www.nature.com/pr

CLINICAL RESEARCH ARTICLE

Growth and body composition trajectories in infants meeting the WHO growth standards study requirements

Efrah I. Yousuf[1,2], Niels Rochow[1,3,4], Jenifer Li[5,6], Julia Simioni[5,6], Elizabeth Gunn[1,2], Eileen K. Hutton[2,5,6] and Katherine M. Morrison[1,2✉]

BACKGROUND: While the World Health Organization (WHO) developed postnatal growth standards for infants, corresponding body composition data remains scarce. This study explores growth and body composition trajectories in infants meeting the WHO Multicentre Growth Reference Study (MGRS) eligibility criteria.
STUDY DESIGN: Infants enrolled in this longitudinal cohort underwent anthropometric and body composition measurement by air displacement plethysmography (ADP) at 6 weeks, 12 weeks, and 5 months postnatally. Age and sex-specific growth and body composition percentiles were generated using GAMLSS, with extrapolated data at 5 months for those exceeding ADP weight limits. We evaluated which anthropometric measure (body mass index (BMI), weight for length or mid upper arm circumference) was most closely related to adiposity.
RESULTS: Of the 225 infants with body composition measures, 187 met the WHO MGRS criteria. Their length and weight curves were comparable with WHO growth curves. Trajectory curves for fat and fat-free mass were developed. Of the anthropometric measures, BMI z score was most closely related to fat mass index z score at all timepoints.
CONCLUSION: This study presents body composition trajectories for infants meeting the WHO growth standard criteria. BMI z score is the best anthropometric metric to estimate adiposity in infants.

Pediatric Research (2022) 92:1640–1647; https://doi.org/10.1038/s41390-022-02005-y

IMPACT:

- While postnatal growth standards derived from the WHO Multicentre Growth Reference Study (MGRS) exist for the routine clinical assessment of infant growth, this study fills the previous gap in the availability of corresponding body composition data for term-born, healthy, breastfed infants meeting the MGRS criteria.
- Extrapolation was used to obtain body composition values for infants exceeding the weight limit of the ADP device, to avoid construction of biased body composition curves.
- Sex-specific growth curves for fat mass, fat-free mass, fat mass index, and fat-free mass index are presented for a population meeting the World Health Organization growth standard criteria.

INTRODUCTION

It has long been hypothesized that fetal and early-life exposures help shape health outcomes across the life course. According to this concept, termed the 'developmental origins of health and disease,' adverse conditions in early development result in metabolic and other changes that may predispose individuals to chronic disease[1]. The increasing prevalence of child overweight and obesity is a growing global concern that has been linked, in part, to growth in infancy. Rapid weight gain in the first 6 months of life is associated with overweight and obesity during childhood[2,3] and adulthood[4,5], and with increased total body fat mass and central fat distribution in later life[6–8]. Measures of body proportionality like weight for length (WFL) and body mass index (BMI) and their z scores have been explored as surrogate measures of adiposity in early infancy, with higher measures associated with obesity in early childhood[3,9]. Additionally, mid-upper arm circumference (MUAC), which is a simple and well-known tool used for the identification of malnutrition in children under 5 years of age[10], has also been used to identify obesity in children and adolescents[11,12].

Weight gain occurs due to increases in both fat mass and fat-free mass (muscle, bone, water, skin, organs). It is important to examine how these compartments, and not just total weight for height, change during early growth to better understand the relationship of anthropometric trajectories and health outcomes. For example, in a study examining early body composition

[1]Department of Pediatrics, McMaster University, Hamilton, ON, Canada. [2]Centre for Metabolism, Obesity and Diabetes Research, McMaster University, Hamilton, ON, Canada. [3]Department of Pediatrics, Paracelsus Medical University, General Hospital, Nuremberg, Bavaria, Germany. [4]Department of Pediatrics, University Hospital, 18057 Rostock, Germany. [5]Department of Obstetrics & Gynecology, McMaster University, Hamilton, ON, Canada. [6]McMaster Midwifery Research Centre, McMaster University, Hamilton, ON, Canada. ✉email: kmorrison@mcmaster.ca

Received: 29 October 2021 Revised: 13 February 2022 Accepted: 15 February 2022
Published online: 16 March 2022

measurement and later obesity development, increased fat mass accrual over the first 8 months of life was more predictive of obesity in mid-childhood than weight gain[13]. In early infant growth, percent fat mass increases for the first 3–6 months of life[14–21] and then declines to 1 year[15,19,20]. Studies examining early growth of fat and fat-free mass have not consistently considered the influence of other important factors known to impact growth including exclusive/predominant breastfeeding for at least 4 months and continued breastfeeding to 12 months, significant perinatal morbidity, maternal smoking, multiple pregnancy, preterm or post-term birth, and health, environmental or economic constraints to infant growth[22,23]. The availability of reference data for body composition indices in early life in healthy, breastfed infants would facilitate such research.

In 2006, the World Health Organization (WHO) Multicentre Growth Reference Study (MGRS) developed and released a child growth standard for infants and young children based on longitudinal measurement. This growth standard included healthy infants, born at full term, who had no exposure to maternal smoking and who were exclusively or predominantly breastfed to 4 months of age. This anthropometric standard has been accepted as the appropriate growth reference for infants in many countries, including Canada[23,24].

Utilizing data from a longitudinal study of healthy, term-born infants with multiple measures of body composition, the aim of this study was to describe body composition changes during the first 5 months of life for infants who met the key MGRS criteria. Growth percentiles for fat mass, fat mass index, fat-free mass, fat-free mass index, and total body fat percent were planned. Further, as measurement of body composition is not always practical, we examined which anthropometric measures, WFL, BMI, or MUAC, were most closely related to fat mass and fat-free mass in healthy infants.

METHODOLOGY

Study outline

Mother-infant pairs were recruited from midwifery practices in the city of Hamilton and surrounding areas in Ontario, Canada, as part of the Baby & Mi study[25]. Inclusion criteria, assessed in the second trimester of pregnancy, included low-risk singleton pregnancy, a planned vaginal birth, and ability to communicate in English in order to provide informed, signed consent. The exclusion criteria of preterm birth were re-assessed after 37 weeks gestation[25]. Recruitment of mother-infant pairs took place between July 1st 2012 and March 9th 2018. Ethics approval for this work was obtained at participating healthcare organizations (Hamilton Integrated Research Ethics Board, St. Joseph's Healthcare Hamilton, Joseph Brant Hospital, Niagara Health System Research Ethics Board, and Brantford Community Healthcare System Research Ethics Committee). Mothers provided written, informed consent for their engagement in the study at the time of enrollment and legal guardians provided written, informed consent for their child.

The study participants for this analysis were the subgroup of infants enrolled in the Baby & Mi study that had completed body composition assessments using an air displacement plethysmography (ADP) device (PEA POD Infant Body Composition System Analysis, Cosmed USA, Inc., Concord, CA); a validated method for non-invasive infant body composition measurements[26]. Measurements collected from infants exposed to maternal smoking during pregnancy, born post-term, with identified health conditions including perinatal morbidities requiring neonatal intensive care stay >24 h or without birth data available, or infants that were completely weaned from breast milk prior to completing the ADP measurements, were excluded from this analysis[22,23,27]. In applying the WHO MGRS definition of breastfeeding[28], at each timepoint, measurements from participating infants were further classified as exclusively/predominantly breastfed (EBF) or, if they were also receiving mixed feeds before or at 4 months, partially breastfed (PBF) for the timepoint that they received mixed feeds and subsequent timepoints. Measurements collected from infants that abided by the WHO MGRS criteria, including classification as EBF, were included in the development of body composition trajectories. In a secondary analysis, the growth of EBF infants was compared to infants that met the WHO MGRS criteria except that they were PBF prior to, or at, 4 months of age. Infants that had incomplete information available at 4 months of age but were PBF at the 5-month visit were placed in the PBF group at 4 months. Similarly, if they were EBF at 5 months, they were considered EBF at 4 months.

Data collection and growth measurements

The Baby and Mi study enrolled women during pregnancy with confirmation of enrollment after 37 weeks gestation when inclusion criteria were confirmed. Mothers self-reported demographic information, pregnancy history, health history, and smoking history, and this data was complemented by pregnancy and birth data collected from the antenatal and birth forms. The antenatal form and maternal and infant hospital records were accessed by the participant's midwife. After birth, parents completed questionnaires on day 3 and day 10 of life and at 4 months of age, and three in-person study visits at 6 weeks, 12 weeks, and 5 months postpartum. Due to low participation, the 6 week in-person visit was adjusted to a parental questionnaire. Thus, the physical measures at 6 weeks were only completed in a subset of the cohort. Household income, maternal education and infant ethnicity were reported by parents at 3 years postpartum. At each study timepoint, questionnaires were completed addressing infant health and breastfeeding history and the introduction and patterns of consumption of other foods and beverages were assessed through food frequency questionnaires[29]. Report of consumption of mixed feeds, such as foods, milk other than breast milk and fruit-based fluids (as detailed by the WHO MGRS study criteria) >2 times per week at any time-point up to and including 4 months of age (i.e., greater than 12 days non-compliance) resulted in the infant being classified as PBF for that time-point and for all subsequent time-points[28].

The in-person visits involving physical measures took place at McMaster University Medical Centre, Hamilton, Canada, and included measurement of infant length, weight, head circumference, MUAC, and body composition by research personnel. Recumbent length was measured once, to the nearest 0.1 cm, using a pediatric length board (Ellard Instrumentation Ltd, WA). Head circumference was measured once, to the nearest 0.1 cm, using standardized paper measuring tape. MUAC was measured in triplicate to the nearest 0.1 cm using standardized paper measuring tape, and values were averaged. Weight (kg), fat mass (kg), fat-free mass (kg) and body fat percentage (%) were assessed using the ADP, according to the manufacturer's recommendations. Body composition was calculated by the ADP according to density values from Fomon[30]. This instrument has a maximum weight capacity of 8 kg. Fat mass index and fat-free mass index were calculated from the measured length and the fat mass and fat-free mass values respectively (kg/m^2).

Statistical analysis

Age, growth, and body composition are presented as means (standard deviations). Postmenstrual age was used instead of chronological age in order to control for differences in gestational age at birth. Differences between male and female infants were analyzed in IBM SPSS Statistics (IBM SPSS Statistics for Windows, Version 27.0.1. Armonk, NY: IBM Corp.) using independent t-tests (normal data) and Mann-Whitney U tests (non-normal data), and normality was assessed using the Shapiro–Wilk test.

Body composition at 5 months of age was extrapolated for EBF infants meeting the WHO eligibility criteria who were missing this data because they exceeded the weight limit of the ADP device (8 kg). One infant weighing 7.96 kg at 5 months was marked as too large for the device, and their values were also extrapolated. Extrapolation is necessary here as ignoring the censored and not randomly missing data would lead to a biased reference curve[31]. Percent fat mass at 5 months of age was extrapolated using a linear model based on sex, weight, length, and head circumference measurements at 5 months of age, as well as percent fat mass at 12 weeks. Infants that did not attend the 5-month visit were not included in the extrapolation.

Weekly percentiles from 45 to 64 weeks postmenstrual age were constructed based on available growth and body composition measures. Percentiles were calculated using the "gamlss" function of the R package GAMLSS version 5.3–4[32]. The generalized additive model for location, scale and shape (GAMLSS) was employed to identify optimal distribution functions, according to Generalized Information Criterion (GIC)[23,33]. This statistical analysis was performed using R, version 4.1.2 (2021-11-01; Vienna, Austria)[34]. Z scores for fat mass index and fat-free mass index were calculated for male and female EBF infants using the optimal distributions identified through GAMLSS. Z scores for WFL, BMI, and MUAC were calculated using the macro "WHO Child Growth Standards SPSS Syntax File (igrowup.sps)." Linear regression models were used in SPSS to examine the association between the body composition z scores and MUAC, as well as WFL, BMI, and MUAC z scores at corresponding time-points. MUAC z score could only be computed for infants after they had reached 3 months of age.

The longitudinal effects of EBF vs PBF on growth and body composition were assessed using linear mixed modeling in R (package lme4, version 1.1–26)[35]. A model was constructed for each anthropometric and body composition outcome, and these models included postmenstrual age, sex, and birth weight as fixed effects due to their influence on growth and body composition, and participant as a random effect in order to control for repeated sampling. For all analyses, a p-value of < 0.05 was considered significant.

RESULTS

Study cohort

Of the 256 infants enrolled in the Baby & Mi study, 225 completed at least one ADP measurement during the study period for a total of 433 measurements. Reasons for infants not completing the ADP measurements at each visit are shown in Supplementary Table 1 but were most often related to parents declining this component of the visit. 45 ADP measures (from 27 infants) were excluded from this analysis for the following reasons: infant exposure to maternal smoking ($n = 9$), post-term birth ($n = 3$), weaning prior to the study visit ($n = 13$), perinatal morbidities including a stay in the NICU extending beyond 24 h or missing birth data ($n = 19$), and machine error in the ADP measurement ($n = 1$). The flowchart of participant inclusion and exclusion is shown in Supplementary Fig. 1. Of the remaining 388 measurements, 347 were collected from infants that were EBF ($n = 187$) up to 4 months of age, thus meeting the WHO criteria, and an additional 30 measurements were extrapolated at the 5-month visit as described in the methods section. All infants that were included were born at term (37–<42 weeks gestation). Demographic characteristics of these infants are shown in Table 1.

Table 1. Demographics of male and female infants who met the WHO MGRS criteria.

	All ($n = 187$)	Males ($n = 86$)	Females ($n = 101$)
Maternal age (years)	32.3 (3.3)	32.4 (3.3)	32.3 (3.2)
Maternal pre-pregnancy BMI (kg/m²)	24.0 (4.3)	24.2 (3.9)	23.8 (4.7)
Parity	1.0 (0.9)	0.9 (0.8)	1.0 (0.9)
Gestational age at birth (weeks)	40.1 (1.0)	40.0 (1.1)	40.1 (0.9)
Infant Ethnicity			
White	147 (78.6%)	64 (74.4%)	83 (82.2%)
Asian	2 (1.1%)	2 (2.3%)	0
Black	1 (0.5%)	0	1 (1.0%)
Hispanic	1 (0.5%)	0	1 (1.0%)
Other (Multi-ethnic)	9 (4.8%)	5 (5.8%)	4 (4.0%)
Unknown	27 (14.4%)	15 (17.4%)	12 (11.9%)
Birth weight (g)	3618 (420)	3684 (428)	3562 (408)
Birth length (cm)	51.9 (2.5)	52.5 (2.6)	51.4 (2.4)
Birth head circumference (cm)	35.1 (1.3)	35.5 (1.2)	34.8 (1.2)
Birth BMI (kg/m²)	13.4 (1.3)	13.4 (1.2)	13.5 (1.4)
Maternal Education			
Highschool/ secondary school	5 (2.7%)	1 (1.2%)	4 (4.0%)
College diploma	28 (15.0%)	14 (16.3%)	14 (13.9%)
Postsecondary apprenticeship or training certificate	3 (1.6%)	1 (1.2%)	2 (2.0%)
University undergraduate degree	64 (34.2%)	30 (34.9%)	34 (33.7%)
Graduate degree	49 (26.2%)	20 (23.3%)	29 (28.7%)
Postgraduate degree	11 (5.9%)	6 (7.0%)	5 (5.0%)
Unknown	27 (14.4%)	14 (16.3%)	13 (12.9%)
Household Income			
\$25,000 to \$49,999	9 (4.8%)	3 (3.5%)	6 (5.9%)
\$50,000 to \$74,999	13 (7.0%)	3 (3.5%)	10 (9.9%)
\$75,000 to \$99,999	33 (17.6%)	11 (12.8%)	22 (21.8%)
\$100,000 to \$124,999	33 (17.6%)	18 (20.9%)	15 (14.9%)
\$125,000 or more	72 (38.5%)	37 (43.0%)	35 (34.7%)
Unknown	27 (14.4%)	14 (16.3%)	13 (12.9%)

[a]Values are presented as mean (standard deviation) for continuous variables and N (%) for categorical variables.

Development of body composition percentiles

Body composition trajectories were developed from 187 infants with a total of 377 ADP measurements. As noted in Fig. 1, sex-specific weight and length percentile curves constructed with data from the EBF infants were comparable to the 2006 WHO child growth curves. Anthropometric and body composition data for male and female EBF infants included in the curve development are presented in Supplementary Table 2 and described below.

As expected, weight (kg) was significantly higher in males compared to females at 6 weeks (5.5 (0.5) vs 5.0 (0.6)), 12 weeks (6.4 (0.6) vs 5.8 (0.6)), and 5 months (7.7 (0.7) vs 6.9 (0.7)). Similar findings were observed when comparing length (cm) between male and female infants at 6 weeks (57.8 (1.9) vs. 55.8 (2.0)), 12 weeks (61.5 (2.1) and 59.6 (1.7)), and 5 months (66.3 (2.0) vs 64.2 (2.2)). BMI (kg/m^2) was higher in males vs females at 12 weeks (17.0 (1.4) vs 16.3 (1.5)) and 5 months of age (17.5 (1.5) vs 16.6 (1.4)). Male and female body composition percentile curves were constructed over postmenstrual age for body fat percentage, fat mass, fat mass index, fat-free mass percentage, fat-free mass, and fat-free mass index from 6 weeks to 5 months of age, or 45 weeks to 64 weeks postmenstrual age, as shown in Fig. 2. Body fat percentage and fat mass index were similar in males and females, while fat mass was higher in males at 12 weeks and 5 months.

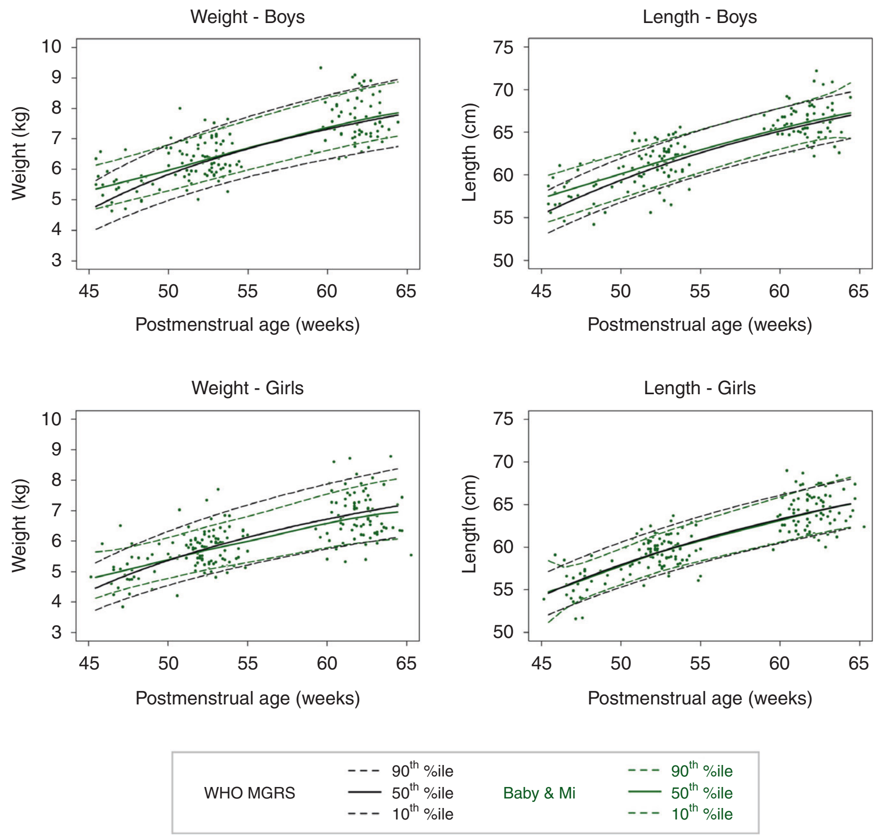

Fig. 1 Percentile curves for weight and length in male and female infants, across postmenstrual age, relative to WHO reference data. Black indicates the WHO growth standards and green indicates the subset of EBF infants from the Baby & Mi study.

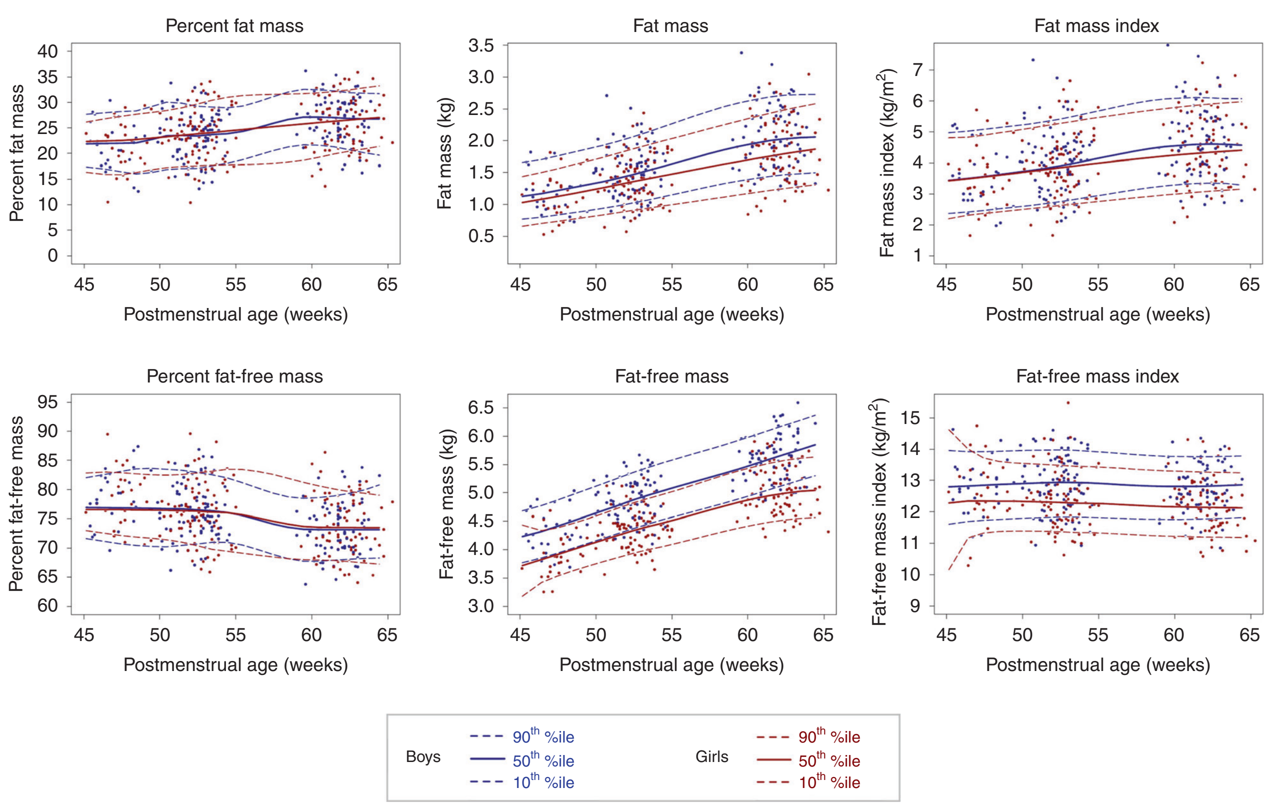

Fig. 2 Percentile curves for body composition in male and female infants. Males are depicted in blue and females are depicted in red.

Average fat-free mass was significantly lower in females across all visits and fat-free mass index was lower in females at 12 weeks and 5 months of age. Males demonstrated increases in fat-free mass of 0.11 kg/week from 6 to 12 weeks and 0.08 kg/week from 12 weeks to 5 months, whereas females accrued fat-free mass at an average rate of 0.09/week and 0.07 kg/week, respectively. In male infants, fat-free mass index was relatively stable and on average increased from 12.84 kg/m^2 at 6 weeks to 12.93 kg/m^2 at 12 weeks, before decreasing to 12.78 kg/m^2 at 5 months, while it declined over time in female infants from 12.56 kg/m^2 to 12.19 kg/m^2 between 6 weeks and 5 months.

It is important to note that the curves depicted in Fig. 2 include extrapolated values at the 5-month timepoint for 30 infants. In total, 44 infants did not complete the ADP measurement at 5 months because they exceeded the machine's maximum capacity (Supplementary Table 1). Of these 44 infants, 30 infants met the WHO criteria and had completed a previous ADP measurement at 12 weeks, enabling extrapolation of their body composition at 5 months based on previous measurements, as described in the Methods section and shown in Supplementary Fig. 2.

Comparison of BMI, WFL, and MUAC to body composition outcomes

In EBF infants, both BMI and WFL z scores at 6 weeks, 12 weeks, and 5 months of age, were associated with fat mass index and fat-free mass index z scores at corresponding time-points (Table 2). While MUAC and MUAC z score were significantly associated with fat mass index z scores, very weak correlations were found between fat-free mass index and MUAC and MUAC z score. Compared to the other anthropometric measures, BMI z score was found to explain a greater percentage of the variance in fat mass index z scores at every time-point (55–68%). Changes in BMI z score were also more closely predictive of positive changes in fat mass index, as evidenced by the generally higher regression coefficients (0.76–0.85), further suggesting that BMI z score is more closely related to fat mass index than other metrics. Conversely, the variance explained by WFL z score for fat-free mass index z score was slightly greater than the variance explained by BMI z score at 12 weeks and 5 months, although the regression coefficients were lower at 6 and 12 weeks.

Comparison between EBF and PBF infants

Though most of the study participants in this cohort met criteria for EBF, 28 infants were classified as PBF during the study period. A total of 41 ADP measurements were collected from PBF infants; 34 measurements were from infants that had received breastmilk and formula prior to 4 months of age and 7 measurements were from infants receiving solids foods. We compared the body composition measures for infants that were PBF to those that were EBF (Table 3). Independent of birth weight, postmenstrual age, and sex, EBF infants had higher weight ($p = 0.01$), but not length ($p = 0.17$). Consistent with previous studies, EBF infants had higher adiposity, including total body fat percentage, fat mass, and fat mass index compared to PBF infants ($p < 0.01$). No significant differences in fat-free mass were noted.

DISCUSSION

Body composition in infancy is an important predictive factor of subsequent growth patterns and metabolic health, but normative data has been scarcely available. While length, weight, and head circumference growth curves for a population based on a growth standard (term born, healthy, breastfed infants not exposed to maternal smoking) collected longitudinally are available, comparable curves for body composition of these healthy infants are lacking. In this study, we explored growth and body composition in infancy by generating age and sex-specific body composition growth curves for a cohort of healthy, breastfed, full term infants that adhere to the eligibility requirements of the WHO child growth standards.

Consistent with sex-based anthropometric differences, sex differences in body composition were also evident. Fat-free mass was significantly lower in females compared to males at all time-points, consistent with previous studies[15,18,20]. Furthermore, fat-free mass index, which accounts for differences in length, was also lower in females at 12 weeks and 5 months of age. While fat-free mass index was generally stable in male infants, increasing slightly

Table 2. Association between WHO z scores and body composition indices z scores at 6 weeks, 12 weeks, and 5 months.

	Fat mass index Z score			Fat-free mass index Z score		
	Regression coefficient	**95% Confidence interval**	R^2	**Regression coefficient**	**95% Confidence interval**	R^2
	6 Weeks					
WFL Z Score (***n*** = 53)	0.56	0.37–0.76	0.40	0.62	0.46–0.78	0.55
BMI Z Score (***n*** = 53)	0.76	0.57–0.95	0.55	0.71	0.53–0.89	0.55
MUAC (***n*** = 53)	0.52	0.28–0.76	0.27	0.14	−0.12–0.40	0.02*
MUAC Z Score (***n*** = 0)						
	12 Weeks					
WFL Z Score (***n*** = 170)	0.69	0.58–0.80	0.49	0.71	0.60–0.83	0.49
BMI Z Score (***n*** = 170)	0.81	0.70–0.92	0.57	0.76	0.63–0.88	0.46
MUAC (***n*** = 170)	0.71	0.58–0.84	0.41	0.24	0.06–0.41	0.04
MUAC Z Score (***n*** = 26)	0.83	0.50–1.17	0.52	0.18	−0.30–0.66	0.03*
	5 Months					
WFL Z Score (***n*** = 154)	0.83	0.74–0.93	0.67	0.67	0.55–0.79	0.46
BMI Z Score (***n*** = 154)	0.85	0.75–0.94	0.68	0.67	0.55–0.79	0.44
MUAC (***n*** = 154)	0.61	0.50–0.72	0.43	0.19	0.04–0.33	0.04
MUAC Z Score (***n*** = 154)	0.74	0.61–0.87	0.46	0.23	0.06–0.40	0.05

[a] Results of the univariate regression models are presented as the unstandardized regression co-efficient, its 95% confidence interval, and the coefficient of determination (R^2). All results were significant ($p < 0.05$), except for univariate regressions between 6 week MUAC and fat-free mass index z score, and 12 week MUAC z score and fat-free mass index z score, as indicated by *($p > 0.05$).

Table 3. Multivariate analysis of predictors of growth and body composition metrics, including: feeding mode, sex, postmenstrual age, and birth weight.

	Feeding mode (EBF)	Sex (Male)	Postmenstrual age (weeks)	Birth weight (kg)
Weight (kg)	0.23 (0.05–0.41)*	0.63 (0.47–0.80)†	0.13 (0.12–0.13)†	0.48 (0.29–0.68)†
Length (cm)	0.44 (−0.18–1.05)	1.94 (1.44–2.44)†	0.53 (0.51–0.55)†	1.40 (0.80–2.00)†
% Body fat	2.61 (1.22–4.01)†	−0.42 (−1.66–0.82)	0.33 (0.29–0.37)†	0.50 (−0.97–1.98)
Fat mass (kg)	0.24 (0.12–0.37)†	0.13 (0.02–0.25)*	0.05 (0.05–0.06)†	0.14 (0.006–0.27)*
Fat mass index (kg/m^2)	0.50 (0.20–0.80)**	0.08 (−0.20–0.36)	0.07 (0.06–0.08)†	0.21 (−0.12–0.54)
Fat-free mass (kg)	0.0006 (−0.11–0.11)	0.50 (0.40–0.60)†	0.08 (0.07–0.08)†	0.35 (0.23–0.46)†
Fat-free mass index (kg/m2)	−0.04 (−0.33–0.25)	0.51 (0.31–0.72)†	−0.02 (−0.03–(−0.005))**	0.34 (0.09–0.58)**

$p < 0.05$*, $p < 0.01$**, $p < 0.001$† [a] Regression data are presented as regression co-efficient estimate (95% confidence interval). [b] Values for feeding mode are expressed as the adjusted difference between EBF infants compared to PBF infants, with positive values indicating higher growth metrics in EBF infants and negative values indicating lower growth metrics in EBF compared to PBF infants. [c] Values for sex are expressed as the adjusted difference between male infants compared to female infants.

between 6 and 12 weeks, and decreasing between 12 weeks and 5 months, females had declining fat-free mass index over time. These early differences in growth patterns between sexes may be related to the hormonal changes that occur within the first few months of life, the so-called "minipuberty"[36]. This involves the transient re-activation of the hypothalamic-pituitary-gonadal (HPG) axis and elevation of gonadotrophin levels, resulting in increased gonadal hormone production[36]. Kiviranta and colleagues (2016) explored the relationship between linear growth velocity and infant sex steroid levels and found significant positive correlations between testosterone levels, which were higher in male infants throughout the study period, and concurrent linear growth velocity during the first 5 months of life[37]. Body composition was not measured by Kiviranta et al., but differences in linear growth velocity may be related to the greater fat-free mass observed in male infants. Maximal sex-based differences in growth velocity in that study were observed within the first 2 months of life, which overlaps with the period of peak HPG axis activity and testosterone levels[37]. Perhaps the slight decline noted in fat-free mass index in our cohort from 12 weeks to 5 months is related to the fall in testosterone levels following the postnatal surge. Thus, the physiological mechanisms underlying sex differences in body composition and their implications for later health outcomes need to be further explored.

To date, two other studies with sample sizes of 59 and 160 infants and conducted in Italy by Roggero et al. and in the United States by Fields et al., respectively, have reported longitudinal body composition data to 6 months of age using ADP for a cohort of exclusively breastfed, term infants whose key criteria for inclusion were based on the WHO MGRS[38,39]. At around 3 months, our values for average body fat percentage were notably lower than both Roggero et al. and Fields et al., which reported greater fat mass and less fat-free mass[38,39]. At 5 months, body fat percentage was more similar to Fields et al., while average body weight, specifically fat mass in males and fat-free mass in males and females, were higher in our cohort compared to both studies. Recently, these two studies were pooled to generate postnatal body composition percentiles to 27 weeks of age[40]. These authors did not present the comparability of the length and weight measurements to the WHO growth standard. This is an important consideration since these previous studies included smaller sample sizes for measurements at 5 and 6 months due to loss to follow-up. Further, as with our study, these studies identified a proportion of babies whose weight exceeded 8 kg. Infants exceeding the PEAPOD weight limit were left out of the curve development in the pooled study, resulting in body composition curves that did not include the heavier babies. The weight limitation of the ADP machine hinders longitudinal studies by preventing the assessment of larger infants at 5–6 months of age, resulting in the under representation of infants with higher weights at these time-points[41]. In constructing the body composition percentiles of EBF infants meeting the WHO growth standards criteria, the present study included extrapolated body composition measurements for 30 infants exceeding the size capacity of the ADP device, and presents median length and weight curves that are similar in shape and trajectory to the WHO growth standards.

As body composition measurement is not feasible in all clinical environments, BMI, WFL, and MUAC have been used as alternative measures – though the relative strength of the relationship of these to fat mass and fat-free mass is uncertain. BMI is used to evaluate the weights of individuals of difference sizes; however, fluctuations in the 50th percentile for BMI are observed throughout infancy, and in adulthood, a normal BMI can range from 18.5 to 24.9 kg/m^2. Similarly, while fat mass over height squared and fat-free mass over height squared have been proposed as height-normalized indices for body composition[42], their role in adjusting for differences in infant size and capturing infant health risk remains unclear. That is why the utilization of age-and sex-specific z scores for body composition are needed, and their relationship with anthropometric measures require exploration. Here, we have demonstrated that BMI z score is more closely related to the fat mass index z score from 6 week to 5 months of age compared to MUAC, MUAC z score, and WFL z score. Similar findings have been reported when comparing BMI and WFL z scores in 293 infants at 1 month of age[43]. Infant BMI z score has also been shown to be a stronger indicator of obesity risk in children[9,44]. Together, these findings suggest that BMI z score may offer greater utility in clinical settings as an estimation of adiposity than WFL, MUAC, or the MUAC z score. In contrast, WFL z score was more tightly correlated to fat-free mass index than BMI z score at 12 weeks and 5 months. Meanwhile, MUAC and MUAC z score were found to be stronger correlates of fat mass than fat-free mass, as previously reported by others[45–47].

In the sub-analysis in which we compared measurements from EBF infants that met the WHO MGRS criteria to infants who met the criteria except that they were PBF by 4 months, differences in body composition were identified. On average, weight, body fat percentage, fat mass, and fat mass index were significantly higher in EBF infants compared to PBF infants after controlling for the effects of postmenstrual age, sex, and birthweight. This finding is consistent with other studies that have reported higher fat mass in exclusively/predominantly breastfed infants compared to formula-fed infants or infants receiving mixed feeds from 3–6 months of age[48,49]. While breastfeeding appears to be related to higher fat mass up to 6 months of age, formula-feeding has been associated with higher fat mass at 1 year[48], as well as an increased risk of overweight and obesity from childhood to early adulthood[50].

Conversely, breastfeeding is believed to exert a protective effect against obesity[51,52]. These findings highlight the importance of body composition measurement and underscore the need for further research to explore whether the effects of infant feeding on later obesity risk are related to body composition differences in infancy.

While this study reports longitudinal sampling of a cohort of 187 infants for the construction of body composition trajectories, there are some limitations. Firstly, the limited number of timepoints for growth assessment, the low rate of ADP completion at the first timepoint, and the separate assessment of body composition for male and female infants, reduce the amount of data available for analysis. This small sample size is partially compensated for by use of the GAMLSS method, which allows for the construction of percentile data from smaller cohorts, but larger sample sizes and more frequent sampling would have led to greater accuracy of the reference body composition curves appropriate for clinical use, similar to the construction of the WHO growth standards. The use of extrapolation in this study allowed for the inclusion of larger infants for analysis, removing the systematic bias presented by using the ADP device, and thereby enabling presentation of a more comprehensive distribution of infant weight and body composition measures at 5 months. However, this did require extrapolation based on infant size and as such may have introduced some uncertainty compared to actual values. Additionally, while ADP is considered a valuable tool for non-invasive body composition assessment, reporting satisfactory reproducibility and accuracy in body fat percent measures in full term infants, concerns have been raised regarding its estimation of body composition in leaner individuals, prompting the need for additional study[53]. Furthermore, the applicability of this data to other environments and populations is uncertain. While the infants included in our study were predominantly White, it is important to note that ethnic differences in body composition have previously been reported, with studies of multi-ethnic cohorts identifying differences in the amount and distribution of fat mass between neonates and infants of different racial/ethnic backgrounds[54,55]. Other cohort characteristics not discussed in this analysis, including maternal parity, obesity, and other maternal metabolic factors and disorders, may also influence infant growth and body composition, and so research on larger cohorts accounting for these factors are needed[56]. Finally, as discussed throughout this paper, the relationship between infant body composition trajectories and clinical outcomes in later life are still under investigation, so the utility of this data in clinical settings for the evaluation of infant growth requires further exploration.

In conclusion, data generated from this study provide age and sex-specific body composition percentile curves from 6 weeks to 5 months of age for infants that meet the inclusion criteria of the WHO MGRS. Notably, our study population reflects similar median weight and length percentiles to WHO growth standards. Extrapolation enabled inclusion of heavier infants with weight exceeding the ADP machine's upper limit—one of the key limitations in the prior literature. These curves will help facilitate future studies aimed at understanding the relationship of early life body composition and subsequent health outcomes. Further, we have demonstrated that BMI z score is more closely related to fat mass index z score than MUAC, WFL z score or MUAC z score at study timepoints.

REFERENCES

1. Inadera, H. Developmental origins of obesity and type 2 diabetes: molecular aspects and role of chemicals. *Environ. Health Prev. Med* **18**, 185–197 (2013).
2. Dubois, L. & Girard, M. Early determinants of overweight at 4.5 years in a population-based longitudinal study. *Int J. Obes.* **30**, 610–617 (2006).
3. Taveras, E. M. et al. Weight status in the first 6 months of life and obesity at 3 years of age. *Pediatrics* **123**, 1177–1183 (2009).
4. Stettler, N., Kumanyika, S. K., Katz, S. H., Zemel, B. S. & Stallings, V. A. Rapid weight gain during infancy and obesity in young adulthood in a cohort of African Americans. *Am. J. Clin. Nutr.* **77**, 1374–1378 (2003).
5. Stettler, N. et al. Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula. *Circulation* **111**, 1897–1903 (2005).
6. Chomtho, S. et al. Infant growth and later body composition: evidence from the 4-component model. *Am. J. Clin. Nutr.* **87**, 1776–1784 (2008).
7. Chandler-Laney, P. C., Gower, B. A. & Fields, D. A. Gestational and early life influences on Infant body composition at 1 year. *Obesity* **21**, 144–148 (2013).
8. Ekelund, U. et al. Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm weight development study (Swedes). *Am. J. Clin. Nutr.* **83**, 324–330 (2006).
9. Smego, A. et al. High body mass index in infancy may predict severe obesity in early childhood. *J. Pediatr.* **183**, 87–93 e81 (2017).
10. Briend, A., Maire, B., Fontaine, O. & Garenne, M. Mid-upper arm circumference and weight-for-height to identify high-risk malnourished under-five children. *Matern Child Nutr.* **8**, 130–133 (2012).
11. Chaput, J. P. et al. Mid-upper arm circumference as a screening tool for identifying children with obesity: a 12-country study. *Pediatr. Obes.* **12**, 439–445 (2017).
12. Jaiswal, M., Bansal, R. & Agarwal, A. Role of mid-upper arm circumference for determining overweight and obesity in children and adolescents. *J. Clin. Diagn. Res* **11**, SC05–SC08 (2017).
13. Koontz, M. B., Gunzler, D. D., Presley, L. & Catalano, P. M. Longitudinal changes in infant body composition: association with childhood obesity. *Pediatr. Obes.* **9**, e141–e144 (2014).
14. Breij, L. M. et al. Impact of early infant growth, duration of breastfeeding and maternal factors on total body fat mass and visceral fat at 3 and 6 months of age. *Ann. Nutr. Metab.* **71**, 203–210 (2017).
15. Butte, N. F., Hopkinson, J. M., Wong, W. W., Smith, E. O. & Ellis, K. J. Body composition during the first 2 years of life: an updated reference. *Pediatr. Res* **47**, 578–585 (2000).
16. Eriksson, B., Lof, M. & Forsum, E. Body composition in full-term healthy infants measured with air displacement plethysmography at 1 and 12 weeks of age. *Acta Paediatr.* **99**, 563–568 (2010).
17. Olhager, E., Flinke, E., Hannerstad, U. & Forsum, E. Studies on human body composition during the first 4 months of life using magnetic resonance imaging and isotope dilution. *Pediatr. Res* **54**, 906–912 (2003).
18. Carberry, A. E., Colditz, P. B. & Lingwood, B. E. Body composition from birth to 4.5 months in infants born to non-obese women. *Pediatr. Res* **68**, 84–88 (2010).
19. Fomon, S. J. & Nelson, S. E. Body composition of the male and female reference infants. *Annu. Rev. Nutr.* **22**, 1–17 (2002).
20. de Fluiter, K. S. et al. Longitudinal body composition assessment in healthy term-born infants until 2 years of age using Adp and Dxa with vacuum cushion. *Eur. J. Clin. Nutr.* **74**, 642–650 (2020).
21. Andersen, G. S. et al. Body composition from birth to 6 mo of age in Ethiopian infants: reference data obtained by air-displacement plethysmography. *Am. J. Clin. Nutr.* **98**, 885–894 (2013).
22. de Onis, M. et al. The WHO multicentre growth reference study: planning, study design, and methodology. *Food Nutr. Bull.* **25**, S15–S26 (2004).
23. WHO Multicentre Growth Reference Study Group. WHO child growth standards based on length/height, weight and age. *Acta Paediatr. Suppl.* **450**, 76–85 (2006).
24. Valérie, M. Promoting optimal monitoring of child growth in Canada: using the new World Health Organization growth charts – executive summary. *Paediatr Child Health* **15**, 77–79 (2010).
25. Simioni, J. et al. A comparison of intestinal microbiota in a population of low-risk infants exposed and not exposed to intrapartum antibiotics: The Baby & Microbiota of the Intestine Cohort Study Protocol. *BMC pediatrics* **16**, 183 (2016).
26. Ellis, K. J. et al. Body-composition assessment in infancy: air-displacement plethysmography compared with a reference 4-compartment model. *Am. J. Clin. Nutr.* **85**, 90–95 (2007).
27. Dewey, K. G., Cohen, R. J., Nommsen-Rivers, L. A. & Heinig, M. J. Implementation of the WHO multicentre growth reference study in the United States. *Food Nutr. Bull.* **25**, S84–S89 (2004).
28. WHO Multicentre Growth Reference Study Group Breastfeeding in the WHO multicentre growth reference study. *Acta Paediatr. Suppl.* **450**, 16–26 (2006).
29. Emmett, P. Dietary assessment in the avon longitudinal study of parents and children. *Eur. J. Clin. Nutr.* **63**, S38–S44 (2009).
30. Fomon, S. J., Haschke, F., Ziegler, E. E. & Nelson, S. E. Body composition of reference children from birth to age 10 years. *Am. J. Clin. Nutr.* **35**, 1169–1175 (1982).
31. Timmerman, M. E., Voncken, L. & Albers, C. J. A tutorial on regression-based norming of psychological tests with Gamlss. *Psychol. Methods* **26**, 357–373 (2021).
32. Rigby, R. A. & Stasinopoulos, D. M. Generalized additive models for location, scale and shape. *J. R. Stat. Soc.: Ser. C* **54**, 507–554 (2005).

33. Rigby, R. A. & Stasinopoulos, D. M. Automatic smoothing parameter selection in Gamlss with an application to centile estimation. *Stat. Methods Med. Res.* **23**, 318–332 (2014).
34. Team, R. C. R.: a language and environment for statistical computing computer program, Version 3.6. 1. (2019).
35. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using Lme4. *J. Stat. Softw.* **67**, 1–48 (2015).
36. Kuiri-Hanninen, T., Sankilampi, U. & Dunkel, L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. *Horm. Res Paediatr.* **82**, 73–80 (2014).
37. Kiviranta, P. et al. Transient postnatal gonadal activation and growth velocity in infancy. *Pediatrics* **138** (2016).
38. Roggero, P. et al. Quality of growth in exclusively breast-fed infants in the first six months of life: an Italian study. *Pediatr. Res.* **68**, 542–544 (2010).
39. Fields, D. A. et al. Longitudinal body composition data in exclusively breast-fed infants: a multicenter study. *Obesity* **19**, 1887–1891 (2011).
40. Norris, T. et al. New charts for the assessment of body composition, according to air-displacement plethysmography, at birth and across the first 6 mo of life. *Am. J. Clin. Nutr.* **109**, 1353–1360 (2019).
41. Hamatschek, C. et al. Fat and fat-free mass of preterm and term infants from birth to six months: a review of current evidence. *Nutrients* **12**, 288–297 (2020).
42. VanItallie, T. B., Yang, M. U., Heymsfield, S. B., Funk, R. C. & Boileau, R. A. Height-normalized indices of the body's fat-free mass and fat mass: potentially useful indicators of nutritional status. *Am. J. Clin. Nutr.* **52**, 953–959 (1990).
43. Roy, S. M. et al. Body mass index is a better indicator of body composition than weight-for-length at age 1 month. *J. Pediatr.* **204**, 77–83 e71 (2019).
44. Roy, S. M. et al. Infant BMI or weight-for-length and obesity risk in early childhood. *Pediatrics* **137**, e20153492 (2016).
45. Chomtho, S., Fewtrell, M. S., Jaffe, A., Williams, J. E. & Wells, J. C. Evaluation of arm anthropometry for assessing pediatric body composition: evidence from healthy and sick children. *Pediatr. Res* **59**, 860–865 (2006).
46. Grijalva-Eternod, C. S. et al. Midupper arm circumference and weight-for-length Z scores have different associations with body composition: evidence from a cohort of Ethiopian infants. *Am. J. Clin. Nutr.* **102**, 593–599 (2015).
47. Jensen, S. M. et al. Validity of anthropometric measurements to assess body composition including muscle mass in 3-year-old children from the SKOT cohort. Matern. *Child Nutr.* **11**, 398–408 (2015).
48. Gale, C. et al. Effect of breastfeeding compared with formula feeding on infant body composition: a systematic review and meta-analysis. *Am. J. Clin. Nutr.* **95**, 656–669 (2012).
49. Rodríguez-Cano, A. M., Mier-Cabrera, J., Allegre-Dávalos, A. L., Muñoz-Manrique, C. & Perichart-Perera, O. Higher fat mass and fat mass accretion during the first six months of life in exclusively breastfed infants. *Pediatr. Res.* **87**, 588–594 (2020).
50. Oddy, W. H. et al. Early infant feeding and adiposity risk: from infancy to adulthood. *Ann. Nutr. Metab.* **64**, 262–270 (2014).
51. Bernardo, Horta, Cesar, Victora & World Health Organization. Long-term effects of breastfeeding: a systematic review. World Health Organization. https://apps.who.int/iris/handle/10665/79198. (2013). accessed 11 March 2022.
52. Patro-Gołąb, B. et al. Nutritional interventions or exposures in infants and children aged up to 3 years and their effects on subsequent risk of overweight, obesity and body fat: a systematic review of systematic reviews. *Obes. Rev.* **17**, 1245–1257 (2016).
53. Mazahery, H., von Hurst, P. R., McKinlay, C. J. D., Cormack, B. E. & Conlon, C. A. Air displacement plethysmography (Pea Pod) in full-term and pre-term infants: a comprehensive review of accuracy, reproducibility, and practical challenges. *Matern Health Neonatol. Perinatol.* **4**, 12 (2018).
54. Paley, C. et al. Body fat differences by self-reported race/ethnicity in healthy term newborns. *Pediatr. Obes.* **11**, 361–368 (2016).
55. Stanfield, K. M., Wells, J. C., Fewtrell, M. S., Frost, C. & Leon, D. A. Differences in body composition between infants of South Asian and European Ancestry: The London Mother and Baby Study. *Int. J. Epidemiol.* **41**, 1409–1418 (2012).
56. Herath, M. P. et al. Determinants of infant adiposity across the first 6 months of life: evidence from the baby-bod study. *J. Clin. Med.* **10**, 1770–1783 (2021).

ACKNOWLEDGEMENTS
We would like to thank participating parents for their contribution to this study, as well as the Baby & Mi research team and participating midwifery practices.

AUTHOR CONTRIBUTIONS
The authors' contributions to the study were as follows—E.K.H., K.M.M., N.R. and E.I.Y.: designed the research and oversaw all tasks; E.G., J.L., J.S. and E.I.Y.: acquired the data; N.R. and E.I.Y.: analyzed the data; N.R. and E.I.Y.: wrote the paper and had primary responsibility for the final content; and all authors: edited and approved the final manuscript.

FUNDING
The study was supported by the Hamilton Academic Health Sciences Organization AFP Innovation Fund; and Canadian Institutes of Health Research (MOP-136811 [to EKH and KMM]).

CONSENT STATEMENT
Mothers provided written, informed consent for their engagement in the study at the time of enrollment and legal guardians provided written, informed consent for their child.

COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41390-022-02005-y.

Correspondence and requests for materials should be addressed to Katherine M. Morrison.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.