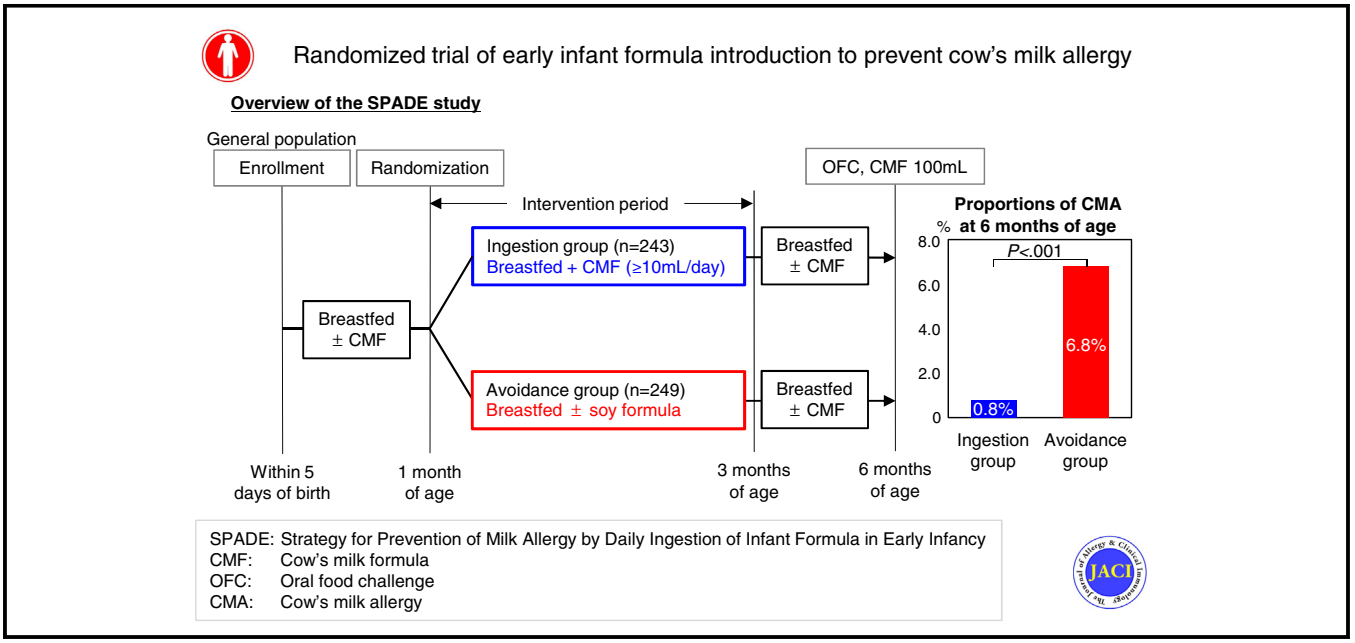
Randomized trial of early infant formula introduction to prevent cow's milk allergy

Tetsuhiro Sakihara, MD,[a] Kenta Otsuji, MD,[b] Yohei Arakaki, MD,[c] Kazuya Hamada, MD,[d] Shiro Sugiura, MD, MPH, PhD,[e] and Komei Ito, MD, PhD[e] *Okinawa and Aichi, Japan*

GRAPHICAL ABSTRACT



Background: Previous research has produced conflicting evidence on the preventive effects of early introduction of cow's milk protein on cow's milk allergy (CMA).
Objective: Through a randomized controlled trial, we sought to determine whether the early introduction of cow's milk formula (CMF) could serve as an effective strategy in the primary prevention of CMA in a general population.
Methods: We recruited newborns from 4 hospitals in Okinawa, Japan. Participants were randomly allocated to ingest at least10 mL of CMF daily (ingestion group) or avoid CMF (avoidance group) between 1 and 2 months of age. In the avoidance group breast-feeding was supplemented with soy formula as needed. Oral food challenge was performed at 6 months of age to assess CMA development. Continuous breast-feeding was recommended for both groups until 6 months of age.
Results: We identified 504 infants for randomization into the 2 groups. In all, the parents of 12 participants declined to receive the intervention, and the study sample comprised 491 participants (242 in the ingestion group and 249 in the avoidance group) for a modified intention-to-treat analysis. There were 2 CMA cases (0.8%) among the 242 members of the ingestion group and 17 CMA cases (6.8%) among the 249 participants in the avoidance group (risk ratio = 0.12; 95% CI = 0.01-0.50; $P < .001$). The risk difference was 6.0% (95% CI = 2.7-9.3). Approximately 70% of the participants in both groups were still being breast-fed at 6 months of age.
Conclusions: Daily ingestion of CMF between 1 and 2 months of age prevents CMA development. This strategy does not compete with breast-feeding. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: *Food allergy, milk allergy, early introduction, prevention, cow's milk, randomized controlled trial, birth cohort, infant formula, cow's milk formula, soy formula*

Cow's milk allergy (CMA) is relatively common in early childhood, with an estimated prevalence of 0.5% to 4.9%[1,2] and approximately 50% of children outgrowing their CMA within the first 5 years of life.[3,4] In Japan, neonates and their mothers usually remain in maternity hospitals for 1 week after birth,

From [a]the Department of Pediatrics, Heartlife Hospital, Okinawa; [b]the Department of Pediatrics, Okinawa Kyodo Hospital; [c]the Department of Pediatrics, Naha City Hospital, Okinawa; [d]the Department of Child Health and Welfare (Pediatrics), Graduate School of Medicine, University of the Ryukyus, Okinawa; and [e]the Department of Allergy, Aichi Children's Health and Medical Center.

This trial is registered with the University Hospital Medical Information Network Clinical Trials Registry (Trial ID: UMIN000025402; Available at https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000029128).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication April 11, 2020; revised June 16, 2020; accepted for publication August 20, 2020.

Corresponding author: Tetsuhiro Sakihara, MD, Heartlife Hospital, 208 Iju, Nakagusuku-son, Nakagami-gun, Okinawa 901-2492, Japan. E-mail: odekorin2000@yahoo.co.jp.

0091-6749/$36.00

© 2020 American Academy of Allergy, Asthma & Immunology

https://doi.org/10.1016/j.jaci.2020.08.021

Abbreviations used
CMA: Cow's milk allergy
CMF: Cow's milk formula
CMP: Cow's milk protein
EAT: Enquiring About Tolerance
FPIES: Food protein–induced enterocolitis syndrome
OFC: Oral food challenge
RCT: Randomized controlled trial
RR: Risk ratio
SPADE: Strategy for Prevention of Milk Allergy by Daily Ingestion of Infant Formula in Early Infancy
SPT: Skin prick test
UMIN-CTR: University Hospital Medical Information Network Clinical Trials Registry

during which time a proportion of neonates are given cow's milk formula (CMF) if required. Because of the general recommendations for exclusive breast-feeding, CMF ingestion is frequently discontinued after hospital discharge. Some of these infants have an elevated risk of developing CMA with the introduction of baby food containing cow's milk protein (CMP) or the reintroduction of CMF. Recent systematic reviews and a meta-analysis of randomized controlled trials (RCTs) have reported that the early introduction of allergenic foods can prevent development of the corresponding allergies.[5,6] Similarly, observational studies have found that early CMP introduction was associated with a lower risk of CMA development.[7-9] In 1 such study, exposure to CMP at age 15 to 94 days was associated with increased odds of CMA development when compared with exposure in the first 14 days of life.[7] However, no RCTs have demonstrated the efficacy of early CMP exposure to prevent CMA development.[5,6]

An RCT from Japan in 2019 showed that avoidance of CMP for the first 3 days of life suppressed milk sensitization and prevented CMA development at 2 years of age.[10] However, previous RCTs have generally been limited by factors such as low adherence to diet regimens,[11] late intervention,[12] and lack of information about the continuation of CMF ingestion.[10] Accordingly, there is insufficient evidence of the CMA-preventive effects of early CMP introduction. To provide better evidence for the possible preventive effects of early CMF introduction, we designed and conducted an RCT that accounted for the timing of CMF introduction, adherence, ingestion duration, and ingestion quantities.

Here, we have reported the results of the Strategy for Prevention of Milk Allergy by Daily Ingestion of Infant Formula in Early Infancy (SPADE) study, which was a randomized controlled intervention study on infants recruited from the general Japanese population. This trial was designed to determine whether the early introduction of CMF could serve as an effective strategy in the primary prevention of cow's milk sensitization and allergy in a general population.

METHODS

Study design and participants

The SPADE study was a multicenter, open-label RCT (Fig 1). The trial recruited newborns within 5 days of birth from 4 hospitals in Okinawa, Japan (Heartlife Hospital, Okinawa Kyodo Hospital, Naha City Hospital, and University of the Ryukyus Hospital). The inclusion criteria were a gestational age of at least 35 weeks and a birth weight of at least 2000 g. Candidate participants were excluded if they had complications of any severe underlying disease (ie, severe neonatal asphyxia, severe respiratory or cardiovascular diseases, and endocrine metabolic diseases). Written informed consent was obtained from each participant's parents at the time of enrollment. Shortly after the birth of the participating infants, all participating families received a standardized baseline questionnaire about the mother's elimination diet during pregnancy, parental smoking, pet ownership, and parental history of atopy (including food allergy, bronchial asthma, atopic dermatitis, and allergic rhinitis). The mothers were advised to follow the Japanese Guidelines for Food Allergy 2017, which do not recommend food elimination as an allergy prevention measure for pregnant and lactating mothers.[13] The investigators checked the daily quantity of CMF consumed by each participant on the basis of his or her medical record during the initial maternity hospitalization. Participants ingested CMF as required to supplement breast-feeding before 1 month of age. Adverse events and the daily quantities of CMF consumed were recorded in an event diary by parents, and physicians checked these diaries during monthly visits up until the participants reached 6 months of age.

Screening OFC

Screening oral food challenge (OFC) was performed on all participants at 1 month of age. Here, participants ingested 20 mL of CMF at 1 time. Participants with a negative test result were randomly assigned to either the CMF ingestion group (designated the ingestion group) or the elimination group (avoidance group).

Randomization and allocation

Participants were randomly assigned to the ingestion group or avoidance group in a 1:1 allocation ratio. The allocation plan was created by an investigator (T.S.) and carried out by using the block randomization method (block size = 4). Assignments were based on a computer-generated random number table, and the results were orally communicated to the treating physicians and the participants' parents.

Intervention

Participants in the ingestion group were assigned to ingest at least 10 mL of CMF (equivalent to 150 mg of CMP) daily between 1 and 2 months of age. This regimen specified a minimal average administration of CMF for at least 20 days per month with a maximum interruption of 1 week during the intervention period until the participant reached 3 months of age. There was no upper limit on CMF intake, and the participants' parents were to procure CMF as needed. Although the type of CMF was not specified, parents were instructed not to use hydrolyzed or amino acid–based formulas. Participants in the avoidance group were assigned to avoid CMF (<10 days per month) between 1 and 2 months of age. The participants' parents were advised to supplement breast-feeding with soy formula when required during the intervention period. Continued breast-feeding throughout the study period was recommended for both groups.

Skin prick tests (SPTs), an open OFC at 3 months of age (first OFC), and an open OFC at 6 months of age (second OFC) were performed to assess cow's milk sensitization and CMA (see the Methods section of the Online Repository at jacionline.org). With the exception of infants with a positive first OFC result, the participants ingested CMF on demand to supplement breast-feeding after reaching 3 months of age. The second OFC was performed in all participants, including those with positive first OFC results. Although there were generally no restrictions on the ingestion of complementary food, participants were not allowed to ingest dairy products except for CMF until the second OFC was performed.

Outcome measures

The primary outcome measure was the proportion of participants with CMA confirmed by the second OFC at 6 months of age. The secondary outcome measures were the proportion of participants with a positive SPT response to cow's milk; diameter of the wheal formed during the cow's milk

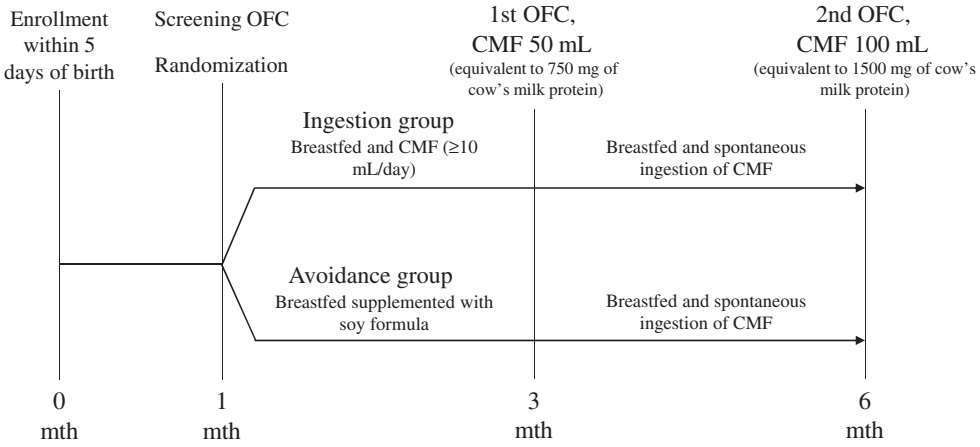


FIG 1. Study design. Infants in the ingestion group ingested at least 10 mL of CMF daily between 1 and 2 months of age. Infants in the avoidance group avoided CMF between 1 and 2 months of age. At 3 and 6 months of age, participants in both groups underwent OFCs with a total of 50 mL (first OFC) and 100 mL (second OFC) of CMF, respectively.

SPT; and serum titers of cow's milk–specific IgE, casein-specific IgE, and casein-specific IgG4 in cow's milk–sensitized infants at 6 months of age.

Sample size

On the basis of published reports, we predicted that the prevalence of IgE-mediated CMA in the general population would be less than 5%.[1,2] We estimated that 344 infants were required in each group (for $\alpha = 0.05$ and $\beta = 0.20$) to detect a 75% reduction in CMA at 6 months of age (4.9% in the avoidance group vs 1.2% in ingestion group). Allowing for a 10% dropout rate during the follow-up period (until the second OFC), we originally aimed to recruit a total of 764 infants.

Statistical analysis

We performed an analysis of all participants who received the intervention and could be assessed for the primary outcome irrespective of whether they had discontinued treatment before the scheduled visit at 6 months of age (modified intention-to-treat analysis). We also analyzed the per-protocol population, which included only those participants who could be assessed for the primary outcome and had adhered to the assigned regimen.

Intergroup comparisons were performed by using the Fisher exact test for categoric variables and the Mann-Whitney *U* test for continuous variables. The risk ratios (RRs) and 95% CIs for the outcome measures were calculated. We set the level of statistical significance at .05 (2 tailed). All statistical analyses were performed by using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[14] The institutional ethics committee of each participating hospital approved the study protocol. This trial is registered with the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR Trial [identifier UMIN000025402]).

Source of infant formula

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. A portion of the CMF was provided free of charge from Meiji Holdings Co, Ltd, and sample packages of soy formula were provided free of charge from Asahi Group Foods Ltd (see the Methods section of the Online Repository).

RESULTS

Study population

The study population is outlined in a Consolidated Standards of Reporting Trials diagram (Fig 2). SPADE study enrollment took place from January 1, 2017, to August 31, 2019. On September 1, 2019, because of funding constraints, enrollment was ceased before the target sample size had been reached. Data collection was completed on March 30, 2020. Of the 518 infants enrolled within 5 days of birth, 12 did not participate because their parents declined their participation before allocation and 2 were excluded because they developed allergic reactions to the screening OFC. One of the excluded infants was diagnosed as having food protein–induced enterocolitis syndrome (FPIES) for CMP. The remaining 504 candidate participants were randomized into 2 groups, but the parents of 12 of them (2.4%) declined to have their infant receive the intervention. The study sample comprised 492 participants (243 in the ingestion group and 249 in the avoidance group). Of these participants, 462 (93.9%) attended their scheduled visit at 6 months of age to undergo the SPT and second OFC. In all, 30 participants were voluntarily withdrawn from both groups; of these withdrawals, 29 were confirmed by a phone call to their parents to have ingested at least 100 mL of CMF with no allergic reaction at 6 months of age. These participants were included in the primary intention-to-treat analysis but excluded from the per-protocol analysis. Because of nonadherence to the regimens, 23 participants in the ingestion group (10.1%) and 40 participants in the avoidance group (17.0%) were excluded from the per-protocol analysis.

Baseline characteristics

The participants' baseline characteristics are summarized in Table I and Table E1 (available in this article's Online Repository at jacionline.org). There were no significant intergroup differences in the baseline demographic and clinical characteristics. None of the mothers had eliminated CMP from their diet during pregnancy. The proportions of CMF avoidance for the first 3 days of life[10] and start of daily CMF ingestion in the first 14 days of life[7] were similar between the groups.

Intervention adherence

In the ingestion group, 89.9% of participants ingested CMF for at least 20 days per month, with a maximum interruption of 1 week during the intervention period. In the avoidance group,

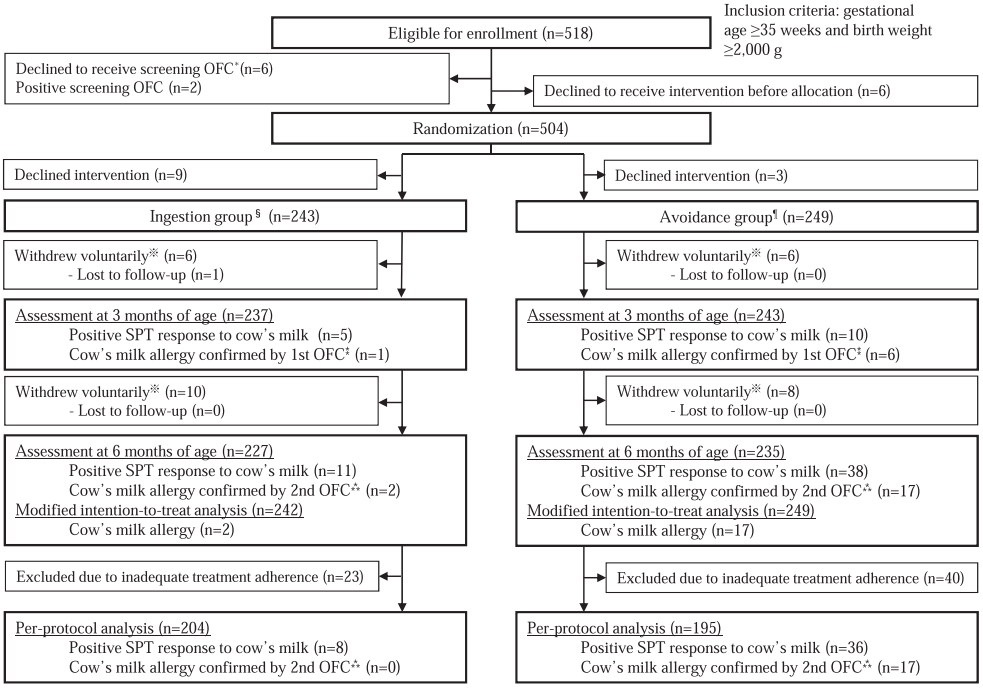


FIG 2. SPADE study enrollment, screening, and participant flow. *At 1 month of age, participants underwent a screening OFC involving a single dose of 20 mL of CMF. §Infants in the ingestion group ingested at least 10 mL of CMF daily between 1 and 2 months of age. ¶Infants in the avoidance group avoided CMF between 1 and 2 months of age. **The first OFC consisted of a cumulative dose of 50 mL of CMF. ***The second OFC consisted of a cumulative dose of 100 mL of CMF.

TABLE I. Baseline characteristics of participants at randomization

	Ingestion group (n = 243)			Avoidance group (n = 249)			
Characteristic	**Missing value**	**No. or median**	**Percentage or IQR**	**Missing value**	**No. or median**	**Percentage or IQR**	***P* value**
Male	0	127	52.3	0	142	57.0	.32
Caesarean section	0	53	21.8	0	62	24.9	.46
Season of birth: spring/summer	0	155	63.8	0	168	67.5	.40
Maternal food allergy	0	39	16.0	0	27	10.8	.11
Maternal bronchial asthma	0	31	12.8	0	33	13.3	.89
Maternal atopic dermatitis	0	24	9.9	0	18	7.2	.34
Maternal allergic rhinitis	0	63	25.9	0	70	28.1	.61
Paternal food allergy	0	39	16.0	2	36	14.5	.71
Paternal bronchial asthma	0	33	13.6	2	30	12.0	.69
Paternal atopic dermatitis	0	19	7.8	2	12	4.8	.20
Paternal allergic rhinitis	0	56	23.0	2	56	22.5	>.99
Amount of CMF intake per day during the neonatal period (mL)	8	80	0 to >200	8	80	0 to >200	.61
Instances of CMF intake per month during the neonatal period	8	27	8-30	8	26	9-30	.94
Avoidance of CMF for the first 3 days of life	8	18	7.4	8	13	5.2	.36
Started daily ingestion of CMF in the first 14 days of life	8	115	47.3	8	117	47.0	>.99

P values were calculated by using the Fisher exact test for categoric variables and the Mann-Whitney *U* test for continuous variables.
IQR, Interquartile range.

83.0% of participants did not ingest CMF for at least 20 days per month during the intervention period (see Fig E1 in this article's Online Repository at jacionline.org).

Clinical outcomes at 6 months of age

The clinical outcomes at 6 months of age are summarized in Table II. The proportion of participants with daily CMF ingestion

TABLE II. Clinical outcomes in the intention-to-treat analysis population

Clinical outcome	Ingestion group (n = 242)			Avoidance group (n = 249)			*P* value
	Missing value	Number or median	Percentage or IQR	Missing value	Number or median	Percentage or IQR	
Amount of CMF intake per day between age 1 mo and 2 mo (mL)	9	106	30 to >200	8	0	0-0	<.001
Instances of CMF intake per month between age 1 mo and 2 mo	9	30	26-30	8	2	0-8	<.001
Amount of CMF intake per day between age 3 mo and 5 mo (mL)	16	>200	0 to >200	15	100	0 to >200	<.001
Frequency of CMF intake per month between age 3 mo and 5 mo	16	30	8.5-30	15	26	0-30	<.001
Daily ingestion of CMF between age 3 mo and 5 mo	16	123	54.2	15	82	35.0	<.001
Continuation of breastfeeding up to age 3 mo	6	212	89.5	6	218	89.7	>.99
Continuation of breastfeeding up to age 6 mo	16	164	72.2	14	159	67.7	.31
Body weight at age 6 mo (g)	26	7560	7130-8220	24	7415	6965-8138	.23
Eczema before age 3 mo	4	121	50.6	5	126	51.6	.86
Eczema between age 3 mo and 5 mo	15	70	30.7	13	75	31.8	.84
Positive SPT response to egg white at age 6 mo	16	64	28.2	14	64	27.2	.84
Positive SPT response to wheat at age 6 mo	16	2	0.9	14	2	0.9	>.99
Positive SPT response to soy at age 6 mo	16	1	0.4	14	0	0.0	.49
Positive SPT response to cow's milk at age 6 mo	16	11	4.9	14	38	16.2	<.001
Cow's milk allergy at age 6 mo*	0	2	0.8	0	17	6.8	<.001

P values were calculated by using the Fisher exact test for categoric variables and the Mann-Whitney *U* test for continuous variables.
IQR, Interquartile range.
*Modified intention-to-treat analysis included participants who had confirmed the intake of dairy products after voluntarily withdrawing from the trial.

between 3 and 5 months of age was significantly higher in the ingestion group than in the avoidance group (54.2% vs 35.0% [$P < .001$]). Approximately 70% of participants in both groups were still being breast-fed at 6 months of age. None of the mothers had eliminated CMP from their diet during lactation. There were no significant intergroup differences in the proportions of clinician-diagnosed eczema (including mild eczema not considered to be atopic dermatitis) and sensitization to egg white, wheat, and soy.

Primary outcome measure

The primary outcomes are shown in Fig 3. In the primary intention-to-treat analysis population, 2 of the 242 ingestion group participants (0.8%) and 17 of the 249 avoidance group participants (6.8%) had OFC-confirmed CMA at 6 months of age (RR = 0.12; 95% CI = 0.01-0.50; $P < .001$). The risk difference was 6.0% (95% CI = 2.7-9.3). For the worst-case scenario analysis (in which participants who withdrew and were lost to follow-up from the ingestion group and the avoidance group were considered positive and negative, respectively, for the primary outcome), 3 (1.2%) of the 243 ingestion group participants had OFC-confirmed CMA compared with 17 (6.8%) of the 249 avoidance group participants ($P < .001$).

In the per-protocol analysis population, none of the 204 ingestion group participants had OFC-confirmed CMA compared with 17 (8.7%) of the 195 avoidance group participants ($P < .001$). The clinical details of participants with positive second OFC results are presented in Table E2 (available in this article's Online Repository at jacionline.org).

Secondary outcome measures

Of the 227 ingestion group participants, 11 (4.8%) had a positive SPT response to cow's milk at 6 months of age, as did 38 (16.2%) of the 235 avoidance group participants (RR = 0.26; 95% CI = 0.12-0.55; $P < .001$). Although the diameter of the wheal formed during the egg white SPT was similar between the groups, the diameter of the wheal formed during the cow's milk SPT was larger in the avoidance group than in the ingestion group ($P < .001$) (Fig 4). The median titer of cow's milk–specific IgE in participants with a positive SPT response to cow's milk at 6 months of age (n = 49) was 0.51 kUA/L (range, <0.10 to 0.78 kUA/L) in the ingestion group and 0.55 kUA/L (range, <0.10 to 21.1 kUA/L) in the avoidance group ($P = .20$). The median titer of casein-specific IgE was less than 0.10 kUA/L (range, <0.10 to 0.33 kUA/L) in the ingestion group and less than 0.10 kUA/L (range, <0.10 to 26.3 kUA/L) in the avoidance group ($P = .40$). The median titer of casein-specific IgG4 was 2.61 mgA/L (range, 0.45-10.46 mgA/L) in the ingestion group and 0.12 mgA/L (range, 0.08-0.33 mgA/L) in the avoidance group ($P = .02$) (Fig 5).

Safety outcomes

No CMF-related adverse events occurred during the study. One avoidance group participant had an FPIES-type reaction (delayed,

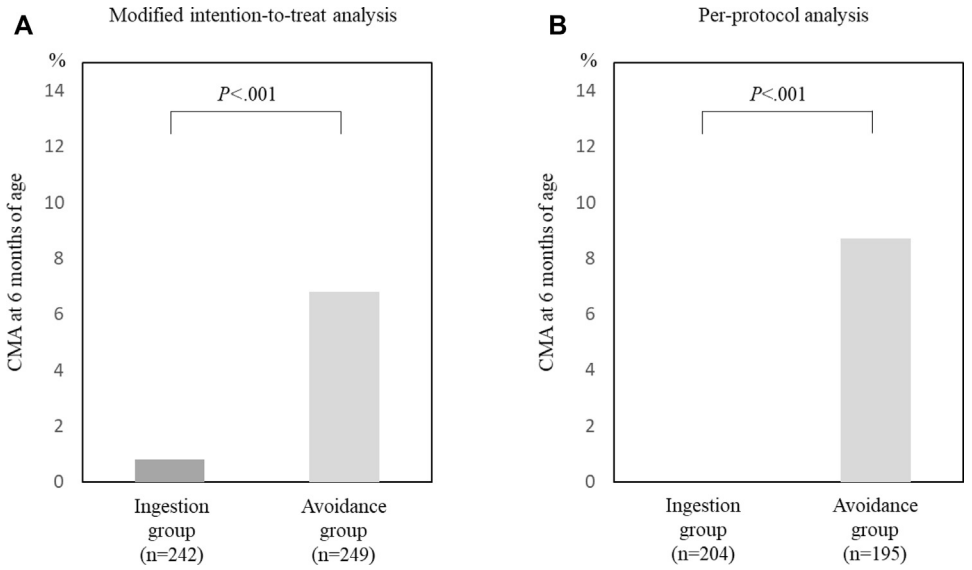


FIG 3. Primary outcome in the modified intention-to-treat analysis and per-protocol analysis. Proportions of positive results in the CMF OFC (cumulative dose of 100 mL) at 6 months of age in the modified intention-to-treat analysis, which included participants who withdrew voluntarily but not those lost to follow-up (**A**), and in the per-protocol analysis, which included only participants who adhered to the assigned regimen (**B**). *P* values were calculated by using the Fisher exact test.

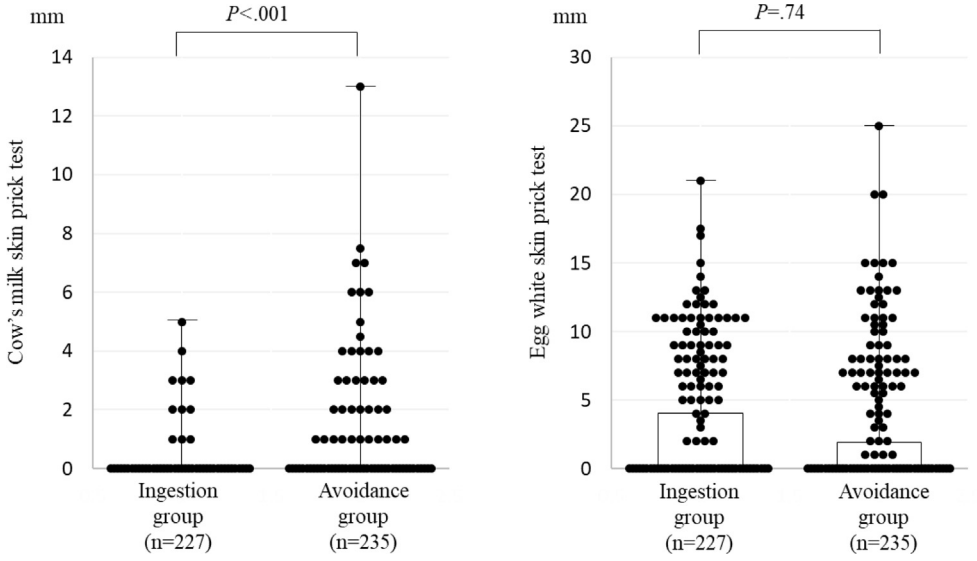


FIG 4. Diameter of wheals formed during the cow's milk and egg white SPTs at 6 months of age. *P* values were calculated by using the Mann-Whitney *U* test.

recurrent, and profuse vomiting) and was advised to stop the assigned regimen. A total of 20 avoidance group participants experienced transient gastrointestinal symptoms (9 had vomiting, 8 had constipation, and 3 had diarrhea) after ingesting soy formula. A total of 30 participants (6.1% [16 in the ingestion group and 14 in the avoidance group]) withdrew from the trial after the intervention began. No participants withdrew on account of adverse reactions caused by the trial formula (see Table E3 [available in the Online Repository at jacionline.org]). We also monitored the hospitalization of participants throughout the study period. The majority of hospitalizations were due to infections, and the proportions of hospitalized cases did not differ significantly between the groups (see Table E4 in this article's Online Repository at jacionline.org).

DISCUSSION

This RCT demonstrated that daily consumption of a small quantity of CMF between 1 and 2 months of age prevented the development of OFC-confirmed CMA in infants recruited from the general population. These findings are clinically important because the strategy does not hinder continued breast-feeding.

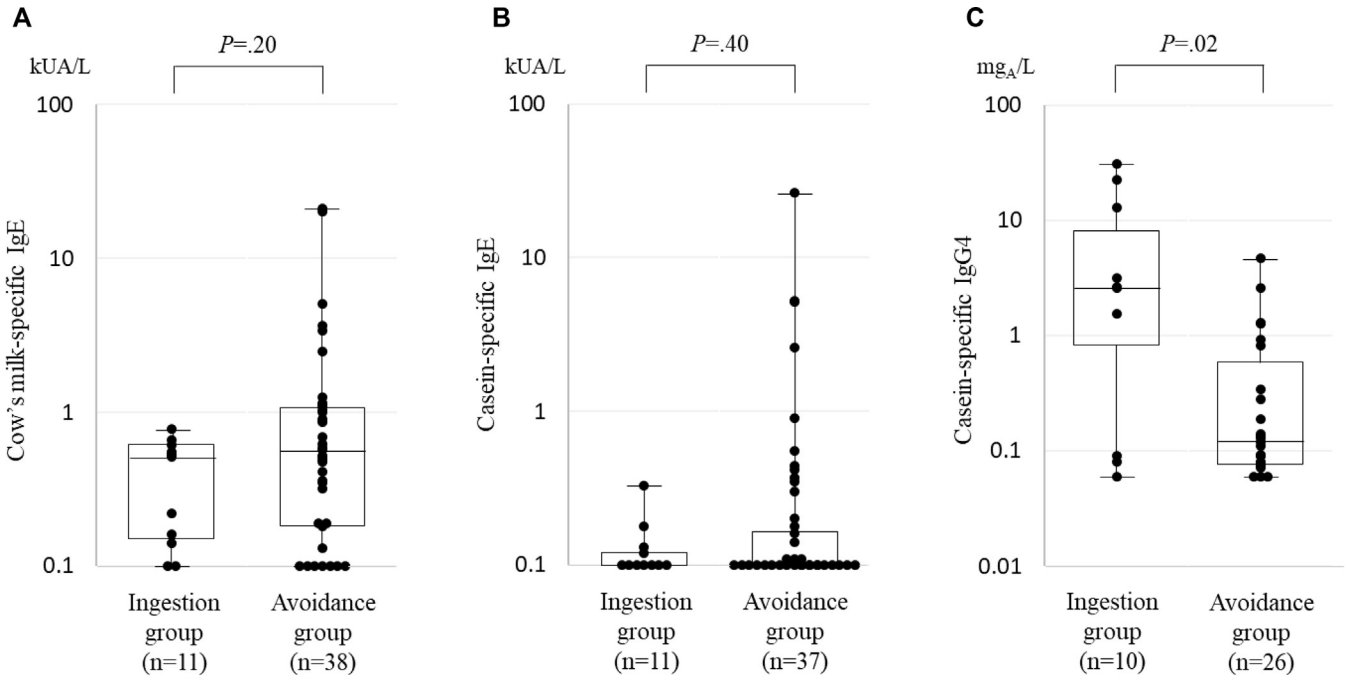


FIG 5. Titers of cow's milk–specific IgE, casein-specific IgE, and casein-specific IgG4 in cow's milk–sensitized infants at 6 months of age. Serum titers of cow's milk–specific IgE (**A**), casein-specific IgE (**B**), and casein-specific IgG4 (**C**) were measured in participants with cow's milk sensitization confirmed by SPT. *P* values were calculated by using the Mann-Whitney *U* test.

Timing of CMF introduction, intervention adherence, ingestion duration, and ingestion quantities

Although some observational studies have shown that early CMF introduction is associated with a lower risk of CMA,[7-9] a meta-analysis of 2 RCTs did not find any significant preventive effects (RR = 0.76; 95% CI = 0.32-1.78).[6] However, the results of those RCTs should be interpreted with several caveats in mind. One RCT had recruited infants at high risk of atopy, and it reported the CMA prevalence within the first 2 years of life to be 4.2% in the soy formula (avoidance) group and 3.1% in the conventional CMF group (odds ratio = 1.36; 95% CI = 0.46-4.00).[11] However, the rate of adherence to the allocated regimen was only 63% at 6 months of age, which may have contributed to the nonsignificant results. The other RCT was based on the Enquiring About Tolerance (EAT) study, which recruited infants from the general population. The prevalence of CMA was 0.67% in the standard introduction group versus 0.53% in the early introduction group (RR = 0.79; 95% CI: 0.18-3.50).[12] However, the EAT study had defined CMP avoidance as a daily formula milk intake less than 300 mL, resulting in the possible early introduction of CMP in both groups. In addition, infants in the early introduction group did not begin CMF ingestion until 3 months of age, which may be too late to effectively prevent CMA development.[15,16]

Because our trial did not perform a screening SPT before the intervention, the participants may have included infants who were sensitized to cow's milk at the time of assignment. Nevertheless, the lack of adverse events during the intervention period indicates that the daily ingestion of 10 mL of CMF is safe. In addition, the relatively low minimum intake of CMF in our strategy is practical and may have facilitated adherence. However, 2 cases showed allergic reactions after ingestion of 20 mL of CMF in the screening OFC. One case involved an FPIES-type reaction, and the other involved development of skin symptoms without anaphylaxis. Early intervention from the neonatal period may be beneficial because IgE-mediated CMA can develop before 1 month of age. Urashima et al recruited newborns with a high risk of atopy and reported that the prevalence of CMA by 2 years of age was 0.7% in infants who were breast-fed for at least the first 3 days of life versus 6.6% in infants who ingested CMF from the first day of life (RR = 0.10; 95% CI = 0.01-0.77).[10] In our study, there were no differences in breast-feeding or CMF feeding patterns between the ingestion and avoidance groups during the neonatal period (Table I and see Fig E1), and none of the 31 participants who avoided CMF for the first 3 days of life developed CMA (see Fig E2 in this article's Online Repository at jacionline.org). A prospective study found that exposure to CMP during the initial maternity hospitalization increased the risk of CMA whereas subsequent or continued exposure may promote tolerance.[15] Among our participants, the ingestion group and avoidance group had a higher and lower proportion, respectively, of daily CMF ingestion after the intervention period; the intervention may have influenced each participant's postintervention feeding pattern.

A dose-response analysis examined the relationship between the ingestion quantities of CMF and CMA development; the analysis showed that none of the participants who consumed at least 70 mL of CMF per week between 1 and 2 months of age developed CMA (see Fig E3 in this article's Online Repository at jacionline.org). These results therefore support our intervention protocol involving a daily minimum of 10 mL of CMF.

Intervention period and breast-feeding

As the randomization of breast-feeding in infants is unethical, we recommended breast-feeding to both groups. Furthermore, limiting CMF supplementation during the neonatal period may be associated with various risks, such as jaundice, hypoglycemia, and poor weight gain. Thus, we set the start of the intervention at 1 month of age. Infant feeding patterns in the first 3 months after birth are important in preventing CMA development,[8,9] and the intervention period was therefore set to end at 3 months after birth. The EAT study detected no difference in the rate of breast-feeding continuation between the early introduction group (starting CMF intake at 3 months of age) and the standard introduction group (starting CMF intake at 6 months of age).[12] Similarly, we did not observe any intergroup difference in our subjects from the standpoint of the proportion of breast-feeding up to 6 months of age. In Okinawa, the proportion of breast-feeding continuation is approximately 70% (comprising similar proportions of exclusive breast-feeding and mixed feeding) at 6 months of age in the general population.[17] Therefore, even the ingestion group participants in our study had higher rates of breast-feeding continuation than the general population did.

Study strengths

The main strength of the SPADE study is that it was designed for the general population, and its findings have a high level of generalizability. In addition, the participants performed the regimens safely with high levels of adherence, and the intervention did not interfere with breast-feeding. RCTs involving the early introduction of infant formula are often impaired on account of the high proportion of mothers who wish to maintain exclusive breast-feeding. In addition, it would be ethically unacceptable to prohibit ingestion of infant formula in the control group, and it is difficult for parents to accept amino acid–based formulas, which have inferior taste and nutritional value. To overcome these problems, our intervention allowed unlimited CMF ingestion during the neonatal period. To facilitate CMP avoidance, the avoidance group was provided with soy formula at no cost between 1 and 2 months of age. In addition, CMA was determined by OFC in all cases (including in infants who disliked CMF ingestion), excluding withdrawers. This ensured the accuracy of CMA incidence at 6 months of age. However, CMA occurred in 6.8% of the avoidance group, which is substantially higher than previously reported prevalences.[1,2] The dose-response analysis showed that all participants who developed CMA ingested less than 70 mL of CMF per week between 1 and 2 months of age (see Fig E3). The higher incidence of CMA among those in the avoidance group may therefore be due to their restricted CMF ingestion, but further analyses are required to understand this finding.

Study limitations and future issues

First, this study did not use masking or allocation concealment. Because it is difficult to blindly and randomly assign infant formula, we used an open-label intervention. Second, we did not perform double-blinded, placebo-controlled OFC to confirm CMA. Therefore, the physicians were aware of each participant's allocated group, which renders the evaluations susceptible to investigator bias. Also, this lack of masking may have influenced the results, as a larger number of participants in the ingestion group declined the intervention than in the avoidance group. However, because of the low prevalence of CMA in the general population,[1,2] we posit that the difference in declined cases would not have a substantial impact on our findings. Furthermore, objective physical findings were used to identify sensitization, and each evaluation was performed by at least 2 investigators. Third, blood samples were drawn only from cow's milk–sensitized infants. However, the SPT is more sensitive for identifying sensitization than analyses of allergen-specific IgE levels are.[13] Fourth, eczema severity was not quantitatively evaluated by using thymus and activation-regulated chemokines or the Scoring Atopic Dermatitis tool. For this reason, we performed a subgroup analysis for the presence or absence of eczema (see Fig E2). Both groups used the same skin care intervention, and there was no difference in the prevalence of eczema or the proportion of participants with food sensitization (excluding CMP). Fifth, mothers in both groups were not instructed to avoid dairy products. It is therefore possible that some avoidance group participants had ingested a small amount of CMP via breast milk between 1 and 2 months of age. However, dietary restrictions for mothers are not recommended. Our results indicate that CMA can be prevented by daily ingestion of CMF in infants without restricting mothers' intake of dairy products.

This study did not examine the effectiveness of probiotics and the role of gut microbiota in preventing CMA. Previous studies have reported an association between gut microbiota and regulatory T-cell induction.[18,19] Feehley et al noted that the transfer of healthy infants' microbiota to mice protected against allergic responses to CMP but the microbiota of infants with CMA had no such effects.[20] It may therefore be important to introduce CMP after the establishment of gut microbiota that can induce regulatory T-cells. Our study showed that cow's milk–sensitized participants in the ingestion group had higher casein-specific IgG4 levels than in the avoidance group, suggesting that CMF ingestion from the first month induces tolerance to CMA. If CMF is ingested within the first 3 days of life, CMA may be prevented by consistent ingestion of CMF from the first month onward.

Conclusions

Daily ingestion of CMF between 1 and 2 months of age prevents CMA development. This strategy does not interfere with breast-feeding.

We are grateful to the members of the SPADE study team, Meiji Holdings Company, and Asahi Group Foods, and we thank the participants and their families for their cooperation with the trial.

Key messages

- **Daily ingestion of at least 10 mL of cow's milk formula between 1 and 2 months of age prevents the development of cow's milk allergy.**
- **This strategy does not compete with breast-feeding.**

REFERENCES

1. Fiocchi A, Brozek J, Schünemann H, Bahna SL, von Berg A, Beyer K, et al. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's

Milk Allergy (DRACMA) guidelines. Pediatr Allergy Immunol 2010;21(suppl 21): 1125.
2. Schoemaker AA, Sprikkelman AB, Grimshaw KE, Roberts G, Grabenhenrich L, Rosenfeld L, et al. Incidence and natural history of challenge-proven cow's milk allergy in European children – EuroPrevall birth cohort. Allergy 2015;70:963-72.
3. Wood RA, Sicherer SH, Vickery BP, Jones SM, Liu AH, Fleisher DM, et al. The natural history of milk allergy in an observational cohort. J Allergy Clin Immunol 2013;131:805-12.
4. Elizur A, Rajuan N, Goldberg MR, Leshno M, Cohen A, Katz Y. Natural course and risk factors for persistence of IgE-mediated cow's milk allergy. J Pediatr 2012;161:482-7.e1.
5. de Silva D, Halken S, Singh C, Antonella M, Angier E, Arasi S, et al. Preventing food allergy in infancy and childhood: systematic review of randomized controlled trials [e-pub ahead of print]. Pediatr Allergy Immunol 10.1111/pai.13273. Accessed May 23, 2020.
6. Ierodiakonou D, Garcia-Larsen V, Logan A, Groome A, Cunha S, Chivinge J, et al. Timing of allergenic food introduction to the infant diet and risk of allergic or autoimmune disease a systematic review and meta-analysis. JAMA 2016;316: 1181-92.
7. Katz Y, Rajuan N, Goldberg MR, Eisenberg E, Heyman E, Cohen A, et al. Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. J Allergy Clin Immunol 2010;126:77-82.
8. Sakihara T, Sugiura S, Ito K. The ingestion of cow's milk formula in the first 3 months of life prevents the development of cow's milk allergy. Asia Pac Allergy 2016;6:207-12.
9. Peters RL, Koplin JJ, Dharmage SC, Tang MLK, McWilliam VL, Gurrin LC, et al. Early exposure to cow's milk protein is associated with a reduced risk of cow's milk allergic outcomes. J Allergy Clin Immunol Pract 2019;7:462-70.
10. Urashima M, Mezawa H, Okuyama M, Urashima T, Hirano D, Gocho N, et al. Primary prevention of cow's milk sensitization and food allergy by avoiding supplementation with cow's milk formula at birth: a randomized clinical trial. JAMA Pediatr 2019;173:1137-45.
11. Lowe AJ, Hosking CS, Bennett CM, Allen KJ, Axelrad C, Carlin JB, et al. Effect of a partially hydrolyzed whey infant formula at weaning on risk of allergic disease in high-risk children: a randomized controlled trial. J Allergy Clin Immunol 2011; 128:360-5.
12. Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, et al. Randomized trial of introduction of allergenic foods in breast-fed infants. N Engl J Med 2016;374: 1733-43.
13. Ebisawa M, Ito K, Fujisawa T. Japanese guidelines for food allergy 2017. Allergol Int 2017;66:248-64.
14. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452-8.
15. Saarinen KM, Juntunen-Backman K, Järvenpää AL, Kuitunen P, Lope L, Renlund M, et al. Supplementary feeding in maternity hospitals and the risk of cow's milk allergy: a prospective study of 6209 infants. J Allergy Clin Immunol 1999;104:457-61.
16. Santos A, Dias A, Pinheiro JA. Predictive factors for the persistence of cow's milk allergy. Pediatr Allergy Immunol 2010;21:1127-34.
17. The Okinawa Society of Child Health. Report on health checkups for infants and toddlers 2018. Available at: http://www.osh.or.jp/h_repo/data/H30/2018sum0.pdf. Accessed December 25, 2019.
18. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science 2011; 331:337-41.
19. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 2013;500:232-6.
20. Feehley T, Plunkett CH, Bao R, Choi Hong SM, Culleen E, Belda-Ferre P, et al. Healthy infants harbor intestinal bacteria that protect against food allergy. Nat Med 2019;25:448-53.

METHODS

OFCs

The first OFC was performed in both groups at 3 months of age and consisted of a cumulative dose of 50 mL of CMF (equivalent to 750 mg of CMP). Infants who had a positive reaction to the cow's milk SPT were administered 5 mL, 15 mL, and 30 mL of CMF orally at 30-minute intervals. Infants who had a negative reaction to the cow's milk SPT ingested 50 mL of CMF at 1 time.

The second OFC was performed in both groups at 6 months of age and consisted of a cumulative dose of 100 mL of CMF (equivalent to 1500 mg of CMP). Infants who had a positive reaction to the cow's milk SPT were administered 5 mL, 15 mL, 30 mL, and 50 mL of CMF orally at 30-minute intervals. Infants who had a negative reaction to the cow's milk SPT ingested 100 mL of CMF at 1 time.

In accordance with the Japanese Guidelines for Food Allergy 2017 criteria,[E1] the OFC results were defined as positive for CMA if the following objective clinical reactions were observed: urticaria, angioedema, vomiting, diarrhea, continuous cough, wheezing, stridor, or a decrease in blood pressure. Participants with positive first OFC results continued to participate in the study and underwent the second OFC at 6 months of age. We also confirmed by phone the intake of dairy products in withdrawers when they reached 6 months of age. If allergic symptoms were observed after intake of dairy products, the affected participant was urged to visit a physician for examination and treatment.

Immune parameters

Blood samples were drawn from participants with positive reactions to the cow's milk SPT. We measured serum titers of cow's milk–specific IgE, casein-specific IgE, and casein-specific IgG4 by using the ImmunoCAP system (Thermo Fisher Diagnostics KK, Tokyo, Japan).

Application of corticosteroid ointment for eczema

We aggressively treated any episode of eczema at entry and maintained control without exacerbation throughout the intervention period. Specifically, topical corticosteroids were applied as proactive treatment for infants with moderate-to-severe eczema. Topical corticosteroids were applied until the eczema had cleared, and they were used intermittently to maintain remission. Monthly clinical assessments, including to determine growth parameters and presence of atopic eczema on examination (based on the Japanese Guidelines for Atopic Dermatitis 2017), were performed up to 6 months of age.[E2]

SPTs

SPTs with commercial allergen extracts of cow's milk, egg white, wheat, and soy (Torii Pharmaceutical Co, Ltd, Tokyo, Japan) were performed by using a standard technique. Histamine dihydrochloride (10 mg/mL) served as a positive control, and a 50% glycerol solution was used as a negative control. A wheal diameter of at least 3 mm larger than that of the negative control or larger than half that of the positive control was regarded as a positive response for sensitization.

Post hoc analyses

We performed *post hoc* subgroup analyses in the primary outcome population (excluding withdrawers) stratified by parental history of atopy, mode of delivery, perinatal (maternal and/or newborns within the first 7 days of life) antibiotic use, eczema in the neonatal period, eczema between 1 and 2 months of age, eczema between 3 and 5 months of age, CMF ingestion in the first 24 hours after birth, CMF ingestion in the first 3 days of life, and daily CMF ingestion between 3 and 5 months of age. We also evaluated the mean weekly ingested quantities of CMF participants with and without CMA between 1 and 2 months of age as a dose-response analysis.

Source of infant formula

Participants in the ingestion group were provided with Meiji Hohoemi RakuRaku Cube infant formula; it was provided free of charge from Meiji Holdings Co, Ltd, Tokyo, Japan. The investigators provided a total of 2000 mL of CMF per 2 months to each ingestion group participant. Participants in the avoidance group were provided with Bonlact i, which is a prepared soymilk powder formula. Sample packages of Bonlact i were provided free of charge from Asahi Group Foods Ltd, Tokyo, Japan. To supplement the sample packages, Heartlife Hospital purchased larger (360-g) cans of Bonlact i cans for distribution to the avoidance group participants as required. The companies that provided the formulas had no role in study design, data collection and analysis, decision to publish, or article preparation.

RESULTS

Post hoc analysis results

We carried out *post hoc* subgroup analyses stratified according to parental history of atopy, mode of delivery, perinatal antibiotic use, presence of eczema, and CMF ingestion outside the intervention period. In all subgroups, the superiority of the ingestion group was maintained (see Fig E2). Participants who avoided CMF for the first 3 days of life and ingested CMF daily between 3 and 5 months of age did not develop CMA at 6 months of age. The dose-response analysis showed that all participants who developed CMA had ingested less than 70 mL of CMF per week between 1 and 2 months of age (see Fig E3).

DISCUSSION

Daily ingestion of CMF between 3 and 5 months of age

As shown in our *post hoc* subgroup analysis, CMA did not occur in infants who ingested CMF daily between 3 and 5 months of age in either group. However, the possibility of reverse causation means that we cannot determine whether CMF introduction from 3 months of age had a preventive effect on the development of CMA. In other words, some participants may have continued to ingest CMF because they did not have CMA.

SUPPLEMENTARY MEMBERS AND STAFF

The membership of the SPADE study team is as follows: clinical support staff: Tomoko Kunishima (Ando), Yutaka Kawamitsu, Yumi Kinjo, Hitomi Abe, Kazunori Sakai, Ryoko Amazumi, and Chiaki Higa; nursing staff: Keiko Adaniya, Keiko Ishihara, Kozue Kinjo, Kumiko Tamanaha, Megumi Hatori, Naomi Asato, and Yuka Nakamatsu; and medical assistant staff: Akane Yamashiro, Akino Uehara, Junko Kudo, Kazuyo Higa, Kikumi Willis, Koko Kinjo, Midori Tanaka, Miki Yasumura, Rina Miyagi, Nanae Unten, Yuki Hoshi, and all other obstetrics and gynecology ward staff.

REFERENCES

E1. Ebisawa M, Ito K, Fujisawa T. Japanese guidelines for food allergy 2017. Allergol Int 2017;66:248-64.

E2. Katayama I, Aihara M, Ohya Y, Saeki H, Shimojo N, Shoji S, et al. Japanese guidelines for atopic dermatitis 2017. Allergol Int 2017;66:230-47.

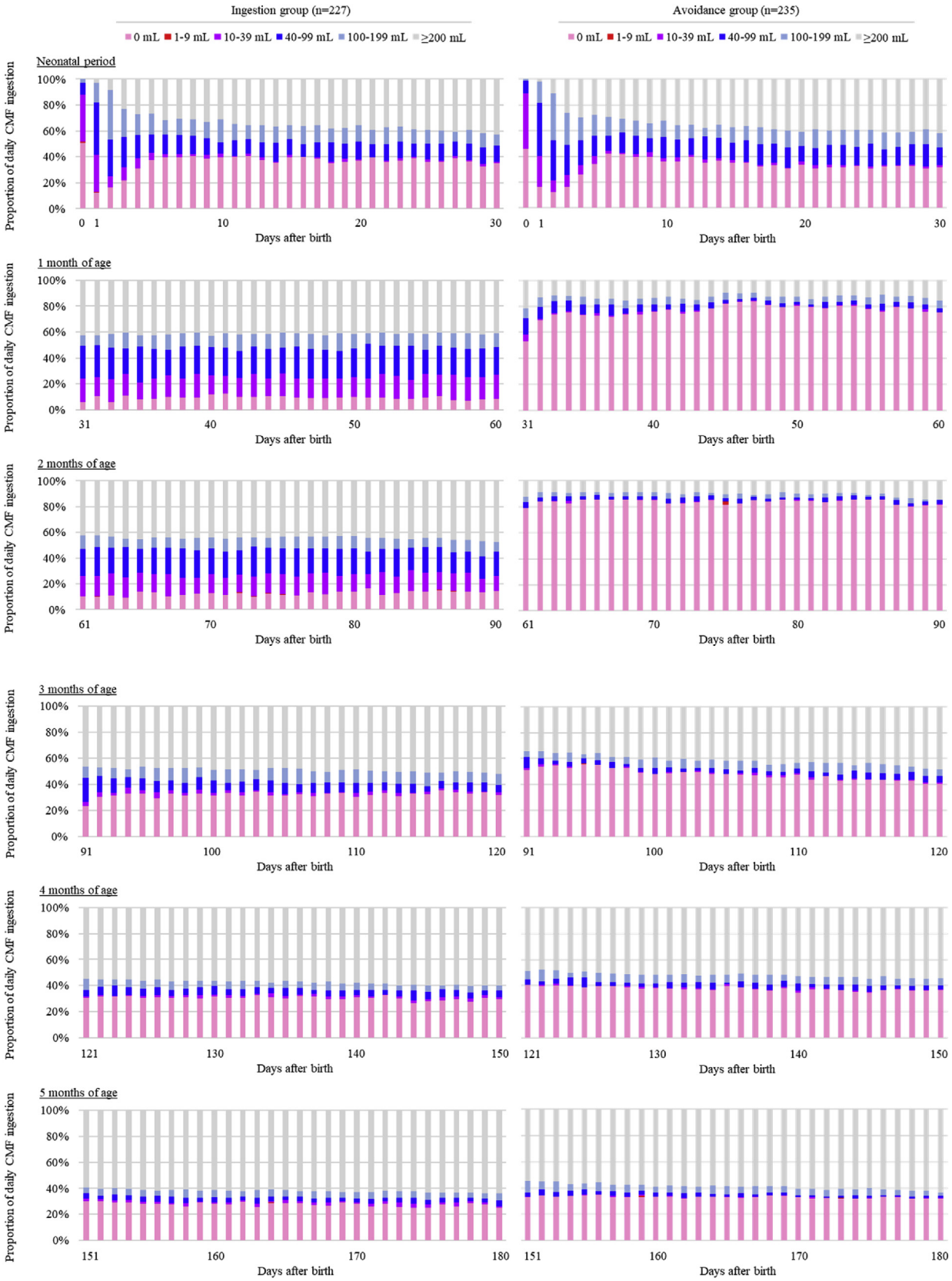


FIG E1. Proportions of daily CMF ingestion quantities. The proportions of daily CMF ingestion quantities throughout the study period are shown. The ingestion quantities were classified into 6 groups: 0 mL, 1 to 9 mL, 10 to 39 mL, 40 to 99 mL, 100 to 199 mL, and at least 200 mL. *C/S*, Caesarean section; *VD*, vaginal delivery.

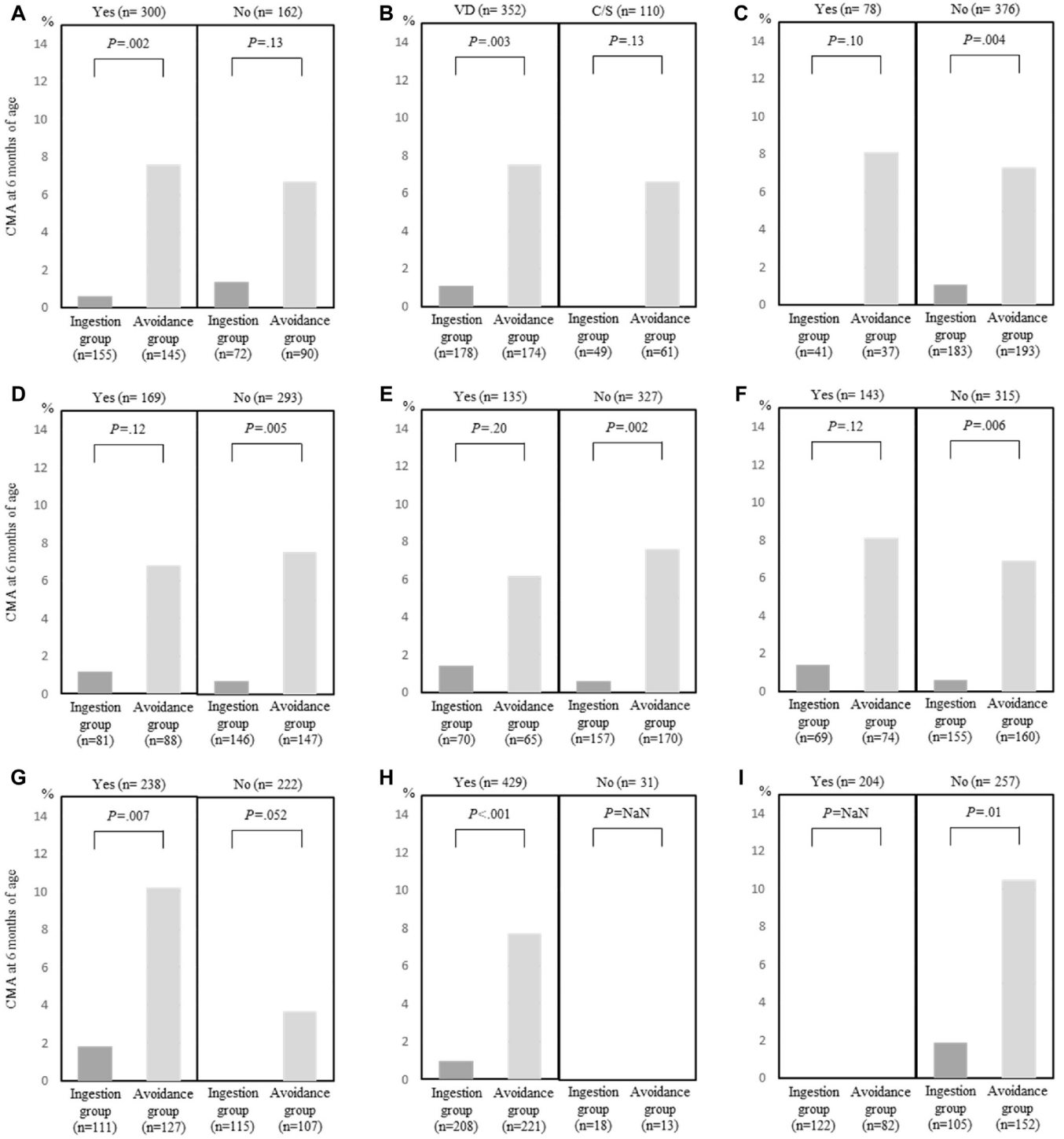


FIG E2. Outcomes of the *post hoc* subgroup analyses. *Post hoc* analyses that evaluated the incidence of CMA between the ingestion group and avoidance group stratified by parental history of atopy (**A**), mode of delivery (**B**), perinatal (maternal and/or newborns within the first 7 days of life) antibiotic use (**C**), eczema in the neonatal period (**D**), eczema between 1 and 2 months of age (**E**), eczema between 3 and 5 months of age (**F**), ingestion of CMF in the first 24 hours after birth (**G**), ingestion of CMF in the first 3 days of life (**H**), and daily ingestion of CMF between 3 and 5 months of age (**I**). *P* values were calculated by using the Fisher exact test. *C/S*, Caesarean section; *NaN*, not a number; *VD*, vaginal delivery.

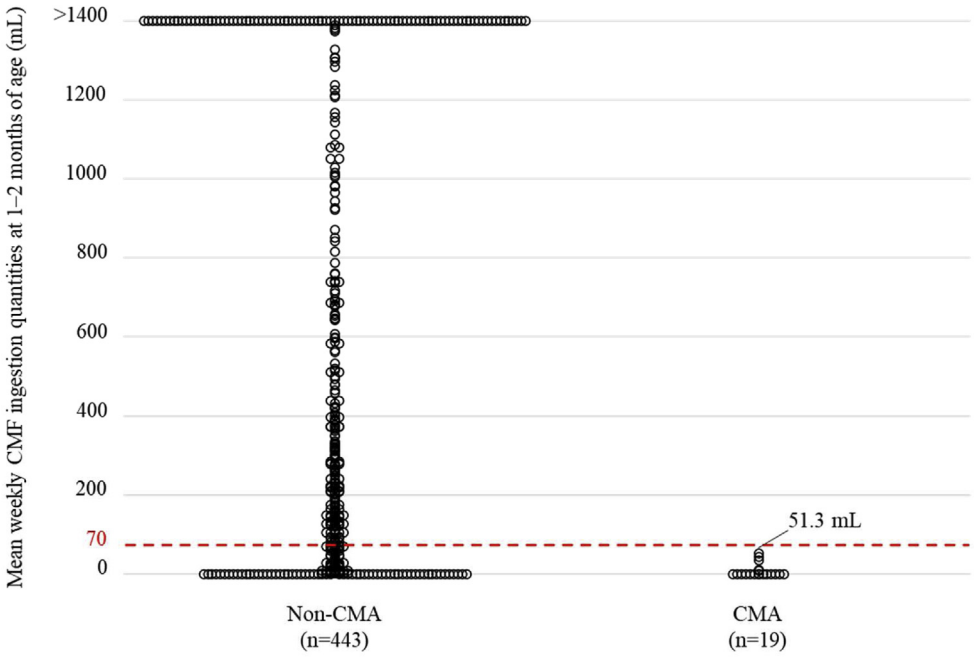


FIG E3. Mean weekly CMF ingestion quantities between 1 and 2 months of age in infants without CMA (non-CMA) and infants with CMA (CMA infants). This dose-response analysis evaluated the mean weekly quantities of CMF ingested by participants with and without CMA from 1 to 2 months of age.

TABLE E1. Additional baseline characteristics of participants at randomization

Characteristic	Ingestion group (n = 243)			Avoidance group (n = 249)			*P* value
	Missing value	No. or median	Percentage or IQR	Missing value	No. or median	Percentage or IQR	
Gestational age (wk)	0	39	38-40	0	39	38-40	.34
Birth weight (g)	0	3026	2805-3276	0	2995	2795-3185	.08
No siblings	0	91	37.4	0	95	38.2	.78
Maternal age (y)	0	32	28-36	0	32	29-36	.60
Maternal body mass index before pregnancy (kg/m^2)	3	20.7	19.2-23.1	2	21.2	19.5-23.4	.11
Maternal weight gain during pregnancy (kg)	5	10.0	8.0-12.6	1	9.7	7.4-11.9	.26
Maternal smoking	0	10	4.1	0	6	2.4	.32
Paternal age (y)	0	33	29-37	2	33	29-37	.86
Paternal smoking	0	81	33.3	2	82	32.9	>.99
Domestic dog exposure at birth	0	41	16.9	0	27	10.8	.07
Domestic cat exposure at birth	0	16	6.2	0	15	6.0	>.99

P values were calculated by using the Fisher exact test for categoric variables and the Mann-Whitney *U* test for continuous variables.
IQR, Interquartile range.

TABLE E2. Clinical details of participants with positive results in the OFC to CMF at 6 months of age

No.	Group allocation	Cumulative CMF (CMP) dosage at first appearance of symptoms, ml (mg)	Symptom	Medication	Total IgE level at 6 age 6 mo (IU/mL)	Cow's milk–specific IgE level at age 6 mo (kUA/L)	Casein-specific IgE level at age 6 mo (kUA/L)	Casein-specific IgG4 level at age 6 mo (mg_A/L)
1*	Ingestion	100 (1500)	LU	None	53.2	0.78	<0.10	0.09
2*	Ingestion	5 (75)	LU	None	9.0	0.53	<0.10	<0.07
3	Avoidance	100 (1500)	LU	None	8.3	1.26	<0.10	0.12
4	Avoidance	20 (300)	LU	None	35.0	0.52	0.20	0.07
5	Avoidance	100 (1500)	LU	None	19.6	0.69	<0.10	<0.07
6	Avoidance	50 (750)	LU	None	23.0	0.90	<0.10	0.19
7	Avoidance	5 (75)	SU	Antihistamine	152	5.04	5.19	0.13
8	Avoidance	50 (750)	LU	None	14.2	3.64	<0.10	<0.07
9	Avoidance	5 (75)	LU	None	32.7	0.48	0.42	0.09
10	Avoidance	20 (300)	SU, V, coughing, wheezing	Antihistamine, salbutamol	156	20.2	5.19	0.09
11	Avoidance	20 (300)	LU	None	11.5	0.57	<0.10	0.08
12	Avoidance	100 (1500)	LU	None	106	1.09	0.30	0.13
13	Avoidance	100 (1500)	LU	None	<5.0	0.49	0.44	NA
14	Avoidance	5 (75)	LU	None	338	3.39	2.60	0.09
15	Avoidance	100 (1500)	LU	None	5.9	0.19	0.16	NA
16	Avoidance	100 (1500)	LU	None	13.9	1.04	0.37	NA
17	Avoidance	50 (750)	LU	None	67.1	1.13	<0.10	NA
18	Avoidance	20 (300)	SU	Antihistamine	152	21.1	26.3	NA
19	Avoidance	20 (300)	LU, V	None	11.8	0.51	<0.10	NA

LU, Localized urticaria referring to noncontact urticarial reactions; *SU*, systemic urticaria; *V*, vomiting.
*Excluded from the per-protocol analysis because of insufficient adherence to the assigned regimen (ie, ingestion of CMF less than 10 days per month in the avoidance group and regular ingestion of CMF for 20 or more days per month with a maximum interruption of 1 week in the ingestion group).

TABLE E3. Details of participants who withdrew from the trial after the intervention

Group allocation	Age at withdrawal (mo)	Reason for withdrawal	Allergic reaction caused by the trial formula
Ingestion	2	Stopped taking CMF because of disliking the taste	None
Ingestion	3	Unknown	Unknown
Ingestion	3	Difficulties in visiting the hospital for appointments	None
Ingestion	3	Difficulties in visiting the hospital for appointments	None
Ingestion	3	Difficulties in visiting the hospital for appointments	None
Ingestion	3	Moved	None
Ingestion	4	Difficulties in visiting the hospital for appointments	None
Ingestion	4	Difficulties in visiting the hospital for appointments	None
Ingestion	4	Difficulties in visiting the hospital for appointments	None
Ingestion	4	Refused to undergo OFC for assessment of the primary outcome	None
Ingestion	5	Moved	None
Ingestion	6	Difficulties in visiting the hospital for appointments	None
Ingestion	6	Difficulties in visiting the hospital for appointments	None
Ingestion	6	Difficulties in visiting the hospital for appointments	None
Ingestion	6	Difficulties in visiting the hospital for appointments	None
Ingestion	6	Difficulties in visiting the hospital for appointments	None
Avoidance	2	Stopped taking the soy formula due to disliking the taste	None
Avoidance	2	Exacerbation of maternal mental illness	None
Avoidance	2	Treatment of a ventricular septal defect	None
Avoidance	3	Stopped taking the soy formula because of disliking the taste	None
Avoidance	3	Difficulties in visiting the hospital for appointments	None
Avoidance	3	Moved	None
Avoidance	4	Difficulties in visiting the hospital for appointments	None
Avoidance	4	Difficulties in visiting the hospital for appointments	None
Avoidance	5	Difficulties in visiting the hospital for appointments	None
Avoidance	5	Difficulties in visiting the hospital for appointments	None
Avoidance	5	Difficulties in visiting the hospital for appointments	None
Avoidance	5	Refused to undergo OFC for assessment of the primary outcome	None
Avoidance	6	Difficulties in visiting the hospital for appointments	None
Avoidance	6	Difficulties in visiting the hospital for appointments	None

TABLE E4. Hospitalization during the study period

	Ingestion group (n=227)	Avoidance group (n=235)	*P* value
Total hospitalizations, no. (%)	17 (7.5%)	11 (4.7%)	.24
Reason for hospitalization (no.)			
Respiratory syncytial virus infection	12	5	
Influenza virus infection	1	3	
Human metapneumovirus infection	1	0	
Acute bronchiolitis	1	0	
Viral pneumonia	1	0	
Viral meningitis	0	1	
Febrile seizure	1	2	

P value was calculated by using the Fisher exact test.