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Original Article

A 60-minute dosing interval is safer than a 30- or 40-minute interval in oral food challenge

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OFC Oral food challenge

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ABSTRACT

Background: The interval between antigen ingestion may influence the safety of oral food challenge tests (OFCs), especially in patients with severe food allergies.**Methods:** This retrospective chart review of OFCs eliciting objective reactions to wheat, egg, and milk that were performed between April 2012 and January 2021 evaluated an equivalent number of low-dose OFCs performed at 30-, 40-, or 60-min intervals. To avoid the influence of the potential allergy severity of the patients, the prediction scores of all intervals were matched. We evaluated the total symptom score (TS), total ingested dose, and the proportions of severe reactions (TS $\geq$ 30) and adrenaline use.**Results:** We analyzed 945 OFCs (wheat, n = 186; egg, n = 561; milk, n = 198). The 60-min OFC had significantly lower TS than the 30- and 40-min OFC methods in wheat ($p < 0.001$ and $p = 0.003$, respectively), egg ($p < 0.001$ for both), and milk ($p < 0.001$ and $p = 0.018$, respectively). The total dose in the 60-min method was significantly lower than in the 30-min method ($p < 0.001$ for all). The proportion of severe reaction (TS $\geq$ 30) in the 60-min method was significantly lower than that in the 30-min method for the egg and milk OFCs ($p = 0.001$ and $p < 0.001$, respectively). There was no difference in the rates of adrenaline injection.**Conclusions:** The 60-min interval is safer than 30- or 40-min intervals in wheat, egg, and milk OFCs in patients with a low threshold dose for food allergy.Copyright © 2021, 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/>).

Introduction

The oral food challenge test (OFC) is the gold standard for diagnosing food allergy.^{1–4} Recently, OFCs have been used for threshold determination before initiating oral immunotherapy in addition to their use in definitive diagnosis.^{5,6} Threshold-determination OFCs in patients with potentially severe food allergy confer a high probability of allergic reaction despite low target doses.^{6,7} Therefore, the importance of safer OFC methods has become increasingly evident in this era of food-allergy treatment.

The interval between doses is an important factor for performing OFCs safely.^{1–3,6} Guidelines recommend a loading interval of 15–30 min,^{1,2,4} but these intervals may be too short as the time

from ingestion to symptom onset was 30 and 60 min for milk and egg, respectively, in a single-dose OFC study^{8,9}; therefore, the optimal dosing interval may vary depending on the allergen.¹⁰ Studies comparing the safety of different dosing intervals were limited to OFCs of moderate to daily doses in patients with relatively mild food allergies. Although wheat is a risk factor for anaphylactic reaction during OFC,¹¹ few studies have evaluated the optimal interval for wheat OFCs.¹²

There may be a trend toward the selection of longer intervals for OFCs in patients with severe food allergy^{13,14}; however, it would be difficult to compare safety outcomes between the groups accurately if the severity of allergy for a patient differs at each interval. A simple comparison may result in a bias toward more severely allergic patients in the 60-min method, thereby leading to more severe symptoms. With this in mind, we designed a study to systematically compare the differences in safety outcomes between different intervals by matching the scores of the 30-, 40-, and 60-min interval groups using the validated scoring system^{15–17} that predicts the potential severity of the OFC results.

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OFCs with longer loading intervals may be burdensome for both healthcare providers and the family members of the patients, and it is worthwhile to evaluate the optimal loading interval. This study aimed to compare the differences in safety outcomes between different intervals in OFCs (30-, 40-, and 60-min) in low-dose wheat, egg, and milk OFCs in patients with severe food allergies to help clinicians optimize safety when conducting OFCs.

Methods

Study design

We conducted a retrospective study based on a chart review of OFC results eliciting objective reactions to wheat, egg, and milk performed at Aichi Children's Health and Medical Center, a tertiary referral center for food allergy, from April 2012 to January 2021. OFCs performed in doubling protocols with 30-, 40-, or 60-min intervals, with a final dose of ≤ 5 g (mL; protein dose; equivalent to 130 mg wheat, 565 mg egg white, or 165 mg milk) were considered for inclusion. To minimize the selection bias of the patients in the three interval groups, we used the validated prediction score^{15–17} to match the potential severity of the participants. Written consent for the OFC and data publication was obtained from the children's guardians. This study was approved by the ethics committee of Aichi Children's Health and Medical Center (2017001).

Prediction score and OFC matching

The prediction score was determined based on the specific immunoglobulin E (IgE) (ImmunoCAP®) levels of wheat,¹⁵ ovomucoid,¹⁶ or milk,¹⁷ in addition to the background factors (i.e., age, food avoidance status, and history of anaphylaxis; [Supplementary Methods and Supplementary Table 1](#)) for a maximum score of 10 points, with a higher score indicating the higher potential severity of the OFC result ([Supplementary Fig. 1](#)).

We reviewed the OFC results and selected candidate OFCs at 30-, 40- and 60-min intervals. If a single patient participated in multiple OFCs during the study period, only the most recent OFC result was included. Each patient was then classified based on the given prediction score from 4 to 10 points. The prediction score was used to select subjects for analysis. [Supplementary Table 2](#) shows the number of OFCs for each food category, challenge interval, and prediction score.

To minimize the selection bias, analysis was performed on an equal number of OFCs from each interval group. The interval group with the fewest positive OFCs dictated the number of OFCs that would be analyzed. For example, when selecting patients with a prediction score of 5 from the wheat OFCs, 30-, 40- and 60-min intervals achieved 19, 15, and 5 OFCs, respectively. Since there were only 5 OFCs in the 60-min interval, we also selected 5 OFCs from the 30- and 40-min interval groups, in reverse chronological order. Ultimately, 62, 187, and 66 OFCs were selected in the wheat, egg, and milk categories, respectively, from a total of 396, 1358, and 864 total OFC results. For every prediction score, the 60-min interval group achieved the fewest OFCs and influenced the number of OFCs that would be analyzed.

OFC and symptom score

Open OFCs of wheat (udon noodle, 2.6% wheat protein), egg white (20 min-boiled, 11.3% egg-white protein), or whole cow's milk (3.3% milk protein) were performed according to guidelines.³ The interval from the first to last ingestion was set at 120 min, with three, four, or five ingestions at 60-, 40-, or 30-

min intervals, respectively, and consecutive doses were selected from 0.1, 0.2, 0.5, 1, 2, and 5 g (mL) depending on the estimated severity of allergy of the patient (described in [Supplementary Methods](#)).

Table 1

Demographics and clinical characteristics of participants in each OFC-interval and food-allergy group.

Wheat	30-min (n = 62)	40-min (n = 62)	60-min (n = 62)	P value
Sex (male)	44 (71%)	39 (63%)	47 (76%)	0.32
Age (years)	5.5 (3.9–7.5)	5.4 (3.4–7.1)	6.2 (4.7–7.7)	0.22
History of immediate reaction to causative food	56 (90%)	60 (97%)	60 (97%)	0.23
History of anaphylaxis to causative food	46 (74%)	41 (66%)	49 (79%)	0.30
Other food allergies	52 (84%)	54 (87%)	52 (84%)	0.90
Atopic dermatitis	50 (81%)	54 (87%)	49 (79%)	0.48
Bronchial asthma	25 (40%)	32 (52%)	27 (44%)	0.47
Allergic rhinitis	19 (31%)	21 (34%)	23 (37%)	0.78
Total IgE (IU/mL)	601 (336–1078)	532 (296–1227)	635 (308–1243)	0.89
Wheat-sIgE (kUA/L)	50.5 (26.8–100)	58.0 (25.7–100)	50.3 (24.9–100)	0.71
ω 5G-sIgE (kUA/L)	4.3 (1.6–11.9)	7.8 (1.5–21.2)	5.6 (1.2–14.5)	0.38
Egg	30-min (n = 187)	40-min (n = 187)	60-min (n = 187)	P value
Sex (male)	124 (66%)	121 (65%)	127 (68%)	0.82
Age (years)	5.5 (3.3–7.6)	5.1 (2.9–7.4)	5.7 (3.9–7.8)	0.20
History of immediate reaction to causative food	147 (79%)	152 (81%)	159 (85%)	0.28
History of anaphylaxis to causative food	52 (28%)	49 (26%)	56 (30%)	0.74
Other food allergies	139 (74%)	127 (68%)	133 (71%)	0.42
Atopic dermatitis	149 (80%)	155 (83%)	143 (76%)	0.33
Bronchial asthma	64 (34%)	63 (34%)	64 (34%)	1.00
Allergic rhinitis	59 (32%)	66 (35%)	62 (33%)	0.76
Total IgE (IU/mL)	567 (220–1384)	621 (404–1505)	641 (261–1359)	0.26
Egg white-sIgE (kUA/L)	27.1 (13.3–55.6)	32.1 (15.0–60.4)	28.9 (12.2–80.0)	0.29
Ovomucoid-sIgE (kUA/L)	15.9 (8.0–43.9)	19.8 (9.4–44.8)	18.6 (7.9–47.6)	0.46
Milk	30-min (n = 66)	40-min (n = 66)	60-min (n = 66)	P value
Sex (male)	42 (64%)	51 (77%)	40 (61%)	0.10
Age (years)	5.8 (3.8–7.4)	5.8 (3.7–8.5)	6.2 (4.3–8.2)	0.48
History of immediate reaction to causative food	58 (88%)	62 (94%)	63 (96%)	0.32
History of anaphylaxis to causative food	18 (27%)	21 (32%)	24 (36%)	0.55
Other food allergies	55 (83%)	60 (91%)	57 (86%)	0.47
Atopic dermatitis	53 (80%)	60 (91%)	60 (91%)	0.16
Bronchial asthma	23 (35%)	31 (47%)	26 (39%)	0.40
Allergic rhinitis	22 (33%)	30 (45%)	27 (41%)	0.38
Total IgE (IU/mL)	649 (189–1351)	735 (383–1214)	626 (242–1507)	0.57
Milk-sIgE (kUA/L)	27.6 (11.9–66.0)	33.7 (11.1–62.1)	22.9 (6.6–64.3)	0.59
Casein-sIgE (kUA/L)	24.4 (11.4–64.5)	34.4 (12.4–62.9)	23.5 (5.7–63.7)	0.64

Data are expressed as n (%) or median values (interquartile ranges).

Each P value is calculated using the Kruskal–Wallis test (continuous variables) or Fisher's exact test (categorical variables).

OFC, oral food challenge; sIgE, specific immunoglobulin E.

The severity of provoked symptoms was determined based on the total score (TS) of Anaphylaxis Scoring Aichi – a quantitative evaluation of the reactions in multiple organs (Supplementary Table 3).¹⁸ Moreover, we used an overall severity indicator “TS/Pro” for the simultaneous expression of severity and the threshold dose of antigen. “Pro” represents the cumulative protein dose of the allergen that provoked the symptoms, and the TS/Pro was obtained by dividing the TS by Pro. The TS, total ingested dose, and proportions of severe reaction ($TS \geq 30$) and adrenaline use were compared among the different interval groups.

Statistical analysis

The Mann–Whitney *U* and Kruskal–Wallis tests were used for continuous variables. Fisher's exact test was used to analyze differences in categorical variables. Bonferroni correction was applied for multiple comparisons. All significance tests were two-sided, and $p < 0.05$ was considered statistically significant. All analyses were performed using EZR, a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 3.5.1).¹⁹

Results

Demographics and clinical characteristics after OFC matching

Table 1 shows the patient characteristics of the 30-, 40-, and 60-min interval groups of the wheat, egg, and milk OFCs. There was no significant intergroup difference in the sex, age, history of immediate reaction or anaphylaxis to causative food, history of other food allergies and allergic diseases, and serum IgE levels.

Symptom score and total ingested dose

The 60-min method had a significantly lower TS than the 30- and 40-min methods in wheat ($p < 0.001$ and $p = 0.003$, respectively), egg ($p < 0.001$ for both), and milk OFCs ($p < 0.001$ and $p = 0.018$, respectively) (Fig. 1). The TS was significantly lower in the 40-min method than in the 30-min method only in the milk OFCs ($p = 0.008$).

The total dose was significantly lower in the 60- and 40-min method than in the 30-min method in wheat ($p < 0.001$ and $p = 0.033$, respectively) and milk OFCs ($p < 0.001$ for both) (Fig. 2). The total dose in the egg OFCs was significantly lower in the 60-min method than in the 30- and 40-min methods ($p < 0.001$ for both). There was no significant difference in the TS/Pro, which indicated

the overall severity of the participants was identical among the three interval groups (Table 2).

Similar results for TS and total dose were obtained in sensitivity analyses that excluded OFCs wherein symptoms were induced by the final dose (Supplementary Results and Supplementary Table 4). The comparison of symptom scores by organs is summarized in Supplementary Results and Supplementary Table 5.

Severe symptoms and adrenaline use

The proportion of severe reactions ($TS \geq 30$; e.g., intermittent coughing, multiple hives, and vomiting) was significantly lower in the 60-min group compared to the 30-min group in the egg and milk OFCs ($p = 0.001$ and $p < 0.001$, respectively), and tended to be lower although the difference was not statistically significant in the wheat OFC ($p = 0.08$) (Table 2). The proportion of adrenaline use did not differ significantly among the OFC interval groups for all foods.

Comparison of the safety outcomes between the higher-risk and lower-risk groups (prediction score: ≥ 7 or ≤ 6 , respectively)

Additional analysis was performed (Table 3) that further divided the OFCs into a higher-risk group (prediction score: 7–10) and a lower-risk group (prediction score: 4–6). The 60-min method had both a significantly lower TS, and a lower total ingested dose than the 30-min method in the higher- and lower-risk groups for all three foods (except for the total ingested dose of wheat OFC in the lower-risk group). The proportion of severe reactions of $TS \geq 30$ to milk was significantly lower in the 60-min method than in the 30-min method in both risk groups ($p = 0.037$ and $p = 0.034$) as well as in the lower-risk group for egg OFC ($p = 0.016$). The proportion of adrenaline use did not significantly differ among the interval groups for any food category, in either risk group.

Symptom-onset duration after ingestion

The time from the final ingestion of food to the first appearance of an objective symptom could not be accurately measured as this study was not a single-dose OFC study. To ensure the best possible analysis, we compared the symptom-onset duration for each of the three foods which was limited to the OFCs that had induced symptoms after the final dose. The symptom-onset duration for milk (median [interquartile range] 21 [9–30] minutes) was significantly shorter than that for egg (28 [15–57] minutes, $p = 0.005$), and wheat (30 [12–44] minutes, $p = 0.031$) (Fig. 3).

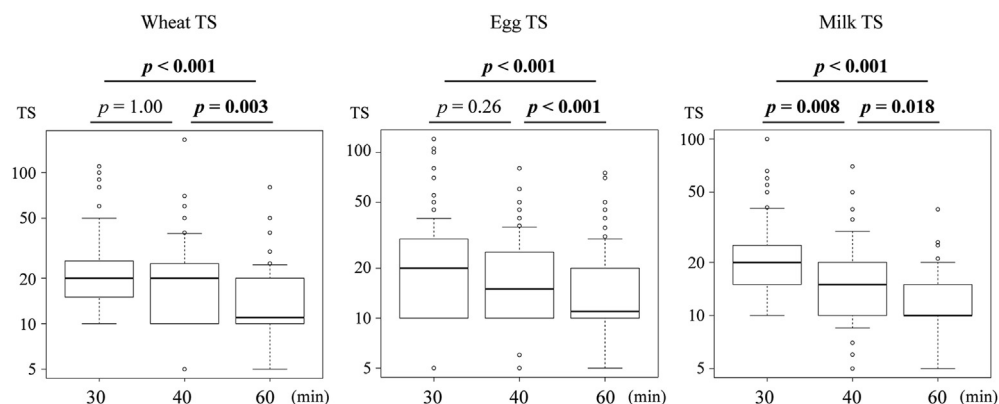


Fig. 1. Symptom score (TS) for each food and OFC interval. The box plots of TS in each food and interval are shown. Horizontal lines represent the median (10th–90th percentile). The 60-min method had significantly lower TS than the 30- and 40-min methods in all foods. Bold indicates statistical significance. TS, total score; OFC, oral food challenge test.

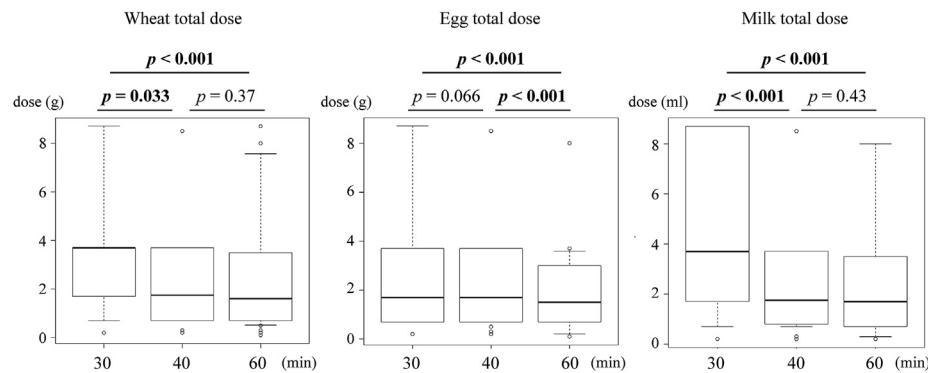


Fig. 2. Total ingested dose for each food and OFC interval. Box-plots of the total ingested dose in each food and OFC interval are shown. Horizontal lines represent the median (10th–90th percentile). The 60-min method had a significantly lower total dose than the 30-min method in all foods. Bold indicates statistical significance.

Table 2
Safety outcomes for each food and OFC interval.

Wheat	30-min (n = 62)	40-min (n = 62)	60-min (n = 62)	P value†	P value‡		
					60 vs. 30	60 vs. 40	40 vs. 30
TS	20 (15–26)	20 (10–25)	11 (10–20)	<0.001	<0.001	0.003	1.00
Total ingested dose (g)	3.7 (1.7–3.7)	1.8 (0.7–3.7)	1.6 (0.7–3.4)	<0.001	<0.001	0.37	0.033
TS/Pro	224 (156–760)	347 (156–1099)	339 (115–769)	0.61			
%TS ≥ 30	15 (24%)	15 (24%)	5 (8.1%)	0.025	0.08	0.08	1.00
Adrenalin use	5 (8.1%)	2 (3.2%)	3 (4.8%)	0.61			
Egg	30-min (n = 187)	40-min (n = 187)	60-min (n = 187)		P value‡		
					60 vs. 30	60 vs. 40	40 vs. 30
TS	20 (11–30)	15 (10–25)	11 (10–20)	<0.001	<0.001	<0.001	0.26
Total ingested dose (g)	1.7 (0.7–3.7)	1.7 (0.7–3.7)	1.5 (0.7–3.0)	<0.001	<0.001	<0.001	0.07
TS/Pro	60 (36–196)	74 (36–190)	104 (32–259)	0.25			
%TS ≥ 30	52 (28%)	34 (18%)	24 (13%)	0.001	0.001	0.60	0.11
Adrenalin use	3 (1.6%)	2 (1.1%)	4 (2.1%)	0.91			
Milk	30-min (n = 66)	40-min (n = 66)	60-min (n = 66)		P value‡		
					60 vs. 30	60 vs. 40	40 vs. 30
TS	20 (15–25)	15 (10–20)	10 (10–15)	<0.001	<0.001	0.018	0.008
Total ingested dose (ml)	3.7 (1.7–8.7)	1.8 (1.0–3.7)	1.7 (0.7–3.5)	<0.001	<0.001	0.43	<0.001
TS/Pro	178 (70–354)	267 (102–476)	202 (101–452)	0.21			
%TS ≥ 30	16 (24%)	9 (14%)	1 (1.5%)	<0.001	<0.001	0.051	0.55
Adrenalin use	2 (3.0%)	1 (1.5%)	1 (1.5%)	1.00			

Data are expressed as n (%) or median values (interquartile ranges). Bold indicates statistical significance. See also Figure 1 and 2 for the box-plots of TS and total ingested dose. TS, Total score of ASCA; TS/Pro, Total score of ASCA/cumulative protein dose.

† Each P value is calculated using the Kruskal–Wallis test (continuous variables) or Fisher's exact test (categorical variables).

‡ Each P value is calculated using the Mann–Whitney test (continuous variables) or Fisher's exact test (categorical variables), and Bonferroni corrections was applied.

Discussion

In this study, we evaluated the safety outcomes of low-dose wheat, egg, and milk OFCs performed at 30-, 40-, and 60-min intervals. The TS was significantly lower with the 60-min method than with the 30- and 40-min methods for all foods. To the best of our knowledge, this is the first study to systematically compare the safety of different intervals for low-dose OFCs in a large number of patients with severe food allergies.

The 60-min method not only had a lower TS but also elicited an objective reaction at a significantly lower total dose than the 30-min method for all foods. The overall severity indicator “TS/Pro” did not differ significantly among the different interval groups for any of the foods. These results suggest that the 60-min interval is overall safer, possibly due to the reduced amount of antigen ingestion prior to symptom provocation.

Shorter dosing intervals may result in higher accumulated doses before symptom onset, which may provoke anaphylactic reactions that could be avoided with longer dose intervals.^{9,12} Single-dose OFCs do not generate this concern²⁰; however, in academic OFCs, such as for the threshold determination prior to

an oral immunotherapy, wherein titrating protocols starting with low doses are often selected.^{4,21} Based on the results of this study, how should we choose the optimal loading interval? The 60-min interval should be selected at least in the egg and wheat OFCs because the 40-min interval did not show significantly greater safety than the 30-min interval. Longer intervals than 60 min or single-dose OFCs, which were not examined in this study, may further increase safety. However, longer-interval OFCs have disadvantages, such as a requirement for longer OFC time or more frequent OFC visits and, therefore, may not translate to a better clinical practice.

Milk was the only food in this study that showed significantly lower TS even in the 40-min method compared to the 30-min method (Fig. 1). It can be inferred that milk tends to elicit an allergic symptom within the shorter duration.^{8,22} The subgroup analysis in the present study focused on the symptom-onset duration after the final dose also showed that milk elicited the symptoms within a significantly shorter duration than that for egg and wheat (Fig. 3). To the best of our knowledge, the data on the longer symptom-onset duration of wheat than milk that we report herein is novel.

Table 3

Comparison between the higher-risk and lower-risk groups (prediction score: 7–10 and 4–6, respectively).

Wheat	Higher-risk group (prediction score 7 to 10)							Lower-risk group (prediction score 4 to 6)						
	30-min	40-min	60-min	P	P value [‡]			30-min	40-min	60-min	P	P value [‡]		
	(n = 49)	(n = 49)	(n = 49)	value [†]	60 vs. 30	60 vs. 40	40 vs. 30	(n = 13)	(n = 13)	(n = 13)	value [†]	60 vs. 30	60 vs. 40	40 vs. 30
TS	20 (15–26)	20 (10–25)	15 (10–20)	0.006	0.005	0.08	1	20 (15–25)	20 (10–30)	10 (10–10)	0.001	0.002	0.016	1
Total dose (g)	3.7 (0.8–8.7)	1.7 (0.7–3.7)	1.5 (0.7–3.0)	0.004	0.005	0.78	0.07	3.7 (3.7–3.7)	3.5 (1.7–3.7)	3.0 (1.5–3.5)	0.11	0.13	0.90	0.78
%TS ≥ 30	12 (25%)	10 (20%)	5 (10%)	0.16				3 (23%)	5 (39%)	0 (0%)	0.065			
Adrenaline use	3 (6.1%)	2 (4.1%)	3 (6.1%)					2 (15%)	0 (0%)	0 (0%)				
Egg	30-min	40-min	60-min		60 vs. 30	60 vs. 40	40 vs. 30	30-min	40-min	60-min		60 vs. 30	60 vs. 40	40 vs. 30
	(n = 88)	(n = 88)	(n = 88)					(n = 99)	(n = 99)	(n = 99)				
TS	20 (14–31)	20 (10–25)	15 (10–25)	0.016	0.015	0.42	0.38	15 (10–25)	15 (10–20)	10 (5–10)	<0.001	<0.001	<0.001	1
Total dose (g)	1.7 (0.7–3.7)	1.7 (0.7–3.7)	0.7 (0.5–1.7)	<0.001	0.002	<0.001	1	3.7 (1.7–6.4)	3.5 (1.7–3.7)	1.7 (0.8–3.5)	<0.001	<0.001	0.007	0.022
%TS ≥ 30	32 (36%)	20 (23%)	18 (21%)	0.044	0.09	1	0.21	20 (20%)	14 (14%)	6 (6.1%)	0.012	0.016	0.29	1
Adrenaline use	0 (0%)	2 (2.3%)	0 (0%)					0 (0%)	0 (0%)	0 (0%)				
Milk	30-min	40-min	60-min		60 vs. 30	60 vs. 40	40 vs. 30	30-min	40-min	60-min		60 vs. 30	60 vs. 40	40 vs. 30
	(n = 30)	(n = 30)	(n = 30)					(n = 36)	(n = 36)	(n = 36)				
TS	21 (15–30)	15 (10–16)	11 (10–16)	<0.001	<0.001	0.69	0.008	16 (10–25)	15 (10–20)	10	<0.001	<0.001	0.037	0.52
Total dose (ml)	1.7 (1.0–3.7)	1.7 (0.7–3.1)	1.2 (0.6–1.7)	0.009	0.012	0.12	0.71	8.7 (3.7–8.7)	2.7 (1.7–3.7)	3 (1.5–8.0)	<0.001	<0.001	1	<0.001
%TS ≥ 30	9 (30%)	5 (17%)	1 (3.3%)	0.023	0.037	0.58	1	7 (19%)	4 (11%)	0 (0%)	0.015	0.034	0.34	1
Adrenaline use	2 (3.0%)	1 (1.5%)	1 (1.5%)					0 (0%)	0 (0%)	0 (0%)				

Data are expressed as n (%) or median values (interquartile ranges). Bold indicates statistical significance.

[†] Each P value is calculated using the Kruskal–Wallis test (continuous variables) or Fisher's exact test (categorical variables).[‡] Each P value is calculated using the Mann–Whitney test (continuous variables) or Fisher's exact test (categorical variables), and Bonferroni corrections was applied.

OFCs to wheat have been identified as an independent risk factor for severe reactions to low-dose antigen.¹¹ In medium-dose OFCs among patients with milder wheat allergy, the 30-min method resulted in significantly lower rates of cardiovascular symptoms and adrenaline administration than the 15-min method.¹² This result was attributed to the longer symptom-onset duration of wheat, and this study adds to the relevant data that the 60-min interval is safer than the 30-min method even in lower-dose wheat OFCs in more severe patients.

OFCs have attendant risks: serious anaphylactic reactions can occur and, rarely, can prove fatal.^{4,6,11} Although only low-dose OFCs were analyzed in this study, 24–28% of the 30-min OFCs had TS ≥ 30, which suggests that reducing the total dose itself is not sufficient to confer safety in OFCs. A study of roasted egg-yolk OFC containing trace amounts of egg white showed that the incidence of anaphylactic reactions was significantly lower in the 60-min method compared to that in the 15-min method.²³ We showed that the 60-min method significantly reduced the proportion of severe reactions compared to the 30-min method (marginally significant in wheat OFCs) (Table 2). Although the proportion of OFCs that required adrenaline administration was not significantly different, this may be due to the limited number of cases that required an adrenaline injection.

The high positive rate of 75% for all OFCs performed at our institution during the same period with the same protocols as this study supports the high-risk characteristics of this study population (Supplementary Table 6). Additional analysis of higher-risk and lower-risk groups (prediction score: 7–10 and 4–6, respectively) showed that the 60-min method had significantly lower TS than the 30-min method in both groups (Table 3), indicating that the 60-min method is safer even in patients with the most severe allergy.

When symptom scores were compared by organ, the 60-min method showed significantly lower skin, gastrointestinal, and respiratory scores for all foods, egg, and wheat and milk OFCs,

respectively (Supplementary Table 5). The frequency of organ symptoms other than skin symptoms varies depending on the food, with egg and milk or wheat being associated with a higher frequency of gastrointestinal and respiratory symptoms, respectively.^{5,8,10,12} In this study, we noted that the organ-specific symptom scores that were lower in the 60-min method were the characteristic allergic symptoms for each food.

As differences in the final doses (due to differences in protocols), rather than differences in the intervals, may have affected the TS and total ingested dose in this study, we performed an additional analysis by excluding the OFC results wherein symptoms were provoked after the final dose intake, which was considered to be the most influential (Supplementary Table 4). For all foods, even if after excluding the group that reached the final dose, the TS and total dose were significantly lower for the 60-min method than for the 30-min method for all foods, as in the original analysis. This suggests that our results are not derived from differences in final doses, but that differences in loading intervals contributed to the results. Since this study included only OFC-positive subjects and no negative subjects, the influence of the differences in the final doses planned for each interval was considered minor.

We also examined whether there was a difference in the diagnostic accuracy of each interval. We confirmed no significant difference in the positive rate of each interval (Supplementary Table 6), or in the regression line between specific IgE level and TS/Pro (Supplementary Fig. 2), indicating that there was no significant difference in the diagnostic accuracy of each interval.

We acknowledge several limitations in this study. First, this was not a prospective randomized controlled study, which is difficult to conduct for OFC intervals in actual clinical practice; however, we believe that we used an innovative approach to simulate randomization using prediction score matching. Second, this study was limited to patients with relatively severe food allergies, and it is not known whether the results are generalizable to patients with less

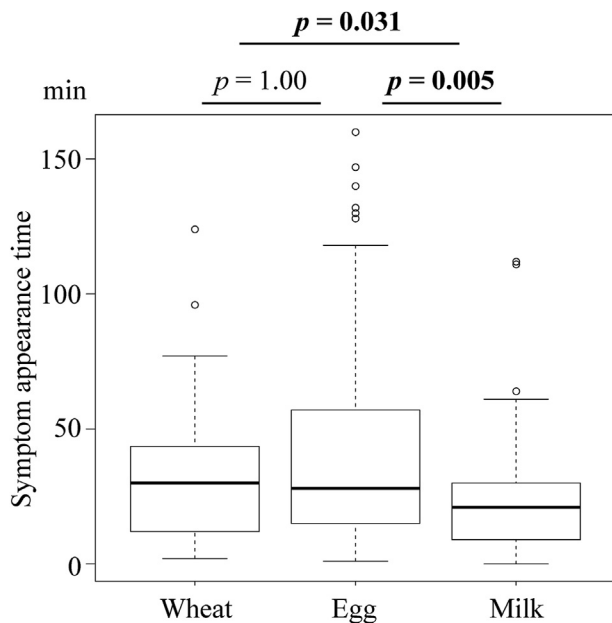


Fig. 3. Time from the ingestion of the final dose to the appearance of the first objective symptom. Box-plots of the time from the ingestion of the final dose to the appearance of the first objective symptom for each food are shown. Horizontal lines represent the median (10th–90th percentile). The symptom-onset duration for milk (median, 21 min; n = 89) was significantly shorter than that for egg (29 min; n = 193) and wheat (30 min; n = 71). Bold indicates statistical significance.

severe food allergies. The other limitation is that titrated protocols may cause a partial desensitization effect during OFC.^{24–26} Although it is unclear which administration interval and dosing method contributed to this effect more, the possibility that this effect may have influenced the results of this study cannot be ruled out. Similarly, complete elimination status before OFC may be associated with immediate symptoms during OFC²⁷; however, the proportion of patients with complete elimination before OFC in this study did not differ among the interval groups for all foods (data not shown), and the influence of this limitation is considered to be negligible.

In conclusion, the 60-min method resulted in significantly lower TS than the 30- and 40-min methods for wheat, egg, and milk OFCs. This study clearly showed that low-dose OFCs can be performed safely even in patients with severe food allergies by increasing the interval time.

Acknowledgements

We would like to thank all the physicians, nutritionists, and nurses who participated in performing OFCs and collecting data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2021.11.005>.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

KK contributed to the study design, analyzed the data, and wrote the manuscript. AM, TM, and YT contributed to the analysis and interpretation of the data. SS and KI contributed to the conception and design of the study and analysis and interpretation of the data. KI is responsible for finalizing the manuscript. All authors read and approved the final manuscript.

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Supplementary data

Supplementary data

Title

A dosing interval of 60 minutes is favorable in comparison to 30 or 40 minutes for a safe oral food challenge

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Supplementary Methods

Oral food challenge (OFC) test protocols

The protocols were 0.1–0.2–0.5–1.0–2.0 g (mL) or 0.2–0.5–1.0–2.0–5.0 g (mL) for the 30-minute method; 0.1–0.2–0.5–1.0 g (mL), 0.2–0.5–1.0–2.0 g (mL), or 0.5–1.0–2.0–5.0 g (mL) for the 40-minute method; and 0.1–0.2–0.5 g (mL), 0.2–0.5–1.0 g (mL), 0.5–1.0–2.0 g (mL), or 1.0–2.0–5.0 g (mL) for the 60-minute method. OFC protocols were determined by discussions among multiple allergists based on medical history, including anaphylaxis, IgE levels, and other clinical information. The aim was to set a protocol to confirm objective symptoms while avoiding unnecessary anaphylaxis or other severe symptoms during OFC. A challenge was determined to be positive only in the case of an objective allergic reaction corresponding to ≥ 5 -point total score (TS) of Anaphylaxis Scoring Aichi (ASCA). Subjective complaints, including pruritus, nausea, or oral symptoms and perioral rash, were not used to define a positive result of the OFC.

Prediction score

This is a simple scoring model to predict severe allergic reaction of OFC, based on the concept of TS/Pro (total score of ASCA/cumulative protein dose) (Supplementary Table 1). For example, the prediction score for wheat OFC is the sum of 1 point per each wheat-specific IgE class, 1 point for a positive omega 5 gliadin-specific IgE, 1 point if total IgE is less than 1000, and 2 points for a history of anaphylaxis to wheat. The maximum score is 10 points. A higher score indicates the higher potential severity of the OFC result (Supplementary Figure 1).

Symptom score

The ASCA was used to determine the severity of the allergic reaction during OFC, especially for a quantitative evaluation of the reactions among multiple organs. ASCA is composed of symptoms according to five organ systems, and each symptom is given a score of 1 to 60 points according to its severity. The highest scores of each organ are summed and described as the total score (TS). For example, if intermittent coughing and local hives are observed, the TS is 15 points.

ImmunoCAP analysis

Serum levels of total and specific immunoglobulin E (IgE; ImmunoCAP system; Phadia/Thermo Fisher Scientific, Uppsala, Sweden) were measured within 6 months of the OFC. In the analysis, sIgE levels of <0.35 kUA/L and ≥ 100 were considered as 0.34 and 100, respectively.

Supplementary Results

Total score and total ingested dose (excluding the OFCs in which symptoms were provoked after the final dose)

Additional analyses were performed after excluding the OFC results in which symptoms were provoked following the ingestion of the final dose. The 60-minute method had significantly lower TS than the 30- and 40-minute methods in wheat ($p=0.042$ and $p=0.023$, respectively), egg ($p<0.001$ and $p=0.028$, respectively), and milk OFCs ($p<0.001$ and $p=0.004$, respectively) (Supplementary Table 4). The total ingested dose was significantly lower in the 60-minute method than in the 30-minute method in OFCs of wheat, egg, and milk ($p<0.001$ for all foods). The proportion of severe reactions of $TS \geq 30$ was significantly lower in the 60-minute method compared to the 30-minute method in milk OFCs ($p=0.003$). The proportion of adrenaline use did not differ significantly among the interval groups for all foods.

Symptom scores by organ

The analysis of symptom scores by organ showed that, for wheat OFCs, the respiratory symptom score was significantly lower in the 60-minute method than in the 30- and 40-minute methods ($p=0.018$ and $p=0.032$, respectively), and the skin symptom score was significantly lower in the 60- and 40-minute methods than in the 30-minute methods ($p<0.003$ and $p=0.019$, respectively) (Supplementary Table 5). In egg OFCs, the gastrointestinal symptom score was significantly lower in the 60-minute method than in the 30-minute method ($p=0.004$), and the skin symptom score was significantly lower in the 60-minute method than in the 30- and 40-minute methods ($p=0.003$ and $p<0.001$, respectively). In milk OFCs, the respiratory symptom score was significantly lower in the

60-minute method than in the 30-minute method ($p=0.014$), and the skin symptom score in the 60-minute method was significantly lower than in the 30-minute method ($p<0.001$).

Overall positive rate and correlation between sIgE and TS/Pro for each OFC interval

This study only included results of positive-OFC. The overall positive rate for all OFCs for wheat, egg, and milk performed at our institution during the same period, using the same protocols, was 75% (Supplementary Table 6).

To examine the correlation between sIgE and TS/Pro for each OFC interval, a correlation diagram between sIgE and TS/Pro was created (Supplementary Figure 2). For each food, the 95% confidence intervals of the regression lines (not drawn in the figure), estimated from the intercept of Y-axis and the regression lines' coefficients, overlapped among the 30-, 40-, and 60-minute intervals, indicating that there was no significant difference in the correlation between sIgE and TS/Pro of each interval ($p<0.05$ for all).

Supplementary Table 1. Prediction scores: scoring model to predict the potential severity of OFC

Wheat	
Wheat-sIgE (class)	1/class
ω5G-sIgE > 0.34	1
Total IgE < 1000	1
Anaphylaxis	2
Egg white	
Ovomucoid-sIgE (class)	1/class
Total IgE < 1000	1
Complete avoidance	2
Aged 5 years or more	1
Milk	
Milk-sIgE (class)	1/class
Total IgE < 1000	2
Complete avoidance	2

Supplementary Table 2. Number of OFCs and matched pairs per prediction scores for each food

Prediction score	Number of OFCs (n)			Selected number in each group	Number of OFCs (%)		
	30-min	40-min	60-min		30-min	40-min	60-min
Wheat							
4	8	1	<u>1</u>	<u>1</u>	5.1	0.6	1.6
5	19	15	<u>5</u>	<u>5</u>	12.1	8.5	8.1
6	42	29	<u>7</u>	<u>7</u>	26.8	16.4	11.3
7	30	51	<u>11</u>	<u>11</u>	19.1	28.8	17.7
8	18	38	<u>15</u>	<u>15</u>	11.5	21.5	24.2
9	24	25	<u>14</u>	<u>14</u>	15.3	14.1	22.6
10	16	18	<u>9</u>	<u>9</u>	10.2	10.2	14.5
Total	157	177	62	62	100.0	100.0	100.0
Egg							
4	43	33	<u>15</u>	<u>15</u>	8.1	5.1	8.0
5	82	67	<u>32</u>	<u>32</u>	15.5	10.5	17.1
6	168	171	<u>52</u>	<u>52</u>	31.7	26.7	27.8
7	139	190	<u>39</u>	<u>39</u>	26.2	29.6	20.9
8	81	127	<u>33</u>	<u>33</u>	15.3	19.8	17.6
9	15	47	<u>15</u>	<u>15</u>	2.8	7.3	8.0
10	2	6	<u>1</u>	<u>1</u>	0.4	0.9	0.5
Total	530	641	187	187	100.0	100.0	100.0
Milk							
4	24	28	<u>8</u>	<u>8</u>	7.5	5.8	12.1
5	57	76	<u>11</u>	<u>11</u>	17.9	15.9	16.7
6	69	89	<u>17</u>	<u>17</u>	21.6	18.6	25.8
7	84	96	<u>14</u>	<u>14</u>	26.3	20.0	21.2
8	73	149	<u>13</u>	<u>13</u>	22.9	31.1	19.7
9	8	32	<u>2</u>	<u>2</u>	2.5	6.7	3.0
10	4	9	<u>1</u>	<u>1</u>	1.3	1.9	1.5
Total	319	479	66	66	100.0	100.0	100.0

OFC, oral food challenge

Supplementary Table 3. Anaphylaxis Scoring Aichi

Score organ	0	① 1 point	① 5 points	② 10 points	② 20 points	③ 40 points	④ 60 points
Respiratory (subjective)	None	Itchy nose	Laryngeal discomfort	Nasal congestion Suffocating breath	Speech disturbance Difficulty in breathing	Loss of voice	
(objective)		Sneeze	Mild transient coughing Runny nose	Intermittent coughing Mild wheezing	Frequent coughing Apparent wheezing Hoarseness	Continuous coughing Strong wheezing Intentional breathing Inspiratory stridor Retraction	Weak breath sounds Strong retraction Cyanosis SpO ₂ ≤ 90%
Skin/Mucosal (subjective)	None	Itch (around mouth) Mild discomfort, Burning sensation	Itch (local and mild)	Itch (whole body)	unbearable itch		
(objective)		<Peri-oral> Hives, Erythema, Swelling, Vesicle	<Local> Eye edema, Bloodshot Hives, Erythema, Swelling, Angioedema	<Multiple> Hives, Erythema, Swelling Angioedema	<Spreading, Generalized> Hives, Erythema, Swelling Angioedema		
Gastrointestinal (subjective)	None	Oral or pharyngeal itch, Hot taste, Sore throat	Mild nausea, Abdominal pain (FS1)	Mild nausea, Abdominal pain (FS2)	Strong abdominal pain (FS3)	Unbearable abdominal pain (FS4)	
(objective)			Increased bowel sounds	Diarrhea, Vomiting	Recurrent vomiting	Dehydration by vomiting	
Psycho- neurological	None	Refusal to eat Mild excitement	Loss of activity Irritation	Sleep, Tendency to lay down Mild excitement	Sleep (not usual) Agitating, Crying	Tend to fall unconscious Uncontrolled panic	Unconsciousness
Cardiovascular	None					Pale, Tachycardia Cold extremities, Cold sweat	Bradycardia
(Blood pressure)						Mild decrease of blood pressure <1 y: <70 mmHg 1–10 y: <70+ (2 x age) mmHg 11–17 y: <90	Low Blood Pressure <1 y: <50 mmHg 1–10 y: <60 mmHg ≥11 y: <70 mmHg

Allergic symptoms are categorized into 5 organ systems, and each organ symptom score (0 - 60 points) is given based on the severity of the symptoms. Total score (TS) is defined as a sum of the highest organ symptom score observed throughout the course of allergic symptom (maximum 240 points). FS, Face scale to express the degree of abdominal pain.

Supplementary Table 4. Safety outcomes (excluding the OFCs in which symptoms were provoked after the final dose)

Wheat	30-min (n=47)	40-min (n=35)	60-min (n=33)	<i>P</i> value †	60 vs. 30	<i>P</i> value ‡ 60 vs. 40	40 vs. 30
TS	20 (15-22.5)	20 (15-30)	10 (10-20)	0.012	0.042	0.023	1.00
Total ingested dose (g)	1.7 (0.75-3.7)	0.7 (0.7-1.7)	0.7 (0.7-1.5)	<0.001	<0.001	1.00	<0.001
%TS ≥ 30	10 (21%)	10 (29%)	5 (15%)	0.43			
Adrenalin use	4 (6.5%)	1 (0.6%)	3 (2.6%)				
Egg	30-min (n=144)	40-min (n=116)	60-min (n=108)		60 vs. 30	60 vs. 40	40 vs. 30
TS	20 (10-30)	20 (10-25)	13.5 (10-25)	0.001	<0.001	0.028	0.87
Total ingested dose (g)	1.7 (0.7-3.7)	1.5 (0.7-1.7)	0.7 (0.3-1.5)	<0.001	<0.001	<0.001	<0.001
%TS ≥ 30	45 (31%)	24 (21%)	21 (19%)	0.055			
Adrenalin use	1 (1.9%)	1 (1.3%)	0 (0%)				
Milk	30-min (n=37)	40-min (n=37)	60-min (n=35)		60 vs. 30	60 vs. 40	40 vs. 30
TS	20 (15-30)	15 (11-21)	10 (10-15)	<0.001	<0.001	0.004	0.049
Total ingested dose (ml)	1.7 (1.7-3.7)	0.7 (0.7-1.7)	0.7 (0.5-1.5)	<0.001	<0.001	0.08	0.010
%TS ≥ 30	11 (30%)	6 (16%)	0 (0%)	0.002	0.003	0.08	1.00
Adrenalin use	0 (0%)	0 (0%)	0 (0%)				

Data are expressed as n (%) or median values (interquartile ranges).
† Each P value is calculated using the Kruskal-Wallis test (continuous variables) or Fisher’s exact test (categorical variables).
‡ Each P value is calculated using the Mann-Whitney test (continuous variables) or Fisher’s exact test (categorical variables), and Bonferroni corrections was applied.

Supplementary Table 5. Symptom scores by organ for each food and OFC interval

Wheat	30-min (n=62)	40-min (n=62)	60-min (n=62)	<i>P</i> value
Respiratory	10 (10-10)	10 (5-10)	10 (5-10)	0.008
Skin	10 (6.3-10)	10 (5-10)	5 (0-10)	< 0.001
Gastrointestinal	0 (0-0)	0 (0-0)	0 (0-0)	0.54
Neurological	0 (0-0)	0 (0-0)	0 (0-0)	0.07
Cardiovascular	0 (0-0)	0 (0-0)	0 (0-0)	0.07
Egg	30-min (n=187)	40-min (n=187)	60-min (n=187)	<i>P</i> value
Respiratory	1 (0-10)	0 (0-10)	0 (0-10)	0.54
Skin	5 (0-10)	5 (0-10)	1 (0-10)	< 0.001
Gastrointestinal	10 (0-10)	5 (0-10)	5 (0-10)	0.005
Neurological	0 (0-10)	0 (0-0)	0 (0-0)	0.12
Cardiovascular	0 (0-0)	0 (0-0)	0 (0-0)	0.15
Milk	30-min (n=66)	40-min (n=66)	60-min (n=66)	<i>P</i> value
Respiratory	10 (5-10)	5 (0-10)	5 (0-10)	0.014
Skin	10 (5-10)	10 (5-10)	5 (5-10)	< 0.001
Gastrointestinal	0 (0-1)	0 (0-1)	0 (0-1)	0.64
Neurological	0 (0-0)	0 (0-0)	0 (0-0)	0.07
Cardiovascular	0 (0-0)	0 (0-0)	0 (0-0)	0.61

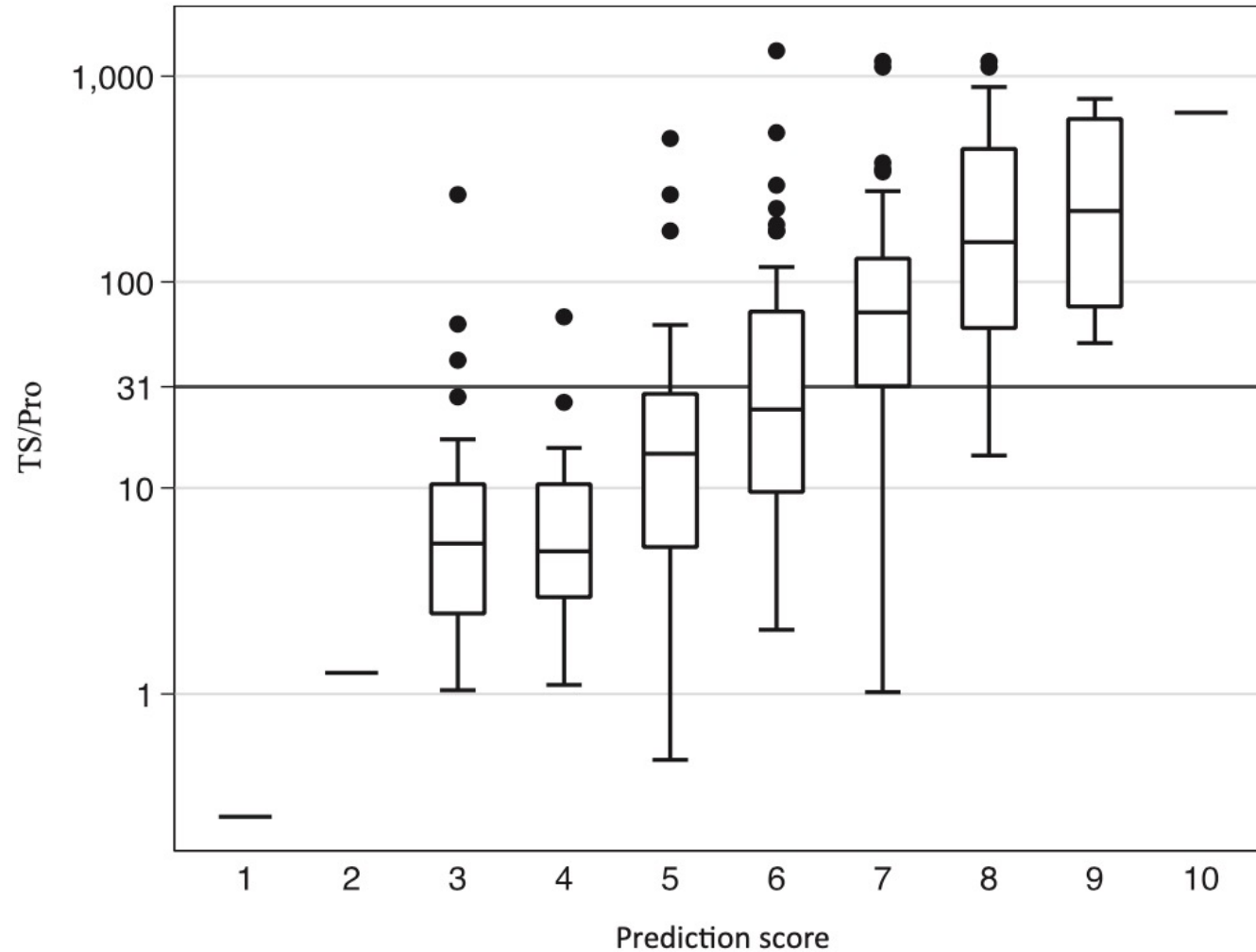
Data are expressed as median values (interquartile ranges).
Each *P* value is calculated using the Kruskal-Wallis test.

Supplementary Table 6. Overall positive rate for all OFCs for wheat, egg, and milk during the study period, using the same protocols

	30-min	40-min	60-min	<i>P</i> value
Wheat	74.1%	71.7%	78.6%	0.44
Egg	74.6%	70.7%	72.4%	0.33
Milk	80.2%	76.0%	71.4%	0.12

Each *P* value is calculated using the Kruskal-Wallis test.

Supplementary Figure 1. Correlation between the prediction score and the TS/Pro

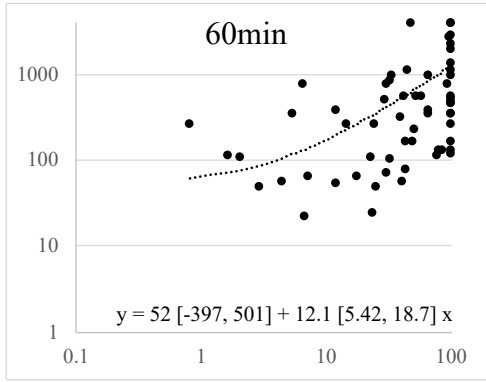
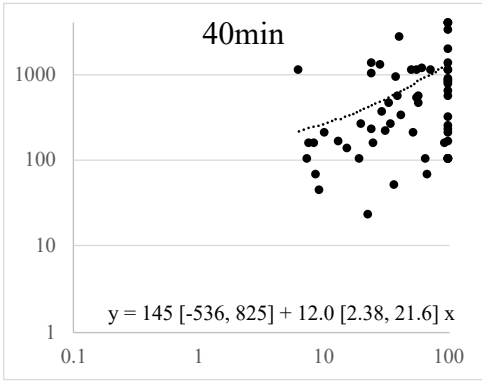
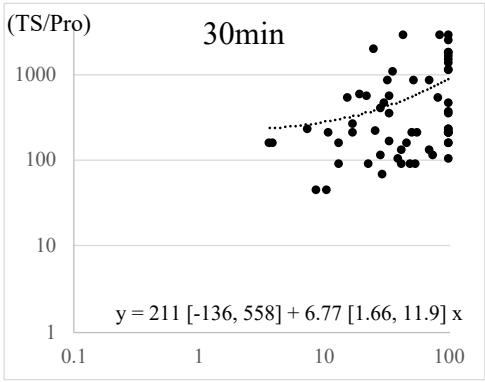


Box-plots of TS/Pro in each prediction score are shown. Adapted from the original data used to develop the Prediction score for boiled egg challenges (reference #16 of the main text). The prediction score significantly correlated to the TS/Pro ($R_s = 0.65$, $p < 0.01$).

TS/Pro is an overall severity indicator for the simultaneous expression of severity and the threshold dose of antigen. “Pro” represents the cumulative protein dose of the allergen that provoked the symptoms, and the TS/Pro was obtained by dividing the TS by Pro.

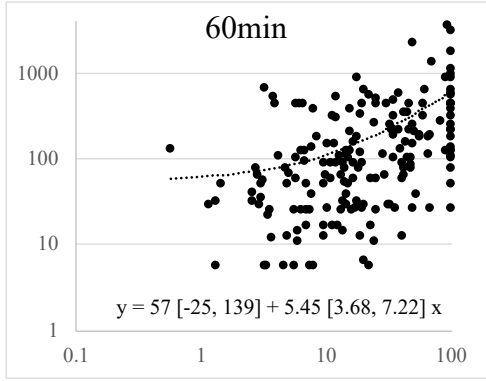
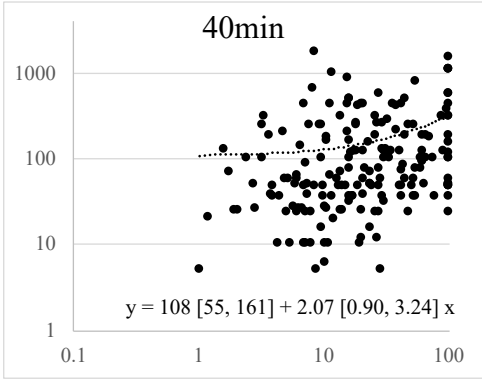
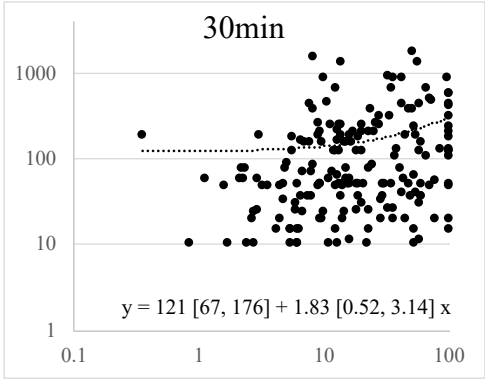
Supplementary Figure 2. Correlation between specific IgE and TS/Pro for each food and OFC interval

Wheat



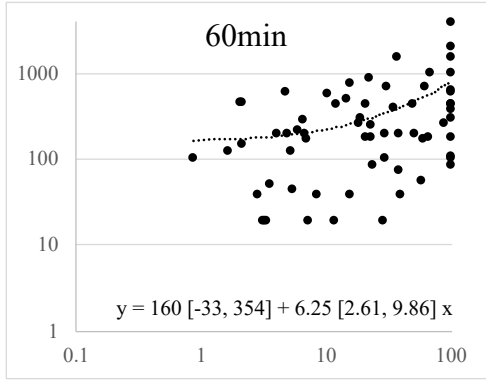
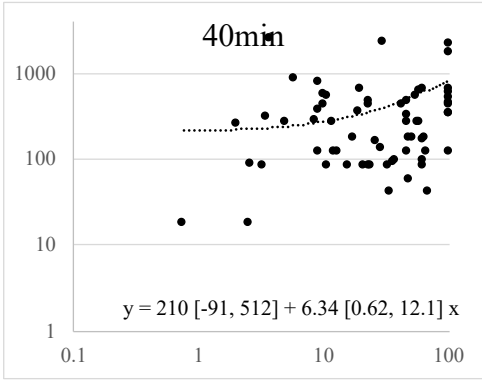
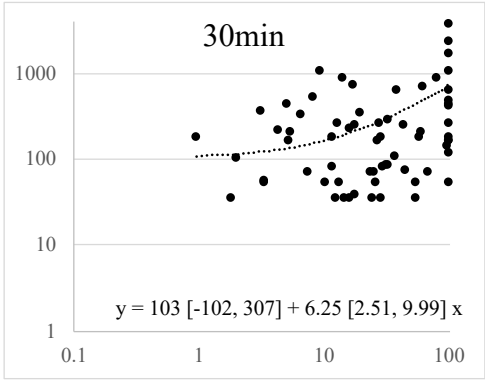
(wheat-sIgE)

Egg



(ovomucoid-sIgE)

Milk



(milk-sIgE)

×

The equation represents the result of linear regression analysis. [] indicates the 95% confidence interval. TS/Pro, total score of ASCA/cumulative protein dose; sIgE, specific-IgE.