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Normative data for Lean Mass and Fat Mass in Healthy predominantly Breast-fed Term Infants from 1 month to 1 Year of Age

Olusola F. Sotunde[1], Sina Gallo[2] Catherine A. Vanstone[1] and Hope A. Weiler[1*]

[1]School of Human Nutrition, McGill University, 21111 Lakeshore Road, Ste-Anne-de-Bellevue, QC, H9X 3V9 Canada

[2]Nutrition and Food Studies, George Mason University Fairfax, VA USA 22030

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Running title: Body composition reference data in infancy

***Corresponding author**: Hope A. Weiler. School of Human Nutrition, McGill University, 21111 Lakeshore Road, Ste-Anne-de-Bellevue, QC, H9X 3V9 Canada. Tel: +1-514-398-7905

ABSTRACT

Background: A leaner body phenotype in infancy plays an important role in the early life prevention of obesity. However, there is a dearth of reference data for body composition in infancy. This study aimed to create a normative reference dataset for lean (LM) and fat (FM) mass and accretion rates in healthy infants.

Methods: Healthy term-born infants (35 boys; 35 girls) were studied at ≤ 1, 3, 6, 9 and 12 mo of age for growth and compared to World Health Organization (WHO) standards. LM (g) and FM (g) were measured using DXA (APEX version 13.3:3, Hologic 4500A) in infant whole-body mode. Sex specific reference curves were generated using the LMS method (LMSchartmaker©, Medical Research Council, UK).

Results: Infants were predominantly white (82.9%), breastfed (98.4% ≥ 3 mo) and grew in length and weight within WHO Z-score ranges for normal growth across infancy. LM accretion was 327.4 ± 12.5 g/mo representing 95% increment in LM. Boys had more LM compared to girls at 12 mo (7807.4 ± 1114.0 vs 6817.4 ± 1016.1 g; p = 0.008). FM accretion was 114.3 ± 12.0 g/mo representing 114% increment in FM with no difference between the sexes.

Conclusions: This data, which is based on a healthy sample of infants, characterises LM and FM accretion during the first year of life and will aid in the interpretation of body composition.

Introduction

Once established, obesity is often persistent and increases the risk of chronic diseases (1). Infants with body mass index (BMI) values exceeding the 85th percentile of the World Health Organization (WHO) growth standards at 6, 12 or 18 months have a two-fold increase risk of obesity at age six years (2). Emerging evidence shows that a leaner body phenotype in infancy plays an important role in the early life prevention of obesity (3-5). The Institute of Medicine (IOM) has identified infancy as a critical time for obesity prevention and has developed infancy-related intervention strategies (6). The early infancy period (0 to 6 months) appears to be a sensitive period within which rapid infant weight gain elevates later obesity risk (7).

Developmental programming during the “critical window” of early infancy can be studied with the aid of longitudinal measures of body composition (8). Growth monitoring is fundamental to the assessment of nutritional status and is used to detect individual abnormalities in growth trajectory in clinical settings, and also to understand trends in child growth as related to public health (9). To this effect, weight-for-height, or BMI-for-age compared to WHO reference datasets, has been used as an index of body composition for decades (10). Limitations, however, include the masking of alterations in body composition as children could have normal BMI despite a higher fat mass (FM) and a lower lean mass (LM) (11). Few reference data on body composition in infants exist and most of the existing studies used air-displacement plethysmography (ADP) to assess FM and fat free mass (FFM) (12, 13), which is not designed for infants who are 6 to 12 months (mo) of age (14). In recent years, dual-energy X-ray absorptiometry (DXA) has provided further clarity on body composition in older pediatric populations

(15, 16). DXA utilises a three-compartment model to assess body composition using the underlying principle that fat, lean and bone tissue attenuate X-rays differently (17, 18). The overall minimal X-ray exposure of DXA in comparison to other procedures involving X-ray methodology has made it an effective method of assessment in several studies in children (18, 19). One of the major advantages of DXA over ADP is its ability to delineate muscle from bone (18). Nevertheless, the use of DXA to assess body composition in infants is still relatively minimal (19) and there is a dearth of reference data for LM in infancy. Hence, the objective of this study is to create LM and FM normative reference data sets for infants from the neonatal period up to 12 mo of age.

Methods

Study design and subjects

This is a secondary analysis of data from a published vitamin D randomized dose-response trial (NCT00381914) in infants (20). Briefly, healthy, term (36-42 weeks) born, singleton, appropriate size for age (5-95th percentile based on Centers for Disease Control and Prevention growth charts), born to healthy mothers (no gestational diabetes, hypertension in pregnancy, chronic alcohol use, or malabsorption syndromes) and breastfeeding infants were recruited ≤1 mo of age from Montréal, Québec Canada between March 2007 and August 2010. The infants were followed at 3, 6, 9 and 12 mo of age. The total cohort included 132 infants but, included in this study were infants with vitamin D sufficiency at baseline, defined as plasma 25-hydroxyvitamin D [25(OH)D] values of ≥ 50 nmol/L (21) as a proxy to further describe healthy nutrition status (n = 70). The 70 infants included in this study were randomised to receive vitamin D dosages of either 400 IU/d (n= 22), 800 IU/d (n = 11), 1200 IU/d (n = 22) and 1600 IU/d (n = 9).

All nine infants on 1600 IU/d dosage were switched to the 400 IU/d standard of care at 6 to 9 mo of age (6 infants at ≤ 6 mo, and 3 infants at 9 mo of age) (20). Demographic information including education, income and race as well as infant feeding practices were self-reported by mothers at baseline. Based on self-reported parental (mother and father) race, infants were categorized as white if both parents were white; all other infants were categorized as non-white. All study visits were carried out at the Mary Emily Clinical Nutrition Research Unit of McGill University (Montréal, Québec, Canada). Ethical approval for secondary data use was obtained from the Faculty of Medicine Institutional Review Board, McGill University. Parents provided written informed consent and were compensated for travel.

Anthropometry and body composition measurements

At all study visits, nude weight was measured to the nearest gram using an electronic weighing scale (model SB 32000, Mettler-Toledo Inc., Greifensee, Switzerland). Length was measured to the nearest 0.1 cm with an infant length board (O'Learly Length Boards, Ellard Instrumentation Ltd., Seattle, Washington). Head circumference was measured using a non-stretchable tape. Weight-for-age (WAZ), height-for-age (HAZ), head circumference-for-age (HCZ) and BMI-for-age (BAZ) Z-scores were calculated using the WHO software (WHO AnthroPlus, Geneva, Switzerland).

Body composition was assessed using DXA (APEX version 13.3:3, Hologic 4500A, Discovery Series, Bedford, Massachusetts) in infant whole-body mode by an International Society for Clinical Densitometry (ISCD) Certified Bone Densitometry Technologist (CBDT). For the scan, infants wore standardised diapers and gowns with no metal clips, were swaddled in a standard cotton blanket and most infants were

asleep during scanning (no sedatives were employed). Infants were scanned in supine position to limit errors even though error obtained due to altered positioning are considered minimal (22). Scans were excluded if there were more than 2 line breaks due to movement artifact. Daily calibration of DXA was performed using a lumbar spine phantom (Hologic phantom No. 14774). The coefficient of variation for quality control measures over the course of the study were 0.327% for bone mineral density and 1% for bone mineral content. Data obtained from the infant whole-body scan included LM (g, %) and FM (g, %). Lean mass index (LMI) was calculated as LM (kg)/length^2 (m) and fat mass index (FMI) as FM (kg)/length^3 (m) (23).

Vitamin D measurements

To establish a healthy newborn cohort, infants were referred to the trial based on physician assessments and additionally, based on a non-fasted capillary blood samples which were collected by heel or finger lance and stored at -80°C until analysed for 25(OH)D concentration. Plasma 25(OH)D concentrations were measured by liquid chromatography tandem mass spectrometry (Warnex Bioanalytical Services, Laval, Quebec, Canada).(20) The intra-assay CVs for 25(OH)D was < 15% and the laboratory was certified by the Vitamin D External Quality Assessment Scheme. The measured 25(OH)D_3 concentrations of the National Institute of Standards and Technology standard reference materials (SRM 968e) were within 7.0% for level 2 and 2.5% for level 3 of the certified values. Sufficient vitamin D status was defined as plasma 25(OH)D concentration of ≥ 50 nmol/L (21).

Sample size estimation

To achieve our primary objective of describing infant body composition over the first year of life using the available sample size, the margin of error was estimated between the observed mean and the population mean. The margin of error was calculated with the equation $W = z^2_{\alpha}S/\sqrt{n}$ (24) where z^2_{α} = 95% confidence level, *S* = standard deviation of LM and FM from previously published trial and *W* = width of the margin of error. Based on respective LM and FM mean ± SD of 3.5 ± 0.4 kg and 1.1 ± 0.3 kg at 1 mo of age from Sudhagoni et al.(25) and our sample size of 70 infants; we are 95% confident that the true LM and FM mean of infants sampled in our study are within 10.3% and 6.8% respectively, of the true population mean.

Statistical analyses

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Continuous variables are presented as mean ± SD while categorical data were analysed with frequency tables and expressed as percentages. Mixed model ANOVA was used to analyse differences in anthropometry and body composition measurements between sexes and across infancy with *post hoc* Tukey-Kramer adjustments. Statistical significance was set at $p < 0.05$, after adjustment for multiple comparisons where applicable. Sex specific reference curves were generated using the LMS method (LMSchartmaker© Light version 2.54, Medical Research Council, UK) (26). The LMS method summarizes the changing distribution of measurements as a function of age, representing the median (M), coefficient of variation (S) and the skewness which is expressed as Box-Cox transformation (L) (27). The LMS method fits the three curves into cubic splines by non-linear regression (27). The degree of freedom (edf) for L, M

and S were 3, 5 and 3 respectively. LM and FM centiles were fitted using actual age at each measurement. The goodness of fit of the generated centiles were graphically examined in comparison to the raw data and by Q-Q plots.

Results

Maternal age at delivery was 32.9 ± 3.7 years, the majority (88.6 %) self-identified their race as white, 92.9% had college/university education and 64% had household income of > 75,000 CAD/annum (Table 1). Infant characteristics at birth (Table 1) reflects the inclusion criteria for eligibility into the study (20). Sex of the infants was equally distributed with similar proportion born across all four seasons (Table 1). The majority of infants (82.9%), mothers (88.6%) and fathers (85.7%) were white. All infants were receiving breast milk at baseline and 93.1% were breastfed for 6 mo or more in addition to other foods. Mean age of introduction to solid foods was 5.1 ± 0.9 mo. There was a high retention rate of ~75% infants until 12 mo follow up, while DXA scans were not available for 9 out of the 52 infants that completed the study due to movement artifact.

Infant weight and length were within WHO Z- score range for normal growth across infancy (Table 2). LM increased with a steady accretion of 327.0 ± 12.5 g/mo representing a 95% increase across infancy. No differences in LM were observed up to 9 mo between sexes, however, boys had significantly higher LM than girls at 12 mo of age (Table 2). There was a decline in LM % from ≤ 1 to 6 mo followed by an increase between 6 and 12 mo (Table 2). This was also demonstrated when LM was adjusted for length in the form of LMI. FM had a steady accretion of 114.3 ± 12.0 g/mo which represented a 114% increase across infancy with no significant differences observed between the sexes (Table 2). FM% increased from ≤ 1 to 6 mo followed by a decline

between 6 and 12 mo. When FM was adjusted for length as FMI, the decline started at 6 mo of age and continued until 12 mo of age (Table 2). There were no vitamin D supplementation dosage effects on infant body composition variables ($p > 0.149$).

Sex specific LMS-based percentiles for LM and FM ≤ 1 -12 mo are presented in Figure 1. Sex specific LMS parameters and percentile distribution are available for LM and FM in Supplementary Tables S1 – S4.

Discussion

This study provides normative data for LM and FM of infants from ≤1 to 12 mo of age using the widely applied LMS method (27). This is of great importance as differentiating tissue compartments provide further elucidation on pathological mechanisms leading to childhood obesity. Furthermore, it will enable clinicians to compare and interpret body composition in infants with various disease states, particularly diseases that may affect body composition.

Despite the differences in methods of assessing body composition in infants, our data is comparable to that of other studies (12, 13, 17). Comparing the FM and FM % of our infants to those of Fields and colleagues who used ADP to assess body composition of exclusively breastfed predominantly white (75.6%) infants (83 males and 76 females), our infants have slightly higher FM (≤ 0.38 kg) and FM% (≤ 3.44 %) (13). However, compared to Butte and colleagues' study on 76 American infants (33 males and females) using DXA and a 4-compartment approach, our female infants have lower FM and FM% across the age span, while our male infants had higher FM and FM% at 6 and 9 mo of age (17). Butte et al. generated reference data for body composition during the

first 2 years using various methods including DXA, however, they did not report on LM as a separate body compartment (17). When comparing our study with a study that assessed the relationship between LM accrual on bone parameters in predominantly white (92.3%) infants aged 1 to 12 mo, our infants had slightly higher LM and lower FM across infancy (25). Of relevance, Butte et al. generated reference data using a similar number of infants as in our study (17), while other studies with a higher number of participants used ADP (12, 13).

Body composition differences between sexes has been shown previously with boys having slightly higher values of FFM and lower FM% compared to girls (17). Sex differences were observed in the present study with LM at 12 mo being higher in boys but no significant sex difference for LM%, FM and FM%. There were no sex differences when we normalised body composition indices by length. These observed differences between our study and others could be an indication that population variability is already evident in early life and such variability could be driven by environmental and genetic factors (9, 28). Furthermore, the infants in the present study maintained 25(OH)D concentration ≥ 50 nmol/L across infancy and were taking a vitamin D supplement of at least 400 IU daily with a tolerable upper intake level of 1000 to 1500 IU/d as per public health recommendations (21, 29). This may be of importance since sufficient vitamin D status has been previously shown to support a leaner body phenotype (3, 4).

In addition to infant sex, a number of sociodemographic factors relate to body composition. For example, studies have shown inverse associations between breastfeeding duration and overweight/obesity in early childhood (30, 31). Body composition differences across race/ethnicity have also been previously documented in

older children with black children having less body fat % than white children at corresponding levels of BMI for age (32). Infants in our study were mostly white and predominantly breastfed with 93.6% receiving breast milk for at least first 6 months of life. Therefore, the body composition curves generated from our infants will be a good reference for similar populations.

All infants grew within the WHO growth standards and thus reflect a healthy infant cohort comparable to WHO's 8,500 children of various ethnic and sociodemographic background (9, 10, 33). The decrease in LM % from ≤ 1 to 6 mo of age with its corresponding FM % increase observed in our study could be partly explained by the rapid growth phase of 0 to 6 mo which usually results in increasing FM % in relation to decreasing LM %. Furthermore, infants are born relatively lean (17).

The well-known timing of gross motor development in infancy could also contribute to the increase in LM from 6 to 12 mo and its corresponding decrease in FM %. Infants often attain use of large muscles needed for advanced milestones like sitting without support, crawling, standing with assistance etc. around 6 mo (34). Most published studies on body composition in infancy stop at 6 mo with more focus on FFM (12, 13), therefore, there is not much data available on LM % in infants beyond 6 mo of age. However, we propose that this observed pattern will become a common report with increase in LM and FM studies in infants.

One of the limitations of our study is being a secondary analysis of a randomized trial (20) and thus the data may not represent all Canadian infants as it is not based on a nationally representative sample and all infants were recruited from a single Canadian city. Furthermore, a high percentage of the mothers had a university education with

household income above average for Quebec and Canada (35). However, infants maintaining 25(OH)D ≥ 50 nmol/L from the neonatal period until 12 mo is a strength as it further classifies them as healthy, a particular important criteria for creating a reference data for future comparison. The limited available reference data on LM further underscores the importance of creating one for monitoring and managing body composition in clinical settings.

In conclusion, these normative LM data provide a much-needed reference to assess normal body composition and nutritional status in infancy. This data also provides a groundwork for future research and will aid in the elucidation of physiologic trends in nutritional status right from infancy.

Conflict of interest

Authors declare no conflicts of interest.

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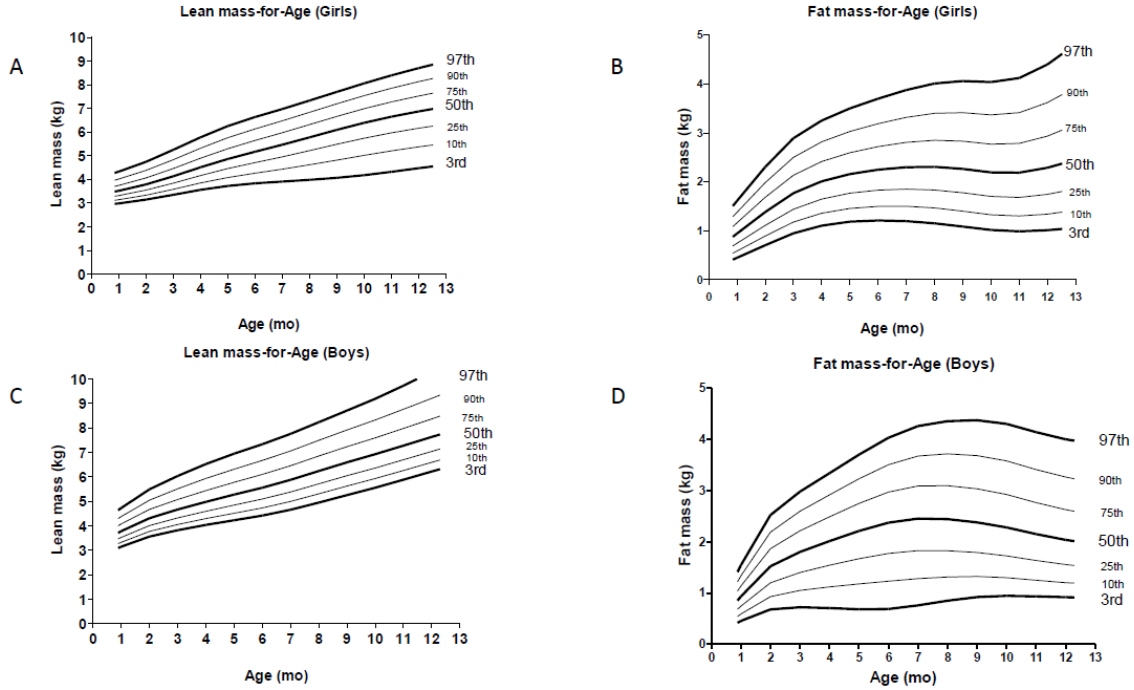

Figure 1: Percentiles of LM for girls (A) & boys(C) and FM for girls (B) & boys (D) reference curves ≤1 to 12 mo. The 3rd, 10th, 25th, 50th, 75th, 90th and 97th percentiles are given in ascending order.

Table 1: Characteristics of participants (n=70)

Variables	Mean ± SD or n (%)
Infants at birth	
Sex	
Male	35 (50.0)
Female	35 (50.0)
Parity (% first born)	25 (35.7)
Race[1] (white)	58 (82.9)
Season of birth	
Spring	20 (28.6)
Summer	17 (24.3)
Fall	19 (27.1)
Winter	14 (20.0)
Gestational age, weeks	39.5 ± 1.1
Weight, g	3539.7 ± 495.9
Weight-for-age Z-score	0.47 ± 0.97
Length, cm	51.8 ± 2.3
Length-for-age Z-score	1.21 ± 1.21
Head circumference, cm	34.7 ± 1.5
Head circumference-for-age Z-score	0.44 ± 1.22
BMI, kg/m^2	13.2 ± 1.3
BMI-for-age Z-score	-0.23 ± 1.09
Infants at ~1 month	
Age, d	34.6 ± 5.8
Weight, g	4635.8 ± 733.9
Weight-for-age Z-score	0.18 ± 0.97
Length, cm	54.9 ± 2.5
Length-for-age Z-score	0.10 ± 1.11
Head circumference, cm	37.9 ± 1.3
Head circumference-for-age Z-score	0.63 ± 0.97
BMI-for-age Z-score	0.18 ± 0.96
Plasma 25(OH)D_3 (nmol/L)[2]	88.2 ± 21.3
Breastfeeding status[3]	
≥ 3 mo	61/62 (98.4)
≥ 6 mo	54/58 (93.1)
Mothers	
Age at delivery, y	32.9 ± 3.7
Race[4] (white)	62 (88.6)
Household income (> $75,000 CAD/annum)	44 (63.8)
Education (University degree)	65 (92.9)

[1]Parental self-reported categories.[2]Sample limited to ≥ 50 nmol/L (defined as sufficiency by IOM); [3]Receiving any breastmilk either exclusively or with formula. [4]Self-reported race; others include Asian (n = 4), Black (n = 1), Hispanic (n = 1), Arabian (n =1) and not disclosed (n =1).

Table 2: Anthropometry and body composition by age across infancy and by sex

	n	Weight (kg)	WAZ	Length (cm)	HAZ	HC (cm)	HCZ	BAZ	LM (kg)	LM%	LMI (kg/m^2)	FM (kg)	FM%	FMI (kg/m^3)
All														
≤ 1 mo (25 – 52 d)	70	4.6 ± 0.7[a]	0.18 ± 0.97	54.9 ± 2.5[a]	0.10 ± 1.11	37.9 ± 1.3[a]	0.63 ± 0.97	0.18 ± 0.96	3.8 ± 0.5[a]	77.0 ± 5.9	12.5 ± 1.0	1.1 ± 0.4[a]	20.9 ± 5.8	6.3 ± 2.0[a]
3 mo (89 – 107 d)	62	6.2 ± 0.7[b]	-0.04 ± 0.92	61.1 ± 2.1[b]	0.13 ± 0.93	40.8 ± 1.2[b]	0.58 ± 0.92	-0.15 ± 0.93	4.5 ± 0.6[b]	69.6 ± 6.8	11.9 ± 1.4[b]	1.8 ± 0.5[b]	28.4 ± 6.8	8.0 ± 2.2[b]
6 mo (164 – 200 d)	58	7.7 ± 0.9[c]	0.03 ± 0.96	66.8 ± 2.4[c]	0.01 ± 1.10	43.7 ± 1.3[c]	0.73 ± 0.92	0.03 ± 0.85	5.4 ± 0.8[c]	68.6 ± 9.1	12.1 ± 1.5	2.3 ± 0.8[c]	29.2 ± 9.0	7.8 ± 2.6[c]
9 mo (270-312 d)	55	8.8 ± 1.0[d]	0.20 ± 1.00	71.3 ± 2.5[d]	0.07 ± 1.08	45.4 ± 1.4[d]	0.71 ± 1.01	0.22 ± 0.94	6.4 ± 0.9[d]	71.5 ± 9.5	12.5 ± 1.5	2.4 ± 1.0	26.3 ± 9.5	6.6 ± 2.7[d]
12 mo (356 – 381 d)	52	9.7 ± 1.2[e]	0.31 ± 1.04	75.5 ± 2.8[e]	0.23 ± 1.13	46.4 ± 1.5[e]	0.69 ± 1.04	0.25 ± 1.02	7.3 ± 1.2[e]	74.7 ± 7.8	12.8 ± 1.4[b]	2.3 ± 0.8	22.9 ± 7.8	5.2 ± 1.9
Girls														
≤ 1 mo (26 – 45 d)	35	4.4 ± 0.5	0.09 ± 0.89	54.0 ± 2.0	-0.01 ± 0.99	37.3 ± 1.1	0.48 ± 0.94	0.13 ± 0.89	3.6 ± 0.3	77.0 ± 5.9	12.2 ± 1.0	1.0 ± 0.4	21.0 ± 5.8	6.2 ± 2.0
3 mo (89 – 107 d)	32	5.9 ± 0.6	-0.02 ± 0.74	60.6 ± 1.7	0.26 ± 0.78	40.4 ± 1.0	0.56 ± 0.78	-0.22 ± 0.64	4.2 ± 0.5	67.9 ± 6.5	11.5 ± 9.0	1.9 ± 0.5	30.0 ± 6.4	8.4 ± 2.1
6 mo (164 – 200 d)	31	7.4 ± 0.6	0.04 ± 0.71	66.2 ± 2.2	0.17 ± 0.97	43.2 ± 1.0	0.70 ± 0.82	-0.07 ± 0.63	5.2 ± 0.8	67.8 ± 8.0	11.7 ± 1.5	2.3 ± 0.6	30.1 ± 7.9	7.9 ± 2.2
9 mo (270-312 d)	28	8.5 ± 0.8	0.22 ± 0.76	70.9 ± 2.1	0.25 ± 0.86	44.8 ± 1.1	0.66 ± 0.85	0.11 ± 0.83	6.1 ± 1.0	70.9 ± 10.5	12.5 ± 1.5	2.4 ± 1.0	26.9 ± 1.0	6.6 ± 2.9
12 mo (356 – 381 d)	26	9.4 ± 1.0	0.30 ± 0.83	75.0 ± 2.3	0.39 ± 0.85	45.7 ± 1.2	0.62 ± 0.88	0.11 ± 0.87	6.8 ± 1.0*	72.3 ± 8.6	12.2 ± 1.6	2.4 ± 0.9	25.3 ± 8.6	5.7 ± 1.9
Boys														
≤ 1 mo (27 – 52 d)	35	4.9 ± 0.8	0.27 ± 1.05	55.8 ± 2.7	0.20 ± 1.22	38.5 ± 1.3	0.78 ± 1.00	0.23 ± 1.03	4.0 ± 0.5	77.0 ± 5.9	12.7 ± 1.0	1.1 ± 0.5	20.9 ± 5.8	6.3 ± 2.1
3 mo (89 – 106 d)	30	6.4 ± 0.8	-0.06 ± 1.08	61.7 ± 2.9	-0.01 ± 1.06	41.3 ± 1.3	0.60 ± 1.06	-0.08 ± 1.18	4.8 ± 0.7	71.4 ± 6.8	12.5 ± 1.5	1.8 ± 0.6	26.6 ± 6.7	7.6 ± 2.2
6 mo (178 – 193 d)	27	8.0 ± 1.0	0.01 ± 1.20	67.4 ± 2.6	-0.16 ± 1.23	44.3 ± 1.3	0.76 ± 1.04	0.15 ± 1.05	5.7 ± 0.8	69.6 ± 10.2	12.5 ± 1.5	2.4 ± 1.0	28.3 ± 10.2	7.6 ± 3.0
9 mo (270 – 287 d)	27	9.2 ± 1.2	0.17 ± 1.21	71.8 ± 2.8	-0.12 ± 1.27	46.0 ± 1.5	0.75 ± 1.18	0.33 ± 1.04	6.7 ± 0.9	72.1 ± 8.5	13.0 ± 1.3	2.5 ± 1.0	25.7 ± 8.4	6.6 ± 2.5
12 mo (357 – 375 d)	26	10.1 ± 1.3	0.33 ± 1.23	75.9 ± 3.2	0.07 ± 1.34	47.0 ± 1.5	0.75 ± 1.20	0.39 ± 1.16	7.8 ± 1.1*	76.8 ± 6.5	13.4 ± 1.0	2.1 ± 0.8	20.8 ± 6.5	4.9 ± 1.8

Data presented as mean ± SD. HC, Head circumference; HAZ, height-for-age Z-score; WAZ, weight-for-age Z-score; HCZ, Head circumference-for-age Z-score; BAZ, BMI-for-age Z-score. LM, lean mass; LMI, lean mass index; FM, fat mass; FMI, fat mass index. Means with different superscript letters indicate statistically significant differences between time points * indicate where there are statistically significant differences between sexes. ($P < 0.05$, post-Tukey adjustment).