**Timing of introduction of complementary foods, breastfeeding, and child cardiometabolic risk: a prospective multi-ethnic Asian cohort study**

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Trial registration number: NCT01174875

URL of registration: https://clinicaltrials.gov/ct2/show/NCT01174875

Sources of support:This work was supported by the Singapore National Research Foundation under its Translational and Clinical Research (TCR) Flagship Programme and administered by the Singapore Ministry of Health’s National Medical Research Council (NMRC), Singapore [NMRC/TCR/004-NUS/2008, NMRC/TCR/012-NUHS/2014]. KMG is supported by the UK Medical Research Council (MC\_UU\_12011/4), the National Institute for Health Research (NIHR Senior Investigator (NF-SI-0515-10042) and NIHR Southampton Biomedical Research Centre (IS-BRC-1215-20004)), the European Union (Erasmus+ Programme ImpENSA 598488-EPP-1-2018-1-DE-EPPKA2-CBHE-JP) and the British Heart Foundation (RG/15/17/3174). Additional funding is provided by the Singapore Institute for Clinical Sciences, Agency for Science Technology and Research, Singapore.

Conflict of interest: KMG and SYC are part of an academic consortium that has received research funding from Nestle. YSL has given lectures in events sponsored by Nestle and Abbott. All other authors have nothing to disclose.

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Data described in the manuscript, code book, and analytic code will be made available upon request, pending application and approval.

Running title: Infant feeding and child cardiometabolic risk

Abbreviations

BF – breastfeeding

BMI – body mass index

CF – complementary feeding

GUSTO – Growing Up in Singapore Towards healthy Outcomes

HDL – high density lipoprotein cholesterol

HOMA-IR – homeostasis model assessment of insulin resistance

SDS – standard deviation score

z-BMI – body mass index z-score

**Abstract**

Background: Timing of introduction of complementary foods and duration of breastfeeding have been independently associated with child overweight and obesity but their combined influence on body fat partitioning and cardiometabolic risk is unclear.

Objective: We investigated associations of timing of introduction of complementary foods, duration of breastfeeding, and their interaction with child adiposity and cardiometabolic risk markers.

Methods: We analyzed data from 839 children in the prospective Growing Up in Singapore Towards healthy Outcomes (GUSTO) cohort. Mothers reported the age at which infants were first fed complementary foods and breastfeeding duration, classified as early (≤4months) vs. typical (>4months) complementary feeding (CF) and short (≤4months) vs. long (>4months) duration of any breastfeeding (BF), respectively. We measured adiposity and cardiometabolic risk markers at age 6 years and examined their associations with infant feeding patterns using multiple regression, adjusting for socio-demographics, parents’ body mass index (BMI), maternal factors, birthweight-for-gestational-age, and infant weight gain.

Results: Of 839 children, 18% experienced early CF while 54% experienced short BF. Short (vs. long) BF and early (vs. typical) CF were independently associated with higher z-BMI [β (95% CI), short BF: 0.18 SDS (-0.01,0.38); early CF: 0.34 SDS (0.11,0.57)] and sum of skinfolds [short BF: 1.83 mm (0.05,3.61); early CF: 2.73 mm (0.55,4.91)]. Children who experienced both early CF and short BF (vs. typical CF-long BF) had synergistically higher diastolic blood pressure [1.41 mmHg (-0.15,2.97), p-interaction=0.023] and metabolic syndrome score [0.81 (0.16,1.47), p-interaction=0.081]. Early CF-long BF (vs. early CF-short BF) was associated with lower systolic blood pressure [-3.74 mmHg (-7.01,-0.48)], diastolic blood pressure [-2.29 mmHg (-4.47,-0.11)], and metabolic syndrome score [-0.90 (-1.80,0.00)].

Conclusions: A combination of early CF and short BF was associated with elevated child adiposity and cardiometabolic markers. Longer breastfeeding duration may protect against cardiometabolic risk associated with early complementary feeding.

Keywords: Timing of complementary foods introduction; Complementary feeding; Breastfeeding; Infant feeding; Child obesity; Adiposity; Cardiometabolic; Metabolic syndrome

Introduction

Childhood obesity is a global public health concern (1), with infant feeding practices implicated in programming later obesity risk (2,3). Guidelines recommend exclusive breastfeeding for at least 6 months (4) and introducing solid or liquid food items other than human milk or infant formula between the ages 4-6 months (5). Early introduction of complementary foods before age 4 months (6–8), never breastfeeding, and short duration of breastfeeding (9–13) are modifiable infant feeding practices that have been linked with child obesity/adiposity.

Some observational studies examining associations of timing of complementary foods introduction or duration of breastfeeding with childhood obesity have yielded conflicting results (7,11,12). The inconsistent findings could reflect limitations, including short study duration, small sample size, cross-sectional study design, inconsistent definitions of infant feeding categories, and lack of adjustment for key confounders such as parental body mass index (BMI), gestational tobacco exposure, and infant weight gain(11–13). Confounding by early infant growth is particularly salient as rapid infant weight gain associated with later obesity risk (14) and can affect parental perceptions of the need for their child to be fed formula milk or complementary foods (15,16) rather than breastfed. Few studies have examined the extent to which timing of introduction of complementary foods or duration of breastfeeding are associated with child body fat partitioning, a stronger predictor of later cardiometabolic disease development than BMI (17). Even fewer studies have assessed associations with cardiometabolic risk markers such as arterial thickness/stiffness and pediatric metabolic syndrome score, which might provide additional insights on the early pathways to the development of cardiometabolic diseases. Furthermore, both complementary feeding and breastfeeding are highly interrelated (18); the interaction between complementary feeding and breastfeeding is important for infant weight gain, child gut microbiota composition, adiposity, and overweight/obesity (19–23). Yet, few studies have examined these associations in Asian cohorts, important given the differing sociocultural norms relative to Western countries related to infant feeding practices (24) and the rising prevalence of childhood obesity in Asia (25).

To address these research gaps, the present study aimed to investigate the associations between the timing of complementary feeding, the duration of breastfeeding, and their multiplicative influence on child adiposity and cardiometabolic risk markers in a prospective multi-ethnic Asian cohort. We considered adiposity and body fat partitioning measurements to be the primary outcomes, and the rest of the cardiometabolic measures to be secondary outcomes. We hypothesized that a combination of early introduction of complementary foods and short breastfeeding duration are associated with not just increased adiposity but also increased cardiometabolic risk markers manifesting in children aged 6 years, and that a longer duration of breastfeeding might ameliorate the associations between early complementary feeding and adiposity/cardiometabolic risk.

Materials and Methods

*Study population*

We studied participants from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) prospective mother-offspring cohort described previously (26). Briefly, we recruited pregnant women in their first trimester from KK Women’s and Children’s Hospital and National University Hospital from June 2009 to October 2010. Eligibility criteria include Singapore citizens/permanent residents aged at least 18 years, of homogenous parental ethnic background, and exclusion criteria include receiving chemotherapy, on psychotropic drugs, or having type 1 diabetes. Of 1180 singleton deliveries, we included 839 children who had infant feeding data (**Supplementary figure 1**). The National Healthcare Group Domain Specific Review Board and SingHealth Centralized Institutional Review Board approved this study and participants provided written informed consent.

*Exposures*

*Breastfeeding –* We obtained information on breastfeeding practices and duration using interviewer-administered questionnaires at 3 weeks postnatally and at 3-monthly intervals from ages 3 to 18 months. We defined “any breastfeeding” as any exposure to breastfeeding (fed directly at the breast or consumed expressed human milk), regardless of what else the child consumed We defined short duration of breastfeeding (BF) as any BF ≤ 4 months and long BF as any BF > 4 months based on previous studies (21,27) as well as the local context – current labor policies in Singapore allow up to ~4 months of maternity leave for mothers (28), and most mothers would not continue breastfeeding beyond 4 months once they have returned to work (29).

*Complementary feeding –* At age 9 months, trained research assistants asked mothers to report the age at which infants were first fed solid foods other than human milk or formula milk on a daily basis via questionnaires. We defined early complementary feeding (CF) as CF initiated at ≤ age 4 months and typical CF as CF initiated at > age 4 months, similar to previous studies (30) and in accordance with the European Society for Pediatric Gastroenterology, Hepatology and Nutrition guidelines (5). We confirm that timing of introduction of complementary feeding was ascertained after the period of exclusive/predominant breastfeeding for all participants as exclusively/predominantly breastfed children should not be consuming any complementary foods.

*Child Adiposity Outcomes at 6 years old*

Trained research assistants measured standing height (SECA213 stadiometer), weight (SECA803 Weighing Scale), abdominal circumference (measuring tape) and skinfold thicknesses (triceps, biceps, subscapular and supra-iliac skinfolds) from the right side of the body using Holtain skinfold calipers (Holtain, Ltd., Crymych, UK). We calculated sex- and age-standardized z-scores of body mass index (z-BMI) using World Health Organization growth standards (31) and calculated the sum of skinfold thicknesses measured at the four sites.

We measured body fat partitioning to the abdominal adipose tissues, liver, and skeletal muscle using standardized protocols described previously (32). Briefly, we performed abdominal magnetic resonance imaging in children without sedation using a Siemens Skyra 3T magnetic resonance scanner to derive volumes of abdominal subcutaneous adipose tissue and visceral adipose tissue by segmentation (33). To ensure good precision, we used stabilization measures (foam pads, straps, etc.) to minimize motion during the scan and conducted a fast 2D imaging sequence which is robust to respiratory motion. Children were informed about the importance of remaining still during the scan and were allowed to watch their favorite movie while in the scanner to further limit their movement. We assessed intramyocellular lipids in the soleus muscle and liver fat by proton magnetic resonance spectroscopy. We measured intramyocellular lipids as a percentage of the water signal which is proportional to lipid accumulation within skeletal muscles, and liver fat as a percentage by weight, averaged across the right and left liver lobe scans.

*Child Cardiometabolic Outcomes at 6 years old*

Using standardized protocols, trained research staff measured peripheral systolic and diastolic blood pressure from the right upper arm (Dinamap CARESCAPE V100, GE Healthcare, Milwaukee, WI) in a quiet room. We also measured carotid intima-media thickness using high resolution B-mode ultrasound (CX-50 XMatrix, Philips Medical Ultrasound Systems at KK Women’s and Children’s Hospital and Aloka at National University Hospital) at the right common carotid artery 1 cm proximal to the carotid bulb. With the child in the supine position, we conducted applanation tonometry (SphygmoCorVx, AtCor Medical, West Ryde, NSW, Australia) to derive carotid-femoral pulse wave velocity from the carotid-femoral path length and carotid-femoral transit time.

We instructed parents to ensure their child undergoes an overnight fast for at least 8 hours prior to the study visit. Before blood collection, we asked parents: “Has your child fasted for at least 8 hours?”; if the answer was “no”, the study visit was rearranged. We drew venous blood to measure fasting plasma glucose (Abbott Architect c8000 analyzer at KK Women’s and Children’s Hospital and Beckman AU5800 analyzer at National University Hospital), serum insulin using a sandwich immunoassay (Beckman DXL800 analyzer, Beckman Coulter), high density lipoprotein cholesterol (HDL) using the enzymatic method (Beckman AU5800 analyzer, Beckman Coulter), and triglycerides using the colorimetric method (Beckman AU5800 analyzer, Beckman Coulter). We calculated the homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula (34): [fasting insulin (mU/L) \* fasting glucose (mmol/L)] / 22.5.

To assess cardiometabolic risk holistically, we calculated a pediatric metabolic syndrome score based on a previously published equation (35). First, we derived cohort-specific and sex-standardized z-scores of abdominal circumference, systolic blood pressure, diastolic blood pressure, HOMA-IR, triglycerides, and HDL. Due to its inverse association with metabolic risk, we inverted the z-score of HDL by multiplying −1 to all values. Subsequently, we calculated the metabolic syndrome score as the sum of z-scores for the four components of cardiometabolic risk: 1) abdominal circumference, 2) mean z-scores of systolic and diastolic blood pressure, 3) HOMA-IR and 4) mean z-scores of triglycerides and inverted HDL.

*Covariates*

We collected socio-demographic data (mother’s age, highest educational attainment, total household income, self-identified ethnicity) and self-reported pre-pregnancy weight at recruitment or the first clinic visit using interviewer-administered questionnaires. Mothers reported their smoking status and environmental (home or work) tobacco exposure through interviewer-administered questionnaires at gestational week 26-28. At the gestational week 26-28 visit, we measured maternal height using a calibrated stadiometer (SECA213 Stadiometer, SECA Corp, Hamburg, Germany) andfasting plasma glucose by colorimetry [Advia 2400 Chemistry system (Siemens Medical Solutions Diagnostics, Deerfield, IL, USA); Beckman LX20 Pro analyser (Beckman Coulter, USA)]. We calculated total gestational weight gain by subtracting self-reported pre-pregnancy weight from last measured prenatal weight (within 4wks before delivery) and classified mothers into categories of gestational weight gain based on Institute of Medicine guidelines (36). We obtained infant birthweight, sex, and parity from medical records, calculated gestational age based on first trimester ultrasound scans conducted by trained ultrasonographers, and derived cohort-specific birthweight percentiles, adjusted for sex and gestational age (37).

During the postnatal year 2 or 3 visit, trained research staff measured father’s height (SECA 213 Stadiometer, SECA Corp., Hamburg, Germany) and weight (SECA 803 Weighing Scale, SECA Corp., Hamburg, Germany) to calculate BMI. At birth and age 3 months, we measured infant weight using a calibrated scale (SECA 334 Weighing Scale, SECA Corp., Hamburg, Germany) and derived sex- and age-standardized weight z-scores (z-weight) using World Health Organization growth standards (31) to calculate infant z-weight gain.

*Statistical analysis*

We performed all analyses using Stata17.0 (StataCorp LP, TX) and considered two-sided p-value < 0.05 as the threshold for significant difference. We performed two-tailed t-tests (for continuous variables, comparing between two groups), one-way analysis of variance (for continuous variables, comparing between four infant feeding groups), and chi-square tests (for categorical variables).

To account for missing data, we used chained equation multiple imputation to impute missing covariates and outcomes (number of missing values for the following outcomes: z-BMI, 30; sum of skinfolds, 127; abdominal circumference, 42; SAT, 390; VAT, 384; liver fat, 406; intramyocellular lipids, 407; blood pressure, 138; carotid intima media thickness, 339; pulse wave velocity, 305; fasting plasma glucose, 285; HOMA-IR, 397; triglycerides, 393; HDL, 393; metabolic syndrome score, 437) among children with at least one outcome measurement – we excluded children with missing data for all outcomes (n=106) as their inclusion would mean that every single outcome measure would be imputed rather than measured, which might induce bias. The imputation model included all exposure, outcome, and covariates under study (38). We generated 50 imputed datasets.

The linearity assumption was satisfied after testing for non-linearity by including spline terms (39). To examine the independent effects of BF and CF on child adiposity and cardiometabolic outcomes, we performed pooled multiple linear regression analyses that mutually adjusted for long (vs. short) BF and early (vs. typical) CF using the “*mi estimate*” command. We adjusted for socio-demographic confounders (maternal age, maternal education, household income, ethnicity, parity), parents’ BMI (father’s BMI, pre-pregnancy BMI), maternal factors (gestational tobacco exposure, fasting plasma glucose, gestational weight gain), and infant characteristics (sex, gestational age, birthweight-for-gestational age). In models investigating blood pressure as the outcome, we additionally adjusted for child’s height at age 6 years (40). Additionally, as infant weight gain may not only be a common cause of early breastfeeding cessation/early introduction of complementary foods (15,16) and later cardiometabolic risk but also a potential mediator where early breastfeeding cessation might lead to increased child adiposity through greater infant weight gain (41), we investigated models with and without adjusting for infant weight gain.

To test for interacting effects, we performed pooled multiple linear regression analyses with the “*mi estimate*” command to investigate the associations of early (vs. typical) CF, long (vs. short) BF, and their interaction term (CF x BF) with child adiposity and cardiometabolic outcomes, adjusted for the same confounders described above. To visualize the effects of this interaction, we used the “*mimrgns*” command to calculate adjusted predicted values of child outcomes for each infant feeding group ‒ typical CF-long BF (reference), typical CF-short BF, early CF-long BF, and early CF-short BF ‒ while holding covariates at the mean, using Rubin’s combination rules to obtain pooled estimates from imputed datasets (42). We made post-hoc comparisons using the “*pwcompare”* command to compare between adjusted predictions of each of the three infant feeding groups and the reference infant feeding group (typical CF-long BF), as well as between the early CF-long BF and early CF-short BF groups.

As sensitivity analyses, we conducted complete case analysis for all regression models to assess the robustness of our findings. We examined the independent effects of BF and CF as continuous variables in addition to the *a priori* defined BF and CF categories.

Results

*Cohort description*

Among 839 included children, the mean (SD) age at cessation of any BF is 7.43 (10.1) months; 1.44 (1.24) months among 454 children with short BF and 14.5 (11.3) months among 385 children with long BF. The mean (SD) age at introduction of CF is 5.67 (1.15) months; 3.86 (0.44) months among 155 children with early CF and 6.08 (0.81) months among 684 children with typical CF (**Supplementary Figure 1**). Children from the early CF-short BF group (vs. typical CF-long BF) had mothers who were younger, with lower education, lower monthly household income, more likely to be of minority ethnic groups (Malays, Indians), with higher gestational tobacco exposure, and higher pre-pregnancy BMI (**Table 1**). Children from the short (vs. long) BF groups as well as early (vs. typical) CF groups had lower infant weight gain from birth to 3 months. Excluded (vs. included) participants were younger, had lower maternal educational attainment, were more likely to be of Malay or Indian ethnicity, had higher tobacco exposure, and lower gestational age (**Supplementary Table 1**). Participants with (vs. without) missing outcome measurements had slightly lower gestational age (**Supplementary Table 2**). We present the frequency distribution of outcomes by BF categories in **Supplementary Figure 2** and CF categories in **Supplementary Figure 3**, as well as the distribution of infant feeding types in the first 6 months in **Supplementary Figure 4**.

*Independent effects of breastfeeding and complementary feeding*

Adjusting for confounders and infant weight gain, short (vs. long) BF was associated with increased z-BMI [β (95% CI), 0.18 SDS (-0.01, 0.38), p=0.064] and sum of skinfolds [1.83 mm (0.05, 3.61)] but not with any other cardiometabolic outcomes (**Table 2**). Early (vs. typical) CF was associated with increased z-BMI [0.34 SDS (0.11, 0.57)], sum of skinfolds [2.73 mm (0.55, 4.91)], abdominal circumference [1.00 cm (-0.06, 2.06), p=0.063], and fasting glucose [0.08 mmol/L (0.00, 0.16), p=0.055] but not with any other cardiometabolic outcomes (**Table 3**).

*Interaction between complementary feeding and breastfeeding*

The interaction between CF and BF reached statistical significance for systolic blood pressure (p=0.020), diastolic blood pressure (p=0.023), and trended toward significance for metabolic syndrome score (p=0.081) (**Supplementary Table 3**). Adjusted differences in cardiometabolic risk markers considering the interactive effects are presented in **Figure 1** (where p-interaction < 0.1) and **Supplementary Figure 5** (where p-interaction ≥ 0.1). Adjusting for confounders and infant weight gain (model 2), early CF-short BF (vs. typical CF-long BF) was associated with increased diastolic blood pressure [1.41 mmHg (-0.15, 2.97), p=0.077] and metabolic syndrome score [0.81 (0.16, 1.47)]. Comparing between the early CF groups, early CF-long BF (vs. early CF-short BF) was associated with lower systolic blood pressure [-3.74 mmHg (-7.01, -0.48)], diastolic blood pressure [-2.29 mmHg (-4.47, -0.11)], and metabolic syndrome score [-0.90 (-1.80, 0.00), p=0.051].

*Sensitivity analyses*

Similar associations were found in complete case analysis (**Supplementary Table 4, Supplementary Table 5**) where early (vs. typical) CF was associated with increased z-BMI [0.42 SDS (0.14, 0.69)], sum of skinfolds [4.35 mm (1.77, 6.94)], and abdominal circumference [1.30 cm (0.08, 2.53)] (). In complete case analysis, adjusting for confounders and infant weight gain, early CF-short BF (vs. typical CF-long BF) was associated with increased diastolic blood pressure [2.08 mmHg (0.16, 4.01)] and metabolic syndrome score [1.20 (0.02, 2.37)], while early CF-long BF (vs. early CF-short BF) was associated with lower systolic blood pressure [-5.22 mmHg (-9.09, -1.35)], diastolic blood pressure [-3.37 mmHg (-5.97, -0.77)], and metabolic syndrome score [-1.87 (-3.31, -0.43)] (**Supplementary Figure 6**). Similar associations were also found when investigating BF (**Supplementary Table 6**) and CF (**Supplementary Table 7**) as continuous variables.

**Discussion**

In a prospective multi-ethnic Asian cohort, we found that short breastfeeding duration and early introduction of complementary foods were independently associated with increased overall child adiposity. We also noted a synergistic combination of both factors in relation to increased blood pressure and metabolic syndrome score at age 6 years – clear elevations in these cardiometabolic risk markers were not seen when children were exposed to only one of either factor alone. Providing novel insights on the early development of cardiometabolic risk associated with infant feeding, we found that being exposed to an adverse combination of early complementary feeding and shorter breastfeeding duration was associated with higher overall adiposity, blood pressure, and metabolic syndrome score without concomitant associations with higher abdominal visceral adipose tissue, liver fat, intramyocellular lipids, arterial thickness/stiffness, triglycerides, or lower HDL. Some of these cardiometabolic risk markers might not be elevated yet at age 6 years, perhaps because the interrelated cluster of adiposity/cardiometabolic risk markers only surface gradually over time. Additionally, the protective effect of breastfeeding on blood pressure and metabolic syndrome score was found only in the early complementary feeding group and not in the typical complementary feeding group. Longer breastfeeding duration might attenuate child cardiometabolic risk associated with the adverse combination of early complementary feeding and short breastfeeding duration.

Our findings on the combined association of early complementary feeding and short breastfeeding duration with child adiposity/cardiometabolic markers are consistent with previous studies (21,22,27). We found concomitant increases in overall adiposity, blood pressure, and metabolic syndrome score in 6-year-old children, which might track to adulthood (43) and be associated with increased odds of developing type 2 diabetes (44) and cardiovascular disease (45). Associations with increased z-BMI and sum of skinfolds were not accompanied by clear elevations in ectopic fat partitioning to the abdominal visceral adipose tissue, liver, or muscles, which are known to be associated with increased cardiometabolic risk beyond the total amount of fat, perhaps because differences might be more pronounced from puberty onwards (46). We reported elevations in blood pressure without significant elevations in markers of subclinical vascular damages at age 6 years, though we postulate that these might emerge later in childhood as higher blood pressure at 6-8 years old has been associated with retinal arteriolar narrowing at 10-12 years old (47) and increased arterial thickness/stiffness in overweight/obese 11-year-old children (48).

In contrast, some studies found no association between early complementary feeding introduction and adiposity (7,49,50), likely due to limitations such as short follow-up duration, lack of adjustment for important confounders such as maternal age, education, household income, pre-pregnancy BMI, and parity, and the heterogeneous effect of early complementary feeding from the different frequencies of complementary feeding (e.g., once every few days or weeks) after it is first introduced. In our study, we considered this issue by specifically asking participants when their child was introduced to complementary feeding “on a daily basis”, and the more frequent exposure to complementary feeding might have resulted in a larger effect size. Therefore, the lack of consistent associations concluded in several systematic reviews (30,51,52) might be due to such limitations and does not necessarily mean that appropriate timing of introduction of complementary feeding is not an important factor in ameliorating child obesity/cardiometabolic risk.

The association between short breastfeeding duration and increased adiposity might be explained by higher infant weight gain, and we would expect that adjustment for infant weight gain would attenuate the effect estimate closer to the null. In our sample, however, children with short (vs. long) breastfeeding had lower, rather than higher, infant weight gain in the first 3 months, which may have explained the strengthening of the effect estimates for the association between short breastfeeding duration and increased child adiposity after additional adjustment for infant weight gain. It is worth noting that these estimates were quite imprecise with wide 95% CI and should be interpreted with caution. If our reported associations are at least in part causal, many interrelated molecular mechanisms, such as alterations in metabolic programming (53,54) and gut microbiota colonization (19,55,56), may be at play. Appetite regulation and metabolic programming are influenced by hormones in human milk (53) as well as plasma ghrelin concentration, which might be influenced by timing of solid food introduction (54). Furthermore, in the first year of life, type of milk feeding, timing of complementary feeding, and the interaction between them play major roles in shaping the infant’s gut microbiota (19,55), which might affect their risk of developing cardiometabolic diseases later in life (56). Hence, although elevations in adiposity/cardiometabolic measures are modest at 6 years old, they are likely to be amplified over time – i.e., altered appetite regulation might lead to chronic overconsumption of food (57,58), while alterations in child gut microbiota might lead to chronic low-grade inflammation which promotes the eventual development of cardiometabolic diseases (56).

Our study has several strengths. The added value of our study is the inclusion of child body fat partitioning measures as well as detailed cardiometabolic risk markers (arterial thickness/stiffness, pediatric metabolic syndrome score, etc.) at age 6 years. This enables us to understand the early development of subclinical cardiometabolic risk. We reduced recall bias through the prospective design and frequently administered infant feeding questionnaires, considered both the independent and the interacting effects between complementary feeding and breastfeeding, and reduced confounding bias by adjusting for important confounders based on recent literature. Limitations of our study include that there might be bias from missing data, which we attempted to reduce by multiple imputation (59). Despite adjusting for confounders including maternal age, education, household income, pre-pregnancy BMI, and parity, there could be residual confounding by unmeasured socioeconomic or maternal health measures which could play a causal role in the decision of mothers to cease breastfeeding or start complementary feeding early. While findings for overall adiposity, blood pressure, and metabolic syndrome score were consistent between the two analytical approaches (multiple imputation vs. complete case analysis), there were slight differences in the magnitudes of effect for the other cardiometabolic risk markers as clearer associations may only emerge later in childhood/adolescence. There is potential for recall bias for the timing of complementary feeding measure because mothers reported this information at the 9-month study visit only and not at other ages. Children might unintentionally break their fast before the collection of fasting blood. However, we believe this limitation would have little impact on our study findings, given that the median (IQR) fasting blood glucose levels in our study [4.55 (4.3-4.8) mmol/L] is consistent with other studies [girls, 4.8 (4.6-5.0) mmol/L; boys, 4.9 (4.7-5.1) mmol/L] (60). Despite utilizing Singapore’s largest birth cohort study to date, the current study may be underpowered for certain exposure categories (e.g., Early CF-Long BF, n=49) which had small sample sizes. We investigated multiple cardiometabolic outcomes, therefore increasing the risk of false-positive results, though the chances of our findings being false-positives are low due to the consistency of associations across related outcomes, consistent findings from literature (2,3,6–8,21,22), and plausible biological mechanisms found (19,53–56,61–65). Therefore, we chose not to adjust for multiple comparisons because we wanted to reduce the risk of type 2 errors (false-negative results). Our study was conducted in a multi-ethnic Asian cohort. Thus, our findings might not be generalizable to other populations. Also, the metabolic syndrome score was derived using cohort-specific z-scores so findings for the metabolic syndrome score may not be generalizable to other populations.

Taken together, our findings are in line with infant feeding guidelines, which recommend introducing complementary foods not before 4 months of age and longer breastfeeding duration beyond the first 4 months (5). The significant interaction between breastfeeding and complementary feeding on child blood pressure and metabolic syndrome score suggests the importance of a more holistic approach in studying interrelated infant feeding practices. While complementary feeding might be introduced early for various reasons such as concerns about iron deficiency and/or increased nutritional needs, future studies may consider continued breastfeeding as a potential intervention strategy for improved child cardiometabolic profiles.

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Acknowledgements

We thank all GUSTO participants as well as the GUSTO study group which includes: Airu Chia, Allan Sheppard, Amutha Chinnadurai, Anna Magdalena Fogel, Anne Eng Neo Goh, Anne Hin Yee Chu, Anne Rifkin-Graboi, Anqi Qiu, Arijit Biswas, Bee Wah Lee, Birit Froukje Philipp Broekman , Bobby Kyungbeom Cheon, Boon Long Quah, Candida Vaz, Chai Kiat Chng, Cheryl Shufen Ngo, Choon Looi Bong, Christiani Jeyakumar Henry, Ciaran Gerard Forde, Claudia Chi, Daniel Yam Thiam Goh, Dawn Xin Ping Koh, Desiree Y. Phua, Doris Ngiuk Lan Loh, E Shyong Tai, Elaine Kwang Hsia Tham, Elaine Phaik Ling Quah, Elizabeth Huiwen Tham, Evelyn Chung Ning Law, Evelyn Xiu Ling Loo, Fabian Kok Peng Yap, Faidon Magkos, Falk Müller-Riemenschneider, George Seow Heong Yeo, Hannah Ee Juen Yong, Helen Yu Chen, Heng Hao Tan, Hong Pan, Hugo P S van Bever, Hui Min Tan, Iliana Magiati, Inez Bik Yun Wong, Ives Yubin Lim, Ivy Yee-Man Lau, Izzuddin Bin Mohd Aris, Jeannie Tay, Jeevesh Kapur, Jenny L. Richmond, Jerry Kok Yen Chan, Jia Xu, Joanna Dawn Holbrook, Joanne Su-Yin Yoong, Joao Nuno Andrade Requicha Ferreira, Johan Gunnar Eriksson, Jonathan Tze Liang Choo, Jonathan Y. Bernard, Jonathan Yinhao Huang, Joshua J. Gooley, Jun Shi Lai, Karen Mei Ling Tan, Keith M. Godfrey, Kenneth Yung Chiang Kwek, Keri McCrickerd, Kok Hian Tan, Kothandaraman Narasimhan, Krishnamoorthy Naiduvaje, Kuan Jin Lee, Leher Singh, Li Chen, Lieng Hsi Ling, Lin Lin Su, Ling-Wei Chen, Lourdes Mary Daniel, Lynette Pei-Chi Shek, Marielle V. Fortier, Mark Hanson, Mary Foong-Fong Chong, Mary Rauff, Mei Chien Chua, Melvin Khee-Shing Leow, Michael J. Meaney, Michelle Zhi Ling Kee, Min Gong, Mya Thway Tint, Navin Michael, Neerja Karnani, Ngee Lek, Oon Hoe Teoh, P. C. Wong, Paulin Tay Straughan, Peter David Gluckman, Pratibha Keshav Agarwal, Priti Mishra, Queenie Ling Jun Li, Rob Martinus van Dam, Salome A. Rebello, Sambasivam Sendhil Velan, Seang Mei Saw, See Ling Loy, Seng Bin Ang, Shang Chee Chong, Sharon Ng, Shiao-Yng Chan, Shirong Cai, Shu-E Soh, Sok Bee Lim, Stella Tsotsi, Stephen Chin-Ying Hsu , Sue-Anne Ee Shiow Toh, Suresh Anand Sadananthan, Swee Chye Quek, Varsha Gupta, Victor Samuel Rajadurai, Walter Stunkel, Wayne Cutfield, Wee Meng Han, Wei Wei Pang, Wen Lun Yuan, Yanan Zhu, Yap Seng Chong, Yin Bun Cheung, Yiong Huak Chan, Yung Seng Lee.

Authors’ contributions to the manuscript: Y.Y.O. designed research, analyzed data, and wrote the paper. W.W.P designed research, conducted research, and revised the manuscript. N.M., I.M.A., M.E.W., and Y.S.L. designed research, contributed to the interpretation of data, and revised the manuscript. S.A.S, M.T.T, J.TL.C, L.H.L., N.K., S.S.V., M.V.F., K.H.T., P.D.G., F.Y., Y.S.C., K.M.G., S.Y.C., J.G.E., and M.FF.C., designed research, provided essential materials, and made critical revisions of the manuscript for important intellectual content. Y.Y.O. and Y.S.L. had primary responsibility for final content. All authors have read and approved the final manuscript.

Data sharing: Data described in the manuscript, code book, and analytic code will be made available upon request pending approval.

Table 1: Characteristics of study participants[[1]](#footnote-1)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Totaln = 839 | Typical CF-long BFn = 336 | Typical CF-short BFn = 348 | Early CF-long BFn = 49 | Early CF-short BFn = 106 | p[[2]](#footnote-2) |
| Maternal age (yr) | 31.6 ± 5.0 | 32.4 ± 4.5 | 31.1 ± 5.2 | 32.2 ± 4.6 | 30.1 ± 5.5 | <0.001 |
| Maternal education |  |  |  |  |  | <0.001 |
| University | 303 (36.3%) | 192 (57.7%) | 72 (20.7%) | 23 (46.9%) | 16 (15.4%) |  |
| Post-secondary | 289 (34.7%) | 94 (28.2%) | 145 (41.7%) | 15 (30.6%) | 35 (33.7%) |  |
| Secondary or lower | 242 (29.0%) | 47 (14.1%) | 131 (37.6%) | 11 (22.4%) | 53 (51.0%) |  |
| Monthly household income |  |  |  |  |  | <0.001 |
| ≥ S$6,000 | 245 (31.2%) | 148 (46.4%) | 71 (22.0%) | 16 (34.8%) | 10 (10.1%) |  |
| S$4,000-5,999 | 192 (24.4%) | 89 (27.9%) | 68 (21.1%) | 14 (30.4%) | 21 (21.2%) |  |
| < S$4,000 | 349 (44.4%) | 82 (25.7%) | 183 (56.8%) | 16 (34.8%) | 68 (68.7%) |  |
| Ethnicity |  |  |  |  |  | <0.001 |
| Chinese | 501 (59.7%) | 235 (69.9%) | 197 (56.6%) | 31 (63.3%) | 38 (35.8%) |  |
| Malay | 199 (23.7%) | 42 (12.5%) | 98 (28.2%) | 11 (22.4%) | 48 (45.3%) |  |
| Indian | 139 (16.6%) | 59 (17.6%) | 53 (15.2%) | 7 (14.3%) | 20 (18.9%) |  |
| Parity |  |  |  |  |  | 0.669 |
| Primiparous | 385 (45.9%) | 150 (44.6%) | 165 (47.4%) | 25 (51.0%) | 45 (42.5%) |  |
| Multiparous | 454 (54.1%) | 186 (55.4%) | 183 (52.6%) | 24 (49.0%) | 61 (57.5%) |  |
| Gestational tobacco exposure |  |  |  |  |  | <0.001 |
| No exposure | 516 (64.7%) | 262 (81.1%) | 184 (56.6%) | 29 (61.7%) | 41 (40.2%) |  |
| Secondhand exposure | 266 (33.4%) | 58 (18.0%) | 132 (40.6%) | 17 (36.2%) | 59 (57.8%) |  |
| Current smoker | 15 (1.9%) | 3 (0.9%) | 9 (2.8%) | 1 (2.1%) | 2 (2.0%) |  |
| Father's BMI (kg/m2) | 25.8 ± 4.5 | 25.7 ± 4.5 | 25.7 ± 4.4 | 26.5 ± 4.4 | 26.1 ± 4.7 | 0.594 |
| Pre-pregnancy BMI (kg/m2) | 22.8 ± 4.4 | 22.0 ± 3.6 | 23.3 ± 4.9 | 22.9 ± 3.7 | 23.9 ± 4.5 | <0.001 |
| Gestational fasting glucose (mmol/L) | 4.36 ± 0.49 | 4.31 ± 0.39 | 4.39 ± 0.52 | 4.32 ± 0.41 | 4.43 ± 0.69 | 0.086 |
| Gestational weight gain |  |  |  |  |  | 0.341 |
| Normal | 266 (35.7%) | 120 (39.6%) | 100 (32.9%) | 17 (37.0%) | 29 (31.5%) |  |
| Inadequate | 214 (28.7%) | 84 (27.7%) | 95 (31.3%) | 13 (28.3%) | 22 (23.9%) |  |
| Excessive | 265 (35.6%) | 99 (32.7%) | 109 (35.9%) | 16 (34.8%) | 41 (44.6%) |  |
| Sex |  |  |  |  |  | 0.540 |
| Female | 410 (48.9%) | 165 (49.1%) | 176 (50.6%) | 24 (49.0%) | 45 (42.5%) |  |
| Male | 429 (51.1%) | 171 (50.9%) | 172 (49.4%) | 25 (51.0%) | 61 (57.5%) |  |
| Gestational age (wk) | 38.8 ± 1.4 | 38.9 ± 1.6 | 38.8 ± 1.3 | 39.0 ± 1.1 | 38.7 ± 1.1 | 0.634 |
| Birthweight-for-gestational age (SDS) | 0.18 ± 1.20 | 0.27 ± 1.12 | 0.10 ± 1.26 | 0.05 ± 1.29 | 0.20 ± 1.23 | 0.280 |
| Infant z-weight gain from 0 to 3 months (SDS) | 0.36 ± 0.93 | 0.55 ± 0.85 | 0.23 ± 1.00 | 0.33 ± 0.77 | 0.21 ± 0.90 | <0.001 |

Table 2: Associations between duration of breastfeeding and cardiometabolic risk markers at 6 years old[[3]](#footnote-3)

|  |  |
| --- | --- |
|  | Short (≤4m) vs. long (>4m) BF |
|  | Unadjusted β (95% CI) | p | Model 1[[4]](#footnote-4)β (95% CI) | p | Model 2[[5]](#footnote-5)β (95% CI) | p |
| Adiposity measures |  |  |  |  |  |  |
| z-BMI (SDS) | 0.20 (0.01, 0.39) | 0.036 | 0.05 (-0.15, 0.25) | 0.605 | 0.18 (-0.01, 0.38) | 0.064 |
| Sum of skinfolds (mm) | 2.37 (0.66, 4.08) | 0.007 | 1.05 (-0.75, 2.84) | 0.253 | 1.83 (0.05, 3.61) | 0.044 |
| Abdominal circumference (cm) | 0.58 (-0.29, 1.45) | 0.190 | 0.12 (-0.80, 1.03) | 0.804 | 0.69 (-0.20, 1.58) | 0.129 |
| Abdominal SAT (cc) | 78.09 (16.16, 140.02) | 0.014 | 22.73 (-42.98, 88.44) | 0.497 | 51.45 (-13.53, 116.43) | 0.120 |
| Abdominal VAT (cc) | 3.71 (-7.49, 14.91) | 0.515 | 1.44 (-10.78, 13.65) | 0.817 | 3.93 (-8.24, 16.10) | 0.525 |
| Liver fat (% by weight) | 0.10 (0.02, 0.18) | 0.012 | 0.05 (-0.04, 0.13) | 0.325 | 0.05 (-0.04, 0.14) | 0.314 |
| Intramyocellular lipids (% of water signal) | 0.01 (-0.04, 0.05) | 0.764 | 0.01 (-0.04, 0.06) | 0.652 | 0.02 (-0.03, 0.07) | 0.433 |
| Cardiometabolic measures |  |  |  |  |  |  |
| Carotid intima media thickness (mm) | 0.00 (-0.01, 0.00) | 0.555 | 0.00 (-0.01, 0.00) | 0.535 | 0.00 (-0.01, 0.00) | 0.644 |
| Pulse wave velocity (m/s) | 0.14 (-0.09, 0.37) | 0.240 | 0.08 (-0.16, 0.31) | 0.525 | 0.08 (-0.15, 0.32) | 0.487 |
| Fasting plasma glucose (mmol/L) | 0.00 (-0.07, 0.07) | 0.968 | -0.01 (-0.08, 0.06) | 0.869 | 0.00 (-0.07, 0.07) | 0.912 |
| HOMA-IR (units) | 0.08 (-0.02, 0.17) | 0.133 | 0.03 (-0.07, 0.14) | 0.521 | 0.03 (-0.07, 0.13) | 0.590 |
| Triglycerides (mmol/L) | 0.08 (0.03, 0.13) | 0.003 | 0.03 (-0.03, 0.08) | 0.351 | 0.03 (-0.03, 0.08) | 0.363 |
| HDL (mmol/L) | 0.00 (-0.05, 0.05) | 0.942 | -0.01 (-0.06, 0.04) | 0.790 | -0.01 (-0.06, 0.04) | 0.674 |

Table 3: Associations between timing of introduction of complementary foods and cardiometabolic risk markers at 6 years old[[6]](#footnote-6).

|  |  |
| --- | --- |
|  | Early (≤4m) vs. typical (>4m) CF |
|  | Unadjusted β (95% CI) | p | Model 1[[7]](#footnote-7)β (95% CI) | p | Model 2[[8]](#footnote-8)β (95% CI) | p |
| Adiposity measures |  |  |  |  |  |  |
| z-BMI (SDS) | 0.44 (0.19, 0.69) | <0.001 | 0.32 (0.08, 0.56) | 0.010 | 0.34 (0.11, 0.57) | 0.004 |
| Sum of skinfolds (mm) | 3.26 (0.97, 5.54) | 0.005 | 2.60 (0.38, 4.82) | 0.022 | 2.73 (0.55, 4.91) | 0.014 |
| Abdominal circumference (cm) | 1.33 (0.19, 2.47) | 0.022 | 0.91 (-0.20, 2.01) | 0.108 | 1.00 (-0.06, 2.06) | 0.063 |
| Abdominal SAT (cc) | 88.11 (6.96, 169.26) | 0.033 | 55.54 (-23.10, 134.19) | 0.166 | 60.45 (-16.81, 137.72) | 0.125 |
| Abdominal VAT (cc) | 4.41 (-10.47, 19.29) | 0.560 | 3.49 (-11.29, 18.27) | 0.642 | 3.92 (-10.79, 18.62) | 0.600 |
| Liver fat (% by weight) | 0.01 (-0.10, 0.12) | 0.877 | -0.02 (-0.14, 0.09) | 0.667 | -0.02 (-0.14, 0.09) | 0.669 |
| Intramyocellular lipids (% of water signal) | 0.01 (-0.05, 0.07) | 0.733 | 0.01 (-0.05, 0.07) | 0.731 | 0.01 (-0.05, 0.07) | 0.694 |
| Cardiometabolic measures |  |  |  |  |  |  |
| Carotid intima media thickness (mm) | 0.00 (-0.01, 0.01) | 0.905 | 0.00 (-0.01, 0.00) | 0.815 | 0.00 (-0.01, 0.00) | 0.832 |
| Pulse wave velocity (m/s) | 0.26 (-0.01, 0.53) | 0.062 | 0.20 (-0.07, 0.47) | 0.137 | 0.21 (-0.06, 0.48) | 0.134 |
| Fasting plasma glucose (mmol/L) | 0.08 (0.00, 0.16) | 0.061 | 0.08 (0.00, 0.16) | 0.061 | 0.08 (0.00, 0.16) | 0.055 |
| HOMA-IR (units) | 0.10 (-0.04, 0.24) | 0.154 | 0.07 (-0.06, 0.20) | 0.312 | 0.07 (-0.07, 0.20) | 0.318 |
| Triglycerides (mmol/L) | 0.02 (-0.05, 0.10) | 0.508 | 0.01 (-0.07, 0.08) | 0.827 | 0.01 (-0.07, 0.08) | 0.831 |
| HDL (mmol/L) | -0.01 (-0.09, 0.06) | 0.703 | -0.01 (-0.08, 0.07) | 0.815 | -0.01 (-0.08, 0.06) | 0.800 |

Figure legend

Figure 1: Adjusted differences in cardiometabolic risk markers between children from different infant feeding groups compared to the reference group (typical CF-long BF) while holding covariates at the mean. Analysis is based on imputed dataset (n=733; typical CF-long BF: n=290; typical CF-short BF: n=311; early CF-long BF: n=42; early CF-short BF: n=90). Model 1: Adjusted for maternal age, maternal education, household income, ethnicity, parity, gestational tobacco exposure, father’s BMI, pre-pregnancy BMI, gestational fasting plasma glucose, gestational weight gain, child’s sex, gestational age, birthweight-for-gestational age, and the interaction between CF and BF. Sex-standardized metabolic syndrome score was not additionally adjusted for sex. Blood pressures were additionally adjusted for child’s height. Model 2: Model 1 + infant z-weight gain. Error bars show the 95% confidence intervals. \* p < 0.05 based on post-hoc pairwise comparisons between each infant feeding group and the reference group, as well as between the early complementary feeding groups. BF, breastfeeding; CF, complementary feeding.

1. Values are means ± SDs (continuous) or n (%) (categorical). BF, breastfeeding; BMI, body mass index; CF, complementary feeding; SDS, standard deviation score. [↑](#footnote-ref-1)
2. P values calculated using one-way analysis of variance (continuous) or chi-square tests (categorical). [↑](#footnote-ref-2)
3. Analysis is based on imputed dataset (n=733; short BF: n=401; long BF: n=332). Multiple linear regression coefficient estimates with 95% confidence intervals are presented, referenced to the long (>4m) breastfeeding duration category. BF, breastfeeding; HDL, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; SAT, subcutaneous adipose tissue; SDS, standard deviation score; VAT, visceral adipose tissue; z-BMI, body mass index z-score. [↑](#footnote-ref-3)
4. Model 1: Adjusted for maternal age, maternal education, household income, ethnicity, parity, gestational tobacco exposure, father’s BMI, pre-pregnancy BMI, gestational fasting plasma glucose, gestational weight gain, child’s sex, gestational age, birthweight-for-gestational age, and timing of complementary feeding categories. Sex-standardized z-BMI was not additionally adjusted for sex. [↑](#footnote-ref-4)
5. Model 2: Model 1 + infant z-weight gain [↑](#footnote-ref-5)
6. Analysis is based on imputed dataset (n=733; early CF: n=132; typical CF: n=601). Multiple linear regression coefficient estimates with 95% confidence intervals are presented, referenced to the typical (>4m) complementary feeding category. CF, complementary feeding; HDL, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; SAT, subcutaneous adipose tissue; SDS, standard deviation score; VAT, visceral adipose tissue; z-BMI, body mass index z-score. [↑](#footnote-ref-6)
7. Model 1: Adjusted for maternal age, maternal education, household income, ethnicity, parity, gestational tobacco exposure, father’s BMI, pre-pregnancy BMI, gestational fasting plasma glucose, gestational weight gain, child’s sex, gestational age, birthweight-for-gestational age, and breastfeeding duration categories. Sex-standardized z-BMI was not additionally adjusted for sex. [↑](#footnote-ref-7)
8. Model 2: Model 1 + infant z-weight gain [↑](#footnote-ref-8)