

Primary Prevention of Cow's Milk Sensitization and Food Allergy by Avoiding Supplementation With Cow's Milk Formula at Birth

A Randomized Clinical Trial

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IMPORTANCE Cow's milk formula (CMF) is used to supplement breastfeeding (BF) at birth without clear clinical evidence to support the practice.

OBJECTIVE To determine whether avoiding supplementation with CMF at birth can decrease risks of sensitization to cow's milk protein and/or clinical food allergy, including cow's milk allergy (CMA), overall and in subgroups stratified by 25-hydroxyvitamin D (25[OH]D) levels.

DESIGN, SETTING, AND PARTICIPANTS The Atopy Induced by Breastfeeding or Cow's Milk Formula (ABC) trial, a randomized, nonblinded clinical trial, began enrollment October 1, 2013, and completed follow-up May 31, 2018, at a single university hospital in Japan. Participants included 330 newborns at risk for atopy; of these, 312 were included in the analysis. Data were analyzed from September 1 through October 31, 2018.

INTERVENTIONS Immediately after birth, newborns were randomized (1:1 ratio) to BF with or without amino acid-based elemental formula (EF) for at least the first 3 days of life (BF/EF group) or BF supplemented with CMF (≥ 5 mL/d) from the first day of life to 5 months of age (BF plus CMF group).

MAIN OUTCOMES AND MEASURES The primary outcome was sensitization to cow's milk (IgE level, ≥ 0.35 allergen units [U_A]/mL) at the infant's second birthday. Secondary outcomes were immediate and anaphylactic types of food allergy, including CMA, diagnosed by oral food challenge test or triggered by food ingestion, with food-specific IgE levels of at least $0.35 U_A$ /mL. Subgroup analysis was prespecified by tertiles of serum 25(OH)D levels at 6 months of age.

RESULTS Of the 312 infants included in the analysis (160 female [51.3%] and 152 male [48.7%]), 151 of 156 (96.8%) in the BF/EF and BF plus CMF groups were followed up until their second birthday. The primary outcome occurred in 24 infants (16.8%) in the BF/EF group, which was significantly fewer than the 46 infants (32.2%) in the BF plus CMF group (relative risk [RR], 0.52; 95% CI, 0.34-0.81). The middle tertile of the 25(OH)D subgroup, but not the low and high tertiles, had a significant interaction with the intervention (RR, 0.19; 95% CI, 0.07-0.50; $P = .02$). The prevalence of food allergy at the second birthday was significantly lower in the BF/EF than in the BF plus CMF groups for immediate (4 [2.6%] vs 20 [13.2%]; RR, 0.20; 95% CI, 0.07-0.57) and anaphylactic (1 [0.7%] vs 13 [8.6%]; RR, 0.08; 95% CI, 0.01-0.58) types.

CONCLUSIONS AND RELEVANCE The evidence suggests that sensitization to cow's milk and food allergy, including CMA and anaphylaxis, are primarily preventable by avoiding CMF supplementation for at least the first 3 days of life.

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 Editorial

 Supplemental content

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Food allergy mediated by IgE is becoming a global concern because its prevalence and severity are worsening.¹⁻³ To overcome this issue, prolonged exclusive breastfeeding (BF) with or without supplementation with hypoallergenic formulas for infants at risk has been recommended.⁴ However, these preventive strategies have recently been called into question.⁵ For example, a cohort study⁶ demonstrated that the frequency of IgE-mediated cow's milk allergy (CMA) was lower in infants who began receiving regular cow's milk formula (CMF) within the first 14 days of life and therefore recommended CMF supplementation at birth. However, the effects of supplementing BF with CMF at birth or within the first 3 days of life on the risk of CMA^{7,8} and atopy⁹⁻¹¹ are still controversial.

Many Japanese maternity wards encourage BF but allow mothers or nurses to supplement BF with CMF—for example, approximately 6 to 10 hours after birth or even earlier—based on maternal preferences but not clinical evidence. However, more than 20 to 30 years ago, sugar water was given instead of CMF supplement at birth. Thus, we hypothesized that early exposure to CMF at birth is, at least in part, associated with the recent increase in children with food allergy. Therefore, a randomized clinical trial (RCT), Atopy Induced by Breastfeeding or Cow's Milk Formula (ABC) trial, was conducted to assess whether the risk of CMF sensitization and food allergy is decreased by avoiding or supplementing with CMF at birth.

Excessive and/or deficient levels of vitamin D during the neonatal and infantile periods were suggested to raise the risk of food allergy.¹²⁻¹⁵ Although vitamin D supplementation for lactating mothers or infants is not common in Japan, a previous RCT¹⁶ demonstrated that food allergy was increased in the offspring of mothers supplemented with vitamin D compared to placebo. Moreover, no consensus exists about cutoff points for low, middle, and high levels of 25-hydroxyvitamin D (25(OH)D). Thus, subgroups stratified by tertiles of serum 25(OH)D levels, a biomarker of vitamin D status, were pre-specified for exploratory analyses.

Methods

Trial Design

At the departments of obstetrics and pediatrics of Jikei University Hospital, Tokyo, Japan, newborns were randomly assigned immediately after birth to avoiding supplementation with CMF for at least the first 3 days of life (ie, BF with or without an amino acid-based elemental formula [EF; BF/EF group]) or adhering to BF supplemented with CMF (≥ 5 mL/d) from the first day of life (BF plus CMF group) and then followed up to their second birthdays. The trial protocol was approved by the ethics committee of the Jikei University School of Medicine, and parents of infants provided written informed consent. This trial used an external data and safety monitoring committee. The full protocol for this trial is available in [Supplement 1](#). This study followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline for RCTs.

Key Points

Question At birth, are the risks of sensitization to cow's milk formula and food allergy decreased by avoiding or by supplementing with cow's milk formula?

Findings In this randomized clinical trial involving 312 newborns, risks of sensitization to cow's milk and immediate-type food allergy, including cow's milk allergy and anaphylaxis, were decreased by avoiding supplementation with cow's milk formula for at least the first 3 days of life.

Meaning Results suggest that sensitization to cow's milk and clinical food allergies may be preventable by avoiding cow's milk formula supplementation at birth, which is easily and immediately applicable to clinical practice worldwide without the cost and time of therapy.

Participants

The inclusion criterion was an infant at risk for atopy, with at least 1 of the father, mother, or siblings having current and/or past atopic diseases (eg, asthma). The exclusion criteria consisted of parents who intended exclusive BF or exclusive CMF before birth or infants who were born at less than 36 weeks' gestational age, had a birth weight less than 2000 g, or had serious congenital anomalies (eg, cleft palate). Pregnant women and their husbands were informed about the trial at child-birth classes during the third trimester of pregnancy.

Randomization and Blinding

The computer-generated and centrally administered randomization used permuted blocks of 4. Participants were randomized in a 1:1 ratio without stratification. Owing to the nature of the interventions, the participants were not blinded. However, levels of cow's milk-specific IgE (CM-IgE) as the primary outcome and other food-specific IgEs were measured by outsourcing to a clinical laboratory testing company (SRL, Inc; Tokyo, Japan), and parts of food allergy as the secondary outcomes were diagnosed by an oral food challenge (OFC) test administered by pediatricians who were blinded to the allocation group. The secondary outcomes were finally determined by the end point committee, who were blinded to the intervention arms.

Intervention

At the maternity ward of Jikei University Hospital, participating mothers were informed of their allocation group. Mothers allocated to the BF/EF group were asked to feed their offspring by BF and continue to the first blood test at 5 months of age, but they were allowed to add amino acid-based EF when they believed that amounts of BF were not enough. If the mother added more than 150 mL/d of EF to BF for 3 consecutive days, EF was switched to CMF after the fourth day. Thus, offspring allocated to BF/EF could avoid CMF for at least the first 3 days of life. On the other hand, mothers allocated to the BF plus CMF group were asked to supplement BF with at least 5 mL of CMF from the first day or within the first 24 hours after delivery and with at least 40 mL/d after 1 month and to continue to the first blood test at 5 months of age or before start-

ing solid food. Both CMF (Hohoemi) and EF (Meiji Elemental formula) were purchased from Meiji Holdings Company, Ltd.

Outcomes

The primary outcome was atopic sensitization to cow's milk protein (ie, serum levels of CM-IgE of ≥ 0.35 allergen units [U_A]/mL at 24 months of age). Serum levels of CM-IgE at 5 and 24 months of age as a continuous variable were used as reference values for the primary outcome. Other atopic sensitizations to egg white, wheat, and mites at levels of at least 0.35 U_A /mL at 24 months of age were also evaluated to be compared with cow's milk sensitization, but not for the primary outcome.

In this trial, the secondary outcomes were clinical food allergies, defined as immediate and anaphylactic types. Immediate types of food allergy presented with at least 1 organ symptom of the skin (eg, urticaria), respiratory tract (eg, wheezing), gastrointestinal tract (eg, vomiting), or circulatory system (eg, loss of consciousness) triggered by an OFC test conducted by pediatricians trained to administer it (eTable in Supplement 2) or by food ingestion in daily life that appeared within minutes to a few hours, in combination with serum levels of the suspected food-specific IgE level (≥ 0.35 U_A /mL). The anaphylactic type of food allergy presented when symptoms were derived from at least 2 organ systems (eg, urticaria and wheezing). These secondary outcomes were evaluated by cumulative incidence if an infant even once experienced an immediate allergic reaction with or without outgrowing it by the second birthday and by prevalence at the second birthday without outgrowing it. Atopic conditions other than food allergy were planned to report separately. Safety outcomes were admission or emergency department visit for any reason and growth retardation (height and weight) at the second birthday. Serum levels of 25(OH)D were measured at 5 months using radioimmunoassay (SRL, Inc), as described.¹⁷

Follow-up

Participating infants were examined at 1, 2, 3, 4, 5, 7 to 8, 9 to 10, 12, 14 to 15, 18, and 24 months of age at the outpatient clinic of Jikei University Hospital. All participants underwent evaluation of blood samples obtained at 5 and 24 months of age, by which total IgE and antigen-specific IgE levels, cow's milk, egg white, wheat, mites, Japanese cedar pollen, dog dandruff, and cat dandruff were measured with specific IgE additive allergens (ImmunoCAP; Thermo Fisher Diagnostics KK) at SRL, Inc. Unscheduled visits were made to Jikei University Hospital, and blood tests were performed when a participating infant showed the immediate type of food allergy (eg, with immediate reaction to walnuts, walnut-specific IgE was measured by blood sampling at the unscheduled visit).

For infants who had food antigen-specific IgE-positive findings (≥ 0.35 U_A /mL) at 5 months of age, the OFC test was performed at approximately 1 year of age at Jikei University Hospital. After blood examination at 5 months of age, if the infants had already taken the allergenic food without clinical allergic signs or, conversely, the infants had already showed a strong allergic or anaphylactic reaction to the allergenic food, the OFC test was not performed.

Sample Size

The primary outcome, CM-IgE level of at least 0.35 U_A /mL at 24 months of age, would be found in 10% in one group and 25% in the other group with a 2-sided type I error of 5%, a power of 90%, and an assumption of 3% loss to follow-up. We estimated that 300 participants with a ratio of 1:1 would be sufficient to detect this difference. Assuming 10 participants per month could participate in this trial, the accrual period was estimated to be 2.5 years to enroll 300 participants. With final participant follow-up 2 years after enrollment, the total duration of the planned trial was 4.5 years.

Statistical Analysis

Data were analyzed from September 1 through October 31, 2018. All participants who underwent randomization were included in this analysis. Outcomes were assessed according to the randomization group even in cases that did not adhere to the intervention. Effects of the intervention, such as avoiding supplementation with CMF at birth, on the risk of outcomes were estimated using risk ratios (RRs) and 95% CIs. Levels of CM-IgE as a continuous variable were compared using the Mann-Whitney test. Subgroups were prespecified according to 25(OH)D levels sampled at 5 months of age into low, middle, and high tertiles. To clarify whether the intervention significantly affected these subgroups, *P* values for interaction were analyzed using the Mantel-Haenszel test. These analyses were not corrected for multiple comparisons. However, because of the potential for type I error due to multiple comparisons, findings for subgroup analyses should be interpreted as explanatory.

All data were analyzed using Stata, version 14.0 (Stata-Corp). Interim analyses were planned twice at reaching 100 and 200 participating infants who had their second birthday. Statistical significance at the interim analysis was set at 2-sided *P* < .001 according to Peto group stopping boundaries.¹⁸

Post Hoc Analysis

Infants in the BF/EF group were further divided into the following 3 subgroups: (1) continued regimen at 5 months of age; (2) switched from EF to CMF on day 15 and after; and (3) switched from EF to CMF on day 14 or before. Levels of CM-IgE were then compared among these 3 groups and infants in the BF plus CMF group (ie, total of 4 groups).

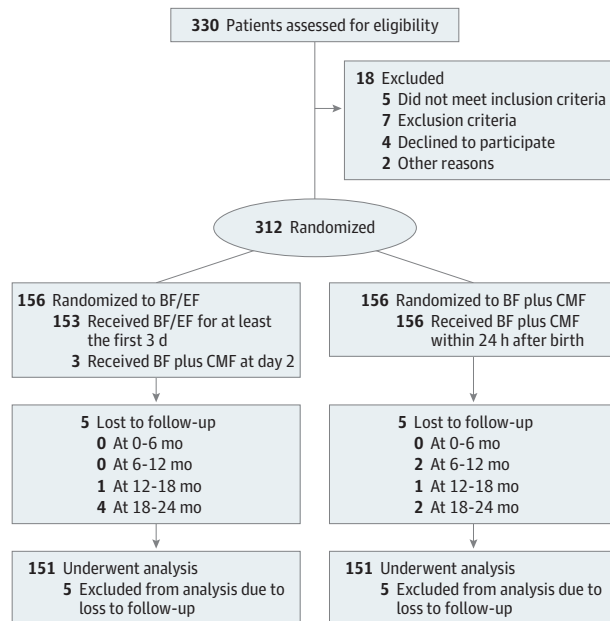
Results

Study Population

Enrollment began October 1, 2013, and follow-up was completed May 31, 2018. After excluding 18 families according to the inclusion and exclusion criteria, 312 of 330 infants (94.5%) (160 female [51.3%] and 152 male [48.7%]) were randomized to the BF/EF (*n* = 156) or the BF plus CMF (*n* = 156) groups at birth (Figure 1). At the second birthday, 151 infants (96.8%) in the BF/EF group and 151 (96.8%) in the BF plus CMF group could be followed up and were included in the analysis.

The participants' characteristics were similar between the 2 groups (Table 1). Because most participating mothers who

Figure 1. Patient Flow Through the Atopy Induced by Breastfeeding or Cow's Milk Formula (ABC) Trial



BF indicates breastfeeding; CMF, cow's milk formula; and EF, elemental formula.

attended the childbirth class were first-time parents, most of the participating infants did not have older sibling(s).

Effects of Avoiding CMF at Birth on Sensitization

To examine atopic sensitization, blood samples were available in 309 of 312 infants (99.0%) at 5 months and in 286 of 302 infants (94.7%) at 24 months of age. Participating infants were divided into the following prespecified subgroups according to tertiles of serum levels of 25(OH)D at 5 months of age: low (6-20 ng/mL [n = 102]), middle (21-36 ng/mL [n = 101]), and high (37-83 ng/mL [n = 100]) (to convert 25[OH]D to nanomoles per liter, multiply by 2.496).

Effects of avoiding CMF at birth on sensitization, reported as food antigen-specific IgE level of at least 0.35 U_A/mL at 24 months of age, are given in Table 2. Significantly fewer infants had the primary outcome, cow's milk sensitization, in the BF/EF group (24 of 143 [16.8%]) than in the BF plus CMF group (46 of 143 [32.2%]; RR, 0.52; 95% CI, 0.34-0.81). In the subgroup of middle 25(OH)D levels, cow's milk sensitization was significantly less frequent in the BF/EF group than in the BF plus CMF group (4 of 44 [9.1%] vs 22 of 45 [48.9%]; RR, 0.19; 95% CI, 0.07-0.50), but it was not significant in the low- or the high-level subgroup. The interaction between the intervention and middle tertile of 25(OH)D levels was significant (P = .02 for interaction).

In contrast, the number of infants with sensitization to egg white or other food antigens was not significantly different between the 2 comparative groups. However, in the subgroup of middle 25(OH)D levels, egg white sensitization was significantly lower in the BF/EF group than in the BF plus CMF group (RR, 0.51; 95% CI, 0.28-0.92), but it was not significant in the

Table 1. Participant Characteristics

Characteristic	Study Group	
	BF/EF (n = 156)	BF Plus CMF (n = 156)
Familial Background		
Age, mean (SD), y		
Paternal	37.0 (6.1)	37.7 (6.8)
Maternal	35.0 (4.5)	35.3 (4.3)
Current/previous atopic disease of mother, No. (%)		
Bronchial asthma	9 (5.8)/24 (15.4)	6 (3.8)/20 (12.8)
Atopic dermatitis	18 (11.5)/32 (20.5)	13 (8.3)/32 (20.5)
Food allergy	28 (17.9)/18 (11.5)	15 (9.6)/15 (9.6)
Allergic rhinitis	36 (23.1)/17 (10.9)	40 (25.6)/13 (8.3)
Pollen allergy	85 (54.5)/12 (7.7)	89 (57.1)/11 (7.7)
Current/previous atopic disease of father, No. (%)		
Bronchial asthma	4 (2.6)/22 (14.1)	11 (7.1)/24 (15.4)
Atopic dermatitis	7 (4.5)/14 (9.0)	13 (8.3)/25 (16.0)
Food allergy	20 (12.8)/4 (2.6)	17 (10.9)/9 (5.8)
Allergic rhinitis	37 (23.7)/11 (7.1)	41 (26.3)/17 (10.9)
Pollen allergy	80 (51.3)/11 (7.1)	79 (50.6)/3 (1.9)
Current/previous atopic disease of siblings, No. (%)^a		
Bronchial asthma	2 (66.7)/1 (33.6)	2 (22.2)/1 (11.1)
Atopic dermatitis	0/0	3 (33.3)/0
Food allergy	1 (33.3)/2 (66.7)	5 (55.6)/1 (11.1)
Allergic rhinitis	2 (66.7)/0	3 (33.3)/0
Pollen allergy	1 (33.3)/0	1 (11.1)/0
Gestational age, median (IQR), wk	39 (38-39)	39 (38-39)
Nonvaginal delivery, No. (%)		
Planned cesarean delivery	16 (10.3)	17 (10.9)
Emergency cesarean delivery	21 (13.5)	20 (12.8)
Female, No. (%)	82 (52.6)	78 (50.0)
Apgar score at 5 min, No. (%)^b		
8 points	4 (2.7)	10 (6.5)
9 points	131 (84.0)	128 (83.7)
10 points	15 (9.6)	15 (9.8)
Placental weight, mean (SD), g	585 (103)	563 (97)
pH of cord blood, mean (SD)	7.28 (0.06)	7.29 (0.08)
Anthropometry at birth, mean (SD)		
Body weight, g	2994 (313)	2994 (314)
Body height, cm	48.7 (1.7)	48.8 (1.8)
Chest circumference, cm	31.5(1.3)	31.6 (1.4)
Head circumference, cm	34.0 (1.3)	34.1 (1.2)

Abbreviations: BF, breastfeeding; CMF, cow's milk formula; EF, elemental formula; IQR, interquartile range.

^a Number of siblings was 3 in the BF/EF group and 9 in the BF plus CMF group.

^b Includes 150 infants in the BF/EF group and 153 in the BF plus CMF group.

Table 2. Effects of Avoiding CMF at Birth on Antigen-Specific IgE Levels^a

Subgroups	Study Group, No. (%) of Infants			RR (95% CI) ^b	P Value for Interaction ^c
	Total	BF/EF	BF Plus CMF		
Cow's Milk-Specific IgE Level ≥ 0.35 U_A/mL					
Overall (n = 286) ^d	70 (24.5)	24 (16.8)	46 (32.2)	0.52 (0.34-0.81)	NA
Subgroups stratified by 25(OH)D levels ^e					
Lower tertile (n = 94)	17 (18.1)	8 (17.0)	9 (19.1)	0.89 (0.38-2.11)	.02
Middle tertile (n = 89)	26 (29.2)	4 (9.1)	22 (49.0)	0.19 (0.07-0.50)	
Higher tertile (n = 96)	26 (27.1)	12 (25.0)	14 (29.2)	0.86 (0.44-1.66)	
Egg White-Specific IgE ≥ 0.35 U_A/mL					
Overall (n = 286) ^d	107 (37.4)	53 (37.1)	54 (37.8)	0.98 (0.73-1.32)	NA
Subgroups stratified by 25(OH)D levels ^e					
Lower tertile (n = 94)	32 (34.0)	16 (34.0)	16 (34.0)	1.00 (0.57-1.78)	.02
Middle tertile (n = 89)	33 (37.1)	11 (25.0)	22 (49.0)	0.51 (0.28-0.92)	
Higher tertile (n = 96)	38 (39.6)	23 (47.9)	15 (31.3)	1.53 (0.92-2.56)	

Abbreviations: BF, breastfeeding; CMF, cow's milk formula; EF, elemental formula; NA, not applicable; RR, risk ratio; 25(OH)D, 25-hydroxyvitamin D; U_A, allergen units.

^a Other antigen-specific IgEs (wheat, mites, Japanese cedar pollen, dog dandruff, and cat dandruff) were not significantly different overall and in the subgroups.

^b Estimated as the risk of the effects of intervention (ie, avoiding supplementation with CMF at birth) on the risk of outcomes.

^c To clarify whether the intervention significantly affected these subgroups, P values for interaction were analyzed using the Mantel-Haenszel test.

^d Blood samples were available in 286 of 302 (94.7%) infants at 24 months of age (143 in each group).

^e Blood samples for measuring 25(OH)D levels were available in 303 infants at 5 months of age, but the number of infants who had data at 5 months and food antigen-specific IgE data at 24 months of age was reduced to 279.

low- or the high-level subgroup ($P = .02$ for interaction). Serum levels of CM-IgE as a continuous variable were lower in the BF/EF group (median, 0.05 U_A/mL [interquartile range {IQR}, 0.05-0.05 U_A/mL]) than in the BF plus CMF group (median, 0.05 U_A/mL [IQR, 0.05-0.10 U_A/mL]) at 5 months of age (Figure 2A). Similar results were found at 24 months of age in the BF/EF group (median, 0.05 U_A/mL [IQR, 0.05-0.16 U_A/mL]) compared with the BF plus CMF group (median, 0.05 U_A/mL [IQR, 0.05-0.48 U_A/mL]) (Figure 2B).

Effects of Avoiding CMF at Birth

The risks of secondary outcomes of each clinical food allergy are shown in Table 3. The immediate type of food allergy occurred in 22 of 151 infants (14.6%) in the BF/EF group, which was significantly less than 45 of 151 infants (29.8%) in the BF plus CMF group. Of a total of 67, 43 infants (64.2%) outgrew the allergy. At their second birthday, 4 infants (2.6%) in the BF/EF group had not yet outgrown the allergy, which was significantly less than the 20 infants (13.2%) in the BF plus CMF group (RR, 0.20; 95% CI, 0.07-0.57). Anaphylaxis developed in 1 infant (0.7%) in the BF/EF group, which was significantly fewer than the infants 13 (8.6%) in the BF plus CMF group (RR, 0.08; 95% CI, 0.01-0.58), all of whom did not outgrow the allergy by the second birthday. The OFC tests were performed in 20 infants in the BF/EF group and 24 in the BF plus CMF group, of whom 1 (5.0% of those tested and 0.7% of total) and 11 (45.8% of those tested and 7.3% of total), respectively, had positive results and were diagnosed as having a food allergy. At the second birthday, all infants with positive OFC findings in the BF/EF group could eat the allergenic food, whereas 9 (6.0%) with OFC-positive findings in the BF plus CMF group could not.

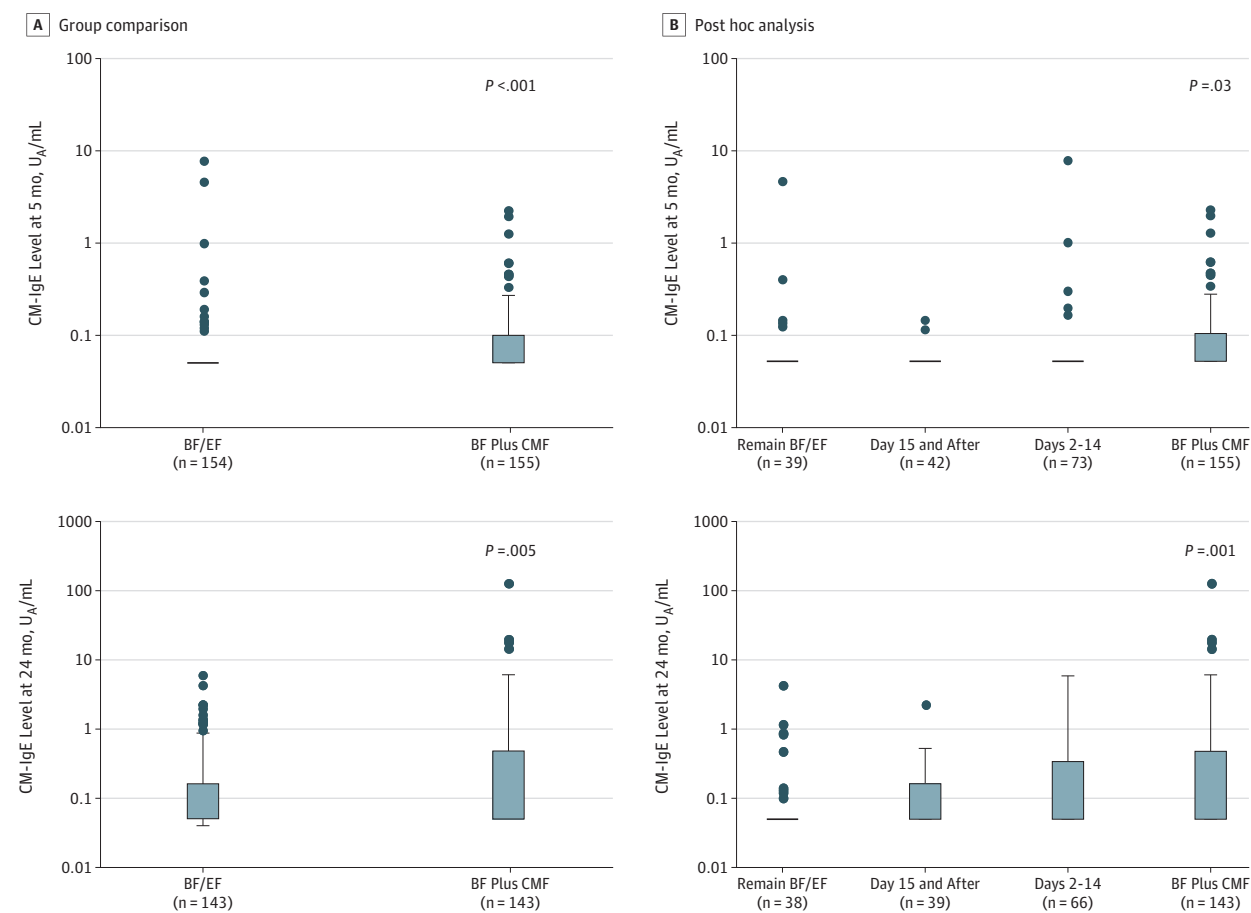
Incidence of CMA was significantly less in the BF/EF group (1 [0.7%]) than the BF plus CMF group (10 [6.6%]). Of the total

11 infants with CMA, 7 (63.6%) outgrew CMA by the second birthday, leaving no infants in the BF/EF group and 4 (2.6%) in the BF plus CMF group with CMA. Similarly, egg allergy occurred significantly less in the BF/EF (17 [11.3%]) than in the BF plus CMF (30 [19.9%]) group. Of the 47 infants with incidence of egg allergy, 38 (80.9%) had outgrown it by the second birthday, leaving 1 (0.7%) in the BF/EF group and 8 (5.3%) in the BF plus CMF group. Incidence of wheat allergy was lower in the BF/EF (1 [0.7%]) than in the BF plus CMF (7 [4.6%]) group, as was prevalence (0 and 5 [3.2%], respectively). The cumulative incidence of other food allergies (eg, walnuts) was 3 (2.0%) in the BF/EF group and 9 (6.0%) in the BF plus CMF group. In the subgroup of middle 25(OH)D levels, fewer immediate food allergies developed in the BF/EF vs BF plus CMF groups (0 vs 8 [17.0%]). Similar results were found for anaphylactic food allergies (0 vs 6 [12.8%]). In both instances, their interactions were not significant.

Post Hoc Analysis

In the BF/EF group, by maternal preferences, 73 infants were switched from BF/EF to BF plus CMF within 14 days and 42, after 14 days of life (total of 115 infants). A residual 39 infants continued to receive BF/EF until 5 months of age. In a post hoc analysis, levels of CM-IgE at 5 months of age did not show a clear trend (Figure 2B, upper panel), whereas those at 24 months of age showed a trend for earlier start of supplementation with CMF to be associated with higher levels of CM-IgE (BF/EF until 5 months of age, median of 0.05 U_A/mL [IQR, 0.05-0.05 U_A/mL]; switched to BF plus CMF after 14 days of life, 0.05 U_A/mL [IQR, 0.05-0.16 U_A/mL]; switched to BF plus CMF within 14 days of life, 0.05 U_A/mL [IQR, 0.05-0.34 U_A/mL]; BF plus CMF group, 0.05 U_A/mL [0.05-0.48 U_A/mL]) (Figure 2B, lower panel). Among these 115 infants, CMF sensitization was en-

Figure 2. Cow's Milk-Specific IgE (CM-IgE) Levels Among Study Groups



A, CM-IgE levels are compared between infants in the groups receiving breastfeeding with or without amino acid-based elemental formula for at least the first 3 days of life (BF/EF group) and BF supplemented with cow's milk formula from the first day of life to 5 months of age (BF plus CMF group). Blood sampling was available at 5 (309 of 312 [99.0%]) and 24 (286 of 302 [94.7%]) months of age. B, As a post hoc analysis, infants in the BF/EF group are further divided into the following 3 subgroups: (1) continued BF/EF; (2) switched from

EF to CMF on day 15 of life and after; and (3) switched from EF to CMF on day 14 of life or before. Subgroups were compared with the BF plus CMF group as adding CMF from the first day of life. Levels of CM-IgE are compared among these 4 groups at 5 and 24 months of age. P values were computed by regression analysis. Error bars indicate 95% CIs. BF indicates breastfeeding; CMF, cow's milk formula; EF, elemental formula; and U_A, allergen units.

hanced, but risks of clinical food allergies (ie, secondary outcomes) were not increased at all compared with 39 infants.

Adverse Events

We obtained the following information from an interview at the outpatient clinic just after the 2-year-old birthday: admission for any reason, emergency department visit for any reason, growth retardation, and height and weight at the second birthday. There was no evidence of a difference between infants in the BF/EF and BF plus CMF groups for safety outcomes (eTable in Supplement 2).

Discussion

In this RCT, avoiding exposure to CMF for at least the first 3 days of life decreased risks of sensitization to cow's milk and CMA compared with supplementing with CMF from the first

day of life. Although the previous cohort study⁶ showed that early exposure to CMF within 14 days after birth reduced the risk of CMA, exposure to small quantities of CMF for the first 3 days of life was not monitored. Thus, the results of that observational study are not necessarily in contrast to those of the present trial. The RCT by Saarinen et al⁷ showed that CMA was detected in 2.4% of infants supplemented with CMF and 1.5% of those supplemented with extensively hydrolyzed whey formula at maternity hospitals, where CMF was started as a supplement at a median of 15 hours. On the other hand, in the present trial, 2.6% in the BF plus CMF group vs 0 in the BF/EF group developed CMA. Thus, the results of both RCTs are considered similar, but amino acid-based EF may be less allergenic than the whey hydrolysate formula, even if extensively hydrolyzed. Moreover, a prospective cohort study⁸ showed that all 39 CMAs developed in 1539 infants who received CMF supplementation during the first 3 days of life, whereas no CMAs occurred in 210 infants

Table 3. Effects of Avoiding CMF at Birth on Immediate and Anaphylactic Types of Food Allergy

Effects	Study Group, No. (%) of Infants ^a			RR (95% CI) ^b	P Value for Interaction ^c
	Total (n = 302)	BF/EF (n = 151)	BF Plus CMF (n = 151)		
Immediate Type of Food Allergy					
Cumulative incidence by the second birthday	67 (22.2)	22 (14.6)	45 (29.8)	0.49 (0.31-0.77)	NA
Prevalence at the second birthday	24 (7.9)	4 (2.6)	20 (13.2)	0.20 (0.07-0.57)	NA
Subgroups stratified by 25(OH)D levels ^d					
Lower tertile (n = 99)	8 (8.1)	2 (4.2)	6 (11.8)	0.35 (0.08-1.67)	.64
Middle tertile (n = 96)	8 (8.3)	0	8 (16.7)	0 (NA)	
Higher tertile (n = 98)	8 (8.2)	2 (4.1)	6 (12.2)	0.33 (0.07-1.57)	
Anaphylactic Type of Food Allergy					
Cumulative incidence by second birthday	14 (4.6)	1 (0.7)	13 (8.6)	0.08 (0.01-0.58)	NA
Prevalence at second birthday	14 (4.6)	1 (0.7)	13 (8.6)	0.08 (0.01-0.58)	NA
Subgroups stratified by 25(OH)D levels ^d					
Lower tertile (n = 99)	5 (5.1)	1 (2.1)	4 (7.8)	0.27 (0.03-2.29)	.55
Middle tertile (n = 96)	6 (6.3)	0	6 (12.5)	0 (NA)	
Higher tertile (n = 98)	3 (3.1)	0	3 (6.1)	0 (NA)	
OFC-Positive Immediate Type of Food Allergy					
Cumulative incidence by second birthday	12 (4.0)	1 (0.7)	11 (7.3)	0.09 (0.01-0.70)	NA
Prevalence at second birthday	9 (3.0)	0	9 (6.0)	0 (NA)	NA
Subgroups stratified by 25(OH)D levels ^d					
Lower tertile (n = 99)	3 (3.0)	0	3 (5.9)	0 (NA)	>.99
Middle tertile (n = 96)	1 (1.0)	0	1 (2.1)	0 (NA)	
Higher tertile (n = 98)	5 (5.1)	0	5 (10.2)	0 (NA)	
Cow's Milk Allergy					
Cumulative incidence by second birthday	11 (3.6)	1 (0.7)	10 (6.6)	0.10 (0.01-0.77)	NA
Prevalence at second birthday	4 (1.3)	0	4 (2.6)	0 (NA)	NA
Subgroups stratified by 25(OH)D levels ^d					
Lower tertile (n = 99)	2 (2.0)	0	2 (3.9)	0 (NA)	>.99
Middle tertile (n = 96)	2 (2.1)	0	2 (4.2)	0 (NA)	
Higher tertile (n = 98)	0	0	0	0 (NA)	
Egg Allergy					
Cumulative incidence by second birthday	47 (15.6)	17 (11.3)	30 (19.9)	0.57 (0.33-0.98)	NA
Prevalence at second birthday	9 (3.0)	1 (0.7)	8 (5.3)	0.13 (0.02-0.99)	NA
Subgroups stratified by 25(OH)D levels ^d					
Lower tertile (n = 99)	4 (4.0)	1 (2.1)	3 (5.9)	0.35 (0.04-3.29)	.68
Middle tertile (n = 96)	2 (2.1)	0	2 (4.2)	0 (NA)	
Higher tertile (n = 98)	3 (3.1)	0	3 (6.1)	0 (NA)	
Wheat Allergy					
Cumulative incidence by second birthday	8 (2.6)	1 (0.7)	7 (4.6)	0.14 (0.02-1.15)	NA
Prevalence at second birthday	5 (1.7)	0	5 (3.3)	0 (NA)	NA
Subgroups stratified by 25(OH)D levels ^d					
Lower tertile (n = 99)	0	0	0	0 (NA)	>.99
Middle tertile (n = 96)	3 (3.1)	0	3 (6.3)	0 (NA)	
Higher tertile (n = 98)	2 (2.0)	0	2 (4.1)	0 (NA)	

Abbreviations: BF, breastfeeding; CMF, cow's milk formula; EF, elemental formula; NA, not applicable; OFC, oral food challenge; RR, risk ratio; 25(OH)D, 25-hydroxyvitamin D.

^a Cumulative incidence and prevalence are calculated using column totals; 25(OH)D subgroups, using row totals.

^b Estimated as the effects of intervention (ie, avoiding supplementation with CMF at birth) on risk of outcomes.

^c To clarify whether the intervention significantly affected these subgroups, P values for interaction were analyzed using the Mantel-Haenszel test.

^d Blood samples for measuring 25(OH)D levels were available in 303 infants at 5 months of age, but the number of infants with data at 5 months and followed up until 24 months of age was reduced to 293.

who did not receive CMF supplementation, findings considered to be consistent with this trial.

Of interest, avoiding CMF for at least the first 3 days of life reduced not only CMA risk but also other food allergy risks in the present trial. Germ-free mice treated with antibiotics or devoid of any bacterial colonization were demonstrated to be highly susceptible to the anaphylactic type of food allergy.^{19,20} On the other hand, gut bacterial flora (eg, *Anaerostipes caccae*) were suggested to protect against food allergy in a murine model by transferring microbiota from healthy and allergic infants.²⁰ Cesarean delivery is a well-known risk factor for food allergy, which may be explained by the evidence that infants born by cesarean delivery acquire nonmaternal vaginal bacteria from the hospital environment.²¹ In addition, a longer duration of BF was associated with a lower prevalence of *Clostridium difficile*,²² colonization of which at 1 month of age was associated with wheeze, eczema, and asthma later in life.²³ Necrotizing enterocolitis, an inflammatory bowel necrosis most commonly affecting preterm infants, can be triggered by feeding of CMF and prevented by BF, which may be further influenced by an altered intestinal microbiome that activates an uncontrolled proinflammatory response.²⁴ Based on these findings, we generated the hypothesis that exposure to a high volume of cow's milk protein with scarce growth of the gut microbiome just after birth may trigger enterocolitis, enhance permeability of gut membrane, facilitate absorption of food allergens, and increase risks of food allergy later in infancy.

In the present trial, the immediate and anaphylactic types of food allergy independent of food type (ie, not only cow's milk but also egg, wheat, and others) were shown to be primarily preventable by avoiding exposure to CMF at birth, although the mechanisms are unknown. Avoiding CMF is easily and immediately applicable to clinical practice throughout the world without incurring the cost and time of therapy. Recent RCTs demonstrated that early introduction of peanuts and heated egg powder among infants with eczema produced marked risk reductions of peanut²⁵ and egg allergy.²⁶ However, avoiding CMF at birth may be safer.

In subgroup analysis, avoiding CMF at birth was effective only in the subgroup of middle 25(OH)D levels. A U-shaped or inverted U-shaped association was reported between 25(OH)D levels and aeroallergen sensitization²⁷ or eczema,²⁸ respectively. These outcomes may suggest not linear but bimodal function of 25(OH)D in the serum on immune functions. However, vitamin D hypotheses in food allergy are still far from conclusions.

Limitations

This trial has several limitations. First, this trial was not double-blinded. For this reason, the primary outcome was set as CM-IgE level, an objective variable, and the OFC tests were performed by pediatricians who were blinded to allocation group. The secondary outcomes were further determined by the end

point committee, which was blinded to the intervention arms. Second, the OFC test was not administered to all participants. The OFC test is the criterion standard for the diagnosis of food allergy, but it is a resource-intensive procedure with some level of risk involved; thus, it was reserved for the equivocal cases predefined in this trial.²⁹ Third, the follow-up period was to the second birthday, which may be too short to evaluate the risks of food allergy because some infants with food allergy can outgrow their allergy after the second birthday, or some infants may show the immediate and anaphylactic types of allergy to certain foods (eg, nuts) after the second birthday. Fourth, the prevalence of each food allergy is different in different countries (eg, egg allergy is highest in Japan, probably because Japanese prefer to eat raw eggs, whereas CMA is highest in other countries).³⁰ Fifth, we cannot differentiate which intervention—avoiding CMF or exposing to EF for the first 3 days—was effective in preventing development of food allergy. However, CMF and EF are fundamentally the same except whether cow's milk protein or amino acids without cow's milk protein is included. Randomization into the BF plus CMF group from the first day of life, BF alone for the first 3 days while avoiding CMF and EF, and BF plus EF for the first 3 days while avoiding CMF is ideal. However, if mothers do not have enough breast milk, it is ethically hard to keep an intervention of BF alone even for 72 hours at birth. Sixth, amounts of CMF or EF added to BF were highly variable, ranging from anywhere to near-exclusive BF or near-exclusive CMF with different duration based on subjective impression of adequate infant intake. Regardless of these variabilities in the intervention, the difference was obvious between the BF/EF and BF plus CMF groups, suggesting that the critical factor may not be the amount of CMF exposure or its duration but timing of the start of adding CMF to BF. Seventh, after 3 days of life, most infants in the BF/EF group were switched from BF/EF to BF plus CMF, whereas all infants in the BF plus CMF group remained in the same group, which may cause differential misclassification. Thus, adding CMF to BF after 3 days of life may have protective effects on the development of food allergy, including CMA. Eighth, cow's milk sensitization as the primary outcome does not always lead to CMA, although CMA develops among infants with cow's milk sensitization. If we focused on OFC-proven CMA as one of the secondary outcomes, statistical power is too small.

Conclusions

This study found that not only sensitization to cow's milk but also clinical food allergies are primarily preventable by avoiding CMF supplementation at birth. This prevention is easily and immediately applicable to clinical practice throughout the world without the cost and time of therapy.

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