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Cohort profile: Mother and Infant Metabolome and Microbiome (MIMM) study, a prospective cohort study of mothers and infants in Boston, Massachusetts

Chloe Andrews ,[1] Saumya Vodapally,[2] Laurie Pane Foster,[2,3] Isabella Lawandy,[2,3] Margot Murphy,[2] Marianna Castellanos,[4] Denisse Moncada,[5] Maria Laura Mourao,[4] Brij Bhushan,[2] Paige K Berger,[2] Raina Fichorova,[6,7] Carmen Monthé-Drèze,[7,8] Steven D Freedman,[7,9] Asimenia Angelidou,[9] Camilia R Martin,[5] Sarbattama Sen[2,3,7,10]

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For numbered affiliations see end of article.

Correspondence to
Sarbattama Sen;
ssen@carene.org

ABSTRACT

Purpose Breastfeeding is beneficial to the health of both the mother and infant. Despite recommendations to breastfeed by organisations including the WHO and the American Academy of Pediatrics, rates of breastfeeding remain below public health goals. The Mother and Infant Metabolome and Microbiome (MIMM) study is a prospective cohort study of healthy mother-term infant dyads designed to comprehensively assess the perinatal, maternal, neonatal and infant factors that are associated with breastfeeding outcomes and human milk composition.

Participants MIMM participants were recruited from two medical centres in Boston, Massachusetts, from 2019 to 2023 and are followed for 2 years. Dyads were included if the mother delivered a singleton infant at ≥37 weeks' gestation, was discharged home <72 hours after vaginal delivery or <6 days after caesarean delivery, spoke English, planned to breastfeed (either exclusively or with formula supplementation) and was willing and able to conduct follow-up through 2 years. Dyads were excluded from the study if the infant was admitted to the neonatal intensive care unit for longer than 72 hours. A total of 156 dyads were enroled in the study; however, eight participants dropped out prior to hospital discharge and will be excluded from all analyses (ie, no data was collected), resulting in a final cohort sample size of 148 mother-infant dyads. Approximately 62% of participants were White, 20% were Black or African American, 11% were Asian and 7% were more than one or unknown race. The cohort was highly educated, with 87% of participants having at least a college degree. Median maternal pre-pregnancy body mass index was 24.8 kg/m^2 and infant gestational age was 39.3 weeks. Approximately 43% of infants were born via caesarean delivery, and 45.5% were female.

Findings to date MIMM study procedures include longitudinal (1) collections of maternal blood, vaginal swab, stool and milk and infant blood and stool samples and (2) assessments of breastfeeding status, child neurodevelopment and growth and maternal health at birth, 6 weeks and 6, 12, 18 and 24 months. Data collection through 18 months is complete. The overall objective of the MIMM study is to identify potential targets to improve breastfeeding outcomes, human milk composition and ultimately, maternal and child health. Preliminary analyses, reported in conference presentations (with ongoing analyses and results manuscripts pending), have found that (1) mothers with higher levels of stress were less likely to be exclusively breastfeeding their infants at 6 weeks; (2) higher breastfeeding intensity was associated with greater postpartum weight loss at 6 weeks; (3) feeding type was a more relevant predictor of feeding frequency and volume compared with feeding mode; (4) infants who received exclusive human milk had

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The MIMM Study is unique in that it has detailed characterisation of breastfeeding practices, human milk composition, maternal and child diet and maternal and child health outcomes.
⇒ The MIMM Study also integrates longitudinal follow-up of the dyad to 2 years with the potential to follow dyads for longer pending additional funding.
⇒ Participants were recruited from two large Boston, Massachusetts-based hospitals with diverse patient populations, and the racial/ethnic composition of our sample closely approximates Massachusetts demographics.
⇒ Our study only included English-speaking mothers and their healthy term infants and was predominantly composed of highly educated and higher-income participants, limiting generalisability of the findings to similar populations.
⇒ Our approach of recruiting in the hospital postpartum (instead of prenatally) precluded direct prenatal assessments; however, we did collect prenatal data through medical record review for participants, which relies on accurate and complete clinical documentation.

higher food enjoyment compared with those who received any formula; and (5) infants of mothers with obesity had higher average feeding volume per feed.

Future plans Data collection for the final 24-month visit is expected to be completed by August 2025. We expect that all sample assays will be completed by December 2025. Findings will continue to be submitted for presentation at scientific conferences, and we expect to publish the first findings from this cohort in manuscript format in 2025.

INTRODUCTION

The American Academy of Pediatrics and WHO recommend that infants be exclusively breastfed for the first 6 months of life with continuation of breastfeeding and introduction of solid foods from 6 months to 2 years and beyond.[1–3] Longer duration of breastfeeding is beneficial to the health of both the mother and infant. For the mother, longer breastfeeding duration has been associated with a decrease in postpartum weight retention, rates of metabolic syndrome and rates of breast and ovarian cancer.[4] For the infant, longer breastfeeding duration has been associated with lower rates of infection, improved cognitive development and lower rates of obesity later in life.[4]

Despite these recommendations and the established benefits of breastfeeding, only 25% of infants in the USA are exclusively breastfed for the first 6 months and only 36% receive any human milk at 1 year.[5] We have previously shown that higher maternal dietary inflammation is associated with early breastfeeding cessation.[6] We have also reported that women with overweight and obesity have shorter duration of any and exclusive breastfeeding, and that dietary and systemic inflammation and caesarean delivery are significant mediators of this association.[7] Others have identified that working full time, lower maternal education, poorer maternal health and anxiety during pregnancy increase the risk of early breastfeeding cessation[8 9]; however, more research is needed to confirm these findings.

Human milk is a complex substance, consisting of macronutrients, micronutrients and many bioactive factors.[10] The composition of human milk is variable and can be influenced by many factors, including maternal nutritional status and diet, infant gestational age and stage of lactation.[11 12] Although studies have examined predictors of specific components of human milk, few have integrated comprehensive data on human milk composition with breastfeeding, maternal and child outcomes within a single cohort.

The Mother and Infant Metabolome and Microbiome (MIMM) study aims to address knowledge gaps in predictors of breastfeeding outcomes (eg, duration, exclusivity and intensity), milk composition and health outcomes of the dyad related to breastfeeding through comprehensive perinatal, maternal, neonatal and infant characterisation and detailed assessment of human milk composition. The ultimate goal of the MIMM study is to identify potential targets to optimise breastfeeding outcomes and human milk composition. The primary aims of the MIMM Study are (1) to determine maternal predictors of breastfeeding outcomes and human milk composition and (2) to examine the associations of human milk composition with child health outcomes.

COHORT DESCRIPTION

Study overview

The prospective MIMM Study nested within the Lactation Lab (www.longwoodlactationlab.org) is a longitudinal observational cohort that recruited 156 full term, healthy postpartum dyads from Brigham and Women's Hospital (BWH) and Beth Israel Deaconess Medical Center (BIDMC) in Boston, Massachusetts. Recruitment occurred from 21 September 2021 to 22 August 2023. Dyads continue to be followed until 2 years of life. The study visits are conducted during the delivery hospitalisation (visit 1a) and at 6 weeks (visit 1b) and 6 (visit 2), 12 (visit 3), 18 (visit 4) and 24 months (visit 5).

Eligibility criteria, recruitment and longitudinal participation

Dyads were included if the mother delivered a singleton infant at ≥37 weeks' gestation, was discharged home <72 hours after vaginal delivery or <6 days after caesarean delivery, spoke English, planned to breastfeed (either exclusively or with formula supplementation) and was willing and able to conduct follow-up through 2 years. Dyads were excluded from the study if the infant was admitted to the neonatal intensive care unit (NICU) for longer than 72 hours.

Postpartum patients were screened by a trained member of the study. If eligible, they were then approached by a member of the study staff during their delivery hospitalisation. Study staff explained the study, asked about any additional inclusion and exclusion criteria (eg, plans to breastfeed or formula feed) and answered any questions the patient or their partner had. If the patient was interested in participating, study staff reviewed the consent form in person and obtained written informed consent from the mother for both themselves and their infant before conducting any study procedures.

Details on the number of patients screened, eligible, approached and enroled are presented in figure 1. A total of 156 mother-infant dyads were enroled. Eight of the 156 participants dropped out prior to hospital discharge and will be excluded from all analyses (ie, no data was collected), resulting in a final cohort sample size of 148 mother-infant dyads. The most common reasons for declining enrollment were related to not being interested in the study, the study being too much of a commitment, moving out of the area and a formula-only feeding plan.

The number of participants who completed visits 1–4 is also presented in figure 1. Participants are still completing visit 5, and we anticipate that all 24-month visits will be completed by August 2025. Our sample size was based on a difference of 1.8 weeks duration of breastfeeding in women of different BMI categories.[7]

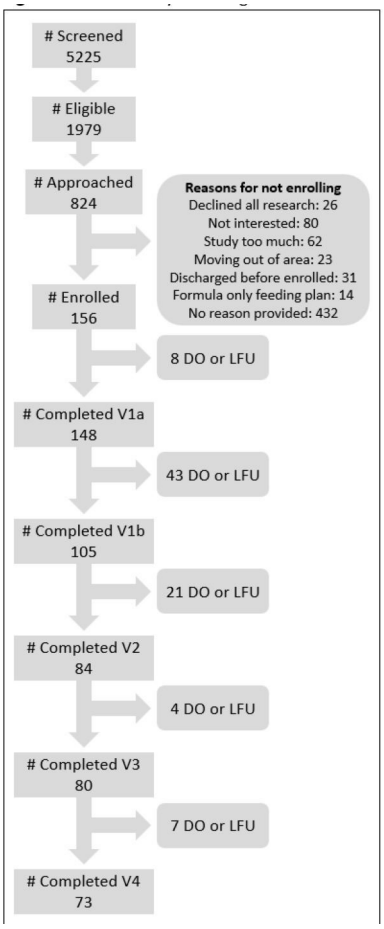

Figure 1 MIMM study flow diagram. DO, drop out; LFU, loss to follow-up.

Data collection

Participants are asked to provide a series of longitudinal samples, including human milk, maternal and infant stool and blood and maternal vaginal swab, complete questionnaires and attend an optional in-person growth and development assessment over the course of the study. Further details on the samples collected and questionnaires administered are presented in table 1. Participants are given the option to opt out of any sample collections.

Sample collections

Human milk

Participants self-collect human milk samples at 6 weeks and 6, 12 and 18 months (or until the mother stops breastfeeding) as an aliquot of a mixed full breast expression. Participants are instructed to wash and dry hands thoroughly, apply a pump to one breast and pump the breast completely (from foremilk to hindmilk) into a collection container that attaches to their pump. After their breast is emptied, participants remove the bottle from the pump, tightly seal it and gently swirl the milk for 30 s to mix the sample. Immediately after mixing, participants pour 15 mL of the full expression into a sterile collection container, record the date and time of collection and store the sample in the refrigerator until pick up by study staff within a few hours of collection.

After samples are returned to the lab, study staff immediately process them for storage. Staff gently mix the human milk samples by inverting 30 times and using a clean pipette, aliquot the parent sample into smaller aliquots for subsequent assays (see Planned Sample Assays section for details) and store at −80°C until time of analysis. One of these aliquots is used to extract and preserve human milk epithelial cells using the following procedure: staff dilute 5 mL of fresh milk in a 1:1 ratio with phosphate-buffered saline (PBS) and centrifuge at 800 x g for 20 min. Staff then transfer the supernatant into a fresh tube with RNAse inhibitor and store it at −80°C. Staff then wash the remaining pellet once with PBS, resuspend the washed pellet in 90% fetal bovine serum and 10% dimethyl sulfoxide, count the suspended cells and make aliquots which are then subjected to slow-freeze cryopreservation and ultimately transferred to −80°C and then liquid nitrogen for storage.

Maternal whole dried blood samples

Participants self-collect whole dried blood samples at 6 weeks and 12 months with the Tasso-M20 device (Tasso, Inc., Seattle, WA) which collects 4 samples of 17.5 µL.[13] Participants are instructed to wash their hands with soap and warm water, warm their arm just below their shoulder and then clean their arm with a provided alcohol pad, letting it air dry. Participants then affix the device to their arm, press the button firmly which pierces the skin and wait 5 min for the blood to collect. After the blood reaches the bottom of the device, participants peel the device off, open the vent to expose the sample pods and pack the device in the provided foil bag with moisture packs. Participants then record the date and time of collection and store the samples at room temperature until pick up by study staff.

After blood samples are returned to the lab, study staff remove the dried blood spot cartridge from the device and place the cartridge in a plastic bag with a silica gel desiccant. The samples are stored at −80°C until the time of analysis.

We conducted an ancillary pilot study with five participants recruited separately from the MIMM cohort to examine the correlation of insulin, c-peptide, c-reactive protein (CRP) and interleukin (IL)-6 from blood samples simultaneously collected with the Tasso-M20 and a venous draw. Samples were stored at −80°C until analysis by Meso Scale Discovery immunoassays (C-peptide and insulin U-plex, IL-6 S-plex and CRP V-plex). We found that the simultaneously collected venous samples and Tasso samples had a high Pearson correlation for insulin (r=0.96), c-peptide (r=0.98), CRP (r=0.92) and IL-6 (r=0.99). All biomarkers were measured within the linearity ranges of the respective immunoassays with excellent reproducibility (quality control intraassay coefficient of variation 6±3%–5.3% for C-peptide, 4.4% for CRP, 3.3% for insulin and 10.3% for IL-6).

Table 1 Samples and data collected throughout the MIMM study

	Delivery	6 weeks	6 months	12 months	18 months	24 months
Samples collected*						
Human milk		X	X	X	X	
Maternal blood		X		X		
Maternal vaginal swab		X	X	X	X	
Maternal stool		X	X	X	X	
Infant stool		X	X	X	X	
Infant blood						X
Anthropometric assessments						
Infant						
Weight	X					X
Length	X					X
Head circumference	X					
Body composition (ADP, SFTs and/or BIA)	X					X*
Maternal						
Body composition (ADP, SFTs, and/or BIA)						X*
Neurodevelopment assessments*						
Bayley IV						X
Spin the Pots						X
Mommies and babies						X
Questionnaires						
Maternal characteristics, stress, diet and health						
Demographics	X					
Holmes-Rahe Life Stress Inventory	X			X		X
Perceived Stress Scale						X
Block 2014 Food Frequency Questionnaire	X					
Maternal Health Questionnaire						X
Infant feeding and feeding behaviour						
Infant Feeding Intentions Scale	X					
Breastfeeding Status Questionnaire		X	X	X	X	X
24-hour feeding log		X				
Infant Feeding Practices Survey		X	X	X		
Baby Eating Behavior Questionnaire			X			
Children's Eating Behavior Questionnaire				X	X	X
Child health						
Child Health Questionnaire		X	X	X	X	X
Child neurodevelopment						
Infant Behaviour Questionnaire – revised very short form			X	X		
Early Childhood Behavior Questionnaire very short form					X	
Ages and Stages Questionnaire						X
Motor and Social Development Questionnaire						X
Parent Report of Children's Abilities-Revised						X
Brief Infant-Toddler Social and Emotional Assessment						X
Behavior Rating Inventory of Executive Function - Preschool						X

*Indicates optional assessments.
ADP, air displacement plethysmography; BIA, bioelectrical impedance analysis; SFT, skinfold thicknesses.

Maternal vaginal swabs

Participants self-collect vaginal swabs at 6 weeks and 6, 12 and 18 months with the OMNIgene·VAGINAL device (DNA Genotek Inc., Ottawa, Ontario, Canada).[14 15] Participants are instructed to wash their hands with soap and warm water prior to collection. After removing the swab

from the packaging, participants insert the swab 3 to 5 cm into the vagina and move the swab in several full circles along the vaginal walls for 20 s, ensuring that the sample is collected on all sides of the swab tip. Participants then immediately insert the swab into the bottom of the tube so it is submerged in the stabilising liquid and tightly screw the cap back onto the tube. Participants then record the date and time of collection and store the samples at room temperature until pick up by study staff.

After samples are returned to the lab, study staff add 5 µL of proteinase K (80 mg/mL) to the original sample collection kit (with the stabilisation solution and swab) and vortex the samples. They then incubate the sample at 50°C for 1 hour in a water bath (or 2 hours in an air incubator). After incubation, staff press and twist the swab against the inner walls of the collection tube to release any liquid contained within the swab and aliquot the sample into cryovials for storage at −80°C until time of analysis.

Maternal and infant stool samples

Participants collect stool samples for themselves and their infants at 6 weeks and 6, 12 and 18 months with the OMNIgene·GUT[16 17] and OMNImet·GUT[18 19] devices (DNA Genotek Inc., Ottawa, Ontario, Canada)). For maternal stool collections, participants are instructed to empty their bladders before collection and collect stool samples free of urine or toilet water. A toilet hat accessory is provided to assist with collection. For infant stool collections, samples are taken from a soiled diaper. Participants use the included spatula to collect a small faecal sample and transfer the faecal sample into the top portion of the tube until it is completely filled. Once filled, the top of the tube is screwed back on and participants vigorously shake the sealed tube for a minimum of 30 s, ensuring that the faecal sample is mixed with the stabilising liquid. Participants then record the date and time of collection and store the samples at room temperature until pick up by study staff.

After samples are returned to the lab, study staff vortex the samples vigorously for 60 s to homogenise the stool further and use a wide-bore pipette tip to aliquot the sample into cryovials for storage at −80°C until time of analysis. If the OMNIgene·GUT samples are too viscous to pipette, the staff incubate the samples at 50°C for 30 min. If samples are still too viscous, Liquefaction Reagent (DNA Genotek Inc., Ottawa, Ontario, Canada) is added.

Infant blood samples

For dyads who opt for the in-person visit at 24 months, an infant blood sample is obtained with the Tasso-M20 device. Staff first place a heating pack on the infant's back above the diaper line for 5 min to warm the area. They then clean the site with an alcohol pad, letting it air dry. Staff then affix the device to the infant's lower back and instruct the parent to press the button firmly which pierces the skin. After 5 min or once the blood is collected, staff remove the Tasso-M20 device. After blood samples are returned to the lab, study staff remove the dried blood spot cartridge from the device and place the cartridge in a plastic bag with a silica gel desiccant. The samples are stored at −80°C until the time of analysis.

Growth and body composition assessments

During the delivery hospitalisation, trained study staff measure infant anthropometry and body composition. Length is measured in duplicate with a recumbent length board and head circumference with a non-stretchable tape. If the two measurements differ by >0.5 cm, a third measurement is taken. Infant weight is measured in duplicate on a calibrated portable digital scale (BIDMC) or once with the PEA POD air displacement plethysmography (ADP) system (Cosmed, Concord, CA) (BWH). If the digital scale measurements differ by >10 g, a third measurement is taken. To assess body fat, study staff measure the suprailiac, thigh, tricep and subscapular skinfold thicknesses (SFTs) in duplicate using a Harpenden skinfold calliper. If SFT measurements differ by >0.5 mm, a third measurement is taken. For infants delivered at BWH only, study staff also use the PEA POD device which uses ADP to measure infant body volume and mass and estimate fat mass and fat-free mass.

At 6 weeks and 6, 12, 18 and 24 months, participants report the date of the closest paediatrician visit and the infant weight and length from that visit.

At 24 months, dyads are given the option to come in for an additional in-person visit. At this visit, trained study staff measure maternal and child anthropometry and body composition. Height is measured in duplicate with the Seca 222 Mechanical Telescopic Measuring Rod (Seca, Hamburg, Germany). Study staff measure maternal and child weight and body composition with the BOD POD ADP device (Cosmed, Concord, CA) and body composition with the Quadscan 4000 bioelectrical impedance analysis (BIA) device (Bodystat, Douglas, Isle of Man).

Infant neurodevelopmental assessments

For dyads who opt for the in-person visit at 24 months, additional neurodevelopmental assessments are performed, including the Bayley Scales of Infant and Toddler Development Fourth Edition (BSID-4),[20] Mommies and Babies,[21] and Spin the Pots.[22 23] The BSID-4 is a formal developmental assessment tool used to diagnose developmental delays in early childhood. The BSID-4 assesses development in five domains, including cognition, motor, language, socio-emotional and adaptive behaviour. However, for this study, we only assess the cognitive, motor and language domains. Mommies and Babies is adapted from the Wisconsin Card Sorting Task and is used to measure executive function,[21] and Spin the Pots assesses working memory.[22 23]

Questionnaires

Questionnaires were administered at each study visit in either paper-based or electronic format, depending on participant preference and whether the visit was in-person

or virtual. Electronic questionnaires were administered through HIPAA-compliant REDCap.

Maternal demographics

At enrollment, participants complete a demographics questionnaire that asks them to self-report information on race, marital status, education level, employment status, income level, health insurance status and smoking status.

Maternal diet and physical activity

Participants also complete the Block 2014 Full-Length Food Frequency Questionnaire (FFQ) and Physical Activity Screener (NutritionQuest, Berkeley, CA) at enrollment, which is used to assess maternal diet and physical activity in the month prior to delivery. The FFQ asks participants to report frequency and portion size consumption of 127 different food and beverage items over the past 1 month. The FFQ also asks participants about consumption of multivitamins, as well as single vitamin and mineral supplements. The Physical Activity Screener asks participants to report their frequency and duration of 11 of the most important energy expenditure sources in the USA and estimates minutes, metabolic equivalent task-minutes and kilocalorie expenditure from activities.[24]

Maternal stress

Participants complete the Holmes-Rahe Life Stress Inventory, at enrollment, 12 months and 24 months, which asks whether 43 different life events occurred during the past year.[25] Each life event is assigned a different 'weight' (ie, score), and each event that occurred is summed to give an overall score, with higher scores indicating higher levels of potential stress.[25 26] Maternal stress is also assessed with the Perceived Stress Scale, a 10-item questionnaire that asks about the mother's feelings and thoughts during the last month, at 24 months. Questionnaire scores range from 0 to 40 and higher scores indicate higher perceived stress.[27]

Maternal health

At 24 months, participants complete a maternal health questionnaire developed by our research team. This questionnaire asks about employment status, general health (eg, alcohol consumption, smoking status, weight), medical diagnoses and medications, supplement intake and reproductive health.

Infant feeding and feeding behaviour

Infant feeding intentions are assessed at delivery with the Infant Feeding Intentions Scale, a 5-item questionnaire that asks questions about the strength of the mothers' intentions to initiate breastfeeding and continue exclusively breastfeeding at 1, 3 and 6 months. The questionnaire is scored with total scores ranging from 0 to 16, and a higher score indicates a higher intention to breastfeed.[28]

At 6 weeks, participants also complete a detailed 24-hour feeding log which asks them to record information for each infant feeding session for an entire 24-hour period. Participants are asked to record start and end times and pre- and post-breastfeeding infant weights (with a scale provided by the study team) for all breastfeeding sessions over a 24-hour period, as well as record any volumes of other milk (formula or donor milk) provided to their infant.

At 6 weeks and 6, 12, 18 and 24 months, participants complete a breastfeeding status questionnaire developed by our research team. This questionnaire asks questions pertaining to whether the mother is still providing their human milk to their infant (including the date their infant last received their human milk), whether their baby is receiving anything other than their milk to drink and whether their baby was ever fed with a bottle. Participants also complete modified versions of the Infant Feeding Practices Survey at 2, 6 and 12 months.[29]

To assess infant and child feeding behaviours, participants complete the Baby Eating Behaviour Questionnaire (BEBQ) at 6 months and the Children's Eating Behaviour Questionnaire (CEBQ) at 12, 18 and 24 months. The BEBQ is an 18-item questionnaire that assesses parent-reported measures of infant appetite during the period that the infant is exclusively receiving milk. The BEBQ provides scores in five distinct appetitive constructs, including 'enjoyment of food,' 'food responsiveness,' 'slowness in eating,' 'satiety responsiveness,' and 'general appetite'.[30] The CEBQ is a 35-item questionnaire that assesses parent-reported measures of eating style in children. The CEBQ is designed to give scores in eight scales, including 'food responsiveness', 'enjoyment of food', 'emotional overeating', 'desire to drink', 'satiety responsiveness', 'slowness in eating', 'emotional undereating' and 'fussiness'.[31]

Child health

Participants complete a child health questionnaire, which was developed by our research team and modelled off other validated questionnaires,[32] at 2, 6, 12, 18 and 24 months. The child health questionnaire asks participants to report their child's height and weight at their paediatrician's appointments, dietary supplements they are receiving, episodes of respiratory illness, diarrhoea, and other infections (as well as medications prescribed for any of these illnesses), and any developmental concerns they or their doctors may have.

Child neurodevelopment

At 6 and 12 months, participants complete the Infant Behavior Questionnaire (revised very short form version) which is a 37-item questionnaire that asks participants to report on their infant's temperament across three domains, including positive affectivity/surgency, negative affectivity and orienting/regulatory capacity.[33] At 18 months, participants complete the Early Childhood Behavior Questionnaire (very short form version), which is a 36-item questionnaire that assesses infant temperament across three broad scales, including negative affect,

surgency and effortful control.[34] At 24 months, participants complete the Ages and Stages Questionnaire (ASQ-3), the Motor and Social Development (MSD) questionnaire, the Parent Report of Children's Abilities-Revised (PARCA-R), the Brief Infant-Toddler Social and Emotional Assessment (BITSEA) and the Behavior Rating Inventory of Executive Function – Preschool Version (BRIEF-P). The ASQ-3 is a developmental screening tool that consists of questions across five domains, including language, gross motor, fine motor, cognitive and personal-adaptive skills.[35] Potential answers to each question include yes (10 points), sometimes (5 points) and not yet (0 points). The total number of points in each domain is summed to give a total score in that domain.[36] The MSD consists of eight components with 15 dichotomous yes/no questions each about whether the child has ever performed certain age-appropriate behaviours. The MSD is scored by summing all of the 'yes' responses.[37] The PARCA-R is used to assess children's cognitive and language development.[38] The cognitive domain includes 34 questions that ask whether or not the child is able to perform certain activities. These questions are summed to provide a total score for the cognitive domain (ranging from 0 to 34). The language domain includes a 100-word vocabulary checklist asking whether or not the child is able to say each word and 18 additional questions to assess grammatical development. These questions are summed to provide a total score for the language development domain (ranging from 0 to 124). The scores for the two domains can also be summed to provide a Parent Report Composite score (ranging from 0 to 158).[39] The BITSEA is a 42-item questionnaire used to screen for social-emotional problems and delays in competence. There are two domains, including a problem scale and a competence scale, and scores are calculated as sums.[40 41] The BRIEF-P is an 83-item questionnaire that measures executive functioning in preschoolers in five clinical scales, including inhibit, shift, emotional control, working memory and plan/organise. The scales yield three composite indexes, including the Inhibitory Self-Control Index, Flexibility Index and Emergent Metacognition Index. There is also an overall composite index, the Global Executive Composite.[42]

Clinical data

Trained study staff abstract clinical data from throughout pregnancy, the delivery hospitalisation and the postpartum period from the medical record. Details on the data collected and derived from the medical record are provided in table 2.

Participant remuneration and benefits

Participants have access to phone-based lactation support from a certified lactation counsellor who is also study staff through the first 6 months of their infant's life. They also receive a detailed analysis of their dietary intake from the FFQ and a report of their infant's neurodevelopmental assessments at the 24-month visit. In addition, participants receive small baby gifts intermittently until study completion and a US$50 gift card after completion of the 24-month visit.

Table 2 Data abstracted and derived from the medical record

Data category/timepoint	Data points collected	Data points derived
General medical history	Medical diagnoses, prescribed medications, supplement intake	N/A
Obstetric history	Gravidity, parity, term/preterm infants, living children, abortions, ectopic pregnancies, multiple pregnancies, outcomes of most recent pregnancy	N/A
Initial prenatal visit	EDC, GA, height, weight, pre-pregnancy weight, blood pressure, EPDS score, labs (routine prenatal labs, haemoglobin A1c, thyroid stimulating hormone, vitamin D), glucose tolerance testing results	BMI at initial prenatal visit, pre-pregnancy BMI
Earliest and last pregnancy ultrasounds	Fetal measurements (*eg,* femur length, abdominal circumference, biparietal diameter) and respective GA at measurements	Estimated fetal weight and weight percentile (Hadlock growth charts)
Last prenatal visit	GA, weight, blood pressure, GBS test results	Weight gain during pregnancy
Delivery admission	Admission/discharge dates, fluids administered during labour, mode of delivery, perinatal complications, Apgar scores, GA at delivery, infant sex, birth measurements, infant diagnoses, NICU admissions	Birth size-for-gestational age percentiles and z-scores (weight, length, head circumference)
Postpartum visit	Maternal weight, blood pressure, problems/concerns, new medications, current infant feeding method, EPDS score, birth control plan, postpartum glucose tolerance testing	BMI at postpartum visit; postpartum weight retention

BMI, body mass index; EDC, expected date of confinement; EPDS, edinburgh postnatal depression scale; GA, gestational age; GBS, group B streptococcus; NICU, neonatal intensive care unit.

Table 3 Planned sample assays

Planned assays*	Methods	Performed by
Human milk		
Energy and macronutrients	Mid-infrared spectroscopy (MIRIS)	Trained study staff
Fatty acids	Gas chromatography–mass spectrometry	Trained study staff
Metabolome	Ultra-performance liquid chromatography/mass spectrometry	Metabolon, Inc.
Oxylipins	Liquid chromatography–mass spectrometry	Trained study staff
Microbiome	16s rRNA sequencing or shotgun sequencing	To be determined
Cytokines (IL-6, IL-8, IL-10, TNFα)	Meso Scale Discovery	Trained study staff
Adipokines (leptin, adiponectin, ghrelin)	Meso Scale Discovery	Trained study staff
HMEC transcriptome	scRNA and bulk RNA sequencing	To be determined
Maternal blood		
Metabolome	Ultra-performance liquid chromatography-mass spectrometry	Metabolon, Inc.
Cytokines (IL-6, IL-8, IL-10, TNFα)	Meso Scale Discovery	Trained study staff
C reactive protein	Meso Scale Discovery/ELISA	Trained study staff
Insulin	Meso Scale Discovery	Trained study staff
IGF	Meso Scale Discovery	Trained study staff
Adipokines (leptin, adiponectin, ghrelin)	Meso Scale Discovery	Trained study staff
Vaginal swabs		
Microbiome	16s rRNA sequencing or shotgun sequencing	To be determined
Maternal and infant stool		
Metabolome	Ultra-performance liquid chromatography-mass spectrometry	Metabolon, Inc.
Microbiome	16s rRNA sequencing or shotgun sequencing	To be determined

*Planned assays are pending funding availability.
HMEC, human milk epithelial cells.

Planned sample assays

Planned sample assays for the human milk, maternal blood, vaginal swabs and maternal and infant stool samples are presented in table 3.

Characteristics of MIMM participants

Baseline characteristics of the 148 mother-infant dyads who were enroled in the MIMM study are presented in table 4.

Ethics approval

This study was approved by the Brigham and Women's Hospital (Protocol #2020P003280) and Beth Israel Deaconess Medical Center (Protocol #2020C001102) Institutional Review Boards. A single IRB was created to maximize efficiency and consistency across participating sites. Mass General Brigham serves as the parent institution. All participants provided written informed consent for themselves and their infants.

Patient and public involvement

None.

FINDINGS TO DATE

Preliminary findings, with ongoing analyses and results manuscripts pending, have been presented at the Pediatric Academic Societies 2024 meeting in Toronto, Canada, and the NUTRITION 2023 meeting in Boston, MA.[43–47] In these preliminary analyses, we found that:

1. Mothers with higher levels of stress were less likely to exclusively breastfeed their infants at 6 weeks and more likely to use provided lactation services.[46]
2. Higher scores on three different breastfeeding intensity instruments at 6 weeks PP (vs. lower scores) were significantly associated with more PP weight loss and greater % of daily human milk intake by infants at 6-weeks. Further, we concluded that different instruments better predict each of these outcomes.[47]
3. Feeding type (exclusive human milk vs any formula) was a more relevant predictor of feeding frequency and volume than feeding mode (exclusive breastfeeding vs any bottle feeding) in a single 24-hour period.[43]
4. In a single 24-hour period, infants who received only breast milk had higher food enjoyment scores vs those who received any formula. Infants who were only

Table 4 Baseline characteristics of enroled mother-infant dyads

	n	n (%) or median (IQR)
Enrolment site	148	
Brigham and Women's Hospital		73 (49.3%)
Beth Israel Deaconess Medical Center		75 (50.7%)
Maternal characteristics		
Race	136	
Asian		15 (11.0%)
Black or African American		27 (19.9%)
White		84 (61.8%)
More than one race		8 (5.9%)
Unknown		2 (1.5%)
Ethnicity	108	
Hispanic or Latino		12 (11.1%)
Marital status	138	
Single		22 (15.9%)
Married		112 (81.2%)
Divorced		2 (1.5%)
Other		2 (1.5%)
Education level	137	
Some high school		4 (2.9%)
High school diploma/GED		5 (3.7%)
Some technical school		1 (0.7%)
Some college/junior college		8 (5.8%)
College degree		34 (24.8%)
Graduate or professional degree		85 (62.0%)
Insurance type	138	
Self-pay		5 (3.6%)
Private insurance/HMO		100 (72.5%)
Medicaid/SSI/Mass Health		33 (23.9%)
Household income	129	
<US$20,000		5 (3.9%)
US$20,000–49,999		15 (11.6%)
US$50,000–69,999		5 (3.9%)
US$70,000–1 00 000		12 (9.3%)
>US$100,000		92 (71.3%)
Smoker	136	3 (2.2%)
Pre-pregnancy BMI, kg/m^2	110	24.8 (22.1–28.1)
Parity	146	1 (1-2)
Infant characteristics		
Born via caesarean delivery	146	62 (42.5%)
Gestational age, weeks	146	39.3 (38.5–40.1)
Female sex	145	66 (45.5%)
Birth weight, grams	146	3390.5 (3125.0–3735.0)

BMI, body mass index; GED, general educational development test; HMO, health maintenance organisation; SSI, social security income.

breastfed also had higher satiety responsiveness scores than those who received any bottle feeding, although this relationship did not reach statistical significance. There were no differences in infant body mass index (BMI) z-score by feeding type or mode at 6 weeks. There were no associations of appetitive traits with BMI z-score at 6 months.[44]

5. In a single 24-hour period, infants of mothers with obesity (vs. normal BMI) had higher average feeding volume (per feed) and exclusively breastfed infants of mothers with obesity (vs normal BMI) had higher average BF volume (per feed), though BF duration did not differ. Maternal BMI category was not associated with odds of exclusive human milk feeding, exclusive breastfeeding or with any infant eating behaviors.[45]

Findings will continue to be submitted for presentation at regional, national and international scientific conferences. We expect to publish the first findings from this cohort in manuscript format in early 2025. Current and planned statistical analyses for the MIMM Study cohort are as follows:

1. Determine the maternal predictors (eg, BMI, diet, mental health) associated with breastfeeding outcomes (eg, duration of exclusive breastfeeding, duration of any breastfeeding).
2. Determine the associations between duration of breastfeeding with short-term women's health outcomes (eg, weight, adiposity, depression, anxiety).
3. Determine the maternal predictors (eg, BMI, diet, mental health) of human milk composition (macronutrients, fatty acids, metabolome, microbiome).
4. Determine the associations between human milk composition (macronutrients, fatty acids, metabolome, microbiome) and infant growth.
5. Determine the associations between human milk composition (macronutrients, fatty acids, metabolome, microbiome) and infant neurodevelopment.
6. Determine the associations between maternal pre-pregnancy BMI with infant feeding type (human milk vs formula), feeding mode (direct breastfeeding vs bottle feeding) and appetitive traits.
7. Determine the associations of infant feeding type (human milk vs formula) and mode (direct breastfeeding vs bottle feeding) with infant appetitive traits and weight trajectories.
8. Determine associations between maternal fibre intake with growth and behaviour outcomes in breastfed in-

fants and examine whether these relationships are mediated by the maternal gut microbiome and human milk composition.
9. Examine the role of maternal health in the maternal gut microbiome, the contribution of maternal gut microbiome to human milk and infant gut microbiome acquisition and the infant gut microbiota in child health outcomes.

STRENGTHS AND LIMITATIONS

The main strengths of the MIMM study include the longitudinal follow up of dyads to 2 years and the detailed characterisation of maternal and child health, breastfeeding practices and human milk composition. The MIMM Study also used electronic medical records to obtain detailed clinical information from prenatal and postpartum visits, as well as the delivery hospitalisation.

The MIMM study did also have limitations as the study population consisted of only English-speaking mothers and their healthy term infants and was comprised mostly of highly educated and higher-income participants, potentially limiting the generalisability and external validity of the findings. Although we used electronic medical records to abstract key data points from clinical visits, including prenatal visits, our approach of recruiting after delivery precluded direct prenatal assessments. In using electronic medical records for this data, we relied on accurate and complete clinical documentation which may have introduced some bias across sites for certain variables. We also had a significant drop-out and loss to follow up rate between the delivery visit and the 6week-study visit. We recorded reasons parents provided for dropping out of the study, which we will take into consideration when planning future studies.

COLLABORATION

Limited data sets from the MIMM study will be made available to investigators after an analysis plan and scope have been agreed upon by the principal investigators and appropriate data use agreements are executed. Metabolome and microbiome data will be made available in a public data repository.

Author affiliations
[1]Brigham and Women's Hospital Pediatric Newborn Medicine, Boston, Massachusetts, USA
[2]Department of Pediatrics, Brigham and Women's Hospital, Boston, Massachusetts, USA
[3]Department of Pediatrics, Women and Infants Hospital, Providence, Rhode Island, USA
[4]Department of Neonatology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA
[5]Weill Cornell Medicine, New York, New York, USA
[6]Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital, Boston, Massachusetts, USA
[7]Harvard Medical School, Boston, Massachusetts, USA
[8]Brigham and Women's Hospital, Boston, Massachusetts, USA
[9]Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA
[10]Brown University Warren Alpert Medical School, Providence, Rhode Island, USA

X Maria Laura Mourao @MariaMouraoMD

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ORCID iD
Chloe Andrews http://orcid.org/0000-0002-4282-8215

REFERENCES

1 America Counts Staff. Massachusetts: 2020 census. U. S. Census Bur; 2021. Available: https://www.census.gov/library/stories/state-by-state/massachusetts-population-change-between-census-decade.html
2 World Health Organization. Breastfeeding. Available: https://www.who.int/health-topics/breastfeeding [Accessed 2025].
3 Meek JY, Noble L. Policy Statement: Breastfeeding and the Use of Human Milk. *Pediatrics* 2022;150:e2022057988.
4 Dieterich CM, Felice JP, O'Sullivan E, *et al*. Breastfeeding and health outcomes for the mother-infant dyad. *Pediatr Clin North Am* 2013;60:31–48.
5 Centers for Disease Control and Prevention/ Agency for Toxic Substances and Disease Registry/ Geospatial Research, Analysis, and Services Program. Breastfeeding report card United States. 2022.
6 Sen S, Rifas-Shiman SL, Shivappa N, *et al*. Dietary Inflammatory Potential during Pregnancy Is Associated with Lower Fetal Growth and Breastfeeding Failure: Results from Project Viva. *J Nutr* 2016;146:728–36.
7 Keyes M, Andrews C, Midya V, *et al*. Mediators of the association between maternal body mass index and breastfeeding duration in 3 international cohorts. *Am J Clin Nutr* 2023;118:255–63.
8 Kehler HL, Chaput KH, Tough SC. Risk Factors for Cessation of Breastfeeding Prior to Six Months Postpartum among a Community Sample of Women in Calgary, Alberta. *Can J Public Health* 2009;100:376–80.
9 Odar Stough C, Khalsa AS, Nabors LA, *et al*. Predictors of Exclusive Breastfeeding for 6 Months in a National Sample of US Children. *Am J Health Promot* 2019;33:48–56.
10 Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. *Pediatr Clin North Am* 2013;60:49–74.
11 Han SM, Derraik JGB, Binia A, *et al*. Maternal and Infant Factors Influencing Human Milk Oligosaccharide Composition: Beyond Maternal Genetics. *J Nutr* 2021;151:1383–93.
12 Miliku K, Duan QL, Moraes TJ, *et al*. Human milk fatty acid composition is associated with dietary, genetic, sociodemographic, and environmental factors in the CHILD Cohort Study. *Am J Clin Nutr* 2019;110:1370–83.
13 TASSO-m20. Tasso Inc. Available: https://www.tassoinc.com/tasso-m20 [Accessed 2025].

14 Bouevitch A, Doukhanine E, LeFrançois B. OMNIgene™•ORAL and OMNIgene™•vaginal devices are validated tools for the collection and stabilization of nucleic acids from oral and vaginal microbiome samples. Ottawa, Ontario, Canada DNA Genotek; 2023.
15 DNA Genotek. Vaginal microbiome dna/rna collection kit. Available: https://www.dnagenotek.com/us/products/collection-microbiome/omnigene-vaginal/OMR-130.html [Accessed 2025].
16 Brown A, Lynch D, Bouevitch A, *et al*. OMNIgene®•GUT provides easy self-collection and stabilization of liquid fecal samples for microbiome profiling. Ottawa, Ontario, Canada DNA Genotek; 2018.
17 DNA Genotek. Gut microbiome dna collection kit from stool/feces. Available: https://www.dnagenotek.com/row/products/collection-microbiome/omnigene-gut/OM-200.html [Accessed 2025].
18 Bruin OMD, Freinkman E, Breton H, *et al*. OMNImet™•GUT enables at-home collection and ambient temperature transport of fecal samples for metabolomics. Ottawa, Ontario, Canada DNA Genotek; 2020.
19 DNA Genotek. Metabolomics microbial collection and stabilization kits. Available: https://www.dnagenotek.com/row/products/collection-metabolomics/omnimet-gut/ME-200.html [Accessed 2025].
20 Balasundaram P, Avulakunta ID. *Bayley scales of infant and toddler development*. Treasure Island (FL): StatPearls Publishing, 2024.
21 Romine CB, Lee D, Wolfe ME, *et al*. Wisconsin Card Sorting Test with children: a meta-analytic study of sensitivity and specificity. *Arch Clin Neuropsychol* 2004;19:1027–41.
22 Zimmermann L, Frank HE, Subiaul F, *et al*. Applying computational modeling to assess age-, sex-, and strategy-related differences in Spin the Pots, a working memory task for 2- to 4-year-olds. *Dev Psychobiol* 2021;63:42–53.
23 Morra S, Gandolfi E, Panesi S, *et al*. A working memory span task for toddlers. *Infant Behav Dev* 2021;63:101550.
24 Questionnaires and screeners, assessment & analysis services. NutritionQuest. Available: https://www.nutritionquest.com/assessment/list-of-questionnaires-and-screeners/ [Accessed 2025].
25 Holmes TH, Rahe RH. The Social Readjustment Rating Scale. *J Psychosom Res* 1967;11:213–8.
26 Noone PA. The Holmes–Rahe Stress Inventory. *Occup Med* 2017;67:581–2.
27 Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav* 1983;24:385–96.
28 Nommsen-Rivers LA, Dewey KG. Development and validation of the infant feeding intentions scale. *Matern Child Health J* 2009;13:334–42.
29 CDC. Questionaires: breastfeeding and infant feeding practices. Cent. Dis. Control Prev; 2023. Available: https://www.cdc.gov/breastfeeding/data/ifps/questionnaires.htm
30 Llewellyn CH, van Jaarsveld CHM, Johnson L, *et al*. Development and factor structure of the Baby Eating Behaviour Questionnaire in the Gemini birth cohort. *Appetite* 2011;57:388–96.
31 Wardle J, Guthrie CA, Sanderson S, *et al*. Development of the Children's Eating Behaviour Questionnaire. *J Child Psychol Psychiatry* 2001;42:963–70.
32 Asher MI, Keil U, Anderson HR, *et al*. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483–91.
33 Putnam SP, Helbig AL, Gartstein MA, *et al*. Development and assessment of short and very short forms of the infant behavior questionnaire-revised. *J Pers Assess* 2014;96:445–58.
34 The early childhood behavior questionnaire (ecbq) | mary rothbart's temperament questionnaires. Available: https://research.bowdoin.edu/rothbart-temperament-questionnaires/instrument-descriptions/the-early-childhood-behavior-questionnaire/ [Accessed 2025].
35 Muthusamy S, Wagh D, Tan J, *et al*. Utility of the Ages and Stages Questionnaire to Identify Developmental Delay in Children Aged 12 to 60 Months: A Systematic Review and Meta-analysis. *JAMA Pediatr* 2022;176:980–9.
36 ASQ-3 scoring help. Ages Stages. Available: https://agesandstages.com/resource/asq-3-scoring-help/ [Accessed 2025].
37 Motor and social development (msd) | national longitudinal surveys. Available: https://www.nlsinfo.org/content/cohorts/nlsy79-children/topical-guide/assessments/motor-and-social-development-msd [Accessed 2025].
38 Johnson S, Bountziouka V, Brocklehurst P, *et al*. Standardisation of the Parent Report of Children's Abilities–Revised (PARCA-R): a norm-referenced assessment of cognitive and language development at age 2 years. Lancet Child Adolesc Health 2019;3:705–12.
39 Johnson S, Bountziouka V. Using the PARCA-R to assess children's cognitive and language development at two years of age. *Infant* 2020;16:159–63.
40 Briggs-Gowan MJ. The Brief Infant-Toddler Social and Emotional Assessment: Screening for Social-Emotional Problems and Delays in Competence. *J Pediatr Psychol* 2004;29:143–55.
41 Hungerford GM, Garcia D, Bagner DM. Psychometric Evaluation of the Brief Infant-Toddler Social and Emotional Assessment (BITSEA) in a Predominately Hispanic, Low-Income Sample. *J Psychopathol Behav Assess* 2015;37:493–503.
42 Sherman EMS, Brooks BL. Behavior Rating Inventory of Executive Function – Preschool Version (BRIEF-P): Test Review and Clinical Guidelines for Use. *Child Neuropsychol* 2010;16:503–19.
43 Andrews C, Monthé-Drèze C, Berger P, *et al*. P18-004-23 Associations of Infant Feeding Type and Mode With Frequency and Volume. *Curr Dev Nutr* 2023;7:101063.
44 Berger P, Andrews C, Monthé-Drèze C, *et al*. P18-007-23 Associations of Feeding Type and Feeding Mode With Infant Appetitive Traits and Body Weight in the First 6 Months. *Curr Dev Nutr* 2023;7:101066.
45 Monthe-Dreze C, Andrews C, Berger P, *et al*. P07-040-23 Associations of Maternal Body Mass Index With Infant Feeding Characteristics and Eating Behaviors. *Curr Dev Nutr* 2023;7:101031.
46 Andrews C, Kalluri NS, Lawandy IJ, *et al*. Associations between maternal stress and social vulnerability with lactation outcomes. 2–6 May 2024, Toronto, Canada.
47 Lawandy IJ, Andrews C, Abdel-Rahman R, *et al*. Comparing instruments for measuring breastfeeding intensity and duration. 2–6 May 2024, Toronto, Canada.