cost you anything. As a thank you for your time, we will provide $15 for each measurement session. We will also give you all of your baby's growth information and your body composition information (approx. $35 value per BodPod measurement). You will receive compensation after each of the data collection sessions. If you decide to withdraw the during one of the data collection points, you will still receive compensation for that session.

Confidentiality

Your confidentiality will be respected. We will give you a unique study number as a participant in this study. Only this number will be used on any research-related information collected about you during the course of this study. Your identity [i.e. your name or any other information that could identify you] will be kept confidential. Only Principal Investigator will have access to any data that contains your personal information (such as this consent form). The list that matches your name to the unique study number will not be removed or released.

Only the research team will view and analyze the information gathered as part of this study. The results of the study may be presented at scientific meetings, published in a scientific journal and used for Master's Theses. If the results are published, only group values will be reported. All data will be kept on a locked database for 5 years and then securely destroyed; milk samples will be stored for 10 years before being destroyed.

Please note that data will be kept confidential within the confines of the law. For instance, if we come across an evidence of abuse in a course of this study, we are obligated to report it to appropriate authorities, such as Halifax Regional Police.

Risks

We do not believe there are any risks involved with participation in this study.

Benefits

You will receive your baby's growth information and your body composition data as a participant of this study. You will have the benefit of contributing to research. We hope that the results of this study can be used to inform future research, public policy and education programs about the feeding of young children.

Questions and further information

Participation in this study is completely voluntary. Also, you have the option to stop participating and withdraw from the study at any time without any problems.

If you have any questions or would like further information about this research, please contact Dr. Kyly Whitfield, the Principal Investigator, at kyly.whitfield@msvu.ca, or by phone at (902) 457-5978.

If you have questions about how this study is being conducted and wish to speak with someone who is not directly involved in the study, you may contact the MSVU Research Office at (902) 457-6350 or via e-mail at research@msvu.ca. You can also contact the UPEI Research Ethics Board at (902) 620-5104, or by email at reb@upei.ca if you have any concerns about the ethical conduct of this study.

Research Results

A summary of research results will be made available online at www.mamalab.ca. The ethical components of this research study have been reviewed by the Research Ethics Boards at Mount Saint Vincent University and the University of Prince Edward Island.

Consent Form for Research Participation

PARTICIPANT AUTHORIZATION:
I have read or had read to me this information and authorization form. I have had the chance to ask questions. My questions have been answered to my satisfaction before moving forward. I understand the nature of the study. I also understand the potential risks. I understand that I have the right to withdraw from the study at any time without any problems. I have received a copy of the Consent Form for future reference.
I freely agree to participate in this research study.

☐ YES ☐ NO

OPTINAL FOLLOW-UP
We would like to keep your contact information in case there are opportunities to follow-up with you and your child for future research. For example, we could contact you asking if you would like your child to use the BodPod after they reach 2 years. There is no obligation to agree to future research participation now. We are simply asking to keep your phone number and email address in a locked database for potential future use. This is in no way required for participation in the current study.
I agree to allow the research team to contact me in the future about other research participation opportunities.

☐ YES ☐ NO

OPTIONAL MILK SAMPLE
We would like to collect milk samples, identified only with your Study ID. These will be stored in our -80°C freezer for future analysis for nutrient content and other compounds such as bioactives and hormones.
I agree to provide milk samples to be entered into a Milk BioBank at MSVU for future analysis.

☐ YES ☐ NO

Participant name: _____

Signature:_____Date: _____

STATEMENT BY PERSON PROVIDING INFORMATION ON STUDY AND OBTAINING CONSENT
I have explained the nature and demands of the research study and judge that the participant named above understands the nature and demands of the study. I have explained the nature of the consent process to the participant and judge that they understand that participation is voluntary and that they may withdraw at any time from participating.

Print name of Person Explaining Consent: _____

Date:_____Signature: _____

Appendix D: 2-month questionnaire

REFINE Study: 2 month questionnaire

Welcome to the first questionnaire of the 'Exploring the growth of infants breast- and bottle-fed human milk' study. This questionnaire will take 20-25 minutes to complete. Please have your baby's growth measurements handy to fill in when prompted.

You will receive $20 via e-transfer for completing this questionnaire. If you have any questions as you are filling out the questionnaire, you can contact us anytime at 902-222-7371 or mama.lab@msvu.ca.

What is your study ID code? (RA-XXX)

*This code is in your text from the MAMA Lab

Eligibility Criteria

Before you start the survey, we want to make sure your eligibility has not changed since you signed up for the study.

* Is your baby mostly fed breast milk (at breast and/or by bottle)? (Maximum 1 formula feed per week)

- ○ Yes
- ○ No

* Do you plan on mostly feeding your baby breast milk until at least 6 months of age?

- ○ Yes
- ○ No

* Does your baby have a condition that impacts feeding? (Example: cleft palate, taking medication for reflux, etc.)

- ○ Yes
- ○ No

* Do you plan to move away from Nova Scotia in the next 4 months?

- ○ Yes
- ○ No

How old are you?

_____ years

What is your marital status?

- ○ Married
- ○ Common-law
- ○ Divorced/Separated
- ○ Single
- ○ Other: _____
- ○ Prefer not to answer

What is your gender?

- ○ Woman
- ○ Man
- ○ Other: _____
- ○ Prefer not to answer

You may belong to one or more racial or cultural groups on the following list. Are you:

*please check all that apply

- ☐ White
- ☐ Chinese
- ☐ South Asian (e.g. East Indian, Pakistani)
- ☐ Black
- ☐ Filipino
- ☐ Latin American
- ☐ Southeast Asian (e.g. Cambodian, Indonesian)

- [ ] Arab
- [ ] West Asian (e.g. Afghan, Iranian)
- [ ] Japanese
- [ ] Korean
- [ ] First Nations
- [ ] Metis
- [ ] Inuk
- [ ] Other
- [ ] Don't know
- [ ] Prefer not to answer

What is your household's total annual income before tax?

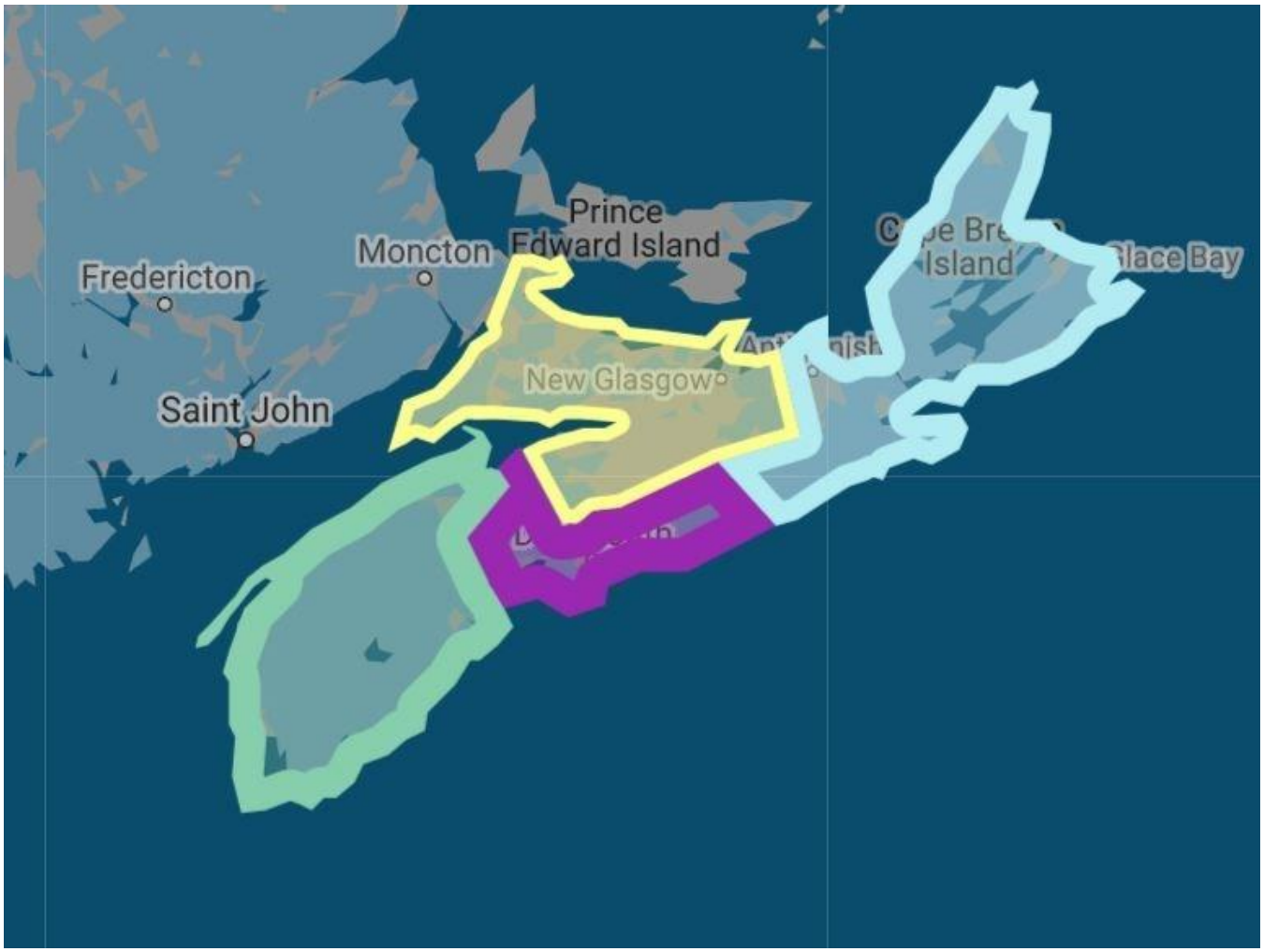
- ○ Less than $10,000
- ○ $10,000 to $19,999
- ○ $20,000 to $29,999
- ○ $30,000 to $39,999
- ○ $40,000 to $49,999
- ○ $50,000 to $59,999
- ○ $60,000 to $69,999
- ○ $70,000 to $79,999
- ○ $80,000 to $89,999
- ○ $90,000 to $99,999
- ○ $100,000 to $149,999
- ○ $150,000 or more
- ○ Prefer not to answer

What is the highest level of education you have completed?

- ○ Some high school education
- ○ High school diploma
- ○ College degree
- ○ Undergraduate degree
- ○ Graduate or professional degree (e.g. MA, JD)
- ○ Other; please describe:

Nova Scotia is split into 4 Health Authority zones (see image below). Which do you live in?

- ○ Central Zone (purple)
- ○ Western Zone (green)
- ○ Eastern Zone (blue)
- ○ Northern Zone (yellow)



How would you describe where you live?

- ○ Large city (e.g. Halifax)
- ○ Medium city (e.g. Truro, Sydney)
- ○ Town (e.g. Kentville, Antigonish)
- ○ Rural area

How many children have you given birth to?

*including your youngest baby

Do you regularly take a multivitamin?

(Note: for mom, not baby)

- ○ Yes
- ○ No
- ○ Other:

What is your height? (Answer in metres OR feet and inches)

Metres

Feet

Inches

The next three questions are about your weight before, during and after pregnancy. Do you have these measurements in kilograms or in pounds?

- ○ kilograms
- ○ pounds

What was your pre-pregnancy weight?

How much weight did you gain during your pregnancy in total?

What is your current weight (2 months postpartum)?

Your baby may belong to one or more racial or cultural groups on the following list. Are they:

Please check all that apply

- [ ] White
- [ ] Chinese
- [ ] South Asian (e.g. East Indian, Pakistani)
- [ ] Black
- [ ] Filipino
- [ ] Latin American
- [ ] Southeast Asian (e.g. Cambodian, Indonesian)
- [ ] Arab
- [ ] West Asian (e.g. Afghan, Iranian)
- [ ] Japanese
- [ ] Korean
- [ ] First Nations
- [ ] Metis
- [ ] Inuk
- [ ] Other
- [ ] Don't know
- [ ] Prefer not to answer

What is your baby's sex?

- ( ) Male
- ( ) Female

Baby's Measurements at Birth

* At birth, was your baby's weight recorded in kilograms or in pounds and ounces?

- ○ kilograms
- ○ pounds and ounces

* What was your baby's weight at birth?
Please answer to at least 1 decimal: e.g. 3.5

kg

* What was your baby's weight at birth?

Pounds:

Ounces:

What was your baby's length at birth?

cm

OR

inches

My health care provider didn't measure length

What was your baby's head circumference at birth?
(measurement shown below)

cm

OR

inches

My health care provider didn't measure head circumference

You recently attended your 2-month follow up visit with your primary care provider. Please answer the folowing questions about this visit.

* What date was the visit?

YYYY-MM-D

What kind of visit did you attend?

- ○ Immunization
- ○ Well Baby visit
- ○ Other:

What kind of health care provider did you visit?

- ○ Family doctor
- ○ Pediatrician
- ○ Public Health Nurse
- ○ Nurse practitioner
- ○ Other:

Weight

How did the health care provider weigh your baby?

- ○ Infant scale (see image below)
- ○ Parent holding baby on adult scale
- ○ Other:

What was your baby wearing when they were weighed?

- ○ Nothing (baby was nude)
- ○ Diaper only
- ○ Clothing and diaper
- ○ Other:

* At this visit, was your baby's weight recorded in kilograms or in pounds and ounces?

- ○ Kilograms
- ○ Pounds and Ounces

* What was your baby's weight at the 2 month visit?
Please answer to at least 1 decimal: e.g. 3.5

kg

* What was your baby's weight at the 2 month visit?

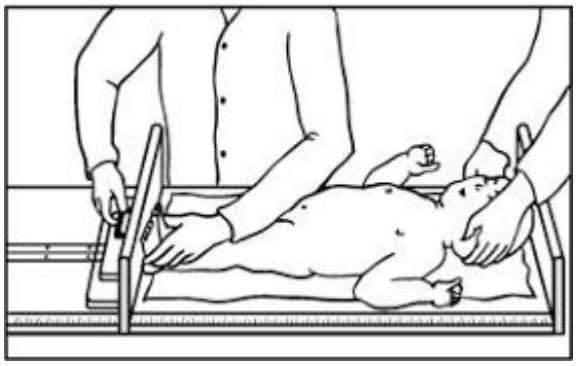
Pounds:

Ounces:

Length

How did the health care provider measure your baby's length?

- ○ Infant length board (see image below)
- ○ Marking your baby's length on paper, then measuring
- ○ Tape measure beside your baby
- ○ My health care provider didn't measure length
- ○ Other, please describe:



What was your baby's length at the 2 month visit?

cm

OR

inches

- ○ My health care provider didn't measure length

Head circumference

How did the health care provider measure your baby's head circumference?

- ○ Using head circumference tape measure (see image below)
- ○ Using a piece of paper
- ○ Using string
- ○ My health care provider didn't measure head circumference
- ○ Other, please describe: _____

What was your baby's head circumference at the 2 month visit?

_____ cm

OR

_____ inches

- ○ My health care provider didn't measure head circumference

Who is your baby's primary caregiver?

- ◯ Yourself
- ◯ Other:

What foods has your baby ever eaten?

(Please check all that apply.)

- ☐ Breast milk
- ☐ Infant formula
- ☐ Vitamin D drops
- ☐ Water
- ☐ Cow's milk
- ☐ Goat's milk
- ☐ Soy milk
- ☐ Almond milk
- ☐ Medicine, such as oral rehydration salts
- ☐ Baby food
- ☐ Other solid food
- ☐ Other:

Which foods did your baby eat in the last week?

(Please check all that apply.)

- ☐ Breast milk
- ☐ Infant formula
- ☐ Vitamin D drops
- ☐ Water
- ☐ Cow's milk

- [ ] Goat's milk
- [ ] Soy milk
- [ ] Almond milk
- [ ] Medicine, such as oral rehydration salts
- [ ] Baby food
- [ ] Other solid food
- [ ] Other:

How often in the last week did your infant consume food or drink other than breastmilk or vitamin D drops?

Answer "0" if baby is exclusively breastfed

instances

Does your baby spit up after feeds?

- ○ After every feed
- ○ After most feeds (75%)
- ○ After some feeds (50%)
- ○ After a few feeds (25%)
- ○ No, or very rarely

Do you pump breastmilk?

- ○ Yes
- ○ No (feed at breast only)

How many feeds of breastmilk does your baby have in a typical day (24 hrs)?

feeds

Of these feeds, how many are directly at breast:

How many are breastmilk from a bottle or cup:

If you feed pumped milk less than once per day, please describe how often you feed pumped breast milk:

Who usually feeds your baby from a bottle?

- Yourself
- Your partner
- An older child
- Other, please describe:
- Not applicable (baby not bottlefed)

How many times do you usually pump milk in a typical day (24 hrs)?

How many feeds of breastmilk does your baby have in a typical day (24 hrs)?

feeds

* The next 18 questions deal with your interactions with your child when you are feeding milk from the breast or from the bottle or cup. Answers range on a scale from "never" to "always". Please select the answer that best describes you and your baby. If you are not certain, make your best guess. Think about the past few weeks.

I feed my baby whenever s/he wants.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby at set times.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I decide when it is time for my baby to have a feed.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I let my baby decide when s/he would like to have a feed.

- ○ Never

- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby to settle her/him, even if he is not hungry.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I offer my baby a feed when s/he is unsettled or crying.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I offer my baby a feed when s/he is hurt.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

When my baby gets unsettled or is crying, feeding her/him is one of the first things I do.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby to make sure that s/he does not get unsettled or cry.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby extra milk just to make sure s/he gets enough.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

If my baby indicates s/he is not hungry, I try to get him to feed anyway.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby extra milk so s/he sleeps longer.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

When deciding how much to feed my baby, I rely on how hungry s/he is.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby for a set time.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I carefully control how much my baby feeds.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I follow a rule about how much my baby should feed.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I let my baby decide how much s/he feeds.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I decide how much my baby feeds.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

Have you scheduled your 4 month visit with your health care provider already?

Please note: this visit may be scheduled a bit before of after 4 months of age.

- ◯ Yes
- ◯ Not yet

Great! Please tell us when your 4 month visit is scheduled.

YYYY-MM-D

No problem! We will reach out to you when you baby is almost 4 months old to remind you about the next questionnaire.

You've made it to the end of the 2 month questionnaire! Thanks so much for your time. We'll be in touch in another 2 months for follow-up. In the meantime, if you have any questions feel free to reach out anytime: mama.lab@msvu.ca or 902-222-7371

We will send you $20 via e-transfer for your participation. Please enter your email:

Please re-enter your email address:

Reminder: This email address will be used for the Interac e-Transfer only, and will not be linked in any way to your data.

* Please type what you see in the box below, so we know you're not a robot!

Thank you so much for your time and interest, but unfortunately you are no longer eligible to take part in our study.

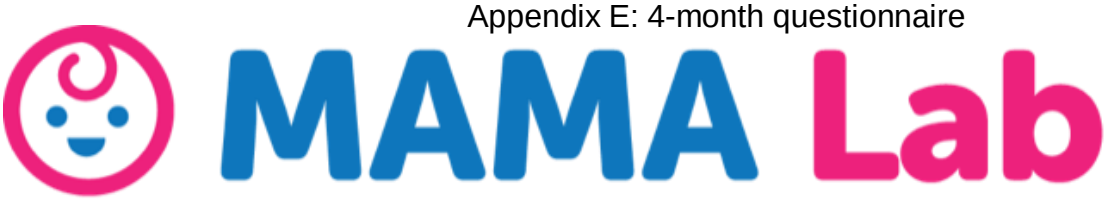
We really appreciate your effort and responsiveness so far and wish you and your family all the best.

Please let us know if you are interested in learning about other study opportunities at the MAMA Lab:

- ○ Yes
- ○ No thanks!

Powered by SimpleSurvey

Appendix E: 4-month questionnaire



REFINE Study: 4 month questionnaire

Welcome to the second questionnaire of the 'Exploring the growth of infants breast- and bottle-fed human milk' study. This questionnaire will take 20-25 minutes to complete. Please have your baby's growth measurements handy to fill in when prompted.

You will receive $20 via e-transfer for completing this questionnaire. If you have any questions as you are filling out the questionnaire, you can contact us at 902-222-7371 or mama.lab@msvu.ca.

What is your study ID code? (RA-XXX)

*This code is in your text from the MAMA Lab

Are you still feeding your baby mostly breast milk? (Maximum one formula feed per week. No solid food.

Note: Answering "no" will not affect your eligibility for the study.

- ○ Yes
- ○ No

Formula

Are you feeding formula more than once per week?

- ○ Yes
- ○ No

If yes, approximately when did you start introducing formula more than once per week?

If no, leave blank.

YYYY-MM-D

If yes, how many formula feeds does your baby typically receive in 24 hrs?

If no, leave blank.

If yes, what is the reason that you started introducing formula more often?

If no, leave blank.

Solid Food

Have you introduced solid food to your baby yet?

- ○ Yes
- ○ No

If yes, approximately when did you start regularly including solid food in your baby's diet?

If no, leave blank.

YYYY-MM-D

If yes, what was the first solid food you introduced to your baby?

Note: regularly included in diet, not just tasted. If no, leave blank.

If yes, what was the reason you started introducing solid food to your baby?

If no, leave blank.

Maternal Measurements

What is YOUR current weight (maternal weight)? Answer in kilograms OR pounds.

Please skip if you do not know your weight or would prefer not to share.

Kilograms:

Pounds:

4-month Primary Care Appointment

You recently attended your 4-month follow up visit with your primary care provider. Please answer the folowing questions about this visit.

* What date was the visit?

YYYY-MM-D

What kind of visit did you attend?

- ○ Immunization
- ○ Well Baby Visit
- ○ Other:

What kind of health care provider did you visit?

- ○ Family doctor
- ○ Pediatrician
- ○ Public Health Nurse
- ○ Nurse practitioner
- ○ Other:

Infant Weight

How did the health care provider weigh your baby?

- ○ Infant scale (see image below)
- ○ Parent holding baby on adult scale
- ○ Other:

What was your baby wearing when it was weighed?

- ○ Nothing (baby was nude)
- ○ Diaper only
- ○ Clothing and diaper
- ○ Other:

* Was your baby's weight recorded in kilograms or in pounds and ounces?

- ○ Kilograms
- ○ Pounds and Ounces

* What was your baby's weight at the 4 month visit?
Please answer to at least 1 decimal: e.g. 3.5

kg

* What was your baby's weight at the 4 month visit?

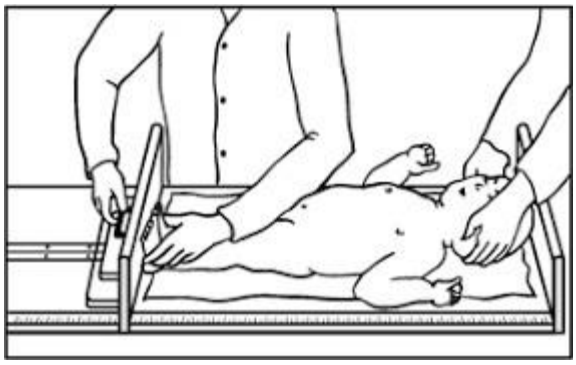
Pounds:

Ounces:

Infant Length

How did the health care provider measure your baby's length?

- ○ Infant length board (see image below)
- ○ Marking your baby's length on paper, then measuring
- ○ Tape measure beside your baby
- ○ My health care provider didn't measure length
- ○ Other, please describe: _____



What was your baby's length at the 4 month visit?

_____ cm

OR

_____ in

- ○ My health care provider didn't measure length

Infant Head Circumference

How did the health care provider measure your baby's head circumference?

- ○ Using head circumference tape measure (see image below)

- ○ Using a piece of paper
- ○ Using string
- ○ My health care provider didn't measure head circumference
- ○ Other, please describe: _____

What was your baby's head circumference at the 4 month visit?

_____ cm

OR

_____ in

- ○ My health care provider didn't measure head circumference

Who is your baby's primary caregiver?

- ○ Yourself
- ○ Other:

Do you regularly take a multivitamin?

(Note: for mom, not baby)

- ○ Yes
- ○ No
- ○ Other:

What foods has your baby ever eaten?

(Please check all that apply.)

- ☐ Breast milk
- ☐ Infant formula
- ☐ Vitamin D drops
- ☐ Water
- ☐ Cow's milk
- ☐ Goat's milk
- ☐ Soy milk
- ☐ Almond milk
- ☐ Medicine, such as oral rehydration salts
- ☐ Baby food
- ☐ Other solid food
- ☐ Other:

Which foods did your baby eat in the last week?

(Please check all that apply.)

- [ ] Breast milk
- [ ] Infant formula
- [ ] Vitamin D drops
- [ ] Water
- [ ] Cow's milk
- [ ] Goat's milk
- [ ] Soy milk
- [ ] Almond milk
- [ ] Medicine, such as oral rehydration salts
- [ ] Baby food
- [ ] Other solid food
- [ ] Other:

How often in the last week did your infant consume food or drink other than breastmilk or vitamin D drops?

Answer "0" if baby is exclusively breastfed

instances

Does your baby spit up after feeds?

- ○ After every feed
- ○ After most feeds (75%)
- ○ After some feeds (50%)
- ○ After a few feeds (25%)
- ○ No, or very rarely

Do you pump breastmilk?

- ○ Yes

- ○ No (feed at breast only)

How many feeds of breastmilk does your baby have in a typical day (24 hrs)?

Of these feeds, how many are directly at breast:

How many are breast milk from a bottle or cup:

If you feed pumped milk less than once per day, please describe how often you feed pumped breast milk:

Who usually feeds your baby from a bottle?

- ○ Yourself
- ○ Your partner
- ○ An older child
- ○ Other, please describe:
- ○ Not applicable (my baby is not bottle fed)

How many times do you usually pump milk in a typical day (24 hrs)?

How many feeds of breastmilk does your baby have in a typical day (24 hrs)?

* The next 18 questions deal with your interactions with your child when you are feeding milk from the breast or from the bottle or cup. Answers range on a scale from "never" to "always". Please select the answer that best describes you and your baby. If you are not certain, make your best guess. Think about the past few weeks.

I feed my baby whenever s/he wants.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby at set times.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I decide when it is time for my baby to have a feed.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I let my baby decide when s/he would like to have a feed.

- ○ Never

- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby to settle her/him, even if he is not hungry.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I offer my baby a feed when s/he is unsettled or crying.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I offer my baby a feed when s/he is hurt.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

When my baby gets unsettled or is crying, feeding her/him is one of the first things I do.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby to make sure that s/he does not get unsettled or cry.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby extra milk just to make sure s/he gets enough.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

If my baby indicates s/he is not hungry, I try to get him to feed anyway.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby extra milk so s/he sleeps longer.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

When deciding how much to feed my baby, I rely on how hungry s/he is.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby for a set time.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I carefully control how much my baby feeds.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I follow a rule about how much my baby should feed.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I let my baby decide how much s/he feeds.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I decide how much my baby feeds.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

* The following questions are about YOU and your eating patterns (not your baby). Answers range on a scale from "strongly disagree" to "strongly agree". Please select the answer that best describes you.

I try to avoid certain foods high in fat, carbohydrates, or calories.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I find myself eating when I'm feeling emotional (e.g., anxious, depressed, sad), even when I'm not physically hungry.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

If I am craving a certain food, I allow myself to have it.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I get mad at myself for eating something unhealthy.

- ○ Strongly disagree
- ○ Disagree

- ○ Neutral
- ○ Agree
- ○ Strongly agree

I find myself eating when I am lonely, even when I'm not physically hungry.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I trust my body to tell me when to eat.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I trust my body to tell me what to eat.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I trust my body to tell me how much to eat.

- ○ Strongly disagree
- ○ Disagree

- ○ Neutral
- ○ Agree
- ○ Strongly agree

I have forbidden foods that I don't allow myself to eat.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I use food to help me soothe my negative emotions.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I find myself eating when I am stressed out, even when I'm not physically hungry.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I am able to cope with my negative emotions (e.g., anxiety, sadness) without turning to food for comfort.

- ○ Strongly disagree

- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

When I am bored, I do NOT eat just for something to do.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

When I am bored, I do NOT turn to food for comfort.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I find other ways to cope with stress and anxiety than by eating.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I allow myself to eat what food I desire at the moment.

- ○ Strongly disagree

- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I do NOT follow eating rules or dieting plans that dictate what, when, and/or how much I eat.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

Most of the time, I desire to eat nutritious foods.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I mostly eat foods that make my body perform efficiently (well).

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I mostly eat foods that give my body energy and stamina.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I rely on my hunger signals to tell me when to eat.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I rely on my fullness (satiety) signals to tell me when to stop eating.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

I trust my body to tell me when to stop eating.

- ○ Strongly disagree
- ○ Disagree
- ○ Neutral
- ○ Agree
- ○ Strongly agree

Have you scheduled your 6 month visit with your health care provider already?

- ○ Yes
- ○ Not yet

Great! Please tell us when your 6 month visit is scheduled.

YYYY-MM-D

No problem! We will reach out to you when you baby is almost 6 months old to remind you about the next questionnaire.

You've made it to the end of the 4 month questionnaire! Thanks so much for your time. We'll be in touch in another 2 months for the final follow-up. In the meantime, if you have any questions feel free to reach out anytime: mama.lab@msvu.ca or 902-222-7371

We will send you $20 via e-transfer for your participation. Please enter your email:

Please re-enter your email:

Reminder: This email address will be used for the Interac e-Transfer only, and will not be linked in any way to your data.

* Please type what you see in the box below, so we know you're not a robot!

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Appendix F: 6-month questionnaire

REFINE Study: 6 month questionnaire

Welcome to the final questionnaire of the 'Exploring the growth of infants breast- and bottle-fed human milk' study. This questionnaire will take 20-25 minutes to complete. Please have your baby's growth measurements handy to fill in when prompted.

You will receive $20 via e-transfer for completing this questionnaire. If you have any questions as you are filling out the questionnaire, you can contact us at 902-222-7371 or mama.lab@msvu.ca.

What is your study ID code? (RA-XXX)

*This code is in your text from the MAMA Lab

Are you still feeding your baby mostly breast milk? (Maximum one formula feed per week. No solid food.)

Note: Answering "no" will not affect your eligibility for the study.

- ○ Yes
- ○ No

Formula

Are you feeding formula more than once per week?

- ○ Yes
- ○ No

If yes, approximately when did you start introducing formula more than once per week?

If no, leave blank.

YYYY-MM-D

If yes, how many formula feeds does your baby typically receive in a typical day (24 hrs)?

If no, leave blank.

If yes, what is the reason that you started introducing formula more often?

If no, leave blank.

Solid Food

Have you introduced solid food to your baby yet?

- ○ Yes
- ○ No

If yes, approximately when did you start regularly including solid food in your baby's diet?

If no, leave blank.

YYYY-MM-D

If yes, what was the first solid food you introduced to your baby?

Note: regularly included in diet, not just tasted. If no, leave blank.

If yes, what was the reason you started introducing solid food to your baby?

If no, leave blank.

Maternal Measurements

What is YOUR current weight (maternal weight)? Answer in kilograms OR pounds.

Please skip if you do not know your weight or would prefer not to share.

Kilograms:

Pounds:

How would you describe your baby's delivery?

- ○ Vaginal delivery
- ○ Caesarean delivery ("c-section")
- ○ Other:

You recently attended your 6-month follow up visit with your primary care provider. Please answer the folowing questions about this visit.

* What date was the visit?

YYYY-MM-D

What kind of visit did you attend?

- ○ Immunization
- ○ Well Baby Visit
- ○ Other:

What kind of health care provider did you visit?

- ○ Family doctor
- ○ Pediatrician
- ○ Public Health Nurse

- ○ Nurse practitioner
- ○ Other:

Infant Weight

How did the health care provider weigh your baby?

- ○ Infant scale (see image below)
- ○ Parent holding baby on adult scale
- ○ Other:

What was your baby wearing when it was weighed?

- ○ Nothing (baby was nude)
- ○ Diaper only
- ○ Clothing and diaper
- ○ Other:

* Was your baby's weight recorded in kilograms or in pounds and ounces?

- ○ Kilograms

- ○ Pounds and Ounces

* What was your baby's weight at the 6 month visit?
Please answer to at least 1 decimal: e.g. 3.5

kg

* What was your baby's weight at the 6 month visit?

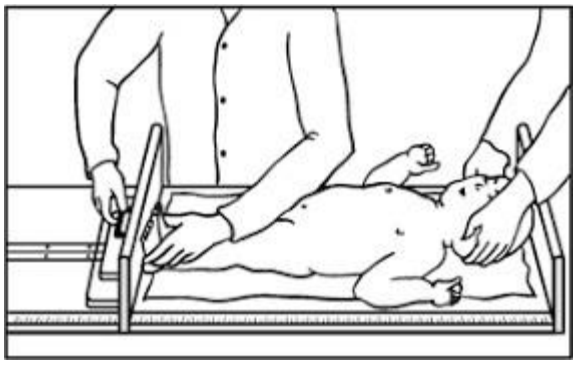
Pounds:

Ounces:

Length

How did the health care provider measure your baby's length?

- ○ Infant length board (see image below)
- ○ Marking your baby's length on paper, then measuring
- ○ Tape measure beside your baby
- ○ My health care provider didn't measure length
- ○ Other, please describe: _____



What was your baby's length at the 6 month visit?

_____ cm

OR

_____ in

- ○ My health care provider didn't measure length

Head circumference

How did the health care provider measure your baby's head circumference?

- ○ Using head circumference tape measure (see image below)

- ○ Using a piece of paper
- ○ Using string
- ○ My health care provider didn't measure head circumference
- ○ Other, please describe: _____

What was your baby's head circumference at the 6 month visit?

_____ cm

OR

_____ in

- ○ My health care provider didn't measure head circumference

Who is your baby's primary caregiver?

- ○ Yourself
- ○ Other:

Do you regularly take a multivitamin?

(Note: for mom, not baby)

- ○ Yes
- ○ No
- ○ Other:

What foods has your baby ever eaten?

(Please check all that apply.)

- ☐ Breast milk
- ☐ Infant formula
- ☐ Vitamin D drops
- ☐ Water
- ☐ Cow's milk
- ☐ Goat's milk
- ☐ Soy milk
- ☐ Almond milk
- ☐ Medicine, such as oral rehydration salts
- ☐ Baby food
- ☐ Other solid food
- ☐ Other:

Which foods did your baby eat in the last week?

(Please check all that apply.)

- [ ] Breast milk
- [ ] Infant formula
- [ ] Vitamin D drops
- [ ] Water
- [ ] Cow's milk
- [ ] Goat's milk
- [ ] Soy milk
- [ ] Almond milk
- [ ] Medicine, such as oral rehydration salts
- [ ] Baby food
- [ ] Other solid food
- [ ] Other:

How often in the last week did your infant consume food or drink other than breastmilk or vitamin D drops?

Answer "0" if baby is exclusively breastfed

instances

Does your baby spit up after feeds?

- ○ After every feed
- ○ After most feeds (75%)
- ○ After some feeds (50%)
- ○ After a few feeds (25%)
- ○ No, or very rarely

Do you pump breast milk?

- ○ Yes

- ○ No (feed only at breast)

How many feeds of breastmilk does your baby have in a typical day (24 hrs)?

feeds

Of these feeds, how many are directly at breast:

How many are pumped breast milk from a bottle or cup:

If you feed pumped milk less than once per day, please describe how often you feed pumped breast milk:

Who usually feeds your baby from a bottle?

- Yourself
- Your partner
- An older child
- Not applicable (my baby does not bottle feed)
- Other, please describe:

How many times do you usually pump milk in a typical day (24 hrs)?

How many feeds of breastmilk does your baby have in a typical day (24 hrs)?

feeds

* The next 18 questions deal with your interactions with your child when you are feeding milk from the breast or from the bottle or cup. Answers range on a scale from "never" to "always". Please select the answer that best describes you and your baby. If you are not certain, make your best guess. Think about the past few weeks.

I feed my baby whenever s/he wants.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby at set times.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I decide when it is time for my baby to have a feed.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I let my baby decide when s/he would like to have a feed.

- ○ Never

- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby to settle her/him, even if he is not hungry.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I offer my baby a feed when s/he is unsettled or crying.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I offer my baby a feed when s/he is hurt.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

When my baby gets unsettled or is crying, feeding her/him is one of the first things I do.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby to make sure that s/he does not get unsettled or cry.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby extra milk just to make sure s/he gets enough.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

If my baby indicates s/he is not hungry, I try to get him to feed anyway.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby extra milk so s/he sleeps longer.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

When deciding how much to feed my baby, I rely on how hungry s/he is.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I feed my baby for a set time.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I carefully control how much my baby feeds.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I follow a rule about how much my baby should feed.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I let my baby decide how much s/he feeds.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

I decide how much my baby feeds.

- ○ Never
- ○ Rarely
- ○ Sometimes
- ○ Often
- ○ Always

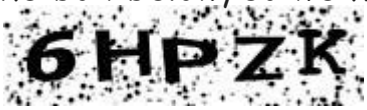
You've made it to the end of the final questionnaire! Thanks so much for your participation, and for your commitment to this important research. Remember, if you want to find out what we learned in this study check www.mamalab.ca in the Spring of 2022 to find a summary of our findings.

We will send you $20 via e-transfer for your participation. Please enter your email:

Please re-enter your email:

Reminder: This email address will be used for the Interac e-Transfer only, and will not be linked in any way to your data.

* Please type what you see in the box below, so we know you're not a robot!



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University Research Ethics Board (UREB)

Certificate of Research Ethics Clearance

☒	Clearance	☐	Secondary Data Clearance	☐	Renewal	☐	Modification	☐	Change to Study Personnel

Effective Date	**June 26, 2020**	**Expiry Date**	**June 25, 2021**

File #:	**2020-008**
Title of project:	Responsive Feeding of Infants with Expressed Milk (REFINE): exploring the growth of infants breast- and bottle-fed human milk
Researcher(s):	Kyly Whitfield
Supervisor (if applicable):	n/a
Co-Investigators:	Melissa Rossiter; Jennifer Brady; Erna Snelgrove-Clarke
Version :	1

The University Research Ethics Board (UREB) has reviewed the above named research proposal and confirms that it respects the *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans* and Mount Saint Vincent University's policies, procedures and guidelines regarding the ethics of research involving human participants. This certificate of research ethics clearance is valid for a period of **one year** from the date of issue.

Researchers are reminded of the following requirements:	
Changes to Protocol	Any changes to approved protocol must be reviewed and approved by the UREB **prior** to their implementation. **Form: REB.FORM.002** **Info: REB.SOP.113** **Policy: REB.POL.003**
Changes to Research Personnel	Any changes to approved persons with access to research data must be reported to the UREB immediately. **Form: REB.FORM.002** **Info: REB.SOP.113** **Policy: REB.POL.003**
Annual Renewal	Annual renewals are contingent upon an annual report submitted to the UREB **prior** to the expiry date as listed above. You may renew up to four times, at which point the file must be closed and a new application submitted for review. **Form: REB.FORM.003** **Info: REB.SOP.116** **Policy: REB.POL.003**
Final Report	A final report is due on or before the expiry date. **Form: REB.FORM.004** **Info: REB.SOP.116** **Policy: REB.POL.003**
Privacy Breach	Researchers must inform the UREB immediately and submit the Privacy Breach form. The breach will be investigated by the REB and the FOIPOP Officer. **Form: REB.FORM.015**
Unanticipated Research Event	Researchers must inform the UREB immediately and submit a report to the UREB within seven (7) working days of the event. **Form: REB.FORM.008** **Info: REB.SOP.115** **Policy: REB.POL.003**
Adverse Research Event	Researchers must inform the UREB immediately and submit a report to the UREB within two (2) working days of the event. **Form: REB.FORM.007** **Info: REB.SOP.114** **Policy: REB.POL.003**

*For more information: http://www.msvu.ca/ethics

[redacted]

Dr. Daniel Séguin, Chair
University Research Ethics Board

To: Kyly Whitfield
Applied Human Nutrition, Mount Saint Vincent University

Protocol Number: REB Ref # 6008074

Title: Feeding infants in Nova Scotia: an exploratory Analysis of Responsive Feeding with Mother's Milk (REFINE Study)

Date Approved: April 15 2019 **End Date:** April 14 2020

This research proposal has been reviewed and approved by the UPEI Research Ethics Board. Please be advised that the Research Ethics Board currently operates according to the Tri-Council Policy Statement 2: Ethical Conduct for Research Involving Humans (2014) and applicable laws and regulations.

It is your responsibility to ensure that the Annual Renewal and Amendment Form for Approved Studies is forwarded to Research Services prior to the renewal date. The information provided in this form must be current to the time of submission and submitted to Research Services not less than 30 days prior to the anniversary of your approval date. The Renewal/Amendment form can be downloaded from the Research Services website (http://www.upei.ca/research/forms).

The Research Ethics Board advises that IF YOU DO NOT return the completed Ethics Renewal form prior to the date of renewal:

- Your ethics approval permit will lapse;
- You will be required to stop research activity immediately;
- You will not be permitted to restart the study until you reapply for and receive approval to undertake the study again.

Lapse in ethics approval may result in the interruption or termination of funding.

Any proposed changes to the study must also be submitted on the same form to the UPEI Research Ethics Board for approval.

Notwithstanding the approval of the REB, the primary responsibility for the ethical conduct of the investigation remains with you.

Sincerely,

[redacted]

Stacey L. MacKinnon, Ph.D.
Acting Vice Chair, UPEI Research Ethics Board