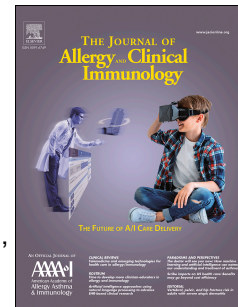


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Randomized trial of early infant formula introduction to prevent cow's milk allergy

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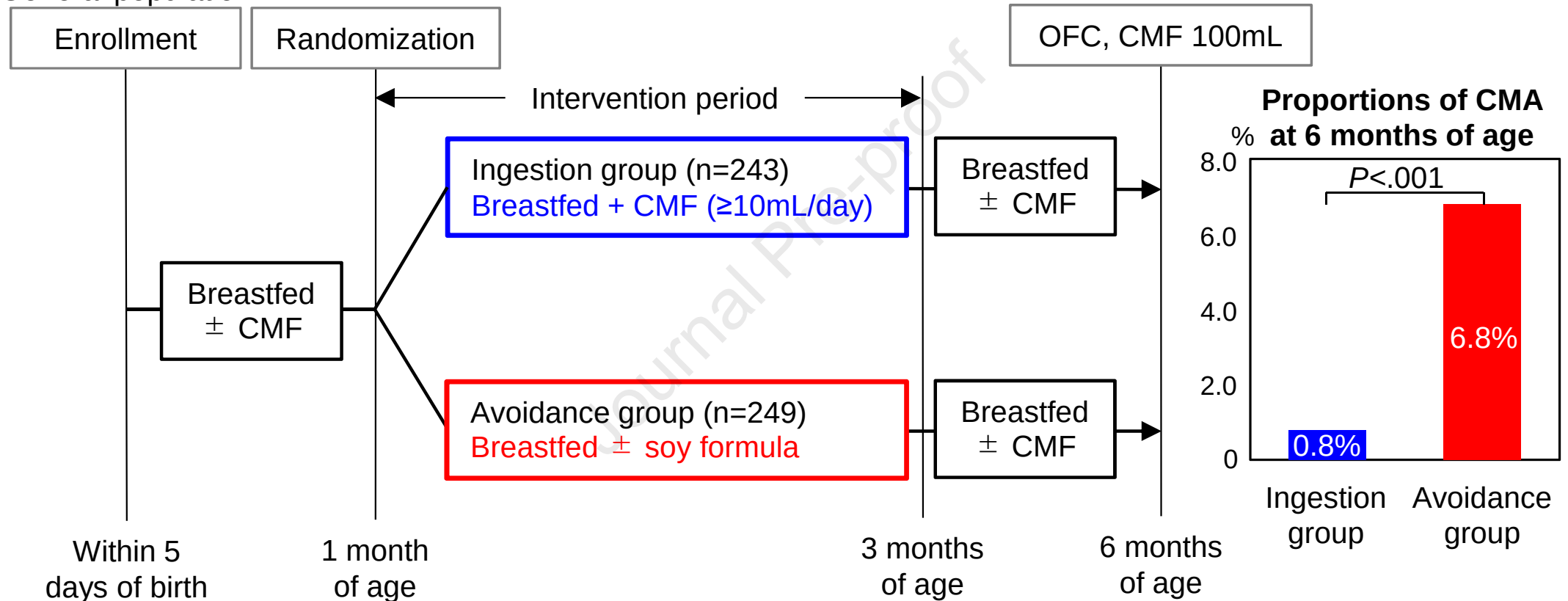
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Randomized trial of early infant formula introduction to prevent cow's milk allergy

Overview of the SPADE study

General population



SPADE: Strategy for Prevention of Milk Allergy by Daily Ingestion of Infant Formula in Early Infancy

CMF: Cow's milk formula
OFC: Oral food challenge
CMA: Cow's milk allergy

1. Title page

Title

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

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2. Abstract

Background: Previous research has produced conflicting evidence on the preventative effects of early cow's milk protein introduction 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 ≥ 10 mL of CMF daily (ingestion group) or avoid CMF (avoidance group) between 1 and 2 months of age. The avoidance group supplemented breastfeeding with soy formula as needed. An oral food challenge was performed at 6 months of age to assess CMA development. Continuous breastfeeding was recommended for both groups until 6 months of age.

Results: We identified 504 infants for randomization into the 2 groups. Twelve participants declined to receive the intervention, and the study sample comprised 491 participants (ingestion group: 242; avoidance group: 249) for a modified intention-to-treat analysis. The number of CMA cases was 2 (0.8%) of 242 in the ingestion group and 17 (6.8%) of 249 in the avoidance group (risk ratio: 0.12; 95% confidence interval: 0.01–0.50; $P < .001$). The risk difference was 6.0% (95% confidence interval: 2.7–9.3). Approximately 70% of participants received breastfeeding at 6 months of age in both groups.

Conclusions: Daily ingestion of CMF between 1 and 2 months of age prevents CMA development. This strategy does not compete with breastfeeding.

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).

3. Key messages

- Daily ingestion of ≥ 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 breastfeeding.

4. Capsule summary

This multicenter randomized controlled trial in infants from the general population demonstrates a safe and practical strategy (daily ingestion of cow's milk formula between 1 and 2 months of age) for preventing cow's milk allergy.

5. Keywords

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

6. Abbreviations

CI (Confidence interval), 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)

7. Text

Introduction

Cow's milk allergy (CMA) is relatively common in early childhood with an estimated prevalence of 0.5–4.9%,^{1,2} and approximately 50% of children outgrow their CMA within the first 5 years of life.^{3,4} In Japan, neonates and their mothers usually remain in maternity hospitals for one week after birth, during which a proportion of neonates are given cow's milk formula (CMF) if required. Due to general recommendations for exclusive breastfeeding, 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) reported that the early introduction of allergenic foods can prevent the 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.^{7,8,9} In one such study, exposure to CMP at age 15–94 days had an 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 the 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 preventative effects of early CMP introduction. In order to provide better evidence for the possible preventative 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 report 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 (Figure 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 ≥ 35 weeks and a birth weight of $\geq 2,000$ g. Candidate participants were excluded if they had complications of any severe underlying disease (i.e., severe neonatal asphyxia, severe respiratory/cardiovascular diseases, and endocrine metabolic diseases). Written informed consent was obtained from each participant's parents upon enrollment. Shortly after birth, 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 based on his/her medical records during the initial maternity hospitalization. Participants ingested CMF as required to supplement breastfeeding 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 till 6 months of age.

Screening oral food challenge

A screening oral food challenge (OFC) was performed on all participants at 1 month of age. Here, participants ingested 20 mL of CMF at one time. Participants with a negative result were randomly assigned to either a CMF ingestion group (designated the "ingestion group") or 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 (TS) and carried out 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 ≥ 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 ≥ 20 days/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/month) between 1 and 2 months of age. The participants' parents were advised to supplement breastfeeding with soy formula when required during the intervention period. Continued breastfeeding throughout the study period was recommended for both groups.

Skin prick tests (SPTs), an open OFC at 3 months of age (1st OFC), and an open OFC at 6 months of age (2nd OFC) were performed to assess cow's milk sensitization and CMA (See Supplementary Methods). With the exception of infants with a positive 1st OFC result, the participants ingested CMF on demand to supplement breastfeeding after reaching 3 months of age. The 2nd OFC was performed in all participants, including those with positive 1st OFC results. While there were generally no restrictions on the ingestion of complementary food, participants were not allowed to ingest dairy products except for CMF until the 2nd OFC was performed.

Outcome measures

The primary outcome measure was the proportion of participants with CMA confirmed by the 2nd OFC at 6 months of age. The secondary outcome measures were the proportion of participants with a positive SPT response to cow's milk; wheal diameter of the cow's milk 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

We predicted that the prevalence of IgE-mediated CMA in the general population would be <5% based on published reports.^{1,2} We estimated that 344 infants were required in each group (for $\alpha = .05$ and $\beta = .20$) to detect a 75% reduction of CMA at 6 months of age (avoidance group: 4.9% vs ingestion group: 1.2%). Allowing for a 10% dropout rate during the follow-up period (until the 2nd 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 only included participants who could be assessed for the primary outcome and had adhered to the assigned regimen.

Inter-group comparisons were performed using Fisher's exact test for categorical variables and the Mann-Whitney *U* test for continuous variables. The risk ratio (RR) and 95% confidence intervals (CI) for the outcome measures were calculated. We set the level of statistical significance at .05 (two-tailed). All statistical analyses were performed 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 ID: UMIN000025402).

Conflicts of interest

This research did not receive any specific grant from funding agencies in the public, commercial, or

not-for-profit sectors. A part of 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 Supplementary Methods).

Results

Study population

The study population is outlined in the CONSORT diagram (Fig 2). SPADE study enrollment took place from January 1, 2017 to August 31, 2019. On September 1, 2019, enrollment was ceased before reaching the target sample size due to funding constraints. Data collection was completed on March 30, 2020. Of 518 infants enrolled within 5 days of birth, 12 declined to participate before allocation and 2 were excluded as 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 12 (2.4%) declined to receive the intervention. The study sample comprised 492 participants (ingestion group: 243; avoidance group: 249). Among these, 462 (93.9%) attended their scheduled visit at 6 months of age to undergo the SPT and 2nd OFC. Thirty participants voluntarily withdrew from both groups; of these, 29 confirmed by phone that they had ingested ≥ 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. Due to non-adherence to the regimens, 23 (10.1%) participants in the ingestion group and 40 (17.0%) participants in the avoidance group were excluded from the per-protocol analysis.

Baseline characteristics

The participants' baseline characteristics are summarized in Table □ and Table E1. There were no significant inter-group 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 life¹⁰ and start of daily CMF ingestion in the first 14 days of life⁷ were similar between the groups.

Intervention adherence

In the ingestion group, 89.9% of participants ingested CMF for ≥ 20 days/month with a maximum interruption of 1 week during the intervention period. In the avoidance group, 83.0% of participants

did not ingest CMF for ≥ 20 days/month during the intervention period (Fig E1).

Clinical outcomes at 6 months of age

The clinical outcomes at 6 months of age are summarized in Table □. The proportion of participants with daily CMF ingestion between 3 and 5 months of age was significantly higher in the ingestion group than the avoidance group (54.2% vs 35.0%, $P < .001$). Approximately 70% of participants were still being breastfed at 6 months of age in both groups. None of the mothers had eliminated CMP from their diet during lactation. There were no significant inter-group differences in the proportion 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 Figure 3. In the primary intention-to-treat analysis population, the number of OFC-confirmed CMA cases at 6 months of age was 2 (0.8%) of the 242 ingestion group participants and 17 (6.8%) of the 249 avoidance group participants (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 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 2nd OFC results are presented in Table E2.

Secondary outcome measures

The number of participants with a positive SPT response to cow's milk at 6 months of age was 11 (4.8%) of the 227 ingestion group participants and 38 (16.2%) of the 235 avoidance group

participants (RR: 0.26, 95% CI: 0.12–0.55, $P<.001$). Although the wheal diameter of the egg white SPT was similar between the groups, the wheal diameter of 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 –0.78 kUA/L) in the ingestion group and 0.55 kUA/L (<0.10 –21.1 kUA/L) in the avoidance group ($P=.20$). The median titer of casein-specific IgE was <0.10 kUA/L (range: <0.10 –0.33 kUA/L) in the ingestion group and <0.10 kUA/L (<0.10 –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 (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, recurrent, and profuse vomiting) and was advised to stop the assigned regimen. Twenty 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 (6.1%) participants (ingestion group: 16; avoidance group: 14) withdrew from the trial after the intervention began. No participants withdrew due to adverse reactions caused by the trial formula (Table E3). 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 significantly differ between the groups (Table E4).

Discussion

This RCT demonstrated that the 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 breastfeeding.

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

While some observational studies have shown that early CMF introduction is associated with a lower risk of CMA,^{7,8,9} a meta-analysis of 2 RCTs did not find any significant preventative 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 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 adherence rate to the allocated regimen was only 63% at 6 months of age, which may have contributed to the non-significant results. The other RCT was based on the Enquiring About Tolerance (EAT) study, which recruited infants from the general population. CMA prevalence was 0.67% in the standard introduction group and 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 <300 mL, resulting in the possible early introduction of CMP in both groups. In addition, infants in the early introduction group only began CMF ingestion from 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 ingesting 20 mL of CMF in the screening OFC. One case involved an FPIES-type reaction, and

the other developed 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 breastfed for at least the first 3 days of life and 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 breastfeeding or CMF feeding patterns during the neonatal period between the ingestion and avoidance groups (Table 1 and Fig E1), and none of the 31 participants who avoided CMF for the first 3 days of life developed CMA (Fig E2). A prospective study found that exposure to CMP during the initial maternity hospitalization increased the risk of CMA, and 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 post-intervention feeding pattern.

A dose-response analysis was performed on the relationship between the ingestion quantities of CMF and CMA development, which showed that none of the participants who consumed ≥ 70 mL of CMF per week between 1 and 2 months of age developed CMA (Fig E3). These results therefore support our intervention protocol involving a daily minimum of 10 mL of CMF.

Intervention period and breastfeeding

As the randomization of breastfeeding in infants is unethical, we recommended breastfeeding 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 breastfeeding continuation rate between the early introduction group (starting CMF intake at 3 months of age) and standard

introduction group (starting CMF intake at 6 months of age).¹² Similarly, we did not observe any inter-group difference in the proportion of breastfeeding up to 6 months of age in our subjects. In Okinawa, the proportion of breastfeeding continuation is approximately 70% (comprising similar proportions of exclusive breastfeeding and mixed feeding) at 6 months of age in the general population.¹⁷ Therefore, even the ingestion group participants in our study had higher breastfeeding continuation rates than the general population.

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 breastfeeding. RCTs involving the early introduction of infant formula are often impaired due to the high proportion of mothers who wish to maintain exclusive breastfeeding. In addition, it would be ethically unacceptable to prohibit infant formula ingestion in the control group, and it is difficult for parents to accept amino acid-based formulas that have inferior taste and nutritional value. To overcome these problems, our intervention allowed unlimited CMF ingestion during the neonatal period. The avoidance group was provided with soy formula at no cost between 1 and 2 months of age to facilitate CMP avoidance. In addition, CMA was determined by OFC in all cases (including 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 (Fig E3). The higher incidence of CMA 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 employ masking or allocation concealment. Because it is difficult to blindly

and randomly assign infant formula, we employed an open-label intervention. Second, we did not perform a 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, due to 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 ≥ 2 investigators. Third, blood samples were only drawn from cow's milk-sensitized infants. However, the SPT is more sensitive than analyses of allergen-specific IgE levels for identifying sensitization.¹³ Fourth, eczema severity was not quantitatively evaluated 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 (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 CMA infants 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 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 breastfeeding.

8. Acknowledgments

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Fig 1. Study design.

Infants in the ingestion group ingested ≥ 10 mL of cow's milk formula (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 oral food challenges (OFCs) with a total of 50 mL (1st OFC) and 100 mL (2nd OFC) of CMF, respectively.

Fig 2. SPADE study enrollment, screening, and participant flow.

* At 1 month of age, participants underwent a screening oral food challenge (OFC) involving a single dose of 20 mL of cow's milk formula (CMF). §Infants in the ingestion group ingested ≥ 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 1st OFC consisted of a cumulative dose of 50 mL of CMF. 'The 2nd OFC consisted of a cumulative dose of 100 mL of CMF. SPT, skin prick test.

Fig 3. Primary outcome in the modified intention-to-treat analysis and per-protocol analysis.

Proportions of positive results in the cow's milk formula oral food challenge (cumulative dose of 100 mL) at 6 months of age in the (A) modified intention-to-treat analysis that included participants who withdrew voluntarily, but not those lost to follow-up; and (B) per-protocol analysis that only included participants who adhered to the assigned regimen. P values were calculated using Fisher's exact test. CMA: cow's milk allergy.

Fig 4. Wheal diameter of cow's milk and egg white skin prick test at 6 months of age.

P values were calculated using the Mann-Whitney U test.

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 (A) cow's milk-specific IgE, (B) casein-specific IgE, and (C) casein-specific IgG4 were measured in participants with cow's milk sensitization confirmed by the skin prick test. *P* values were calculated using the Mann-Whitney U test.

SUPPLEMENTARY TABLES

Table E1. Additional baseline characteristics of participants at randomization

Characteristics	Ingestion group (n=243)			Avoidance group (n=249)			P value
	Missing value	Number or median	Percentage or IQR	Missing value	Number or median	Percentage or IQR	
Gestational age (weeks)	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	0	32	28 - 36	0	32	29 - 36	.60
Maternal body mass index before pregnancy	3	20.7	19.2 - 23.1	2	21.2	19.5 - 23.4	.11
Maternal weight gain during pregnancy	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	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 using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. IQR, interquartile range.

Table E2. Clinical details of participants with positive results in the oral food challenge to cow's milk formula at 6 months of age

No.	Group allocation	Cumulative CMF (CMP) dosage at first appearance of symptoms, ml (mg)	Symptoms	Medication	Total IgE at 6 months of age (IU/mL)	Cow's milk-specific IgE at 6 months of age (kUA/L)	Casein-specific IgE at 6 months of age (kUA/L)	Casein-specific IgG4 at 6 months of age (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

CMF, cow's milk formula; CMP, cow's milk protein; LU, localized urticaria referring to noncontact urticarial reactions; SU, systemic urticaria; V, vomiting. [†]Excluded from the per-protocol analysis due to insufficient adherence to the assigned regimen (i.e., ingestion of CMF <10 days a month in the avoidance group and regular ingestion of CMF for ≥20 days a 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 (months)	Reason for withdrawal	Allergic reaction caused by the trial formula
Ingestion	2	Stopped taking cow's milk formula due to 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 oral food challenge 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 due to 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 oral food challenge 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)	<i>P</i> value
Total number of hospitalizations	17 (7.5%)	11 (4.7%)	.24
Reason for hospitalization			
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	

Data are presented as patient number (%). *P* value was calculated using Fisher's exact test.

Table I. Baseline characteristics of participants at randomization

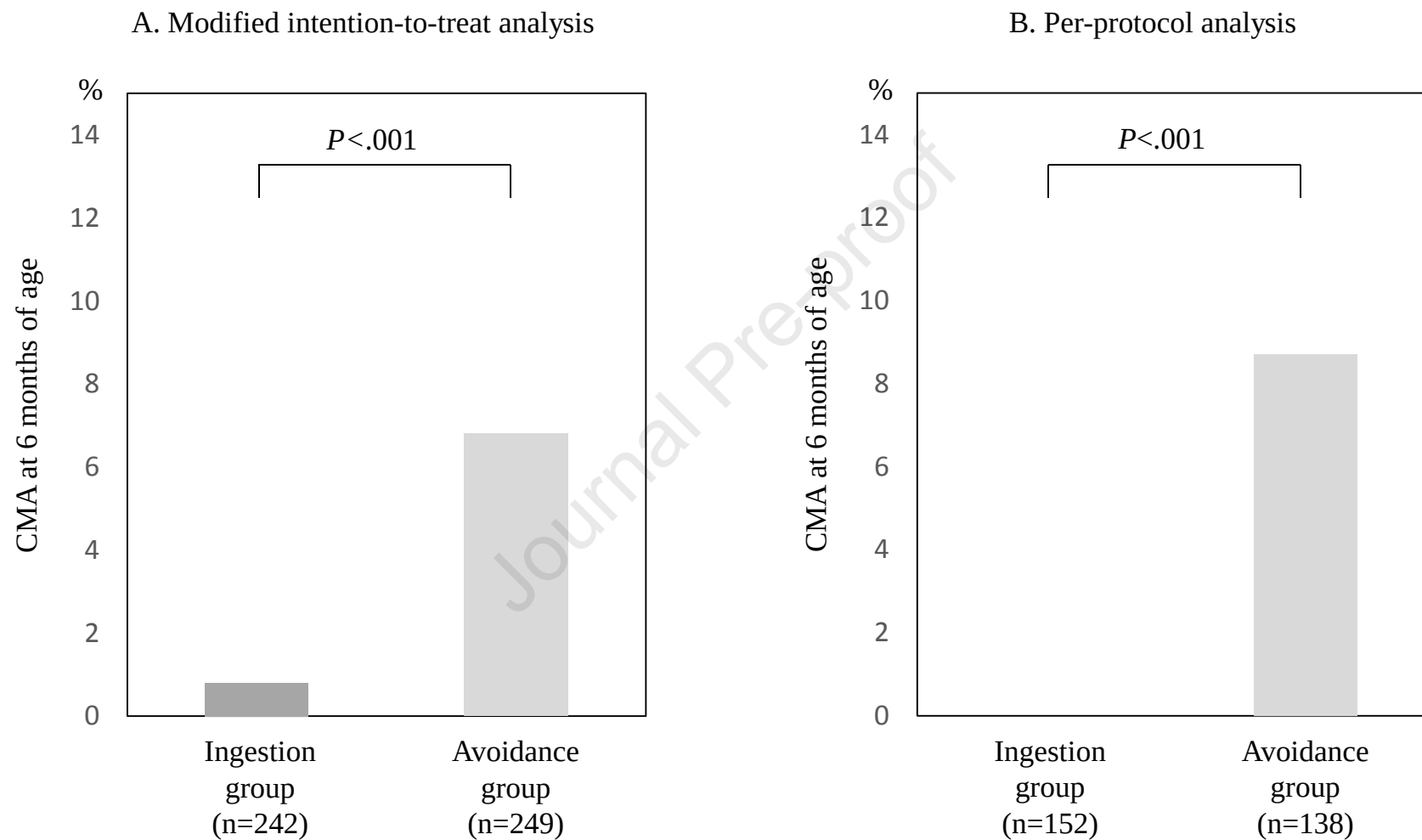
Characteristics	Ingestion group (n=243)			Avoidance group (n=249)			P value
	Missing value	Number or median	Percentage or IQR	Missing value	Number or median	Percentage or IQR	
Male	0	127	52.3	0	142	57.0	.32
Caesarean section	0	53	21.8	0	62	24.9	.46
Season of birth: spring/summer	0	155	63.8	0	168	67.5	.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 - >200	8	80	0 - >200	.61
Frequency 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

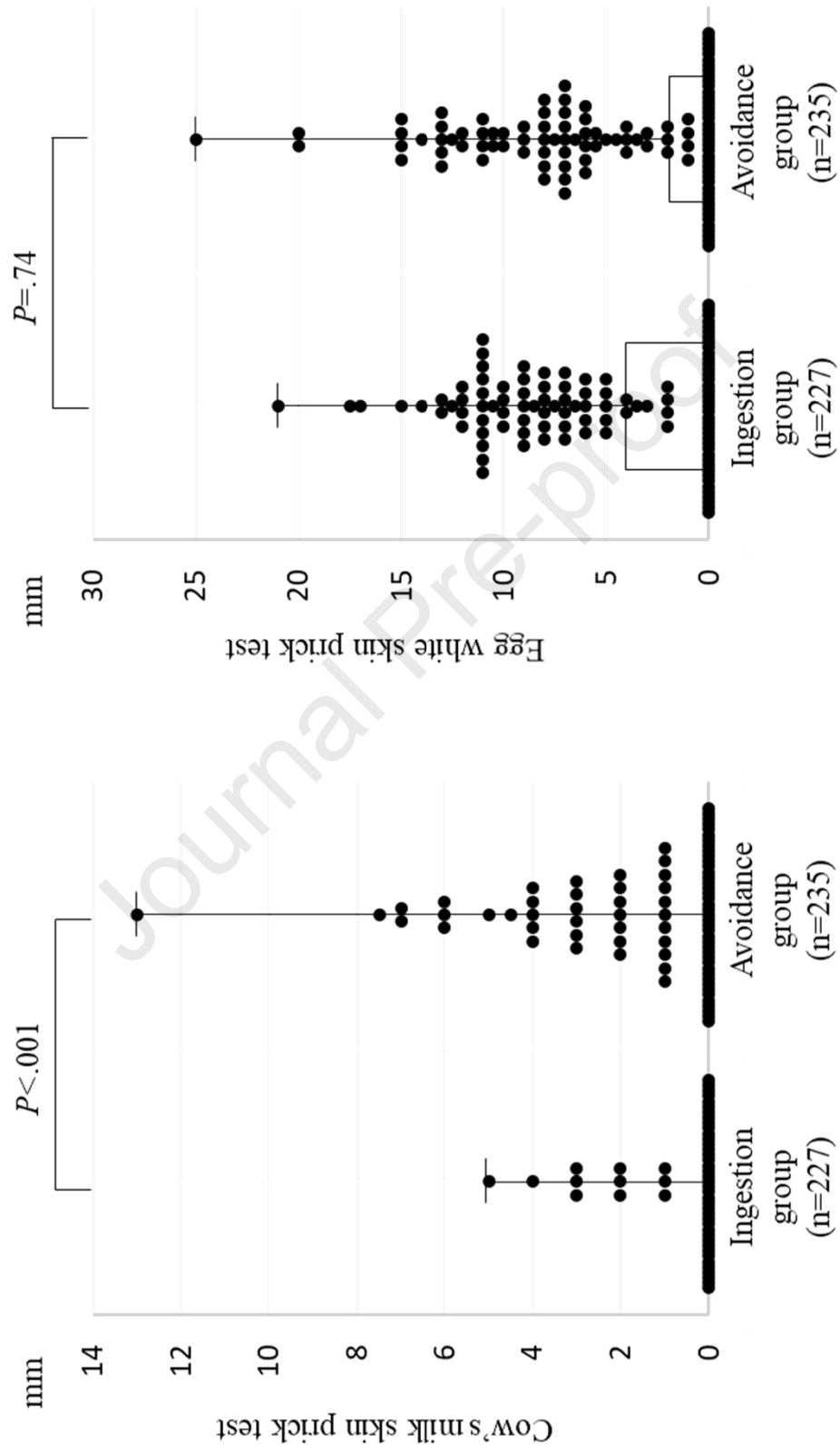
P values were calculated using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. IQR, interquartile range; CMF, cow's milk formula.

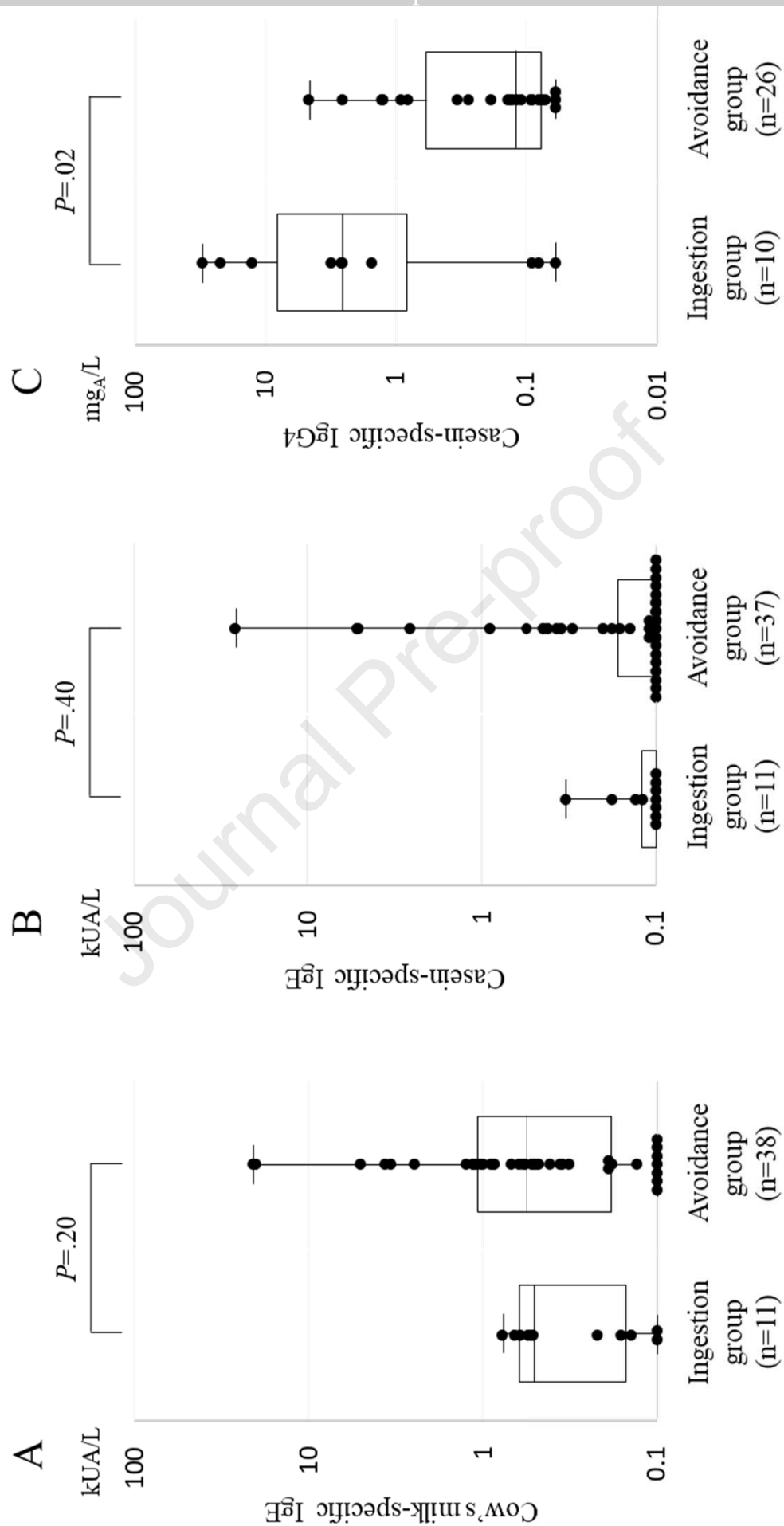
Table 1. Clinical outcomes in the intention-to-treat analysis population

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

P values were calculated using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. †Modified intention-to-treat analysis included participants who had confirmed the intake of dairy products after voluntarily withdrawing from the trial. IQR, interquartile range; CMF, cow's milk formula; SPT, skin prick test.







SUPPLEMENTARY FIGURES

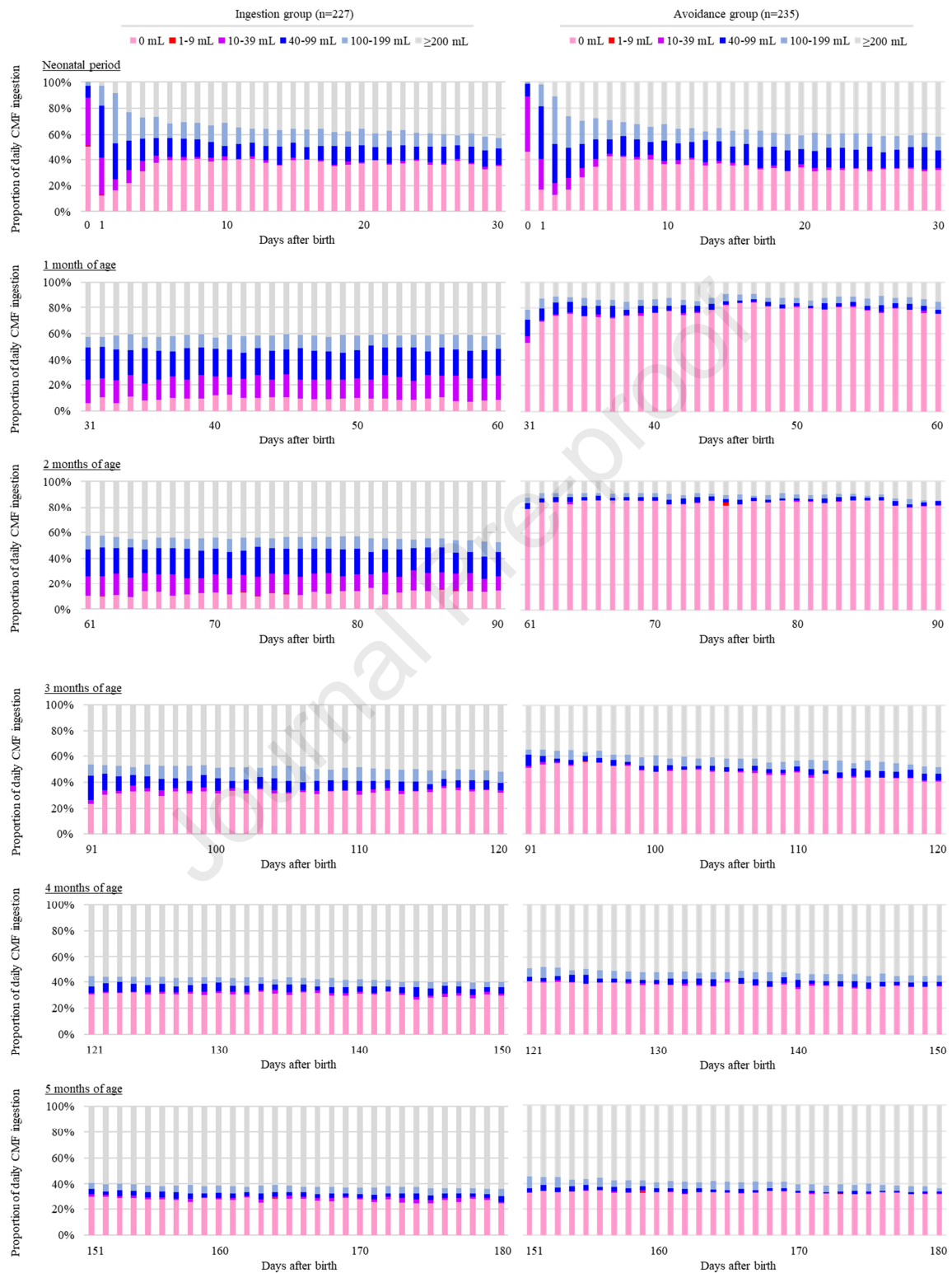


Fig E1. Proportions of daily CMF ingestion quantities

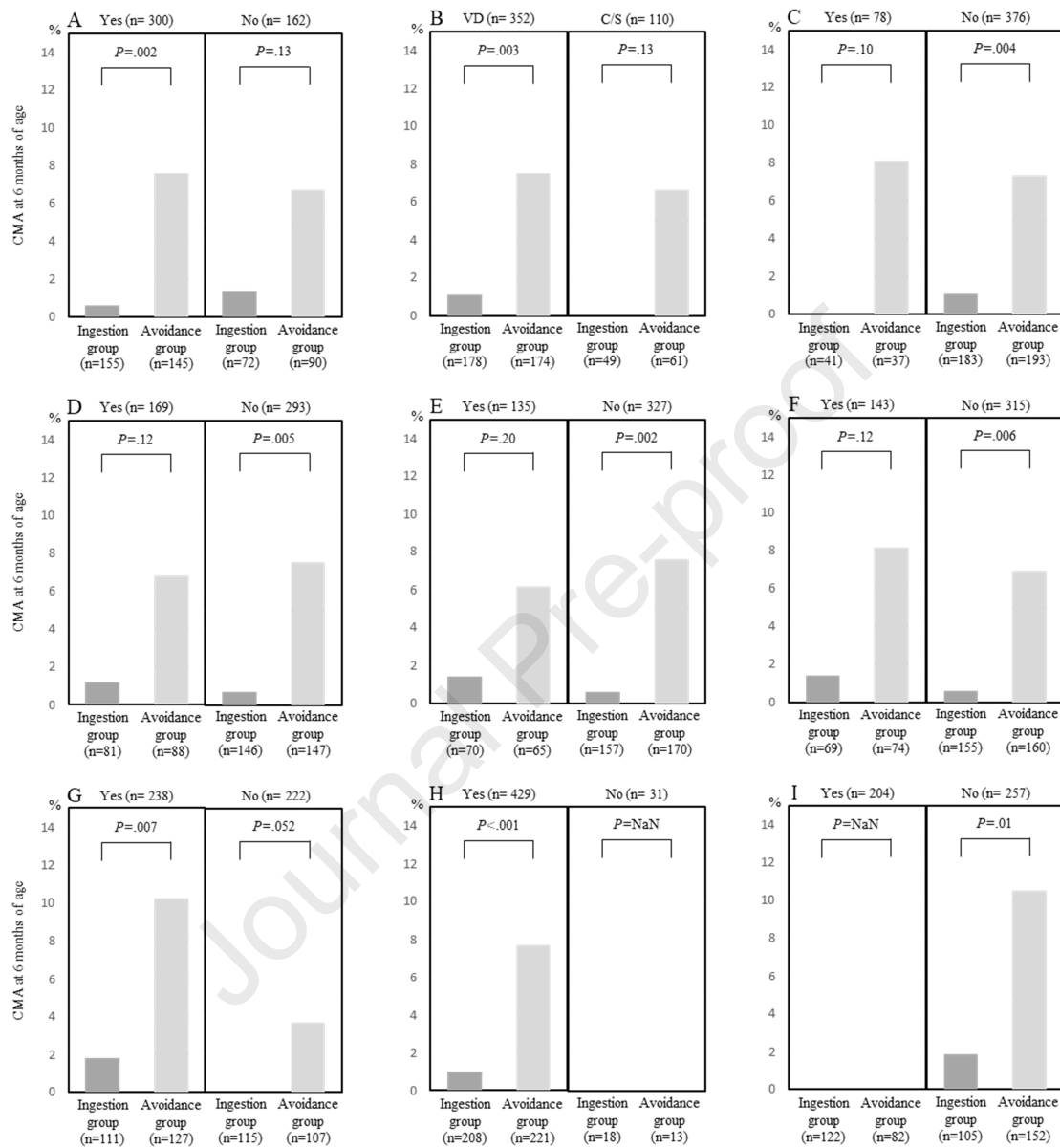


Fig E2. Outcomes of the post-hoc subgroup analyses

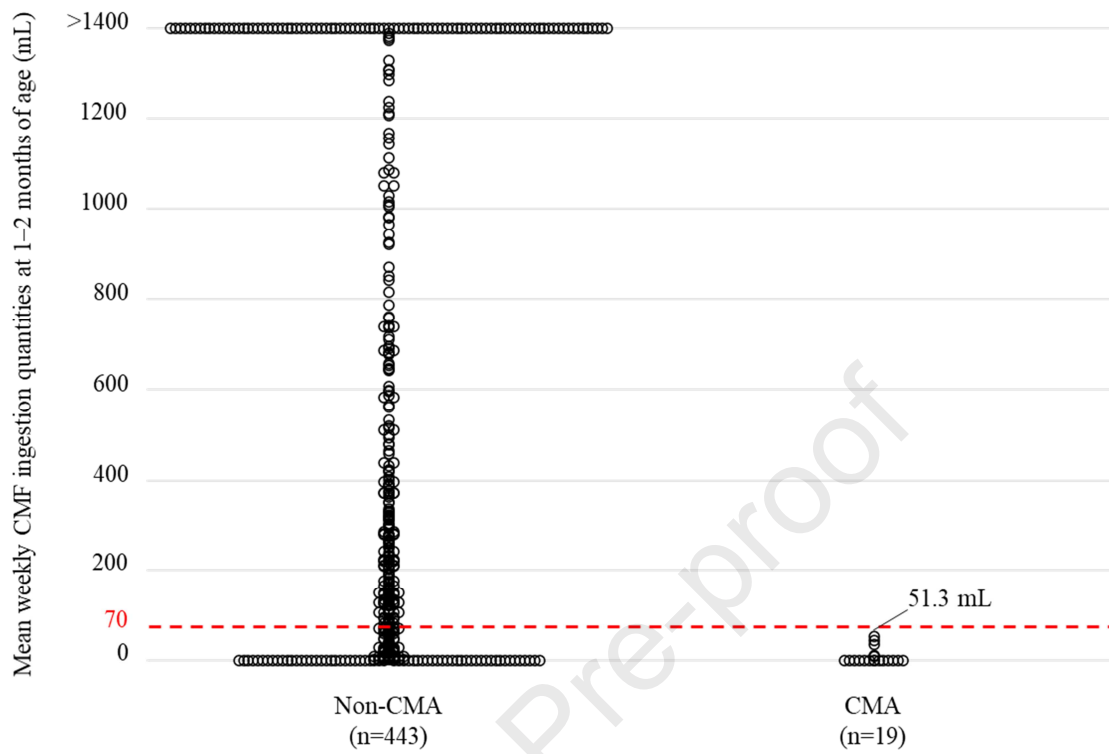
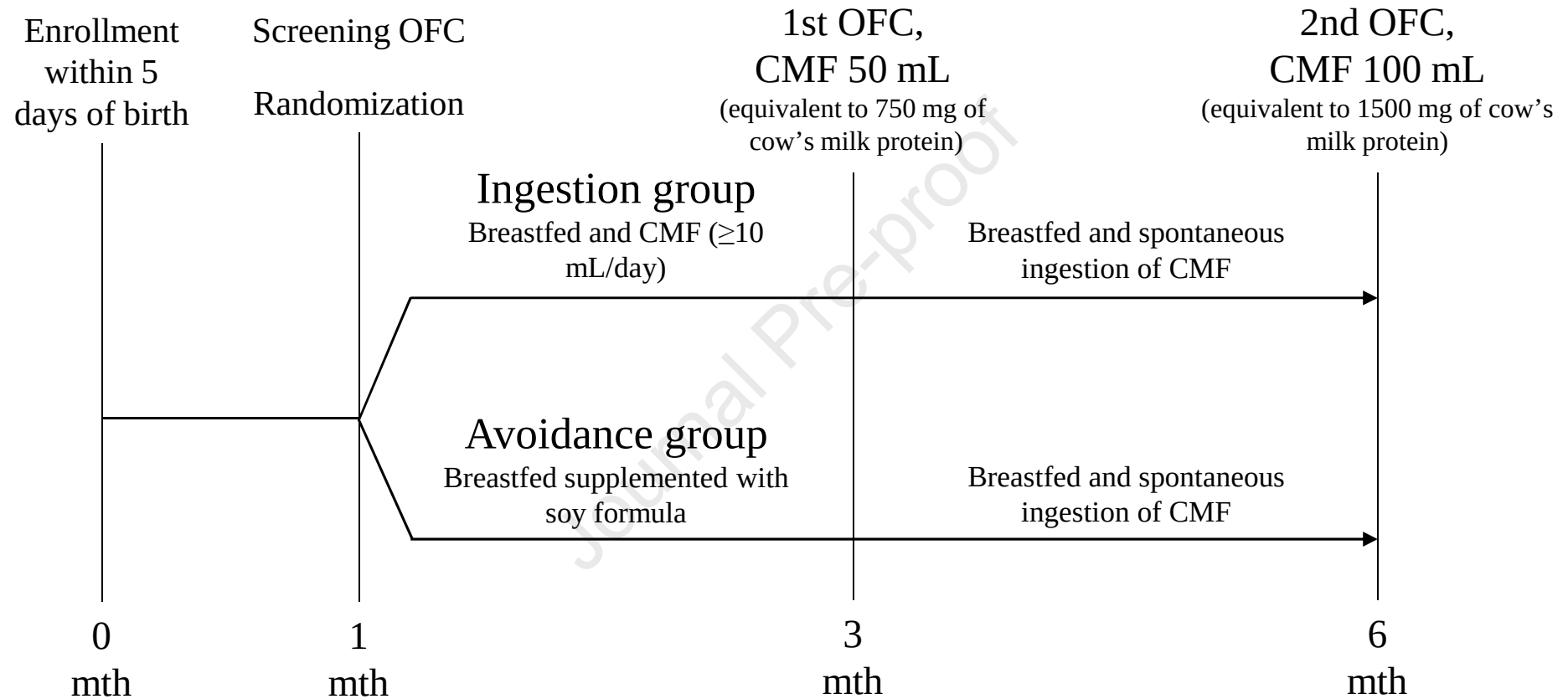
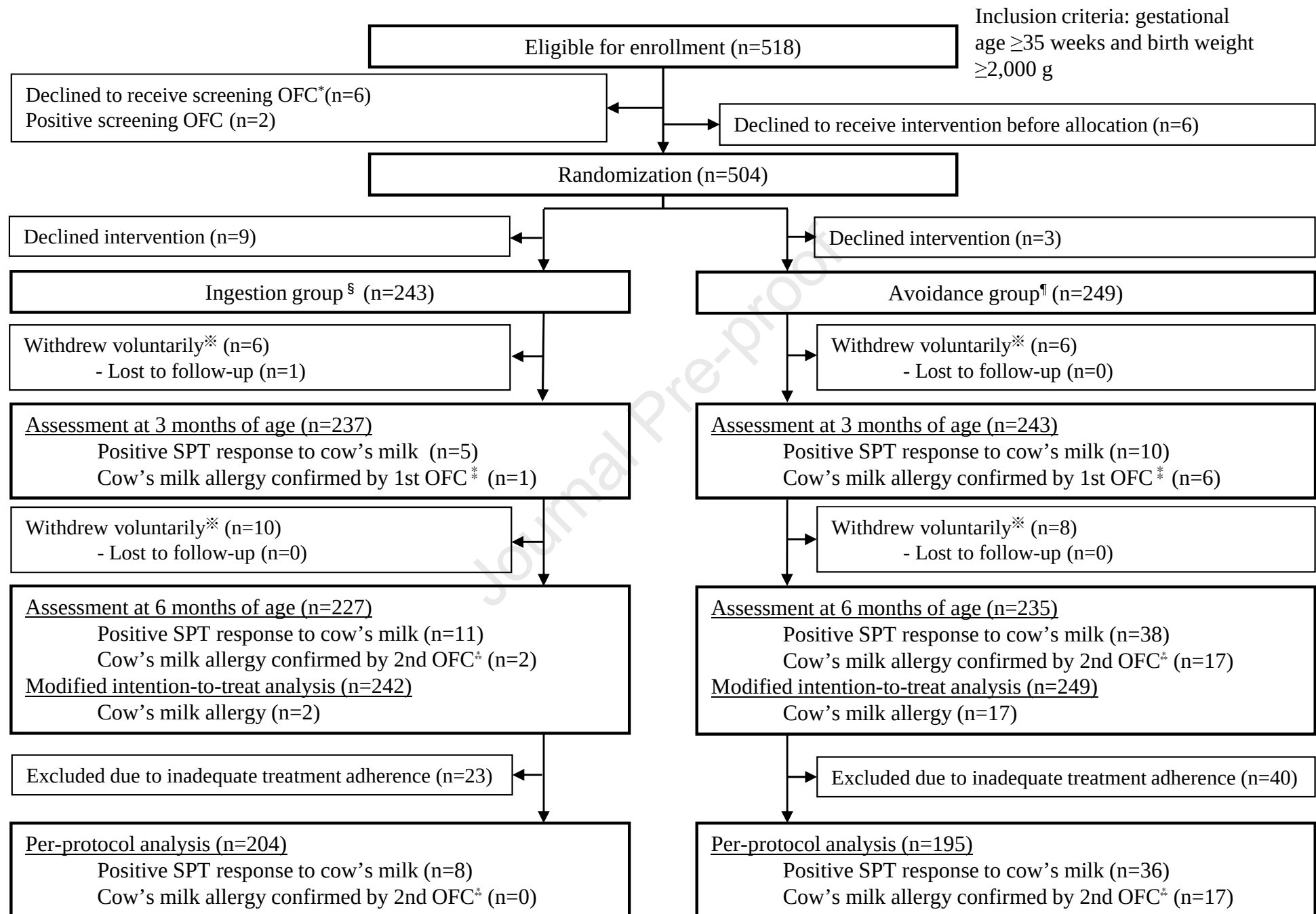


Fig E3. Mean weekly CMF ingestion quantities between 1 and 2 months of age in non-CMA and CMA infants





SUPPLEMENTARY METHODS

Oral food challenges

The 1st oral food challenge (OFC) was performed in both groups at 3 months of age, and consisted of a cumulative dose of 50 mL of cow's milk formula (CMF; equivalent to 750 mg of cow's milk protein). Infants who had a positive reaction to the cow's milk skin prick test (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 one time.

The 2nd 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 cow's milk protein). 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 one time.

In accordance with the Japanese Guidelines for Food Allergy 2017 criteria,^{E1} the OFC results were defined as positive for cow's milk allergy (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 1st OFC results continued to participate in the study, and underwent the 2nd 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 using the ImmunoCAP® system (Thermo Fisher Diagnostics K.K., 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 eczema had cleared, and were used intermittently to maintain remission. Monthly clinical assessments, including 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}

Skin prick tests

SPTs with commercial allergen extracts of cow's milk, egg white, wheat, and soy (Torii Pharmaceutical Co. Ltd., Tokyo, Japan) were performed using a standard technique. Histamine dihydrochloride (10 mg/mL) served as a positive control, and 50% glycerol solution was used as a negative control. A wheal diameter ≥ 3 mm larger than the negative control or larger than half 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 ingestion quantities of CMF between 1 and 2 months of age in CMA and non-CMA participants as a dose-response analysis.

Conflicts of interest

Participants in the ingestion group were provided "Meiji Hohoemi RakuRaku Cube®" infant formula, which was provided free of charge from Meiji Holdings Co. Ltd., Tokyo, Japan. The investigators provided a total of 2,000 mL of CMF per 2 months to each ingestion group participant. Participants in the avoidance group were provided "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 Bonlact i® 360 g 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, the decision to publish, or manuscript preparation.

SUPPLEMENTARY 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 of the intervention period. In all subgroups, the superiority of the ingestion group was maintained (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 ingested <70 mL of CMF per week between 1 and 2 months of age (Fig E3).

SUPPLEMENTARY 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 if CMF introduction from 3 months of age had a preventative effect on the development of CMA. In other words, some participants may have continued to ingest CMF because they did not have CMA.

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Fig E1. Proportions of daily CMF ingestion quantities

The figure shows the proportions of daily CMF ingestion quantities throughout the study period. The ingestion quantities were classified into 6 groups: 0 mL, 1–9 mL, 10–39 mL, 40–99 mL, 100–199 mL, and ≥ 200 mL. CMF, cow's milk formula.

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 (A) parental history of atopy, (B) mode of delivery, (C) perinatal (maternal and/or newborns within the first 7 days of life) antibiotic use, (D) eczema in the neonatal period, (E) eczema between 1 and 2 months of age, (F) eczema between 3 and 5 months of age, (G) ingestion of CMF in the first 24 hours after birth, (H) ingestion of CMF in the first 3 days of life, and (I) daily ingestion of CMF between 3 and 5 months of age. *P* values were calculated using Fisher's exact test. CMA, cow's milk allergy; CMF, cow's milk formula; VD, vaginal delivery; C/S, caesarean section; NaN, not a number.

Fig E3. Mean weekly CMF ingestion quantities between 1 and 2 months of age in non-CMA and CMA infants

This dose-response analysis evaluated the mean weekly CMF ingestion quantities from 1 to 2 months of age in participants with and without CMA. CMF, cow's milk formula; CMA, cow's milk allergy.