



Original Investigation | Pediatrics

Effect of Avoiding Cow's Milk Formula at Birth on Prevention of Asthma or Recurrent Wheeze Among Young Children Extended Follow-up From the ABC Randomized Clinical Trial

Hiroshi Tachimoto, MD, PhD; Eiji Imanari, MD; Hidetoshi Mezawa, MD, PhD; Mai Okuyama, MD, PhD; Takashi Urashima, MD, PhD; Daishi Hirano, MD, PhD; Noriko Gocho, MD; Mitsuyoshi Urashima, MD, PhD, MPH

Abstract

IMPORTANCE Children with food allergies may develop asthma or recurrent wheeze.

OBJECTIVE To evaluate whether asthma or recurrent wheeze among children were changed by avoiding supplementing breastfeeding (BF) with cow's milk formula (CMF) in the first 3 days of life.

DESIGN, SETTING, AND PARTICIPANTS This randomized, unmasked, clinical trial was conducted at 1 university hospital in Japan beginning October 2013 with follow-up examinations occurring until January 2020. A total of 312 newborns at risk for atopy were randomized and assigned to either BF with or without amino acid–based elemental formula (EF) or BF with CMF, with follow-up examinations for participants showing signs of atopy conducted at 24 months. Follow-up examinations ran through January 2020.

INTERVENTIONS Immediately after birth, newborns were randomly assigned (1:1 ratio) to either breastfeeding with or without amino acid–based elemental formula for at least the first 3 days of life (no CMF group) or breastfeeding supplemented with CMF (≥ 5 mL/d) from the first day of life to 5 months of age (CMF group).

MAIN OUTCOMES AND MEASURES Asthma or recurrent wheeze diagnosed by the pediatric allergy specialists of this trial; subgroups were stratified by serum levels of 25-hydroxyvitamin D and IgE.

RESULTS Of 312 infants (156 [50.0%] randomized to the no CMF group), 302 (96.8%) were followed up at their second birthday: 77 of 151 (51.0%) in the no CMF group and 81 of 151 (53.6%) in the CMF group underwent extended follow-up because of having atopic conditions. Asthma or recurrent wheeze developed in 15 (9.9%) of the children in the no CMF group, significantly less than the children in the CMF group (27 [17.9%]; risk difference, -0.079 ; 95% CI, -0.157 to -0.002). In participants with vitamin D levels above the median at 5 months of age, asthma or recurrent wheeze developed in 5 (6.4%) children in the no CMF group, significantly less than in the children in the CMF group (17 [24.6%]; risk difference, -0.182 ; 95% CI, -0.298 to -0.067 ; P for interaction = .04). In the highest quartile group of total IgE at age 24 months, asthma or recurrent wheeze developed in 2 children (5.3%) in the no CMF group, significantly less than the children in the CMF group (14 [43.8%]; risk difference, -0.385 ; 95% CI, -0.571 to -0.199 ; P for interaction = .004).

CONCLUSIONS AND RELEVANCE The findings of this study suggest that avoiding CMF supplementation in the first 3 days of life has the potential to reduce the risk of asthma or recurrent wheeze in young children, especially among those with high vitamin D or high IgE levels.

(continued)

Key Points

Question Is the risk of asthma or recurrent wheeze in young children decreased by avoiding supplementation with small amounts of cow's milk formula at birth?

Findings This randomized clinical trial of 312 children at risk for atopy examined risks of food allergy by the second birthday in extended follow-up examinations. Asthma or recurrent wheeze developed in significantly fewer children breastfed with or without amino acid–based elemental formula for at least the first 3 days of life than in children breastfed with supplementation from cow's milk formula (≥ 5 mL/d) from the first day.

Meaning The findings of this study suggest that asthma or recurrent wheeze can be prevented by avoiding cow's milk formula supplementation at birth.

+ Visual Abstract

+ Supplemental content

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*Abstract (continued)***TRIAL REGISTRATION** [umin.ac.jp/ctr](https://www.umin.ac.jp/ctr) Identifier: [UMIN000011577](https://www.umin.ac.jp/ctr)*JAMA Network Open.* 2020;3(10):e2018534. doi:[10.1001/jamanetworkopen.2020.18534](https://doi.org/10.1001/jamanetworkopen.2020.18534)

Introduction

Asthma is among the most common chronic diseases in the world, and its prevalence has been increasing during the past 60 years.¹ Asthma with onset in early adulthood may have its origins in early childhood.^{2,3} Therefore, the primary prevention of childhood asthma may be key in mitigating the burden of this disease. A 1989 study of patients with hay fever⁴ showed an increase in the prevalence of atopic disease can in part be explained by the hygiene hypothesis that smaller family size, improvements in household amenities, and higher standards of personal cleanliness in wealthier households have reduced the opportunity for cross-infection in young families. Gupta et al⁵ reported that eczema and having a lower number of siblings were associated with increased risks of childhood food allergy and asthma. However, this study reported that food allergy was associated with skin infection, whereas asthma was associated with respiratory syncytial virus infection. A 2016 meta-analysis⁶ demonstrated that food sensitization in the first 2 years of life was associated with an increased risk of asthma or recurrent wheeze. A 2018 study⁷ found that food allergy in infancy is also associated with an increased risk of asthma or recurrent wheeze in toddlers, irrespective of whether the early food allergy resolves.

The World Health Organization recommends breastfeeding (BF) for at least 6 months after birth because of general health benefits for the child. However, a 2010 cohort study⁸ demonstrated that the incidence of cow's milk allergy was lower in infants who began receiving regular cow's milk formula (CMF) within the first 14 days of life, and thus, the authors of this study recommended CMF supplementation at birth. Many Japanese maternity wards encourage BF, but some (including Jikei University Hospital, where this trial was performed) allow mothers or nurses to supplement BF with small amounts of CMF (eg, ≥ 5 mL) approximately 6 to 10 hours after birth (or earlier in some cases) depending on maternal preferences.

We previously conducted a randomized clinical trial (RCT) (the Atopy Induced by Breastfeeding or Cow's Milk Formula [ABC] trial), in which newborns were assigned to 1 of the following groups immediately after birth: (1) BF with or without an amino acid–based elemental formula (EF), avoiding supplementation with CMF for at least the first 3 days of life (ie, the no CMF group), or (2) BF supplemented with a small amount of CMF (≥ 5 mL/d) from the first day of life (ie, the CMF group).⁹ Participants then underwent follow-up until their second birthday. Because asthma and recurrent wheeze tend to be diagnosed later than food allergy in infants, participants who needed medical care for atopic conditions (eg, eczema, atopic dermatitis, food allergy, recurrent wheeze, and/or atopic sensitization) underwent extended follow-up to a maximum age 6 years. Selection bias would be expected because asthma or recurrent wheeze was originally set as 1 of the prespecified exploratory outcomes and was planned to be reported separately. Therefore the aim was to examine whether asthma or recurrent wheeze is preventable by avoiding CMF for at least the first 3 days of life with extended follow-up of participants in the ABC trial.

Methods

Trial Design

Newborn infants at risk for atopy born in Jikei University Hospital, with at least 1 of the father, mother, or siblings having current and/or past atopic diseases (eg, asthma, atopic dermatitis, food allergy, allergic rhinitis, or hay fever), were randomly assigned immediately after birth to avoid supplementation with CMF for at least the first 3 days of life (the no CMF group), or to BF

supplemented with CMF (≥ 5 mL/d) (the CMF group). Each group adhered to these conditions until age 5 months and then followed up to their second birthdays. There was no restrictions on the mother's diet during the intervention to age 5 months. Although the ABC trial initially ended when the last participant reached her second birthday, participants who needed medical care for atopic conditions (eg, eczema, atopic dermatitis, food allergy, recurrent wheeze, and/or atopic sensitization) at their second birthday underwent extended follow-up to a maximum age of 6 years. Thus, a diagnosis of asthma or recurrent wheeze during preschool age was set as 1 of the prespecified exploratory outcomes in the protocol of the ABC trial. Serum levels of 25-hydroxyvitamin D (25[OH]D),¹⁰ total IgE, and antigen-specific IgE levels were measured at age 5 months and 24 months. The trial protocol was approved by the ethics committee of the Jikei University School of Medicine, and parents of infants provided written informed consent. 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.

Outcome

The primary outcome of this study was the incidence of asthma or recurrent wheeze based on the asthma predictive index.¹¹ This outcome had been prespecified in the original protocol as follows: (1) frequent wheezing, and (2) either 1 of 3 major risk factors (ie, parental history of asthma; eczema diagnosed by pediatricians involved in this trial and evaluated using the Severity Scoring of Atopic Dermatitis index¹²; or sensitization with elevated mite-specific IgE ≥ 0.10 U_A/mL) or 2 of 3 minor risk factors (ie, eosinophilia [$\geq 4\%$]; wheezing diagnosed when inhalation with a β -stimulant improved the clinical signs of wheeze and dyspnea effectively; or allergic rhinitis diagnosed, if signs and symptoms of rhinitis continued for 1 month and mite-specific IgE ≥ 0.10 U_A/mL, by the pediatricians involved in this trial). The pediatrician who diagnosed asthma or recurrent wheeze was an allergy specialist and totally masked to the allocation group.

Follow-up

Enrollment began in October 2013, and follow-up for all participants to assess the risk of food allergy initially ended on May 31, 2018. Follow-up for participants without atopic conditions was ended at their second birthday. However, participants who needed medical care for atopic conditions (eg, eczema, atopic dermatitis, food allergy, recurrent wheeze, and/or atopic sensitization) underwent extended follow-up at the outpatient clinic of Jikei University Hospital after their second birthday until January 4, 2020. After excluding 18 families, 312 of 330 infants (94.5%) were randomized to the no CMF group (156 infants) or the CMF group (156 infants) in the first 3 days of life (**Figure 1**). One hundred fifty-one children (96.8%) in the no CMF group and 151 children (96.8%) in the CMF group could be followed-up at their second birthday. In the no CMF group, 77 infants (51.0%) developed atopy and underwent extended follow-up due to having atopic conditions for a median of 3.0 years (interquartile range [IQR], 2.0-4.2 years; maximum, 5.6 years). In the CMF group, 81 infants (53.6%) developed atopy and underwent extended follow-up for a median of 3.3 years (IQR, 2.1-4.3 years; maximum, 6.0 years). These differences were not statistically significant.

Statistical Analysis

All participants who underwent randomization and were followed-up to at least their second birthdays were included in this analysis. Asthma or recurrent wheeze was assessed according to the randomization group even in cases that did not adhere to the intervention as an intention-to-treat analysis. Skewness and kurtosis tests were used to assess the normality of the distributions. Parametric and nonparametric continuous variables with normal and nonnormal distributions were compared between 2 groups and among 4 groups by the *t* test and the Mann-Whitney test, and analysis of variance and the Kruskal-Wallis test, respectively. Dichotomous variables were compared between the groups by the χ^2 test. Spearman rank correlation coefficient (ρ) with linear regression was used to quantify the strengths of associations between 2 continuous variables (strong, $\rho \geq 0.4$;

moderate, $p < 0.4$ and ≥ 0.2 ; and weak, $p < 0.2$). Effects of the intervention on the cumulative incidence of asthma or recurrent wheeze were estimated using risk differences (RDs) and risk ratios (RRs) with 95% CIs. When both sides of the 95% CI of RD were more or less than 0 or both sides of the 95% CI of RR did not include 1, the risk estimate was considered to be significant. Prespecified subgroups were stratified at the median and tertiles of 25(OH)D levels sampled at age 5 and 24 months (ie, 25[OH]D < 29 ng/mL at 5 months, ≥ 29 ng/mL at 5 months, < 21 ng/mL at 24 months, and ≥ 21 ng/mL at 24 months; to convert 25[OH]D to nanomoles per liter, multiply by 2.496). To clarify whether the intervention significantly affected these subgroups, P values for interaction were analyzed using the Mantel-Haenszel test. All reported P values were 2-sided and $P < 0.05$ was considered significant. These analyses were not corrected for multiple comparisons. All data were analyzed using Stata version 14.0 (StataCorp).

In post hoc analysis, subgroup analyses were performed. Infants were divided by quartile of serum levels of total IgE at age 24 months (ie, ≤ 5 IU/mL, 6-13 IU/mL, 14-49 IU/mL, and ≥ 50 IU/mL), by the median of total IgE levels at age 5 months (ie, < 10 IU/mL and ≥ 10 IU/mL), and by positivity (≥ 0.1 U_A/mL) for allergen-specific IgE at age 5 and 24 months.

Infants in the no CMF group were further divided into 3 subgroups: (1) remained on BF with or without EF at age 5 months; (2) switched from EF to CMF on day 15 or after; and (3) switched from EF to CMF on day 14 or before. The incidences of asthma or recurrent wheeze were then compared among these 3 groups.

Results

Effects of Avoiding CMF at Birth on Asthma or Recurrent Wheeze

The characteristics of the 302 participants who underwent randomization and were followed up to at least their second birthdays were compared among 4 groups: (1) no CMF without atopic conditions; (2) no CMF with atopic conditions; (3) CMF without atopic conditions; and (4) CMF with atopic conditions (Table). Participants' characteristics were not different among these 4 groups, except that placental weight was heavier in infants in the no CMF group with atopic conditions than in the no CMF group without atopic conditions (mean [SD] weight, 615 [109] g vs 553 [86] g). Total IgE levels were not different between the no CMF (median [IQR], 13 [6-46] IU/mL) and the CMF

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

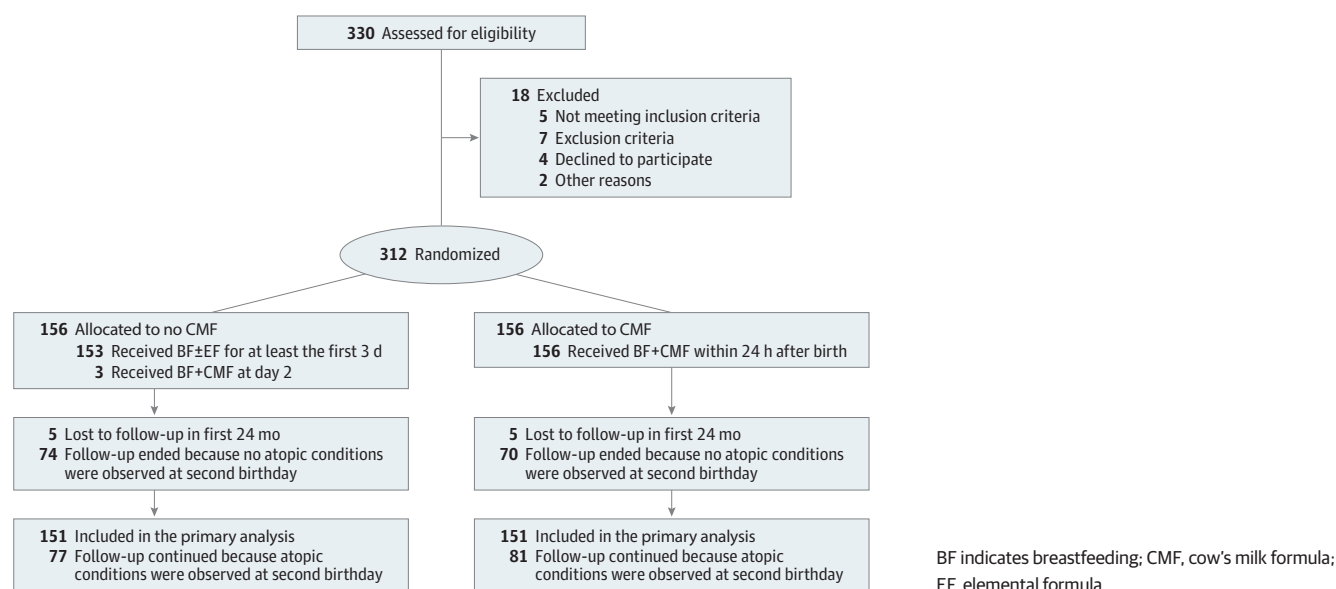


Table. Participants' Characteristics

Perinatal variables before or at randomization	Participants, No. (%)			
	No CMF group (n = 151)	CMF group (n = 151)	Without atopy (n = 70) ^a	With atopy (n = 81) ^b
Parental age, mean (SD), y				
Father	37.4 (5.4)	36.8 (6.8)	37.7 (6.9)	37.5 (6.8)
Mother	35.4 (4.2)	34.8 (4.9)	35.0 (4.5)	35.4 (4.2)
Atopic disease of mother				
Bronchial asthma				
Current	5 (6.8)	4 (5.2)	3 (4.3)	3 (3.7)
Previous	9 (12.2)	14 (18.2)	5 (7.1)	15 (18.5)
Atopic dermatitis				
Current	9 (12.2)	9 (11.7)	4 (5.7)	9 (11.1)
Previous	11 (14.9)	20 (26.0)	13 (18.6)	18 (22.2)
Food allergy				
Current	15 (20.3)	13 (16.9)	3 (4.3)	10 (12.4)
Previous	6 (8.1)	12 (15.6)	7 (10.0)	8 (9.9)
Allergic rhinitis				
Current	18 (24.3)	18 (23.4)	16 (22.9)	23 (28.4)
Previous	6 (8.1)	11 (14.3)	6 (8.6)	7 (8.6)
Pollen allergy				
Current	42 (56.8)	41 (53.3)	41 (58.6)	46 (56.8)
Previous	8 (10.8)	3 (3.9)	4 (5.7)	7 (8.6)
Atopic disease of father				
Bronchial asthma				
Current	3 (4.1)	0	6 (8.6)	4 (4.9)
Previous	10 (13.5)	12 (15.6)	10 (14.3)	13 (16.1)
Atopic dermatitis				
Current	1 (1.4)	6 (7.8)	5 (7.1)	8 (9.9)
Previous	5 (6.8)	9 (11.7)	10 (14.3)	17 (17.3)
Food allergy				
Current	12 (16.2)	8 (10.4)	7 (10.0)	10 (12.4)
Previous	3 (4.1)	1 (1.3)	5 (7.1)	4 (4.9)
Allergic rhinitis				
Current	19 (25.7)	18 (23.4)	19 (27.1)	19 (23.5)
Previous	7 (9.5)	4 (5.2)	8 (11.4)	9 (11.1)
Pollen allergy				
Current	40 (54.1)	36 (46.8)	37 (52.9)	39 (48.2)
Previous	4 (5.4)	5 (6.5)	1 (1.4)	2 (2.5)
Perinatal status				
Gestational time, median (IQR), wk	39 (38-39)	39 (38-39)	39 (38-39)	39 (38-39)
Cesarean delivery				
Planned	10 (13.5)	7 (9.1)	8 (11.4)	7 (8.6)
Emergency	9 (12.2)	11 (14.3)	9 (12.9)	12 (14.8)
Female sex	41 (55.4)	36 (46.8)	35 (50.0)	41 (50.6)
Apgar score at 5 min, points				
8	2 (2.9)	2 (2.7)	3 (4.4)	6 (7.5)
9	62 (88.6)	66 (88.0)	60 (88.2)	65 (81.3)
10	6 (8.6)	7 (9.3)	5 (7.4)	9 (11.3)

(continued)

Table. Participants' Characteristics (continued)

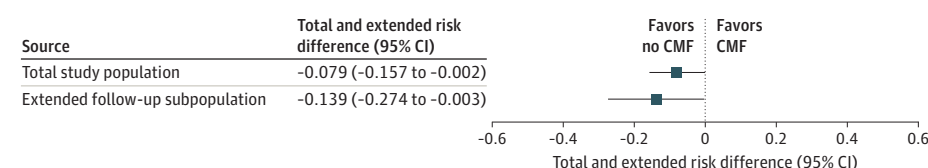
Perinatal variables before or at randomization	Participants, No. (%)			
	No CMF group (n = 151)		CMF group (n = 151)	
	Without atopy (n = 74) ^a	With atopy (n = 77) ^b	Without atopy (n = 70) ^a	With atopy (n = 81) ^b
Placental weight, mean (SD), g	553 (86)	615 (109)	561 (94)	569 (102)
pH of cord blood, mean (SD)	7.28 (0.07)	7.28 (0.06)	7.29 (0.06)	7.29 (0.09)
Anthropometry at birth, mean (SD)				
Weight, g	2969 (284)	3029 (343)	2979 (303)	3007 (328)
Height, cm	48.7 (1.5)	48.8 (1.9)	48.8 (1.7)	48.9 (1.8)
Circumference, cm				
Chest	31.4 (1.3)	31.6 (1.3)	31.4 (1.4)	31.7 (1.4)
Head	33.9 (1.2)	34.0 (1.4)	34.2 (1.1)	34.0 (1.2)

Abbreviations: CMF, cow's milk formula; IQR, interquartile range.

^a Because infants did not have atopic conditions (eg, atopic dermatitis, food allergy, asthma or recurrent wheeze, or high IgE), their follow-ups ended at age 24 months.

^b Because infants had atopic conditions (eg, atopic dermatitis, food allergy, asthma or recurrent wheeze, or high IgE), their follow-ups were extended maximally to age 6 years.

Figure 2. Effects of Avoiding CMF at Birth on Asthma or Recurrent Wheeze



CMF indicates cow's milk formula.

(median [IQR], 15 [6-46] IU/mL; $P = .45$) groups nor between the no CMF with atopic conditions (median [IQR], 22 [6-69] IU/mL) and the CMF with atopic conditions (median [IQR], 30 [11-91] IU/mL; $P = .11$) groups, whereas they were significantly different in each group between participants with and without atopic conditions (no CMF group difference: median [IQR], 26 [9-79] IU/mL; CMF group difference: median, 7 [4-24.5] IU/mL; $P < .001$).

Asthma or recurrent wheeze was diagnosed in 42 infants (13.9%) at a median of 2.8 (IQR, 2.0-3.7; range, 1.2-4.6) years of age. To determine whether avoiding supplementation with CMF in the first 3 days of life decreases the risk of asthma or recurrent wheeze, the analyses were performed in the total study population and in the subpopulation with extended follow-up (**Figure 2**). In the overall study population, asthma or recurrent wheeze developed in 15 of 151 infants (9.9%) in the no CMF group, significantly less than in the CMF group (27 of 151 infants [17.9%]; RD, -0.079; 95% CI, -0.157 to -0.002; RR, 0.556; 95% CI, 0.308 to 1.002). Similarly, in subgroups of infants who had atopic conditions at age 2 years and continued follow-up examination to a maximum of age 6 years, the outcome was observed in 15 of 77 infants (19.5%) in the no CMF group, significantly less than in the CMF group (27 of 81 infants [33.3%]; RD, -0.139; 95% CI, -0.274 to -0.003; RR, 0.584; 95% CI, 0.338 to 1.012). The median (IQR) age at diagnosis of asthma or recurrent wheeze was 2.77 (2.02-3.73) years in the no CMF group and 2.85 (1.97-3.59) years in the CMF group, which was not significantly different.

Levels of 25(OH)D at Age 5 and 24 Months

Both blood sampling and 25(OH)D measurements were available in 303 of 312 infants (97.1%) at age 5 months (median [IQR], 29 [17-39] ng/mL; range, 6-83 ng/mL) and in 278 of 302 infants (92.0%) at age 24 months (median [IQR] ng/mL, 21 [19-25]; range, 10-74). There was a weak association between 25(OH)D levels at age 5 months and 24 months (p , 0.18) (eFigure 1 in [Supplement 2](#)). There were no differences in 25(OH)D levels at age 5 and 24 months among the 4 groups (eFigure 2 in [Supplement 2](#)).

Interaction Between 25(OH)D Levels and Avoiding CMF at Birth

To assess the interaction between 25(OH)D levels and the intervention on the risk of asthma or recurrent wheeze, analysis of subgroups stratified by the median 25(OH)D level was performed (Figure 3). In the subgroup of infants with 25(OH)D levels higher than the median at age 5 months, avoiding CMF at birth significantly reduced the incidence of asthma or recurrent wheeze: 5 of 78 infants (6.4%) in the no CMF group vs 17 of 69 infants (24.6%) in the CMF group (RD, -0.182; 95% CI, -0.298 to -0.067; RR, 0.260; 95% CI, 0.101-0.668). In the subgroup with lower 25(OH)D levels, avoiding CMF did not significantly reduce asthma or recurrent wheeze: 8 of 68 infants (11.8%) in the no CMF group vs 9 of 78 infants (11.5%) in the CMF group (RD, 0.002; 95%CI, -0.102 to 0.107; RR, 1.020; 95% CI, 0.417-2.496). There was a significant interaction between 25(OH)D levels and the intervention (*P* for interaction = .04). On the other hand, there was no such interaction between 25(OH)D levels at age 24 months and the intervention for the risk of asthma or recurrent wheeze.

Subgroup analysis based on tertiles of serum levels of 25(OH)D at age 5 months and 24 months was performed (eTable 1 in Supplement 2). In the subgroup of infants with middle tertile levels of 25(OH)D at 24 months of age (ie, the 103 participants with levels between 20 and 23 ng/mL), the outcome occurred in 2 participants (3.9%) in the no CMF group, which was significantly less than in the CMF group (10 [19.2%]), but the *P* value for interaction was not significant.

Interaction Between Total IgE Levels and Avoiding CMF at Birth

To assess the interaction between total IgE levels and the intervention on asthma or recurrent wheeze, subgroups were stratified by the median total IgE level at 5 months because almost half were not detectable at age 5 months (range, <3 to 239 IU/mL) and by quartiles of the total IgE level at age 24 months, because levels ranged broadly from less than 3 to 1250 IU/mL (Figure 4). There was no statistically significant interaction between total IgE levels at age 5 months and the intervention. However, in the subgroup of infants with the highest quartile level of total IgE at age 24 months (ie, ≥50 IU/mL), avoiding CMF at birth significantly reduced the incidence of asthma or

Figure 3. Effects of Avoiding Cow's Milk Formula (CMF) at Birth on Asthma or Recurrent Wheeze Subgroups Stratified by 25-Hydroxyvitamin D (25[OH]D) Levels

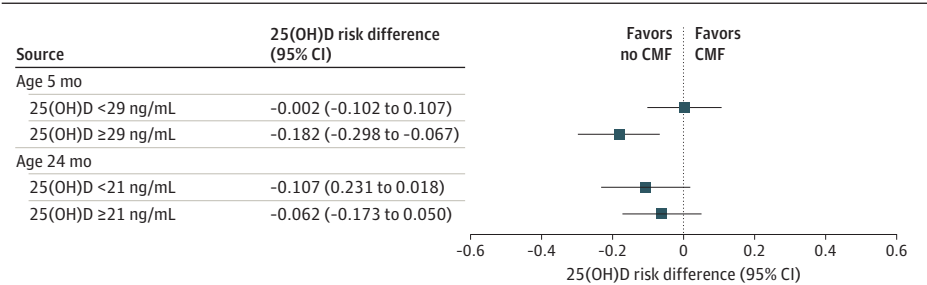
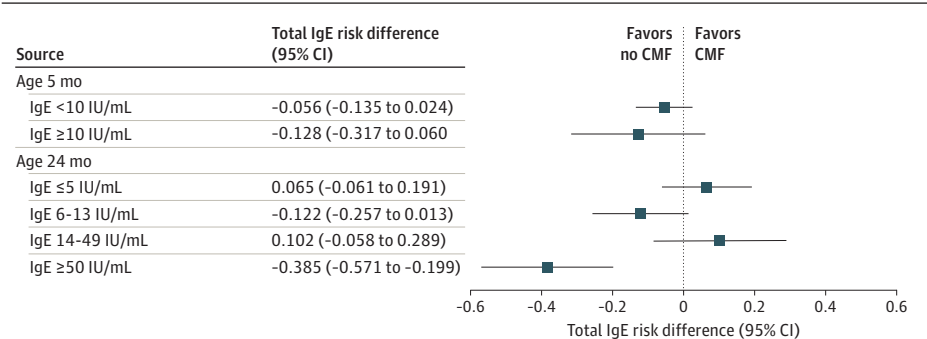


Figure 4. Effects of Avoiding Cow's Milk Formula (CMF) at Birth on Asthma or Recurrent Wheeze Subgroups Stratified by Total IgE Levels



recurrent wheeze: 2 of 38 infants (5.3%) in the no CMF group vs 14 of 32 infants (43.8%) in the CMF group (RD, -0.385 ; 95% CI, -0.571 to -0.199 ; RR, 0.120 ; 95% CI, 0.030 - 0.490 ; P for interaction = $.004$) but not in the other quartiles of total IgE.

Interaction Between Allergen-Specific IgE Levels and Intervention

To assess the interaction between allergen-specific IgE levels and the intervention on asthma or recurrent wheeze, subgroups stratified by positivity, defined as having greater than the lower limit of detection (≥ 0.10 U_A/mL) of each allergen-specific IgE level at age 24 months, further analysis was performed (eTable 2 in [Supplement 2](#)). In subgroups positive for IgE specific to mites, milk, and wheat (but not egg white), avoiding CMF at birth significantly reduced the risk of asthma or recurrent wheeze.

Incidence of Asthma or Recurrent Wheeze by Duration of Adherence to the BF/EF Intervention

In the no CMF group, 40 (12.5%) mothers adhered to BF with or without EF supplementation for infants to age 5 months, 41 (7.3%) mothers switched the feeding pattern from no CMF to CMF after 14 days, and 70 (7.3%) mothers switched within 14 days after birth. The incidence of asthma or recurrent wheeze was not different among these 3 groups (eTable 3 in [Supplement 2](#)).

Discussion

In this RCT, by avoiding exposure to CMF for at least the first 3 days of life, the risk of asthma or recurrent wheeze appeared to have been decreased compared with supplementing with CMF from the first day of life. A systematic review of 21 observational studies suggested that no BF or BF for short durations is associated with higher childhood asthma risk.¹³ In contrast, a 2002 long-term prospective cohort study¹⁴ demonstrated that more children who were breastfed reported current asthma at each assessment between ages 9 and 26 years than those who were not. On the other hand, a birth cohort examined in Copenhagen¹⁵ indicated that exclusive BF does not affect asthma or recurrent wheeze at age 7 years in at-risk children. Similarly, a large cluster RCT involving 17 046 mother-infant pairs conducted in Belarus¹⁶ showed that exclusive and prolonged BF did not reduce the risk of asthma at age 6.5 years.

As for the specific mechanisms behind this association, human milk oligosaccharides as well as secretory immunoglobulins, lactoferrin, and bacterial microbiota may play a role in preventing pathogenic bacterial adhesion while also providing nutrition for the microbiome.¹⁷ Multiple human viruses were more abundant in stool samples from infants who were exclusively fed on CMF, whereas *Bifidobacterium* and *Lactobacillus* were richer in those fed partially or fully on BF,¹⁸ suggesting their potential preventive effects on asthma or recurrent wheeze through gut microbiota. However, these mechanisms remain essentially unknown and further research is necessary.

A 2010 prospective cohort study of 13 019 infants⁸ demonstrated that the odds ratio for the development of IgE-mediated cow's milk allergy in infants supplemented with CMF after 14 days of life was 19.3 compared with those supplemented within 14 days of life and thus recommended supplementation at birth with CMF to promote its tolerance. However, the authors of this study commented that because of the way their data were collected they could not exclude neonatal exposure to small quantities of CMF in the newborn nursery that was either forgotten by the mother or done without her knowledge. Other studies listed previously also cannot exclude the possibility of exposure to small amounts of CMF at birth. Thus, the results of these previous studies do not necessarily contradict those of the present trial. Moreover, about a quarter of the infants in the no CMF group adhered to BF with or without EF to age 5 months, but others switched the feeding pattern from BF with or without EF to supplementing with CMF either before or after 14 days of life. The incidence of asthma or recurrent wheeze did not differ by the duration of adherence to the no

CMF intervention. This post hoc result implies that the first 3 days of life may be an important period for determining allergy or anergy.

This preventive effect on asthma or recurrent wheeze of avoiding CMF in the first 3 days of life was significant in the subgroup of infants with high 25(OH)D levels (≥ 29 ng/mL) at age 5 months, but not in those with low 25(OH)D levels (< 29 ng/mL), suggesting an interaction between the intervention and high 25(OH)D levels. In RCTs, vitamin D supplementation during pregnancy prevented the development of asthma or recurrent wheeze in those with initial 25(OH)D levels greater than 30 ng/mL¹⁹ but not in the total study population.²⁰⁻²²

Avoiding supplementation with CMF effectively reduced the risk of asthma or recurrent wheeze only in the subgroup of infants with the highest quartile levels of total IgE (ie, ≥ 50 IU/mL) at age 24 months and in the subgroups of those with positive mite-, milk-, and wheat-specific IgE. Because it has been suggested that infants with elevated serum IgE levels have a predisposition to asthma at age 6 years,²³ results of subgroup analyses implied that this intervention reduced the risk of allergic asthma²⁴ but not recurrent wheeze induced by viral infection.^{25,26}

Limitations

This trial has several limitations. First, only participants with atopic conditions at their second birthdays were followed to the maximum age of 6 years. Asthma or recurrent wheeze could have developed in participants whose follow-up ended at their second birthday, which would cause bias and be the most critical issue in this study. Thus, although this was a secondary analysis of an RCT, the conclusions of this study require further investigation. Second, the sample size was originally calculated for cow's milk-specific IgE at age 24 months. Thus, the sample size may be small for detecting risks of asthma or recurrent wheeze. In fact, the main result was marginally significant. Due to the small sample size and the weak difference, results obtained in this trial require further testing, especially from a biological plausibility or mechanistic level. Third, subgroup analyses were performed in this study, which may increase the probability of type I error due to multiple comparisons. Fourth, the present study was conducted in a single center in Japan, so that the results may not be relevant for other racial/ethnic groups and countries with different food cultures. Fifth, this trial was performed in the central area of Tokyo. Participants tended to be in a high socioeconomic class, and their children may have a high risk of atopic disease such as food allergy. Therefore, the results obtained in this trial may not be applicable to rural areas.

Conclusions

In this study, BF with or without EF for the first 3 days or more of life appeared to decrease the risk of asthma or recurrent wheeze in young children compared with BF plus a small amount of CMF from the first day of life. These results need to be further examined.

ARTICLE INFORMATION

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Corresponding Author: Mitsuyoshi Urashima, MD, PhD, MPH, Division of Molecular Epidemiology, Jikei University School of Medicine, Nishi-shimbashi 3-25-8, Minato-ku, Tokyo 105-8461, Japan (urashima@jikei.ac.jp).

Author Affiliations: Division of Molecular Epidemiology, Jikei University School of Medicine, Tokyo, Japan (Tachimoto, Mezawa, Okuyama, T. Urashima, Hirano, Gocho, M. Urashima); Department of Pediatrics, Jikei University School of Medicine, Tokyo, Japan (Imanari, Okuyama, M. Urashima).

Author Contributions: Dr Urashima had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Imanari and Tachimoto contributed equally to the study.

Concept and design: Tachimoto, Mezawa, T. Urashima, M. Urashima.

Acquisition, analysis, or interpretation of data: Tachimoto, Imanari, Okuyama, Hirano, Gocho, M. Urashima.

Drafting of the manuscript: Tachimoto, Okuyama, T. Urashima, M. Urashima.

Critical revision of the manuscript for important intellectual content: Imanari, Mezawa, Hirano, Gocho.

Statistical analysis: Tachimoto, Imanari, Mezawa, M. Urashima.

Obtained funding: Tachimoto, M. Urashima.

Administrative, technical, or material support: Imanari, Okuyama, Gocho.

Supervision: Okuyama, T. Urashima.

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SUPPLEMENT 1.

Trial Protocol and Statistical Analysis Plan

SUPPLEMENT 2.

eFigure 1. Histograms of 25(OH)D levels at 5 months and 24 months of age

eFigure 2. Comparison of 25(OH)D levels at 5 and 24 months of age among 4 groups

eTable 1. Interaction between tertiles of 25(OH)D levels and avoiding CMF at birth

eTable 2. Interactions between each allergen-specific IgE level at 5 months of age and the intervention

eTable 3. Incidence of asthma or recurrent wheeze by duration of adherence to the BF/EF intervention

SUPPLEMENT 3.

Data Sharing Statement

Trial Protocol and Statistical Analysis Plan (SAP)

Original Trial Protocol and Statistical Analysis Plan (SAP) in English p2 ~ p25

Final Trial Protocol and Statistical Analysis Plan (SAP) in English p26 ~ p50

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Summary of changes to the protocol approved by the ethics committee p59 ~ p61

***The statistical analysis plan is contained within the protocol files.**

Trial Protocol and Statistical Analysis Plan (SAP)
(original)

Title: Randomized clinical trial in preventing food allergy Breast feeding vs. breast feeding plus formula milk

ABC (Atopy prevention by Breastfeeding or Cow's milk formula)

29 **Steering committee**

30

31 **Lead principal investigator**

32 Mitsuyoshi Urashima MD, PhD, MPH

33 Professor of Molecular Epidemiology

34 The Jikei University School of Medicine

35 Tokyo, JAPAN

36

37 **Co-investigator**

38 Hiroshi Tachimoto MD, PhD

39 Associate Professor of Pediatrics

40 The Jikei University School of Medicine

41 Tokyo, JAPAN

42

43 **Endpoint committee members**

44 a. Hidetoshi Mezawa, M.D., Ph.D.,

45 b. Takashi Urashima, M.D., Ph.D.,

46 c. Daishi Hirano, M.D., Ph.D

47

48 Department of Pediatrics

49 The Jikei University School of Medicine

50 Tokyo, JAPAN

51

52 **Data monitoring**

53 a. Akio Nakashima, MD, PhD

54 b. Haruka Wada

55 Division of Molecular Epidemiology

56 The Jikei University School of Medicine

57 Tokyo, JAPAN

58

59 **Safety and data monitoring committee**

- 60 a. Motohiro Ebisawa, MD, PhD
- 61 b. Sakura Sato, MD, PhD
- 62 c. Kiyotake Ogura, MD, PhD
- 63 Department of Pediatrics
- 64 Sagamihara National Hospital
- 65 Kanagawa, JAPAN

66

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Protocol synopsis

Official title of the study

Randomized clinical trial in preventing food allergy Breast feeding vs. breast feeding plus formula milk

Brief title

Food allergy prevention trial

Region

Japan

Objectives

Compare atopic sensitization at 5-6 months and 2 years of age between breastfeeding with or without amino acid milk (=avoiding supplementation with cow's milk formula (CMF) for at least the first 3 days of life) and breastfeeding with CMF (=adhering to BF supplemented with CMF ≥ 5 ml/day from the first day of life to 1 month and ≥ 40 ml/day after 1 month until weaning) (Efficacy)

Primary outcomes

Atopic sensitization

Cow's milk sensitization, i.e., Cow's milk-specific IgE at 24 months of age, which will be compared with other allergens sensitization, e.g., egg-white.

Key secondary outcomes

I. Immediate type of food allergy (FA) including anaphylaxis

II. Worsening of atopic dermatitis

III. Desensitization of FA

IV. Atopic sensitization alone

V. Asthmatic bronchitis

VI. Allergic rhinitis

Study design

Basic design: Parallel

Randomization: YES

Randomization unit: Individual

Blinding: Prospective, randomized, open, blinded-endpoint (PROBE) design

Control: Active

Stratification: NO

Dynamic allocation: NO

Institution consideration: NOT considered as an adjustment factor

Intervention

No. of arms: 2

Purpose of intervention: Primary prevention of food allergy (FA)

Type of intervention: Food

1. **Intervention 1:** Breastfeeding (BF) with or without amino acid-based elemental formula (EF), called the BF±EF group (if the amount of breast milk is not sufficient, EF will be added), until 5 months of age. When the mother needs to add EF of more than 150 ml/day for 3 consecutive days to BF because of a low breast milk amount, EF is switched to cow's milk formula (CMF). Thus, the offspring allocated to BF±EF can avoid CMF for at least the first 3 days of life.

2. **Intervention 2:** BF plus CMF (≥ 5 ml/day from the first day of life to 1 month and ≥ 40 ml/day after 1 month until weaning), called the BF+CMF group, until 5 months of age or starting solid food.

Participants

Age: Neonates at birth

Gender: Male and female

Key inclusion criteria

1. Be in a high-risk group for atopic sensitization: at least one of the father, mother, brothers,

or sisters of infants has current and/or past atopic diseases (asthma, atopic dermatitis, food allergy, allergic rhinitis, hay fever)

2. Obtain written informed consent.

a. Participants who understand randomization into two groups: BF with or without amino acid milk and BF with cow's formula milk.

b. Blood examination at 5-6 months and 2 years of age is required.

3. Be available to visit the outpatient clinic at 1 month, 2 months, 3 months, 4 months, 5 months, 7~8 months, 9~10 months, 12 months, 14~15 months, 18 months, and 24 months of age.

Key exclusion criteria

1. Less than 36 weeks of gestational age at birth or birth weight less than 2000 g.

2. Having serious congenital anomalies.

3. Being a triplet or more; twins may participate.

4. Abnormal delivery such as asphyxia (Apgar score 6 or less)

Target sample size

Total, n = 300;

BF±EF group, n = 150;

BF+CMF group, n = 150.

Location of the trial

Tokyo, Japan

a. Patient care: Outpatient clinic of Jikei University Hospital

b. Data monitoring and analysis: Division of Molecular Epidemiology, Jikei University School of Medicine

Study period

Accrual, if the number of randomized participants reaches >300, then entry is stopped.

The trial ends when the last participant is followed up after the 2-year-old birthday.

Follow-up

Participating parent(s) are interviewed, and their children are examined from 1 to 24 months of age at the outpatient clinic of Jikei University Hospital. All infants undergo blood examinations to measure serum levels of total IgE and antigen-specific IgE at 5 and 24 months of age. Unscheduled visits and blood examinations are performed at the clinic when a participating infant has atopic symptoms, particularly when FA is suspected.

Statistical analysis

I. Primary outcome: Cow's milk-specific IgE

- A) Antigen-specific IgE ≥ 0.35 U_A/ml at 24 months of age as a dichotomous variable, risk ratio (RR) and 95% confidence intervals (95% CI)
- B) Serum levels of cow's milk-specific IgE as a continuous variable at 5 and 24 months of age: Mann-Whitney test between the comparative groups.

II. Secondary outcomes

Clinical food allergy: RR, and 95% CI are used to evaluate the secondary outcomes.

Ethics

1. Private information is carefully protected.
2. All personal information about the participants is rendered innominate in a linkable fashion at Jikei University Hospital.
3. Liability insurance is obtained to compensate participants for side effects of interventions.

Funding

The Jikei University School of Medicine

Background

The prevalence of food allergy (FA) has been growing over the last 25 years. Especially, the frequency of anaphylaxis, which may be fatal, has increased 5 times since 1990 in developed countries.¹⁻³ In 2000, the American Academy of Pediatrics (AAP) made the following recommendations: Infants at high risk for developing allergy, identified by a strong (one or both parents, sibling) family history of allergy, may benefit from the following: (1) Breastfeeding (BF) mothers should continue BF for the first year of life or longer. During this time, for infants at risk, hypoallergenic formulas can be used to supplement BF; (2) Mothers should eliminate peanuts and tree nuts (e.g., almonds, walnuts, etc.) and consider eliminating eggs, cow's milk, fish, and perhaps other foods from their diets while nursing; (3) Solid foods should not be introduced into the diet of high-risk infants until 6 months of age, with dairy products delayed until 1 year, eggs until 2 years, and peanuts, nuts, and fish until 3 years of age.⁴ However, in 2008, AAP revised the recommendations as follows: (1) For infants at high risk of developing atopic disease, there is evidence that exclusive BF for at least 4 months compared with feeding intact CMF decreases the cumulative incidence of atopic dermatitis and cow's milk allergy (CMA) in the first 2 years of life. Comparative studies of the various hydrolyzed formulas have also indicated that not all formulas have the same protective benefit. Extensively hydrolyzed formulas may be more effective than partially hydrolyzed formulas for the prevention of atopic disease; (2) At the present time, antigen avoidance during lactation does not prevent atopic disease, although more data are needed to substantiate this conclusion; (3) Although solid foods should not be introduced before 4 to 6 months of age, there is no current convincing evidence that delaying their introduction beyond this period has a significant protective effect on the development of atopic disease regardless of whether infants are fed cow's milk protein formula or human milk.⁵

Although WHO recommends BF for the first 6 months of life to improve childhood morbidity and mortality,⁶ supplementation of BF with cow's milk formula (CMF) is quite common in many countries including Japan. In contrast to WHO's recommendation, a population-based prospective cohort study in Israel demonstrated that the frequency of IgE-mediated CMA can be dramatically lowered if infants start receiving regular CMF within the first 14 days of

life (0.05%) compared with beginning CMF between 105 and 194 days (1.75%). They thus recommended CMF supplementation at birth to promote oral tolerance to cow's milk.⁷ Although such studies do not eliminate the possibility of reverse causality as an explanation for this finding (high-risk infants with eczema are deliberately breast-fed longer) due to the nature of observational studies, they raise the question as to whether exposure to CMF as a supplement to BF within 14 days of life may play a role in preventing allergic disease. Indeed, the German Infant Nutritional Intervention Study Group disagreed because they could not reproduce the risk reduction by early exposure to CMF in post-hoc analysis of their previous randomized controlled trial.⁸

Sometimes during the first 3 days of life, the amounts of breast milk are small or postpartum mothers are too tired to breast feed. Consequently, newborn infants are quite often supplied CMF in addition to BF during hospital admission. Indeed, many Japanese maternity wards basically recommend BF, but allow mothers or nurses to help by supplying CMF as a supplement to BF after feeding colostrum, e.g., 6 to 10 hours after birth. In addition to the controversy regarding CMF supplementation before or after day 14, whether supplementary feeding with CMF during the first 3 days of life is protective,⁹ neutral,¹⁰ or rather enhances¹¹ risks of atopic diseases is also controversial, as shown in randomized controlled trials. In 1988, a cohort study in Denmark showed that all 39 CMAs developed in 1,539 infants who had been supplied CMF during the first 3 days of life, whether breast fed or not, after discharge from the maternity hospital, whereas no CMAs were observed in 210 infants who had not been supplied CMF.¹² In the discussion of the cohort study in Israel, the authors stated: "Because of the way our data were collected, we cannot exclude neonatal exposure to small quantities of CMP formula in the newborn nursery either forgotten by the mother or done without her knowledge. However, the role of a brief intermittent early exposure to milk in the neonatal unit is controversial^{10,11} and might have a low effect, if any, on the development of atopy. Accordingly, we found it appropriate not to consider such intermittent exposures to CMP in this study".

Hypotheses

1. Does avoiding exposure to CMF for the first 3 days of life prevent development of FA later?

This is the main hypothesis of this study and was explained in the 'Background'.

Many Japanese maternity wards encourage BF, but allow mothers or nurses to supplement BF with CMF, e.g., approximately 6 to 10 hours after birth or even earlier, based on maternal preferences, but not based on 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 may be associated with the recent increase in children with FA. Therefore, a randomized clinical trial (RCT) was conducted to assess whether the risk of CMF sensitization and FA is decreased by either avoiding or supplementing CMF at birth.

2. Vitamin D hypotheses

Refer to the review article by Dr. G. Lack.¹³

The vitamin D hypothesis takes two forms: the vitamin D excess hypothesis argues that increases in vitamin D levels have led to increased allergies. The vitamin D deficiency hypothesis argues the opposite.

Objectives

Specific Aim 1: To compare atopic sensitization at 5-6 months and 2 years of age between BF with or without amino acid milk and BF with CMF.

Specific Aim 2: Subgroup analysis based on serum levels of 25-hydroxyvitamin D (25OHD) at 5 months, e.g., tertile, may be performed.

Study design

Randomized, controlled, parallel two-group trial at a single institution

Pregnant women during the third trimester are randomly assigned to either BF with or without amino acid-based EF, called the BF±EF group, or BF plus CMF (≥5 ml/day from the first day of life to 1 month and ≥40 ml/day after 1 month until weaning), called the BF+CMF group, in a 1:1 ratio. Infants are followed until they are at least 2 years of age. Follow-up is extended 1 year if participating infants have FA. When the mother needs to add more than 150 ml/day EF for 3 consecutive days to BF because of a low amount of breast milk, EF is switched to CMF. Thus, the offspring allocated to BF±EF can avoid CMF for at least the first 3 days of life.

Subgroup

1. Tertiles of 25(OH)D levels at 5 months of age

Measure 25OHD levels in the serum obtained at 5 months of age.

Research Implementation System

I. Clinical examination

a. Pediatrician: Dr. Mitsuyoshi Urashima: Enrollment and participants' clinical examination before allergic signs. If participating infants have any allergic signs, i.e., food-specific IgE ≥0.35 U_A/ml blood sampling at 5 months of age, then they are referred to Dr. Tachimoto to diagnose atopic diseases and for follow-up.

At the outpatient clinic of The Maternal and Child Center in Jikei University Hospital

b. Pediatrician: Dr. Hiroshi Tachimoto: Clinical examination after allergic signs and diagnosis of atopic diseases with or without the oral food challenge (OFC) test, management, and follow-up.

At the outpatient clinic of Pediatrics in Jikei University Hospital

II. Endpoint committee

Among the following five members who do not know the allocation group and will not examine these participating infants at outpatient clinics, if ≥ 2 recognize the outcome as positive, the outcomes are finally determined to be positive.

1. Hidetoshi Mezawa, M.D., Ph.D.
2. Takashi Urashima, M.D., Ph. D.
3. Daishi Hirano, M.D., Ph.D.

Department of Pediatrics
The Jikei University School of Medicine
Tokyo, Japan

What the committee is expected to determine.

These five members determine whether the infant has had an episode of FA or not.

0. No FA

1. Immediate allergic reaction
2. Anaphylaxis

For 1 (and 2) above, they determine the status at the second birthday by reviewing detailed episodes, results of the OFC test, and levels of food-specific IgE.

1. Sustained unresponsiveness = outgrow
Cow's milk allergy, the infant can drink raw milk ≥ 50 ml
Egg allergy, the infant can eat baked egg ≥ 30 g,
Wheat allergy, the infant can eat udon noodle ≥ 15 g
2. Probable unresponsiveness, e.g., in the process of increasing dose of allergenic food, but not yet reach to predefined dose of unresponsiveness mentioned above.
3. Not yet unresponsive, when the infant eats, he or she still reacts to the allergenic food.
4. Not sure because the infant has not consumed the food allergen

The infants are still state of either 2, 3, or 4 above, they are considered not yet outgrow = remain food allergy.

III. Data monitoring

A) Randomization using a block of four by Dr. Akio Nakashima who does not interview participating families and does not examine their infants.

B) Data monitoring

If the frequency of adverse events deviates in either intervention group, the committee reports the data to the Safety and Data Monitoring Committee as well as the lead investigator.

a. Akio Nakashima, M.D., Ph.D. (physician and nephrologist)

b. Ms. Haruka Wada (secretary at Molecular Epidemiology)

Division of Molecular Epidemiology

The Jikei University School of Medicine

Tokyo, Japan

IV. Safety and Data Monitoring Committee

a. Judging from data of interim analysis, this committee may recommend stopping or continuing the trial.

b. Responding to a report of adverse events, the committee may recommend stopping the trial or allowing the trial to continue.

① Motohiro Ebisawa, M.D., Ph.D.

② Sakura Sato, M.D., Ph.D.

③ Kiyotake Ogura, M.D., Ph.D.

Department of Pediatrics

Sagamihara National Hospital

Kanagawa, Japan

Participants

Inclusion criteria

1. Be in a high-risk group for atopic sensitization: at least one of the father, mother,

brothers, or sisters of infants has atopic diseases (asthma, atopic dermatitis, FA, allergic rhinitis, hay fever)

2. Obtained written informed consent.

a. Participants who understand randomization into two groups: BF with or without amino acid milk and BF with CMF.

b. Blood examinations at 5-6 months and 2 years of age are required.

3. Be available to visit the outpatient clinic at 1 month, 2 months, 3 months, 4 months, 5 months, 7~8 months, 9~10 months, 12 months, 14~15 months, 18 months, and 24 months of age.

Exclusion criteria

1. Less than 36 weeks of gestational age at birth or birth weight less than 2000 g.

2. Having serious congenital anomalies.

3. Being a triplet or more: twins may participate.

4. Abnormal delivery such as asphyxia (Apgar score 6 or less at 5 minutes after birth)

Interventions

1. BF±EF

Mothers in this group are asked to maintain exclusive BF. However, if the amount of breast milk is not sufficient, then EF may be added until starting solid food. If the mother needs to supply EF more than 150 ml/day for 3 consecutive days in addition to BF because of a low amount of breast milk, EF is switched to CMF. Thus, the offspring allocated to BF±EF can avoid CMF for at least the first 3 days of life.

2. BF+CMF

Mothers in this group are asked to maintain exclusive BF, but are also asked to simultaneously supply a small amount of CMF ≥ 5 ml/day from the first day of life until 1 month, and ≥ 40 ml/day after 1 month until weaning. When infants are born after 18:00, CMF may be added on the 2nd day.

Both CMF (Hohoemi[®]) and EF (Meiji Elemental formula[®]) are purchased from Meiji Holdings Co. Ltd. (Chuo-ku, Tokyo, Japan).

EF (Meiji Elemental formula®)

For infants with FA, total proteins are replaced with refined amino acids. However, other fats, carbohydrates, vitamins, and minerals are basically the same as in CMF. Energy is 66 kcal/100 ml (17%) and 391 kcal/100 g powder.

Blinding to allocation group

- a. Participating family: Not blinded
- b. Dr. M.U.: Not blinded, because he needs to confirm compliance with the intervention by interviewing participating families at the outpatient clinic in the Maternal and Child Center of Jikei University Hospital. When an infant has food-specific IgE ≥ 0.10 U_A/ml at 5 months blood test, the infant is referred to Dr. H.T. at the outpatient clinic of Pediatrics without informing Dr. H.T. about the allocation group.
- c. Dr. H.T.: Blinded, who is a specialist in pediatric allergic diseases, diagnoses FA, without knowing the allocation group.
- d. Endpoint committee: Blinded and no contact with the participants.

Outcome Measures

Primary outcome

1. Atopic sensitization:

The primary outcome was atopic sensitization to cow's milk protein, i.e., serum levels of cow's milk-specific IgE (CM-IgE) ≥ 0.35 UA/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 primary outcome. Other atopic sensitization to egg white and wheat ≥ 0.35 UA/ml at 24 months of age were also evaluated to be compared with cow's milk sensitization. Outsourced for measuring with ImmunoCAP Specific IgE® (Thermo Fisher Diagnostics K.K., Minato-ku, Tokyo, Japan) to SRL Inc. and compared as both continuous and dichotomous (CM-IgE: ≥ 0.35 UA/ml) variable between BF±EF and BF+CMF group.

Secondary outcomes

1. Immediate type of FA

Presenting with one organ symptom of skin (urticaria, angioedema, erythema, pruritus, periorbital edema, etc.), respiratory (dry staccato cough, hoarseness, wheezing, etc.), gastrointestinal (nausea, vomiting, colicky abdominal pain, etc.), or circulatory (tachycardia, hypotension, loss of consciousness, etc.) problems triggered by the food ingestion that appeared within a few hours, in combination with blood examination measuring serum levels of the suspected food-specific IgE: ≥ 0.35 U_A/ml by means of a fluorescence enzyme immunoassay.

In this trial, symptoms triggered by skin contact directly with food or indirectly with food-contaminated hands are not included.

The OFC test (Appendix 1) is performed around 1 year of age for infants who are sensitized to certain foods but for whom clinical reactivity after ingestion of the food allergen is unknown. Thus, immediate allergic reaction is a food-specific IgE: ≥ 0.35 U_A/ml plus either a FA episode or positive OFC test. FA is re-evaluated regarding whether the infant could eat the causative food allergen without an allergic reaction at the 2-year-old birthday.

2. Anaphylactic type of FA

When symptoms are derived from multiple organs, e.g., urticaria and wheezing, the condition is defined as an anaphylactic reaction.

3. Worsening of atopic dermatitis

Check the SCORAD index¹⁴ at the 1- and 3-month check-ups.

4. Desensitization of food allergy (= unresponsiveness)

If infants, who had been once diagnosed as immediate type of FA especially with cow's milk allergy, egg allergy, and wheat allergy, can eat the allergenic food (raw milk ≥ 50 ml, baked egg 30g, udon noodle 15g) without allergic signs by 2nd birthday, then they are defined as 'sustained unresponsiveness = outgrow' to the food

allergen in this trial.

5. Atopic sensitization alone

Although serum levels of food-specific IgE are positive (≥ 0.35 U_A/ml), the infant never had allergic signs and symptoms even by eating the allergenic food.

6. Asthmatic bronchitis

A stringent index that includes frequent wheezing during the first 3 years of life and either one major risk factor (parental history of asthma or eczema) or two of three minor risk factors (eosinophilia, wheezing without colds, and allergic rhinitis).¹⁵

Major Criteria

1. Parental asthma†
2. Eczema diagnosed by a medical doctor‡
3. Sensitization with elevated mite- or house dust-specific IgE ≥ 0.10 U_A/ml

Minor Criteria

1. Allergic rhinitis diagnosed by a medical doctor§
2. Wheezing apart from colds
3. Eosinophilia ($\geq 4\%$)

7. Allergic rhinitis

- a. Hay fever, e.g., Sugi pollen allergy: sugi pollen-specific IgE ≥ 0.10 U_A/ml
- b. Allergic rhinitis: Rhinitis signs and symptoms for 1 month and mite- or house dust-specific IgE ≥ 0.10 U_A/ml

Safety outcomes

The following are obtained from an interview at the outpatient clinic just after the 2-year-old birthday.

1. Admission for any reason
2. Emergency room visit for any reason
3. Growth retardation: Height and weight at 2-year-old birthday

Flow of participants

1. Informed consent and registration

- a. Pregnant women and their husbands are informed about the trial in detail at a childbirth class during late pregnancy through a talk by Dr. M. Urashima and the informed consent document. Written, informed consent is sent to the data monitoring center at the Molecular Epidemiology Division of Jikei University School of Medicine.
- b. Current or previous family history of atopic diseases (asthma, atopic dermatitis, FA, allergic rhinitis, hay fever) is obtained through questionnaires as well as parental demographic information such as maternal age and body weight/height. Only pregnant women who are confirmed to have a family history of atopic diseases are included.
- c. Food questionnaires of mothers (Appendix 3) are taken during the third trimester of pregnancy and at 1, 3, and 5 months of age after birth.

2. At the maternity ward of Jikei University Hospital

- a. Some included pregnant women are excluded after delivery, due to, e.g., cleft palate.
- b. Perinatal information, e.g., gestational weeks and birth weight, are taken from charts.
- c. Nursing staff is informed of the participants' names and to which group they are allocated.
- d. Mothers are informed to which group they belong after delivery; thus, mothers are not blinded to the allocation group.
- e. Mothers belonging to the BF+CMF group are asked to supplement BF with CMF at least 5 ml from the first day of delivery. However, if the delivery time is after 18:00, they are allowed to supply CMF in addition to BF starting at midnight of the 2nd day.
- f. Mothers belonging to the BF±EF group may add amino acid-based EF when they feel that BF is not enough. If the mother adds more than 150 ml/day EF to BF for 3 consecutive days, EF is switched to CMF. Thus, offspring allocated to BF±EF can avoid CMF for at least the first 3 days of life.

- g. Nurses at the maternity ward are allowed to feed babies instead of their mothers.

3. At the outpatient clinic in the Maternal and Child Center of Jikei University Hospital

- a. Participating parent(s) are interviewed, and their children are examined at least seven times from 1, (2,) 3, (4,) 5-6, (7-8,) 9-10, 12, (14-15,) 18, and 24 months of age at the outpatient clinic of Jikei University Hospital by a study pediatrician (M.U.) who is not blinded to the allocation group.
- b. If infants have exanthema, the pediatrician provides their parents clear guidance about aggressive skin care such as how to wash their face and body using soap and a shower by showing them a video.
- c. Blood is sampled from all infants at 5-6 and 24 months.

4. At the outpatient clinic in Pediatrics of Jikei University Hospital

In case of at least one antigen-specific IgE is 0.10 IU_A/ml or more, the infant is referred to a study specialist of pediatric allergy (H.T.) at the same Jikei University Hospital who does not know the infant's allocation group.

5. Pediatrics Ward of Jikei University Hospital

The OFC test is performed at admission (Appendix 1) by pediatricians who are blinded to the allocation group.

6. Efforts to reduce loss to follow-up

- a. Using email, we try to contact participants who move to distant places regarding whether their infants have FA and make efforts to reduce the number of infants lost to follow-up, from whom IgE data are not obtained at 5 or 24 months.
- b. If participants do not visit the outpatient clinic after their 2-year-old birthday and we receive no responses to the email, they are considered lost to follow-up.

7. Blood sampling

All infants undergo blood examinations to measure serum total and antigen-specific IgE.

- a. At the 5- to 6-month check-up prior to first solid food introduction:
Egg white, cow's milk, wheat, mites.
- b. At 2 years of age:
Egg white, cow's milk, wheat, mites, Japanese cedar pollen, dog dandruff, cat dandruff.
- c. Unscheduled visits are made to the clinic when participating infants have atopic symptoms, particularly suspicious of FA.
e.g., exanthema just after eating an egg dish.

8. Randomization

a. EF and CMF

Both CMF (Hohoemi[®]) and EF (Meiji Elemental formula[®]) are purchased from Meiji Holdings Co. Ltd. (Chuo-ku, Tokyo, Japan).

b. Randomization

Pregnant women that meet the inclusion criteria are randomly assigned to either the BF+CMF or BF±EF group according to an identification number generated by computer based on permuted blocks of four that are determined beforehand (Appendix 2). Staff members at the maternity ward are informed of which intervention applies to each pregnant participant before hospitalization for childbirth.

c. Blinding

According to the nature of the intervention, we do not use double blinding. However, we try to minimize bias using the following strategies:

Primary outcome: Cow's milk-specific IgE as an objective indicator.

Secondary outcomes: determine by prospective, randomized, open, blinded-endpoint (PROBE), determined by the endpoint committee members who do not examine the infants or are blinded to the allocation group'.

9. Compliance with CMF or EF

1. At the maternity ward, every day's CMF or EF intake is monitored by each

596 participant (mother). The nurse may help with the intervention and monitoring.
597 2. At the outpatient clinic, mothers are asked whether they are using CMF, EF, or
598 exclusive BF.

599

600 **10. Monitoring of secondary outcomes**

601 **a. Scheduled**

602 At either the Maternal and Child Center or Pediatrics of Jikei University Hospital,
603 participating infants are examined frequently.

604

605 **b. Unscheduled**

606 If the infants have any allergic signs, e.g., urticaria after eating allergenic food, they
607 are advised to call Jikei University Hospital. H.T. or other pediatricians try to
608 examine the infants on the day or next day and perform a blood examination.

609

610 **Statistical analysis**

611

612 **1. Sample size**

613 300

614 **2. Sample size calculation**

615 300 pregnant women and their offspring

616 We hypothesized that cow's milk sensitization at 2 years of age, i.e., cow's milk-specific IgE
617 ≥ 0.35 U_A/ml, would be present in 10% of one group of infants and 25% of the other group,
618 with a type I error (two-sided) of 5% and a power of 90%, on the assumption of 3% loss to
619 follow-up. Therefore, 1:1 divided samples of approximately 300 participants were calculated
620 as sufficient to detect a significant difference.

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623

624 Estimated sample size for two-sample comparison of proportions

625

626 Test Ho: $p_1 = p_2$, where p_1 is the proportion in population 1

627 and p2 is the proportion in population 2

628 Assumptions:

629

630 alpha = 0.0500 (two-sided)

631 power = 0.9000

632 p1 = 0.1000

633 p2 = 0.2500

634 n2/n1 = 1.00

635

636 Estimated required sample sizes:

637

638 n1 = 146

639 n2 = 146

640 n = 292

641 Assuming 3% loss to follow-up, 300 are needed to enroll.

642

643 **3. Study period**

644 We assume that 100 pregnant women per year will participate in this trial. Therefore, the
645 accrual period will be 3 years. The last infant is followed up 2 years later. Thus, a total of
646 5 years is predicted to be needed to accomplish this trial.

647

648 **4. Interim analysis**

649 Interim analyses are planned two times at reaching 100 and 200 participating infants
650 who had their second birthday. The P value for significance at the interim analysis is
651 set as <0.001 according to the Peto's stopping boundaries.

652

653 **5. Planned methods of analysis**

654 Outcomes are analyzed by intention-to-treat analyses between the two comparative
655 groups.

656 a. Serum levels of cow's milk-specific IgE at 5 months and 24 months of age as a
657 continuous variable are compared using Mann-Whitney test.

- b. Risk of cow's milk-specific IgE ≥ 0.35 U_A/ml will be analyzed with a 2x2 table with the chi-square test and represented by the risk ratio (RR), risk difference (RD) and 95% confidence interval (95% CI).
- c. Secondary outcomes will be compared with RR, RD, and 95% CI.
- d. Safety outcomes will be compared with RR, RD and 95% CI or Student's t test.
- e. Subgroup analysis will be done according to levels of 25(OH)D.
- f. All reported P values will be two-sided.
- g. Values with $P < 0.05$ will be considered statistically significant.
- h. All data will be statistically analyzed using Stata 14.0 (StataCorp LP., College Station, TX, USA).

6. Subgroup analyses

To clarify whether BF $\pm$ EF or BF+CMF significantly affects the subgroups of 25(OH)D (tertile), P for interaction ($P_{\text{interaction}}$) is computed by creating multiplicative variables. The results of these analyses are not corrected for multiple comparisons.

Safety

Many Japanese maternity wards recommend BF, but allow mothers or nurses to help by supplying CMF to supplement BF after feeding colostrum, e.g., 6 to 10 hours after birth. This is a food intervention trial. Thus, basically no safety concerns exist. However, the following are compared.

- a. Admission due to any disease
- b. Emergency visit or admission for any reason
- c. Body height and weight at 24 months of age

Early withdrawal

Unknown serious adverse events.

Compensation

Mitsui Sumitomo Insurance Fire provides liability insurance (NB41027726) for the principal investigator, to compensate for costs of treating disability as a result of side

effects caused by consuming milk.

The maximum amount of compensation is 100,000,000 yen per person and 300,000,000 yen per trial.

Consideration concerning the protection of human rights and privacy

1. Written informed consent is obtained after sufficient explanation.
2. Participants can withdraw from the trial after providing written, informed consent.
3. Withdrawal is not considered a disadvantage for participants.
4. Private information is exchanged with study ID at the Jikei University School of Medicine; therefore, private information cannot leak from there (linkable anonymizing).

Publication of research results

We aim to publish the results of this trial in a scientific journal or present them at academic conferences. Personal information will be carefully concealed when we present the results.

Research funds

- 1) The Jikei University School of Medicine

Attribution of intellectual property rights

If intellectual property rights such as patent rights become relevant, such rights will be attributable to the investigator.

712

2017.12.25

713

714 **Trial Protocol and Statistical Analysis Plan (SAP)**
715 **(final)**

716

717

718 **Title: Randomized clinical trial in preventing food allergy Breast**
719 **feeding vs. breast feeding plus formula milk**

720

721

722 **ABC (Atopy prevention by Breastfeeding or Cow's milk formula)**

723

724 **Steering committee**

725

726 **Lead principal investigator**

727 Mitsuyoshi Urashima MD, PhD, MPH

728 Professor of Molecular Epidemiology

729 The Jikei University School of Medicine

730 Tokyo, JAPAN

731

732 **Co-investigator**

733 Hiroshi Tachimoto MD, PhD

734 Associate Professor of Pediatrics

735 The Jikei University School of Medicine

736 Tokyo, JAPAN

737

738 **Endpoint committee members**

739 a. Hidetoshi Mezawa, M.D., Ph.D.,

740 b. Takashi Urashima, M.D

741 c. Daishi Hirano, M.D., Ph.D

742 d. Mai Okuyama, M.D

743 e. Noriko Gocho, M.D.

744

745 Department of Pediatrics

746 The Jikei University School of Medicine

747 Tokyo, JAPAN

748

749 **Data monitoring**

750 a. Akio Nakashima, MD, PhD

751 b. Haruka Wada

752 Division of Molecular Epidemiology

753 The Jikei University School of Medicine

754 Tokyo, JAPAN

755

756 **Safety and data monitoring committee**

757 a. Motohiro Ebisawa, MD, PhD

758 b. Sakura Sato, MD, PhD

759 c. Kiyotake Ogura, MD, PhD

760 Department of Pediatrics

761 Sagamihara National Hospital

762 Kanagawa, JAPAN

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766 Ver. 3. 2017.12.25

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Protocol synopsis

Official title of the study

Randomized clinical trial in preventing food allergy Breast feeding vs. breast feeding plus formula milk

Brief title

Food allergy prevention trial

Region

Japan

Objectives

Compare atopic sensitization at 5-6 months and 2 years of age between breastfeeding with or without amino acid milk (=avoiding supplementation with cow's milk formula (CMF) for at least the first 3 days of life) and breastfeeding with CMF (=adhering to BF supplemented with CMF ≥ 5 ml/day from the first day of life to 1 month and ≥ 40 ml/day after 1 month until weaning) (Efficacy)

Primary outcomes

Atopic sensitization

Cow's milk sensitization, i.e., Cow's milk-specific IgE at 24 months of age, which will be compared with other allergens sensitization, e.g., egg-white.

Secondary outcomes

I. Immediate type of food allergy (FA) including anaphylaxis

II. Desensitization of FA (= sustained unresponsiveness, or outgrow)

Exploratory outcomes

I. Atopic dermatitis

II. Asthmatic bronchitis

III. Sensitization to Japanese cedar pollen

IV. Allergic rhinitis

Study design

Basic design: Parallel

Randomization: YES

Randomization unit: Individual

Blinding: Prospective, randomized, open, blinded-endpoint (PROBE) design

Control: Active

Stratification: NO

Dynamic allocation: NO

Institution consideration: NOT considered as an adjustment factor

Intervention

No. of arms: 2

Purpose of intervention: Primary prevention of FA

Type of intervention: Food

1. **Intervention 1:** Breastfeeding (BF) with or without amino acid-based elemental formula (EF), called the BF±EF group (if the amount of breast milk is not sufficient, EF will be added), until starting solid food. When the mother needs to add EF of more than 150 ml/day for 3 consecutive days to BF because of a low breast milk amount, EF is switched to cow's milk formula (CMF). Thus, the offspring allocated to BF±EF can avoid CMF for at least the first 3 days of life.

3. **Intervention 2:** BF plus CMF (≥5 ml/day from the first day of life to 1 month and ≥40 ml/day after 1 month until weaning), called the BF+CMF group, until 5 months of age or starting solid food.

Participants

Age: Neonates at birth

Gender: Male and female

Key inclusion criteria

1. Be in a high-risk group for atopic sensitization: at least one of the father, mother, brothers, or sisters of infants has current and/or past atopic diseases (asthma, atopic dermatitis, food allergy, allergic rhinitis, hay fever)
2. Obtain written informed consent.
 - a. Participants who understand randomization into two groups: BF with or without amino acid milk and BF with cow's formula milk.
 - b. Blood examination at 5-6 months and 2 years of age is required.
3. Be available to visit the outpatient clinic at 1 month, 2 months, 3 months, 4 months, 5 months, 7~8 months, 9~10 months, 12 months, 14~15 months, 18 months, and 24 months of age.

Key exclusion criteria

1. Less than 36 weeks of gestational age at birth or birth weight less than 2000 g.
2. Having serious congenital anomalies.
3. Being a triplet or more; twins may participate.
4. Abnormal delivery such as asphyxia (Apgar score 6 or less)

Target sample size

- Total, n = 300;
BF±EF group, n = 150;
BF+CMF group, n = 150.

Location of the trial

- Tokyo, Japan
- a. Patient care: Outpatient clinic of Jikei University Hospital
 - b. Data monitoring and analysis: Division of Molecular Epidemiology, Jikei University School of Medicine

Study period

Accrual, if the number of randomized participants reaches >300, then entry is stopped.

The trial ends when the last participant is followed up after the 2-year-old birthday.

Follow-up

Participating parent(s) are interviewed, and their children are examined from 1 to 24 months of age at the outpatient clinic of Jikei University Hospital. All infants undergo blood examinations to measure serum levels of total IgE and antigen-specific IgE at 5 and 24 months of age. Unscheduled visits and blood examinations are performed at the clinic when a participating infant has atopic symptoms, particularly when FA is suspected.

Statistical analysis

I. Primary outcome: Cow's milk-specific IgE

A) Antigen-specific IgE ≥ 0.35 U_A/ml at 24 months of age as a dichotomous variable, risk ratio (RR) and 95% confidence intervals (95% CI)

B) Serum levels of cow's milk-specific IgE as a continuous variable at 5 and 24 months of age: Mann-Whitney test between the comparative groups.

II. Secondary outcomes

Clinical food allergy: RR, and 95% CI are used to evaluate the secondary outcomes.

Ethics

1. Private information is carefully protected.
2. All personal information about the participants is rendered innominate in a linkable fashion at Jikei University Hospital.
3. Liability insurance is obtained to compensate participants for side effects of interventions.

Funding

1. JSPS KAKENHI Grant Number JP16K09074
2. The Practical Research Project for Allergic Disease and Immunology of the Japan Agency for Medical Research and Development, AMED (15ek0410019h0101)

895 3. Dairy Products Health Science Council and Japan Dairy Association
896

Background

The prevalence of food allergy (FA) has been growing over the last 25 years. Especially, the frequency of anaphylaxis, which may be fatal, has increased 5 times since 1990 in developed countries.¹⁻³ In 2000, the American Academy of Pediatrics (AAP) made the following recommendations: Infants at high risk for developing allergy, identified by a strong (one or both parents, sibling) family history of allergy, may benefit from the following: (1) Breastfeeding (BF) mothers should continue BF for the first year of life or longer. During this time, for infants at risk, hypoallergenic formulas can be used to supplement BF; (2) Mothers should eliminate peanuts and tree nuts (e.g., almonds, walnuts, etc.) and consider eliminating eggs, cow's milk, fish, and perhaps other foods from their diets while nursing; (3) Solid foods should not be introduced into the diet of high-risk infants until 6 months of age, with dairy products delayed until 1 year, eggs until 2 years, and peanuts, nuts, and fish until 3 years of age.⁴ However, in 2008, AAP revised the recommendations as follows: (1) For infants at high risk of developing atopic disease, there is evidence that exclusive BF for at least 4 months compared with feeding intact CMF decreases the cumulative incidence of atopic dermatitis and cow's milk allergy (CMA) in the first 2 years of life. Comparative studies of the various hydrolyzed formulas have also indicated that not all formulas have the same protective benefit. Extensively hydrolyzed formulas may be more effective than partially hydrolyzed formulas for the prevention of atopic disease; (2) At the present time, antigen avoidance during lactation does not prevent atopic disease, although more data are needed to substantiate this conclusion; (3) Although solid foods should not be introduced before 4 to 6 months of age, there is no current convincing evidence that delaying their introduction beyond this period has a significant protective effect on the development of atopic disease regardless of whether infants are fed cow's milk protein formula or human milk.⁵

Although WHO recommends BF for the first 6 months of life to improve childhood morbidity and mortality,⁶ supplementation of BF with cow's milk formula (CMF) is quite common in many countries including Japan. In contrast to WHO's recommendation, a population-based prospective cohort study in Israel demonstrated that the frequency of IgE-mediated CMA can be dramatically lowered if infants start receiving regular CMF within the first 14 days of

life (0.05%) compared with beginning CMF between 105 and 194 days (1.75%). They thus recommended CMF supplementation at birth to promote oral tolerance to cow's milk.⁷ Although such studies do not eliminate the possibility of reverse causality as an explanation for this finding (high-risk infants with eczema are deliberately breast-fed longer) due to the nature of observational studies, they raise the question as to whether exposure to CMF as a supplement to BF within 14 days of life may play a role in preventing allergic disease. Indeed, the German Infant Nutritional Intervention Study Group disagreed because they could not reproduce the risk reduction by early exposure to CMF in post-hoc analysis of their previous randomized controlled trial.⁸

Sometimes during the first 3 days of life, the amounts of breast milk are small or postpartum mothers are too tired to breast feed. Consequently, newborn infants are quite often supplied CMF in addition to BF during hospital admission. Indeed, many Japanese maternity wards basically recommend BF, but allow mothers or nurses to help by supplying CMF as a supplement to BF after feeding colostrum, e.g., 6 to 10 hours after birth. In addition to the controversy regarding CMF supplementation before or after day 14, whether supplementary feeding with CMF during the first 3 days of life is protective,⁹ neutral,¹⁰ or rather enhances¹¹ risks of atopic diseases is also controversial, as shown in randomized controlled trials. In 1988, a cohort study in Denmark showed that all 39 CMAs developed in 1,539 infants who had been supplied CMF during the first 3 days of life, whether breast fed or not, after discharge from the maternity hospital, whereas no CMAs were observed in 210 infants who had not been supplied CMF.¹² In the discussion of the cohort study in Israel, the authors stated: "Because of the way our data were collected, we cannot exclude neonatal exposure to small quantities of CMP formula in the newborn nursery either forgotten by the mother or done without her knowledge. However, the role of a brief intermittent early exposure to milk in the neonatal unit is controversial^{10,11} and might have a low effect, if any, on the development of atopy. Accordingly, we found it appropriate not to consider such intermittent exposures to CMP in this study".

Hypotheses

1. Does avoiding exposure to CMF for the first 3 days of life prevent development of FA later?

This is the main hypothesis of this study and was explained in the 'Background'.

Many Japanese maternity wards encourage BF, but allow mothers or nurses to supplement BF with CMF, e.g., approximately 6 to 10 hours after birth or even earlier, based on maternal preferences, but not based on 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 may be associated with the recent increase in children with FA. Therefore, a randomized clinical trial (RCT) was conducted to assess whether the risk of CMF sensitization and FA is decreased by either avoiding or supplementing CMF at birth.

3. Vitamin D hypotheses

Refer to the review article by Dr. G. Lack.¹³

The vitamin D hypothesis takes two forms: the vitamin D excess hypothesis argues that increases in vitamin D levels have led to increased allergies. The vitamin D deficiency hypothesis argues the opposite.

4. Dietary fat hypothesis

Refer to the review article by Dr. G. Lack.¹³

The dietary fat hypothesis argues that reduction in consumption of animal fats and a corresponding increase in the use of margarine and vegetable oils have led to the increase in allergies. The argument is that there has been an increase in the consumption of ω -6 polyunsaturated fatty acids, such as linoleic acid, and similarly that through reduced consumption of oily fish, there has been a reduction in ω -3

polyunsaturated fatty acids, such as eicosapentaenoic acid. ω -6 fatty acids lead to the production of prostaglandin E2 (PGE2), whereas ω -3 fatty acids inhibit synthesis of PGE2. PGE2 reduces interferon- γ production by T lymphocytes, thus resulting in increased IgE production by B lymphocytes. This has been proposed to explain the increase in the prevalence of asthma, eczema, and allergic rhinitis.

Objectives

Specific Aim 1: To compare atopic sensitization at 5-6 months and 2 years of age between BF with or without amino acid milk and BF with CMF.

Specific Aim 2: Subgroup analysis based on tertiles of serum levels of 25-hydroxyvitamin D (25OHD) at 5 months will be performed.

Exploratory Aim 1: To determine the relationships between serum levels of free fatty acids and FA and other atopic diseases.

Study design

Randomized, controlled, parallel two-group trial at a single institution

Pregnant women during the third trimester are randomly assigned to either BF with or without amino acid-based EF, called the BF $\pm$ EF group, or BF plus CMF ($\geq$ 5 ml/day from the first day of life to 1 month and $\geq$ 40 ml/day after 1 month until weaning), called the BF+CMF group, in a 1:1 ratio. Infants are followed until they are at least 2 years of age. Follow-up is extended 1 year if participating infants have FA. When the mother needs to add more than 150 ml/day EF for 3 consecutive days to BF because of a low amount of breast milk, EF is switched to CMF. Thus, the offspring allocated to BF $\pm$ EF can avoid CMF for at least the first 3 days of life.

Subgroup

1. Tertiles of 25(OH)D levels at 5 months of age

Measure 25OHD levels in the serum obtained at 5 months of age.

Exploratory variables

1. Fatty acid levels in the serum, which are planned to report as another clinical study.

Measure fatty acid levels in the serum obtained at 5 months of age.

Twenty-four kinds of fatty acids such as arachidonic acid will be measured by high-performance liquid chromatography, which may be outsourced to SRL, using residual serum samples obtained at 5 months of age.

Research Implementation System

I. Clinical examination

a. Pediatrician: Dr. Mitsuyoshi Urashima: Enrollment and participants' clinical examination before allergic signs. If participating infants have any allergic signs, e.g., food-specific IgE ≥ 0.35 UA/ml blood sampling at 5 months of age, then they are referred to Dr. Tachimoto to diagnose atopic diseases and for follow-up.

At the outpatient clinic of The Maternal and Child Center in Jikei University Hospital

b. Pediatrician: Dr. Hiroshi Tachimoto: Clinical examination after allergic signs and diagnosis of atopic diseases with or without the oral food challenge (OFC) test, management, and follow-up.

At the outpatient clinic of Pediatrics in Jikei University Hospital

II. Endpoint committee

Among the following five members who do not know the allocation group and will not examine these participating infants at outpatient clinics, if ≥ 3 recognize the outcome as positive, the outcomes are finally determined to be positive.

1. Hidetoshi Mezawa, M.D., Ph.D.
2. Takashi Urashima, M.D., Ph. D.
3. Daishi Hirano, M.D., Ph.D.

4. Mai Okuyama, M.D.

5. Noriko Gocho, M.D.

Department of Pediatrics

The Jikei University School of Medicine

Tokyo, Japan

What the committee is expected to determine.

These five members determine whether the infant has had an episode of FA or not.

0. No FA

1. Immediate allergic reaction

2. Anaphylaxis

For 1 (and 2) above, they determine the status at the second birthday by reviewing detailed episodes, results of the OFC test, and levels of food-specific IgE.

1. Sustained unresponsiveness

Cow's milk allergy, the infant can drink raw milk ≥ 50 ml

Egg allergy, the infant can eat baked egg ≥ 30 g,

Wheat allergy, the infant can eat udon noodle ≥ 15 g

2. Probable unresponsiveness, e.g., in the process of increasing dose of allergenic food, but not yet reach to predefined dose of unresponsiveness mentioned above.

3. Not yet unresponsive, when the infant eats, he or she still reacts to the allergenic food.

4. Not sure because the infant has not consumed the food allergen

The infants are still state of either 2, 3, or 4 above, they are considered not yet outgrow = remain food allergy.

III. Data monitoring

A) Randomization using a block of four by Dr. Akio Nakashima who does not interview participating families and does not examine their infants.

B) Data monitoring

If the frequency of adverse events deviates in either intervention group, the committee reports the data to the Safety and Data Monitoring Committee as well as the lead investigator.

a. Akio Nakashima, M.D., Ph.D. (physician and nephrologist)

b. Ms. Haruka Wada (secretary at Molecular Epidemiology)

Division of Molecular Epidemiology

The Jikei University School of Medicine

Tokyo, Japan

IV. Safety and Data Monitoring Committee

a. Judging from data of interim analysis, this committee may recommend stopping or continuing the trial.

b. Responding to a report of adverse events, the committee may recommend stopping the trial or allowing the trial to continue.

① Motohiro Ebisawa, M.D., Ph.D.

② Sakura Sato, M.D., Ph.D.

③ Kiyotake Ogura, M.D., Ph.D.

Department of Pediatrics

Sagamihara National Hospital

Kanagawa, Japan

Participants

Inclusion criteria

1. Be in a high-risk group for atopic sensitization: at least one of the father, mother, brothers, or sisters of infants has atopic diseases (asthma, atopic dermatitis, FA, allergic rhinitis, hay fever)

2. Obtained written informed consent.

a. Participants who understand randomization into two groups: BF with or without

amino acid milk and BF with CMF.

b. Blood examinations at 5-6 months and 2 years of age are required.

3. Be available to visit the outpatient clinic at 1 month, 2 months, 3 months, 4 months, 5 months, 7~8 months, 9~10 months, 12 months, 14~15 months, 18 months, and 24 months of age.

Exclusion criteria

1. Less than 36 weeks of gestational age at birth or birth weight less than 2000 g.
2. Having serious congenital anomalies.
3. Being a triplet or more: twins may participate.
4. Abnormal delivery such as asphyxia (Apgar score 6 or less at 5 minutes after birth)

Interventions

1. BF±EF

Mothers in this group are asked to maintain exclusive BF. However, if the amount of breast milk is not sufficient, then EF may be added until starting solid food. If the mother needs to supply EF more than 150 ml/day for 3 consecutive days in addition to BF because of a low amount of breast milk, EF is switched to CMF. Thus, the offspring allocated to BF±EF can avoid CMF for at least the first 3 days of life.

2. BF+CMF

Mothers in this group are asked to maintain exclusive BF, but are also asked to simultaneously supply a small amount of CMF ≥ 5 ml/day from the first day of life until 1 month, and ≥ 40 ml/day after 1 month until weaning. When infants are born after 18:00, CMF may be added on the 2nd day.

Both CMF (Hohoemi[®]) and EF (Meiji Elemental formula[®]) are purchased from Meiji Holdings Co. Ltd. (Chuo-ku, Tokyo, Japan).

EF (Meiji Elemental formula[®])

For infants with FA, total proteins are replaced with refined amino acids. However, other fats, carbohydrates, vitamins, and minerals are basically the same as in CMF. Energy is

66 kcal/100 ml (17%) and 391 kcal/100 g powder.

Blinding to allocation group

- a. Participating family: Not blinded
- b. Dr. M.U.: Not blinded, because he needs to confirm compliance with the intervention by interviewing participating families at the outpatient clinic in the Maternal and Child Center of Jikei University Hospital. When an infant has food-specific IgE ≥ 0.10 U_A/ml at 5 months blood test, the infant is referred to Dr. H.T. at the outpatient clinic of Pediatrics without informing Dr. H.T. about the allocation group.
- c. Dr. H.T.: Blinded, who is a specialist in pediatric allergic diseases, diagnoses FA, without knowing the allocation group.
- d. Endpoint committee: Blinded and no contact with the participants.

Outcome Measures

Primary outcome

1. Atopic sensitization:

The primary outcome was atopic sensitization to cow's milk protein, i.e., serum levels of cow's milk-specific IgE (CM-IgE) ≥ 0.35 UA/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 primary outcome. Other atopic sensitization to egg white and wheat ≥ 0.35 UA/ml at 24 months of age were also evaluated to be compared with cow's milk sensitization. Outsourced for measuring with ImmunoCAP Specific IgE® (Thermo Fisher Diagnostics K.K., Minato-ku, Tokyo, Japan) to SRL Inc. and compared as both continuous and dichotomous (CM-IgE: ≥ 0.35 UA/ml) variable between BF±EF and BF+CMF group.

Secondary outcomes

1. Immediate type of FA

Presenting with at least one organ symptom of skin (urticaria, angioedema, erythema, pruritus, periorbital edema, etc.), respiratory (dry staccato cough,

hoarseness, wheezing, etc.), gastrointestinal (nausea, vomiting, colicky abdominal pain, etc.), or circulatory (tachycardia, hypotension, loss of consciousness, etc.) problems triggered by the food ingestion that appeared within a few hours, in combination with blood examination measuring serum levels of the suspected food-specific IgE: ≥ 0.35 U_A/ml by means of a fluorescence enzyme immunoassay.

In this trial, symptoms triggered by skin contact directly with food or indirectly with food-contaminated hands are not included.

The OFC test (Appendix 1) is performed around 1 year of age for infants who are sensitized to certain foods but for whom clinical reactivity after ingestion of the food allergen is unknown. Thus, immediate allergic reaction is a food-specific IgE: ≥ 0.35 U_A/ml plus either a FA episode or positive OFC test. FA is re-evaluated regarding whether the infant could eat the causative food allergen without an allergic reaction at the 2-year-old birthday.

2. Anaphylactic type of FA

When symptoms are derived from multiple (at least two of above) organs, e.g., urticaria and wheezing, the condition is defined as an anaphylactic reaction.

3. Desensitization of FA (= Sustained unresponsiveness or outgrow)

If infants, who had been once diagnosed as immediate type of FA especially with cow's milk allergy, egg allergy, and wheat allergy, can eat the allergenic food (raw milk ≥ 50 ml, baked egg 30g, udon noodle 15g) without allergic signs by 2nd birthday, then they are defined as 'sustained unresponsiveness = outgrow' to the food allergen in this trial.

4. Each food allergy

- a. Cow's milk allergy was defined allergic reaction by OFC-test or cow's milk intake with or without heated or processed with cow's milk-specific IgE are positive (≥ 0.35 U_A/ml).

b. Egg allergy was defined allergic reaction by OFC-test or egg intake with or without heated or processed with egg white-specific IgE are positive (≥ 0.35 U_A/ml).

c. Wheat allergy was defined allergic reaction by OFC-test or wheat intake with or without heated or processed with wheat-specific IgE are positive (≥ 0.35 U_A/ml).

d. Other food allergy was defined allergic reaction by OFC-test or a certain food intake with or without heated or processed with the food-specific IgE are positive (≥ 0.35 U_A/ml).

Exploratory outcomes: which are planned to report in another opportunity.

1. Atopic dermatitis

Check the SCORAD index¹⁴ at the 1- and 3-month check-ups.

2. Asthmatic bronchitis

A stringent index that includes frequent wheezing during the first 3 years of life and either one major risk factor (parental history of asthma or eczema) or two of three minor risk factors (eosinophilia, wheezing without colds, and allergic rhinitis).¹⁵

Major Criteria

1. Parental asthma†

2. Eczema diagnosed by a medical doctor‡

3. Sensitization with elevated mite- or house dust-specific IgE ≥ 0.10 U_A/ml

Minor Criteria

1. Allergic rhinitis diagnosed by a medical doctor§

2. Wheezing apart from colds

3. Eosinophilia ($\geq 4\%$)

3. Sensitization to Japanese cedar pollen

Japanese cedar pollen-specific IgE ≥ 0.10 U_A/ml

4. Allergic rhinitis

Allergic rhinitis: Rhinitis signs and symptoms for 1 month and mite-specific IgE ≥ 0.10 U_A/ml

Safety outcomes

The following are obtained from an interview at the outpatient clinic just after the 2-year-old birthday.

1. Admission for any reason
2. Emergency room visit for any reason
3. Growth retardation: Height and weight at 2-year-old birthday

Flow of participants

1. Informed consent and registration

- a. Pregnant women and their husbands are informed about the trial in detail at a childbirth class during late pregnancy through a talk by Dr. M. Urashima and the informed consent document. Written, informed consent is sent to the data monitoring center at the Molecular Epidemiology Division of Jikei University School of Medicine.
- b. Current or previous family history of atopic diseases (asthma, atopic dermatitis, FA, allergic rhinitis, hay fever) is obtained through questionnaires as well as parental demographic information such as maternal age and body weight/height. Only pregnant women who are confirmed to have a family history of atopic diseases are included.
- c. Food questionnaires of mothers (Appendix 3) are taken during the third trimester of pregnancy and at 1, 3, and 5 months of age after birth.

2. At the maternity ward of Jikei University Hospital

- a. Some included pregnant women are excluded after delivery, due to, e.g., cleft palate.
- b. Perinatal information, e.g., gestational weeks and birth weight, are taken from

- 1270 charts.
- 1271 c. Nursing staff is informed of the participants' names and to which group they are
1272 allocated.
- 1273 d. Mothers are informed to which group they belong after delivery; thus, mothers are
1274 not blinded to the allocation group.
- 1275 e. Mothers belonging to the BF+CMF group are asked to supplement BF with CMF at
1276 least 5 ml from the first day of delivery. However, if the delivery time is after 18:00,
1277 they are allowed to supply CMF in addition to BF starting at midnight of the 2nd day.
- 1278 f. Mothers belonging to the BF±EF group may add amino acid-based EF when they
1279 feel that BF is not enough. If the mother adds more than 150 ml/day EF to BF for 3
1280 consecutive days, EF is switched to CMF. Thus, offspring allocated to BF±EF can
1281 avoid CMF for at least the first 3 days of life.
- 1282 g. Nurses at the maternity ward are allowed to feed babies instead of their mothers.
1283

1284 **3. At the outpatient clinic in the Maternal and Child Center of Jikei**
1285 **University Hospital**

- 1286 a. Participating parent(s) are interviewed, and their children are examined at least
1287 seven times from 1, (2,) 3, (4,) 5-6, (7-8,) 9-10, 12, (14-15,) 18, and 24 months of
1288 age at the outpatient clinic of Jikei University Hospital by a study pediatrician
1289 (M.U.) who is not blinded to the allocation group.
- 1290 b. If infants have exanthema, the pediatrician provides their parents clear guidance
1291 about aggressive skin care such as how to wash their face and body using soap
1292 and a shower by showing them a video.
- 1293 c. Blood is sampled from all infants at 5-6 and 24 months.
1294

1295 **4. At the outpatient clinic in Pediatrics of Jikei University Hospital**

1296 In case of at least one antigen-specific IgE is 0.1 IU_A/ml or more, the infant is referred to
1297 a study specialist of pediatric allergy (H.T.) at the same Jikei University Hospital who
1298 does not know the infant's allocation group.
1299

1300 **5. Pediatrics Ward of Jikei University Hospital**

1301 The OFC test is performed at admission (Appendix 1) by pediatricians who are blinded

to the allocation group.

6. Efforts to reduce loss to follow-up

- a. Using email, we try to contact participants who move to distant places regarding whether their infants have FA and make efforts to reduce the number of infants lost to follow-up, from whom IgE data are not obtained at 5 or 24 months.
- b. If participants do not visit the outpatient clinic after their 2-year-old birthday and we receive no responses to the email, they are considered lost to follow-up.

7. Blood sampling

All infants undergo blood examinations to measure serum total and antigen-specific IgE.

- a. At the 5- to 6-month check-up prior to first solid food introduction:
Egg white, cow's milk, wheat, mites.
- b. At 2 years of age:
Egg white, cow's milk, wheat, mites, Japanese cedar pollen, dog dandruff, cat dandruff.
- c. Unscheduled visits are made to the clinic when participating infants have atopic symptoms, particularly suspicious of FA.
e.g., exanthema just after eating an egg dish.

8. Randomization

a. EF and CMF

Both CMF (Hohoemi[®]) and EF (Meiji Elemental formula[®]) are purchased from Meiji Holdings Co. Ltd. (Chuo-ku, Tokyo, Japan).

b. Randomization

Pregnant women that meet the inclusion criteria are randomly assigned to either the BF+CMF or BF±EF group according to an identification number generated by computer based on permuted blocks of four that are determined beforehand (Appendix 2). Staff members at the maternity ward are informed of which intervention applies to each pregnant participant before hospitalization for childbirth.

c. Blinding

According to the nature of the intervention, we do not use double blinding. However, we try to minimize bias using the following strategies:

Primary outcome: Cow's milk-specific IgE as an objective indicator.

Secondary outcomes: determine by prospective, randomized, open, blinded-endpoint (PROBE), determined by the endpoint committee members who do not examine the infants or are blinded to the allocation group'.

9. Compliance with CMF or EF

1. At the maternity ward, every day's CMF or EF intake is monitored by each participant (mother). The nurse may help with the intervention and monitoring.
2. At the outpatient clinic, mothers are asked whether they are using CMF, EF, or exclusive BF.

10. Monitoring of secondary outcomes

a. Scheduled

At either the Maternal and Child Center or Pediatrics of Jikei University Hospital, participating infants are examined frequently.

b. Unscheduled

If the infants have any allergic signs, e.g., urticaria after eating allergenic food, they are advised to call Jikei University Hospital. H.T. or other pediatricians try to examine the infants on the day or next day and perform a blood examination.

Statistical analysis

1. Sample size

300

2. Sample size calculation

300 pregnant women and their offspring

We hypothesized that cow's milk sensitization at 2 years of age, i.e., cow's milk-specific IgE ≥ 0.35 U_A/ml, would be present in 10% of one group of infants and 25% of the other group, with a type I error (two-sided) of 5% and a power of 90%, on the assumption of 3% loss to follow-up. Therefore, 1:1 divided samples of approximately 300 participants were calculated as sufficient to detect a significant difference.

. sampsi .1 .25

Estimated sample size for two-sample comparison of proportions

Test Ho: $p_1 = p_2$, where p_1 is the proportion in population 1
and p_2 is the proportion in population 2

Assumptions:

alpha = 0.0500 (two-sided)

power = 0.9000

$p_1 = 0.1000$

$p_2 = 0.2500$

$n_2/n_1 = 1.00$

Estimated required sample sizes:

$n_1 = 146$

$n_2 = 146$

$n = 292$

Assuming 3% loss to follow-up, 300 are needed to enroll.

3. Study period

We assume that 100 pregnant women per year will participate in this trial. Therefore, the accrual period will be 3 years. The last infant is followed up 2 years later. Thus, a total of 5 years is predicted to be needed to accomplish this trial.

4. Interim analysis

Interim analyses are planned two times at reaching 100 and 200 participating infants who had their second birthday. The P value for significance at the interim analysis is set as <0.001 according to the Peto's stopping boundaries.

5. Planned methods of analysis

Outcomes are analyzed by intention-to-treat analyses between the two comparative groups.

- a. Serum levels of cow's milk-specific IgE at 5 months and 24 months of age as a continuous variable are compared using Mann-Whitney test.
- b. Risk of cow's milk-specific IgE ≥ 0.35 U_A/ml will be analyzed with a 2x2 table with the chi-square test and represented by the risk ratio (RR), and 95% confidence interval (95% CI).
- c. Secondary outcomes will be compared with RR, and 95% CI.
- d. Safety outcomes will be compared with RR, RD and 95% CI or Student's t test.
- e. Subgroup analysis will be done according to tertiles of 25(OH)D.
- f. All reported P values will be two-sided.
- g. Values with $P < 0.05$ will be considered statistically significant.
- h. All data will be statistically analyzed using Stata 14.0 (StataCorp LP., College Station, TX, USA).

6. Subgroup analyses

To clarify whether BF $\pm$ EF or BF+CMF significantly affects the subgroups of 25(OH)D (e.g., tertile, quartile, quintile), P for interaction ($P_{\text{interaction}}$) is computed by creating multiplicative variables. The results of these analyses are not corrected for multiple comparisons.

Safety

Many Japanese maternity wards recommend BF, but allow mothers or nurses to help by supplying CMF to supplement BF after feeding colostrum, e.g., 6 to 10 hours after birth.

This is a food intervention trial. Thus, basically no safety concerns exist. However, the following are compared.

- a. Admission due to any disease
- b. Emergency visit or admission for any reason
- c. Body height and weight at 24 months of age

Early withdrawal

Unknown serious adverse events.

Compensation

Mitsui Sumitomo Insurance Fire provides liability insurance (NB41027726) for the principal investigator, to compensate for costs of treating disability as a result of side effects caused by consuming milk.

The maximum amount of compensation is 100,000,000 yen per person and 300,000,000 yen per trial.

Consideration concerning the protection of human rights and privacy

1. Written informed consent is obtained after sufficient explanation.
2. Participants can withdraw from the trial after providing written, informed consent.
3. Withdrawal is not considered a disadvantage for participants.
4. Private information is exchanged with study ID at the Jikei University School of Medicine; therefore, private information cannot leak from there (linkable anonymizing).

Publication of research results

We aim to publish the results of this trial in a scientific journal or present them at academic conferences. Personal information will be carefully concealed when we present the results.

Research funds

1. JSPS KAKENHI Grant Number JP16K09074

- 1457 2. the Practical Research Project for Allergic Disease and Immunology of the Japan
1458 Agency for Medical Research and Development, AMED (15ek0410019h0101)
1459 3. Dairy Products Health Science Council and Japan Dairy Association
1460

1461 **Attribution of intellectual property rights**

1462 If intellectual property rights such as patent rights become relevant, such rights will be
1463 attributable to the investigator.

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Appendix 1

Table 1. Oral Food Challenge (OFC) test

Oral Food Challenge (OFC) test			
Hen's egg	STEP①	egg yolk x 1 (As pumpkin cake)	70 g
	STEP②	whole egg x 1/2 (As pumpkin cake)	70 g
	STEP③	whole egg x 1 (As scrambled egg)	45 g
Milk and its products	STEP①	Heated milk (As pumpkin cake)	1 ml
	STEP②	Heated milk (As pumpkin cake)	10 ml
	STEP③	Heated milk (As potage soup)	50 ml
	STEP④	Yogurt	48 ml
Wheat	STEP①	Flour (As pumpkin cake)	1 g
	STEP②	Flour (As pumpkin cake)	5 g
	STEP③	Flour (As pumpkin cake)	15 g
	STEP④	Flour (As pumpkin cake)	30 g
		Udon dried noodles 3g = Flour 1g = wheat protein 0.08g	
		Udon dried noodles 15g = Flour 5g = wheat protein 0.4g	
		Udon dried noodles 50g = Flour 15g = wheat protein 1.3g	
		Udon dried noodles 100g = Flour 30g = wheat protein 2.6g	
Soy bean	STEP①	Tofu	100 g
peanuts	STEP①	Peanuts	2 g
	STEP②	Peanuts	10 g
almond	STEP①	Almond	2 g
	STEP②	Almond	10 g
walnuts	STEP①	Walnuts	2 g
	STEP②	Walnuts	10 g
Cashew Nuts	STEP①	Cashew Nuts	2 g
	STEP②	Cashew Nuts	10 g
Sesame (other kinds of nuts)	STEP①	Sesame	2 g
	STEP②	Sesame	10 g
Soba	STEP①	Soba	15 g
	STEP②	Soba	50 g
	STEP③	Soba	100 g
Meat		Boiled meat	50 g
Fish		Boiled Fish	40 g
Crustacean Soft body		Shrimp, Crab, Squid, Octopus (served as Hamburger)	15 g
Shellfish		Clams, Scallop, etc. (served as hamburger)	20 g
fish egg		Baked Tarraco	20 g
		Salmon roe	10 g
Potatoes		Boiled Potato Soup	100 g
corn		Corn compote	30 g
fruits		Strawberry Puree	120 ml

1516 **Appendix 2**

1517 Table 2. Permuted blocks of four

1518

1	2	3	4	5	6
A	A	P	P	A	P
A	P	A	P	P	A
P	P	A	A	A	P
P	A	P	A	P	A

1519

1520 **Appendix 3**

1521 **Questionnaires about food intake**

1522 If you obtained written informed consent from the participant, please send the following information by FAX.

1523

1524 Date: _____ year, _____ month, _____ day

1525 Person who filled out the form:

1526 ✎ During the last month, how often did you eat each kind of food per week? Please check (☑).

	Rarely	Once per week	Several times per week	Once per day	Twice per day	Three times per day
Example	☐	☐	☒	☐	☐	☐
Place to eat meal						
Restaurant (eating out)	☐	☐	☐	☐	☐	☐
Ramen shop (eating out)	☐	☐	☐	☐	☐	☐
Fast food (hamburger)	☐	☐	☐	☐	☐	☐
Fast food (rice bowl)	☐	☐	☐	☐	☐	☐
Fast food (soba, udon)	☐	☐	☐	☐	☐	☐
Lunch box bought at convenience store	☐	☐	☐	☐	☐	☐
Cafeteria at work place	☐	☐	☐	☐	☐	☐
At home	☐	☐	☐	☐	☐	☐
Foods						

Butter	☐	☐	☐	☐	☐	☐
Margarine	☐	☐	☐	☐	☐	☐
Vegetable oil	☐	☐	☐	☐	☐	☐
Vegetable oil Please check the most appropriate answer	Safflower oil	Canola oil	Sesame oil Soy oil Sunflower oil	Peanut oil, Coconut oil, Corn oil, Palm oil	others	
Olive oil	☐	☐	☐	☐	☐	☐
Biscuits ^{*1}	☐	☐	☐	☐	☐	☐
Junk food (potato chips, etc.)	☐	☐	☐	☐	☐	☐
Chocolate	☐	☐	☐	☐	☐	☐
Mayonnaise ^{*2}	☐	☐	☐	☐	☐	☐
Plain bread	☐	☐	☐	☐	☐	☐
Pastry	☐	☐	☐	☐	☐	☐
Croissant	☐	☐	☐	☐	☐	☐
Other kinds of bread						
Instant noodles	☐	☐	☐	☐	☐	☐
Fried food at home (tempura, fried chicken, fried potatoes, etc.)	☐	☐	☐	☐	☐	☐
Fried food outside (tempura, fried chicken, fried potatoes, etc.)	☐	☐	☐	☐	☐	☐
Beef (including hamburger) ^{*3}	☐	☐	☐	☐	☐	☐
Processed meat (sausage, salami, hot dogs, bacon, etc.)	☐	☐	☐	☐	☐	☐

Cow's milk	☐	☐	☐	☐	☐	☐
Cheese	☐	☐	☐	☐	☐	☐
Creams (include cream for coffee)	☐	☐	☐	☐	☐	☐
Ice cream	☐	☐	☐	☐	☐	☐
Pork	☐	☐	☐	☐	☐	☐
Chicken	☐	☐	☐	☐	☐	☐
Heated eggs	☐	☐	☐	☐	☐	☐
Raw eggs	☐	☐	☐	☐	☐	☐
Fish eggs (salmon roe, flying fish roe, roe, sea urchin, dried mullet roe, caviar, etc.)	☐	☐	☐	☐	☐	☐
Soy (including soy food, natto, tofu, etc.)	☐	☐	☐	☐	☐	☐
Soba	☐	☐	☐	☐	☐	☐
Tuna (canned tuna, sashimi, etc.)	☐	☐	☐	☐	☐	☐
Swordfish	☐	☐	☐	☐	☐	☐
Salmon (including canned salmon)	☐	☐	☐	☐	☐	☐
Mackerel, sardine, horse mackerel, Pacific saury (including canned)	☐	☐	☐	☐	☐	☐
White fish (red snapper, etc.)	☐	☐	☐	☐	☐	☐
Peanuts (including peanut butter)	☐	☐	☐	☐	☐	☐
Walnuts	☐	☐	☐	☐	☐	☐
Almonds	☐	☐	☐	☐	☐	☐
Cashew nuts	☐	☐	☐	☐	☐	☐
Macadamia nuts	☐	☐	☐	☐	☐	☐

Hazelnuts	☐	☐	☐	☐	☐	☐
Coconut (including processed food)	☐	☐	☐	☐	☐	☐
Other kinds of nuts						
Supplement						

1527 *1. Biscuits include cream puffs, sponge cake, donuts, etc.

1528 *2. Mayonnaise includes creamy salad dressing.

1529 *3. Beef includes heart, liver, diaphragm, and stomach.

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1535 **Summary of changes to**

1536 **the Trial Protocol and Statistical Analysis Plan (SAP)**

1537

1538 Ver. 1. 2013.8.1

1539 Ver. 2. 2016.7.22

1540 Ver.3. 2018.4.1

1541

1542 Ver. 2: certified on 2016.7.22 by the Jikei ethics committee.

1543

1544 **1. Measurement of serum levels of free fatty acids**

1545 As exploratory analyses, we decided to analyze serum levels of free fatty acids using the

1546 residual serum samples stocked in deep freezers (−80°C).

1547

1548 Associations among food intake questionnaires, serum levels of free fatty acids, and FA are

1549 analyzed, as exploratory study.

1550

1551 We added newly followings in SAP.

- 1552 a. Associations between free fatty acids and FA are analyzed.
- 1553 b. Associations between answers to questionnaires on food intake and FA risks are
- 1554 explored.
- 1555 c. Associations between answers to questionnaires on food intake and free fatty
- 1556 acids are explored.
- 1557 d. Associations between 25(OH)D levels and growth at the 2-year-old birthday will be
- 1558 compared.
- 1559

1560 **2. Key secondary outcomes were rearranged.**

1561

1562 Definition was not changed but separated into secondary outcomes and exploratory

1563 outcomes. In addition, each food allergy was made clear.

1564

1565 From;

1566 Key secondary outcomes

- 1567 1. immediate type of food allergy including anaphylaxis
- 1568 2. worsening of atopic dermatitis
- 1569 3. desensitization of food allergy
- 1570 4. atopic sensitization alone
- 1571 5. asthmatic bronchitis

1572

1573 To;

1574 Secondary outcomes: food allergy related

- 1575 I. Immediate type of food allergy (FA)
- 1576 II. Anaphylactic type of FA
- 1577 II. Desensitization of FA (= Sustained unresponsiveness or outgrow)
- 1578 III. Atopic sensitization alone
- 1579 III. Each food allergy
 - 1580 a. Cow's milk allergy
 - 1581 b. Egg allergy
 - 1582 c. Wheat allergy
 - 1583 d. Other food allergy

1584

1585 Exploratory outcomes: atopic conditions other than food allergy

- 1586 I. Atopic dermatitis
- 1587 II. Asthmatic bronchitis
- 1588 III. Sensitization to Japanese cedar pollen
- 1589 IV. Allergic rhinitis

1590

1591 Ver. 3: certified on 2018.4.1, to increase the credibility of the secondary outcomes.

1592

1593 **1. New members in the endpoint committee**

1594 We added one general pediatrician and one specialist in pediatric allergies to the endpoint
1595 committee.

1596

1597 From;

- 1598 a. Hidetoshi Mezawa, M.D., Ph.D.,
- 1599 b. Takashi Urashima, M.D., Ph.D.,
- 1600 c. Daishi Hirano, M.D., Ph.D

- 1601
- 1602 To;
- 1603 a. Hidetoshi Mezawa, M.D., Ph.D.,
- 1604 b. Takashi Urashima, M.D
- 1605 c. Daishi Hirano, M.D., Ph.D
- 1606 d. Mai Okuyama, M.D
- 1607 e. Noriko Gocho, M.D.

Supplementary Online Content

Tachimoto H, Imanari E, Mezawa H, et al. Effect of avoiding cow's milk formula at birth on prevention of asthma or recurrent wheeze among young children: extended follow-up from the ABC randomized clinical trial. *JAMA Netw Open*. 2020;3(10):e2018534.
doi:10.1001/jamanetworkopen.2020.18534

eFigure 1. Histograms of 25(OH)D levels at 5 months and 24 months of age

eFigure 2. Comparison of 25(OH)D levels at 5 and 24 months of age among 4 groups

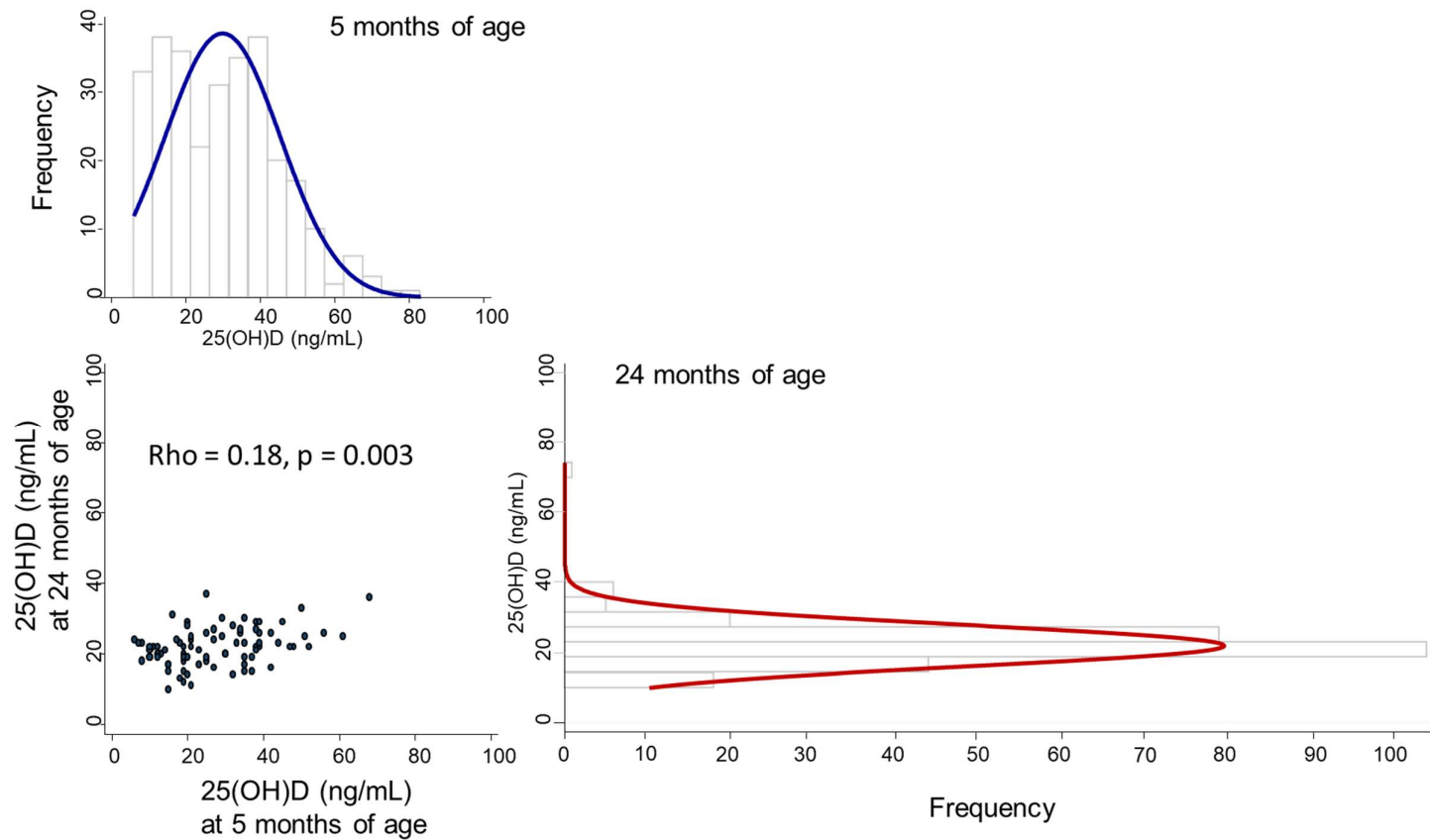
eTable 1. Interaction between tertiles of 25(OH)D levels and avoiding CMF at birth

eTable 2. Interactions between each allergen-specific IgE level at 5 months of age and the intervention

eTable 3. Incidence of asthma or recurrent wheeze by duration of adherence to the BF/EF intervention

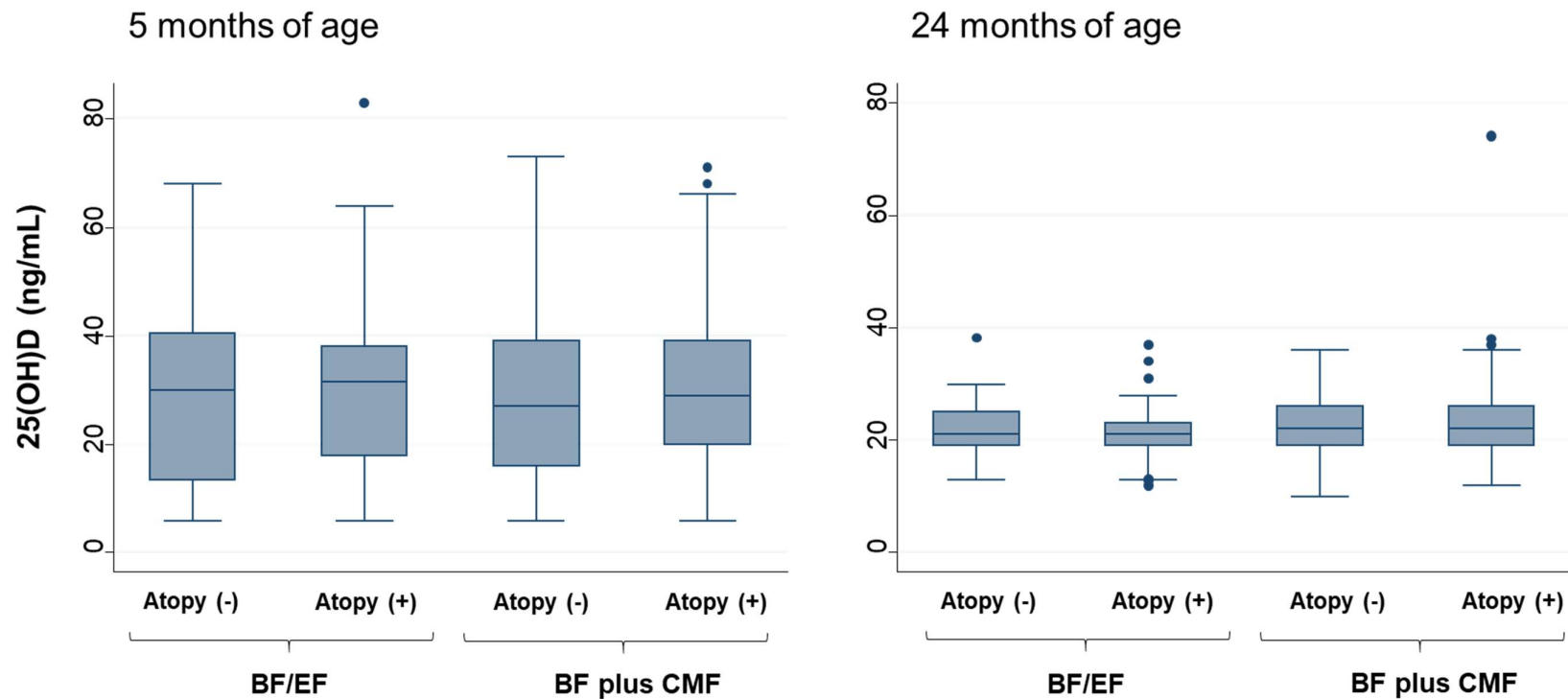
This supplementary material has been provided by the authors to give readers additional information about their work.

eFigure 1. Histograms of 25(OH)D levels at 5 months and 24 months of age



Spearman's rank correlation coefficient (ρ) with linear regression was used to quantify the strengths of associations between two continuous variables: $\rho \geq 0.4$, strong; $0.4 > \rho \geq 0.2$, moderate; and $\rho < 0.2$, weak.

eFigure 2. Comparison of 25(OH)D levels at 5 and 24 months of age among 4 groups



1) BF/EF without atopic conditions; 2) BF/EF with atopic conditions; 3) BF plus CMF without atopic conditions; 4) BF plus CMF with atopic conditions. The differences were assessed by the Kruskal-Wallis test.

eTable 1. Interaction between tertiles of 25(OH)D levels and avoiding CMF at birth

	BF/EF	BF plus CMF	RD (95% CI)	RR (95% CI)	P for interaction
25(OH)D level at 5 months of age					.65
Tertile 1, 6-20 ng/mL, n = 99	5 (10.4)	7 (13.7)	-0.03 (-0.16 to 0.09)	0.76 (0.26-2.23)	
Tertile 2, 21-36 ng/mL, n = 96	3 (6.1)	8 (17.0)	-0.11 (-0.24 to 0.02)	0.36 (0.10-1.27)	
Tertile 3, 37-83 ng/mL, n = 98	5 (10.2)	11 (22.4)	-0.12 (-0.27 to 0.02)	0.45 (0.17-1.21)	
25(OH)D levels at 24 months of age					.25
Tertile 1, 10-19 ng/mL, n = 89	8 (16.3)	9 (22.5)	-0.06 (-0.23 to 0.10)	0.73 (0.31-1.71)	
Tertile 2, 20-23 ng/mL, n = 103	2 (3.9)	10 (19.2)	-0.15 (-0.27 to -0.03)	0.20 (0.05-0.89)	
Tertile 3, 24-74 ng/mL, n = 86	5 (12.8)	7 (15.2)	-0.02 (-0.17 to 0.12)	0.84 (0.29-2.44)	

When both sides of the 95% CI of the RD are more or less than 0 or both sides of the 95% CI of the RR do not include 1, the risk estimate is considered to be significant and is indicated as bold.

eTable 2. Interactions between each allergen-specific IgE level at 24 months of age and the intervention

	BF/EF	BF plus CMF	RD (95% CI)	RR (95% CI)	P for interaction
Mite-specific IgE					.03
Low (<0.1 U _A /mL), n = 225	13 (11.5)	15 (12.8)	-0.02 (-0.11 to -0.07)	0.86 (0.43 to 1.72)	
High (≥0.1 U _A /mL), n = 59	2 (6.7)	12 (41.4)	-0.35 (-0.55 to -0.15)	0.16 (0.04 to 0.66)	
Milk-specific IgE					.03
Low (<0.1 U _A /mL), n = 168	11 (11.8)	7 (9.3)	0.02 (-0.07 to 0.12)	1.27 (0.52 to 3.11)	
High (≥0.1 U _A /mL), n = 117	4 (8.2)	20 (29.4)	-0.21 (-0.35 to -0.08)	0.28 (0.10 to 0.76)	
Egg white-specific IgE					.68
Low (<0.1 U _A /mL), n = 125	3 (4.8)	7 (11.1)	-0.21 (-0.35 to -0.08)	0.44 (0.12 to 1.61)	
High (≥0.1 U _A /mL), n = 161	12 (14.8)	20 (25.0)	-0.10 (-0.22 to 0.02)	0.59 (0.31 to 1.13)	
Wheat-specific IgE					.04
Low (<0.1 U _A /mL), n = 219	15 (10.1)	23 (16.1)	-0.01 (-0.09 to 0.08)	0.94 (0.44 to 2.00)	
High (≥0.1 U _A /mL), n = 56	0 (0.0)	4 (57.1)	-0.36 (-0.56 to -0.15)	0.18 (0.04 to 0.71)	

When both sides of the 95% CI of the RD are more or less than 0 or both sides of the 95% CI of the RR do not include 1, the risk estimate is considered to be significant and is indicated as bold.

eTable 3. Incidence of asthma or recurrent wheeze by duration of adherence to the BF/EF intervention

	BF/EF n = 151		
Day of starting the addition of CMF to BF after birth	Remained on BF/EF	Day 15 or after	Days 2 ^{*1} - 14
Total, n	n = 40	n = 41	n = 70
Asthma and recurrent wheeze, n (%)	5 (12.5)	3 (7.3)	7 (10.0)
95% confidence interval, %	4.2 to 26.8	1.5 to 19.9	4.1 to 19.5

*1. Three mothers in the BF/EF group mistakenly gave CMF to their infants from the second day after birth instead of the 4th day as the earliest start.

Data Sharing Statement

Data

Data available: Yes

Data types: Deidentified participant data

How to access data: urashima@jikei.ac.jp

When available: With publication

Supporting Documents

Document types: None

Additional Information

Who can access the data: Mitsuyoshi Urashima

Types of analyses: individual participants data meta analysis

Mechanisms of data availability: after approval of a proposal