



Contents lists available at ScienceDirect

Allergy International

journal homepage: <http://www.elsevier.com/locate/alit>

Original Article

Early introduction of very small amounts of multiple foods to infants: A randomized trial

Tatsuo Nishimura^{a, *}, Mitsuru Fukazawa^b, Keisuke Fukuoka^c, Teruo Okasora^d, Shinichi Yamada^e, Shigeharu Kyo^f, Makoto Homan^g, Takuma Miura^h, Yasuyuki Nomuraⁱ, Shinya Tsuchida^j, Shigehiro Yajima^k, Satoshi Aoki^l, Yutaka Nakamura^m, Taisuke Hosakaⁿ, Hirokazu Hidaka^o, Hiroyuki Yamamori^p, Akira Inoue^q, Jiro Morimoto^r

^a Nishimura Pediatric Clinic, Osaka, Japan^b Fukazawa Pediatric Clinic, Fukuoka, Japan^c Fukuoka Pediatric and Allergy Clinic, Ehime, Japan^d Okasora Children's Clinic, Tottori, Japan^e Yamada Children's Clinic, Tokushima, Japan^f Miyahara Kid's Clinic, Saitama, Japan^g Ohisama Kodomo Clinic, Kagoshima, Japan^h Oozora Children's Clinic, Ibaraki, Japanⁱ Nomura Pediatric Clinic, Shiga, Japan^j Tsuchida Children's Clinic, Fukui, Japan^k Yajima Children's Clinic, Gifu, Japan^l Aoki Children's Clinic, Nara, Japan^m Yutaka Children's Clinic, Hyogo, Japanⁿ Hosaka Kid's Clinic, Osaka, Japan^o Hidaka Children's Clinic, Aichi, Japan^p Yamamori Pediatric Clinic, Saitama, Japan^q Pediatrics and Dermatology Clinic of Inoue, Saitama, Japan^r Kitaba Pharmacy, Osaka, Japan

ARTICLE INFO

Article history:

Received 7 October 2021

Received in revised form

28 January 2022

Accepted 5 February 2022

Available online xxx

Keywords:

Allergenic food
Early intervention
Infant
Powder
Weaning babies

Abbreviations:

AD, atopic dermatitis; EAT, inquiring about tolerance; FA, food allergy; MP, mixed powder; OFC, oral food challenge; POEM, patient-oriented eczema measure;

ABSTRACT

Background: We investigated whether multiple food allergies could be safely prevented by simultaneously administering very small amounts of multiple foods.

Methods: Infants 3–4 months old with atopic dermatitis from 14 primary care pediatric clinics in Japan were enrolled in this randomized, placebo-controlled trial. The infants were administered either mixed allergenic food powder (MP) containing egg, milk, wheat, soybean, buckwheat, and peanuts, or placebo powder (PP). The amount of powder was increased in a stepwise manner on weeks 2 and 4, and continued until week 12. The occurrence of food allergy episodes after powder intervention was assessed at 18 months old. This trial was registered with the University Hospital Medical Information Network Clinical Trials Registry (number UMIN000027837).

Results: A total of 163 participants were randomly allocated to either the MP group ($n = 83$) or the PP group ($n = 80$). The incidence of food allergy episodes by 18 months was significantly different between the MP and PP groups (7/83 vs. 19/80, respectively; risk ratio 0.301 [95% CI 0.116–0.784]; $P = 0.0066$). Egg allergies were reduced in the MP group. In addition, food allergy episodes from any of the other five foods were significantly reduced, although the reductions in those due to individual foods were not significant.

* Corresponding author. Nishimura Pediatric Clinic, 3-9-3, Kokubu Hommachi, Kashiwara, Osaka 582-0021, Japan.

E-mail address: tatsuo172460@gmail.com (T. Nishimura).

Peer review under responsibility of Japanese Society of Allergy.

<https://doi.org/10.1016/j.alit.2022.03.001>

1323-8930/Copyright © 2022, Japanese Society of Allergy. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

PP, placebo powder; RCT, randomized controlled trial; RR, risk ratio; TARC, thymus and activation-regulated chemokine; UMIN-CTR, University Hospital Medical Information Network Clinical Trials Registry

Conclusions: Gradually increasing the intake of very small amounts of multiple foods in early infancy can safely reduce the incidence of egg allergies. Other foods may also suppress food allergies, but no definitive conclusions could be reached.

Copyright © 2022, Japanese Society of Allergology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Feeding allergenic foods earlier during weaning has been reported to prevent food allergies (FA).^{1–4} Several randomized controlled trials (RCTs) have suggested a possible protective role of the early introduction of individual allergenic foods, such as eggs, milk, and peanuts.^{5–7} However, some RCTs suggested that this approach may be problematic as certain infants may be sensitized to foods early and develop allergic reactions.^{7–10} Moreover, children can be allergic to many foods,¹¹ so it is necessary to establish safe ways to prevent allergies to multiple foods. The Enquiring About Tolerance (EAT) study¹² was the first trial in which six allergenic foods were given from the early weaning period. However, the intention-to-treat analysis did not show a significant difference in the onset of FA, because only 30% of the intervention group complied with the protocol. This may have been because it was a burden for parents to give their baby six allergic foods several times a week.

Therefore, a method to avoid the induction of allergic reactions in infancy and to facilitate the administration of multiple foods to infants is required. Here, we used a mixed powder (MP) containing very small amounts of six allergic foods that are easy for babies to eat. This study was performed to evaluate the efficacy of incremental administration of MP for prevention of FA.

Methods

Study design and participants

This was a multi-center, randomized, controlled trial conducted in Japan. Infants 3–4 months old with eczema diagnosed with atopic dermatitis (AD) according to the U.K. Working Party's Diagnostic Criteria¹³ at 14 primary care clinics in Japan were recruited into this trial.

From the literature,⁶ we estimated that the incidence of FA in infants of similar age would be 20% and expected a reduction of 13% in the incidence by administration of allergenic foods. By setting the α and β errors as 0.05 and 0.20, respectively, we estimated that 92 infants per group were needed for the trial. Therefore, we planned to recruit 200 infants, allowing a loss to follow-up of about 10%. The exclusion criteria were birth before gestational week 37, birth weight <2500 g, history of anaphylaxis reaction, diagnosis of congenital immunodeficiency syndrome, refusal of treatment with steroid ointment, and complications of any severe disease. Written informed consent was obtained from the parents of the participants at the time of enrollment. The study was approved by the institutional review board of Umezu Clinic. This trial was registered with the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR Trial, identifier UMIN000027837). The protocol is available from: http://nishimura-syounika.com/MP_Protocol.pdf.

Randomization and masking

Participants were randomly assigned to the MP or placebo powder (PP) groups in a 1:1 ratio. A block randomization method with a block size of 4 was adopted. A four-digit number was

assigned according to each facility number and administration order by TN using a computer. The MP/PP powder, packed in an opaque black plastic bag with the assignment number, was sent by TN to each participating clinic.

The attending physician and the parents of the participants were not informed of the assignment information during the study period, and the assignment was disclosed only after completion of all clinical trials. KF managed the data from the attending physician and MF assessed the primary and secondary outcomes. In this way, participants, their caregivers, interveners, and outcome assessors were masked from all assignment information during the trial.

MP and PP

MP containing very small amounts of six allergenic foods (pasteurized dried egg white, milk powder, wheat, soybeans, buckwheat, and peanuts) and three gut flora preparations, i.e., Biofermin Powder® (*Streptococcus faecalis*, *Bacillus subtilis*), Miya-BM Powder® (*Clostridium butyricum*), and Lac-B Granular Powder® (*Bifidobacterium*) were used. Gut flora preparations were used as a base material, because they are safe, have a pleasant taste, and are easy to administer.

MP-1, MP-2, and MP-3 contained 2.5 mg, 7.5 mg, and 20 mg of each allergenic food, respectively. PP-1, PP-2, and PP-3 contained only incremental amounts of gut flora preparations. Table 1 shows the amounts of proteins contained in MPs, and Supplementary Table 1 shows the amounts of foods corresponding to the proteins contained in MP-1. As MP and PP are slightly different in color, they were distributed to each clinic in opaque black plastic bags. MP and PP were produced by JM according to the prescription by TN.

Table 1

The compositions of mixed food and placebo powder.

Mixed powder (MP)		MP-1	MP-2	MP-3
Content, mg	Egg (meringue powder)	2.5 (2.2)	7.5 (6.6)	20 (17.6)
(protein, mg)	Milk (infant formula)	2.5 (0.3)	7.5 (0.9)	20 (2.4)
	Wheat (flour)	2.5 (0.2)	7.5 (0.6)	20 (1.6)
	Soybeans (roasted soybean flour)	2.5 (0.9)	7.5 (2.7)	20 (7.2)
	Buckwheat (buckwheat flour)	2.5 (0.3)	7.5 (0.9)	20 (2.4)
	Peanuts (peanuts flour)	2.5 (0.6)	7.5 (1.8)	20 (4.8)
	Biofermin Powder®	30	37.5	40
	Miya-BM Powder®	30	37.5	20
	Lac-B Granular Powder®	25	30	20
	Total	100	150	200
Placebo powder (PP)		PP-1	PP-2	PP-3
Content, mg	Biofermin Powder®	35	54	100
	Miya-BM Powder®	35	54	50
	Lac-B Granular Powder®	30	42	50
	Total	100	150	200

MP containing small amounts of six foods (egg, milk, wheat, soybeans, buckwheat, and peanuts) and three gut flora preparations, i.e., Biofermin Powder® (*Streptococcus faecalis*, *Bacillus subtilis*), Miya-BM Powder® (*Clostridium butyricum*), and Lac-B Granular Powder® (*Bifidobacterium*), was used. PP contained only gut flora preparations.

MP, mixed powder; PP, placebo powder.

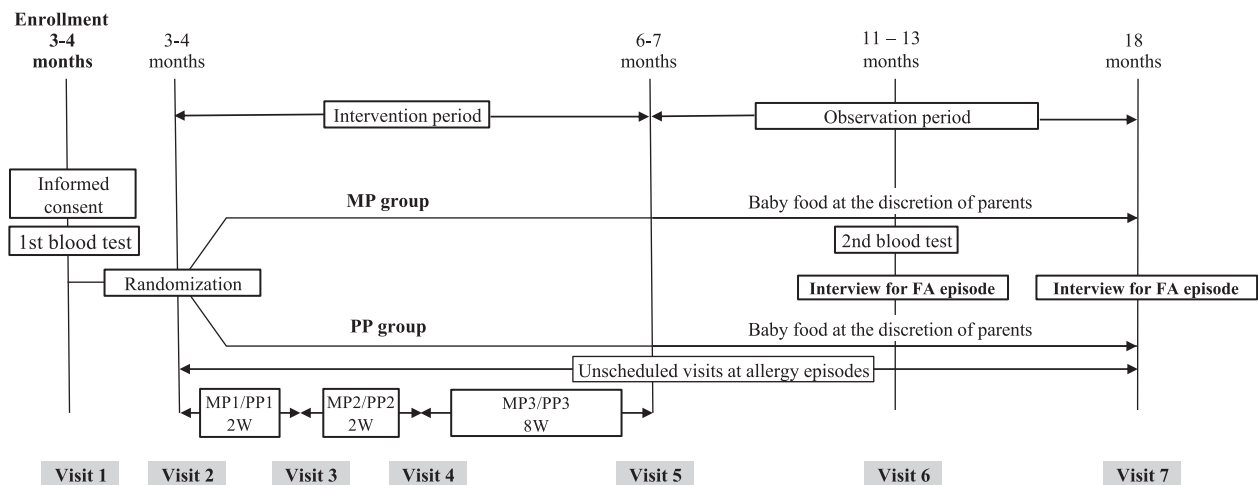


Fig. 1. Study design. Participants consumed the allocated MP-1/PP-1 powder orally from 3 to 4 months of age daily for 2 weeks, MP-2/PP-2 powder for the next 2 weeks, and then MP-3/PP-3 powder for additional 8 weeks. After the intervention period, the parents were instructed to feed allergenic foods carefully and to consult a doctor if symptoms suspected of food allergies were observed. MP, mixed powder; PP, placebo powder; FA, food allergy.

Procedures (Fig. 1)

Visit 1: When babies aged 3–4 months were brought to the clinic with a chief complaint of eczema, the attending physicians determined whether they were eligible to participate in the study after an interview with their parents and medical checks. Written informed consent was obtained from the parents of the participants at the time of enrollment. The attending physicians then contacted TN, and performed the first blood test. They also recorded each participant's skin condition using the patient-oriented eczema

measure (POEM),¹⁴ and then started treatment for eczema following the method of Natsume *et al.*⁶ Participants were assigned a four-digit number based on the facility and entry order.

Visit 2 (Week 0): The attending physicians explained the results of the blood tests to the caregivers and continued to treat the eczema. The facility staff, other than the attending physicians, matched the opaque black bags with the corresponding number among the pre-sent bags. In the other rooms, MP-1/PP-1 from the opaque black bags was then given to the babies and observed for 30 min under the supervision of the attending physicians. The

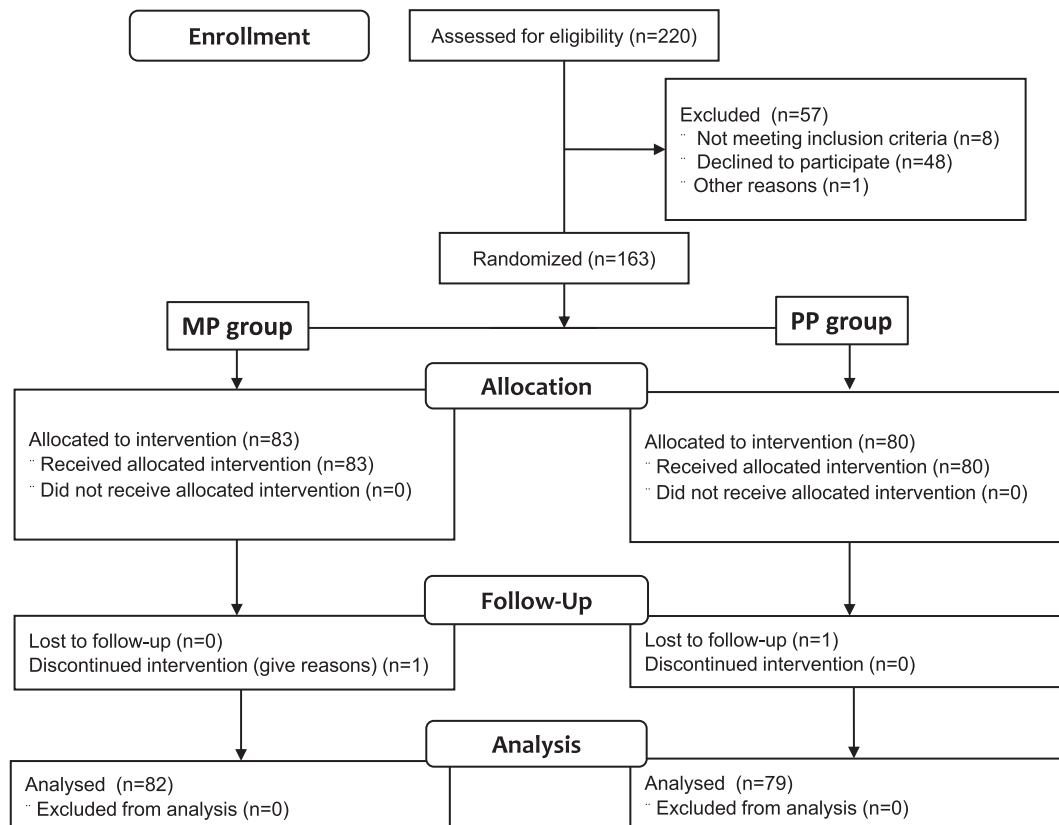


Fig. 2. Study enrollment, screening, and participant flow. MP, mixed powder; PP, placebo powder.

caregivers were instructed to take the bag of the powder home and feed the baby MP-1/PP-1 powder at home for the next 2 weeks unless serious adverse health effects occurred, such as serious anaphylaxis or severe infections. Caregivers were instructed to record subsequent symptoms in the event diaries (Supplementary Fig. 1) and not to give egg, milk, wheat, soybeans, buckwheat, and peanuts during the intervention period. However, infant formula was not restricted if breast milk was scarce.

Visit 3 (Week 2): The attending physicians checked the event diaries, and the studies were continued if 80% or more of the powders had been taken. Participants were given the MP-2/PP-2 powder and observed for 30 min under the supervision of the attending physicians. The caregivers were instructed to feed the babies MP-2/PP-2 powder at home for the next 2 weeks.

Visit 4 (Week 4): The attending physicians checked the event diaries, and the studies were continued if 80% or more of the powders had been taken. Participants were given the MP-3/PP-3 powder and observed for 30 min under the supervision of the attending physicians. The caregivers were then instructed to feed the babies MP-3/PP-3 powder at home for the next 8 weeks.

Visit 5 (Week 12): At the end of the intervention, the attending physicians performed clinical evaluation of the participants, reviewed the event diaries, and recorded the POEM score. In addition, parents were instructed to carefully increase the amount of baby food in a stepwise manner and were given a pamphlet on how to proceed with baby food (Supplementary Fig. 2). The starting amount of weaning stated on the pamphlet and the target intake amounts at 12 and 18 months of age were determined with reference to the Japanese guidelines for bottle-feeding and weaning

(Supplementary Fig. 3).¹⁵ Parents were also instructed to consult their doctor if symptoms of suspected FA appeared.

Visit 6 (11–13 months): At the age of 11–13 months, participants were brought to each clinic for clinical evaluation and a second blood test. Caregivers were interviewed about whether they had fed their children the six allergenic foods in the weaning process and to describe any suspected FA symptoms.

Visit 7 (18 months): The participants were brought to each clinic again at 18 months old for clinical assessments. The attending physician interviewed the families who visited the clinic or called the families who did not visit to investigate whether the children had symptoms of presumed FA.

Unscheduled visit: If the participants had symptoms suggesting an immediate allergic reaction during the study period, such as urticaria, persistent cough, wheezing, vomiting, or diarrhea, the families were invited to make an unscheduled clinic visit. Participants underwent blood tests and/or the open Oral Food Challenge (OFC) test as needed to confirm the diagnosis of FA.

Blood test and food sensitization

We measured complete blood count, serum concentrations of TARC, total IgE, and IgE specific to egg white, milk, wheat, soybeans, buckwheat, and peanuts with ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) at Visits 1 and 6. Food-specific IgE was also measured during an unscheduled visit if deemed necessary by the attending physician. The sensitization status for each food was determined when specific IgE levels were >0.1 kUA/L, based on the EAT study.¹⁶

Table 2
Baseline characteristics of participants.

Compounds	MP group (n = 83)			PP group (n = 80)			P value
	Missing value	No. or median	Percentage or IQR	Missing value	No. or median	Percentage or IQR	
Gestational age (weeks)	1	39	38–40	3	39	37–40	0.35
Birthweight (g)	0	3122	2902–3360	1	3166	2936–3396	0.58
Caesarean section	2	13	15.7%	3	19	23.8%	0.18
Male sex	0	53	63.9%	4	46	57.5%	0.67
Season of birth: spring/summer	0	43	51.8%	0	36	45.0%	0.39
Breastfeeding during the neonatal period [†]	1	56	67.5%	3	45	56.3%	0.20
Have pets	0	15	18.1%	2	22	27.5%	0.13
Age at first administration (days)	0	110	99–132	0	111	97–128	0.77
Weight at first administration (g)	0	6365	5806–6923	0	6360	5945–6935	0.73
Predominant breastfeeding	2	65	80.2%	4	53	69.7%	0.13
Maternal food allergy	0	4	4.8%	0	7	8.8%	0.32
Maternal bronchial asthma	0	4	4.8%	0	10	12.5%	0.08
Maternal atopic dermatitis	0	15	18.1%	0	22	27.5%	0.15
Paternal food allergy	0	7	8.4%	0	11	13.8%	0.28
Paternal bronchial asthma	0	17	20.5%	0	11	13.8%	0.26
Paternal atopic dermatitis	0	19	22.9%	0	11	13.8%	0.13
POEM score	0	9	4–14	1	9	5–15	0.44
White blood cell count (/μl)	7	10,200	8750–11,850	5	10,000	8475–12,250	0.99
Eosinophil count (/μl)	16	702	465–1222	13	678	382–1060	0.28
IgE (IU/mL)	4	13	5–27	3	14	5–36	0.71
TARC (pg/mL)	2	4370	2377–7650	3	4325	2490–8434	0.96
Egg white-specific IgE (≥ 0.1 kUA/L)	1	44	53.0%	0	34	42.5%	0.12
Cow's milk-specific IgE (≥ 0.1 kUA/L)	5	23	27.7%	4	28	35.0%	0.20
Wheat-specific IgE (≥ 0.1 kUA/L)	6	7	8.4%	3	5	6.3%	0.41
Soybeans-specific IgE (≥ 0.1 kUA/L)	6	5	6.0%	3	2	2.5%	0.24
Peanuts-specific IgE (≥ 0.1 kUA/L)	6	5	6.0%	3	2	2.5%	0.24
Buckwheat-specific IgE (≥ 0.1 kUA/L)	8	4	4.8%	4	1	1.3%	0.20
Sensitization to 1 or more foods (≥ 0.1 kUA/L)	8	51	61.4%	5	45	56.3%	0.30

IQR, interquartile range; MP, mixed powder; PP, placebo powder; POEM, patient-oriented eczema measure; TARC, thymus and activation-regulated chemokine.

[†] Exclusively breastfeeding at home regardless the induction of infant formula several times after birth.

Table 3

Details of cases with FA events.

Case no	age (month)	food	Non-symptomatic feeding before the episode	Symptoms
MP				
1	7	Egg white	Tiny amounts	Partial urticaria
2	8	Peanuts	Not fed	Partial erythema
3	9	Milk	Tiny amounts	Partial urticaria
	14	Egg white	Egg yolk only	Wheezing
4	10	Egg white	Tiny amounts	Partial urticaria
5	12	Egg white	Not fed	Partial urticaria
6	12	Milk	Not fed	Partial urticaria
	12	Egg white	Not fed	Partial urticaria
7	12	Soybean	Not fed	Wheezing
PP				
1	6	Wheat	Tiny amounts	Partial urticaria
2	6	Milk	Not fed	Systemic urticaria, wheezing
3	7	egg white	Egg yolk only	Partial urticaria, vomiting
4	7	Wheat	Not fed	Partial urticaria
	9	Egg white	Not fed	Partial urticaria
5	7	Egg white	Not fed	Partial urticaria
6	8	Egg white	Not fed	Partial urticaria
7	8	Egg white	Egg yolk only	Systemic urticaria
8	8	Egg white	Not fed	Partial urticaria
9	8	Milk	Not fed	Systemic urticaria
10	8	Milk	Not fed	Systemic erythema
11	8	Egg white	Not fed	Partial urticaria
12	8	Egg white	Tiny amounts	Vomiting
13	9	Milk	Not fed	Partial urticaria, vomiting
	9	Egg white	Not fed	Partial urticaria, cough, vomiting
14	9	Peanuts	Not fed	Systemic urticaria
15	11	Wheat (bread)	Udon only	Systemic urticaria, cough
	12	Egg white	Tiny amounts	Partial urticaria
16	12	Milk	Tiny amounts	Systemic urticaria
17	12	Egg white	Eating normally according to age	Vomiting
	14	Peanuts	Not fed	Partial urticaria, vomiting
18	12	Milk	Tiny amounts	Partial urticaria
	12	Egg white	Tiny amounts	Partial urticaria
19	13	Egg white	Tiny amounts	Partial urticaria

Some participants had up to two allergic episodes with different foods, they had no repeated episodes caused by the same food. There were no participants who had allergic episodes with three or more food items. FA, food allergy; MP, mixed powder; PP, placebo powder.

Outcomes

Primary outcome: Comparison of FA episodes from the end of the intervention to 18 months of age between the two groups.

The diagnosis of FA was made when there were food sensitizations and any of the following: episodes in which a causal relationship was evident from the time of feeding and the time of onset of symptoms; episodes with reproducibility; and/or episodes confirmed by OFC.

Secondary outcomes: Comparison of specific IgE levels for egg white, milk, wheat, soybeans, buckwheat, and peanuts measured at 11–13 months of age between the two groups.

Safety outcomes: Comparison of adverse events during the intervention period between the two groups.

Statistical analysis

The per-protocol population included participants who consumed MP/PP at least 80% of the planned quantity.

We performed intention-to-treat and per-protocol analyses. Between-group comparisons were performed using Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. The risk ratios (RRs) and 95% CIs for the outcome measures were calculated. In all analyses, $P < 0.05$ was taken to indicate statistical significance. All statistical analyses were performed using R software (version 2.9.2; R Foundation for Statistical Computing, Vienna, Austria).

Results

Study population

The study population is outlined in a Consolidated Standards of Reporting Trials diagram in Figure 2. Study enrollment took place from July 1, 2017, to June 30, 2019. Data collection was completed on September 30, 2020, as originally planned. Parents of 163 infants agreed to participate in the study. Participants were randomized into two groups (MP group, $n = 83$; PP group, $n = 80$). The parents of one infant in the MP group refused to continue providing powder due to inconvenience, and one infant in the PP group dropped out during the follow-up period. A total of 161 (98.8%) participants completed the 12-week intervention.

A total of 142 (87.1%) participants attended their scheduled visits at 11–13 months of age to undergo the blood test. With the exception of the two who dropped out, the parents of all participants when the infant was 18 months old were asked at the clinic (155 cases) or by telephone (6 cases) about any FA episodes attributable to egg, milk, wheat, soybeans, buckwheat, and peanuts.

Baseline characteristics

The participants' baseline characteristics are summarized in Table 2. There were no significant differences in the baseline clinical characteristics or blood test data between the two groups.

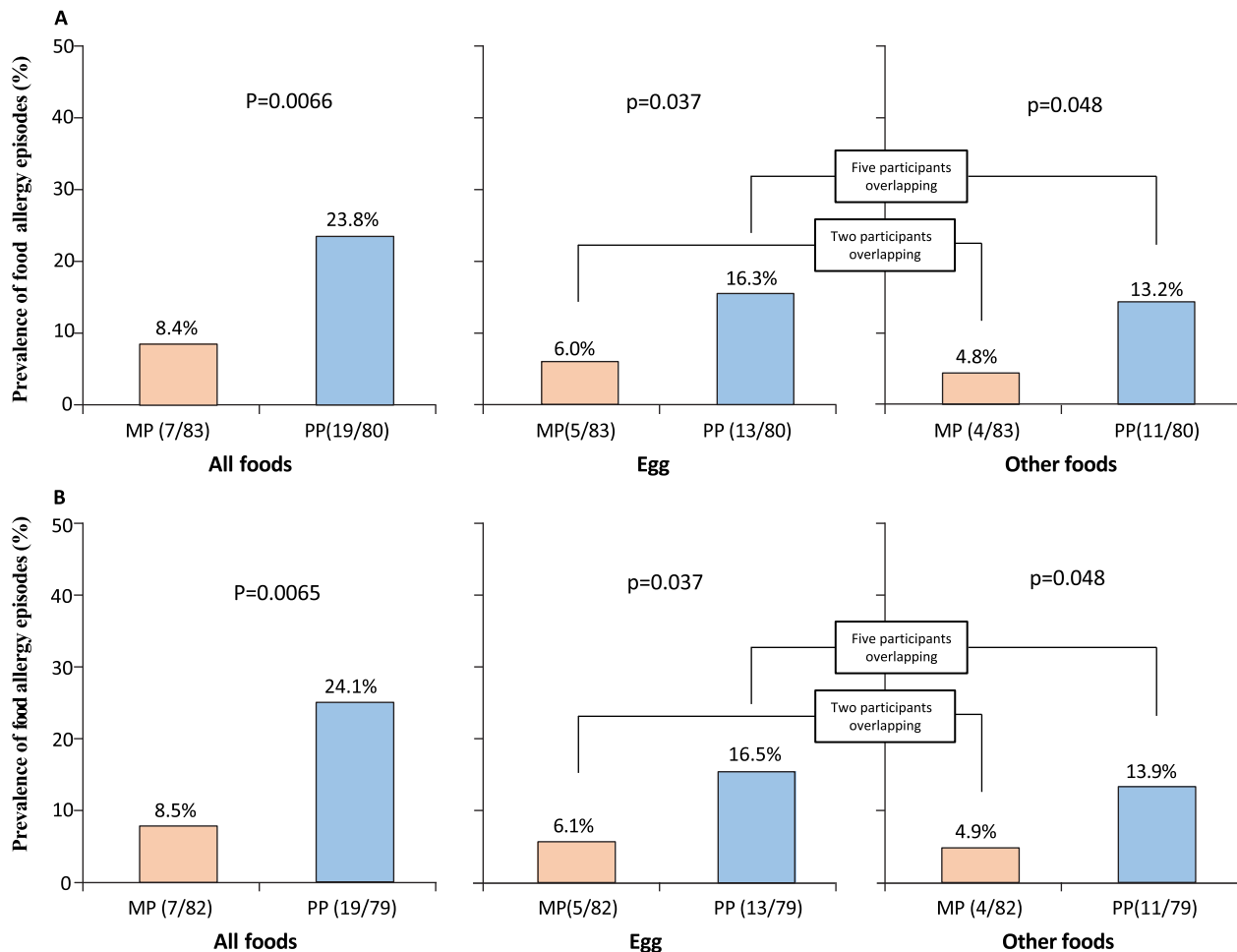


Fig. 3. Prevalence of FA. (A) Intention-to-treat analysis (B) per-protocol analysis. The left panels show all food allergy episodes, the middle panels show egg allergy episodes, and the right panels show allergy episodes other than egg. MP, mixed powder; PP, placebo powder; FA, food allergy.

Primary outcome measures: comparison of FA episodes between the two groups

FA episodes were counted from the induction of solid food after the intervention, but not from the intake of experimental powder. Of the 49 episodes suspected to be related to food intake, 16 were diagnosed as nonspecific reactions unrelated to FA based on the criteria described in the Methods section. Thirty-three episodes in 26 participants were diagnosed as FA as follows: 9 episodes with a clear time correlation between feeding and onset, 13 episodes with reproducibility, and 11 episodes with open OFC.

Overall, there were 9 episodes in 7 participants in the MP group and 24 episodes in 19 participants in the PP group. Details of the allergic episodes were as follows: 22 episodes of partial urticaria or erythema, 7 episodes of more extensive urticaria or erythema, 6 episodes of vomiting, and 5 episodes of respiratory symptoms, such as coughing and wheezing (Table 3). The primary outcomes are shown in Figure 3. In the intention-to-treat analysis, 7 of the 83 participants in the MP group (8.4%) and 19 of the 80 participants in the PP group (23.8%) developed one or more FA episodes as confirmed by the attending physicians. The RR was 0.301 [95% CI 0.116–0.784]; $P = 0.0066$. The foods that caused FA in the MP group were egg white in five cases, milk in two cases, and peanuts and soybeans in one case each. Those in the PP group were egg white in 13 cases, milk in 6 cases,

wheat in three cases, and peanuts in 2 cases. All infants who developed milk allergies were exclusively breastfed. There were no cases with serious symptoms requiring hospitalization.

Fifty-one participants in the MP group (61.4%) and 45 participants in the PP group (56.3%) were considered to have sensitization to one or more foods at enrollment (Table 2). Figure 4 shows a comparison of the incidences of FA with regard to sensitization status. The incidence of FA was significantly lower in the MP group than the PP group among the sensitized cases. The RR was 0.294 [95% CI 0.116–0.745]; $P = 0.0046$. On the other hand, there was no significant difference in the incidence of FA between the groups among nonsensitized cases.

Secondary outcome measures: comparison of six food-specific IgE levels between the two groups

The characteristics of participants at 11–13 months old are summarized in Table 4. There were no differences between the two groups regarding food status at weaning or other parameters, except egg white-specific IgE level. We investigated changes from baseline in six food-specific IgE levels in participants aged 11–13 months and the differences between the two groups. Sensitization rates at 11–13 months showed a significant increase for all six foods in both the MP and PP groups compared to baseline. The median

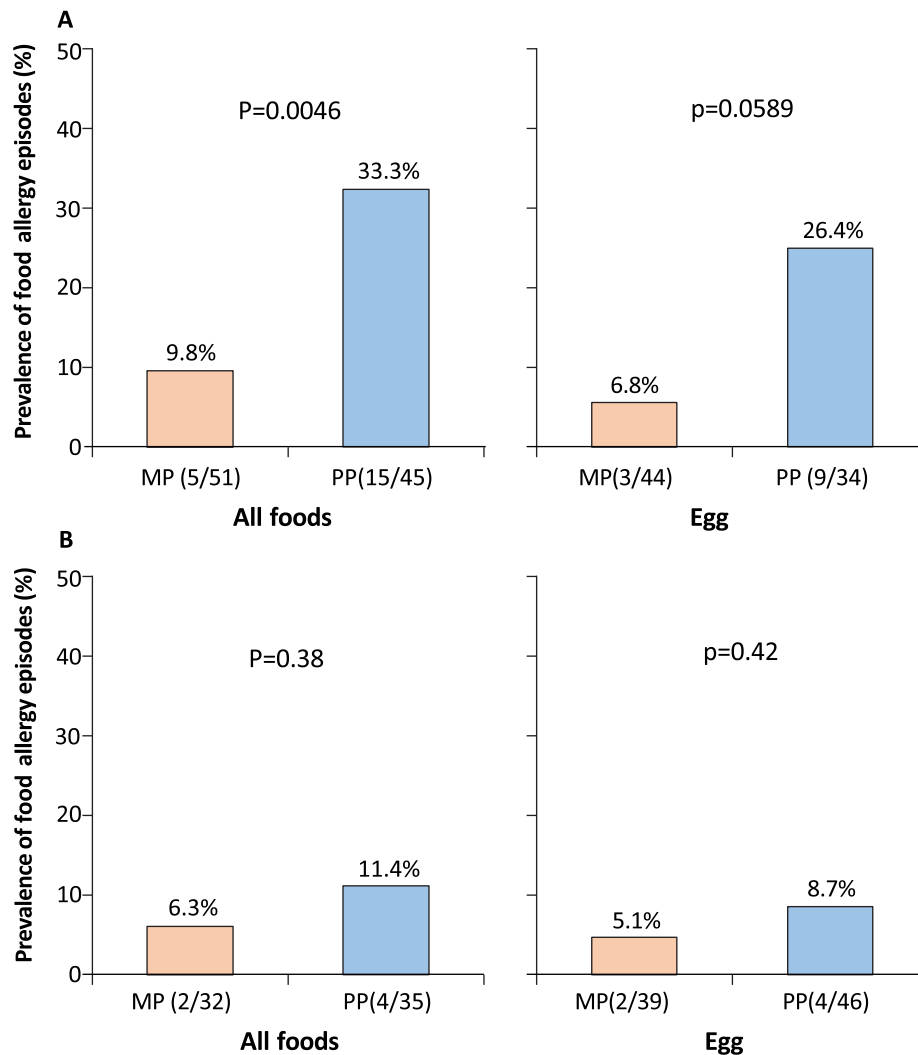


Fig. 4. Prevalence of FA among participants in the intention-to-treat analysis population. **(A)** Participants with sensitization at enrollment (specific IgE ≥ 0.1 kUA/L). **(B)** Participants without sensitization. The left panels show all food allergy episodes. The right panels show egg allergy episodes in participants with and without sensitization to egg white. MP, mixed powder; PP, placebo powder; FA, food allergy.

egg white-specific IgE levels were significantly lower in the MP group than the PP group (Fig. 5), but there were no significant differences in levels of IgE specific for the other five foods (Supplementary Fig. 4).

Safety outcomes

The MP group had a significantly higher incidence of adverse responses at the first trial of MP-1/PP-1 than the PP group (10/83 [12%] vs. 3/80 [3.75%], respectively, $P = 0.046$). The responses consisted of mild redness or small wheals a few millimeters in diameter on the skin of the face or body while under supervision of the attending physicians in the clinics. The same symptoms were observed several times at home, but no further strong symptoms were observed. These symptoms resolved after several repeated doses at home. The same reaction occurred when the powder was changed, but no strong symptoms were observed. Therefore, there was no need to interrupt the study due to allergic reactions.

There was no difference in the number of non-routine medical checkups of the participants during the intervention period between the MP and PP groups (Supplementary Table 2). During the study period up to 18 months, three participants in the PP group (RS

virus infection at 3 months of age, Kawasaki disease at 6 months of age, and febrile seizure at 1 year 2 months of age) and three participants in the MP group (poor feeding due to fever at 4 months of age, rotavirus infection at 6 months of age, and urinary tract infection at 11 months of age) were hospitalized.

Discussion

This study showed that starting feeding very small amounts of multiple foods for 12 weeks from 3 to 4 months of age can safely reduce the incidence of FA in high-risk children with AD.¹⁷ A safe, easy, and effective method to prevent multiple FA in children is required, and our findings showed promise for the development of such a preventive method.

Safety considerations

Feeding allergic foods to babies at high risk of FA can cause strong reactions, and extreme precautions should be taken to ensure safety. In fact, raw egg powder caused a strong allergic reaction in some RCTs, and so the intervention was discontinued.^{8,18} To avoid the risk, we started the intervention with very small amounts of antigenic

Table 4
Characteristics of participants at 11–13 months of age.

Characteristic	MP group (n = 83)			PP group (n = 80)			P value
	Missing value	No. or median	Percentage or IQR	Missing value	No. or median	Percentage or IQR	
Weight (g)	4	8800	8200–9480	7	9117	8575–9675	0.051
POEM score	3	3	0–6	3	2	0–7	0.86
Start of weaning							
Egg	1	82	98.8%	1	79	98.8%	0.74
Milk	1	82	98.8%	1	79	98.8%	0.74
Wheat	1	82	98.8%	1	79	98.8%	0.74
Soybeans	1	82	98.8%	1	79	98.8%	0.74
Buckwheat	1	25	30.1%	1	29	36.3%	0.25
Peanuts	1	30	36.1%	1	30	37.5%	0.49
White blood cell count (/μl)	9	10,800	8775–13,325	6	11,500	9650–13,350	0.53
Eosinophil count (/μl)	15	318	200–443	13	299	188–494	0.86
IgE (IU/mL)	12	41	17–88	10	53	18–138	0.11
TARC (pg/mL)	10	1094	794–1730	8	1253	861–1780	0.24
Egg white-specific IgE (≥0.1 kUA/L)	7	62	74.7%	6	66	82.5%	0.15
Cow's milk-specific IgE (≥0.1 kUA/L)	9	44	53.0%	9	44	55.0%	0.46
Wheat-specific IgE (≥0.1 kUA/L)	8	37	44.6%	9	36	45.0%	0.58
Soybeans-specific IgE (≥0.1 kUA/L)	9	34	40.1%	10	29	36.3%	0.32
Peanuts-specific IgE (≥0.1 kUA/L)	9	24	28.9%	9	22	27.5%	0.49
Buckwheat-specific IgE (≥0.1 kUA/L)	11	25	30.1%	9	19	23.8%	0.23
Sensitization to 1 or more foods (≥0.1 kUA/L)	7	65	78.3%	6	68	85.0%	0.18

IQR, interquartile range; POEM, patient-oriented eczema measure; TARC, thymus and activation-regulated chemokine.

protein with reference to the threshold doses for allergenic foods reported by Taylor *et al.*¹⁹ No serious complications occurred in our study, probably due to the use of low doses of allergenic foods.

Multiple food allergies

As children may be allergic to various food allergens,¹¹ it is important to develop a simple method to prevent allergies to many foods. The EAT study¹² was the first trial to give many types of food antigens from the early weaning period. In the study, healthy breast-fed babies were given six types of food, i.e., milk, egg, peanuts, sesame, fish, and wheat, and the incidences of FA that developed between the ages of 1 and 3 years were examined. Intention-to-treat analysis did not show significant differences in the onset of FA. However, per-protocol analysis showed that the

group with early consumption of the six allergenic foods had a significantly lower incidence of FA than the standard food intake group in the UK. This may have been because the interventions in the study were very burdensome for parents and only 30% of the intervention group complied with the protocol. In our study, in contrast, 98% of participants were able to continue the intervention to the end. This seemed to be because the powder in the bag could be given easily to the baby every day. Thus, the main advantage of our FA prevention method was the ease of compliance.

Intervention period

In a study by Natsume *et al.*⁶ for the prevention of egg allergy, participants in the egg group consumed 50 mg of heated egg powder (containing 25 mg of egg protein) orally per day from 6 to 9

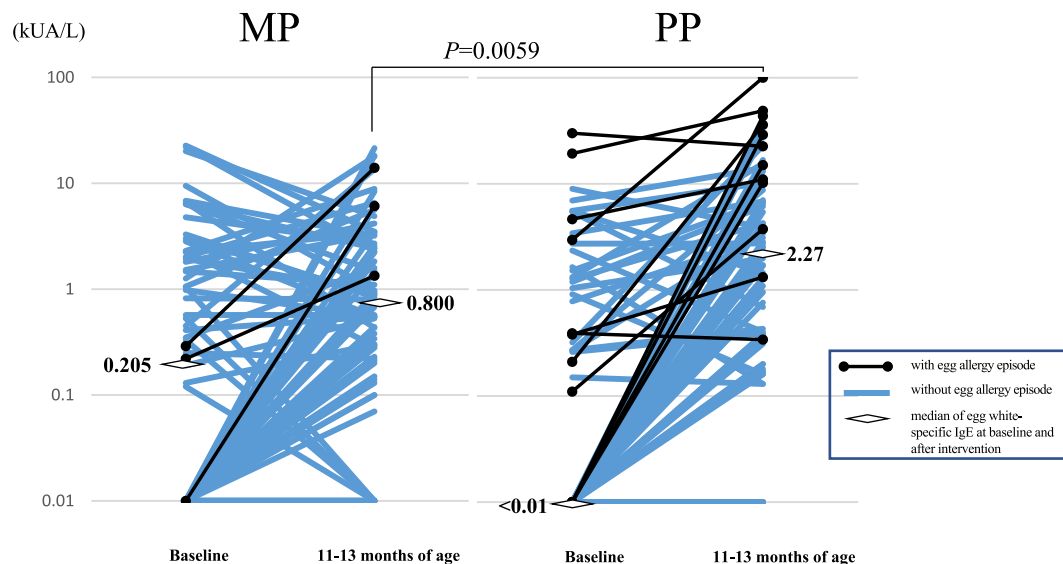


Fig. 5. Egg white-specific IgE levels before or after intervention. The changes of egg white-specific IgE levels before and after the intervention in the MP and PP groups. P values were calculated using the Mann–Whitney U test for the difference in egg-specific IgE after intervention between MP and PP groups. MP, mixed powder; PP, placebo powder.

months of age and 250 mg per day thereafter until 12 months of age. Therefore, the total intervention period was 6 months. The EAT study¹² required 4 g of each allergenic food protein per week from 5 months until 1 year of age. In a study by Du Toit *et al.*⁷ for the prevention of peanut allergy, the intervention involved giving at least 6 g of peanut protein per week until 60 months of age, with an intervention period of ≥ 50 months. In the present study, egg protein was given in amounts ranging from 2.2 to 17.6 mg, milk from 0.3 to 2.4 mg, and peanuts from 0.6 to 4.8 mg for 12 weeks. Thus, our study showed that the interventions with even lower dose and shorter duration than previous studies were also effective. This provided useful insights for preventive interventions against FA.

Intervention target

The effectiveness of early introduction in the EAT study was strongly confirmed in specific groups of infants with sensitization to one or more foods at enrollment. Our study also showed that the effect of MP was more pronounced in the sensitized group. This is a useful finding for selecting children for FA prophylaxis.

Conclusions

Gradual increases in intake of very small amounts of multiple foods over a 12-week period in early infancy can safely reduce the incidence of egg allergies and egg white-specific IgE. Other foods may also suppress food allergies, but there were no differences in specific IgE and no definitive conclusions could be reached. This FA prophylaxis may be particularly effective in children with early food sensitization.

Study limitations and future issues

This study had several limitations. First, this study was underpowered because we could not meet the planned sample size. Therefore, we cannot reach definitive conclusions regarding whether MP is invalid for foods that did not differ significantly. Second, because the frequencies of occurrence of five types of FA other than egg were low, the number of FA episodes in this study was too small to determine the effect of the intervention. Third, OFC were not performed due to difficulty of implementation to all six foods and the diagnosis of FA was made at the discretion of the attending physician. Thus, the diagnosis of FA may have differed between attending physicians.

Further studies in larger numbers of subjects are necessary along with reexamination of the effectiveness of introducing small amounts of individual foods.

Acknowledgments

We are grateful to the participants and their families for their cooperation with the trial, and we thank the staff of the participating facilities. We conducted this study with our own funding, either without public or private support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2022.03.001>.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

TN, MF, and KF designed the trial. TN and KF conceived and designed the contents of the trial powders. TN handled the trial powder to conceal assignment and managed the randomization and concealment of allocation. KF managed the data. MF analyzed the data. TO, SYam, SK, MH, TM, YNo, ST, SYaj, SA, YNa, TH, HH, HY, and AI recruited patients and collected data. JM prepared the mixed allergenic food powder and placebo powder used in the tests. All authors discussed interpretation of the results and are responsible for the accuracy and completeness of the report.

References

- Koplin JJ, Osborne NJ, Wake M, Martin PE, Gurrin LC, Robinson MN, et al. Can early introduction of egg prevent egg allergy in infants? A population-based study. *J Allergy Clin Immunol* 2010;**126**:807–13.
- 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.
- Roduit C, Frei R, Depner M, Schaub B, Loss G, Genuneit J, et al. Increased food diversity in the first year of life is inversely associated with allergic diseases. *J Allergy Clin Immunol* 2014;**133**:1056–64.
- Kull I, Bergstrom A, Lilja G, Pershagen G, Wickman M. Fish consumption during the first year of life and development of allergic diseases during childhood. *Allergy* 2006;**61**:1009–15.
- Sakihara T, Otsuji K, Arakaki Y, Hamada K, Sugiura S, Ito K. Randomized trial of early infant formula introduction to prevent cow's milk allergy. *J Allergy Clin Immunol* 2020;**6749**:31225–2.
- Natsume O, Kabashima S, Nakazato J, Yamamoto-Hanada K, Narita M, Kondo M, et al. Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema (PETIT): a randomised, double-blind, placebo-controlled trial. *Lancet* 2017;**389**:276–86.
- Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med* 2015;**372**:803–13.
- Palmer DJ, Metcalfe J, Makrides M, Gold MS, Quinn P, West CE, et al. Early regular egg exposure in infants with eczema: a randomized controlled trial. *J Allergy Clin Immunol* 2013;**132**:387–92.e1.
- Palmer DJ, Sullivan TR, Gold MS, Prescott SL, Makrides M. Randomized controlled trial of early regular egg intake to prevent egg allergy. *J Allergy Clin Immunol* 2017;**139**:1600–7.
- Bellach J, Schwarz V, Ahrens B, Trendelenburg V, Aksünger Ö, Kalb, et al. Randomized placebo-controlled trial of hen's egg consumption for primary prevention in infants. *J Allergy Clin Immunol* 2017;**139**:1591–9.
- Ebisawa M, Ito K, Fujisawa. Japanese guidelines for food allergy 2020. *Allergol Int* 2020;**69**:370–86.
- 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.
- Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. working party's diagnostic criteria for atopic dermatitis. III. Independent hospital validation. *Br J Dermatol* 1994;**131**:406–16.
- Charman CR, Venn AJ, Williams HC. The patient-oriented eczema measure: development and initial validation of a new tool for measuring atopic eczema severity from the patients' perspective. *Arch Dermatol* 2004;**140**:1513–9.
- The Japanese Guidelines for Nursing/Bottle Feeding, and for Baby Food]. <https://www.mhlw.go.jp/content/11908000/000496257.pdf>. [Accessed 1 February 2021] (in Japanese).
- Perkin MR, Logan K, Bahnson HT, Marrs T, Radulovic S, Craven J, et al. Efficacy of the Enquiring about Tolerance (EAT) study among infants at high risk of developing food allergy. *J Allergy Clin Immunol* 2019;**144**:1606–14.
- Flohr C, Perkin M, Logan K, Marrs T, Radulovic S, Campbell LE, et al. Atopic dermatitis and disease severity are the main risk factors for food sensitization in exclusively breastfed infants. *J Invest Dermatol* 2014;**134**:345–50.
- Wei-Liang Tan J, Valerio C, Barnes EH, Turner PJ, Van Asperen PA, Kakakios AM, et al. Beating Egg Allergy Trial (BEAT) Study Group. A randomized trial of egg introduction from 4 months of age in infants at risk for egg allergy. *J Allergy Clin Immunol* 2017;**139**:1621–8.
- Taylor SL, Hefle SL, Bindslev-Jensen C, Bock SA, Burks Jr AW, Christie L, et al. Factors affecting the determination of threshold doses for allergenic foods: how much is too much? *J Allergy Clin Immunol* 2002;**109**:24–30.

Supplementary Table 1. Protein content and equivalent foods of MP-1

	Protein	Equivalent food
Dried egg white	2.2mg	1/1900 of one whole raw egg (60g)
Infant formula	0.3mg	Infant formula 0.02ml
Wheat	0.2mg	Udon noodle 8mg
Soybeans	0.9mg	Soy milk 0.02ml
Buckwheat	0.3mg	Soba noodle (50% buckwheat) 12.5mg
Peanuts	0.6mg	1/240 of one peanut (0.6g)

※Based on STANDARD TABLES OF FOOD COMPOSITION IN JAPAN - 2015

(Seventh Revised Edition)

Food manufacturers are listed below.

Meringue Powder: Cotta pasteurized dried egg whites[®]

Infant formula: Morinaga Hagukumi[®]

Flour: Nisshin Cooking flower[®]

Roasted soybean flour: Nakamura Foods Hokkaido Whole Grain Kinako[®]

Buckwheat flour: Obinata Sobako[®]

Peanuts flour: Cotta peanut powder[®]

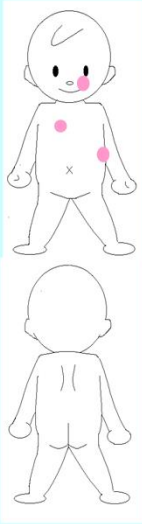
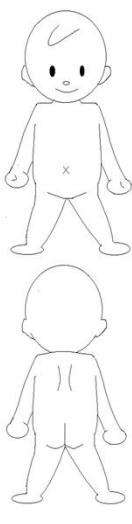
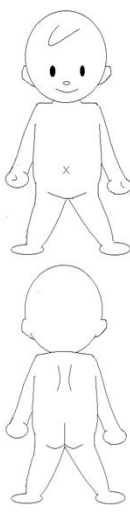
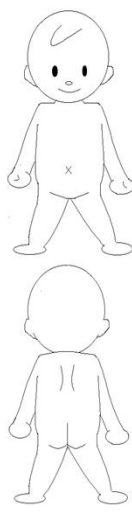
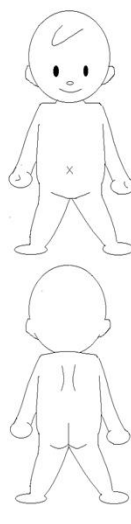
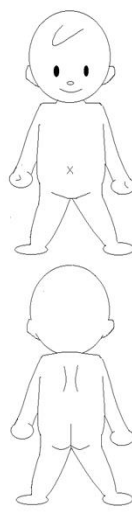
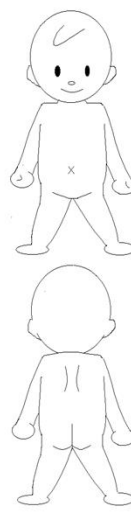
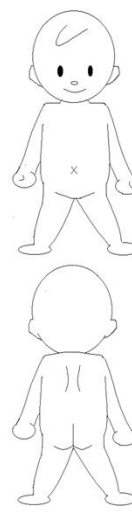
Dried egg white is pre-pasteurized. Wheat flour, soybean flour, buckwheat flour, and peanuts flour were heat-treated twice at 500 W for 40 seconds in a microwave oven, and then mixed. MP / PP powder were produced in a controlled dispensing room, transported in a refrigerator box and stored in the refrigerator.

Microbiological examination of this mixed foods confirmed that general viable bacterial counts were less than 5,000 / g and that E. coli, Staphylococcus aureus, and Salmonella were not detected.

MP: Mixed powder, PP: Placebo powder

Supplementary Table 2. Number of clinic unscheduled visits for illness during the

Type of illness	MP(n=83)	PP(n=80)	<i>P value</i>
Respiratory tract infections	32	42	
Skin infections	4	1	
Gastrointestinal infections	8	5	
Worsening of eczema	7	14	
Urticaria	1	1	
Others	8	8	
Total	36	34	.91

	Example	event dairies						
Date	7/12 (Mon)	/ ()	/ ()	/ ()	/ ()	/ ()	/ ()	/ ()
start	9:00 a.m.							
5 min								
10 min								
20 min								
30 min								
1 hour								
2 hour								
3 hour								
4 hour								
5 hour								
6 hour								
symptoms	urticaria							
details								

Supplementary Fig 1. Event dairies

Caregivers were instructed to record subsequent symptoms in the event dairies and limit egg, milk, wheat, soybeans, buckwheat, and peanuts during the intervention period

How to start weaning food

Weaning foods after the end of the clinical

The following foods should be given after the powder is finished. When feeding for the first time, start with a small amount of only one food per day, and increase the amount gradually. Contact your doctor if you experience allergic symptoms such as urticaria, vomiting, diarrhea, coughing, or wheezing after feeding these foods.

Soybeans:

Start with 1 teaspoon (5g) of tofu and gradually increase. It is recommended to eat at least 1/12 (25g) of tofu at 1 year of age and 1/6 (50g) at 1.5 years of age.

Milk:

Babies who have no allergic symptoms after drinking formula can drink the same amount of yogurt or cow's milk without worrying about allergies. For babies who are fully breastfed and have never had formula, start with 1/4 teaspoon (1.25g) of unsweetened plain yogurt and increase gradually. It is recommended to eat at least 35g of yogurt at 1 year old and 70g at 1.5 years old. ※Most yogurts on the market in Japan weigh 70g per cup.

wheat

Start with 2cm of udon noodle (1g) and gradually increase the amount. It is recommended to eat at least 1/8 of a ball (35g) of udon at 1 year old, and 1/4 of a ball (70g) at 1.5 years old.

Egg

Initially, grind the hard-boiled yolk and give 1/4 teaspoon (1.25g) mixed with porridge. When the child can eat one egg yolk (about 15 g), chop the egg white into small pieces and add it to the hard-boiled yolk. Start with 1/4 teaspoon (1.25g) of egg white and gradually increase. It is recommended to eat at least 1/2 boiled egg at 1 year old and 1 egg at 1.5 years old.

Peanuts:

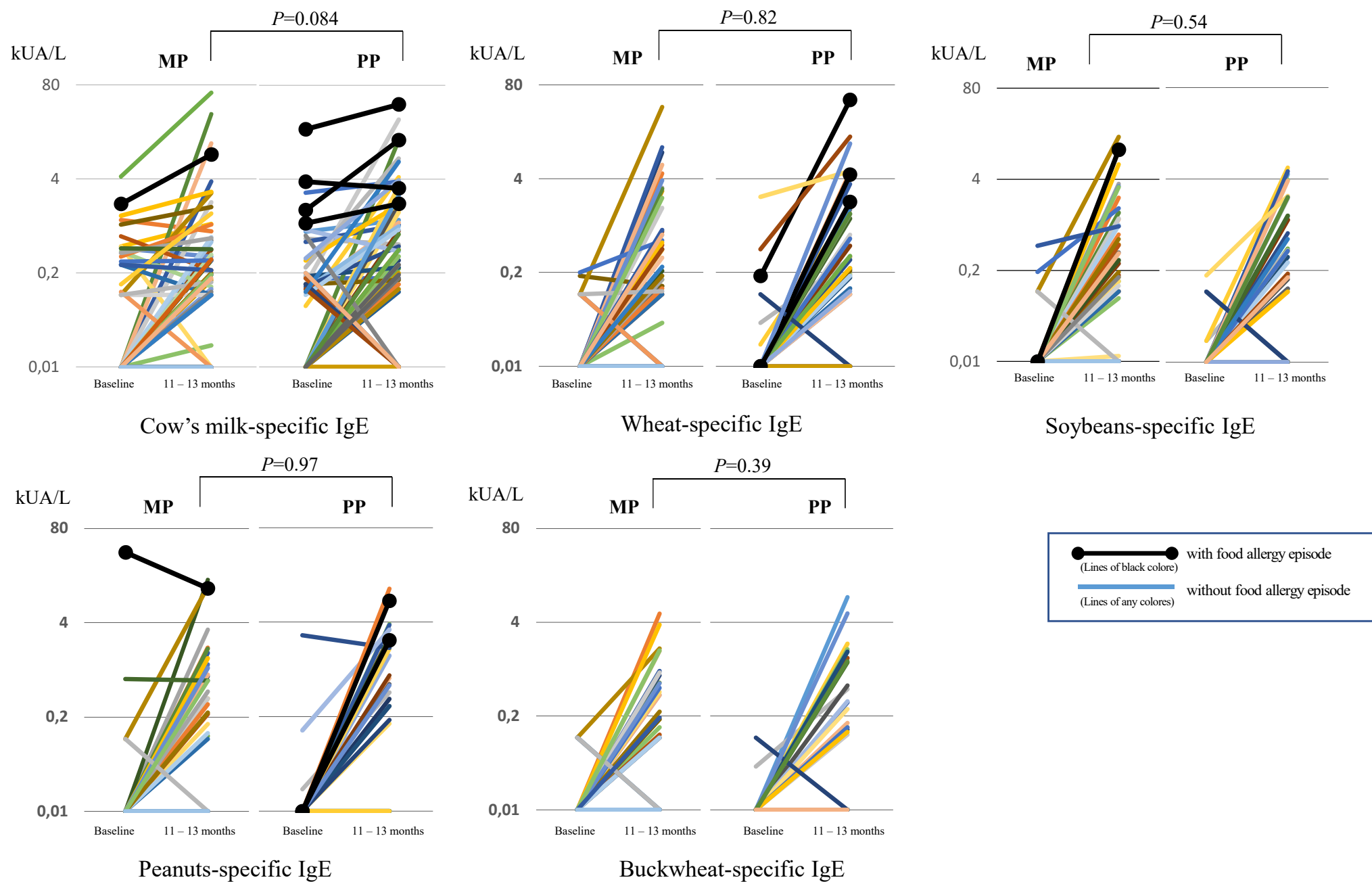
Start with a small amount of peanut cream, about the size of a matchstick head, and gradually increase the amount. It is recommended to eat at least ¼ teaspoon (1.25 g) of peanut cream at 1 year of age, and at least 1 teaspoon (5 g, equivalent to 2 peanuts) at 18 months of age.

Supplementary Fig 2. Pamphlet

Caregivers were given a pamphlet on how to start allergenic foods.

		Start Weaning → Weaning Completed			
		Initial stage (5-6 months)	Middle stage (7-8 months)	Latter stage (9-11 months)	Final stage (12-18 months)
		Recommended amount of food per meal			
I	Cereals (g)	Begin with mashed rice porridge. Also, try mashed vegetables. When the baby is used to these, try mashed tofu, white fish/egg yolk, etc.	Rice Porridge 50~80	Rice Porridge 90 ~ Soft boiled rice 80	Soft boiled rice 90
					~Rice 80
II	Vegetables / Fruit (g)		20~30	30~40	40~50
III	Fish (g)		10~15	15	15~20
	Meat (g)		10~15	15	15~20
	Tofu (g)		30~40	45	50~55
	Egg (g)		Egg yolk 20~ whole egg 20	Whole egg 30	Whole egg 30-40
	Dairy (g)		50~70	80	100

Supplementary Fig 3. Schema of Japanese guidelines for nursing/bottle feeding, and for baby food



Supplementary Fig 4. Changes in food-specific IgE levels per patient

The changes of food-specific IgE levels before and after the intervention in the MP and PP groups. P values were calculated using the Mann-Whitney U test for the difference in food-specific IgE after intervention.

MP: Mixed powder, PP: Placebo powder.