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Association of long-duration breastfeeding and dental caries estimated with marginal structural models

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ABSTRACT

Purpose: To estimate the association between breastfeeding 24 months or beyond and severe early childhood caries (S-ECC).
Methods: Within a birth cohort ($n = 715$) from low-income families in Porto Alegre, Brazil, the age 38-month prevalence of S-ECC ($\geq$4 affected tooth surfaces or $\geq$1 affected maxillary anterior teeth) was compared over breastfeeding duration categories using marginal structural models to account for time-dependent confounding by other feeding habits and child growth. Additional analyses assessed whether daily breastfeeding frequency modified the association of breastfeeding duration and S-ECC. Multiple imputation and censoring weights were used to address incomplete covariate information and missing outcomes, respectively. Confidence intervals (CIs) were estimated using bootstrap resampling.
Results: Breastfeeding 24 months or beyond was associated with the highest adjusted population-average S-ECC prevalence (0.45; 95% CI, 0.36 to 0.54) compared with breastfeeding less than 6 months (0.22; 95% CI, 0.15 to 0.28), 6–11 months (0.38; 95% CI, 0.25 to 0.53), or 12–23 months (0.39; 95% CI, 0.20 to 0.56). High-frequency breastfeeding enhanced the association between long-duration breastfeeding and caries (excess prevalence due to interaction: 0.13; 80% CI, −0.03 to 0.30).
Conclusions: In this population, breastfeeding 24 months or beyond, particularly if frequent, was associated with S-ECC. Dental health should be one consideration, among many, in evaluating health outcomes associated with breastfeeding 24 months or beyond.

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Introduction

The World Health Organization (WHO) recommends continued breastfeeding up to age 2 years or beyond [1], and failure to breastfeed is associated with poor health consequences for both mother and child [2,3]. However, the nature of the relationship between dental caries and the age to which children are breastfed remains uncertain. Caries is among the most common diseases worldwide and often goes untreated, particularly in low-resource settings [4–6], with negative quality of life implications [7]. Some laboratory models suggest that human milk can cause caries [8,9], particularly in combination with added sugars [10], whereas some report no demineralization of tooth material by human milk alone [11]. The epidemiologic literature [12] includes studies that support a positive association between long-duration breastfeeding and early childhood caries (ECC) [13–16] and others that do not [17,18].

Breastfeeding timing relative to other feeding habits complicates study of breastfeeding duration and ECC. Early breastfeeding cessation might accelerate the introduction of particular foods [19,20], and the foods consumed early in life likely influence caries development [21–23]. In turn, early-life food experiences might also influence the duration to which a breastfeeding child continues nursing [19]. Regression modeling is problematic in the presence of such time-dependent confounding, in which a variable (e.g., early-life food experiences) can be part of a causal pathway between an earlier aspect of exposure (e.g., early breastfeeding) and the outcome, whereas simultaneously operating as confounder with respect to a later aspect of exposure (e.g., continued breastfeeding). Marginal structural models (MSMs), in contrast, have been used to make causal inference from observational data in the presence of time-varying covariates [24–28]. Such techniques are particularly relevant for exposures, such as breastfeeding, that cannot be easily assigned as a randomized intervention.

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We aimed to estimate the association between long-duration breastfeeding (≥24 months) and severe-ECC (S-ECC) in a birth cohort of urban, low-income Brazilian children. We hypothesized that long-duration breastfeeding is associated with greater caries occurrence. We secondarily hypothesized that the association between long-duration breastfeeding and S-ECC is stronger if daily breastfeeding episodes are more frequent.

Methods

Participants

We followed a birth cohort nested in a cluster-randomized trial in Porto Alegre, Brazil. The community water supply is optimally fluoridated [29], and 52 public health care centers provide primary medical services predominantly to low-income residents. A stratified random sample ($n = 20$ health centers) was selected from 31 eligible clinics for participation in the original trial of health care worker training [30,31].

In 2008, 715 of 736 eligible pregnant women with appointments at participating clinics agreed to enroll their children in a cohort to track health outcomes (Fig. 1). The trial had provided intervention clinics with health care worker training that promoted healthful infant complementary feeding for incorporation into maternal counseling. After 3 years, the intervention did not extend the total duration of breastfeeding (hazard ratio for breastfeeding cessation, 0.94; 95% confidence interval, 0.79 to 1.11), although the mean duration of exclusive breastfeeding was increased [30]. S-ECC was not lowered significantly among children born to intervention group clinic attendees [31].

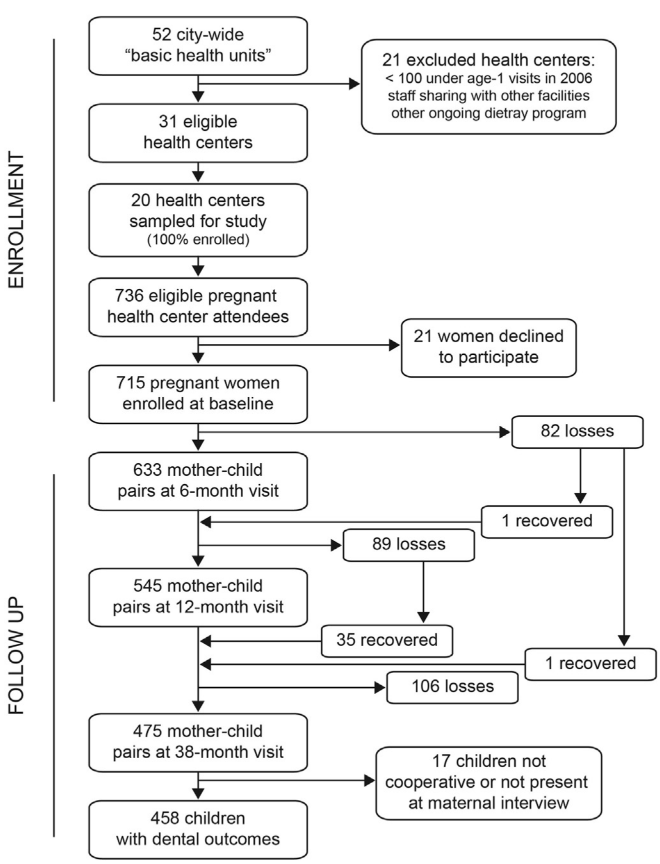

Fig. 1. Flow of participants. Pregnant women were recruited from 20 municipal health centers in the city of Porto Alegre, Brazil and followed to a mean child age of 38 mo.

Baseline variables

Trained fieldworkers collected baseline (during pregnancy) sociodemographic information via structured questionnaires. Data included maternal age, household size, maternal education (≤8 years), maternal smoking (current vs. never/former smoker), indoor bathroom (yes/no), city region (indicators for eight geo-administrative districts), parity (first child yes/no), maternal partner status (married or partnered vs. single, separated, or widowed), household income (≤1500 Brazilian reais monthly; approximately $900 US in 2008), outside income source (e.g., government support), social class (Brazilian Association of Economic Research Institutes classification ≤ C), and low body mass index (BMI) (≤18, based on measured height and self-reported pre-pregnancy weight). Child sex and birth date were collected at age 5–9 months.

Time-varying behaviors and anthropometry

Infant growth and feeding habits were recorded at each of three home visits, corresponding to mean ages of 6 months (range: 5–9), 12 months (range: 11–15), and 38 months (range: 31–46). Infant length and child height were collected following standard protocol and converted to height/length-for-age Z-scores using WHO standards [32]. At each visit, mothers were asked whether they had ever breastfed and whether they were currently breastfeeding. Breastfeeding duration represented the age to which any breastfeeding continued, regardless of complementary feeding. Breastfeeding mothers were asked how frequently they nursed daily (0, 1, 2–3, or "many times," separately for day and night). Mothers no longer breastfeeding were asked at what age (in months) breastfeeding ceased.

At the 6-month assessment, the number of feeding bottles consumed in the preceding day was recorded (later categorized 0, 1–3, ≥4). Sugar in the bottle corresponded to consuming one or more bottle containing any sweet additive: table sugar, powdered or liquid artificial chocolate, soft drinks, or powdered artificial juice. Questionnaires addressed use of commercially prepared infant formula and the age of introduction of 32 specific foods (e.g., fruits, beans, soft drinks, candies). At the 12-month assessment, the questionnaire posed whether 29 specific items were consumed in the previous month and the weekly consumption frequency of five complementary foods (fruits, vegetables, beans, meats, organ meats). Two feeding indices measured dietary patterns to account for foods consumed in combination and to increase the efficiency of the analysis [33]. The indices were created specifically for this analysis due to a lack of existing diet indices specific to cariogenic feeding behaviors in comparable populations. The first, referred to here as the food introduction index, was the count of low nutrient-density and/or presumably cariogenic foods introduced before age 6 months: added sugar, candy, chips, chocolate, chocolate milk, cookies, fruit-flavored drink, gelatin, honey, ice cream, soft drinks, and sweet biscuits. The second, termed here as the first-year feeding index, summed the food introduction index with the count of the following foods recorded at the 12-month assessment: added sugar in a drink, candy, cake, chips, chocolate, chocolate milk, cookies, creamed caramel, fruit-flavored drink, gelatin, honey, ice cream, other confection, soft drinks, and sweet biscuits.

At the 38-month assessment, data were collected regarding bottle use, height-for-age Z-scores, and tooth brushing with fluoride dentifrice. Although these variables are likely associated with S-ECC, we did not consider them confounders because our cutpoint for defining the exposure (breastfeeding ≥24 months) temporally preceded these measures. However, we estimated separate models that included these variables as a sensitivity check.

Dental caries

Dental status was evaluated at 38 months following WHO protocol [34], with noncavitated (white-spot) lesions also recorded. Assessments took place in participants' homes, aided by a lighted intraoral mirror. Teeth were brushed and dried with gauze. S-ECC was defined as 1 or more affected maxillary anterior teeth or 4 or more decayed, missing due to caries, or filled tooth surfaces (dmfs ≥4) [35]. Two dentist-examiners completed the evaluations following identical protocol (interrater unweighted kappa = 0.75; intrarater unweighted kappa = 0.83 for both examiners).

$$SW^{T} = \frac{\Pr[A<6\text{months}]I[A<6\text{months}]}{\Pr[A_6=1|w]} + \frac{\Pr[A=6-11\text{months}]I[A=6-11\text{months}]}{\Pr[A_6=1|w]\Pr[A_{12}=1|A_6=1,W,L_6]} + \frac{\Pr[A=12-23\text{months}]I[A=12-23\text{months}]}{\Pr[A_6=1|w]\Pr[A_{12}=1|A_6=1,W,L_6]\Pr[A_{24}=0|A_{12}=1,W,L_6,L_{12}]} + \frac{\Pr[A\geq 24\text{months}]I[A\geq 24\text{months}]}{\Pr[A_6=1|w]\Pr[A_{12}=1|A_6=0,W,L_6]\Pr[A_{24}=1|A_{12}=1,W,L_6,L_{12}]} \quad (1)$$

Statistical methods

The proportion of children with S-ECC was compared across four breastfeeding duration categories: less than 6 months, 6–11 months, 12–23 months, and 24 months or beyond. Three marginal structural models were fit. The weights for estimating unadjusted models incorporated only clinic allocation status to account for the nested study design. Adjusted models additionally accounted for baseline sociodemographic variables: maternal age, education, parity, pre-pregnancy BMI, smoking status, social class, and child age and sex. Fully adjusted models included those variables, and time-varying bottle use, feeding habits, and length-for-age Z-scores (see the following sections).

In estimating MSMs, inverse probability weighting was used to generate a "pseudo-population" representative of a hypothetical population in which breastfeeding duration (A) had been allocated independently of confounding variables. Weights were assigned inversely to the predicted probability of observed exposure, given baseline characteristics (W) and longitudinally recorded variables (L_t), giving the greatest weight to observations with exposure and confounder combinations least represented in the sample, relative to what would have been observed under random exposure allocation. Figure 2 depicts the assumed relationships among variables as a directed acyclic graph. Exposure probabilities were estimated using Super Learner, a data-adaptive machine-learning tool [36].

To account for time-dependent confounding, weights were based on treatment models for three probabilities: the probability of breastfeeding at 6 months ($\Pr[A_6 = 1]$); the probability of breastfeeding at 12 months, given breastfeeding at 6 months ($\Pr[A_{12} = 1 \mid A_6 = 1]$); and the probability of breastfeeding at 24 months, given breastfeeding at 12 months ($\Pr[A_{24} = 1 \mid A_{12} = 1]$). Each treatment model was estimated while incorporating temporally appropriate putative confounders: in fully adjusted models, the 6-month treatment model included clinic allocation status and baseline sociodemographic variables only; the 12-month treatment model included these variables and added the 6-month bottle use variables, formula use, food introduction index, and 6-month length-for-age Z-scores; the 24-month treatment model replaced the food introduction index and 6-month Z-scores with the first-year feeding index and 12-month Z-scores, respectively, and added complementary food frequency. To stabilize the weights, we multiplied by the marginal probability of the observed exposure category [25].

Equation 1 gives the stabilized treatment weights, where indicators (I) take a value of 1 when the exposure category was observed and 0 otherwise.

For each model, missing variables and missing or incomplete breastfeeding histories were multiply imputed from probabilities estimated using Super Learner, corresponding to 2.6% of data among children with observed outcomes. Censoring weights, equal to the inverse probability of having an observed outcome, given exposure and covariates, upweighted observations most resembling those with missing outcomes. The probability of an observed outcome ($\Pr[C = 0]$) was estimated via Super Learner using predictor variables clinic allocation status, maternal age, education, partner status, parity, smoking in pregnancy, and pre-pregnancy BMI, household income, indoor bathroom, number of inhabitants, outside income source, city region, and social class, and child breastfeeding duration, first-year feeding index, height-for-age Z-score, and sex. Stabilized censoring weight numerators were the product of the probability of having outcome data, given breastfeeding duration category, and a 1/0 indicator for having outcome data (equation 2).

$$\text{SW}^{C} = \frac{\Pr[C=0|A]I[C=0]}{\Pr[C=0|A,W,L_t]} \quad (2)$$

Final MSM weights were the product of stabilized treatment weights and stabilized censoring weights: $\text{SW} = \text{SW}^T \times \text{SW}^C$. In the fully adjusted model, nonzero stabilized weights had a mean of 0.997 (minimum: 0.28, maximum: 8.90).

For each model, point estimates (prevalence ratio, PR; prevalence difference) were averaged over 200 multiple imputations. Percentile-based 95% confidence intervals (CIs) were estimated as the 2.5 and 97.5 quantiles from 5000 bootstrap iterations to account

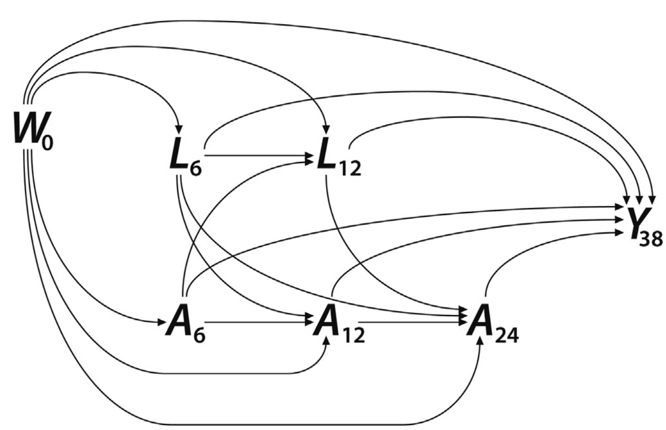

Fig. 2. Directed acyclic graph. The graph depicts the assumed relationships between study variables. W_0 = baseline sociodemographic characteristics; A_t = breastfeeding at time t months; L_t = time-varying feeding behaviors and anthropometry at time t months; Y_{38} = S-ECC at 38 months. Time-dependent confounding occurs through L_{12} variables, which are part of a directed path from A_6 to Y_{38} but a back-door path from A_{24} to Y_{38}.

for variance from sampling, imputation, and weighting. For comparison, an analogous complete-case regression analysis was completed using log-linear models and robust variance. Analyses were completed in R version 3.0.1 (http://www.r-project.org).

Secondarily, we examined whether frequent daytime breastfeeding (≥4 daily episodes) intensified the association of breastfeeding duration and S-ECC, restricting the analysis to children breastfed 6 months or more, the earliest age at which frequency data were collected. High frequency daytime breastfeeding and a long-duration high-frequency interaction term were included as MSM covariates, and frequent breastfeeding was added to the treatment models. We defined the excess prevalence due to interaction (EPI) as a departure from additivity, following an example proposed for the relative risk [37]. If D and F represent the presence of long-duration and high-frequency breastfeeding, respectively, and $\overline{D}$ and $\overline{F}$, the absence of these factors, then the $\mathrm{EPI} = \mathrm{PD}(\mathrm{DF}) - \mathrm{PD}(\mathrm{D\overline{F}}) - \mathrm{PD}(\mathrm{\overline{D}F})$. As a sensitivity check, we also estimated models in which frequency strata were defined by high-frequency breastfeeding in either the day or night versus high-frequency breastfeeding in neither. Nighttime breastfeeding was not used alone to define strata because high-frequency daytime breastfeeding was common (>50%) when nighttime high-frequency breastfeeding was absent. Because tests for statistical interaction may have low power [38], 80% CIs were provided.

Ethics

This study proposal received ethical approval from committees at the Federal University of Health Sciences of Porto Alegre (UFCSPA) and the University of California Berkeley and is in accordance with the Declaration of Helsinki. Informed consent was reached with mothers on behalf of their children. Children with caries or suspected anemia, undernutrition or overweight status were referred to their local health center.

Results

Table 1 demonstrates selected characteristics of the study population. The fraction of mothers interviewed at the 6-month assessment to report initiating any breastfeeding was 0.99 (627/633); the fraction who breastfed to 12 months was 0.47 (282/598). Exclusive breastfeeding continued to mean age 2.1 months. Nearly half the children were introduced to commercially prepared infant formula by 6 months (0.49, 309/632), but few children used formula at 12 months (0.03, 18/539). Bottle use and soft drink consumption were common, whereas inadequate length-for-age was rare (Table 1). S-ECC prevalence at 38 months was 0.34 (157/458); the prevalence of at least one affected tooth was 0.55 (250/458). Caries was most common among children breastfed for 24 months or beyond (Fig. 3).

The highest S-ECC prevalence was associated with breastfeeding 24 months or beyond in all marginal structural models (Table 2). Findings were robust to the decision to exclude the 38-month variables (bottle use, fluoride toothpaste, height-for-age) from the main analysis. In the sensitivity analysis that included 38-month variables in the fully adjusted model, estimates were not appreciably altered. For example, the prevalence ratio comparing breastfeeding 24 months or beyond to less than 6 months changed to 2.11 (95% CI, 1.50 to 3.30) from 2.10 (95% CI, 1.50 to 3.25).

Compared with breastfeeding 6–23 months, breastfeeding 24 months or beyond was associated with elevated S-ECC prevalence, although not statistically significant (unadjusted PR, 1.31; 95% CI, 0.97 to 1.79; adjusted PR, 1.22; 95% CI, 0.89 to 1.66; fully adjusted PR, 1.17; 95% CI, 0.85 to 1.78). However, breastfeeding 24 months or beyond was more strongly associated with S-ECC when daytime breastfeeding was frequent (fully adjusted PR, 1.38; 95% CI, 0.97 to 2.16) (Table 3). The EPI was 0.13 (80% CI, −0.03 to 0.30), suggesting positive interaction between frequent daytime nursing and breastfeeding 24 months or beyond. Results were similar in analyses that defined high frequency based on frequent nursing in either the day or night versus neither day or night (fully adjusted PR with frequent breastfeeding, 1.43; 95% CI, 1.01 to 2.18); the EPI increased to 0.23 (80% CI, 0.03 to 0.41).

Table 1
Characteristics of participants

Characteristic	Value	Number of observations*
Sociodemographic characteristics		
Maternal age at expected delivery date, mean (SD), y	26.0 (6.7)	715
Mother has ≤ 8 y of formal education, *n* (%)	340 (47.6)	715
Household income ≤ 3 times minimum salary†, *n* (%)	565 (81.9)	690
Social class C or lower by ABIPEME index‡, *n* (%)	569 (79.8)	713
Self-identified maternal race white, *n* (%)	395 (55.2)	715
Self-identified maternal race black, mixed, or other, *n* (%)	320 (44.8)	715
Male child, *n* (%)	333 (52.4)	635
Anthropometry		
Length-for-age *Z*-score at age 5–9 mo, mean (SD)	−0.13 (1.2)	631
Length-for-age *Z*-score ≤2 at age 5–9 mo, *n* (%)	31 (4.9)	631
Length-for-age *Z*-score at age 11–15 mo, mean (SD)	−0.03 (0.9)	527
Length-for-age *Z*-score ≤2 at age 11–15 mo, *n* (%)	4 (0.8)	527
Feeding habits		
Introduced to soft drinks before age 6 mo, *n* (%)	192 (30.3)	633
Introduced to any sweets before age 6 mo, *n* (%)	557 (90.8)	613
Consumed soft drinks in prior month at age 11–15 mo, *n* (%)	413 (76.6)	539
Consumed vegetables ≥4 d/wk at age 11–15 mo, *n* (%)	341 (63.5)	537
Ever initiated breastfeeding	627 (98.9)	633
Duration exclusive breastfeeding, mean (SD), mo	2.1 (1.6)	633
Exclusive breastfeeding to age ≥4 mo, *n* (%)	152 (24.0)	633
Breastfeeding duration to age <6 mo, *n* (%)	216 (34.1)	633
Breastfeeding duration to age 6–11 mo, *n* (%)	100 (16.7)	598
Breastfeeding duration to age 12–23 mo, *n* (%)	65 (12.1)	537
Breastfeeding duration to age ≥24 mo, *n* (%)	156 (29.1)	537
Consuming sweet substances in bottle at age 5–9 mo, *n* (%)	198 (32.3)	614
Consuming sweet substances in bottle at age 2–3 y, *n* (%)	312 (68.4)	456
Dental caries experience at age 2–3 y		
Any affected tooth, *n* (%)	250 (54.6)	458
S-ECC, *n* (%)	157 (34.3)	458
dmfs (any decay), mean (SD)	3.2 (6.1)	458
dmfs (cavitated decay only), mean (SD)	2.6 (5.9)	458

ABIPEME = Brazilian Association of Economic Research Institutes; dmfs = decayed missing filled surfaces index; SD = standard deviation.

* Number of observations differ for some variables due to missing data and/or losses to follow-up.

† Monthly income of ≤1500 Brazilian reais; approximately $900 US in 2008.

‡ Socioeconomic classification scale based on material possessions and education, A = highest status, E = lowest status.

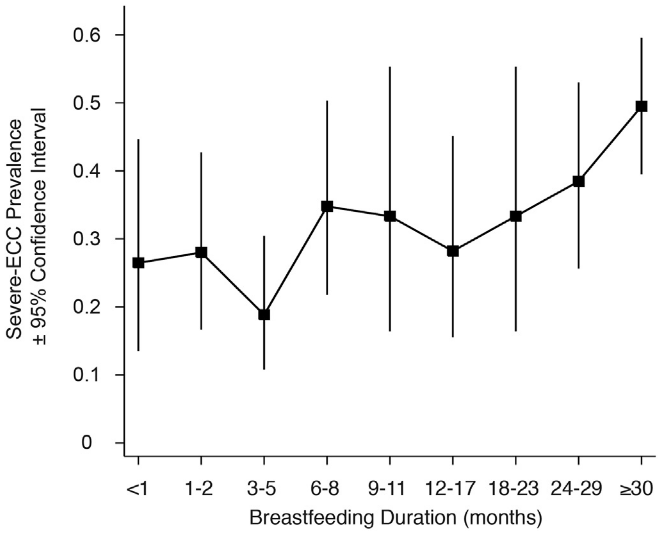

Fig. 3. Observed prevalence of S-ECC at age 38 months by categories of breastfeeding duration. The unadjusted (crude) prevalence of S-ECC by categories of breastfeeding duration is shown for the 439 children with complete observed data for both breastfeeding duration and dental health.

Complete-case regression analysis yielded modest differences in estimates (Table 4). However, findings were qualitatively consistent with MSM results, supporting a positive association between S-ECC and breastfeeding 24 months or beyond.

Discussion

In this population of low-income Brazilian families, we estimated an increase in S-ECC prevalence with breastfeeding 24 months or beyond. Although the overall health benefits of breastfeeding are considerable, this work adds evidence that, in some contexts, very extended and frequent breastfeeding might increase caries risk. In addition to exposing teeth to bacterially fermentable milk sugars, prolonged breastfeeding might enhance the fidelity with which caries-causing oral bacteria are transmitted from mothers [39].

Several investigations have reported positive associations between breastfeeding duration and caries when using cut points exceeding 18 months to define the uppermost duration category [13–15,40,41]. Studies that reported no association between caries and breastfeeding generally used earlier cut points to define long-duration breastfeeding (i.e., ≥8 or ≥13 months) and have featured populations in which breastfeeding to age 2 years is uncommon, such as in Germany [42], Italy [17], and the United States [18]. A large hospital-based breastfeeding promotion intervention in Belarus did not affect caries prevalence at age 6 years [43]; however, the study did not directly compare caries outcomes among children who were or were not breastfed for extended durations (e.g., ≥24 months), which was an uncommon behavior in that trial population.

Daily breastfeeding frequency was associated with S-ECC in a previous study of Brazilian preschoolers, in which breastfeeding frequency, but not duration ≥12 months, maintained statistical significance in multivariable models [21]. A combined measure of breastfeeding duration and frequency was strongly associated with ECC in Mayanmar [44], and a measure of nighttime breastfeeding burden, which was based on frequency, was positively associated with ECC in Iran [45], although not statistically significant.

An important strength of this study was the longitudinal, prospective collection of feeding information. We use weighting estimators to respect the temporal sequence of exposure and covariate information, accounting for time-dependent confounding by early-life feeding habits [46]. These methods have not been broadly used in oral health epidemiology.

Causal interpretation of marginal structural model results depends on unverifiable assumptions, specifically, positivity, exchangeability, and correct specification of the treatment models used to generate the weights [25,28]. In this analysis, the distribution of the weights was not extreme, important socioeconomic and nutritional confounders were prospectively collected, and Super Learner estimation reduced the reliance on parametric model-building assumptions [36]. These strengths give credence to causal interpretations, however, as with any observational study, residual confounding cannot be ruled out. For instance, our main estimates do not adjust for earlier oral hygiene habits. However, adjustment for 38-month toothbrushing habits did not affect estimates, and we have no evidence that oral hygiene habits would be associated with breastfeeding duration. Also, while we adjust for the age of

Table 2
Unadjusted and adjusted associations of breastfeeding duration and S-ECC in preschoolers

Breastfeeding duration	Marginal prevalence S-ECC*	95% CI	Prevalence ratio	95% CI	Prevalence difference	95% CI
Unadjusted† model (mo)						
<6 (reference)	0.23	0.16 to 0.30	1		0	
6–11	0.38	0.27 to 0.50	1.66	1.06 to 2.57	0.15	0.02 to 0.29
12–23	0.31	0.20 to 0.43	1.35	0.81 to 2.16	0.08	−0.05 to 0.22
≥24	0.45	0.38 to 0.53	1.98	1.44 to 2.87	0.22	0.12 to 0.33
Adjusted‡ model (mo)						
<6 (reference)	0.22	0.15 to 0.28	1		0	
6–11	0.39	0.27 to 0.53	1.79	1.13 to 2.80	0.17	0.03 to 0.32
12–23	0.35	0.22 to 0.49	1.59	0.97 to 2.65	0.12	−0.01 to 0.29
≥24	0.45	0.38 to 0.53	2.06	1.51 to 3.00	0.23	0.14 to 0.34
Fully adjusted§ model (mo)						
<6 (reference)	0.22	0.15 to 0.28	1		0	
6–11	0.38	0.25 to 0.53	1.77	1.12 to 2.85	0.17	0.03 to 0.32
12–23	0.39	0.20 to 0.56	1.82	0.85 to 3.20	0.18	−0.03 to 0.42
≥24	0.45	0.36 to 0.54	2.10	1.50 to 3.25	0.24	0.13 to 0.36

* Population-average prevalence of S-ECC at given categories of breastfeeding duration, as estimated from marginal structural models.
† Includes allocation status from nesting intervention study only.
‡ Includes allocation status from nesting intervention study and maternal age, education, parity, pre-pregnancy BMI, smoking status, social class, and child age and sex.
§ Includes all adjusted model variables and time-varying bottle use variables, feeding habits, and height-for-age *Z*-scores.

Table 3
Unadjusted and adjusted associations of breastfeeding ≥24 mo and S-ECC, stratified by frequency of daytime breastfeeding

Breastfeeding duration and frequency	Marginal prevalence S-ECC*	95% CI	Prevalence ratio	95% CI	EPI	80% CI
Unadjusted† model						
Duration 6–23 mo and low frequency	0.38	0.25 to 0.51	1			
Duration ≥24 mo and low frequency	0.37	0.22 to 0.52	0.97	0.53 to 1.68		
Duration 6–23 mo and high frequency	0.31	0.22 to 0.42	1			
Duration ≥24 mo and high frequency	0.48	0.39 to 0.57	1.53	1.06 to 2.30	0.18	−0.07 to 0.43
Adjusted‡ model						
Duration 6–23 mo and low frequency	0.38	0.25 to 0.51	1			
Duration ≥24 mo and low frequency	0.36	0.20 to 0.53	0.94	0.51 to 1.67		
Duration 6–23 mo and high frequency	0.33	0.23 to 0.44	1			
Duration ≥24 mo and high frequency	0.47	0.38 to 0.56	1.42	0.99 to 2.12	0.16	−0.10 to 0.41
Fully adjusted§ model						
Duration 6–23 mo and low frequency	0.36	0.24 to 0.49	1			
Duration ≥24 mo and low frequency	0.37	0.20 to 0.55	1.01	0.52 to 1.81		
Duration 6–23 mo and high frequency	0.35	0.23 to 0.45	1			
Duration ≥24 mo and high frequency	0.48	0.38 to 0.58	1.38	0.97 to 2.16	0.13	−0.03 to 0.30

* Population-average prevalence of S-ECC at given categories of breastfeeding duration, as estimated from marginal structural models.
† Includes allocation status from nesting intervention study only.
‡ Includes allocation status from nesting intervention study and maternal age, education, parity, pre-pregnancy BMI, smoking status, social class, and child age and sex.
§ Includes all adjusted model variables and time-varying bottle use variables, feeding habits, and height-for-age *Z*-scores.

introduction and weekly frequency of selected foods, not every aspect of the diet was recorded. Conservatively, we consider our estimates to represent meaningful associations, for which definitive causal claims await confirmation from future studies.

Losses to follow-up were relatively high but not unusual among cohort studies in low-resource settings, where participants frequently change address and contact information. Inverse probability censoring weights can account for losses, and, in this study, weighted estimates were similar to those from complete-case regression. However, losses remain a limitation, as strategies to account for missing data introduce additional assumptions [47]. EPI estimates were imprecise because the relatively small number of children breastfed both infrequently and to long durations limited the statistical power to confirm interactions. Finally, this study population, featuring a relatively high prevalence of breastfeeding and of caries, might not be representative of the breastfeeding-caries relationship in all historical, geographical, and socioeconomic contexts.

A critical question raised by our findings is why breastfeeding, a normative and otherwise health-promoting human behavior, would be associated with deleterious dental outcomes. One possibility is that the mechanism through which repeated, prolonged exposure to human milk could enhance caries progression might operate differently under the near universal availability of highly refined sugars in the modern diet versus historically. Laboratory studies demonstrating greater cariogenic potential of human milk with the addition of outside sugars support this hypothesis [10,11]. Future research is recommended to better define the relationship between particular breastfeeding practices and dental caries in the context of a high-sugar food supply. Our results in no way suggest that breastfeeding itself be discouraged but are congruent with guidelines from professional dental organizations, which

Table 4
Unadjusted and adjusted associations from regression models of breastfeeding duration and S-ECC

Model variables	Unadjusted model, $n = 439$		Adjusted model, $n = 422$		Fully adjusted model, $n = 338$	
	Prevalence ratio	95% CI	Prevalence ratio	95% CI	Prevalence ratio	95% CI
Breastfeeding <6 mo (reference)	1		1		1	
Breastfeeding 6–11 mo	1.45	0.93–2.23	1.52	0.99–2.33	1.45	0.83–2.53
Breastfeeding 12–23 mo	1.28	0.80–2.05	1.44	0.89–2.32	1.39	0.73–2.64
Breastfeeding ≥24 mo	1.96	1.40–2.73	2.04	1.45–2.85	1.85	1.11–3.08
Clinic allocation (intervention)	0.85	0.65–1.09	0.89	0.69–1.15	0.95	0.71–1.27
Maternal age (y)			0.98	0.96–1.01	0.98	0.96–1.01
Maternal education (≤8 y)			1.24	0.93–1.65	1.35	0.97–1.89
Maternal smoking (current)			1.49	1.13–1.95	1.12	0.81–1.55
Parity (has previous child)			1.18	0.85–1.63	1.22	0.87–1.71
Social class (C or lower)			1.09	0.77–1.54	1.06	0.74–1.52
Pre-pregnancy BMI ≤18			1.43	1.06–1.93	1.44	1.03–2.01
Child age at dental assessment (y)			1.25	0.64–2.42	0.99	0.46–2.12
Child sex (male)			1.18	0.91–1.53	1.34	1.01–1.77
Length-for-age *Z*-score at 11–15 mo (per SD)					1.05	0.90–1.23
First-year feeding index (per unit)					1.05	1.01–1.09
Daily bottles at 5–9 mo (1–3)					0.62	0.38–1.02
Daily bottles at 5–9 mo (≥4)					0.84	0.47–1.52
Added sugar in bottle at 5–9 mo					1.46	0.94–2.26
Ever formula fed					0.82	0.59–1.14
Frequency of fruits at 11–15 mo					0.95	0.90–1.01
Frequency of vegetables at 11–15 mo					1.05	0.98–1.12
Frequency of beans at 11–15 mo					1.02	0.94–1.10
Frequency of meat at 11–15 mo					1.04	0.97–1.12
Frequency of organ meat at 11–15 mo					1.17	0.98–1.39

SD = standard deviation.

recommend supporting a mother's decision to breastfeed but avoiding *ad libitum* breastfeeding after tooth eruption [48].

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