

Early Exposure to Cow's Milk Protein Is Associated with a Reduced Risk of Cow's Milk Allergic Outcomes



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What is already known about this topic? Early introduction of allergenic foods, particularly peanut, into the infant's diet is now recommended in food allergy prevention guidelines internationally. However, the optimal timing of exposure for different allergens, including cow's milk, is not known.

What does this article add to our knowledge? Exposure to cow's milk protein, through the use of infant formula, in the first 3 months of life was associated with a reduced risk of cow's milk sensitization and allergy. This association was specific to cow's milk atopic outcomes only, not other challenge-confirmed food allergies.

How does this study impact current management guideline? These findings are from an observational study and clinical trials are warranted to further assess this association.

BACKGROUND: Overall early exposure to allergenic foods in the infant's diet is a new strategy for preventing food allergy to that allergen, but the optimal timing of exposure for different allergens is not known.

OBJECTIVES: We aimed to examine the relationship between exposure to cow's milk protein in the first 3 months of life and risk of cow's milk allergy at age 12 months.

METHODS: HealthNuts is a longitudinal population-based food allergy study that recruited 5,276 twelve-month-old infants. Skin prick testing to cow's milk was conducted on the second half of the cohort ($n = 2,715$) and sensitization defined as a wheal ≥ 2 mm. Cow's milk allergy was defined as a parent-reported reaction to cow's milk consistent with IgE-mediated allergy together with evidence of sensitization. Early exposure to

cow's milk protein was captured through parental questionnaire administered at 1 year of age and defined as consumption of cow's milk-based infant formula during the first 3 months of life. **RESULTS:** Forty-two percent of infants were exposed to cow's milk in the first 3 months of life ($n = 1,977/4,712$) and 87% of these infants were also breastfed. Early exposure to cow's milk protein was associated with a reduced risk of cow's milk sensitization (adjusted odds ratio [aOR] 0.44, 95% confidence interval [CI] 0.23-0.83), parent-reported reactions to cow's milk (aOR 0.44, 95% CI 0.29-0.67), and cow's milk allergy (aOR 0.31, 95% CI 0.10-0.91) at age 12 months. Age at exposure to cow's milk protein was not associated with the risk of other food allergies. **CONCLUSIONS:** Exposure to cow's milk protein in the first 3 months of life was associated with a reduced risk of cow's milk

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Abbreviations used

aOR-Adjusted odds ratio
CI-Confidence interval
EAT-Enquiring About Tolerance
GI-Gastrointestinal
LEAP-Learning Early About Peanut
OR-Odds ratio
PHF-Partially hydrolyzed formula
RCT-Randomized controlled trial
SPT-Skin prick testing

allergy. These findings are from an observational study and clinical trials are warranted to further assess this association before any recommendations to infant feeding guidelines can be made. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;7:462-70)

Key words: Cow's milk protein; Cow's milk allergy; Food allergy; Infant feeding; Allergen exposure

The timing of introduction of specific allergenic foods into the diet of infants for allergy prevention has been the subject of great interest in the research and clinical community. Following publication and widespread media interest in the Learning Early About Peanut (LEAP) allergy randomized controlled trial (RCT), which showed that the introduction of peanut into the diet of high-risk infants between 4 and 11 months reduced the risk of peanut allergy, infant feeding guidelines around the world have been updated to advise that allergenic foods can be included in the infant's diet from once introduction of solid foods has commenced, and that the avoidance of these foods is no longer recommended for allergy prevention.¹ There have been 5 recent RCTs on early dietary egg exposure for preventing egg allergy, with meta-analysis suggesting an overall protective association.²⁻⁷ However, knowledge gaps remain with regard to the optimal timing of early allergen exposure to induce tolerance for other foods and whether this window of opportunity differs between foods.

Cow's milk allergy is a common food allergy in childhood although it is frequently outgrown early in life.⁸ A recent systematic review and meta-analysis of RCTs found that early introduction of cow's milk was not associated with the risk of cow's milk sensitization (3 studies) or allergy (2 studies); however, there was heterogeneity between studies and how early versus late introduction of cow's milk was defined.⁷ Evidence from individual RCTs has provided conflicting evidence on the role of early cow's milk exposure in the first few days of life for allergic outcomes in childhood.⁹⁻¹¹ In an observational cohort of 13,019 infants, exposure to cow's milk protein through the use of infant formula between age 15 and 194 days compared with exposure in the first 15 days of life was associated with increased odds of developing cow's milk allergy (odds ratio [OR] 19.3, 95% confidence interval [CI] 6.0-62.1).¹² In addition, the timing of exposure to cow's milk has been associated with cow's milk allergy or sensitization in other case-control and observational studies,^{13,14} although another study reported conflicting findings.¹⁵ The Enquiring About Tolerance (EAT) study, an RCT of early dietary introduction of 6 allergenic foods in breast-fed infants, found no protective effect of cow's milk introduction between 3 and 6 months of age, compared with introduction

from 6 months of age against cow's milk allergy, although the early introduction group had significantly less cow's milk sensitization than the standard introduction group at age 3 years in the per protocol but not intent-to-treat analysis (relative risk [RR] 0.12, 95% CI 0.02-0.89 and 0.57, 95% CI 0.21-1.53, respectively).¹⁶ Whether exposure to cow's milk protein in the first 3 months is associated with the development of cow's milk allergy remains unknown.

Cow's milk protein is often one of the first foreign proteins that some infants are exposed to through infant formula and the use of infant formula for allergy prevention has been controversial. There is inconclusive evidence regarding a preventative benefit of partially hydrolyzed formula (PHF) compared with standard cow's milk formula for any atopic outcome including food allergy.¹⁷ In light of recent advances on inducing oral tolerance through early allergen exposure, observational studies on early exposure to cow's milk through the use of infant formula may shed some light on optimal timing of allergen exposure and be useful for informing future interventional studies.

In the HealthNuts population-based study of food allergy, we have previously reported that there was no evidence of an association between duration of breastfeeding and food allergy at 1 year of age, nor was there an association between the use of PHF compared with standard cow's milk infant formula and food allergy at 1 year of age.¹⁸ However, age at first exposure to cow's milk was not specifically examined in this analysis. In the present study, we aim to determine whether exposure to cow's milk protein through the use of cow's milk-based infant formula in the first 3 months of life is associated with the risk of developing cow's milk allergy.

METHODS

The HealthNuts study methods have been described in detail previously.¹⁹ Between 2007 and 2011, 5,276 infants were recruited from council-run immunization sessions and offered skin prick testing (SPT) to 4 common food allergens. All infants underwent SPT to egg white, peanut, and sesame; the first half of the cohort received an additional SPT to shrimp, whereas the second half received SPT to cow's milk. Initially, cow's milk SPT was not conducted because of failure to gain ethics committee approval due to fears that a high prevalence of milk sensitization would be observed in infants already tolerating cow's milk, leading to unnecessary anxiety among parents and possibly extending Royal Children's Hospital Allergy Department waiting lists. This ruling was subsequently reversed when a pilot study found low cow's milk sensitization rates at age 12 months, and SPT for cow's milk replaced shellfish in October 2009. SPTs were administered with a single-tine lancet (Stallergenes, Antony, France) on the infant's back using allergens from ALK-Abello (Madrid, Spain), along with a positive control (10 mg/mL histamine) and a negative control (saline). Wheal size was measured after 15 minutes and calculated by subtracting the negative control from the average of the longest diameter and the diameter perpendicular to it.

After the SPT was administered but before parents received the results, a questionnaire was completed that captured detailed information on infant feeding, including age and type of infant formula used, food reactions, and other demographic and environmental exposures. Any infant with evidence of sensitization to egg, peanut, or sesame was invited to the Royal Children's Hospital in Melbourne for an oral food challenge (OFC) to that food. OFC were not conducted to cow's milk due to insufficient resources. Cow's milk

allergy was determined based on parental report of a reaction to cow's milk consistent with IgE-mediated symptoms and a positive cow's milk skin prick test (see definitions).

Definitions of cow's milk atopic outcomes

Cow's milk sensitized: SPT ≥ 2 mm (second half of cohort only; a lower SPT threshold of sensitization was used because evidence suggests that skin reactivity is lower in infants^{20,21}).

Parent-reported reaction to cow's milk: parent report of reaction to milk consistent with IgE-mediated allergy within 1 hour of ingestion (skin rash, hives, angioedema, vomiting, difficulty breathing).

Cow's milk allergy: parent-reported reaction to milk consistent with IgE-mediated allergy and milk sensitized.

We additionally considered as a separate outcome infants who were reported to have gastrointestinal (GI) reactions to cow's milk including vomiting, diarrhea, constipation, bloating, abdominal pains, and blood/mucus in stool, excluding infants from this category who were identified as having reactions to cow's milk consistent with IgE-mediated food allergy.

Early cow's milk protein exposure

Timing of first exposure to cow's milk protein was ascertained through parental questionnaires at recruitment when the infant was 12 months of age. Parents were asked at what age the infant began using infant formula and the type of infant formula used. For this analysis, early exposure to cow's milk protein was defined as the use of a cow's milk formula (including PHF) in the first 3 months of life. We chose the age of <3 months because it was before solids and whole cow's milk or cow's milk containing foods are likely to have been introduced into the infant's diet, and the age that cow's milk containing foods was introduced into the diet was not collected. Infants who were exposed to only an extensively hydrolyzed ($n = 3$), amino acid-based ($n = 4$), lactose-free ($n = 14$), or reflux/colic formula ($n = 28$) in the first 3 months were included in the early exposure to cow's milk protein category. Infants who used a goat or sheep's milk-based formula in the first 3 months (or if an age was not specified) were excluded because of potential cross-reactivity of the goat's/sheep milk proteins and cow's milk proteins ($n = 11$).

Ethics

Ethics approval was obtained for the HealthNuts study from the Victorian State Government Office for Children (reference no. CDF/07/492), the Victorian State Government Department of Human Services (reference no. 10/07), and the Royal Children's Hospital Human Research Ethics Committee (reference no. 27047).

Statistical methods

A separate logistic regression model for each of the 3 cow's milk atopic outcomes was used to quantify the association between early exposure to cow's milk protein and cow's milk allergy, expressed as ORs with 95% CIs. The following variables were adjusted for *a priori* in all models: immediate family history of any allergy (eczema, asthma, food allergy, or hay fever), parent's country of birth (both Australia, one or both Asia and other) socioeconomic status, child's sex, and presence of older siblings. The following variables were considered as potential confounders and only retained if they altered the magnitude of association between the exposure and outcome by more than 10%: premature birth, mode of delivery, pet dog exposure, and childcare attendance in the first year of life. Effect modification was assessed by fitting an interaction term between the early cow's milk protein exposure variable and other exposure variables listed above using likelihood ratio tests; a P value of $<.10$ was

considered evidence of effect modification. Parent report of a doctor diagnosis of eczema before 3 months of age was considered in the relationship between early cow's milk protein exposure and cow's milk atopic outcomes as it may lie on a casual pathway, alter parental behavior, or share common causes with food allergy, and models were constructed with and without adjusting for age of eczema diagnosis. The associations reported did not appear to be accounted for by the presence of eczema as further adjustment for eczema did not materially alter the findings. Consequently, we report results from the models without adjustment for eczema.

To further explore the role of early milk protein exposure, several variations on the primary exposure were considered. Although we could not formally test for an interaction between early cow's milk protein exposure and breastfeeding, we additionally considered the role of breastfeeding by comparing infants who were exclusively formula fed and those who received both breast milk and a cow's milk formula. PHF was not associated with food allergy risk in this cohort,¹⁸ but at the time this study was conducted, parents may have chosen to use a PHF believing it would reduce their child's risk of food allergy. We therefore conducted a separate analysis using a 3-category exposure of no cow's milk protein exposure, only a standard cow's milk formula, or only PHF exposure in the first 3 months (excluding infants who received both types of formula in the first 3 months). A sensitivity analysis was also conducted excluding children who changed formula type in the first 3 months of life as they may have reacted to the first formula.

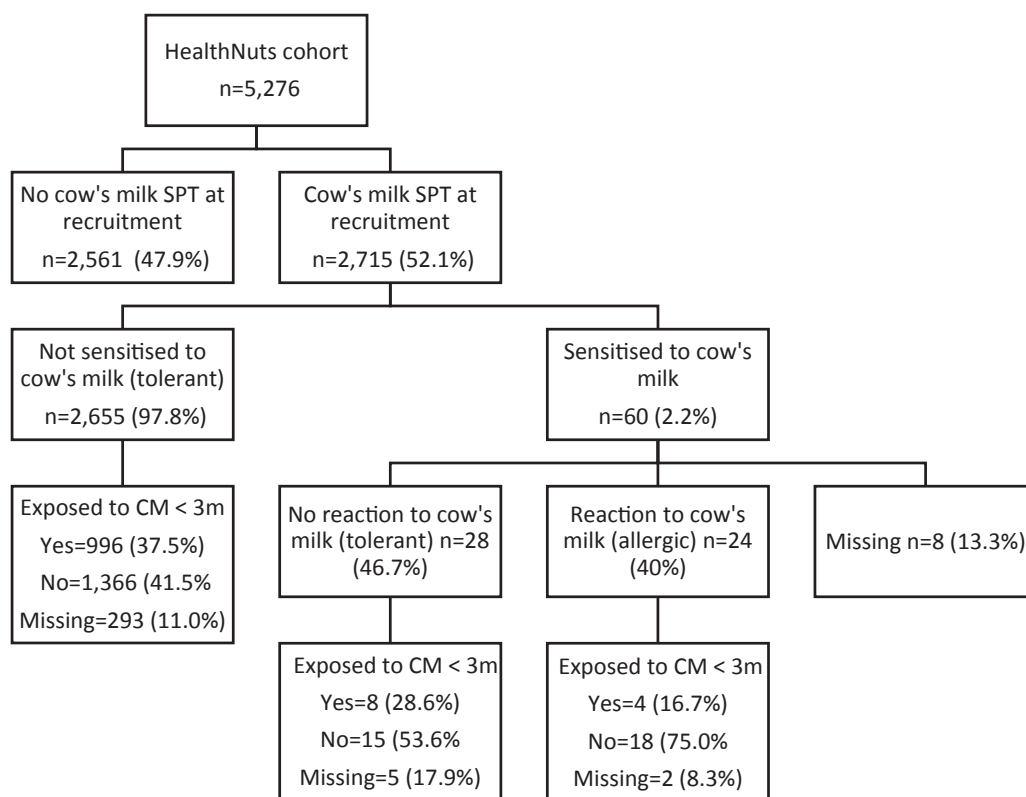
Finally, we assessed whether early exposure to cow's milk protein was associated with the risk of challenge-confirmed allergy to other foods (egg, peanut, and sesame). This analysis was adjusted for all *a priori* confounders, exposure to pet dogs in the first year, age that eczema was diagnosed, and age at introduction to egg (egg and any food allergy outcomes only) as these are important risk factors for food allergy in this cohort.^{22,23} After adjustment, an inverse association between age of cow's milk protein exposure and egg allergy was observed; however, this did not persist after inclusion of cow's milk sensitization in the model, which indicates that the association between cow's milk protein exposure and egg allergy was not independent of pathways related to cow's milk sensitization and that it may be an intermediate factor rather than confounder.

RESULTS

Participation and cow's milk allergy outcomes

A total of 5,276 twelve-month-old infants participated in the HealthNuts study (74% participation). Participation in cow's milk SPT including determination of cow's milk allergy status and proportion of the sample with data on early exposure to cow's milk protein is presented in Figure 1, A. Cow's milk SPT was only conducted on the second half of the cohort and 2.2% (95% CI 1.7-2.8%, $n = 60/2,715$) were sensitized at the population level. Responses to parent-reported reactions to cow's milk were available for 5,140 infants. Of these, 359 reported a reaction to cow's milk but only 149 of these reactions were consistent with IgE-mediated cow's milk allergy; the prevalence of parent-reported reactions to cow's milk consistent with IgE-mediated allergy was 3.0% (95% CI 2.6-3.5%, $n = 149/4,930$). Infants with reactions that were unlikely to be IgE mediated were excluded from this outcome ($n = 210$). Cow's milk allergy was only determined among infants who had SPT to cow's milk and was present in 0.9% (95% CI 0.6-1.3%, $n = 24/2,707$). Complete data on early life feeding history including age

A Cow's milk sensitisation and allergy



B Parent-report reaction to cow's milk

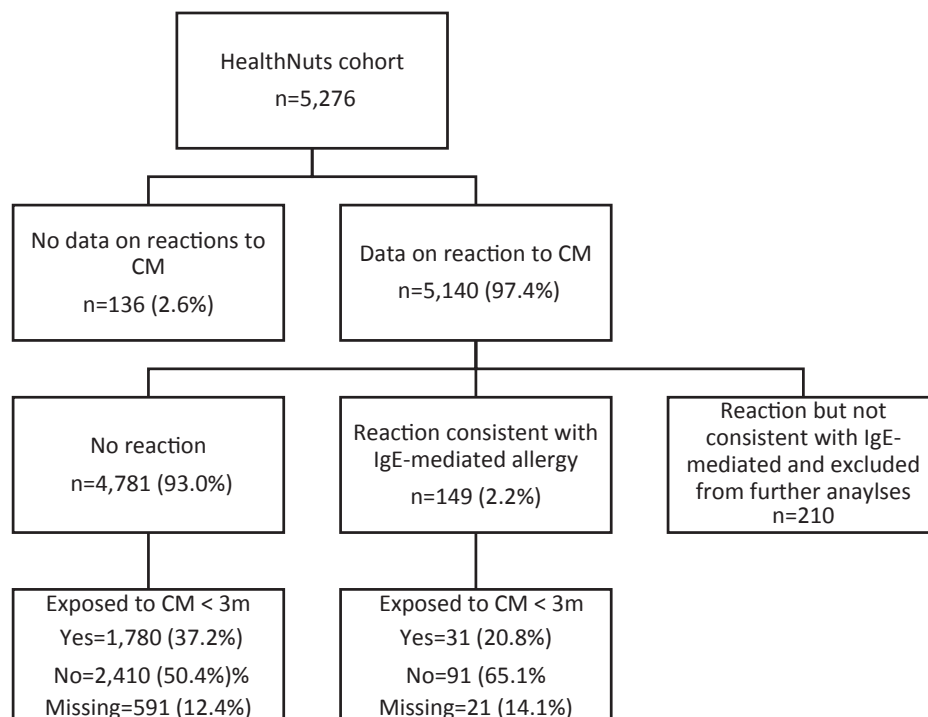


FIGURE 1. Participation in the HealthNuts study and infants included in the final analysis to (A) cow's milk sensitization and allergy and (B) parent-report milk reaction. CM, Cow's milk; SPT, skin prick test.

TABLE I. Key outcome and exposure definitions and demographic characteristics of the HealthNuts cohort

	SPT group (n = 2715)	Parent-report reaction (n = 4930)
	% (n/N)	% (n/N)
Age at recruitment (mo), mean (SD)	12.6 (0.73)	12.7 (0.73)
Sex (female)	50.6% (1,367/2,702)	49.3% (2,419/4,903)
Premature birth <37-wk gestation	5.8% (150/2,597)	6.1% (286/4,682)
Caesarean delivery	33.1% (892/2,698)	33.5% (1,646/4,906)
Any siblings	49.2% (1,333/2,707)	50.1% (2,462/4,913)
Family history of any allergy	69.4% (1,885/2,715)	69.1% (3,404/4,930)
Parent's country of birth—both Australian	59.1% (1,590/2,690)	59.1% (2,885/4,885)
One or both East Asia	11.6% (311/2,690)	11.6% (568/4,885)
Other	29.3% (789/2,690)	29.3% (1,432/4,885)
No eczema diagnosis*	75.6% (1,936/2,560)	75.4% (3,483/4,618)
Eczema diagnosis ≤3 mo old	10.9% (279/2,560)	11.0% (507/4,618)
Eczema diagnosis >3 mo old	13.5% (345/2,560)	13.6% (628/4,618)
Socioeconomic status quintile 1	21.0% (568/2,707)	20.5% (1,006/4,917)
Quintile 2	18.4% (497/2,707)	20.3% (998/4,917)
Quintile 3	22.2% (600/2,707)	21.0% (1,034/4,917)
Quintile 4	20.6% (557/2,707)	19.3% (951/4,917)
Quintile 5	17.9% (485/2,707)	18.9% (928/4,917)
Childcare attendance in first year	26.3% (712/2,706)	24.4% (1,197/4,910)
Pet dog	30.8% (835/2,715)	30.7% (1,517/4,930)

*Parent report of doctor diagnosis of eczema and age that eczema was diagnosed.

TABLE II. Association between early cow's milk protein exposure and cow's milk atopic outcomes

	% (n) allergic	Unadjusted OR	P	Adjusted OR*	P
Reaction to milk					
No cow's milk protein 0-3 mo	3.9% (97/2,507)	1.0	—	1.0	—
Cow's milk protein exposure 0-3 mo	1.7% (31/1,811)	0.43 (0.29-0.65)	<.001	0.44 (0.29-0.67)	<.001
Milk sensitization					
No cow's milk protein 0-3 mo	2.8% (39/1,405)	1.0	—	1.0	—
Cow's milk protein exposure 0-3 mo	1.3% (13/1,009)	0.46 (0.24-0.86)	.015	0.44 (0.23-0.83)	.012
Milk allergy					
No cow's milk protein 0-3 mo	1.3% (18/1,399)	1.0	—	1.0	—
Cow's milk protein exposure 0-3 mo	0.4% (4/1,008)	0.31 (0.10-0.91)	.032	0.31 (0.10-0.91)	.034

OR, Odds ratio.

*All models adjusted for sex, family history of allergy, any siblings, socioeconomic status, and parent's country of birth. The cow's milk reaction model was additionally adjusted for premature birth. Sensitivity analysis including age of eczema onset as a confounding variable did not materially alter the results.

of infant formula use were available for 4,712 infants. Overall, 42.0% (1,977/4,712) were exposed to cow's milk protein through the use of infant formula in the first 3 months of life, and of those who received an infant formula in the first 3 months, 86.6% also received breast milk. Demographic characteristics of the sample with complete information on early exposure to cow's milk protein are presented separately for those who underwent SPT at recruitment and those with parent-reported reactions to cow's milk, in [Table I](#).

Association between early exposure to cow's milk protein and cow's milk atopic outcomes

All cow's milk atopic outcomes, including sensitization, parent-reported reactions, and allergy, occurred less frequently among infants who were exposed to cow's milk protein in the first 3 months of life ([Table II](#)). After adjustment for confounding variables, an inverse association for early cow's milk

protein exposure was observed for all the cow's milk atopic outcomes. Early exposure to cow's milk protein was associated with a reduced risk of cow's milk reactions by an adjusted odds ratio (aOR) of 0.44 (95% CI 0.29-0.67), cow's milk sensitization by 0.44 (95% CI 0.23-0.83), and cow's milk allergy by 0.31 (95% CI 0.10-0.91). There was no evidence of effect modification between early cow's milk protein exposure and other exposure variables considered in this analysis (data not shown).

Association between early exposure to cow's milk protein with or without breastfeeding, and cow's milk atopic outcomes

We additionally assessed the role of breastfeeding in the relationship between early exposure to cow's milk protein and cow's milk atopic outcomes by comparing separately infants who were exclusively formula fed from birth to those who received both infant formula and breast milk in the first 3 months of life, to

TABLE III. Association between early cow's milk protein exposure with or without breastfeeding and cow's milk atopic outcomes

	% (n) allergic	Unadjusted OR	P	Adjusted OR*	P
Reaction to milk					
No cow's milk protein exposure 0-3 mo	3.8% (97/2,507)	1.0	—	1.0	—
Mixed breast and formula feeding 0-3 mo	1.8% (28/1,554)	0.46 (0.30-0.70)	<.001	0.45 (0.29-0.71)	<.001
Exclusive formula feeding from 0 mo	1.2% (3/245)	0.31 (0.10-0.98)	.046	0.36 (0.11-1.16)	.086†
				P-trend	.002
Milk sensitized					
No cow's milk protein exposure 0-3 mo	2.8% (39/1,405)	1.0	—	1.0	—
Mixed breast and formula feeding 0-3 mo	1.5% (13/884)	0.52 (0.28-0.98)	.045	0.49 (0.26-0.93)	.03
Exclusive formula feeding from 0 mo	0% (0/121)	—	.025‡	—	
Milk allergy					
No cow's milk protein exposure 0-3 mo	1.3% (18/1,399)	1.0	—	1.0	—
Mixed breast and formula feeding 0-3 mo	0.5% (4/883)	0.35 (0.12-1.04)	.058	0.34 (0.11-1.02)	.054
Exclusive formula feeding from 0 mo	0% (0/121)	—	.084‡	—	

OR, Odds ratio.

*All models adjusted for sex, family history of allergy, any siblings, socioeconomic status, and parent's country of birth. The cow's milk reaction model was additionally adjusted for premature birth. Sensitivity analysis including age of eczema onset as a confounding variable did not materially alter the results.

†P value comparing exclusive formula feeding to mixed breast and formula feeding in first 3 months is 0.690.

‡Fisher's exact test.

TABLE IV. Association between early cow's milk protein exposure via a standard cow's milk formula or PHF and cow's milk atopic outcomes

	% (n) allergic	Unadjusted OR	P	Adjusted OR*	P†
Reaction to milk					
No cow's milk protein exposure 0-3 mo	3.9% (98/2,556)	1.0	—	1.0	—
Cow's milk formula only 0-3 mo	1.6% (24/1,474)	0.42 (0.26-0.65)	<.001	0.42 (0.26-0.67)	<.001
PHF only 0-3 mo	0.7% (2/278)	0.18 (0.04-0.74)	.017	0.19 (0.05-0.80)	.023
Milk sensitized					
No cow's milk protein exposure 0-3 mo	2.8% (39/1,405)	1.0	—	1.0	—
Cow's milk formula only 0-3 mo	1.3% (10/781)	0.45 (0.23-0.92)	.027	0.43 (0.21-0.88)	.021
PHF only 0-3 mo	1.2% (2/169)	0.42 (0.10-1.75)	.234	0.42 (0.10-1.75)	.231
Milk allergy					
No cow's milk protein exposure 0-3 mo	1.3% (18/1,399)	1.0	—	1.0	—
Cow's milk formula only 0-3 mo	0.4% (3/784)	0.30 (0.09-1.01)	.051	0.30 (0.09-1.02)	.053
PHF only 0-3 mo	0.6% (1/169)	0.46 (0.06-3.44)	.447	0.45 (0.06-3.43)	.441

OR, Odds ratio; PHF, partially hydrolyzed formula.

*All models adjusted for sex, family history of allergy, any siblings, socioeconomic status, and parent's country of birth. The cow's milk reaction model was additionally adjusted for premature birth. Sensitivity analysis including age of eczema onset as a confounding variable did not materially alter the results.

†P value comparing standard cow's milk formula only with PHF use only 0.344 for milk reaction, 0.989 for milk sensitization, and 0.740 for milk allergy.

the comparison group of no cow's milk protein exposure (exclusive breastfeeding) in the first 3 months (Table III). Overall (n = 4,653), 58.8% of infants were exclusively breastfed for the first 3 months, 35.8% were exposed to both breast milk and cow's milk formula in the first 3 months of life, and 5.4% were exclusively formula fed from birth. There were no infants in the exclusively formula fed from birth group who were sensitized or allergic to cow's milk at age 12 months. There was an inverse association between early exposure to cow's milk protein during breastfeeding for all the cow's milk atopic outcomes. This association trended toward significance amongst infants who were exclusively formula fed from birth for cow's milk reactions only. There was no evidence of a difference in risk between infants who were exclusively formula fed compared with mixed breast and formula feeding in first 3 months (P = .690).

Association between early exposure to cow's milk protein via standard cow's milk formula or PHF

The association between early cow's milk protein exposure and the cow's milk atopic outcomes was additionally examined among infants who were exposed to only a standard cow's milk formula or only a PHF, excluding infants who received both types of formula in the first 3 months (n = 71). The inverse association between early cow's milk protein exposure and cow's milk allergy was maintained when restricted to only those infants who received a standard cow's milk formula in the first 3 months of life (Table IV). Although infants who received only a PHF were less likely to have cow's milk sensitization and allergy at age 12 months than those who were not exposed to cow's milk protein in the first 3 months of life, there was little evidence from the adjusted models that the risk was different, which may be attributable to the limited statistical power for comparisons in

TABLE V. Association between early cow's milk protein exposure and challenge-confirmed allergy to other foods

	% (n) allergic	Unadjusted OR	P	Adjusted OR*	P
Peanut allergy					
No cow's milk protein 0-3 mo	3.1% (43/1,383)	1.0	—	1.0	—
Cow's milk protein exposure 0-3 mo	3.6% (35/985)	1.15 (0.73-1.81)	.551	1.08 (0.65-1.80)	.777
Egg allergy					
No cow's milk protein 0-3 mo	10.2% (137/1,349)	1.0	—	1.0	—
Cow's milk protein exposure 0-3 mo	8.0% (78/978)	0.77 (0.57-1.03)	.074	0.77 (0.54-1.08)	.124
Any food allergy†					
No cow's milk protein 0-3 mo	11.1% (149/1,349)	1.0	—	1.0	—
Cow's milk protein exposure 0-3 mo	9.8% (94/964)	0.87 (0.66-1.14)	.317	0.86 (0.62-1.18)	.354

OR, Odds ratio; SPT, skin prick test.

*All models adjusted for sex, family history of allergy, any siblings, age at eczema diagnosis socioeconomic status, parent's country of birth, cow's milk sensitization, and exposure to pet dogs. The egg and any food allergy analyses were additionally adjusted for age of introduction to egg. This analysis was restricted to the second half of the cohort who underwent cow's milk SPT as there was evidence to indicate that the association between early cow's milk exposure and other food allergies was not independent of milk sensitization.

†Challenge confirmed allergy to egg, peanut, or sesame at age 1 year.

these smaller subgroups. An association between early exposure to PHF was observed only for the outcome of parent-reported milk reactions (aOR 0.19, 95% CI 0.05-0.80); however, there was no evidence of a difference in risk between infants who received standard cow's milk formula compared with PHF ($P = .344$).

Association between early exposure to cow's milk protein and GI symptoms

Overall, 0.9% of infants ($n = 44/4,825$) were reported to have reactions to cow's milk resulting in GI symptoms (excluding infants who were identified as having reactions consistent with IgE-mediated cow's milk allergy). Infants who were exposed to cow's milk protein in the first 3 months of life, compared with those who were not exposed, were less likely to have parent-reported GI reactions to cow's milk (0.54% and 1.25%, respectively, aOR 0.45, 95% CI 0.22-0.92, $P = .029$).

Association between early exposure to cow's milk protein and other food allergies

In an adjusted model, an inverse association between early exposure to cow's milk protein and egg allergy was observed; however, this did not persist after inclusion of cow's milk sensitization in the model. This indicates that the association between cow's milk protein exposure and egg allergy is not independent of pathways related to cow's milk sensitization and that it may be an intermediate factor rather than confounder. Therefore, the relationship between early cow's milk protein exposure and other food allergies is presented among the second half of the cohort who underwent cow's milk SPT so that this factor could be included in the model. There was no evidence of an association between early exposure to cow's milk protein and other challenge-confirmed food allergies (Table V).

Sensitivity analyses

We conducted a sensitivity analyses excluding infants who changed to another infant formula in the first 3 months of life as they may have changed formula because of a reaction to the first formula ($n = 189$). This did not materially alter our results (data not shown). To further remove any residual confounding effect of family history of allergy, we stratified the analysis. When

restricted to infants with no sibling history of food allergy and/or eczema and no maternal history of food allergy and/or eczema, the associations were maintained (see Tables E1 and E2, available in this article's Online Repository at www.jaci-inpractice.org).

DISCUSSION

In this population-based cohort, early exposure to cow's milk protein in the first 3 months of life was associated with a reduced risk of all cow's milk atopic outcomes considered, including sensitization, parent-reported reactions, and presumed allergy at age 1 year, even after adjusting for confounding variables including family history of allergy.

The strengths of this study are the large sample size, high participation fraction, and good internal and external validity.¹⁹ Sensitization to cow's milk was objectively measured via SPT on all consecutively recruited infants in the second half of the cohort. A weakness of this study is that oral food challenges were not used to assess cow's milk allergy; rather it was defined as a parent report of reactions consistent with IgE-mediated symptoms in conjunction with a positive SPT, both recorded at age 12 months. It is possible that some infants were allergic but developed tolerance before they were assessed at age 12 months. Transfer of cow's milk proteins into human breast milk may occur²⁴⁻²⁶ and infants who did not consume cow's milk formula in the first 3 months may have been exposed to small amounts of cow's milk protein via breast milk. However, as cow's milk consumption is ubiquitous, it is unlikely that this exposure was substantially different between the 2 groups although we did not collect sufficient data to comprehensively assess this. In addition, questionnaires were conducted when the infant was 12 months of age and there is a possibility of recall bias. Data on the frequency or quantity of formula ingestion (other than exclusivity) were not collected and we were unable to comprehensively assess a dose-response relationship between regularity of cow's milk protein exposure and cow's milk atopic outcomes, other than the analysis presented comparing exclusive formula feeding from birth to infants who received both formula and breast milk. The analysis of the effect of dose of cow's milk protein exposure is complex as this will be contaminated by history of reactions because allergic reactions after the first exposure (provided a

sufficient threshold dose has been achieved) will affect decisions on whether to continue exposure to cow's milk. Finally, these findings are from an observational study not an RCT that would be required if further recommendations were to be made.

Breastfeeding is the optimal form of infant nutrition for many reasons including mother and infant bonding, transfer of immune factors, and protection against infections.^{27,28} If breastfeeding is not possible, then an appropriate infant formula should be used. This study showed that early exposure to cow's milk protein through infant formula was associated with a reduced risk of cow's milk atopic outcomes at age 12 months. The majority of infants in this study were also breastfed during the first 3 months, and there was no greater protective association observed among those who were exclusively formula fed compared with those who received both infant formula and breast milk. Our findings show that early exposure to cow's milk protein does not prevent food allergies *per se*; rather the association was specific to cow's milk atopic outcomes only, indicating that early exposure to the allergen is important for inducing oral tolerance to that allergen only. This is consistent with other research that oral tolerance development is antigen specific and there are no data to date showing that exposure to one allergenic food influences the development of allergy from different allergenic food.⁷

Although PHF has been promoted as reducing the risk of allergies, a recent meta-analysis found that there was insufficient evidence to support a protective effect for PHF compared with standard cow's milk formula for reducing the risk of food allergy or eczema.¹⁷ We have previously reported in the HealthNuts study that neither duration of breastfeeding nor the use of PHF compared with standard cow's milk formula was associated with the risk of food allergy.¹⁸ In the present study, infants who were exposed to cow's milk protein through either a standard cow's milk formula or PHF were both associated with a reduced risk of parent-reported reactions to cow's milk. There was no evidence of a difference in risk between infants who received standard cow's milk formula compared with PHF; however, the study was not powered to detect such a difference.

A recent systematic review and meta-analysis found that early introduction of cow's milk was not associated with the risk of cow's milk sensitization (RR 0.72, 95% CI 0.40-1.27 from intervention studies and OR 1.80, 95% CI 0.37-8.70 from observational studies), nor was it associated with the risk of cow's milk allergy (RR 0.76, 95% CI 0.32-1.78 from intervention studies and OR 0.40, 95% CI 0.09-1.76 from observational studies).⁷ However, the studies included in this review varied by the age at which milk was introduced into the infant's diet. Several RCTs have assessed the use of cow's milk formula in the first few days of life and the risk of allergic outcomes with 2 studies finding no association and a third reporting an increased risk of milk allergy.⁹⁻¹¹ The EAT study reported that exposure to cow's milk from 3 to 6 months of age compared with after 6 months of age did not reduce the risk of cow's milk allergy (RR 0.79, 95% CI 0.18-3.50), but it was associated with a reduced risk of cow's milk sensitization at age 3 years in the per protocol but not intent-to-treat analysis (RR 0.12, 95% CI 0.02-0.89 and 0.57, 95% CI 0.21-1.53, respectively).¹⁶ These RCTs did not specifically assess exposure to cow's milk protein in the first 3 months of life, and our findings, although observational, add to the knowledge base. Our findings are consistent with other observational studies.¹²⁻¹⁴

The role of timing of allergen exposure to induce oral tolerance is increasingly acknowledged. However, the mechanisms involved, optimal timing of allergen exposure, and whether this window of opportunity differs depending on the allergen are not well understood.²⁹ The LEAP study demonstrated that exposure to peanut between ages 4 and 11 months in high-risk infants was important for preventing peanut allergy.¹ Early introduction of egg from 4 to 6 months onward has shown a limited protective effect on egg allergy in several RCTs that may be related to reduced statistical power as a meta-analysis of several studies supports an overall protective effect.⁷ Our study adds to the body of evidence and suggests that the window of opportunity for cow's milk protein exposure to induce tolerance may be earlier than for other allergens. However, early exposure to allergens may only be one piece of the puzzle. Other components that require investigation are optimizing conditions for critical periods of immune system development that promote a tolerogenic environment, such as gut microbial colonization and reducing proinflammatory factors.^{30,31}

In conclusion, this population-based study provides further evidence for the role of early allergen exposure in the prevention of IgE-mediated food allergies and these hypothesis-generating findings suggest that RCTs are required to evaluate this further.

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TABLE E1. The association between early cow's milk protein exposure and cow's milk atopic outcomes stratified by infants with and without a sibling history of food allergy and/or eczema

	No sibling history FA/eczema			Sibling history FA/eczema		
	% allergic	Adjusted OR*	P	% allergic	Adjusted OR*	P
Reaction to cow's milk						
No cow's milk protein 0-3 mo	3.5% (74/2,113)	1.0	—	5.4% (24/443)	1.0	—
Cow's milk protein exposure 0-3 mo	1.5% (24/1,625)	0.40 (0.25-0.65)	<.001	3.3% (8/241)	0.66 (0.29-1.54)	.272
Milk SPT $\geq$ 2 mm						
No cow's milk protein 0-3 mo	2.3% (27/1,167)	1.0	—	5.0% (12/238)	1.0	—
Cow's milk protein exposure 0-3 mo	1.1% (10/888)	0.45 (0.21-0.94)	.033	2.5% (3/121)	0.44 (0.12-1.62)	.216
Cow's milk allergy						
No cow's milk protein 0-3 mo	1.0% (12/1,162)	1.0	—	2.6% (6/237)	1.0	—
Cow's milk protein exposure 0-3 mo	0.3% (3/887)	0.31 (0.09-1.11)	.071	0.8% (1/121)	0.31 (0.04-2.65)	.282

FA, Food allergy; OR, odds ratio; SPT, skin prick test.

*All models adjusted for sex, family history of allergy, any siblings, socioeconomic status, and parent's country of birth. The cow's milk reaction model was additionally adjusted for premature birth. Sensitivity analysis including age of eczema onset as a confounding variable did not materially alter the results.

TABLE E2. The association between early cow's milk protein exposure and cow's milk atopic outcomes stratified by infants with and without a maternal history of food allergy and/or eczema

	No maternal history FA/eczema			Maternal history FA/eczema		
	% allergic	Adjusted OR*	P	% allergic	Adjusted OR*	P
Reaction to cow's milk						
No cow's milk protein 0-3 mo	3.5% (72/2,079)	1.0	—	5.5% (26/477)	1.0	—
Cow's milk protein exposure 0-3 mo	1.6% (25/1,565)	0.48 (0.30-0.78)	.003	2.3% (7/301)	0.32 (0.13-0.81)	.015
Milk SPT $\geq$ 2 mm						
No cow's milk protein 0-3 mo	2.8% (31/1,114)	1.0	—	2.8% (8/291)	1.0	—
Cow's milk protein exposure 0-3 mo	1.2% (10/839)	0.40 (0.19-0.82)	.013	1.8% (3/170)	0.61 (0.16-2.38)	.479
Cow's milk allergy						
No cow's milk protein 0-3 mo	1.1% (12/1,108)	1.0	—	2.1% (6/291)	1.0	—
Cow's milk protein exposure 0-3 mo	0.1% (1/838)	0.10 (0.01-0.81)	.030	1.8% (3/170)	0.82 (0.20-3.39)	.779

FA, Food allergy; OR, odds ratio; SPT, skin prick test.

*All models adjusted for sex, family history of allergy, any siblings, socioeconomic status, and parent's country of birth. The cow's milk reaction model was additionally adjusted for premature birth. Sensitivity analysis including age of eczema onset as a confounding variable did not materially alter the results.