Timing of infant formula introduction in relation to body mass index and overweight at ages 1 and 3 years

BIGCS Study Group

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Timing of Infant Formula Introduction in Relation to Body Mass Index and Overweight at Ages 1 And 3 Years: the Born in Guangzhou Cohort Study (BIGCS)

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Abstract

Mounting evidence suggests that the first few months of life are critical for the development of obesity. The relationships between the timing of solid food introduction and the risk of childhood obesity have been examined previously, however, evidence for the association of timing of infant formula introduction remains scarce. This study aimed to examine whether the timing of infant formula introduction is associated with growth z-scores and overweight at ages 1 and 3 years. This study included 5733 full term (≥37gestational weeks) and normal birthweight (≥2500g and <4000g) children in the Born in Guangzhou Cohort Study, a prospective cohort study with data collected at 6 weeks, 6, 12, and 36 months. Compared with infant formula introduction at 0-3 months, introduction at 4-6 months was associated with the lower BMI, weight-for-age and weight-for-length z-scores at 1 and 3 years old. Also, introduction at 4-6 months was associated with the lower odds of at-risk of overweight at age 1 (adjusted odds ratio (OR) 0.72, 95%CI 0.55, 0.94) and 3 years (adjusted OR 0.50, 95% CI 0.30, 0.85). Introduction at 4-6 months also decreased the odds of overweight at age 1 year (adjusted OR: 0.42, 95% CI: 0.21, 0.84), but not at age 3 years. Based on our findings, compared with introduction within the first 3 months, introduction at 4-6 months has a reduction on later high BMI risk and at-risk of overweight. However, these results need to be replicated in other well-designed studies before more firm recommendations can be made.

Introduction

The prevalence of being overweight in children under 5 years old has risen from 6.3% to 11.9% between 1990 and 2016 in China [1]. By 2015, China had the largest number of children with obesity aged 5 years or under in the world [2]. Epidemiological studies have reported that early obesity was a significant predictor of obesity later in life and the development of cardiometabolic disorders during adulthood [3-6].

Mounting evidence suggests that the first few months of life are critical for the development of obesity [7]. The relationships between early solid food introduction (before age 4 months) and risk of childhood obesity have been examined previously, however, findings were inconsistent [8]. Several cohort studies reported that the association between the timing of solid food introduction and adiposity varied by milk feeding status (formula-fed or breastfed) [9-11]. They found the effects of early solid food introduction on later obesity were more significant in formula-fed infants. Infant formula was usually introduced earlier than solid food. A population-based birth cohort study of China, involving 98097 maternal-infant pairs, showed that in the first month, infant formula exposure rate was 58.8%; in the third month, the exposure rate was 66.6%; in the sixth month, the exposure rate reached up to 72.0% [12]. The association between formula feeding practice and excess weight gain in early childhood has been examined [13]. The explored mechanisms included overfeeding formula, putting a baby to bed with bottle, and compositions (higher protein) in formula [13]. However, to our knowledge, there were no studies primarily focusing on the association between the timing of infant formula introduction and early growth. Though cessation of exclusive breastfeeding is essentially correlated with the introduction of infant formula, it is also related to the introduction of solid foods. Therefore, it is necessary to examine the effects of the timing of infant formula and solid food introduction on later growth separately.

This study has examined the hypothesis that introduction of infant formula at early stage is associated with a higher risk of overweight in early childhood with considering the timing of solid food introduction and breastfeeding duration in a large scale prospective birth cohort.

Methods

Study Population

This is a longitudinal study on singleton births between February 2012 and December 2015 in the Born in Guangzhou Cohort Study (BIGCS), a large-scale prospective observational study in Guangzhou, China. Pregnant women were recruited before 20 gestational weeks from two campuses of Guangzhou Women and Children's Medical Center (GWCMC) from February 2012. Details of the BIGCS cohort with full inclusion and exclusion criteria can be found in the published protocol [14]. This study was approved by the Institutional Ethics Committee of the GWCMC. All participants signed a consent form at the time of recruitment. The baseline questionnaire was completed before week 20 of pregnancy. Follow-up of the children took place at pediatric clinics at the age of 6 weeks, 6, 12, and 36 months after birth and involved questionnaires (completed by the mother or guardian) and physical examinations. Although those who were unable to attend the appointments in person were interviewed on the telephone, they were excluded from the present analysis due to the lack of information on anthropometric measurements. Children with incomplete feeding data were also excluded (**Figure 1**). In addition, all analyses were performed by restricting to full-term (≥37 weeks) infants with normal birth weight (≥2500g and <4000g).

Exposures

Feeding Information

The age of first introduction infant formula and other food, and duration of breastfeeding was defined from several variables reported in the self-administered questionnaire at age 6 weeks as well as 6, 12, and 36 months. At each time point, if the response to the question "Has your child been fed infant formula?" was affirmative, the mother was asked to state the type of infant formula (standard cow's milk formula, hydrolyzed formula, preterm formula, other types of formulas) and the age when the child first had the infant formula. If the child has been fed any food other than milk (cereal, rice porridge, vegetables, fruits, meat, offal, fish, other seafood, egg yolk, egg white), the age when he/she first ate the food was recorded and taken as the age of solid food introduction.

Infant Outcomes

Anthropometric measurements were undertaken at each follow-up visit by trained fieldworkers. Abdomen circumference and upper arm circumference, in centimeters (cm), were measured in a supine position, using a measuring tape to the nearest 0.1 cm. Length (cm) was measured in a supine position using a length board (Shekel Healthweigh™) to the nearest 0.1 cm. Bodyweight, in kilograms (kg), was measured without shoes and with light clothing (single layer) in a supine position, using a stadiometer (Shekel Healthweigh™) to the nearest 0.01 kg. To account for additional weight due to clothes worn, 200 grams was subtracted from the weight of each child. Body mass index (BMI) was calculated using the formula kg/m^2. Children's sex and age-specific z-scores of BMI were calculated using SAS (WHO-source-code.sas) based on the 2006 WHO growth standards [15]. According to the WHO classifications for overweight and obesity in younger children (0–5 years), at-risk of overweight was defined as BMI-for-age z-score above +1 SD and less than +2 SDs, overweight was defined as above +2 SDs and less than +3 SDs, and obesity was defined as above+3 SDs [16]. Since the number of children with overweight and obesity was insufficient for reliable analyses, these high BMI statuses were analyzed as a single category in this study (overweight). Z-scores for a child's sex and age for weight and height (length-for-age z-score, weight-for-age z-score, weight-for-length z-score) based on the WHO Growth Charts were also calculated. The cutoffs for extreme z-scores (biologically implausible values) were used as <-5 and >5 according to the WHO criteria [15]. The extreme values were excluded from data analysis.

Covariates

Socio-demographic characteristics and potential confounders including maternal age, maternal educational level, maternal smoking and passive smoking status during pregnancy, maternal pre-pregnant BMI, paternal BMI, and other health-related factors, were obtained by the baseline questionnaire before 20 weeks of gestation. Obstetrics-related variables, including delivery date, mode of delivery, gestational age, birth weight, and infant sex, were extracted from the hospital clinical records.

Statistical Analysis

All statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC, USA). The timing of infant formula introduction was categorized in to 3 groups, including ≤3 months, 4-6 months, and >6 months or never received infant formula during the study period. The participants' characteristics were stratified by the timing of infant formula introduction (≤3 months, 4-6 months, and >6 months). The information on characteristics was presented as means and standard deviation (SD) for continuous variables or as percentages for categorical variables. The overall associations of timing of infant formula introduction with abdomen circumference, upper arm circumference, BMI z-score, length-for-age z-score, weight-for-age z-score, and weight-for-length z-score were analyzed with linear regression models and presented as β and 95% confidence intervals (95% CI). The associations between timing of infant formula introduction and risk of overweight were analyzed with logistic regression models and presented as odds ratio (OR) and 95% confidence intervals (95% CI). Results were adjusted for potential confounders including maternal age at delivery (≤25, 26-30, 31-35, >35 years of age), maternal education (high school or below, vocational/technical college, undergraduate, postgraduate), maternal pre-pregnancy BMI and paternal BMI (<18.5, 18.5-23.9, 24-27.9, ≥28 kg/m^2) [(17)], maternal smoking during pregnancy (yes, no), passive smoking during pregnancy (yes, no), parity (primiparous, multiparous), mode of delivery (vaginal labour, cesarean delivery), birth weight (g, continuous value) , infant sex, the duration of breastfeeding (0-6, >6 months) and age at first introduction to solid foods (≤3 months, 4-6 months, >6 months). For the analysis of children's length-for-age and weight-for-length z-scores, maternal and paternal heights were also adjusted. A two-tailed *P*-value <0.05 was considered statistically significant. Furthermore, a sensitivity analysis was performed by only including the children with longer breastfeeding duration (>6 months), because the longer duration of breastfeeding was associated with a lower risk of excess weight gain during early life [18].

Given the proportion of missing data on confounder variables were from 0.1% to 10.3%, analyses based on complete cases may be biased. Thus, we used multiple imputation (MI) analysis to cope with missing data [19]. We used the fully conditional method (FCS) iterative method for imputation by using SAS 9.4. The following variables were imputed: pre-pregnancy BMI, paternal BMI, maternal smoking during pregnancy, passive smoking during pregnancy, parity, mode of delivery, the duration of breastfeeding, and the timing of introduction of any solid food. For the analysis of children's length-for-age and weight-for-length z-scores, maternal and paternal heights were also imputed. Exposure and outcome variables of each model were considered as observed covariates and used in the models to impute these variables. For each imputation model, 10 imputations were run. We ran a procedure call proc mianalyze which combines all the estimates (coefficients and standard errors) across all the imputed datasets and outputs one set of parameter estimates for the model of interest [20]. The fraction of missing information (FMI) analysis was performed to determine potential efficiency gains from MI (**see online Supplementary Table S1 and Table S2**). Values of FMI range between 0 and 1. A smaller FMI (close to 0) indicates low variability between imputed data sets, which means observed data in the imputation model provide much information about the missing values [21].

Results

Characteristics of the study population

Characteristics of the participants included in the 1 year analysis based on the three infant formula introduction groups are shown in **Table 1.** Compared with infant formula introduction at 0-3 months group, the mothers in infant formula introduction after 6 months group were more likely to be younger and primiparous. The children in the later introduction group were more likely to be female, breastfed longer than 6 months, and introduced to solid food later. For the population included in the 3-year analysis, compared with the mothers who introduced formula feeding at 0-3 months, those who introduced formula after 6 months group were more likely to be better educated and multiparous, but experience higher levels of passive smoking during pregnancy. The children receiving formula later were, as expected, more likely to be breastfed longer than 6 months, and be introduced to solid food later (**see online Supplementary Table S3**). Characteristics of included and excluded participants of this study are also shown in **online Supplementary Table S4**. Compared with excluded population, included children were more likely to have higher educated and multiparous mothers, higher birthweight, and earlier formula introduction.

Table 2 shows the anthropometric outcomes including abdomen circumference (cm), upper arm circumference (cm), BMI, the prevalence of at-risk of overweight and overweight, at 1 year and 3 years old, respectively. The means of BMI at 1 year and 3 years old were different between the three infant formula introduction groups. The differences also existed in the prevalence of at-risk of overweight at 1 year and 3 years old between these groups. Furthermore, we provided the proportions of children were introduced to infant formula at each month after birth, including who had never received formula by 3 years in the supplementary file (**see online Supplementary Table S5**).

Timing of introduction of infant formula and anthropometric outcomes

Table 3 presents the associations between the timing of infant formula introduction and anthropometric outcomes at 1 year and 3 years of age. Compared with formula introduction within the first 3 months, introduction at 4-6 months was associated with lower upper arm circumference (adjusted β -0.15, 95%CI -0.26, -0.05), BMI z-score (adjusted β -0.18, 95% -0.26, -0.09), length-for-age z-score (adjusted β -0.09, 95% CI

-0.17, -0.01), weight-for-age z-score (adjusted β -0.21, 95% CI -0.29, -0.13), and weight-for-length z-score (adjusted β -0.19, 95%CI -0.27, -0.10) at 1 year of age in multiple imputation models. Compared with formula introduction at 0-3 months, introduction after 6 months was associated with lower length-for-age z-score (adjusted β -0.08, 95% CI -0.17, 0.00), and weight-for-age z-score (adjusted β -0.10, 95% CI -0.18, -0.01).

For 3 years outcome, compared with infant formula introduction within the first 3 months, introduction at 4-6 months of age was associated with lower BMI z-score (adjusted β -0.14, 95%CI -0.24, -0.03), weight-for-age z-score (adjusted β -0.11, 95%CI -0.21, -0.01), and weight-for-length z-score (adjusted β -0.14, 95%CI -0.25, -0.04) in multiple imputation models (**Table 3**).

Timing of introduction of infant formula and weight statuses during the first 3 years

Compared with infant formula introduction within the first 3 months of life, introduction at 4-6 months was associated with the lower odds of at-risk of overweight at 1 year (adjusted OR 0.73, 95% CI 0.55, 0.95) and 3 years old (adjusted OR 0.52, 95% CI 0.31, 0.87) in multiple imputation models (**Table 4**). Infant formula introduction at 4-6 months was associated with a lower risk of overweight at 1 year old (adjusted OR 0.43, 95% CI 0.22, 0.85), but the association was not significant for overweight at 3 years of age (adjusted OR 0.45, 95% CI 0.14, 1.47). These findings were similar when we restricted the analysis to children with longer breastfeeding duration (>6 months) (**see online Supplementary Table S6**).

Discussion

Results of this prospective longitudinal cohort indicated that compared to infant formula introduction within the first 3 months, introduction at 4-6 months was associated with the lower z-scores for BMI, weight-for-age and weight for length at ages 1 and 3 years. We also found that later formula introduction at 4-6 months of age was associated with the lower odds of at-risk of overweight at 1 and 3 years of age, and decreased odds of overweight at age of 1 year, but not at age 3 years. However, no significant association was observed between later introduction of formula after 6 months and the risk of overweight at ages 1 or 3 years.

Previous studies examining the associations between the timing of solid food introduction and risk of overweight or obesity found that the effects of the timing of solid food introduction on later development of obesity were different between breastfed and formula-fed infants [9, 11]. These results suggested the effects of solid food introduction on later weight status may be affected by formula feeding. However, studies focusing on examining the effects of timing of infant formula introduction on later overweight or obesity are limited. A longitudinal cohort study indicated that the risk of overweight or obesity was significantly higher among infants who were introduced to infant formula or solids during the first 4 months of life compared to those introduced later [22]. Nevertheless, they did not distinguish the effects between solid and infant formula introduction. Infant formula usually was introduced to infants much earlier than other solid foods. In our study population, over 80% infants were introduced to infant formula within the first 3 months of life, highlighting that infant formula is widespread used in this urban area of China. We found that infants who were introduced to infant formula at 4-6 months of age were more likely to have lower BMI, weight-for-age and weight-for-length z-scores at both 1 year and 3 years old than those introduced earlier (≤3 months), independent of the timing of solid food introduction.

Potential mechanisms of infant formula introduction at early stage on later weight status might be associated with the immature intestinal ecosystem and immune system during the first few months of life. The establishment and interactive development of early gut microbiota are driven and modulated by specific compounds present in breast milk [23]. Evidence showed that the Bifidobacteria and Lactobacillus were predominant in breastfed infants, whereas the Ruminococcus was predominant in formula-fed infants[24]. In children, a high concentration of Bifidobacteria during the early stage of life has been reported to have protective effects on later obesity [25]. The bacteria in the gut ferment dietary fibres into short-chain fatty acids (SCFAs), whose interaction with G-protein-coupled receptors (GPCRs) influences insulin sensitivity in several tissues, including liver, muscle, and adipose tissue, thus regulating energy metabolism[26]. Therefore, introduction of infant formula at early stage of infancy might influence the composition and ecosystem of the gut microbiome, which links to the development of childhood overweight or obesity. Furthermore, infant formula feeding induces uncontrolled excessive protein intake, which overacts the infant's mammalian target of rapamycin complex 1 (mTORC1) signaling pathways[27]. Overactivated mTORC1 enhances S6K1-mediated adipocyte differentiation. Thus, early formula feeding is considered to be associated with the development of mTORC1-driven metabolic disease, including obesity [27].

In our study, no significant differences in outcomes between ≤3 months and >6 months formula introduction groups were observed. The recommended timing for solid foods introduction is not earlier than 4 months or later than 6 months of life [28]. Late solid food introduction (≥7 months of age) was found to be associated with an increased risk of later childhood overweight/obesity among exclusively breastfed children (exclusive breastfeeding over 6 months)[29]. After 6 months of age, it is difficult to distinguish the impacts of later introduction of infant formula or solid foods on weight status. Formula feeding and complementary feeding might be not independent decisions, and may jointly explain variances in later obesity.

Although breast milk is recommended for all infants, preterm formulas are alternative sources of enteral nutrition for preterm or low birth weight infants when sufficient maternal breast milk and donor human milk are not available [30]. In addition, the association between feeding practice and excess weight during the early stage might be modified by birth weight [31]. Therefore, we performed the analysis by limiting it to full-term infants with normal birth weight.

Mothers participating in BIGCS are likely to be more affluent, older and have higher education than the contemporary pregnant women in Guangzhou, hence limiting the generalizability of the findings [14]. However, in this study, a relatively wide spread across all social-economic status indicators can be still observed within the participants of BIGCS, hence enabling us to explore the differences in health consequences across different social-economic status strata. Our data showed that a large proportion of infants were given infant formula as a supplement to breast milk (mix feeding) during the early months of life. And the main findings of this study remained significant in children with longer breastfeeding duration (over 6 months), though the longer breastfeeding was suggested to protect against subsequent obesity [18]. The aggressive marketing for infant formula and other breast milk substitutes has influenced the parents' preferences in China [32]. A study reporting the factors associated with Chinese mothers' decision to formula feeding showed that the majority of women choosing formula feeding thought they had insufficient breast milk [33]. The authors also found that some mothers had the belief that formula is more nutritious than breast milk [33]. This belief has also been described in some studies from other countries [34, 35]. In addition, child-related factors, such as weight and appetite in early life, can influence the parental feeding practices. A cohort study from UK reported that mothers of lower birth weight or lower appetite infants, or those perceiving their children are at risk of underweight tend to overfeed the infant in order to achieve greater weight gain [36]. The government and health professionals should provide more information on the differences in health benefits between breast milk and infant formula to let the parents understand that there's no better early food than breast milk for their young infants during the early stage.

One strength of our study is the longitudinal study design with a large number of participants, which enabled us to measure the associations with adequate statistical power. A further strength is the repeated assessment of feeding practice, at 6 weeks, 6 and 12 months, allowing specific descriptions of feeding patterns during infancy. A wide range of confounders was adjusted or controlled for in our multiple imputation models while assessing our exposure-outcome relationship. However, the associations might be confounded by some potential factors for which we did not adjust, such as the amount and the duration of infant formula consumption. Limitations of this study should be considered. First, the population size in 3 years age group was smaller than 1 year age group. Thus, there was a relatively lower power to detect differences at 3 years of age as opposed to 1 year of age. Second, the assessment of feeding practices was based on parental self-report, but recall of infant feeding practices is regarded as sufficiently accurate [37]. Third, a proportion of the cohort did not have anthropometry measured at 1 (37%) and 3 (51%) years, which limits the generalizability of our findings. In addition, a lower proportion (71.8%) of children without anthropometry data at 1 year were introduced to formula within the first 3 months, compared to that of those with anthropometry data (82.2%) (**see online Supplementary Table S3**). Based on our findings, it is possible that the included population is at a higher risk of overweight than those in the full cohort due to the higher proportion of children who have infant formula exposure at early stage. However, it is difficult to specify whether this difference would affect the associations that we found due to the absence of outcomes in the excluded population. Therefore, these results need to be replicated in other cohort studies before more firm recommendations can be made. Fourth, the information on the specific quantity of infant formula and solid food introduced was absent in this analysis. We also have no information on whether the consumption of infant formula was sustained after the introduction. Further evidence is needed to explore the short-term and long-term effects of the timing and quantity of infant formula introduction on the risk of overweight or obesity in later life.

Conclusion

Overall, compared with infant formula introduction within the first 3 months, introduction at 4-6 months was associated with the lower z-scores for BMI, weight-for-age and weight for length at both 1 year and 3 years old. Also, introduction after 3 months was associated with decreased odds of at-risk overweight at ages of 1 and 3 years. Although the results need to be replicated in other well-designed studies before more firm recommendations can be made, avoiding unnecessary infant formula introduction, particularly in the first 3 months, should be promoted to reduce the possibility of excess or rapid weight gain during early childhood.

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Conflict of Interest Disclosures

None.

Authorship

MY, GNT, XQ, and KKC conceived the study design; MY, ML, YG, JL, JH, SS and DW collected the data; MY conducted the data analysis and wrote the initial manuscript. YG, JL, KBHL and JH assisted with the statistical analysis. All authors contributed to data interpretation and the writing of the manuscript and critically reviewed and approved the final manuscript.

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Table 1 Baseline characteristics of participants in different timing of infant formula introduction groups in the BIGCS study

Characteristics	Timing of any infant formulas introduction, months			*P*-Value	No. of cases missing out of 5733 (%)
	≤3 m (n=4698)	4-6 m (n=522)	>6 m[a] (n=513)		
Mother					
Age at delivery (years), mean (SD)	29.4 (3.4)	29.1 (3.0)	29.0 (3.2)	0.030	0 (0)
Educational level, n (%)				0.066	0 (0)
High school or below	367 (7.8)	37 (7.1)	34 (6.6)		
Vocational/technical college	1193 (25.4)	119 (22.8)	112 (21.8)		
Undergraduate	2611 (55.6)	289 (55.4)	296 (57.7)		
Postgraduate	527 (11.2)	77 (14.8)	71 (13.8)		
Pre-pregnancy BMI, n (%)				0.373	137 (2.4)
<18.5 kg/m^2	1139 (24.9)	121 (23.6)	109 (21.6)		
18.5-23.9 kg/m^2	3004 (65.6)	338 (65.9)	352 (69.7)		
24-27.9 kg/m^2	370 (8.1)	42 (8.2)	36 (7.1)		
≥28kg/m^2	65 (1.4)	12 (2.3)	8 (1.6)		
Height (cm), mean (SD)	160.0 (4.9)	159.7 (4.9)	160.0 (4.7)	0.550	41 (0.7)
Parity, n (%)				0.012	3 (0.1)
Primiparous	4173 (88.9)	474 (90.8)	437 (85.2)		
Multiparous	522 (11.1)	48 (9.2)	76 (14.8)		
Delivery mode, n (%)				0.561	1(0)
Vaginal labor	3064 (65.2)	348 (66.7)	345 (67.3)		
Cesarean delivery	1633 (34.8)	174 (33.3)	168 (32.7)		
Smoking during pregnancy, n (%)	25 (0.5)	4 (0.8)	3 (0.6)	0.792	40 (0.7)
Passive smoking during pregnancy, n (%)	1458 (31.3)	169 (32.6)	149 (29.2)	0.475	38 (0.7)
Father					
BMI, n (%)				0.668	592 (10.3)
<18.5 kg/m^2	182 (4.3)	25 (5.5)	14 (2.9)		
18.5-23.9 kg/m^2	2361 (56.1)	253 (55.9)	276 (57.9)		
24-27.9 kg/m^2	1351 (32.1)	143 (31.6)	151 (31.7)		
≥28kg/m^2	317 (7.5)	32 (7.1)	36 (7.6)		
Height (cm), mean (SD)	172.7 (5.3)	172.7 (5.1)	172.8 (5.2)	0.874	102 (1.8)
Child					

Child's gender, n (%)				0.006	
Male	2498 (53.2)	241 (46.2)	257 (50.1)		0 (0)
Female	2200 (46.8)	281 (53.8)	256 (49.9)		
Birth weight (g), mean (SD)	3219.0 (328.5)	3197.4 (321.0)	3219.4 (327.6)	0.356	0 (0)
Duration of any breastfeeding, n (%)				<0.001	161 (2.8)
≤6 months	1304 (28.7)	98 (18.8)	2 (0.4)		
>6 months	3234 (71.3)	424 (81.2)	510 (99.6)		
Timing of solid food introduction				<0.001	8 (0.1)
≤3 m	501 (10.7)	33 (6.3)	34 (6.6)		
4-6 m	4094 (87.3)	476 (91.2)	460 (89.8)		
>6 m	96 (2.1)	13 (2.5)	18 (3.5)		

[a]The children who had never received infant formula during the study period were combined into >6 months group.

Table 2 Anthropometric outcomes in different timing of infant formula introduction groups in the BIGCS study

Anthropometric outcomes	Timing of any infant formulas introduction, months			*P*-Value
	≤3 m	4-6 m	>6 m[a]	
at 1 year of age, mean (SD)	**n=4698**	**n=522**	**n=513**	
Abdomen circumference (cm)	42.9 (4.0)	42.7 (3.5)	42.9 (3.9)	0.749
Upper arm circumference (cm)	14.5 (1.2)	14.3 (1.1)	14.4 (1.2)	0.006
BMI (kg/m^2)	17.0 (1.4)	16.7 (1.4)	17.0 (1.4)	<0.001
Overweight, n (%)	180 (3.9)	9 (1.8)	14 (2.8)	0.019
At-risk of overweight, n (%)	992 (21.6)	82 (16.3)	104 (20.8)	0.021
at 3 years of age, mean (SD)	**n=2955**	**n=327**	**n=292**	
Abdomen circumference (cm)	46.3 (4.5)	46.1 (4.3)	46.2 (4.2)	0.896
Upper arm circumference (cm)	14.9 (1.1)	14.7 (1.1)	14.9 (1.2)	0.113
BMI (kg/m^2)	15.5 (1.3)	15.3 (1.1)	15.5 (1.2)	0.011
Overweight, n (%)	62 (2.2)	3 (0.9)	5 (1.8)	0.193
At-risk of overweight, n (%)	349 (12.2)	20 (6.3)	30 (10.7)	0.007

BMI: Body Mass Index

[a]The children who had never received infant formula during the study period were combined in to >6 months group.

Table 3 Linear regression models to evaluate the associations between timing of formula introduction and anthropometric outcomes at 1 year and 3 years of age (Before and after multiple imputation)

Anthropometric outcomes	Timing of formula introduction	Outcomes at 1 year of age				Outcomes at 3 years of age (n=3898)			
		Crude β (95% CI)	Adjusted β before MI (95% CI)	Adjusted β after MI (95% CI)	P-Value[‡]	Crude β (95% CI)	Adjusted β before MI (95% CI)	Adjusted β after MI (95% CI)	P-Value[c]
Abdomen circumference[a]		n=5590	n=4555	n=5590		n=3473	n=2768	n=3473	
	≤3 m	Ref.	Ref.	Ref.		Ref.	Ref.	Ref.	
	4-6 m	-0.14 (-0.52 ; 0.23)	0.13 (-0.27 ; 0.53)	-0.03 (-0.41 ; 0.34)	0.857	-0.13 (-0.66 ; 0.40)	0.17 (-0.42 ; 0.75)	-0.04 (-0.56 ; 0.48)	0.874
	>6 m[d]	-0.01 (-0.38 ; 0.36)	-0.05 (-0.45 ; 0.34)	-0.04 (-0.42 ; 0.33)	0.824	-0.01 (-0.58 ; 0.55)	-0.23 (-0.85 ; 0.38)	-0.05 (-0.62 ; 0.52)	0.869
Upper arm circumference[a]		n=5382	n=4543	n=4543		n=3338	n=2794	n=3338	
	≤3 m	Ref.	Ref.	Ref.		Ref.	Ref.	Ref.	
	4-6 m	-0.17 (-0.28 ; -0.06)	-0.15 (-0.27 ; -0.04)	-0.15 (-0.26 ; -0.05)	0.004	-0.11 (-0.24 ; 0.02)	-0.07 (-0.20 ; 0.07)	-0.09 (-0.22 ; 0.04)	0.155
	>6 m[d]	-0.05 (-0.16 ; 0.06)	-0.05 (-0.17 ; 0.06)	-0.04 (-0.15 ; 0.06)	0.424	0.08 (-0.06 ; 0.21)	-0.01 (-0.15 ; 0.13)	0.05 (-0.09 ; 0.19)	0.472
BMI z-score[a]		n=5590	n=4715	n=5590		n=3473	n=2905	n=3473	
	≤3 m	Ref.	Ref.	Ref.		Ref.	Ref.	Ref.	
	4-6 m	-0.17 (-0.26 ; -0.08)	-0.17 (-0.26 ; -0.07)	-0.18 (-0.26 ; -0.09)	<0.001	-0.16 (-0.27 ; -0.05)	-0.13 (-0.25 ; -0.02)	-0.14 (-0.24 ; -0.03)	0.014
	>6 m[d]	-0.01 (-0.10 ; 0.08)	-0.06 (-0.16 ; 0.03)	-0.05 (-0.14 ; 0.04)	0.270	-0.02 (-0.13 ; 0.10)	-0.09 (-0.21 ; 0.04)	-0.03 (-0.14 ; 0.09)	0.647
Length-for-age z-score[b]		n=5652	n=4838	n=5652		n=3492	n=2979	n=3492	
	≤3 m	Ref.	Ref.	Ref.		Ref.	Ref.	Ref.	
	4-6 m	-0.12 (-0.21 ; -0.03)	-0.09 (-0.18 ; -0.01)	-0.09 (-0.17 ; -0.01)	0.028	-0.05 (-0.16 ; 0.06)	-0.04 (-0.15 ; 0.06)	-0.05 (-0.14 ; 0.05)	0.358
	>6 m[d]	-0.11 (-0.20 ; -0.02)	-0.06 (-0.15 ; 0.02)	-0.08 (-0.17 ; 0.00)	0.045	0.05 (-0.06 ; 0.17)	-0.01 (-0.12 ; 0.11)	0.03 (-0.08 ; 0.13)	0.600
Weight-for-age z-score[a]		n=5641	n=4758	n=5641		n=3518	n=2944	n=3518	
	≤3 m	Ref.	Ref.	Ref.		Ref.	Ref.	Ref.	
	4-6 m	-0.21 (-0.30 ; -0.13)	-0.21 (-0.30 ; -0.12)	-0.21 (-0.29 ; -0.13)	<0.001	-0.11 (-0.24 ; 0.01)	-0.10 (-0.21 ; 0.00)	-0.11 (-0.21 ; -0.01)	0.030

	>6 m[d]	-0.08 (-0.16 ; 0.01)	-0.10 (-0.19 ; -0.01)	-0.10 (-0.18 ; -0.01)	0.021	0.04 (-0.09 ; 0.17)	-0.02 (-0.13 ; 0.10)	0.03 (-0.08 ; 0.14)	0.581
Weight-for-length z-score[b]		n=5590	n=4785	n=5590		n=3483	n=2970	n=3483	
	≤3 m	Ref.	Ref.	Ref.		Ref.	Ref.	Ref.	
	4-6 m	-0.19 (-0.28 ; -0.10)	-0.17 (-0.27 ; -0.08)	-0.19 (-0.27 ; -0.10)	<0.001	-0.17 (-0.28 ; -0.06)	-0.14 (-0.25 ; -0.02)	-0.14 (-0.25 ; -0.04)	0.008
	>6 m[d]	-0.03 (-0.11 ; 0.06)	-0.07 (-0.16 ; 0.02)	-0.06 (-0.15 ; 0.02)	0.153	-0.01 (-0.13 ; 0.10)	-0.09 (-0.20 ; 0.03)	-0.03 (-0.14 ; 0.09)	0.638

BMI: Body Mass Index; MI: Multiple Imputation

[a]Adjusted for maternal age at delivery, maternal education, maternal pre-pregnancy BMI and paternal BMI, maternal smoking during pregnancy, passive smoking during pregnancy, parity, mode of delivery, infant sex, birth weight, the duration of breastfeeding, and age at first introduction to solid foods

[b]Adjusted for maternal age at delivery, maternal education, maternal pre-pregnancy BMI, maternal height, paternal BMI, paternal height, maternal smoking during pregnancy, passive smoking during pregnancy, parity, mode of delivery, infant sex, birth weight, the duration of breastfeeding, and age at first introduction to solid foods

[c]Adjusted p-values for multiple imputation models

[d]The children who had never received infant formula during the study period were combined in to >6 months group.

Table 4 Logistic regression models to evaluate the associations of timing of formula introduction with at-risk overweight and overweight at 1 year and 3 years of age

Timing of introduction, months	Case （n%）	Crude OR (95% CI)	Adjusted before MI OR (95% CI)	Adjusted after MI OR (95% CI)	*P*-Value[a]
At-risk of overweight					
1 year of age		n=5373	n=4595	n=5373	
≤3 m	806 (18.3)	1	1	1	
4-6 m	69 (14.1)	0.73 (0.56-0.95)	0.73 (0.54-0.98)	0.73 (0.55-0.95)	0.015
>6 m[b]	86 (17.8)	0.97 (0.76-1.23)	0.91 (0.69-1.18)	0.89 (0.69-1.15)	0.367
3 years of age		n=3403	n=2904	n=3403	
≤3 m	287 (10.2)	1	1	1	
4-6 m	17 (5.4)	0.50 (0.30-0.83)	0.55 (0.32-0.95)	0.52 (0.31-0.87)	0.014
>6 m[b]	25 (9.1)	0.88 (0.57-1.35)	0.81 (0.49-1.32)	0.93 (0.60-1.45)	0.752
Overweight					
1 year of age		n=5590	n=4715	n=5373	
≤3 m	180 (3.9)	1	1	1	
4-6 m	9 (1.8)	0.45 (0.23-0.88)	0.43 (0.21-0.89)	0.43 (0.22-0.85)	0.016
>6 m[b]	14 (2.8)	0.70 (0.41-1.22)	0.60 (0.32-1.09)	0.60 (0.33-1.07)	0.085
3 years of age		n=3473	n=2905	n=3473	
≤3 m	62 (2.2)	1	1	1	
4-6 m	3 (0.9)	0.43 (0.13-1.38)	0.56 (0.17-1.81)	0.45 (0.14-1.47)	0.188
>6 m[b]	5 (1.8)	0.82 (0.33-2.06)	0.41 (0.10-1.74)	0.89 (0.34-2.29)	0.802

MI: multiple imputation

Adjusted for maternal age at delivery, maternal education, maternal pre-pregnancy BMI and paternal BMI, maternal smoking during pregnancy, passive smoking during pregnancy, parity, mode of delivery, infant sex, birth weight, the duration of breastfeeding, and age at first introduction to solid foods.

[a] P-values for multiple imputation models

[b]The children who had never received infant formula during the study period were combined in to >6 months group.

Figure 1 Flow chart of the study population