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Authors
Kale, A
Deardorff, J
Lahiff, M
et al.

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Breastfeeding Versus Formula-Feeding and Girls' Pubertal Development

Aarti Kale · Julianna Deardorff · Maureen Lahiff · Cecile Laurent · Louise C. Greenspan · Robert A. Hiatt · Gayle Windham · Maida P. Galvez · Frank M. Biro · Susan M. Pinney · Susan L. Teitelbaum · Mary S. Wolff · Janice Barlow · Anousheh Mirabedi · Molly Lasater · Lawrence H. Kushi

Abstract To examine the association of breastfeeding or its duration with timing of girls' pubertal onset, and the role of BMI as a mediator in these associations. A population of 1,237 socio-economically and ethnically diverse girls, ages 6–8 years, was recruited across three geographic locations (New York City, Cincinnati, and the San Francisco Bay Area) in a prospective study of predictors of pubertal maturation. Breastfeeding practices were assessed using self-administered questionnaire/interview with the primary caregiver. Girls were seen on at least annual basis to assess breast and pubic hair development. The association of breastfeeding with pubertal timing was estimated using parametric survival analysis while adjusting for body mass index, ethnicity, birth-weight, mother's education, mother's menarcheal age, and family income. Compared to formula fed girls, those who were mixed-fed or predominantly breastfed showed later onset of breast development [hazard ratios 0.90 (95 % CI 0.75, 1.09) and 0.74 (95 % CI 0.59, 0.94), respectively]. Duration of breastfeeding was also directly associated with age at onset of breast development (p trend = 0.008). Associations between breastfeeding and pubic hair onset were not significant. In stratified analysis, the association of breastfeeding and later breast onset was seen in Cincinnati girls only. The association between breast feeding and pubertal onset varied by study site. More research is needed about the environments within which breastfeeding takes place in order to better understand whether infant feeding practices are a potentially modifiable risk factor that may influence age at onset of breast development and subsequent risk for disease in adulthood.

Keywords Puberty · Puberty-early onset · Breastfeeding · Body mass index

A. Kale (✉) · J. Deardorff · M. Lahiff
School of Public Health, University of California, Berkeley, Berkeley, CA, USA
e-mail: kale.aarti@gmail.com

C. Laurent · A. Mirabedi · M. Lasater · L. H. Kushi
Division of Research, Kaiser Permanente Northern California, Oakland, CA, USA

L. C. Greenspan
Department of Pediatrics, Kaiser Permanente San Francisco, San Francisco, CA, USA

R. A. Hiatt
Department of Epidemiology and Biostatistics and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA

G. Windham
California Department of Public Health, Richmond, CA, USA

M. P. Galvez
Department of Community and Preventive Medicine, Mount Sinai School of Medicine, New York, NY, USA

F. M. Biro
Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

S. M. Pinney
University of Cincinnati College of Medicine, Cincinnati, OH, USA

S. L. Teitelbaum · M. S. Wolff
Mount Sinai School of Medicine, New York, NY, USA

J. Barlow
Zero Breast Cancer, San Rafael, CA, USA

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Introduction

The documented decline in girls' age at onset of puberty and its clinical and social consequences is a growing public health concern [1–4]. Early pubertal maturation not only affects adolescent behavioral and emotional health, but some pubertal events, such as age at menarche, are established risk factors for cancer later in life [2, 4]. In addition, studies have shown that early pubertal timing is associated with endocrine-related disorders, breast cancer [4], metabolic syndrome, psychosocial and other health problems [5]. The timing of puberty is known or suspected to be influenced by various factors including genetics, body mass index (BMI), endocrine disrupting chemicals, nutritional factors, and socio-cultural determinants including race/ethnicity [6–8]. Moreover, peri-natal factors such as maternal smoking during pregnancy, low birth weight, and infant weight gain have been found to influence the timing of pubertal onset [9, 10].

Early infant nutrition and feeding practices may also influence age at pubertal onset. These are some of the few potentially modifiable targets for early intervention; however, research in this area has been scarce. One recent study conducted in Hong Kong found no association between breastfeeding and onset of breast development in Chinese girls [11]. In another study, an association of exclusive breastfeeding with later attainment of menarche was reported in a prospective birth cohort of Filipino girls [12]. A retrospective study in the UK observed a direct association of delayed menarche among women who were breastfed as infants [13]. Others have shown that girls who are formula fed have higher body fat deposition and earlier attainment of menarche compared to those who were breastfed [14]. As breastfeeding has been associated with a decrease in overweight and obesity in infancy and early childhood [15, 16], and overweight is linked to younger onset of puberty [17], it is reasonable to suggest that breastfeeding may be associated with later onset of puberty. In addition, alternative pathways may exist that can affect pubertal timing such as protective factors in breast milk composition [18, 19], or associations of breastfeeding with behavior patterns in mothers that promote health.

This study is the first known longitudinal study of girls in the US that estimates associations between early infant feeding and their timing of pubertal onset, as determined by physical examination. The objectives were (1) to assess whether breastfeeding or its duration was associated with timing of girls' pubertal onset, and (2) to examine the role of BMI as a mediator in these associations. We hypothesized that girls who were predominantly breastfed, and those who were breastfed for longer durations, would enter puberty at later ages, compared to girls who were formula-fed or were breastfed for shorter durations. We also expected that breastfeeding would be associated with girls' lower BMI, which in turn, would be associated with later pubertal onset.

Methods

Data originated from the Puberty Studies of the Breast Cancer and the Environment Research Program (BCERP), a consortium of three collaborative prospective studies examining predictors of early sexual maturation in girls [20]. In these studies, 1,237 socio-economically and ethnically diverse sample of girls, ages 6–8 years, were enrolled between 2004 and 2007 from three locations led by the following institutions: (1) Mount Sinai School of Medicine (MSSM), with recruitment through clinics, schools, and neighborhood centers from East Harlem, New York; (2) Cincinnati Children's Hospital Medical Center, with recruitment through public and parochial schools in the Cincinnati metropolitan area and through the Breast Cancer Registry of Greater Cincinnati; and (3) Kaiser Permanente Northern California (KPNC), with recruitment of Kaiser Health Plan members in the San Francisco Bay Area. Informed consent was obtained from the primary caregiver and child assent from the study participant. Study procedures were approved by the Institutional Review Boards at each participating institution. Eligibility criteria included age (6–8 years), female sex, no underlying endocrine medical conditions, and at MSSM, black or Hispanic race/ethnicity. This collaborative project is an ongoing prospective cohort study, and the present analyses use data from the first 6 years.

Data Collection and Variable Definition

Data were collected annually in California and New York and semi-annually in Cincinnati during in-person clinic visits that included child anthropometry and pubertal assessment. Collection of information on other factors using interviews or self-administered questionnaire with the caregiver was conducted annually at all three sites.

Pubertal Onset

Girls' development was assessed using Marshall and Tanner criteria for breast maturation and pubic hair stages [21] conducted by trained staff at each in-person clinic visit, using a five-stage classification scheme for describing the onset and progression of breast and pubic hair changes by inspection and palpation. Details of training, certification and assessment procedures are reported elsewhere [22]. For analytic purposes, breast and pubic hair pubertal stages were characterized as "no onset" (stage 1) or "onset" (stage 2 or above) for each visit in this investigation.

Infant Feeding Practices

At baseline, the primary caregiver answered questions related to infant feeding history, including whether the

study participant was breast fed and/or formula fed and duration of breastfeeding. Study participants were categorized into three groups based on feeding practices: (1) predominantly breastfed, defined as girls who were exclusively breastfed or received formula no more than 30 days in their lifetime; (2) mixed fed, defined as girls who were breastfed and also received formula for more than 30 days in their lifetime; and (3) formula-fed, defined as girls who were never breastfed and were exclusively formula-fed. *Duration of breastfeeding* was also examined and was categorized as: never breastfed; <3 months; 3–6 months; 6–12 months; and 12 months or longer.

Other Covariates

Body mass index at baseline was calculated as weight (kg)/height (m^2), and the age-specific BMI percentiles based on Centers for Disease Control and Prevention (CDC) standards [23] were estimated for the age in months. Weight and height were measured in clinic using calibrated scales and fixed stadiometers during the baseline examination. BMI percentiles were categorized into overweight (from the 85th up to the 95th percentile), obese (≥the 95th percentile), and normal (<85th percentile).

Girls' race and ethnicity was reported by primary caregivers using census-based categories as well as more detailed information regarding country of origin for parents and grandparents. Caregivers could respond to multiple race or ethnicity categories. These data were categorized into four mutually-exclusive groups: African-American, Hispanic, Asian, and non-Hispanic White. Annual household income at baseline was categorized as <$25,000; $25,000–$50,000; $50,000–$100,000; and >$100,000. For 84 % of girls, the primary caregiver was their biological mother. Mother's educational attainment was classified into five categories: less than high school; high school graduate; some college or associate's degree; bachelor's degree; and master's/professional degree. Birth weight was reported by the primary caregiver at baseline and categorized as low (<2,500 g), normal (2,500–4,000 g), and high (>4,000 g). Mothers' age at menarche was coded as a categorical variable: early (<12 years), normal (12–14 years), and late (>14 years).

Statistical Analysis

Initial analyses focused on describing potential differences in covariate distributions among infant feeding practice categories. Those that appeared to show a difference were then treated as covariates in subsequent analyses.

Primary analyses examined associations for infant feeding practices (breastfeeding or formula feeding) and breastfeeding duration with age at onset of breast or pubic hair development. Analyses used proportional hazards survival methods with left-, interval-, and right-censoring, where the outcome of interest was time (i.e., age of girls) until an event (i.e. pubertal onset). Observations were interval censored because the exact point within the time interval in which the transition to pubertal stage 2 occurred was unknown. To accommodate censoring, one upper and one lower boundary of the interval (girls' age) were considered in every case to estimate the "likelihood contribution" of each observation. The interval in which pubertal onset occurred was defined as between two clinic visit dates: (1) the earlier date with pubertal stage 1 and no prior visit with stage 2 or greater; and (2) the later date as the first clinic visit with pubertal stage 2 or greater and no subsequent stage 1 visit. Right-censored girls had not yet experienced pubertal onset by the final wave included, and so the later date was set as missing. About 14 % of girls already had experienced pubertal onset at the baseline exam. For these left-censored girls, the earlier date defining the interval was set at age 60 months (5 years), based on the assumption that girls would not have begun breast or pubic hair development before that age.

Survival analyses were conducted using STATA version 11, using the Weibull parametric survival model. Interval-censored data requires the use of parametric survival models rather than the Cox proportional hazards model, which only allows for right censoring. The Weibull model is a widely-used and flexible parametric survival model [24]. This model allows for accelerating failure times, which is appropriate in the context of pubertal development, as the older a girl is, the more likely she is to begin pubertal development. Breast and pubic hair onset were treated as separate outcomes. Length of time interval within which puberty began, as defined above, was used as the outcome variable. A final parametric survival model included variables (race/ethnicity, BMI percentile, mother's menarche age, mother's education and family income) that altered coefficient estimates by >10 % or that were significant at a probability level <0.05. Secondary analyses included testing for trend in breastfeeding duration, stratifying analyses by recruitment site (New York City, Greater Cincinnati, and San Francisco Bay Area) and testing for mediation of breastfeeding-puberty association by BMI using the Baron and Kenny steps [25]. We also examined interactions of site with breastfeeding.

Results

Characteristics of the 1,237 girls in the cohort are presented in Table 1. Breastfeeding rates varied across the three sites. Almost two-thirds of San Francisco Bay Area girls were breastfed for at least 6 months, while <7 % were never

Table 1 Characteristics of the BCERP Puberty Studies cohort according to infant feeding practices, BCERP Puberty Studies, 2004–2011

Characteristic	*p*	Category	Predominantly breastfed		Mixed fed		Formula-fed		Overall	
			N	%	N	%	N	%	N	%
Site	<0.001	San Francisco Bay Area	148	33.4	266	60.0	29	6.5	444	35.9
		Greater Cincinnati	44	12.9	187	54.8	110	32.2	377	30.5
		New York City	56	13.8	231	56.8	120	29.5	416	33.6
Race/ethnicity	<0.001	African-American	38	10.2	204	55.1	128	34.6	390	31.5
		Hispanic	72	19.8	232	63.9	59	16.2	372	30.0
		Asian	15	27.8	33	61.1	6	11.1	57	4.6
		White	123	30.4	215	53.2	66	16.3	418	33.8
BMI (percentile)	0.098	Normal (<85)	178	22.3	452	56.7	167	21.0	826	66.7
		Overweight (85–94)	41	22.1	105	56.8	39	21.1	193	15.6
		Obese (≥ 95)	29	13.9	127	60.8	53	25.4	218	17.6
Mother's age at menarche (years)	0.008	<12	55	19.9	170	61.6	51	18.5	278	26.1
		12–13.9	124	23.3	283	53.1	126	23.6	537	50.4
		≥14	46	18.4	165	66.2	38	15.2	249	23.4
Birth-weight (g)	0.004	<2,500	11	9.9	66	59.5	34	30.6	112	9.6
		≥2,500	229	21.9	598	57.1	219	21.0	1,055	90.4
Mother's education	<0.001	<High school graduate	30	16.9	103	58.2	44	24.9	180	15.3
		High school graduate	25	13.8	94	51.9	62	34.2	182	15.4
		Some college	46	12.4	218	58.7	107	28.8	372	31.6
		Bachelor's degree	81	28.6	174	61.5	28	9.9	285	24.2
		Prof./doctoral degree	61	39.6	83	53.9	10	6.5	158	13.4
Family income ($)	<0.001	<25,000	41	15.6	137	52.1	85	32.3	267	21.9
		25,000–49,999	29	12.3	141	59.7	66	27.9	239	19.6
		50,000–99,999	78	24.9	177	56.5	58	18.5	318	26.1
		≥100,000	90	31.2	175	60.8	23	8.0	291	23.8
		Unknown	10	11.0	54	59.3	27	29.6	104	8.5

breastfed, compared to 34 % never breast fed in Cincinnati, and 30 % in New York City. The prevalence of predominant breastfeeding was lowest among African-Americans. Among obese girls, 13 % were reported to be predominantly breastfed, while almost 25 % were formula-fed. Higher breastfeeding rates were found among mothers with higher education and higher household income. Table 2 shows the characteristics of the cohort by pubertal onset. Pubertal onset was noted to occur earlier in girls from Cincinnati and NYC compared to California, in overweight/obese girls compared to those with normal BMI, in African American girls, and in those with lower family income.

Breastfeeding and Onset of Breast Development

Overall, in crude analyses, using formula-fed girls as the reference group, hazard ratios for breast onset were 0.82 (95 % CI 0.69, 0.97) for mixed fed girls and 0.64 (95 % CI 0.52, 0.78) for predominantly breastfed girls. After adjustment for covariates, these associations were somewhat attenuated, but similar associations remained. Hazard ratios from adjusted analyses were 0.90 (95 % CI 0.75, 1.09) and 0.74 (95 % CI 0.59, 0.94) for mixed fed and predominantly breastfed girls, respectively. Table 3 shows site-specific crude and adjusted associations of breastfeeding and onset of breast development.

There was an inverse association of breastfeeding duration with younger age at breast development. In unadjusted analyses, in comparison to girls who were not breastfed, the hazard ratios for increasing duration of breast feeding were 0.84 (0.67, 1.05), 0.85 (0.68, 1.07), 0.92 (0.76, 1.13), and 0.62 (0.51, 0.75) for <3, 3–6, 6–12, and 12 months and more, respectively. After adjustment for covariates, the hazard ratios were 0.84 (0.66, 1.06) for <3 months, 0.98 (0.76, 1.26) for 3–6 months, 1.12 (0.89, 1.40) for 6–12 months, and 0.72 (0.57, 0.90) for 12 months and more. The test for trend (breastfeeding duration) was statistically significant in both crude and adjusted models. Table 4 shows site-specific crude and adjusted associations of breastfeeding and onset of breast development.

Table 2 Characteristics by pubertal onset (crude), BCERP Puberty Studies, 2004–2011

Characteristic	N	Breast onset HR (95 % CI)	Pubic hair onset HR (95 % CI)
Site			
San Francisco Bay Area	444	1.0	1.0
Greater Cincinnati	377	1.7 (1.4, 1.9)	1.1 (0.9, 1.3)
New York City	416	1.5 (1.3, 1.8)	1.5 (1.2, 1.7)
Race/ethnicity			
African-American	390	1.7 (1.4, 2.0)	2.4 (2.0, 2.8)
Hispanic	372	1.2 (1.01, 1.4)	1.3 (1.1, 1.5)
Asian	57	0.7 (0.5, 1.01)	0.5 (0.4, 0.8)
White	418	1.0	1.0
BMI (percentile)			
Normal (<85)	826	1.0	1.0
Overweight (85–94)	193	1.9 (1.5, 2.2)	1.5 (1.2, 1.8)
Obese (≥95)	218	2.2 (2.9, 2.7)	1.7 (1.5, 2.1)
Mother's age at menarche (years)			
<12	278	1.0	1.0
12–13.9	537	0.8 (0.7, 1.01)	0.8 (0.7, 0.9)
≥14	249	0.7 (0.6, 0.8)	0.6 (0.5, 0.8)
Birth-weight (g)			
<2,500	112	0.9 (0.7, 1.1)	1.0 (0.8, 1.3)
≥2,500	1,055	1.0	1.0
Mother's education			
<High school graduate	180	1.0	1.0
High school graduate	182	0.8 (0.6, 1.0)	1.1 (0.8, 1.4)
Some college	372	1.1(0.9, 1.3)	1.3 (1.1, 1.6)
Bachelor's degree	285	0.8 (0.7, 1.0)	1.0 (0.8, 1.2)
Prof./doctoral degree	158	0.8 (0.6, 1.0)	0.9 (0.7, 1.1)
Family income ($)			
<25,000	267	1.0	1.0
25,000–49,999	239	1.1 (0.9, 1.3)	1.3 (1.0, 1.6)
50,000–99,999	318	0.8 (0.7, 0.9)	0.9 (0.8, 1.1)
≥100,000	291	0.6 (0.5, 0.7)	0.6 (0.5, 0.7)

Breastfeeding and Onset of Pubic Hair Development

Unadjusted associations of infant feeding practices with pubic hair onset were qualitatively similar to those for breast onset. In unadjusted bivariate models, using formula-fed girls as the reference group, hazard ratios for breast onset were 0.85 (95 % CI 0.72, 1.01) for mixed fed girls and 0.67 (95 % CI 0.55, 0.83) for predominantly breastfed girls (Table 5). However, adjusted models were more markedly attenuated and did not show statistically-significant associations between predominant breastfeeding and the onset of pubic hair development. There was also an association with duration of breastfeeding in unadjusted models, which was attenuated after adjustment (Table 6).

Table 3 Association of breastfeeding with age at breast onset, stratified by site, BCERP Puberty Studies, 2004–2011

Breastfeeding category	N	Crude HR (95 % CI)	Adjusted[a] HR (95 % CI)
San Francisco Bay Area			
Formula-fed	29	1.0	1.0
Mixed fed	266	0.95 (0.59, 1.51)	1.73 (1.02, 2.96)
Predominantly breastfed	148	0.80 (0.49, 1.29)	1.43 (0.82, 2.47)
Greater Cincinnati			
Formula-fed	110	1.0	1.0
Mixed fed	187	0.70 (0.53, 0.91)	0.56 (0.41, 0.75)
Predominantly breastfed	44	0.60 (0.40, 0.89)	0.55 (0.35, 0.86)
New York City			
Formula-fed	120	1.0	1.0
Mixed fed	231	1.25 (0.95, 1.65)	1.18 (0.87, 1.59)
Predominantly breastfed	56	1.07 (0.73, 1.57)	1.04 (0.68, 1.61)

[a] Adjusting for BMI, ethnicity, mother's age at menarche, girls' birth weight, mother's education, and family income

In adjusted models, BMI was not found to mediate the effect of breastfeeding on pubertal onset. The association of BMI (being overweight or obese) with younger age at breast development was significant (HR 2.12, $p < 0.0005$). Although somewhat associated in crude models as seen in Table 1 ($p = 0.098$), breastfeeding (or its duration) and BMI were no longer associated after adjusting for other covariates ($p > 0.5$). In addition, there was no evidence of mediation by BMI after stratifying by site in individual site-specific models.

The breastfeeding by site interaction term was significant for onset of breast development ($p < 0.005$), but not for pubic hair ($p > 0.5$). Site-specific analyses examining the associations between infant-feeding practices and breastfeeding (Tables 3, 4, 5, 6) demonstrated substantial variation across sites, and adjustment for covariates did not account for this variation. The overall direct association of breastfeeding with age at onset of breast development was seen in Cincinnati girls only. However, the other sites—in particular, the San Francisco Bay Area girls—had relatively limited variation in breast feeding practices, as described below. We also examined effect modification by race/ethnicity and the coefficients did not vary depending on girls' race/ethnicity.

Discussion

This study examined the effect of breastfeeding versus formula feeding, as well as duration of breastfeeding, on girls' breast and pubic hair onset. Predominant breastfeeding and

Table 4 Association of breastfeeding duration with age at breast onset, stratified by site, BCERP Puberty Studies, 2004–2011

Breastfeeding duration (months)	N	Crude HR (95 % CI)	Adjusted[a] HR (95 % CI)
San Francisco Bay Area			
Never breastfed	29	1.0	1.0
<3	49	1.17 (0.67, 2.04)	1.69 (0.91, 3.10)
3–6	63	0.80 (0.47, 1.37)	1.48 (0.79, 2.76)
6–12	118	1.06 (0.65, 1.72)	2.13 (1.21, 3.76)
≥12	179	0.79 (0.49, 1.27)	1.48 (0.85, 2.60)
p trend		0.093	0.93
Per month (continuous)		0.97 (0.94, 1.00)	0.98 (0.95, 1.02)
Greater Cincinnati			
Never breastfed	112	1.0	1.0
<3	51	0.58 (0.40, 0.85)	0.57 (0.38, 0.87)
3–6	50	0.79 (0.54, 1.14)	0.59 (0.39, 0.89)
6–12	73	0.85 (0.61, 1.19)	0.72 (0.49, 1.04)
≥12	45	0.50 (0.33, 0.73)	0.38 (0.24, 0.61)
p trend		0.014	0.001
Per month (continuous)		0.98 (0.94, 1.01)	0.97 (0.93, 1.01)
New York City			
Never breastfed	122	1.0	1.0
<3	72	1.14 (0.80, 1.64)	1.04 (0.70, 1.54)
3–6	57	1.54 (1.03, 2.29)	1.55 (1.01, 2.40)
6–12	59	1.80 (1.23, 2.62)	1.72 (1.13, 2.61)
≥12	99	0.95 (0.69, 1.32)	0.88 (0.61, 1.27)
p trend		0.749	0.63
Per month (continuous)		0.97 (0.94, 1.00)	0.98 (0.94, 1.01)

[a] Adjusting for BMI, ethnicity, mother's age at menarche, girls' birth weight, mother's education, and family income

Table 5 Association of breastfeeding with age at pubic hair onset, overall and stratified by site, BCERP Puberty Studies, 2004–2011

Breastfeeding category	N	Crude HR (95 % CI)	Adjusted[a] HR (95 % CI)
Total population			
Formula-fed	259	1.0	1.0
Mixed fed	684	0.85 (0.72, 1.01)	0.95 (0.79, 1.15)
Predominantly breastfed	248	0.67 (0.55, 0.83)	0.84 (0.67, 1.06)
San Francisco Bay Area			
Formula-fed	29	1.0	1.0
Mixed fed	266	0.75 (0.48, 1.17)	0.92 (0.57, 1.49)
Predominantly breastfed	148	0.64 (0.40, 1.01)	0.84 (0.51, 1.39)
Greater Cincinnati			
Formula-fed	110	1.0	1.0
Mixed fed	187	0.85 (0.64, 1.12)	0.82 (0.60, 1.12)
Predominantly breastfed	44	0.86 (0.57, 1.27)	0.97 (0.63, 1.50)
New York City			
Formula-fed	120	1.0	1.0
Mixed fed	231	1.04 (0.79, 1.36)	1.08 (0.80, 1.46)
Predominantly breastfed	56	0.65 (0.43, 0.95)	0.7 (0.45, 1.07)

[a] Adjusting for BMI, ethnicity, mother's age at menarche, girls' birth weight, mother's education, and family income

longer breastfeeding duration were associated with a later age at onset of breast development when data were combined across all sites. Compared to formula-fed girls, the ages of breast onset were older for mixed-fed girls and even more so for predominantly breast-fed girls, which indicates a possible dose-dependent effect of breastfeeding on later pubertal onset. This is also supported by the observed inverse association between duration of breastfeeding and early onset of breast development. However, there were notable site differences in results from stratified analyses. These associations were evident only in Cincinnati, indicating that there may be unmeasured modifying variables that varied across sites.

Given the temporal sequence of the events examined (breastfeeding—BMI—pubertal onset); we hypothesized that breastfeeding might influence BMI, which in turn, would influence pubertal onset. Thus, we examined BMI as a mediator of the relationship between breastfeeding and onset of pubertal events. The inverse association of breastfeeding with childhood obesity is widely documented [15, 16]. One possible mechanism involves the higher plasma concentration of insulin found in formula-fed infants as compared to breastfed infants [26]. Since insulin enhances cell glucose uptake and inhibits lipolysis (fat breakdown), greater deposition of subcutaneous adipose tissue has been seen in bottle-fed infants [26]. However, there was no evidence of BMI mediation in the combined data or for each site separately. This may be attributed to the factors other than breastfeeding such as early childhood BMI, physical activity, nutrition, etc. [27].

In addition to BMI, alternative pathways to delayed puberty are plausible, such as protective factors in breast milk composition (such as bioactive nutrients) [18, 19], or associations of breastfeeding with behavior patterns in mothers that promote health. For example, mothers who are more likely to breastfeed may also be more likely to have their daughters engage in physical activity, which may in turn delay pubertal onset. Another potential mechanism, based on evolutionary theories, is that breastfeeding might be a proxy for infant attachment security, which has shown to predict later pubertal onset [28].

It is important to note that infant feeding practices across the sites were considerably distinct, as seen by the

Table 6 Association of breastfeeding duration with age at pubic hair onset, overall and stratified by site, BCERP Puberty Studies, 2004–2011

Breastfeeding duration (months)	N	Crude HR (95 % CI)	Adjusted[a] HR (95 % CI)
Total population			
Never breastfed	263	1.0	1.0
<3	172	0.91 (0.72, 1.14)	0.93 (0.73, 1.19)
3–6	170	0.98 (0.78, 1.23)	1.01 (0.78, 1.29)
6–12	250	0.81 (0.66, 0.99)	0.97 (0.77, 1.21)
≥12	323	0.67 (0.55, 0.82)	0.81 (0.65, 1.01)
p trend		0.000	0.059
Per month (continuous)		0.96 (0.95, 0.98)	0.98 (0.96, 1.00)
San Francisco Bay Area			
Never breastfed	29	1.0	1.0
<3	49	0.81 (0.47, 1.37)	0.84 (0.48, 1.48)
3–6	63	0.77 (0.46, 1.28)	0.97 (0.55, 1.71)
6–12	118	0.71 (0.44, 1.12)	0.93 (0.56, 1.55)
≥12	179	0.67 (0.43, 1.05)	0.92 (0.55, 1.51)
p trend		0.000	0.94
Per month (continuous)		0.98 (0.95, 1.01)	1.00 (0.97, 1.04)
Greater Cincinnati			
Never breastfed	112	1.0	1.0
<3	51	0.73 (0.49, 1.08)	0.77 (0.50, 1.19)
3–6	50	1.16 (0.79, 1.72)	0.91 (0.58, 1.42)
6–12	73	0.93 (0.66, 1.31)	0.92 (0.63, 1.34)
≥12	45	0.66 (0.44, 0.99)	0.72 (0.45, 1.14)
p trend		0.151	0.32
Per month (continuous)		0.98 (0.95, 1.02)	0.99 (0.95, 1.03)
New York City			
Never breastfed	122	1.0	1.0
<3	72	1.25 (0.87, 1.79)	1.10 (0.75, 1.64)
3–6	57	1.17 (0.80, 1.71)	1.11 (0.73, 1.69)
6–12	59	1.02 (0.70, 1.48)	1.13 (0.75, 1.72)
≥12	99	0.67 (0.48, 0.93)	0.75 (0.52, 1.08)
p trend		0.002	0.07
Per month (continuous)		0.94 (0.91, 0.97)	0.96 (0.92, 0.99)

[a] Adjusting for BMI, ethnicity, mother's age at menarche, girls' birth weight, mother's education, and family income

numbers of girls who fell into the different exposure categories, which may in part account for differences across the sites. A substantial majority of San Francisco Bay Area girls was breastfed and for longer durations, whereas only a minority of girls in New York City was predominantly breastfed, thus limiting variability in the exposure for these two sites. Participants in New York City may be exposed to other risk factors for earlier pubertal onset such as environmental exposures, or lifestyle factors. These associations may have a much stronger impact on pubertal onset that the counter effect of breastfeeding may not have been observable This may also help explain why past studies, originating from different parts of the world, that examine breast feeding and menarche have yielded inconsistent or equivocal results.

This study had several limitations. Participants were recruited from three different areas of the US with diverse socio-economic and ethnic backgrounds; however, our sample is not generalizable to the entire US population. For example, sites were predominantly urban therefore participants from rural areas were not represented. Participants had to voluntarily go to the clinic site, adding a possible selection bias. Also, we did not gather detailed data about early infant feeding practices, such as details about exclusivity of breastfeeding, age of formula/solids introduction. Maternal age at menarche, a proxy for genetic contributions to daughter's age at breast or pubic hair onset, may not be sufficient to control for potential genetic confounders. The differences noted across the three sites may appear to be a limitation. However, by combining data across the sites, we thus have greater and more stable variation in our main exposures of interest and other key covariates. This could thus be considered a marked strength of this multi-site study. Of note, variation in the sampling strategies across the sites may be considered a limitation. In future cross-site studies, consistent sampling approaches should be employed to minimize potential unmeasured confounding.

Despite limitations, our study addresses some distinct gaps in this area of research. For example, studies with cross-sectional designs [2] or those using logistic regression models fail to address the key analytical issue of censoring, potentially creating significant bias in estimates of pubertal onset. Many studies [12, 14] focus mainly on a single pubertal marker, menarche, which occurs relatively late in pubertal development and is thus a weak and indirect measure of pubertal onset. Some studies [11] fail to include BMI, despite its documented association with pubertal timing. Other studies [9] use self-report or parent-report for pubertal stage estimates. This method is not as reliable as pubertal staging by standardized in-clinic examination with palpation, the method used in this study [29].

The unique strengths of this study outweigh its limitations. This is the first prospective study on a multiethnic US cohort to examine the association between breastfeeding and girls' pubertal onset using clinical Tanner staging to assess pubertal markers. The longitudinal study design, with annual/semiannual follow-up using physical examination and palpation, allowed for better estimates of age at pubertal onset compared to past studies. We assessed the

role of BMI as a possible mediator of the association of breastfeeding and pubertal onset, which has not been tested in other studies. In addition, this study is one of the first to use parametric survival analysis models with interval censoring, incorporating more appropriate analytic methods for analyzing longitudinal data with time to onset of puberty as the outcome.

Breastfeeding has been linked to many health benefits across the lifespan for both mothers and their children [30]. Such benefits include reduced morbidity due to gastrointestinal and respiratory infections, reduced rates of sudden death syndrome in infants; and lower cardiovascular disease risk and obesity later in adulthood. In this study, an additional potential benefit of breastfeeding was explored, an inverse association with timing of pubertal onset. Our principal findings are that breastfeeding and its duration may be associated with later age at onset of breast development in certain contexts. This study may viewed as a pivot to the research on this topic, with a future representative national sample further exploring the roles of ethnic origins, BMI, maternal factors, environmental factors. Our findings suggest that future research should focus on potential modifiers of these effects and whether these modifying factors may vary systematically across contexts. From a clinical perspective, such research could provide important information for pediatricians, pediatric nurse practitioners and family physicians, who play a role in education and promotion of breastfeeding and work nationally across diverse contexts. If further research confirms that breastfeeding is associated with delayed puberty within certain contexts, then targeted interventions [31] may confer additional health benefits to delay onset of puberty among certain sets of girls and may promote subsequent beneficial psychosocial and health effects throughout the life-span.

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