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Early, continuing exposure to cow's milk formula and cow's milk allergy

The COMEET study, a single center, prospective interventional study

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ABSTRACT

Background: Cow's milk allergy (CMA) is a common food allergy among infants. Information regarding the best timing for first exposure to cow's milk formula (CMF) is controversial and more evidence is required. Few randomized control trials have tried to accurately assess the timing and preventive effect of exposure to CMF on small cohorts.

Objective: This study assessed the association between early, continuing exposure to CMF on the basis of the parents' preferences and the development of immunoglobulin E (IgE)–mediated CMA in a large birth cohort.

Methods: Newborns were prospectively recruited shortly before birth and divided into 2 groups according to parental feeding preference for the first 2 months of life: (1) exclusive breastfeeding (EBF); or (2) at least 1 meal of CMF (with or without breastfeeding) daily. Infants were followed up monthly until the age of 12 months.

Results: Among 1992 infants participating in the study, 1073 (53.86%) were in the EBF group until 2 months of age. IgE-mediated CMA was confirmed in 0.85% (n = 17); all were in the EBF group. Within this group, the prevalence of IgE-mediated CMA was 1.58% compared with 0 in the other groups (relative risk, 29.98; $P < .001$). Post hoc analysis revealed IgE-mediated CMA prevalence of 0.7% in the per-protocol EBF group vs 3.27% among breastfed infants who were exposed to a small amount of CMF during the first 2 months of life. A family atopic background did not affect the results.

Conclusion: Early, continuing exposure to CMF from birth has the potential to prevent the development of IgE-mediated CMA and should be encouraged. However, the exposure needs to be consistent because occasional exposure increases the risk of developing IgE-mediated CMA and should be avoided.

Introduction

Except for breast milk, cow's milk (CM) formula is usually the first food infants are exposed to and is one of the most common food allergies among young children.[1,2] The estimated prevalence of CM allergy (CMA) is 0.5% to 3%.[1–5] CMA is differentiated into immunoglobulin E (IgE) and non-IgE–mediated. IgE-mediated CMA is defined as an immediate reaction after exposure to CM, presenting as urticarial rash, shortness of breath, cough, and vomiting. Non-IgE–mediated CMA includes food protein-induced enterocolitis (FPIES) and food protein-induced allergic proctocolitis (FPIAP).[6] Approximately 50% of patients with IgE-mediated CMA outgrow their allergy during the first decade of life,[7,8] but the resolution rate decreases over time.[9] Desensitization to CM protein (CMP) may sometimes be performed, but there is no widely-accepted, effective curative therapy for IgE-mediated CMA.[1] Thus, preventive strategies are of foremost importance.

Previous studies on peanuts and eggs have reported that early exposure to these products reduces the development of IgE-mediated allergy.[10–14] The current guidelines regarding peanuts and eggs encourage exposure from 4 months of age for high-risk infants and 6 months for others.[15–17] The value of early exposure to CMP on the development of IgE-mediated CMA is debated.[15] A few randomized

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control trials (RCTs) have tried to ascertain the timing and preventive effect of early exposure to CMF and reported conflicting results, and as of today, the conclusions of these trials have not been translated into guidelines.[3,15,18–21] As recently stated, the optimal time for introducing CMF has not been established.[22] This might be because of, in part, the inability of RCTs to imitate varied real-life experiences. Daily practices and parental feeding preferences do not follow randomization and may be affected by various factors, such as the existence of atopic diseases in the family, thus, potentially limiting the ability of randomization to mirror real life.

On the basis of previously published data, we hypothesized that early, continuing exposure to CMF could significantly lower the chances of developing IgE-mediated CMA. The goal of the Cow's Milk Early Exposure Trial (COMEET) was to prospectively evaluate whether continuing exposure to CMF from birth is associated with decreased occurrence of IgE-mediated CMA.

Methods

This single-center, prospective interventional study followed infants from birth to 12 months of age. Participants were enrolled in the delivery room before the delivery between May 2018 and May 2021 and included the general population from urban, suburban, and rural areas. Participating parents chose the feeding method for their infant, either exclusive breastfeeding (EBF) or exposure to CMF from birth. Propensity scores analysis was performed to compensate for nonrandomization.

Inclusion Criteria

Term and near-term infants, born beyond 36 plus 0 gestational weeks, with an appropriate birth weight according to their gestational age, and without known congenital anomalies were eligible. Parents of all ethnic groups who were fluent local language speakers were invited to participate.

Study Design

Participants were recruited shortly before labor and followed up monthly by phone or E-mail surveys until 12 months of age. Participants were divided into 1 of 2 groups according to the parents' feeding preferences for the first 2 months of life, as expressed at hospital discharge: group 1 being EBF and group 2 being infants with exposure to CMF from birth (exposure group). The latter was divided into 2 subgroups: subgroup 2.1 included breastfeeding (BF) infants with at least 1 bottle of CMF per day (BF + CMF), whereas subgroup 2.2 included CMF only (eFigure 1). The first 2 months of life were considered the intervention period, during which, the parents were requested to follow their feeding preferences. The infants' diet during the days in the hospital after birth was recorded and the mothers were asked specifically on CMF exposure in both groups; however, it did not define the study groups or exclude them from the study. These deviations were considered in the posthoc analysis. The parents and newborns were invited for a physical examination and a skin prick test (SPT) with cow's milk extract, casein, and fresh milk at the age of 2 months (the SPT procedure is detailed in eMethods 1).

After giving informed consent, the parents completed a preliminary questionnaire regarding demographic data and family history of atopic diseases (asthma, atopic dermatitis, allergic rhinitis, and food allergy). The parents answered a monthly questionnaire that included information regarding exposure to CMF or dairy products, suspected allergic reactions, and accompanying atopy expressed as the use of β-agonist inhalations and physician diagnosis of atopic dermatitis. Infants who required inhalation of medications other than sodium chloride were classified as having hyperreactive airway disease. The questionnaires relating to the first 2 months included questions regarding protocol deviations.

When parents reported a suspected allergic reaction to CMF or CM products, they were interviewed by the primary investigator. When symptoms were compatible with IgE-mediated CMA or FPIES, they were invited for an SPT and an oral challenge test (OCT) in case of either inconclusive anamnesis or SPT results (the OCT procedure is detailed in eMethods 2). Symptoms that were suggestive of IgE-mediated CMA or FPIES include immediate skin rash and vomiting (none of the parents reported coughing, wheezing, or shortness of breath). Reports of bloody stool, diarrhea, abdominal pain, colic, or delayed rash were suspected to indicate FPIAP or nonallergic symptoms, and the parents were instructed to continue CMP exposure or avoidance for a month followed by reintroduction in case of suspected FPIAP.

Cow's Milk Allergy Definition

IgE-mediated CMA was defined according to the accepted guidelines.[9] Briefly, it was defined as the presence of at least 2 of the following: (1) symptoms suggestive of an immediate allergic reaction including urticaria, cough, wheezing, and vomiting occurring within 1 hour after exposure; (2) positive SPT to CM extracts (wheal size >3 mm); and (3) objective reaction in supervised open OCT with CMF. Non–IgE-mediated CMA was defined as follows: FPIES was defined as the appearance of vomiting 1 hour or more after CMF ingestion after ruling out other reasons for the vomiting, and the disappearance of symptoms after CMP avoidance; FPIAP was defined as the presence of bloody stool or diarrhea and disappearance of symptoms after CMF was eliminated or after eliminating CMP from the mother's diet in the case of a breastfed infant. CMA evaluation was done by the study principal investigator (allergy and immunology specialist) according to accepted diagnostic methods.

Study Outcomes

The primary outcome was a proven IgE-mediated allergic reaction to CMP during the first year of life. The secondary outcome was a non-IgE–mediated allergic reaction to CMP.

Calculated Sample Size

On the basis of previous studies,[1,3–5] we estimated the prevalence of IgE-mediated CMA in the entire cohort as 0.5% to 2%. Our assumption was that the EBF group (group 1) would have 1.5% to 2.5% infants with IgE-mediated CMA compared with 0.5% to 1% in the exposure group (group 2). A sample size of approximately 1250 infants in each group (α = 0.05, power = 0.8) with 5% lost to follow-up was calculated as sufficient for obtaining significant results. The sample size was calculated conservatively using the formula for a 2-sided binomial test, but the final testing approach differs according to the research assumption and the observational nature of this study (see below).

Statistical analyses

Only infants completing 12 months of follow-up were included in the data analysis. The characteristics of the study groups were compared using either the $\chi 2$ test (binary and categorical variables) or *t* test (continuous variables). *P* values are reported without correction for multiplicity. Analysis was conducted according to the study group assigned at hospital discharge (intention-to-treat). The primary analysis used a propensity score-based randomization test[23] for formal hypothesis testing (eMethods 3). A priori confounding variables that

were considered were atopic comorbidities of the parents and siblings, and infant delivery mode (vaginal vs cesarean section). Relative risks were calculated using Haldane correction (adding 0.5 to all cells in a table having an empty cell).

Posthoc Analysis

The EBF group (group 1) was divided into subgroups according to protocol fulfillment or deviations, as follows: (1) subgroup 1.1, which included per-protocol infants who were not exposed to CMF until at least 2 months of age, not even during the first 48 hours at the hospital after birth; subgroup 1.2, those with protocol deviation including subgroup 1.2.1, which included EBF infants who were not exposed to more than 5 meals of CMF during the first 2 months of life; and subgroup 1.2.2, which included EBF infants with occasional exposure to CMF during the first 2 months of life (more than 5 meals) or switched to BF plus CMF or CMF only. Subgroups 1.1 and 1.2.1 were analyzed posthoc.

In addition, characteristics of infants with and without IgE-mediated CMA were compared using a 2-sided Fisher's test to find subgroups having a higher prevalence of IgE-mediated CMA. The study was approved by the local Ethics Committee and registered at the National Institutes of Health clinical trials registry. Informed consent was signed by 1 or both participating parents before labor.

Results

Study Population

During the study period of May 2018 to May 2021, 2252 infants from the local general population were enrolled including 20 pairs of twins. The results of 1992 infants were analyzed; another 260 infants (11.55%) were excluded: 97 were lost to follow-up, 158 withdrew consent, and 5 were excluded because of the infant's medical problems (eMethods 4, eTable 1, Fig 1). A total of 1073 infants were EBF, defined as group 1 (53.86%). Another 919 (46.14%) were in group 2, the CM exposure group. Among them, 617 were in subgroup 2.1 (BF + CMF) (67.14%) and 302 (32.86%) in subgroup 2.2 (CMF only) (Fig 2A). Within group 1, a total of 507 infants (47%) reported protocol deviations (Fig 2B). In group 2, a total of 23 infants (2.50%) switched to EBF within the first 2 months of life, all in the BF plus CMF subgroup. For data analysis, these patients were included in their original study group.

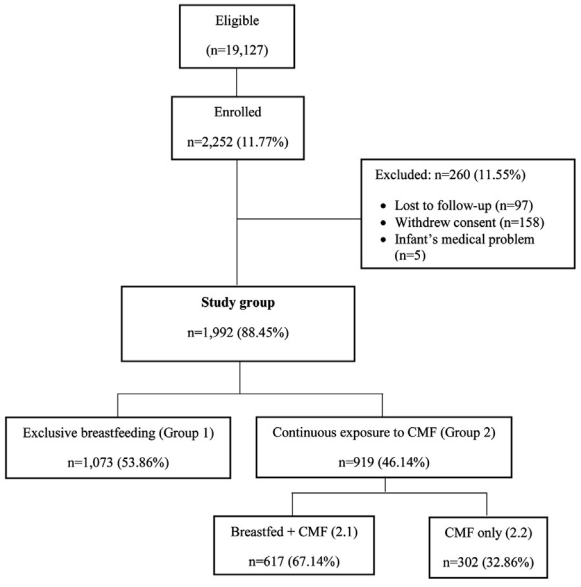

Figure 1. Study population. Participation in the study was offered to all women who arrived for delivery, who fulfilled the inclusion criteria, and were available for recruitment. The eligible population included all the deliveries registered in the delivery room during the recruitment period. CMF, cow's milk formula.

Demographics and Family Background

Demographic data are detailed in Table 1 and eTable 2. A total of 772 infants had at least 1 family member with an atopic comorbidity (38.7%), of which 272 family members (13.6%) had atopic dermatitis. Details of the different types of atopic comorbidities and family members are presented in Table 1 and eFigure 2.

Exposure to Cow's Milk Formula or Dairy Products

At 12 months of age, 1943 infants (97.5%) were exposed routinely either to CMF or dairy products without any suspected allergic reaction. The other 46 infants included infants with CM allergy (IgE and non-IgE–mediated). Only 5 out of 46 infants who were not exposed routinely to dairy products were in the BF plus CMF group (group 2.1)—the others belonged to the EBF group. The reasons for nonexposure of those 5 infants included FPIAP, vegan family, and suspected CMA that was subsequently ruled out.

Cow's Milk Allergy

Intention-to-Treat Analyses

A total of 124 patients reported suspected allergic reaction to CMP. After a detailed interview by the principal investigator, 39

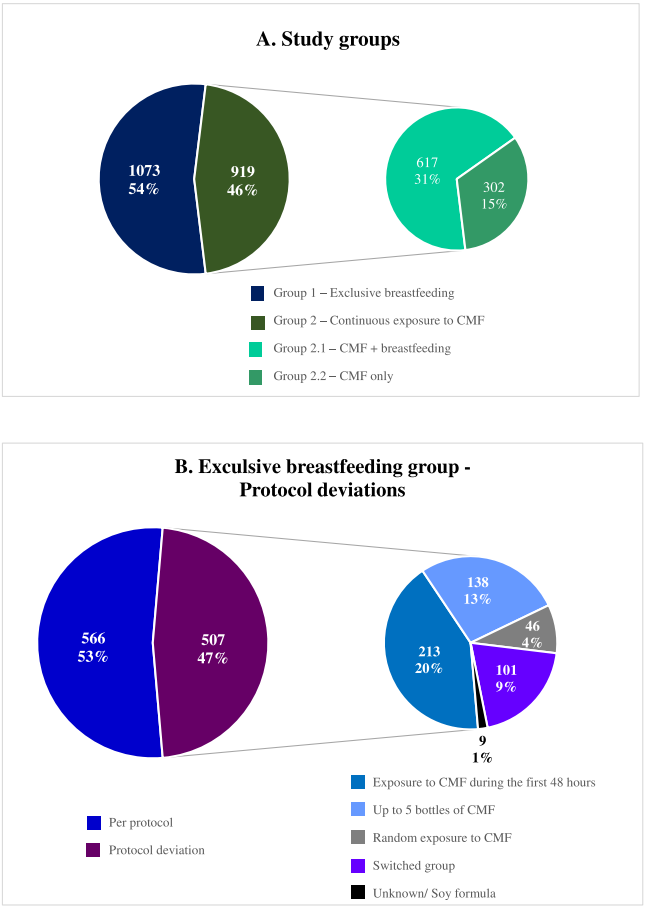

Figure 2. Study groups. (A) Major study groups. (B) Exclusive breastfeeding group per protocol vs protocol deviation. CMF, cow's milk formula.

Table 1
Study Population Demographics

	Characteristics	Breastfeeding only (n = 1073)	CMF continuous exposure (n = 919)	*P* value
Sex (female)		565 (52.66%)	432 (47.06%)	<.05
Ethnicity	Jewish	996 (56.2%)	775 (43.8%)	<.01
	Arab	77 (34.8%)	144 (65.2%)	
Socioeconomic status[a]		7 ± 2	7 ± 2	NS
Mothers age ± SD		32.8 ± 4.4	33.5 ± 5.2	NS
Mothers' education	Academic degree	829 (84.9%)	572 (69%)	<.01
	Postgraduate	72 (7.4%)	109 (13.1%)	
	High school	75 (7.7%)	148 (17.8%)	
	Missing data	97	90	
Mode of delivery (vaginal)		1001 (93.3%)	799 (86.9%)	<.01
Siblings	0	302 (28.1%)	350 (38.1%)	<.001
	1	362 (33.7%)	297 (32.3%)	NS
	≥ 2	409 (38.1%)	272 (29.6%)	<.001
At least 1 family member with atopic disease		458 (42.7%)	314 (34.2%)	<.001
At least 1 family member with atopic dermatitis		167 (15.6%)	105 (11.4%)	<.01
Maternal	Any atopic disease	205 (19.1%)	162 (17.6%)	NS
	Asthma	71 (6.6%)	63 (6.7%)	
	Atopic dermatitis	45 (4.2%)	26 (2.8%)	
	Allergic rhinitis	109 (10.2%)	81 (8.8%)	
	Food allergy[b]	34 (3.2%)	28 (3%)	
Paternal	Any atopic disease	159 (14.8%)	108 (11.7%)	NS
	Asthma	68 (6.3%)	51 (5.5%)	NS
	Atopic dermatitis	33 (3.1%)	9 (1.0%)	<.01
	Allergic rhinitis	89 (8.3%)	56 (6.1%)	NS
	Food allergy[b]	18 (1.7%)	14 (1.5%)	NS
Siblings	Any atopic disease	216 (20.1%)	151 (16.4%)	<.05
	Asthma	92 (8.6%)	65 (7.1%)	NS
	Atopic dermatitis	110 (10.2%)	77 (8.4%)	NS
	Allergic rhinitis[c]	12 (1.1%)	10 (1.1%)	NS
	Food allergy[b]	58 (5.4%)	35 (3.8%)	NS

Abbreviations: CMF, cow's milk formula; NS, nonsignificant.
NOTE. Significant difference, $P < .05$ using the $\chi 2$ test.
[a]Socioeconomic status in a scale of 1 to 10.
[b]Food allergy among family members includes food allergies that wane over.
[c]Prevalence of allergic rhinitis in siblings was low compared with the general population prevalence because of the young age of the siblings.

infants underwent an SPT to CMP (30 from group 1 [EBF] and 9 from group 2 [CM exposure]). Any type of CMA, including both IgE- and non-IgE–mediated, was found in 47 infants (2.36%). Among these, 17 had IgE-mediated CMA (0.85%), 7 infants had FPIES (0.35%) and 23 had FPIAP (1.15%). Overall, 35 infants were in the EBF group (3.26%) vs 12 infants in the CM exposure group (1.31%) (relative risk [RR], 2.3; 95% confidence interval [CI], 1.22-4.33) (Fig 3). The clinical and demographic characteristics of all infants who developed CMA are presented in eTable 3.

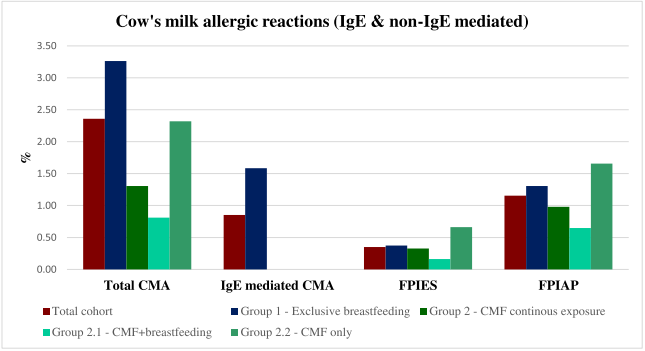

Figure 3. Type of allergic reaction according to study group. Significant differences were found only for IgE-mediated CMA between the exclusive breastfeeding group (group 1) and the CMF continuing exposure group (Group 2), $P < .001$. CMA, cow's milk allergy; CMF, cow's milk formula; FPIAP, food protein-induced allergic proctocolitis; FPIES, food protein-induced enteropathy; IgE, immunoglobulin E.

The diagnosis of IgE-mediated CMA was based on suspected anamnesis and positive SPT result to CM in 12 infants and allergic reaction during OCT with CMF in 5 infants (eTable 3). All 17 infants with IgE-mediated CMA were in the EBF group (1.58%, RR = 29.98 [using Haldane's correction], $P < .001$). The mean age of the first allergic reaction was 6.1 months (range 2-10 months).

There were no significant differences between infants with or without IgE-mediated CMA in sex, family atopic background, and atopic comorbidities (Table 2). There were no significant differences in the prevalence of FPIES or FPIAP between the study groups: 4 (0.37%) and 14 (1.3%), respectively, in the EBF group (group 1) vs 3 (0.33%) and 9 (0.98%), respectively, in the CM exposure group (group 2). The mean age of the first FPIES reaction was 2 months (range 1-5 months). The mean age of the first FPIAP reaction was 2 months (range 0-5 months). Infants in the EBF group developed FPIAP at an older age compared with the CM exposure group (2.5 vs 1.2 months). In the EBF group, only 2 cases of FPIAP happened after CMF exposure and the rest were during EBF. All FPIES and FPIAP were resolved by 12 months of age.

Posthoc Analyses According to Per-Protocol And Protocol Deviations

Immunoglobulin E–mediated CMA was found in 4 of 567 infants in the per-protocol EBF group (0.7%) vs none of 825 infants in the per-protocol exposure group (RR = 13.08 using Haldane's correction, $P = .11$). Among 1073 infants in the EBF group, 507 had protocol deviations because of exposure to CMF during the first 2 months of life. Among them, 397 were exposed to small amounts of CMF: 213 were only exposed during the first 48 hours of life, 138 were exposed to a maximum of 5 meals during the intervention period, and 46 had random exposures to more than 5 meals (Fig 2B). A total of 13 of the 397

Table 2
Atopic Comorbidities Within the Study Groups and CMA Infants

	Characteristics	Breastfeeding only (n = 1073)	IgE-mediated CMA[a] (n = 17)	*P* value
Sex - female		565 (52.6%)	10 (58.8%)	NS
Ethnicity	Jewish	996 (56.2%)	17 (100%)	NS
	Arab	77 (34.8%)	0 (0%)	
At least 1 family member with atopic disease		458 (42.7%)	9 (52.9%)	NS
At least 1 family member with atopic dermatitis		167 (15.6%)	4 (23.5%)	NS
Maternal	Any atopic disease	205 (19.1%)	5 (29.4%)	NS
	Asthma	71 (6.6%)	1 (5.9%)	NS
	Atopic dermatitis	45 (4.2%)	1 (5.9%)	NS
	Allergic rhinitis	109 (10.2%)	2 (11.8%)	NS
	Food allergy	34 (3.2%)	3 (17.6%)	.01
Paternal	Any atopic disease	159 (14.8%)	4 (23.5%)	NS
	Asthma	68 (6.3%)	2 (11.8%)	NS
	Atopic dermatitis	33 (3.1%)	3 (17.6%)	.01
	Allergic rhinitis	89 (8.3%)	1 (5.9%)	NS
	Food allergy	18 (1.7%)	0	NS
Siblings	Any atopic disease	216 (20.1%)	3 (17.6%)	NS
	Asthma	92 (8.6%)	1 (5.9%)	
	Atopic dermatitis	110 (10.2%)	2 (11.8%)	
	Allergic rhinitis	12 (1.1%)	0	
	Food allergy	58 (5.4%)	0	

Abbreviations: CMA, cow's milk allergy; CMF, cow's milk formula; IgE, immunoglobulin E; NS, nonsignificant.
NOTE. Significant differences $P < .05$ using a 2-sided Fisher's exact test.
[a]All IgE-mediated CMA infants are exclusively breastfed; *P* values were calculated for the differences between the allergic infants and their original study group.

infants exposed to small amounts of CMF developed IgE-mediated CMA. This prevalence is not significantly higher compared with the per-protocol EBF group (3.27% vs 0.7%, respectively, RR, 4.64; 95% CI, 1.52-14.15; $P = .20$), but it is significantly higher than in the exposure group (group 2) (3.27% vs 0, respectively, RR, 62.41 using Haldane's correction, $P = .01$).

Skin Prick Test at 2 Months of Age

A total of 183 infants (9.2%) came for clinical evaluation and SPT for CMP at 2 months of age. The proportions of the study groups who had SPT were similar to their percentages in the study cohort (56.3% EBF, 33.3% BF + CMF, and 10.4% CMF only). The SPT was negative in 178 infants (97.3%). Only 2 of 5 infants with at least 1 positive SPT were diagnosed with IgE-mediated CMA (the clinical details of these 5 infants with positive SPT are in the eMethods 5).

Discussion

This current report of the COMEET study revealed that early continuing exposure to CMF since birth for at least 2 months is associated with the prevention of the development of IgE-mediated CMA. None of the infants who were continuously exposed to CMF developed IgE-mediated CMA. On the other hand, random exposure to CMF during the first 2 months of life was found to be the highest risk factor for developing IgE-mediated CMA. The influence of early exposure to CMF on the occurrence of IgE-mediated CMA corresponds with the consensus established on the effect of early exposure to peanuts and eggs.[10,11] Early exposure to CMF did not influence the risk for non-IgE–mediated CMA. Almost all infants in the study (97.5%) had been exposed routinely to dairy products by the age of 12 months without clinical allergic symptoms.

Therefore, we can conclude, with high probability, that they do not have IgE-mediated CMA. The others developed CMA or belonged to the EBF group, except for 1 vegan infant. Food allergic reactions, especially to dairy products, are a basic part of an infant's diet and are not easily missed by a caregiver. Because all participants answered a monthly questionnaire, which includes suspected allergic reaction, it is unlikely that the infants experienced allergic reactions to dairy products that were not detected or diagnosed.

Most available data regarding the influence of CMF exposure have been obtained from RCTs. Although the advantages of RCTs are well-established, they have 2 major disadvantages that relate to IgE- mediated CMA prevention studies: (1) it is unethical to decide for mothers whether to breastfeed or not, and (2) RCTs do not reflect real-world practices.[15,24] Moreover, according to previous studies on the effect of CMF exposure on the occurrence of IgE-mediated CMA, the parents in the study groups who were randomly assigned to avoid CMF were instructed to give the infants breastmilk, extensively hydrolyzed formula,[19] or soy-based formula.[18] The actual percentage of real EBF was not reported in either study. The influence of the addition of other formulas to the infants' diet on the occurrence of IgE-mediated CMA is controversial.[15,25,26] After drawing suggestions from RCTs regarding the ability of cow's milk ingestion to affect the development of IgE-mediated CMA by RCTs, the COMEET study increases the strength of conclusions on the influence of early and continuous exposure to CMF on the prevalence of IgE-mediated CMA in daily practice and does this without the interference of other factors.

Our results are consistent with previous studies. As early as 1988, Host et al,[27] in their prospective study of 1749 infants, suggested that occasional exposure to CMF during the first days of life is a risk factor for developing IgE-mediated CMA. Complementing this, Katz et al[3] reported that exposure to CMF during the first 15 days of life decreases the risk for IgE-mediated CMA. However, in this observational study, continuing or random exposure was not addressed. The current study reinforces and expands the conclusion that cow's milk exposure must be early and continuous to inhibit IgE-mediated CMA development. Sakihara et al[18] reported that early exposure to CMF decreased the rate of IgE-mediated CMA. All infants in their study were exposed to CMF for the first month of life and only then the avoidance group started to avoid CMF.

The early introduction of CMF and then CMF avoidance might explain the exceedingly high (6.8%) incidence of IgE-mediated CMA they found in the avoidance group. Our results suggest that introducing and then avoiding CMF during this specific period increases the prevalence of IgE-mediated CMA. These findings are similar to the results reported by Sakihara et al[28] who have reported this recently in a subanalysis of their primary data. The main difference between our current study and that of Sakihara et al[28] was in the size of the groups. They had only 43 infants who discontinued CMF exposure during the first 2 months of life compared with 397 infants in our

study who were exposed to small amounts of CMF during the same period. They concluded that exposure to CMF only during the first 3 days of life was associated with the development of CMA. We expand on this conclusion by suggesting that random exposure during the first 2 months of life is a risk factor, and not just during the first 3 days.

On the other hand, in a study of 312 infants, Urashima et al[19] reported that avoiding exposure to CMF at least during the first 3 days of life decreased the risk for IgE-mediated CMA. This conclusion differs from our results. In our study, which included a much larger cohort of 1992 infants, we found that infants who were exposed routinely and continuously to CMF from birth did not develop IgE-mediated CMA, whereas infants who were inconsistently exposed to small amounts of CMF during the first 2 months of life, had the highest risk of developing IgE-mediated CMA. We conclude that continuing exposure to CMF rather than CM avoidance during the first 3 days of life, as suggested by Sakihara et al,[28] may be protective against the development of IgE-mediated CMA.

The current study addressed the general population of newborns. The risk of developing IgE-mediated CMA was independent of known risk factors, including a family history of atopy and the infant`s accompanying atopic diseases. This is in contrast with previously published studies[29] of food allergy prevention that included only so-called “high-risk” infants.[10,11,19] The Enquiring about Tolerance study could not confirm the efficacy of early exposure to CMF on the incidence of IgE-mediated CMA in low-risk infants.[20] In their study, CMF was introduced after 3 months of age. We suggest that the failure of the Enquiring about Tolerance study to prove that early exposure to CM prevents IgE-mediated CMA is owing to the timing of the introduction of CM and not to the low-risk characteristics of their study population. As noted by Brough et al,[30] intervention might need to be started within the first few weeks of life, not in the first few months.

One theory explaining the effect of early and occasional exposure to CMF during the first days or weeks of life on the development of IgE-mediated CMA is that the gastrointestinal tract of newborns is immature and therefore, more permeable to macronutrients such as proteins.[31] Macromolecules can cross paracellularly, whereas, after maturation, absorption is mainly transcellular and more selective.[32] The paracellular pathway might be more responsible for developing sensitization.[32,33] Kuitunen et al[31] measured levels of human α-lactalbumin and bovine β-lactoglobulin in the bloodstream of term infants. They found that the amount of both proteins decreased dramatically toward 8 weeks of age, suggesting maturation of the intestinal epithelium. At the same time, the neonatal immune system, which, during fetal life and for the first month after birth is polarized toward T helper cell type 2,[34] is also immature. We suggest that random exposure to CMF causes the immune system to recognize CM proteins as foreign molecules and, in susceptible individuals, to become sensitized to them. This might explain why infants who were occasionally exposed to small amounts of CMF during the first months of life had a higher incidence of IgE-mediated CMA.

It could also explain the high incidence of IgE-mediated CMA in the study by Sakihara et al.[18] This theory complements the dual-allergen exposure hypothesis.[35] When exposure to food allergens occurs through especially eczematous skin, complete proteins cross the barrier, are exposed directly to the immune system and cause sensitization, similar to what occurs in the immature intestinal epithelial barrier. The immature healthy intestinal epithelial barrier may act similarly to inflamed eczematous skin. Our data also suggest that the quantity and frequency of oral exposure to CM proteins are important determinants of sensitization vs tolerance induction; continuing exposure to CMF tolerized the immature immune system, exposure to small amounts, either while drinking CMF or owing to cow’s milk components that exist in the mother’s breast milk, can induce sensitization. This hypothesis needs to be investigated further.

Limitations

A major limitation of this study was the assignment to study groups on the basis of the parents’ preferences rather than randomization. Randomization in studies involving infant feeding is problematic for ethical reasons. It is unethical to implement initial randomization and decide for the mothers whether to breastfeed or not. Nevertheless, even after this selection, group sizes were comparable. Randomization tests that were applied to both intention-to-treat and per-protocol analyses compensated for selection biases and revealed significant differences between the groups in the intention-to-treat analysis.

Other confounders not measured in this study that may affect both the choice of feeding type and IgE-mediated CMA include the following: urban vs rural living environments, birth order, and animal exposures. These should be explored further in future studies.

Another limitation was the reliance on self-reported questionnaires regarding atopic comorbidities. Nevertheless, the final diagnosis of IgE-mediated CMA was determined by an allergy specialist after SPT and food challenge if needed.

The allergy specialist was not blinded to the patients’ study group. The OCT was open and not blinded. However, only objective symptoms (urticarial rash, vomiting, wheezing) caused cessation of the OCT and diagnosis of IgE-mediated CMA. In group 1 (EBF), the rate of protocol deviation was 47.3%. Although this proportion is high, it is not unusual in studies dealing with breastfeeding and allergen exposure. For example, the Enquiring about Tolerance study had 65.8% protocol deviations,[20] and Urashima et al[19] had 76% protocol deviations. Even after excluding the infants who deviated from the study, the EBF cohort was larger than any other published study on this topic. Moreover, because we knew what the deviation was, the deviated group gave us the opportunity to understand the influence of occasional exposure to CMF better.

The representation of the Arab population in the EBF group was significantly low. Therefore, we could not state whether they suffer less from IgE-mediated CMA. On the other hand, we can speculate that the high prevalence of continuous exposure to CMF in this population protects them from developing IgE-mediated CMA. Further studies on this specific population are needed. These limitations were at least partially compensated for by the size and similarity between the groups.

Our results are controversial and contradict the current approach and guidelines of the World Health Organization and the recommendations of the American Academy of Pediatrics of EBF for the first 6 months of life.[36,37] Because of this and the clinical importance of IgE-mediated CMA, we believe that additional studies and evidence are required to draw a firm conclusion on whether to continue or alter the current recommendations.

Conclusion

Early and continuing exposure to CMF is associated with a beneficial effect on preventing the development of IgE-mediated CMA in low- and high-risk infants. Although breastfeeding is the best nutrition for infants, on the basis of the results of our study, regular exposure to at least 1 meal per day of CMF from birth might be considered. On the other hand, occasional and infrequent exposure to CMF is associated with a higher risk of IgE-mediated CMA and should be discouraged.

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In memory of my grandfather, Dr. David Miguel Kurlat, an extraordinary allergologist with wisdom and patience that looked at his patients as a whole world and not just a collection of symptoms. Dr. Kurlat passed away on January 1995.[38]

Supplementary materials

Supplementary data related to this article can be found at doi:10.1016/j.anai.2022.10.013

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Supplementary Data

eMethods 1. Skin Prick Test Procedure

Skin tests were performed by trained staff on the volar aspect of the forearm, with commercial CM extracts, casein (ALK-Abello Pharm. Inc), and fresh CM (3% fat). Positive (histamine 1 mg/mL) and negative controls (0.9% normal saline) were performed as well. Skin tests were defined as positive when the wheal diameter was at least 3 mm larger than the wheal size of the negative control after 15 minutes. CM, cow's milk.

eMethods 2. Oral Challenge Test Procedure

The OCT was done in case of inconclusive anamnesis and/or SPT. The OCT was conducted using CMF (contained 1.6-2.6g protein per 100 mL). The OCT is done as an open challenge. It starts with 0.5 mL CMF and the dosage is doubled every 30 minutes to 2, 5, 10, 20, 40, 80 mL of CMF. After the last dose, the infants stay for at least 2 hours for observation. CMF, cow's milk formula; OCT, oral challenge test; SPT, skin prick test.

eMethods 3. Randomization Test

We used the testing approach suggested in reference 23 to account for possible biases in selecting the feeding group. The number of IgE-mediated allergic reactions to CMP in the EBF group was used as the test statistic, but instead of using the simple Fisher Exact test, we calculated *P* values using propensity scores sampling. To this end, we first fitted logistic regression with the outcome the feeding group (EBF/CMF) and confounders: atopic comorbidities and atopic dermatitis of the parents or siblings and a categorical covariate for type of delivery. We then calculated propensity scores and used them to simulate the samples. The 1-sided *P* value was calculated by the proportion of samples in which the test statistic was equal to or larger than that obtained in the original data. CMF, cow's milk formula; CMP, cow's milk protein; EBF, exclusive breastfeeding; IgE, immunoglobulin E.

eMethods 4. Excluded Infants

From the total of 2252 infants, 260 infants (11.55%) were excluded for the following reasons detailed below:

- Lost to follow-up – the parents did not answer the E-mails or the telephone during the first 2 months of life (97 infants).
- Withdrew consent – after signing consent before labor, the parents removed their consent (158 infants).
- Infants' medical problems – medical problems were discovered in 5 infants during the first months of life: patient 209 underwent a surgical intervention; patient 483 was diagnosed with severe combined immunodeficiency at 3 months of age; patient 925 was diagnosed with cleft palate; patient 1642 suffered from respiratory failure immediately after birth and was intubated and admitted to the neonatal intensive care unit; and patient 2063 needed abdominal surgery 24 hours after birth.

Details of the demographics and atopic comorbidities were available for 110 infants from this group. eTable 1 summarizes the differences between the study group and the excluded infants.

eMethods 5. Skin Prick Test at 2 Months of Age

All participants were invited for clinical evaluation and SPT to cow's milk (commercial CM extract, casein, and fresh milk). Among them, 183 (9.2%) were evaluated. In 178/183 cases (97.3%), all 3 SPT's did not exhibit sensitization. The details of the other 5 infants are described below:

1. Patient 668 was on breastfeeding plus CMF since birth. SPT was positive only for casein extract. He continued routine exposure to CMF and from 8 months of age was exposed routinely to dairy products without adverse symptoms.
2. Patient 676 was on EBF since birth. However, she was exposed to CMF during the first 48 hours postbirth. A few days before the clinical visit and SPT, the mother started to combine CMF routinely without any clinical symptoms. SPT was positive for all 3 extracts. She was instructed to continue the routine exposure, and from 8 months of age she was also exposed to dairy products (not just CMF) without any clinical symptoms.
3. Patient 781 was on EBF since birth. During the first month of life, he was exposed twice to CMF and developed delayed vomiting on both occasions. SPT was positive only to fresh milk and he was invited for OCT. Minutes after 5cc of CMF he vomited.
4. Patient 880 was on EBF without deviation from the protocol. SPT was positive with casein and fresh milk. He was invited to OCT, and skin rash appeared after 0.5cc of CMF.
5. Patient 1893 was on EBF since birth. However, she was exposed to CMF during the first 48 hours. A few days before the clinical visit and SPT, the mother started to combine CMF routinely without any clinical symptoms. SPT was positive for casein and fresh milk. She was instructed to continue the routine exposure, and from 6 months of age she was also exposed to dairy products (not just CMF) without any clinical symptoms.

eTable 1
Comparison Between the Study Cohort and the Excluded Infants

	Characteristics	Total (n = 1992)	Excluded infants[a] (n = 110)	*P* value
Sex (female)		997 (50.1%)	88 (53.2%)	NS
Mothers' age ± SD		33.2 ± 4.82	30.7 ± 5	NS
Mothers' education	**Academic**	1401 (77.6%)	49 (62%)	<.001
	Postgraduate	181 (10%)	10 (12.7%)	
	High school	223 (12.4%)	20 (25.3%)	
	Missing data	187 (9.4%)	31 (28.2%)	
Mode of delivery (vaginal)		1800 (90.4%)	99 (90%)	NS
Siblings	**0**	652 (32.7%)	43 (39%)	NS
	1	659 (33.1%)	28 (25.7%)	
	2≤	681 (34.2%)	38 (34.5%)	
At least 1 family member with atopic disease		566 (38.87%)	22 (20%)	<.001
At least 1 family member with atopic dermatitis		204 (14.01%)	11 (10%)	NS
Maternal	**Any atopic disease**	367 (18.4%)	11 (10%)	<.05
	Asthma	134 (6.7%)	3 (2.7%)	NS
	Atopic dermatitis	71 (3.6%)	5 (4.5%)	NS
	Allergic rhinitis	190 (9.5%)	4 (3.6%)	NS
	Food allergy	62 (3.1%)	2 (1.8%)	NS
Paternal	**Any atopic disease**	260 (13%)	9 (8.2%)	NS
	Asthma	119 (6%)	8 (7.3%)	
	Atopic dermatitis	42 (2.1%)	3 (2.7%)	
	Allergic rhinitis	145 (7.3%)	3 (2.7%)	
	Food allergy	32 (1.6%)	1 (0.9%)	
Siblings	**Any atopic disease**	367 (18.4%)	6 (5.4%)	<.01
	Asthma	157 (7.9%)	3 (2.7%)	NS
	Atopic dermatitis	187 (9.4%)	3 (2.7%)	<.05
	Allergic rhinitis	22 (1.1%)	0	NS
	Food allergy	93 (4.7%)	2 (1.8%)	NS

Abbreviation: NS, nonsignificant differences.
[a]This includes data only on infants that were excluded from the study for whom we had information on their family background.

eTable 2
Comparison Between the Cow's Milk Formula Continuous Exposure Subgroups

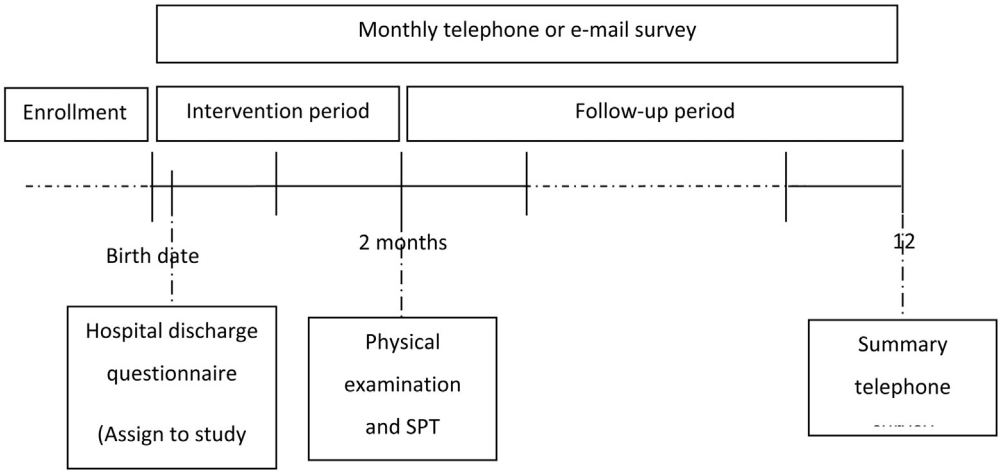
	Characteristics	Breastfeeding + CMF (n = 617)	CMF only (n = 302)	*P* value
Sex (female)		287 (46.6%)	145 (48%)	NS
Mothers' age ± SD		34.1 ± 5.2	32.8 ± 4.9	
Mothers' education	**Academic**	403 (72.6%)	169 (61.7%)	
	Postgraduate	75 (13.5%)	34 (12.4%)	
	High school	77 (13.9%)	71 (25.9%)	
	Missing data	62	28	
Mode of delivery (vaginal)		529 (85.7%)	270 (89.4%)	
Siblings	**0**	254 (41.2%)	96 (31.8%)	
	1	197 (31.9%)	100 (33.1%)	
	2 ≤	166 (26.9%)	106 (35.1%)	
At least 1 family member with atopic disease		209 (33.9%)	105 (34.8%)	
At least 1 family member with atopic dermatitis		71 (11.5%)	34 (11.3%)	
Maternal	**Any atopic disease**	104 (16.9%)	58 (19.2%)	
	Asthma	40 (6.5%)	23 (7.6%)	
	Atopic dermatitis	21 (3.4%)	5 (1.7%)	
	Allergic rhinitis	51 (8.3%)	30 (9.9%)	
	Food allergy	18 (2.9%)	10 (3.3%)	
Paternal	**Any atopic disease**	76 (12.3%)	32 (10.6%)	
	Asthma	35 (5.7%)	16 (5.3%)	
	Atopic dermatitis	6 (1%)	3 (1%)	
	Allergic rhinitis	41 (6.6%)	14 (5%)	
	Food allergy	10 (1.6%)	4 (1.3%)	
Siblings	**Any atopic disease**	100 (16.2%)	51 (16.9%)	
	Asthma	38 (6.2%)	27 (8.9%)	
	Atopic dermatitis	50 (8.1%)	27 (8.9%)	
	Allergic rhinitis	6 (1%)	4 (1.3%)	
	Food allergy	27 (4.4%)	8 (2.6%)	

Abbreviation: NS, nonsignificant differences.

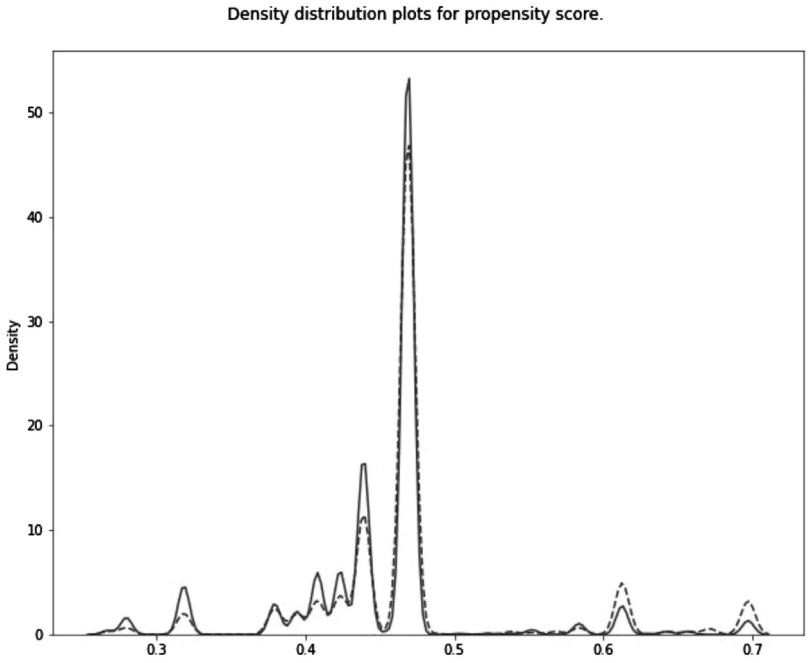
eTable 3
Characterization of Patients With CMA (IgE And Non-IgE–Mediated)

No.	Study group		Cow's milk allergic reaction details							Other atopic comorbidities			Family history of atopic diseases	Sex	Month of birth
	Study group	Per protocol	Age at first exposure to CMF	Age at first reaction (month)	Symptoms	SPT results			OFC						
						CE	casein	FM		AD	HRAD (use of inhalation)	Other food allergy			
IgE-mediated CMA															
312	1	1.2.1H	0	6	rash	Neg	Neg	Pos	failed	No	Yes	No	No	F	February
352	1	1.2.1H	0	6	rash	Pos	Pos	Pos	failed	No	No	No	Asthma	F	April
537	1	1.1		9	rash	Pos	Pos	Pos	ND	Yes	No	Yes	AD	F	June
643	1	1.2.2	0	8	Rash	Pos	Pos	Pos	ND	No	No	No	FA, Asthma, AD, AR	M	August
781	1	1.2.1R	1	2	vomiting	Neg	Neg	Pos	failed	No	No	No	FA	M	October
800	1	1.2.1H	0	10	Rash	Pos	Neg	Pos	ND	No	No	No	No	F	October
880	1	1.1			Rash	Neg	Pos	Pos	Failed	Yes	No	Yes	AD	M	November
931	1	1.2.1R	0	6	Rash	Pos			ND	No	No	No	No	F	November
1199	1	1.2.1R	1	7	Rash	Pos	Pos		ND	No	No	Yes	No	M	February
1306	1	1.2.2	0	7	Rash	Pos	Neg	Pos	ND	Yes	Yes	Yes	No	F	May
1536	1	1.1	6	6	vomiting, rash	Pos	Neg	Pos	ND	No	No	No	FA	M	July
1656	1	1.2.1H	0	5	Rash	Pos	Pos		ND	No	Yes	No	No	F	September
1669	1	1.1	8	8	Rash	Pos	Pos		ND	No	No	No	No	M	September
1832	1	1.2.1H	0	3	Rash	Neg	Neg	Neg	Failed	No	Yes	No	No	M	December
1918	1	1.2.1	0	2	Vomiting	Neg	Neg	Pos	ND	No	No	No	Asthma	F	January
2174	1	1.2.1H	0	8	Vomiting	Pos			ND	No	No	No	AD, AR	F	April
2177	1	1.2.1H	0	5	Rash	Pos	Pos	Pos	ND	No	No	Yes	Asthma, AR	F	April
Food protein-induced enterocolitis															
52	2.2		0	1	Vomiting	Neg	Neg	Neg	ND	No	No	No	FA	F	September
760	2.2		0	3	Vomiting				ND	No	yes	No	Asthma, AD	F	September
808	1	1.2.1R	0	1	vomiting	Neg	Neg	Neg	ND	No	No	No	Asthma, FA (CMA)	M	October
871	2.1		0	1	vomiting	Neg	Neg	Neg	ND	Yes	Yes	No	Asthma, AD	F	November
1147	1	1.2.1R	0	2	Vomiting				ND	No	Yes	No	Asthma	M	January
1949	1	1.2.1H	0	5	Vomiting	Neg	Neg	Neg	ND	No	No	No	No	M	January
2110	1	1.2.1R	0	1	Vomiting	Neg	Neg	Neg	ND	No	No	No	Asthma	M	March
Food protein-induced allergic proctocolitis															
70	2.1		0	1	Bloody stool	Neg	Neg	Neg	ND	No	No	No	AD	F	October
112	1	1.1		1	Bloody stool	Neg	Neg	Neg	ND	Yes	Yes	No	No	M	October
176	1	1.2.1R	0	0	Bloody stool	Neg	Neg	Neg	ND	Yes	Yes	No	AD, AR	M	December
289	1	1.2.1H	0	1	Bloody stool				ND	No	No	No	AR	F	February
484	2.2		0	1	Diarrhea				ND	No	yes	No	No	F	June
584	1	1.2.3	0	2	Bloody stool				ND	Yes	yes	No	AR	F	July
682	1	1.2.1H	0	3	Bloody stool				ND	No	No	No	AD	F	August
969	1	1.1		2	Bloody stool				ND	No	No	No	No	F	December
1035	1	1.2.1R	1	3	Bloody stool			Neg	ND	No	No	Soy (FPIAP)	No	M	December
1464	1	1.2.1H	0	3	Bloody stool				ND	No	No	No	AR, AD, FPIAP	M	June
1569	2.2		0	1	Diarrhea				ND	No	yes	No	FA (CMA)	F	August
1584	2.1		0	1	Bloody stool	Neg	Neg	Neg	ND	No	yes	No	No	M	August
1699	2.2		0	2	Bloody stool				ND	No	Yes	No	No	F	October
1758	2.1		0	2	Bloody stool				ND	No	Yes	No	No	M	October
1764	2.2		0	1	Bloody stool				ND	No	No	No	No	F	November
1797	1	1.2.1R	0	4	Diarrhea				ND	No	No	No	Asthma	F	November
1846	1	1.1		3	Bloody stool				ND	No	No	No	No	F	December
1849	1	1.1		5	Bloody stool	Neg	Neg	Neg	ND	No	No	No	No	F	December
1934	2.1		0	1	Diarrhea				ND	No	Yes	No	Asthma	F	January
1990	1	1.2.1H		1	Bloody stool				ND	Yes	No	No	AR, AD	F	February
1994	2.2		0	1	Diarrhea				ND	No	No	No	No	M	February
2150	1	1.1	4	4	Bloody stool				ND	No	No	No	AR	F	March
2206	1	1.1		3	Bloody stool	Neg	Neg	Neg	Succeed	Yes	No	Yes	Asthma	M	April

Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; CE, cow's milk commercial extract; CMA, cow's milk allergy; CMF, cow's milk formula; FA, food allergy; FM, fresh milk; FPIAP, food protein-induced allergic proctocolitis; HRAD, hyperreactive airway diseases defined as use of inhalations; OFC, oral food challenge; SPT, skin prick test.
NOTE. Study group: 1, exclusive breastfeeding; 2.1, breastfeeding + cow's milk formula; 2.2, cow's milk formula only. Per protocol: 1.1, per protocol; 1.2.1H, hospital exposure only; 1.2.1R, exposure to maximally 5 bottles during the intervention period; 1.2.2, irregular exposure to CMF; 1.2.3, switch to another group.



eFigure 1. Study design. SPT, skin prick test.



eFigure 2. Propensity score. The propensity score was determined for the following variables: parents (maternal and/or paternal) atopic comorbidities, parents (maternal and/or paternal) atopic dermatitis, siblings' atopic comorbidities, siblings' atopic dermatitis, and mode of delivery. Dashed line illustrates exposure to cows' milk formula group whereas continuous line denotes exclusive breastfeeding.