

Safe egg yolk consumption after a negative result for low-dose egg oral food challenge

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Running title: Egg yolk consumption after low-dose egg OFC

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Safe egg yolk consumption after a negative result for low-dose egg oral food challenge

Conflicts of interest

Motohiro Ebisawa serves on the clinical medical advisory board of DBV Technologies. Sato

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All other authors declare no conflicts of interest.

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Abstract

Background: Hen's egg is one of the most common allergens causing infantile food allergy. Consuming heated egg yolk slightly contaminated with egg white (EY with scEW) improves diet quality. Most children with egg allergies can safely consume 1/25 of a heated whole egg (low-dose egg). Although low-dose egg has similar antigenicity to EY with scEW, clinical reproducibility is unknown. We aimed to examine the safety of EY with scEW consumption after a negative result of low-dose egg oral food challenge (OFC).

Methods: In this prospective study, children aged <18 years with a history of immediate reaction to eggs were enrolled. We advised children and guardians to consume EY with scEW after a negative result of low-dose egg OFC and to record symptoms, if any.

Results: We followed 276 children with negative results for low-dose egg OFC who had previously shown reactivity to eggs. The median age was 1.2 years. Boys accounted for 188 (68%) of children. The median egg white-specific immunoglobulin E (IgE) level was 11.7 kU_A/L. At home, six children experienced mild symptoms. Skin symptoms were the most common. Among the 6 children, 5 were confirmed to continue the consumption of EY with scEW; 1 developed mild respiratory symptoms and continued to avoid eating eggs.

Conclusion: Although a few children with egg allergies experience mild symptoms, most of them can ultimately consume EY with scEW. Consumption of EY with scEW after low-dose egg OFC seems safe and may improve their quality of life by making egg yolk products available.

Keywords: child, egg, food allergy, egg yolk, oral food challenge

1 | INTRODUCTION

Hen's egg is one of the most common allergens for infantile food allergy.^{1, 2} Among infants in Western countries, the estimated prevalence rate of egg allergy is around 2%.^{3, 4} The major allergens in eggs are ovalbumin and ovomucoid,⁴ and egg yolk products include a little egg white containing ovalbumin and ovomucoid.⁵ Oral food challenges (OFCs) with heated egg yolk can help most children with egg allergies to consume products containing heated egg yolk slightly contaminated with egg white (EY with scEW) safely (e.g., cookies, seasoning, and bread),⁵ and the food aspect of the overall quality of life can be improved.^{5, 6} Furthermore, daily consumption of EY with scEW may contribute to earlier tolerance to egg.^{7, 8} Nevertheless, the challenge foods used in egg yolk OFC have the disadvantage of a variable amount of egg white,⁵ because egg yolk is separated manually from the whole egg.⁹ This disadvantage is covered by performing an OFC with 1/25 of a heated whole egg (low-dose egg) as egg juice, a product with a stable protein dose that varies only by 10%.^{10, 11} Almost 80% of children with egg allergies can safely consume low-dose egg.¹¹ *In vitro* data of enzyme-linked immunosorbent assay (ELISA) showed that low-dose egg juice has antigenicity (265.8 mg) similar to that of pumpkin cake, containing EY with scEW (213.2 mg).¹¹ Nevertheless, whether children who tolerate low-dose egg OFC can safely consume EY with scEW is unknown. This study aimed to examine the safety of EY with scEW consumption after negative results for low-dose egg OFC.

2 | METHODS

2.1 | Study design

As a part of a multicenter prospective study, we collected children's data for stepwise OFC, starting from low-dose egg OFC (250 mg egg protein, equivalent to 1/25 of a heated whole egg) (UMIN000013026) (Online Repository Text, Figure S1_SupInfo, Table S1). The study was undertaken in Sagami-hara National Hospital as a part of a multicenter study

between 2017 and 2018, as previously described.¹¹

2.2 | Eligibility criteria

Among children aged <18 years with a history of immediate reaction to eggs, children who passed the low-dose egg OFC participated in this study (Figure 1). Children with missing laboratory data or clinical information were excluded. Children who showed obvious or uncertain symptoms during OFC were also excluded.

2.3 | Materials

The challenge food was egg juice, which included 1 g of cooked egg product and 39 mL of apple juice (Table S1). We provided the children's guardians with a recipe for egg yolk pumpkin cake (Table S1). Egg yolk pumpkin cakes were cooked by the guardians. We examined the protein doses of the challenge foods in advance (Table S1). Experimentally, the amount of egg white separated from whole egg was measured with and without chalaza or egg separator at Beppu University.

2.4 | Protocol

We advised children and their guardians to consume EY with sCEW at home, starting with 1/8 of an egg yolk pumpkin cake and gradually increasing to 1/4, 1/2, and one egg yolk (home dosing). If ingestion of egg yolk provoked obvious symptoms, we advised guardians to stop home dosing. If ingestion of egg yolk provoked mild or uncertain symptoms, we advised them to confirm the reproducibility of symptoms by repeating the consumption of the same dose.¹² Guardians were required to record symptoms, if any, on a chart.

2.5 | Outcomes

The primary outcome of this study was the proportion of symptoms provoked by home dosing after a negative result for low-dose egg OFC. The secondary outcomes were the details of provoked symptoms at home (presence of skin, respiratory, gastrointestinal, neurological, or cardiovascular symptoms) and treatments used for provoked symptoms.

2.6 | Sample size

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We hypothesized that the expected proportion of reaction during home dosing is 0.9% and that the width of the confidence interval is 2.7%, based on a previous retrospective study.⁵

The whole sample size was calculated as 188.

2.7 | Statistical analysis

The Mann-Whitney U test was used for the analyses of continuous variables. The incidence of events was analyzed by the chi-squared test or Fisher's exact test, as appropriate. A p-value <0.05 was considered statistically significant. Continuous data were expressed as medians and incidence of events was expressed as proportion. All analyses were performed using SPSS software (version 24.0 SPSS Inc., Chicago, IL, USA).

2.8 | Ethical considerations

This study adhered to the principles of the Declaration of Helsinki. This study was approved by the Ethics committee of Sagamihara National Hospital (Approval number: 2016-2-18).

Written consent was obtained from the guardians of all children. Further details of the methods are shown in the Online Repository Text.

3 | RESULTS

3.1 | Study enrollment

Among 495 children with egg allergies who previously reacted to eggs, 75 children were excluded due to missing laboratory data or clinical information (Figure 1). The children who showed obvious symptoms (n = 89) and uncertain symptoms (n = 65) were excluded. We followed 276 children who passed the low-dose egg OFC (Table 1).

3.2 | Study population

The median age of children was 1.2 years. One hundred eighty-eight (68%) of the children were male. Twenty-two (8%) had history of anaphylaxis in response to eggs. The median egg white-specific IgE level was 11.7 kU_A/L.

3.3 | Measurement of egg white

Experimentally, the amount of egg white separated from whole egg was measured with (n =

5) and without chalaza (n = 5), or without chalaza using egg separator (n = 5). The amount of egg white separated from the whole egg varied from 0.5 to 1.9 g (equivalent to 62.5-237.5 mg egg white protein) (Figure 1). Removal of chalaza reduced the median amount of residual egg white significantly, from 1.7 g to 0.8 g ($p = 0.016$). Additionally, use of egg separator reduced the median amount of egg white 0.8 g to 0.5 g significantly ($p = 0.032$).

3.4 | Outcomes

Following home dosing, 6 children (2.2%) experienced mild symptoms (Figure 2). There were no significant differences in background between the 6 children who reacted and the 270 asymptomatic children ($p = 0.218$, Table 2).

Among the 6 children, 5 children were finally confirmed to continue the consumption of EY with scEW within 1 month after OFC, and mild skin symptoms were reproduced in one (0.4%) child who continued to avoid egg completely (Table 3). Immediate skin symptoms after ingestion were the most common for the 5 children who continued consumption, and none of the children experienced chronic symptoms. No children needed any treatment for the symptoms observed at home.

4 | DISCUSSION

Our study confirmed that most children with egg allergies can safely consume EY with scEW at home after negative results for low-dose egg OFC. This safety data will help to improve their quality of life regarding daily egg consumption.

A previous report showed that only 0.9% of children who passed egg yolk OFC reacted to EY with scEW at home dosing of egg yolk.⁵ Similarly, in our study, only 6 children (2.2%) reacted to home dosing and only 1 child (0.4%) continued to avoid egg completely. Although a significant difference was not observed, children who had symptoms at home showed higher ovomucoid levels (14.9 kU_A/L) than those without symptoms (5.2 kU_A/L). Therefore, we should pay attention to children with high ovomucoid sIgE levels with regard to

consumption of EY with scEW at home. Based on *in vitro* data, the antigenicities of low-dose egg juice and egg yolk-containing pumpkin cake are similar.¹¹ This study is the first to confirm that clinical reproducibility is also similar between these two products.

Although mild symptoms might occur, moderate to severe symptoms were not seen.

Furthermore, most children who showed mild symptoms could eventually consume EY with scEW. This result is compatible with that of a previous report that showed that around 80% of children with mild uncertain symptoms can ultimately consume causative foods.¹² Therefore, we can safely recommend that children who passed the low-dose egg OFC consume EY with scEW. Although children with egg allergies are often sensitized to both egg yolk and egg white, and the reactivities to egg yolk and egg white have heterogeneous patterns, most children react to egg white.¹⁰ Although the egg yolk OFC is safe and useful,¹³ our study also confirmed that the amount of contaminating egg white in egg yolk OFC varies occasionally. Low-dose OFC overcomes the disadvantage of egg yolk OFC, because the protein dose of low-dose egg juice OFC varies only by 10%. Most children with egg allergies can consume low-dose egg¹¹ and thereby improve their quality of life by safe consumption of low-dose egg product.¹⁴ Moreover, our study confirmed that most children could consume EY with scEW after low-dose egg OFC. Therefore, our findings suggest that the low-dose egg juice OFC can replace egg yolk OFC.

This study has several limitations. First, this study recruited only children who had previously reacted to eggs. Children sensitized to eggs may react less than those in this study. Second, in this study, there were no severe symptoms. Severe symptoms may be provoked in a study with a larger sample size. Unequal group sizes of children showing reactions and asymptomatic children could also be a potential limitation. Therefore, other studies with larger sample size may be required. Third, dietary practices and prevalences of wheat allergy differ among countries. Although wheat is the third common food allergen in Japan,¹⁵ in a country with a low prevalence of wheat allergy, a wheat matrix may be used for home dosing.

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None of the cases reacted to heated whole egg at home. To confirm the reproducibility of negative OFC results, a further prospective study should evaluate the same heated whole egg powder. Finally, this study was not a double-blind placebo-controlled food challenge (DBPCFC). None of the enrolled children showed any symptoms during the OFC at the hospital. Therefore, open OFC may not have affected our result.

In conclusion, among children with egg allergies who passed the low-dose egg OFC, a few children may experience mild symptoms after home dosing, but most of them can ultimately consume EY with scEW. Therefore, we can safely recommend that children can consume EY with scEW after passing the low-dose egg OFC. To confirm the safety of this approach, we are now starting a further nationwide multicenter larger sample-size study including children sensitized to eggs, supported by Japanese Society of Pediatric Allergy and Clinical Immunology.

Author contributions:

NY conceived the study, wrote the manuscript, analyzed the data, and generated figures. SS, KT, TA, KN, KO, and ME revised this manuscript and critiqued the manuscript and figures. NY, SS, KT, TA, KN, and KO collected data. KT created the study database. NT experimented with protein doses of eggs. ME advised regarding the study design and helped conceive the study. All authors read and approved the final manuscript.

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foods. We would like to thank Editage (www.editage.com) for English language editing.

Key Message:

We can safely recommend children with egg allergies to consume heated egg yolk slightly contaminated with egg white after negative results for 1/25 of a heated whole egg OFC.

Data availability statement

On reasonable request, the datasets used to support the findings of this trial are available from the corresponding author.

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- Accepted Article
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Table 1. Background characteristics of the participants

Background	(n = 276)
Sex (male)	188 (68%)
Age (years)	1.2 (0.9–2.2)
History of anaphylaxis in response to eggs	22 (8%)
Other food allergy	130 (47%)
Atopic dermatitis, current	156 (57%)
Bronchial asthma, current	14 (5%)
Allergic rhinitis, current	7 (3%)
Egg white sIgE (kU _A /L)	11.7 (5.4–26.5)
Egg yolk sIgE (kU _A /L)	1.8 (0.7–4.0)
Ovomucoid sIgE (kU _A /L)	5.3 (0.8–17.3)
Total IgE (IU/mL)	104 (46.3–367)

Data are expressed as n (%) or median values, with 25% to 75% interquartile ranges provided in parentheses.

Data of egg yolk sIgE levels were missing in 88 children.

Atopic dermatitis was well controlled in this study population.

sIgE, specific immunoglobulin E

Table 2. Differences in the background characteristics of the participants

Background	No symptoms with home dosing (n = 270)	Mild symptoms with home dosing (n = 6)	<i>p</i> value
Sex (male)	184 (68%)	4 (67%)	>0.999
Age (years)	1.2 (0.9–2.3)	2.0 (1.4–3.3)	0.432
History of anaphylaxis in response to eggs	21 (8%)	1 (17%)	0.395
Other food allergy	125 (46%)	5 (83%)	0.103
Atopic dermatitis, current	152 (56%)	4 (67%)	0.700
Bronchial asthma, current	14 (5%)	0 (0%)	>0.999
Allergic rhinitis, current	7 (3%)	0 (0%)	>0.999
Egg white sIgE (kU _A /L)	11.6 (5.3–26.7)	20.3 (12.6–91.6)	0.409
Egg yolk sIgE (kU _A /L)	1.8 (0.7–4.1)	0.9 (0.1–0.9)	0.310
Ovomucoid sIgE (kU _A /L)	5.2 (0.8–17)	14.9 (1.9–99.4)	0.218
Total IgE (IU/mL)	104 (45.4–368)	196.9 (61.3–1998.3)	0.148

Data are expressed as n (%) or median values, with 25% to 75% interquartile ranges provided in parentheses.

Data of egg yolk sIgE levels were missing in 88 children.

Atopic dermatitis was well controlled in this study population.

sIgE, specific immunoglobulin E

Table 3. Detail of symptoms induced by home dosing

Case	Age (years)	Sex	History of anaphylaxis to eggs	Egg white sIgE (kU _A /L)	Ovomucoid sIgE (kU _A /L)	Avoidance of egg yolk 1 month after OFC	Dose of home consumption	Detail of symptoms and treatment
1	1.6	Female	(-)	18.2	24.1	(+)	One egg yolk (pumpkin cake)	Mild skin symptom, vomiting, and cough/no treatment
							One egg yolk (pumpkin cake)	Mild skin symptom on the back 10 hour after ingestion/no treatment
2	0.9	Male	(-)	22.4	2.18	(-)	1/2 egg yolk (pumpkin cake)	Mild skin (rip)/no treatment
3	2.0	Female	(+)	16.4	5.7	(-)	One egg yolk (pumpkin cake)	Mild skin symptom (rip)/no treatment

4	2.0	Male	(-)	25.1	26.5	(-)	One egg yolk	Mild skin symptom (rip)
							(pumpkin cake)	Left eye swelling/no treatment
							1/8 egg yolk	Mild skin symptom on the
							(pumpkin cake)	trunk/no treatment
							1/4 egg yolk	Mild skin symptom on the
5	2.8	Male	(-)	1.06	1.12	(-)	(pumpkin cake)	trunk/no treatment
							1/4 egg yolk	Mild skin symptom on the
							(pumpkin cake)	trunk/no treatment
6	4.9	Male	(-)	291	318	(-)	1/2 egg yolk	Mild skin symptom on the
							(pumpkin cake)	trunk/no treatment
							One egg yolk	Mild diarrhea/no treatment
							(pumpkin cake)	
							One egg yolk	Oral discomfort/no treatment
							(hamburger steak)	

1/8 egg yolk

Mild skin symptoms (rip) / no

(pumpkin cake)

treatment

sIgE, specific immunoglobulin E

OFC, oral food challenge

Figure legends

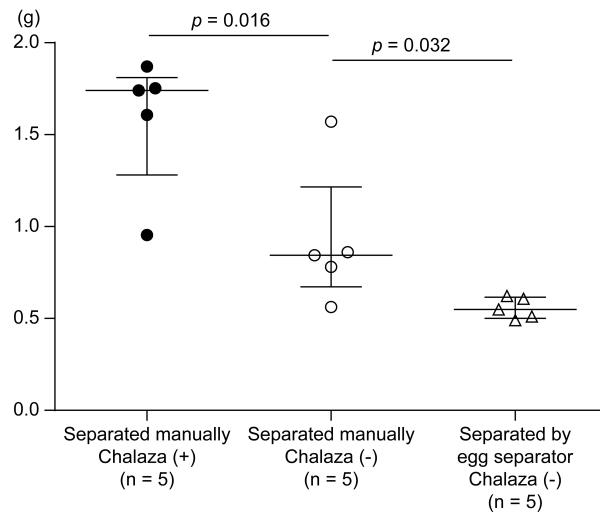
Figure 1. The amount of egg white contaminating the separated egg yolk.

Egg yolk was separated manually with chalaza (n = 5), without chalaza (n = 5), and with egg separator (n = 5) from one whole egg (approximately 50 g).

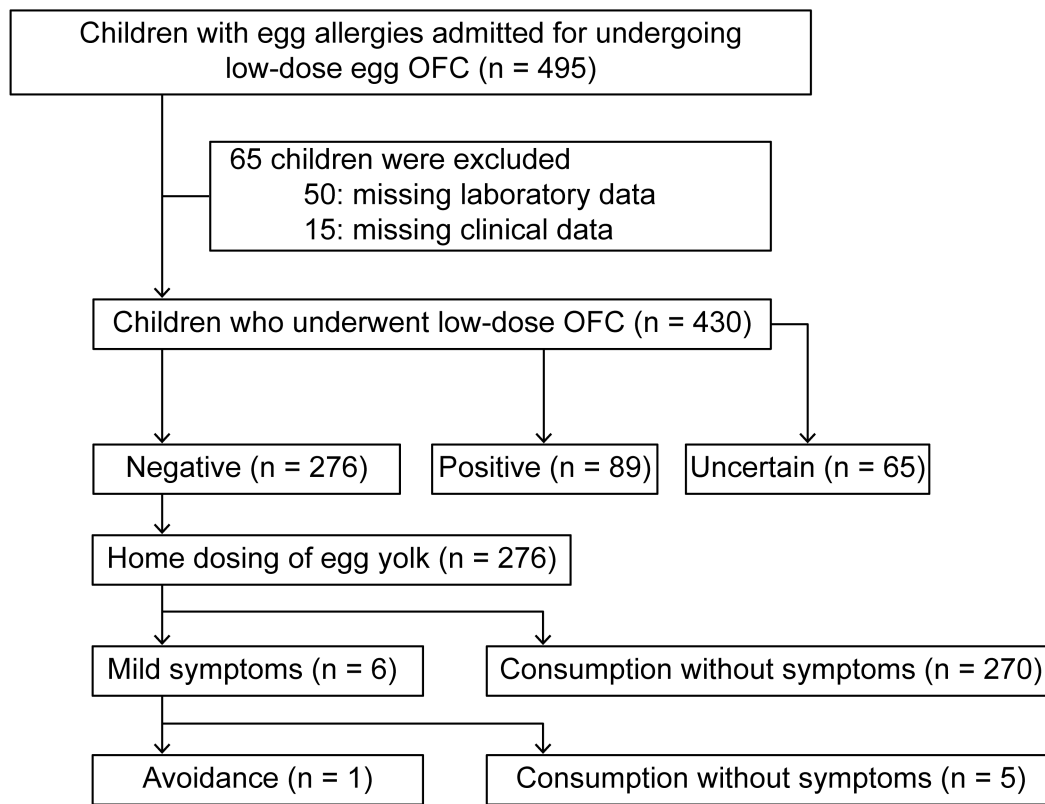
The amount of egg white was 0.5–1.9 g (equivalent to 62.5–237.5 mg egg white protein).

Figure 2. Patient enrollment.

Low-dose oral food challenge (OFC) was administered. Among 495 children with egg allergies who previously reacted to eggs, 75 were excluded due to missing laboratory data or clinical information. The children who showed obvious symptoms (n = 89) and uncertain symptoms (n = 65) were excluded. We followed 276 children with negative results for low-dose egg OFC who had previously reacted to eggs.



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ONLINE REPOSITORY

Methods

Study period

This study prospectively evaluated data from children who had conclusive oral food challenge (OFC) results for heated egg yolk, documented in clinical records at the Department of Pediatrics at the National Hospital Organization Sagamihara National Hospital between January 2017 and December 2018 ^{E1}. Follow-up data were collected between January 2017 and December 2019. Data were analyzed in 2020.

Intervention

Participants consumed egg yolk at home after low-dose OFC. Children ate egg yolk slightly contaminated with egg white (e.g., pumpkin cake; Table S1). Children and guardians were required to record symptoms, if any, on a chart.

Inclusion/Exclusion criteria

Among children aged less than 18 years with a history of immediate reaction to heated hen's egg, children with negative results for low-dose egg OFC participated. We excluded children with missing clinical information or laboratory data. Moreover, we excluded children with a previous positive result for low-dose egg OFC to avoid bias from a second positive result in these children. The following specific immunoglobulin E (sIgE) titers (ImmunoCAPTM; Thermo Fisher Scientific/Phadia, Uppsala, Sweden) were measured within 6 months of OFC: sIgE to egg white, ovomucoid, and egg yolk. OFCs were not performed for children with comorbid symptoms, such as severe eczema

or respiratory symptoms, that would affect the determination of OFC results. History of anaphylaxis was not established as an exclusion criterion, nor were any exclusion criteria established considering the sIgE antibody titers to the above antigens. The term “anaphylaxis” was defined as decreased blood pressure or severe multi-organ symptoms, as described in the World Allergy Organization’s Anaphylaxis Guidelines ^{E2}. Children without a clinical history of allergic reaction to whole hen’s egg were excluded because we could not assess how they would actually react to whole hen’s egg.

Outcomes

The primary outcome of this study was the proportion of any provoked symptom at home after negative results for the low-dose egg OFC. The secondary outcomes were the details of provoked symptoms at home (presence of skin, respiratory, gastrointestinal, neurological, or cardiovascular symptoms) and treatments used for provoked symptoms. We also examined the background data of children (e.g., sex, age, complications of allergic disease, and laboratory data).

Oral food challenge

Low-dose egg OFC was administered to diagnose food allergy or confirm tolerance acquisition safely under hospitalization at Sagamihara National Hospital from 2017 to 2018. Low-dose OFC was administered according to the European Academy of Allergy and Clinical Immunology (EAACI) Guidelines ^{E3}. The OFC has been previously described in detail ^{E1}. Challenge foods were provided as liquid drinks (Tables S1 in the Online Repository). Egg juice was made using 1 g of cooked egg product and 39 mL of apple juice (total 40 mL). Cooked egg product was made from 0.5 g of cooked egg

powder and 0.5 g of sweet potato powder. The heated egg powder has the same allergenicity as a hard-boiled egg. This egg powder is commercially available from Tamakona, Ltd. (Kobe, Japan). Drugs that could potentially affect OFC results, such as antihistamines and leukotriene receptor antagonists, were discontinued 72 h before the OFC. OFCs were implemented while the children's allergic diseases (e.g., atopic dermatitis, asthma, and allergic rhinitis) were well controlled.

Treatment for induced symptoms

We administered appropriate treatment corresponding to symptom severity, including fluid resuscitation, oxygenation, antihistamines, steroids, β_2 stimulant inhalation, and adrenaline injections. We used intramuscular adrenaline injections for severe gastrointestinal symptoms, severe respiratory symptoms, hypotension, loss of consciousness, and when respiratory symptoms persisted after β_2 stimulant inhalation. Study participants who exhibited OFC-induced allergic symptoms (i.e., had a positive OFC result) were treated according to the EAACI guidelines^{E3}. The symptoms and associated severity are described in Table S2 in the Online Repository^{E4}. We included children who first underwent a low-dose OFC for egg (250 mg). We monitored the children for at least 3 h after the final administration. We did not divide the challenge food, but administered it all at the same time. We monitored symptoms for 2 h after children consumed the challenge foods.

Instructions to children with negative OFC results

Children with negative OFC results were instructed to eat foods containing a small amount of whole egg, such as bread or hamburger, at home to confirm the negative OFC

results. We allowed them to use one cooked egg yolk that had been manually separated from whole eggs; trace amounts of egg white were included with the yolk. We also provided the recipe for pumpkin cake with egg yolk (Tables S1 in the Online Repository). We advised children and their guardians to consume egg yolk with slightly contaminating egg white at dosing after negative result of low-dose egg OFC, starting from 1/8 of egg yolk pumpkin cake and gradually increasing to 1/4, 1/2, and 1 egg yolk (home dosing). Additionally, we allowed children with negative OFC results to consume heated egg yolk (with approximately 1/20 of the egg white) or a trace amount of heated whole egg (equal to 1/60 whole egg, e.g., a seasoned powder for sprinkling over rice) with written instructions. If ingestion of egg yolk provoked mild or uncertain symptoms, we advised them to confirm the reproducibility of symptoms by repeating the same dose consumption. If children showed moderate or severe symptoms, appropriate measures were implemented by guardians: antihistamine administration, intramuscular adrenaline injection, and emergency hospital visit. Children' reports on the amount of daily egg consumed after OFC and whether they could safely consume heated egg yolk slightly contaminated with egg white at home were collected by a doctor at the first hospital visit within 1 month after negative OFC.

ImmunoCAP analysis

Children' levels of serum specific immunoglobulin E (sIgE) against egg white, and ovomucoid, (ImmunoCAPTM) were measured within 6 months of OFC. If the sIgE for causative foods was >100 kU_A/L, dilution measurement was applied.

Experiment for the amount of egg white protein

97 Experimentally, the amount of egg white separated from whole egg was measured
98 with and without chalaza or egg separator. Those experiments were held in Beppu
99 University.

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112 Food Allergy TJSOPA, Clinical Immunology TJSOA. Japanese guidelines for food
113 allergy 2017. *Allergol Int* 2017;66:248-264.

114 **Table S1.** Oral food challenge with the amount of protein and amounts of detected egg protein in the challenge foods

Challenge food	Egg protein used	Egg protein measured	
Low-dose egg OFC (Egg juice)	250 mg of whole egg protein (175 mg of egg white protein and 75 mg of egg yolk protein)	265.8 mg	Egg juice was made using 1 g of cooked egg product and 39 mL of apple juice (total 40 mL). Cooked egg product was made from 0.5 g of cooked egg powder and 0.5 g of sweet potato powder. Cooked egg powder (Kewpie Corporation, Tokyo, Japan) was produced by boiling hen's egg at 95 °C for 15 min, followed by pasteurization at 65 °C for 20 min and spray-drying.
Pumpkin cake containing one egg yolk (70 g)	53–165 mg of egg white protein and	213.2 mg	Prepare pumpkin (50.0 g), 1 egg yolk (16.0 g), and sugar (4.0 g)

231 mg of egg yolk
protein

(Total 70.0 g)

Microwave pumpkin in a bowl.

Mix all the ingredients thoroughly using a
hand mixer.

Measure 70 g of the mixture for one portion,
place in a silicone cup, and heat in a
microwave (1000 W) for 90 s.

115 Challenge foods were prepared in the nutrition management room. The amounts of egg protein were measured using the FASTKIT version
116 III enzyme-linked immunosorbent assay egg kit from NH Foods, Ltd. (Osaka, Japan) that detects whole egg protein. In the OFC, the
117 challenge foods were administered all at once or in two portions (1/4 and 3/4, 1 hour interval).
118 OFC, oral food challenge

119

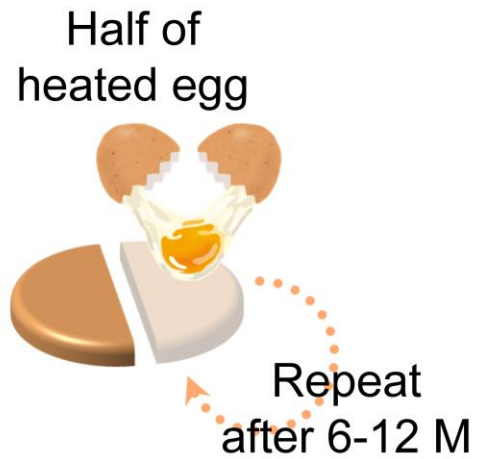
120 **Table S2.** Severity of symptoms during the oral food challenge

Grade	1 (Mild)	2 (Moderate)	3 (Severe)
Skin	Localized urticaria, exanthema, wheal, pruritus	Generalized urticaria, exanthema, wheal, pruritus	-
	Swollen eyelid or lip	Swollen face	-
Gastrointestinal tract	Pruritus of the throat or oral cavity	Throat pain	-
	Mild abdominal pain	Moderate abdominal pain	Cramps
	Nausea, emesis, diarrhea	Recurrent emesis, diarrhea	Continuous emesis, loss of bowel control
Respiratory tract	Intermittent cough, nasal congestion, sneezing, rhinorrhea	Repetitive cough	Persistent cough, hoarseness, “barking” cough

	-	Chest tightness, wheezing detectable on auscultation	Audible wheezing, dyspnea, cyanosis, saturation <92%, swallowing or speaking difficulties, throat tightness, respiratory arrest
Cardiovascular	-	Pale face, mild hypotension, tachycardia (increase of >15 beats/min)	Hypotension, dysrhythmia, severe bradycardia, cardiac arrest
Neurological	Change in activity level, tiredness	Light-headedness, feeling of “pending doom,” somnolence, headache	Confusion, loss of consciousness, incontinence

121 The severity score is based on the organ system that was most affected by the symptoms. Hypotension is defined as systolic blood pressure
122 <70 mmHg for children aged 1 month to 1 year, $<70 + (2 \times \text{age})$ mmHg for children aged 1–10 years, and <90 mmHg for children aged 11
123 years to adulthood. Mild hypotension is defined as systolic blood pressure <80 mmHg for children aged 1 month to 1 year, $<80 + (2 \times \text{age})$
124 mmHg for children aged 1–10 years, and <100 mmHg for children aged 11 years to adulthood. Wheezing detectable on auscultation with a
125 stethoscope is defined as mild wheezing, whereas wheezing detected without a stethoscope is defined as audible wheezing. This severity
126 score has been defined in the anaphylaxis guidelines for Japan ^{E4}.

Traditional

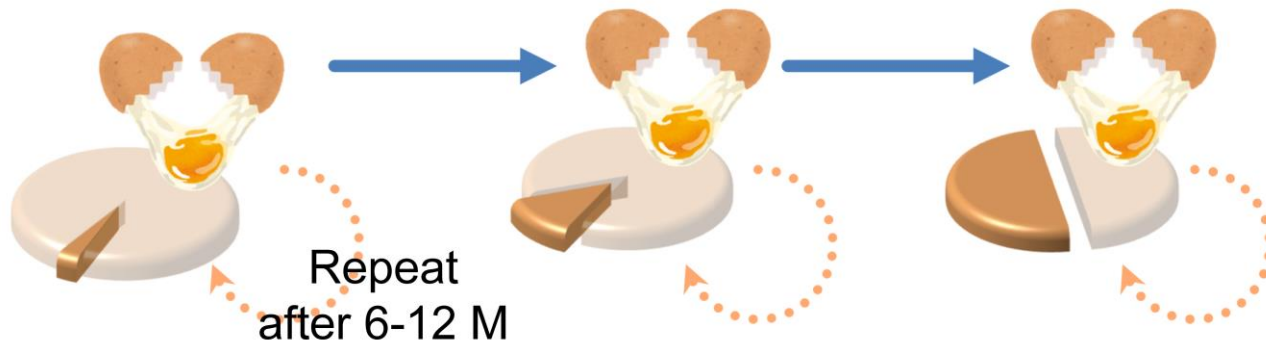


Stepwise

1/25 of
heated egg

1/8 of
heated egg

Half of
heated egg



129 **Figure S1_SuppInfo.** Stepwise oral food challenge (OFC)

130 Patients did not increase their doses beyond the low doses consumed at home. After confirming safe consumption of low doses at home,

131 patients received medium-dose OFC (1/8 to 1/2 of a whole egg), and after confirming safe consumption of medium doses, patients received

132 high-dose OFC (full serving).

133