Sexually dimorphic response to feeding mode in the growth of infants[1,2]

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ABSTRACT

Background: The relation between infant feeding and growth has been extensively evaluated, but studies examining sex differences in the influence of infant milk feeding on growth are limited.

Objective: We examined the interaction of infant feeding and sex in relation to infant growth and compared growth trajectories in breastfed and formula-fed boys and girls.

Design: In 932 infants in a Singapore mother–offspring cohort, feeding practices in the first 6 mo were classified into the breastfeeding group (BF), mixed feeding group (MF), and formula feeding group (FF). Infant weight and length were measured and converted to WHO standards for weight-for-age *z* scores (WAZs) and length-for-age *z* scores (LAZs). Differences in WAZ and LAZ from birth to 6 mo, 6 to 12 mo, and 12 to 24 mo of age were calculated. Three-way interactions were examined between feeding mode, sex, and age intervals for WAZ and LAZ changes, with adjustment for confounders.

Results: The interaction between feeding mode, sex, and age intervals was significant for LAZ changes (P = 0.003) but not WAZ changes (P = 0.103) after adjustment for potential confounders. Compared with BF girls, BF boys showed similar LAZ gain (+0.28 compared with +0.39, P = 0.544) from 0 to 6 mo of age but greater LAZ gain from 6 to 12 mo of age (+0.39 compared with −0.10, P = 0.008). From 0 to 6 mo of age, FF boys and girls showed greater LAZ gains than their BF counterparts; from 6 to 12 mo of age, FF girls showed higher LAZ gain (+0.25 compared with −0.10, P = 0.031) than BF girls, which was not seen in boys.

Conclusions: During infancy, there is a sexually dimorphic growth response to the mode of infant milk feeding, raising questions about whether formula feeding ought to remain sex neutral. However, further investigations on sex-specific feeding and infant growth are warranted before a conclusive message can be drawn based on our current findings. This trial was registered at www.clinicaltrials.gov as NCT01174875.

Am J Clin Nutr doi: 10.3945/ajcn.115.115493.

Keywords: gender, breastfeeding, formula feeding, infant, growth, interaction

INTRODUCTION

Breast milk from healthy mothers is the best source of nourishment for infants. The WHO recommends breast milk as a nutrition standard for infants in the first 6 mo of life (1). Breast milk contains all the essential nutrients necessary for optimal growth and neurodevelopment in the early stages of lactation. The caloric density of breast milk also varies as the infant grows (2). The lower energy content of colostrum (53.6 kcal/100 mL) is replaced by transition milk (57.7 kcal/100 mL) from day 6, whereas mature milk (65.2 kcal/100 mL) is produced only after 14 d of life (2). From 30 to 120 d after birth, caloric density of breast milk produced for boys continued to rise (67.8–77.6 kcal/100 mL) but not for girls (62.6–64.0 kcal/100 mL) in a Singapore study (3). At 120 d, caloric density of breast milk for boys was higher by 24% than that for girls (3). These data indicate that breast milk is not only age-dependent but also sex-specific. However, current infant formula substitutes share constant energy content from birth to 6 mo of age (67 kcal/100 mL) (2), without consideration for sex. As suggested

[1] Supported by the Singapore National Research Foundation under its Translational and Clinical Research Flagship Programme and administered by the Singapore Ministry of Health's National Medical Research Council (Singapore-NMRC/TCR/004-NUS/2008 and NMRC/TCR/012-NUHS/2014) and by the Singapore Institute for Clinical Sciences, Agency for Science Technology and Research, Singapore. Also supported by the National Institute for Health Research through the Southampton Biomedical Research Centre and by the European Union's Seventh Framework Programme (FP7/2007-2013), project EarlyNutrition under grant agreement no. 289346 (KMG), and the Ministry of Health's National Medical Research Council, Singapore (NMRC/CSA/043/2012) (JKYC).

[2] Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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Received May 19, 2015. Accepted for publication November 24, 2015.

doi: 10.3945/ajcn.115.115493.

in a meta-analysis, the higher energy content of formula milk may expose formula-fed infants to energy-dense milk in early life, which may affect subsequent growth outcomes (2). We speculate that this causal process may be more apparent in formula-fed girls.

In the past 2 decades, infant growth studies mainly focused on assessing the differences between various feeding modes and comparing growth to existing references and standards, such that it is now well established that formula-fed infants gain more weight than breastfed counterparts (2, 4–6). There is also recognition that healthy breastfed infants tend to grow more rapidly than formula-fed peers in the first 2 mo of life and less rapidly from 3 to 12 mo of age (7). However, differences in growth trajectories between breastfed boys and girls are still unclear, because few studies have addressed infant feeding mode and growth interactions in sex subgroups. Moreover, it remains uncertain whether the greater growth in formula-fed infants is sex specific or whether it occurs in both sexes, because data from previous studies are limited and inconsistent (5, 8, 9).

In this study, we hypothesized that there is a sexually dimorphic infant growth response to mode of infant milk feeding. We aimed to do the following: *1*) examine the interaction of feeding mode and sex in relation to longitudinal infant growth, *2*) describe and compare length and weight growth trajectories in breastfed boys and girls, and *3*) compare the growth of formula-fed infants with infants fed breast milk.

METHODS

Study design and participants

Participants were taken from the Growing Up in Singapore Toward healthy Outcomes (GUSTO)[18] birth cohort study. The GUSTO study was designed as a population-based observational study to collect detailed information on pregnant women and characteristics of their infants from birth onward in Singapore. The study was established to investigate the effects of early life events on the risk of developing metabolic diseases in later life. Details of the GUSTO study are provided elsewhere (10).

A total of 1152 pregnant women aged ≥18 y and attending antenatal clinics at a gestational age of <14 wk (based on ultrasound scan) were recruited from 2 major public maternity units in Singapore, namely, KK Women's and Children's Hospital and National University Hospital, between June 2009 and September 2010. The recruited women were Singapore citizens or permanent residents who were of Chinese, Malay, or Indian ethnicity with homogeneous parental ethnic background. Women who were on chemotherapy or psychotropic drugs or who had type 1 diabetes mellitus were excluded from the study. Written informed consent was obtained from each participant.

This study was approved by the Centralised Institutional Review Board of SingHealth (reference 2009/280/D) and the Domain Specific Review Board of the Singapore National Healthcare Group (reference D/09/021).

[18] Abbreviations used: BF, breastfeeding group; FF, formula feeding group; GUSTO, Growing Up in Singapore Toward healthy Outcomes; LAZ, length-for-age *z* score; MF, mixed feeding group; MGRS, Multicentre Growth Reference Study; WAZ, weight-for-age *z* score.

Maternal and infant characteristics

Demographic characteristics and past medical history (e.g., diabetes mellitus and hypertension) of pregnant women were recorded with the use of interviewer-administered questionnaires at the recruitment visit and at 26–28 wk of gestation. An oral-glucose-tolerance test (75 g) was performed at 26–28 wk of gestation to diagnose gestational diabetes mellitus with the use of WHO criteria (fasting glucose ≥7.0 mmol/L or 2-h glucose ≥7.8 mmol/L) (11). Maternal weight before pregnancy was based on self-reported data, whereas height was measured at 26–28 wk of gestation with a stadiometer (Seca 206). The mother's BMI before pregnancy was computed from weight (kilograms) divided by height (meters squared). After delivery, infant characteristics such as sex and gestational age were obtained from the hospital case notes by trained health personnel.

Infant feeding assessments

Infant feeding modes for the preceding 3 mo were ascertained at ages 3 and 6 mo with the use of interviewer-administered questionnaires. Based on the WHO definition, infant feeding modes were classified as follows: *1*) exclusive breastfeeding, in which the infant received only breast milk and no other liquids or solids with the exception of drops or syrups consisting of vitamins, mineral supplements, or medicines (12); *2*) predominant breastfeeding, in which the infant received mainly breast milk and some water-based drinks (12); *3*) partial breastfeeding, in which the infant received a combination of breast milk and formula milk (12); and *4*) formula feeding, in which the infant was fed exclusively formula milk. Mothers were asked to classify their infant feeding modes at ages 1, 2, and 3 mo during the 3-mo postnatal visit, and their infant feeding modes at ages 4, 5, and 6 mo during the 6-mo postnatal visit. All interviewers followed protocol by not providing any feeding advice to mothers throughout the study period. The age of weaning (solid food introduction to infants on a daily basis) was determined at the 9-mo interview.

Infant milk feeding classification

Because exclusive breastfeeding during the first 6 mo is accepted as the nutrition standard for infants (1), infants in the current analysis were classified into the following groups: the breastfeeding group (BF), representing infants fed with the use of methods *1* and/or *2* for the entire first 6 mo of life; the formula feeding group (FF), representing infants fed with the use of method *4* for the entire first 6 mo of life; and the mixed feeding group (MF), representing infants who were fed with the use of method *3* only or methods *1* and/or *2* with *3* and/or *4* or methods *3* and *4* in the first 6 mo.

Infant anthropometric measurements

Infant anthropometric measurements were taken by 23 trained staff on day 1 of life and at 6, 12, and 24 mo of age with the use of standardized techniques (13). All infants were randomly assigned to 11 clinic staff members for all clinic visits but matched to 12 home visitors based on participants' preferred language for home visits. Before the measurements could be conducted, all staff had received rigorous training and had their technique calibrated against the gold standard of an experienced anthropometrist who endorsed their competency. To ensure standardization of measuring techniques throughout the study period, intra- and intermeasurer assessments were also performed on a quarterly basis. Retraining was provided for staff whose measurements did not meet standards.

Each infant's anthropometric factors were measured by one staff member who was not intentionally blinded to the infant feeding mode. However, to minimize possible bias, standardized procedures (13) were employed when conducting measurements. Weight at birth and 6 and 12 mo of age was recorded to the nearest 0.001 kg with the use of a SECA 334 Mobile Digital Baby Scale, and at 24 mo of age to the nearest 0.01 kg with the use of a SECA 803 Digital Flat Scale. Recumbent crown–heel length at birth and 6, 12, and 24 mo of age were measured to the nearest 0.1 cm with the use of a SECA 210 Mobile Measuring Mat. When length measurement at 24 mo of age was not possible, height was measured with the use of a SECA 213 portable stadiometer and converted to length by adding 0.7 cm (n = 71) (14). All measuring equipment was calibrated on a yearly basis according to the specific protocols (15).

Z scores and changes in *z* scores

Z scores rather than absolute measurements were computed to allow comparison across age and sex, as well as to quantify longitudinal changes in growth. Weight-for-age *z* score (WAZ) and length-for-age *z* score (LAZ) were derived from infant weight and length based on WHO Child Growth Standards 2006 with the use of WHO Anthro software (version 3.2.2). To assess longitudinal growth, interval growth changes were determined by obtaining the *z* score differences in WAZ and LAZ from birth to 6 mo, 6 to 12 mo, and 12 to 24 mo of age.

Statistical analyses

Fisher's exact tests for categorical variables and independent sample t tests for continuous variables were performed to explore differences in maternal and infant characteristics between sexes and feeding mode (BF and MF against FF to understand the impact of formula milk exposure).

To test the effect of feeding mode and sex on changes in WAZ and LAZ, linear mixed-effect modeling was used to analyze repeated measurements and adjust for potential confounders. The covariates were ethnicity, maternal age, education, marital status, monthly household income, active smoking before and/or during pregnancy, passive smoking before and/or during pregnancy, diabetes mellitus, hypertension, and WAZ or LAZ at birth. An additional covariate, the mother's BMI before pregnancy, was adjusted for in further analysis. Passive smoking was defined as daily cigarette smoke exposure at work and/or at home. Diabetes mellitus included women with pre-existing type 2 diabetes and gestational diabetes mellitus. Hypertension referred to chronic hypertension, gestational hypertension, preeclampsia, and eclampsia. WAZ or LAZ at birth was included in the covariate adjustment, because rapid postnatal growth has been associated with smaller size at birth (16). Continuous covariates including maternal age and WAZ or LAZ at birth were centered at their mean values in the multivariate model. The basic model included feeding mode (BF, MF, or FF), sex, and age intervals (0–6 mo, 6–12 mo, and 12–24 mo of age), and interactions between feeding mode, sex, and age intervals. All P values were 2-tailed, with $P < 0.05$ considered to be statistically significant. Statistical analyses were performed with the use of Stata 13.1.

RESULTS

Study participants

For this study, only singleton infants with a known feeding history in the first 6 mo of life were included. Based on the inclusion criteria for this study, 932 infants were included in the final analysis; this comprised 62 BF boys, 63 BF girls, 302 MF boys, 276 MF girls, 120 FF boys, and 109 FF girls. Of 578 MF infants, only 97 (16.8%) were exclusively and/or predominantly breastfed from 0 to 3 mo of age.

Characteristics of mothers

The comparison of maternal characteristics by feeding mode and sex is shown in **Table 1**. In the comparison by sex, there were no differences in maternal characteristics for all 3 feeding groups. With respect to feeding modes, FF boys and girls had mothers with lower educational levels and family income compared with both the BF and MF boys and girls; FF infants had greater exposure to cigarette smoke before and/or during pregnancy.

Characteristics of infants

A comparison of infant characteristics by feeding modes and sex is shown in **Table 2**. Overall, boys and girls in this study were shorter and lighter at birth than the 2006 WHO Child Growth Standards. FF boys were shorter and lighter at birth compared with BF and MF boys, but these differences were not statistically significant in girls. No significant difference between feeding modes and sex was observed in the weaning age.

Overall growth patterns

Infants in all feeding mode and sex groups showed accelerated growth over the first 6 mo in comparison with the WHO Child Growth Standards. The greatest growth difference between sexes based on feeding mode was observed in the 6–12-mo interval. From 12 to 24 mo of age, WAZ and LAZ were stable in all groups. These findings were similar after adjustment for covariates (**Figure 1** and **Table 3**).

Interaction effect between feeding mode, sex, and age intervals

The adjusted mean changes in WAZ and LAZ of infants are shown in Table 3. Overall, 3-way interactions between feeding mode, sex, and age intervals were significant for LAZ changes (P = 0.003) but not WAZ changes (P = 0.103) after adjustment for potential confounders. From 0 to 6 mo, BF boys and girls showed similar LAZ gain (+0.28 compared with +0.39, P = 0.544). From 6 to 12 mo of age, BF boys had greater LAZ gain than BF girls (+0.39 compared with −0.10; P = 0.008). From 12 to 24 mo of age, LAZ gain was stable in both BF boys and girls (LAZ: +0.05 compared with +0.03, P = 0.919).

Growth differences between BF, MF, and FF infants were also observed at different time intervals. In the first 6 mo of life, both FF boys (+0.72 compared with +0.28, P = 0.006) and girls (+0.72 compared with +0.39, P = 0.038) showed greater LAZ gains compared with their BF counterparts.

From 6 to 12 mo of age, FF girls showed higher LAZ gain (+0.25 compared with −0.10, P = 0.031) than BF girls, whereas FF boys

TABLE 1
Comparison of maternal characteristics by feeding practices and sex[1]

	Breastfeeding			Mixed feeding			Formula feeding						
Demographic characteristics	Boys (n = 62)	Girls (n = 63)	P	Boys (n = 302)	Girls (n = 276)	P	Boys (n = 120)	Girls (n = 109)	P	P^2	P^3	P^4	P^5
Ethnicity, n (%)			0.407			0.815			0.370	<0.001	0.178	0.001	0.827
Chinese	52 (83.9)	47 (74.6)		153 (50.7)	146 (52.9)		67 (55.8)	54 (49.5)					
Malay	4 (6.5)	5 (7.9)		85 (28.1)	77 (27.9)		37 (30.8)	33 (30.3)					
Indian	6 (9.7)	11 (17.5)		64 (21.2)	53 (19.2)		16 (13.3)	22 (20.2)					
Maternal education, n (%)			0.799			0.432			0.692	<0.001	<0.001	<0.001	<0.001
None/primary/secondary	8 (13.1)	10 (16.1)		75 (25.3)	61 (22.3)		61 (50.8)	58 (53.7)					
Tertiary	53 (86.9)	52 (83.9)		222 (74.7)	213 (77.7)		59 (49.2)	50 (46.3)					
Marital status, n (%)			1.000			1.000			0.299	0.017	0.006	0.416	0.190
Single/divorced	0 (0.0)	1 (1.6)		7 (2.4)	6 (2.2)		11 (9.2)	5 (4.8)					
Married	61 (100.0)	60 (98.4)		288 (97.6)	265 (97.8)		109 (90.8)	100 (95.2)					
Household monthly income (Singapore dollars), n (%)			0.185			0.780			0.345	<0.001	<0.001	<0.001	<0.001
≤1999 (equivalent to 1414 US$)	1 (1.8)	4 (6.5)		32 (11.1)	28 (11.0)		30 (27.8)	36 (35.3)					
2000–5999 (equivalent to 1415–4244 US$)	22 (38.6)	30 (48.4)		163 (56.4)	150 (59.1)		64 (59.3)	58 (56.9)					
≥6000 (equivalent to 4245 US$)	34 (59.6)	28 (45.2)		94 (32.5)	76 (29.9)		14 (13.0)	8 (7.8)					
Active smoking before/during pregnancy, n (%)	1 (1.6)	5 (7.9)	0.207	26 (8.7)	21 (7.6)	0.651	36 (30.5)	23 (21.7)	0.171	<0.001	<0.001	0.020	<0.001
Passive smoking before/during pregnancy, n (%)	15 (24.6)	8 (12.9)	0.110	112 (38.1)	110 (40.4)	0.605	77 (65.8)	66 (62.3)	0.675	<0.001	<0.001	<0.001	<0.001
Diabetes mellitus, n (%)	10 (16.1)	17 (27.6)	0.192	45 (15.0)	47 (17.2)	0.496	16 (13.3)	12 (11.0)	0.688	0.657	0.760	0.011	0.159
Hypertension, n (%)	3 (4.8)	1 (1.6)	0.365	14 (4.6)	12 (4.3)	1.000	10 (8.3)	7 (6.4)	0.623	0.547	0.162	0.261	0.435
Mother's age, y	31.56 ± 4.50[6]	30.48 ± 4.39	0.174	31.19 ± 4.66	31.24 ± 5.12	0.908	29.31 ± 5.75	29.20 ± 5.75	0.889	0.004	0.002	0.105	0.001
Mother's height, cm	158.33 ± 5.18	158.31 ± 5.54	0.983	158.22 ± 5.55	157.99 ± 5.84	0.619	157.93 ± 5.50	158.80 ± 5.96	0.265	0.638	0.631	0.604	0.232
Mother's BMI, kg/m^2	21.69 ± 3.06	22.09 ± 3.58	0.517	22.54 ± 3.86	22.95 ± 4.57	0.260	22.68 ± 4.65	23.10 ± 5.34	0.549	0.106	0.775	0.166	0.790

[1]Fisher's exact tests for categorical variables and independent-samples t tests for continuous variables were used to compare the 2 groups.
[2]Comparison between breastfed boys and formula-fed boys.
[3]Comparison between mixed-fed boys and formula-fed boys.
[4]Comparison between breastfed girls and formula-fed girls.
[5]Comparison between mixed-fed girls and formula-fed girls.
[6]Mean ± SD (all such values).

TABLE 2
Comparison of infant characteristics by feeding practices and sex[1]

	Breastfeeding			Mixed feeding			Formula feeding						
Demographics	Boys (n = 62)	Girls (n = 63)	P	Boys (n = 302)	Girls (n = 276)	P	Boys (n = 120)	Girls (n = 109)	P	P^2	P^3	P^4	P^5
Gestational age, wk	38.55 ± 0.90[6]	38.40 ± 1.17	0.419	38.34 ± 1.54	38.38 ± 1.40	0.771	38.16 ± 1.32	38.27 ± 1.40	0.549	0.020	0.254	0.532	0.483
Birth weight, kg	3.22 ± 0.36	3.06 ± 0.39	0.020	3.21 ± 0.45	3.04 ± 0.42	<0.001	3.07 ± 0.43	3.04 ± 0.45	0.624	0.020	0.011	0.794	0.980
Birth length, cm	49.50 ± 2.99	48.70 ± 1.95	0.027	49.11 ± 1.95	48.16 ± 1.97	<0.001	48.39 ± 1.93	48.07 ± 2.06	0.253	<0.001	0.001	0.057	0.731
Weight-for-age z score by age													
Birth	−0.29 ± 0.76	−0.42 ± 0.91	0.405	−0.34 ± 0.95	−0.47 ± 0.97	0.109	−0.62 ± 0.94	−0.47 ± 1.04	0.243	0.018	0.005	0.740	0.970
6 mo	0.37 ± 1.04	−0.07 ± 0.87	0.013	0.05 ± 1.04	−0.01 ± 0.95	0.513	−0.27 ± 1.11	0.00 ± 0.91	0.047	<0.001	0.007	0.633	0.947
12 mo	0.05 ± 1.00	−0.20 ± 0.81	0.130	−0.02 ± 1.04	0.03 ± 0.94	0.524	−0.30 ± 1.06	0.13 ± 0.91	0.002	0.041	0.021	0.022	0.402
24 mo	0.06 ± 1.20	−0.23 ± 0.83	0.148	0.03 ± 1.06	−0.02 ± 0.96	0.601	−0.27 ± 1.13	0.12 ± 1.20	0.019	0.091	0.020	0.053	0.260
Length-for-age z score by age													
Birth	−0.23 ± 1.05	−0.27 ± 1.06	0.827	−0.45 ± 1.02	−0.57 ± 1.08	0.185	−0.84 ± 1.03	−0.62 ± 1.16	0.130	<0.001	0.001	0.057	0.714
6 mo	0.02 ± 1.29	0.07 ± 0.95	0.827	0.11 ± 1.21	0.18 ± 1.16	0.494	−0.09 ± 1.23	0.10 ± 1.09	0.237	0.566	0.144	0.875	0.526
12 mo	0.27 ± 1.34	−0.09 ± 1.01	0.111	0.24 ± 1.27	0.19 ± 1.18	0.623	−0.12 ± 1.23	0.24 ± 1.15	0.031	0.068	0.011	0.071	0.746
24 mo	0.29 ± 1.23	−0.07 ± 0.93	0.079	0.26 ± 1.16	0.17 ± 1.05	0.378	−0.08 ± 1.11	0.18 ± 1.10	0.102	0.057	0.013	0.148	0.945
Weaning age, mo	5.99 ± 0.92	5.86 ± 0.92	0.446	5.61 ± 1.12	5.59 ± 1.20	0.847	5.67 ± 1.22	5.68 ± 1.23	0.965	0.075	0.669	0.337	0.556

[1]Independent-samples t tests for continuous variables were used to compare the 2 groups.
[2]Comparison between breastfed boys and formula-fed boys.
[3]Comparison between mixed-fed boys and formula-fed boys.
[4]Comparison between breastfed girls and formula-fed girls.
[5]Comparison between mixed-fed girls and formula-fed girls.
[6]Mean ± SD (all such values).

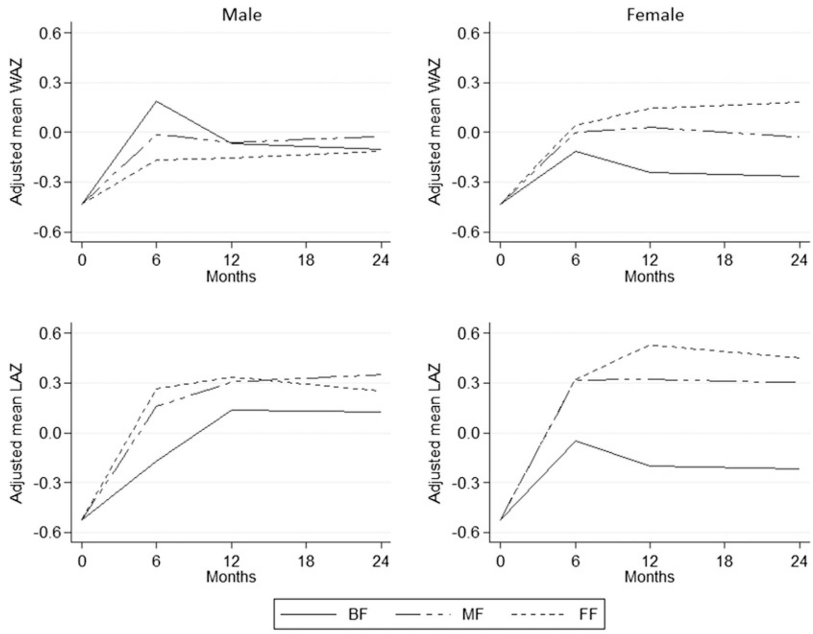

FIGURE 1 Adjusted mean WAZ and LAZ of infants from birth to 24 mo of age for BF, MF, and FF groups. Analyses were performed with the use of linear mixed-effect modeling and adjusted for ethnicity, maternal age, education, marital status, monthly household income, active smoking before and/or during pregnancy, passive smoking before and/or during pregnancy, diabetes mellitus, hypertension, and WAZ or LAZ at birth. BF, breastfeeding; FF, formula feeding; LAZ, length-for-age *z*-score; MF, mixed feeding; WAZ, weight-for-age *z* score.

tended to have less LAZ gain than BF boys, although this was not statistically significant (+0.06 compared with +0.39, $P = 0.055$). From 12 to 24 mo of age, boys and girls from both the BF and FF showed similar changes in LAZ (boys: +0.05 compared with −0.09, $P = 0.435$; girls: +0.03 compared with −0.01, $P = 0.823$). To further understand the effect of formula milk exposure, MF and FF infants were compared. Across all time intervals, no significant differences in LAZ gains were observed between MF and FF infants for boys and girls. All findings remained similar after further adjustment for maternal BMI before pregnancy **(Supplemental Table 1)**. When additional analysis was performed for weight-for-length *z* scores, a significant 3-way interaction between feeding mode, sex, and age intervals after adjustment for potential confounders was found ($P < 0.001$) **(Supplemental Table 2)**.

DISCUSSION

This study supports the hypothesis that there is a sexually dimorphic infant growth response to the mode of infant milk feeding. We observed a significant 3-way interaction for length

TABLE 3
Adjusted mean changes in WAZs and LAZs of infants[1]

	WAZ change[2] (n = 824)		LAZ change[3] (n = 791)	
Age interval	Boy	Girl	Boy	Girl
Breastfeeding, mo				
Birth–6	0.62 (0.43, 0.82)	0.32 (0.13, 0.51)	0.28 (0.01, 0.54)*	0.39 (0.13, 0.64)*
6–12	−0.25 (−0.45, −0.04)	−0.16 (−0.34, 0.03)	0.39 (0.11, 0.68)**	−0.10 (−0.36, 0.15)*
12–24	−0.03 (−0.24, 0.18)	−0.03 (−0.22, 0.17)	0.05 (−0.23, 0.34)	0.03 (−0.23, 0.30)
Mixed feeding, mo				
Birth–6	0.37 (0.26, 0.48)	0.38 (0.28, 0.49)	0.58 (0.44, 0.72)	0.78 (0.63, 0.92)
6–12	−0.07 (−0.18, 0.04)	0.01 (−0.10, 0.12)	0.21 (0.07, 0.36)	0.02 (−0.13, 0.17)
12–24	0.00 (−0.11, 0.11)	−0.08 (−0.20, 0.03)	0.11 (−0.04, 0.26)	0.01 (−0.15, 0.17)
Formula feeding, mo				
Birth–6	0.29 (0.13, 0.45)	0.41 (0.25, 0.57)	0.72 (0.51, 0.93)	0.72 (0.51, 0.93)
6–12	−0.03 (−0.19, 0.14)	0.08 (−0.08, 0.24)	0.06 (−0.16, 0.28)	0.25 (0.03, 0.47)
12–24	−0.02 (−0.20, 0.15)	0.00 (−0.17, 0.17)	−0.09 (−0.32, 0.15)	−0.01 (−0.24, 0.23)

[1]Values are means with 95% CIs in parentheses. Analyses were performed with the use of linear mixed-effect modeling and adjusted for ethnicity, maternal age, education, marital status, monthly household income, active smoking before and/or during pregnancy, passive smoking before and/or during pregnancy, diabetes mellitus, hypertension, and WAZ or LAZ at birth. *Significantly different from formula-fed boys and girls at the same time interval ($P < 0.05$). **Significantly different from breastfed girls at the same time interval ($P < 0.05$). LAZ, length-for-age *z* score; WAZ, weight-for-age *z* score.

[2]Three-way interaction: feeding practices × sex, age; $P = 0.103$.

[3]Significant 3-way interaction: feeding practices × sex, age; $P = 0.003$.

but not weight changes in relation to mode of feeding, sex, and age intervals, after adjustment for confounders. Boys compared with girls who were exclusively/predominantly breastfed in the first 6 mo had greater length gain from 6 to 12 mo. When measurements from breastfed infants were used as a reference for normal growth, we found greater length gain from 6 to 12 mo of age in formula-fed girls compared with their breastfed counterparts; however, such differences were not noted between breastfed and formula-fed boys.

We highlight the importance of understanding sex differences in growth even in breastfed infants, because the growth of breastfed infants is used as a yardstick for normal growth. Sex differences in this study are relative to the sex differences found in the WHO growth standards. Our breastfed boys gained 1.64 cm (adjusted mean 0.38 *z* score) more length than girls, whereas the WHO Multicentre Growth Reference Study (MGRS) breastfed boys gained 1.00 cm more in length than girls in the first 12 mo of life (17). Greater length gain in boys than girls may be attributable to substantially higher calories consumed by boys than girls (18), as a result of a greater caloric density in human milk for boys than girls that was reported in 2 previous studies (3, 18). A Singapore study demonstrated that human milk for boys compared with that for girls in the first 4 mo of life was 14.8 kcal/100 mL higher in caloric density (3), whereas a study from Massachusetts showed that caloric density in human milk for boys was 24.68 kcal/100 mL higher than that for girls between 2 and 5 mo of age, after adjustment for time since last feeding (18). This difference in caloric density was contributed by higher lipid content in human milk secreted for boys (3). Such a sex difference in milk composition has been proposed to be sufficient to account for differences in growth between boys and girls in early life (2, 18). Additional evidence that the sex of the offspring influences the quality of maternal milk can be found in landmark animal studies (19, 20).

Our findings are consistent with a study that also demonstrated greater growth in formula-fed girls than in boys (8). Whereas a significant 3-way interaction between feeding mode, sex, and time interval for weight gain was noted (8), our study showed significant interaction for length gain instead. In contrast, other studies reported that differences in weight and length gain between breastfed and formula-fed infants were more notable in boys than in girls (5, 9). Comparing against National Center for Health Statistics-WHO growth reference, an Italian study showed that weight and length gains from birth to 4 mo of age between breastfed (36 boys; 37 girls) and formula-fed (35 boys; 30 girls) infants were significantly different in boys but comparable in girls (5). Similarly, a Japanese study that used local references found significantly greater growth in breastfed ($n = 31$) than in formula-fed boys ($n = 19$) but little impact of feeding mode on the growth of girls (9). The inconsistencies between these studies, including ours, may be due to sample size and methodologic differences such as the use of growth references rather than standards. We compared our infants with WHO MGRS infants, because they provide a single international standard that represents the best description of physiologic growth for children aged 0–5 y with the use of breastfed infants as the normative growth model.

This study showed that formula-fed infants gained more length than those who were breastfed, which may be the result of higher energy density (2), protein (21), and micronutrient content (22) in formula than in breast milk. Rapid growth is of particular concern because it has been linked with increased risk of developing obesity and chronic disease (23, 24). Although the infants in this study were 0.53 *z* score shorter at birth than those in the WHO MGRS, rapid growth in the context of shorter birth length can be explained. First, shorter birth length is unlikely to be influenced by maternal undernutrition, given Singapore's affluent environment. Second, the mean height of Singaporean mothers was 158.2 cm compared with 161.0 cm in WHO MGRS, and the positive association between maternal anthropometric measurements and birth size in offspring is well known (25). Finally, the effect of birth length on postnatal growth had been controlled for in multivariate analysis. Therefore, these suggest a postnatal nutritional explanation for the greater growth seen in formula-fed infants.

Of particular interest is the observation that formula-fed girls but not boys continued to gain more length compared with their breastfed counterparts in the second 6 mo after accelerated length gain from birth to 6 mo of age, which suggests that they may have received comparatively greater nutrition from milk. Because the reported caloric density in breast milk for girls remained at 62.6–64.0 kcal/100 mL by 1 mo after birth (3), constantly feeding girls with relatively higher calorie formula milk (67 kcal/100 mL) may contribute to greater growth (18). In contrast with that for girls, the caloric density of breast milk for boys (67.8–77.6 kcal/100 mL) (3) is similar to or exceeds that in formula milk (2), which may partly explain the comparable length gains between breastfed and formula-fed boys in this cohort. Another explanation for the greater growth in formula-fed girls may be related to decreases in serum leptin (26). Because leptin is present in breast milk but not formula milk, exclusive formula feeding can lead to an increase in appetite and energy intake, leading to greater growth (27, 28). Yet another possible explanation for our findings relates to sex and feeding mode differences in the population of gut microbes, which have been associated with obesity (29). We recommend further investigations in these areas.

The strengths of this study include a large sample size, longitudinal approach, and standardized measurements by trained and regularly audited personnel, which lead to increased power of analyses and precision of findings. Although data collection on feeding mode and anthropometric measurements was not completely blinded, clinic staff and home visitors were blinded to group assignment. The present findings, however, should be interpreted cautiously. Reverse causality, that infant growth can influence the choice and feeding mode by caregivers, has previously been described (30) and may account for the reported observations. A total of 220 (19.1%) participants were excluded from the analysis and had different maternal and infant characteristics from those included in the analysis; however, these variables were adjusted for in the multivariable analyses. We were unable to compare changes in total caloric intake and nutrient composition of infants throughout the first 2 y of life, because both quality and quantity of breast milk and formula milk were not examined in this study. In addition, infant body composition was not serially measured from 0 to 24 mo of age to consolidate our findings. Nonetheless, the continuous follow-up of anthropometric measurements within a cohort study allows us to better understand long-term growth and size changes in relation to early feeding practices and sex.

In conclusion, our study found differential infant growth responses to feeding mode according to sex, raising questions about whether formula feeding ought to remain sex neutral. Unlike boys, formula-fed girls had greater length gain than breastfed girls from 6 to 12 mo of age, potentially exposing them to a higher risk of future metabolic diseases. However, further investigations on sex-specific feeding and infant growth are warranted before a conclusive message can be drawn based on our current findings. Whether the association between feeding mode, sex, and growth will hold up in other populations remains to be seen. Further investigations on the quality of breast milk across different populations will inform whether sex differences in breast milk composition are indeed universal.

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We thank Taara Madhavan (Associate, Clinical Sciences, Duke-NUS Medical School) for medical writing assistance, and the GUSTO study group and the funding organizations.

The authors' responsibilities were as follows—Y-SC, YSL, and FY: conceived of the GUSTO cohort study; NL and FY: conceived of this substudy; TSC: performed the data analysis; TSC, SLL, JKYC, NL, and FY: interpreted the findings and drafted the manuscript; YBC: advised on the statistical analysis and participated in the interpretation; and all authors: participated in the critical review, revision, and approval of the final manuscript. KMG, PDG, Y-SC, and YSL have received reimbursement for speaking at conferences sponsored by companies selling nutritional products. KMG, PDG, and Y-SC are part of an academic consortium that has received research funding from Abbott Nutrition, Nestlé, and Danone. None of the other authors reported a conflict of interest related to the study.

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