

## Predictive power of ovomucoid and egg white specific IgE in heated egg oral food challenges

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### Clinical Implications

- After controlling for confounding variables, the predictive importance of ovomucoid specific IgE is greater than that of egg white specific IgE in heated egg oral food challenges.

### TO THE EDITOR:

Hen's egg white (EW) contains ovomucoid (OVM), ovalbumin (OVA), and many other glycoproteins. OVA is the most abundant protein in a hen's egg; however, OVM is the dominant allergen in heated eggs. This is because OVA is easily affected by heat and protease digestion, whereas OVM is largely heat-resistant. Hence, OVM-specific IgE (sIgE) is thought to be more important than crude EW-sIgE in predicting heated egg tolerance.<sup>1,2</sup> However, some recent studies have reported contrasting viewpoints.<sup>3,4</sup>

Because OVM-sIgE and EW-sIgE share some cross-reactivity,<sup>2,3</sup> the predictive power of EW-sIgE in a heated egg oral food challenge (OFC) could come from a spurious relationship with OVM-sIgE. Therefore, statistical analysis controlling for confounding variables is required to determine the truly useful parameter. The aim of our study was to compare the importance of EW-sIgE and OVM-sIgE in predicting the result of a heated egg OFC after statistical adjustment.

This was a retrospective, cross-sectional study that examined the association between OVM-sIgE, EW-sIgE, and the results of a heated egg OFC. A retrospective chart review was conducted for all patients who had undergone an inpatient OFC of half a heated egg at Sagami-hara National Hospital between January 2011 and August 2014. The exclusion criteria were (1) unavailability of laboratory data from within 120 days of the OFC and (2) patients who did not complete the OFC.

In total, 1264 children underwent inpatient OFCs. This study ultimately included 511 males and 260 females (median age, 3.80 years; interquartile range, 2.17-6.19). The excluded patients were significantly older (see [Methods](#), [Figure E1](#), and [Table E1](#) in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)) because older patients had longer intervals between blood tests. Median levels of total IgE, EW-sIgE, and OVM-sIgE in the overall study group were 570 IU/mL (interquartile range, 211-1340), 15.8 kU<sub>A</sub>/L (6.37-33.4), and 7.16 kU<sub>A</sub>/L (1.95-18.2), respectively. Correlation coefficients are presented in [Table E2](#) in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org).

Among all 771 subjects, 395 (51.2%) had a history of immediate reaction to egg, while the remaining underwent OFC because their EW-sIgE levels were high. And 397 (51.5%) had

undergone heated egg yolk OFC before this OFC. As a result, 389 (50.5%) failed the OFC (ie, had a positive reaction to the food tested). Patients with positive OFCs were older and had higher levels of total IgE, EW-sIgE, and OVM-sIgE than those in the negative group ( $P < .001$ ). Crude odds ratios (ORs) of total IgE, EW-sIgE, and OVM-sIgE were 2.02 (95% CI, 1.58-2.59), 3.08 (2.31-4.10), and 4.62 (3.53-6.04), respectively, for every 1.0-unit increase in the decimal logarithm. In model 1, unlike the crude ORs, the adjusted ORs of EW-sIgE and OVM-sIgE were 0.60 (95% CI, 0.38-0.95) and 4.62 (3.53-6.04), respectively. In model 2, the adjusted ORs of EW-sIgE and OVM-sIgE were 1.01 (0.60-1.71) and 5.85 (3.86-8.88), respectively ([Table 1](#)). EW-sIgE and OVM-sIgE were correlated to some extent, but evidence for multicollinearity was absent, because no variance inflation factor in these models exceeded 5.0. The results of internal validations are presented in [Table E3](#) in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org).

Probability curves were drawn on the basis of EW-sIgE and OVM-sIgE levels ([Figure 1](#); see [Figure E2](#) in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)). We plotted receiver operating characteristic curves for EW-sIgE, OVM-sIgE, and models 1 and 2 (see [Figure E3](#) in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)). The area under the curve of OVM-sIgE (0.764; 95% CI, 0.731-0.797) was significantly larger than that of EW-sIgE (0.659; 95% CI, 0.621-0.697) ( $P < .001$ ).

In this study, we aimed to determine the importance of OVM-sIgE in predicting the results of heated egg OFCs and have successfully shown that it should be used instead of EW-sIgE. To our knowledge, this is the largest study to date comparing the utility of EW-sIgE and OVM-sIgE, with more than 4 times as many cases as in any previous report.<sup>1-5</sup> Previous studies<sup>1,3,5</sup> with probability curves for heated egg challenges included samples with lower levels of allergen-sIgE than those included in our study. Therefore, our curves have good reliability at high serum levels of sIgE.

The results of the logistic regression analysis indicate that the predictive importance of OVM-sIgE is greater than that of EW-sIgE in predicting OFC results. This supports the hypothesis that the predictive power of EW-sIgE comes from a spurious relationship with OVM-sIgE. Therefore, if there is a discrepancy between OVM-sIgE and EW-sIgE levels in a patient, OFC should be considered on the basis of his or her OVM-sIgE levels. This cannot be distinguished by solely comparing the areas under the curve.

Our results disagree with the results of 2 previously reported studies<sup>3,4</sup> in 2 ways. First, the subjects included in our study are younger and have higher titers of EW-sIgE and OVM-sIgE than did the subjects in the previous studies. The distribution of sIgE changes with age; thus, age could influence the contribution ratio of EW-sIgE and OVM-sIgE to OFC results. Second, the food prepared for our OFCs was different from that used in the other studies. Caubet et al<sup>4</sup> used muffins and waffles, and Bartnikas et al<sup>3</sup> used muffins and cupcakes baked in an oven. We used a microwave oven to prepare pumpkin cakes and confirmed that the core temperature of the cakes reached approximately 100°C after 90 seconds of microwaving. We used no wheat flour to

TABLE I. Results of logistic regression analysis

| Characteristic (n = 771)       | OFC Positive (n = 389) | OFC Negative (n = 382) | P value | Crude OR         | Model 1          |          |      | Model 2          |          |      |
|--------------------------------|------------------------|------------------------|---------|------------------|------------------|----------|------|------------------|----------|------|
|                                |                        |                        |         |                  | Adjusted OR      | P value* | VIF  | Adjusted OR      | P value* | VIF  |
| Sex                            |                        |                        |         |                  |                  |          |      |                  |          |      |
| Male                           | 259 (66%)              | 252 (66%)              | .857†   | 1.00 (reference) | —                | —        | —    | 1.00 (reference) | .574     | 1.05 |
| Female                         | 130 (34%)              | 130 (34%)              |         | 0.97 (0.72-1.31) |                  |          |      | 0.90 (0.63-1.29) |          |      |
| Age (y)                        | 5.11 (3.07-6.80)       | 2.91 (1.88-4.79)       | <.001‡  | 1.19 (1.13-1.25) | —                | —        | —    | 1.17 (1.09-1.27) | <.001    | 1.72 |
| Total IgE (IU/mL)§             | 705.0 (311.0-1510.0)   | 415.5 (136.2-1100.0)   | <.001‡  | 2.02 (1.58-2.59) | —                | —        | —    | 0.43 (0.28-0.66) | <.001    | 2.43 |
| EW-sIgE (kU <sub>A</sub> /L)§  | 21.6 (9.60-42.9)       | 11.2 (4.16-23.7)       | <.001‡  | 3.08 (2.31-4.10) | 0.60 (0.38-0.95) | .029     | 2.23 | 1.01 (0.60-1.71) | .968     | 2.77 |
| OVM-sIgE (kU <sub>A</sub> /L)§ | 12.9 (5.45-28.4)       | 3.33 (0.63-9.08)       | <.001‡  | 4.62 (3.53-6.04) | 4.62 (3.53-6.04) | <.001    |      | 5.85 (3.86-8.88) | <.001    | 2.27 |
| Comorbid allergic disease      |                        |                        |         |                  |                  |          |      |                  |          |      |
| Bronchial asthma               | 100 (26%)              | 66 (17%)               | 0.004†  | 1.53 (1.06-2.21) | —                | —        | —    | 1.18 (0.78-1.80) | .435     | 1.11 |
| Atopic dermatitis              | 268 (69%)              | 231 (60%)              | 0.014†  | 1.22 (0.88-1.68) | —                | —        | —    | 1.19 (0.83-1.70) | .347     | 1.07 |
| Allergic rhinoconjunctivitis   | 67 (17%)               | 31 (8%)                | <0.001† | 1.99 (1.25-3.18) | —                | —        | —    | 1.32 (0.78-2.24) | .305     | 1.10 |

VIF, Variance inflation factor.

Data are expressed as values (ratios) or median values (interquartile ranges). ORs are expressed with 95% CIs.

\*Wald test.

† $\chi^2$  test.

‡Mann-Whitney U test.

§ORs are for every 1.0-unit increase in the decimal logarithm ( $\log_{10}$ ).

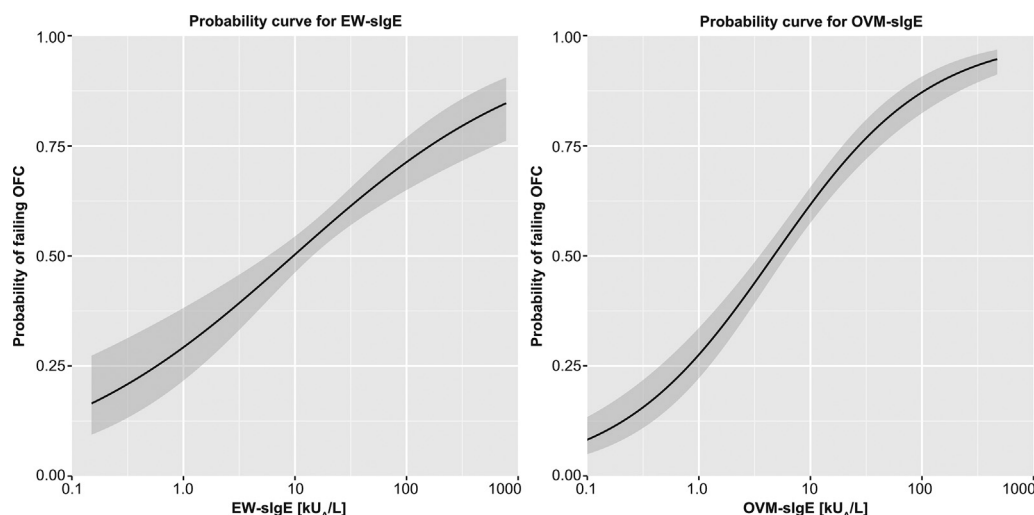


**FIGURE 1.** Probability curves for EW-sIgE and OVM-sIgE. Gray band indicates 95% confidence region.

produce the pumpkin cakes. Bartnikas et al<sup>3</sup> noted that cooking eggs with wheat flour leads to OVM insolubility, potentially reducing its antigenicity due to intermolecular disulfide bonds created between OVM and the wheat during heating.<sup>6</sup> We confirmed that OVA levels in our pumpkin cakes, measured by ELISA, were much lower than those of OVM (see Table E4 this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)). However, Watanabe et al<sup>7</sup> reported that OVA in cupcakes remains detectable at levels as high as 4 times those of OVM, even after baking at 180°C for 20 minutes in an oven. Therefore, residual antigenicity of OVA in the foods used in these studies may have skewed the OFC results, and EW-sIgE levels may be much more important for foods with OVA-antigenicity—dominant products.

In conclusion, the predictive importance of OVM-sIgE is greater than that of EW-sIgE in heated egg OFCs.

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## ONLINE REPOSITORY

### METHODS

#### Oral food challenge

All OFCs were performed as open challenges according to the Japanese guidelines for food allergy<sup>E1</sup> with a prepared pumpkin cake containing half an egg (Table E5). Patients who were diagnosed as having, or were suspected to have, allergies to hen's egg underwent heated egg OFC. OFCs were performed in a stepwise approach.<sup>E2</sup> Patients who reacted to hard-boiled egg yolk or foods containing a heated egg yolk did not undergo OFC with foods containing half an egg.

OFCs were discontinued at the first objective sign of reaction. If symptoms were limited to only mild, subjective symptoms or a negative reaction, patients were encouraged to consume the causative foods at their homes on another day to reach a final judgment.

#### Allergen-sIgE analysis

Serum allergen-sIgE was analyzed using a commercially available fluorescent enzyme immunoassay (ImmunoCAP; Thermo Fisher Scientific/Phadia, Uppsala, Sweden). If the serum allergen-sIgE concentration exceeded 100.0 kU/L, the test was repeated with dilution. Levels of sIgE less than the traditional cutoff level of 0.35 kU/L were treated as negative.

#### Statistical methods

All statistical analyses were performed using R version 3.4.4.<sup>E3</sup> Logistic regression analysis, the  $\chi^2$  test, the Mann-

Whitney *U* test, DeLong test,<sup>E4</sup> and Spearman correlation coefficient were used for statistical analyses. EW-sIgE, OVM-sIgE, and total IgE were log-transformed for conformity with a linear gradient.

To compare the usefulness of EW-sIgE and OVM-sIgE, 2 models were created for logistic regression analysis. The variables in model 1 were EW-sIgE and OVM-sIgE. Total IgE, age, sex, and comorbid allergic diseases were considered in model 2 as additional parameters to calculate ORs after simultaneously controlling for these potential confounders. A variance inflation factor exceeding 5.0 was regarded as indicating serious multicollinearity in the logistic regression model.<sup>E5</sup> Probability values of less than 0.05 were considered statistically significant. Data are expressed as median values and interquartile ranges (25-75th percentiles; interquartile range) unless otherwise noted. Probability curves were plotted using results from the logistic regression analysis.

A sample size calculation<sup>E6</sup> was performed beforehand to estimate the number of required samples, using estimated event rate ( $P_1$ ) = 0.5,  $\alpha$  = 0.05, power = 0.8, OR = 1.5, and a correlation coefficient ( $R$ ) of log OVM- and EW-sIgE = 0.8 based on previous studies. The sample size required for the desired effect size was 531 patients in all groups.

#### Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review boards of Sagami-hara National Hospital. Written, informed consent was obtained from all participants.

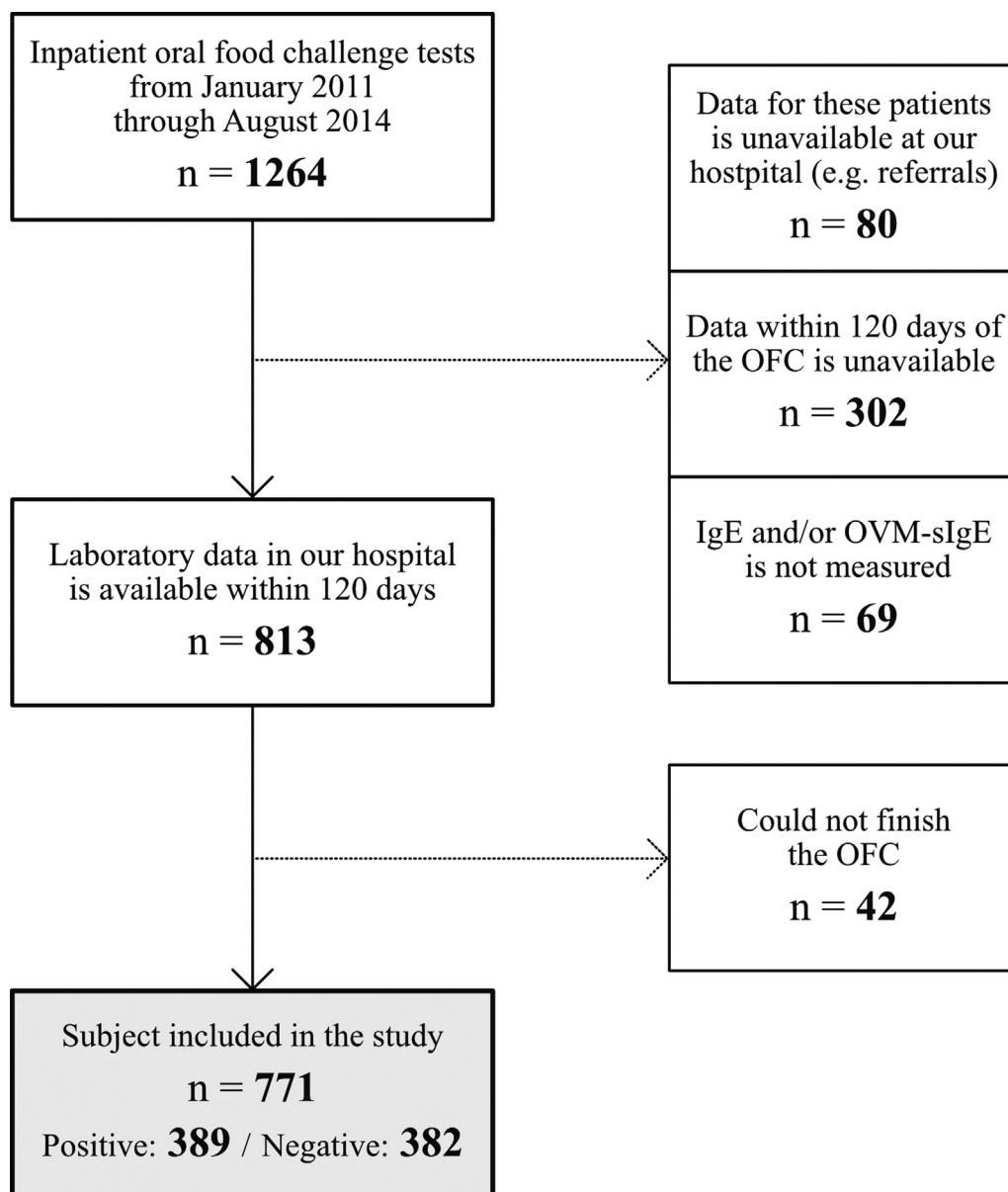


FIGURE E1. Study design and enrolled subjects.

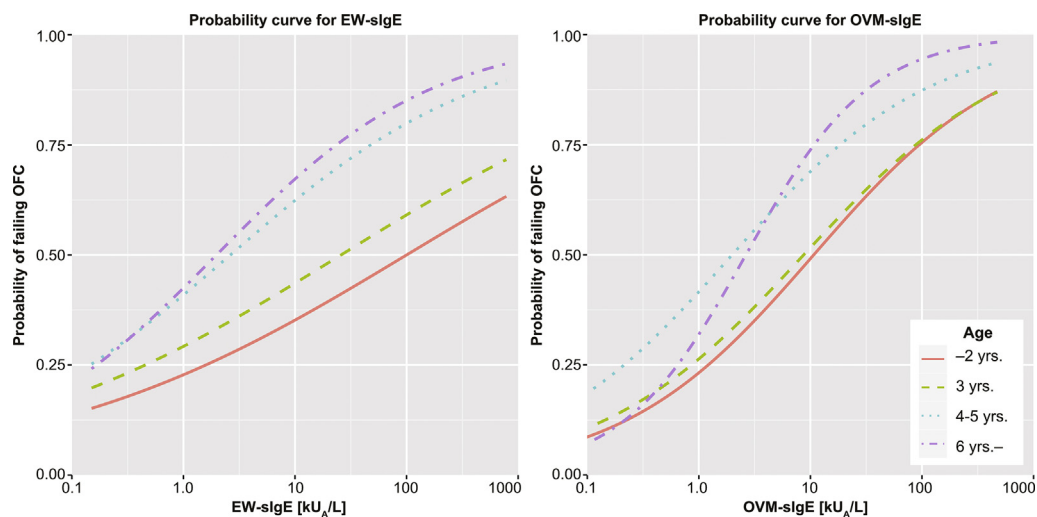


**FIGURE E2.** Probability curve for EW-sIgE and OVM-sIgE (grouped by age).

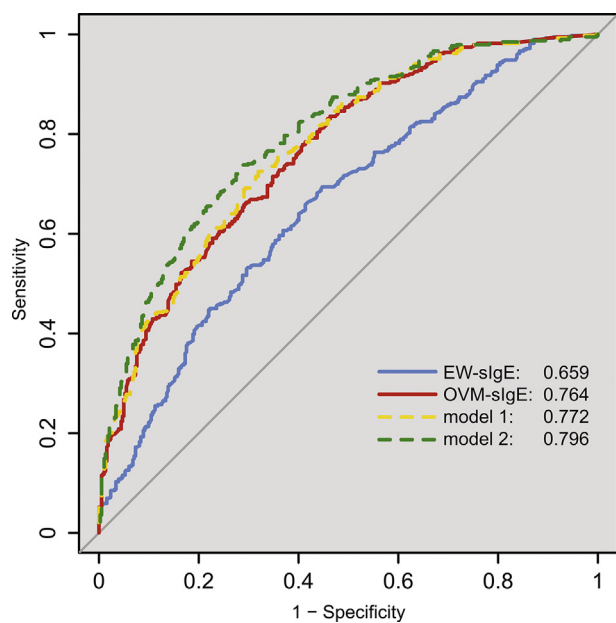


**FIGURE E3.** ROC curves of EW-sIgE, OVM-sIgE, and models 1 and 2 in predicting food challenge results. *ROC*, Receiver operating characteristic.

**TABLE E1.** Patients excluded from this study

| Characteristic            | n = 493          | <i>P</i> value (compared with subjects included in the study) |
|---------------------------|------------------|---------------------------------------------------------------|
| Sex                       |                  |                                                               |
| Male                      | 309 (68.1%)      | .926*                                                         |
| Age (y)                   | 4.42 (2.18-6.89) | .028 <sup>†</sup>                                             |
| Comorbid allergic disease |                  |                                                               |
| BA                        | 96 (20%)         | .657*                                                         |
| AD                        | 249 (51 %)       | .045*                                                         |
| ARC                       | 71 (14%)         | .055*                                                         |

AD, Atopic dermatitis; ARC, allergic rhinoconjunctivitis; BA, bronchial asthma.  
Data are expressed as values (ratios) or median values (interquartile ranges).

\* $\chi^2$  test.

<sup>†</sup>Mann-Whitney *U* test.

**TABLE E2.** Correlation coefficient matrix

| Pearson's <i>r</i> (95% CI)  | Age                 | log <sub>10</sub> (IgE) | log <sub>10</sub> (EW-sIgE) | log <sub>10</sub> (OVM-sIgE) |
|------------------------------|---------------------|-------------------------|-----------------------------|------------------------------|
| Age                          | 1.00                | —                       | —                           | —                            |
| log <sub>10</sub> (IgE)      | 0.495 (0.440-0.547) | 1.00                    | —                           | —                            |
| log <sub>10</sub> (EW-sIgE)  | 0.100 (0.029-0.170) | 0.598 (0.551-0.642)     | 1.00                        | —                            |
| log <sub>10</sub> (OVM-sIgE) | 0.266 (0.199-0.330) | 0.548 (0.497-0.596)     | 0.708 (0.671-0.742)         | 1.00                         |

**TABLE E3.** Result of bootstrap method (internal validation of models 1 and 2)

| Characteristic                      | Model 1                |       | Model 2                |        |
|-------------------------------------|------------------------|-------|------------------------|--------|
|                                     | β (95% CI)             | Bias  | β (95% CI)             | Bias   |
| Sex                                 |                        |       |                        |        |
| Male                                | —                      |       | 1 (reference)          |        |
| Female                              |                        |       | −0.04 (−0.43 to 0.36)  | >−0.01 |
| Age (y)                             | —                      |       | 0.18 (0.09 to 0.29)    | <0.01  |
| log <sub>10</sub> (total IgE)(kU/L) | —                      |       | −1.02 (−1.52 to −0.55) | −0.03  |
| log <sub>10</sub> (EW-sIgE) (kU/L)  | −0.82 (−1.40 to −0.28) | 0.01  | −0.19 (−0.86 to 0.45)  | >−0.01 |
| log <sub>10</sub> (OVM-sIgE) (kU/L) | 2.14 (1.65 to 2.71)    | −0.01 | 2.10 (1.56 to 2.70)    | 0.04   |
| Comorbid allergic disease           |                        |       |                        |        |
| BA                                  | —                      |       | 0.21 (−0.25 to 0.66)   | >−0.01 |
| AD                                  | —                      |       | 0.22 (−0.20 to 0.61)   | 0.01   |
| ARC                                 | —                      |       | 0.22 (−0.32 to 0.80)   | 0.02   |

AD, Atopic dermatitis; ARC, allergic rhinoconjunctivitis; BA, bronchial asthma.

**TABLE E4.** Pumpkin cake used in our OFCs

| Form    | Pumpkin cake                         |
|---------|--------------------------------------|
| Protein | 1.3 g from egg yolk<br>1.8 g from EW |
| OVA     | ELISA: 41.0 mg                       |
| OVM     | ELISA: 310.5 mg                      |

ELISA, measured by Setsuko Ito (Department of Food Science and Nutrition, Doshisha Women's College of Liberal Arts, Kyoto, Japan).

**TABLE E5.** Pumpkin cake recipe

|                                                                |
|----------------------------------------------------------------|
| Ingredients                                                    |
| 40 g pumpkin                                                   |
| 1/2 whole egg                                                  |
| 1 tsp sugar                                                    |
| Directions                                                     |
| 1. Preheat pumpkin in microwave oven                           |
| 2. Mix all ingredients using a hand mixer and fill muffin cups |
| 3. Microwave on high (1000 W) for 90 s                         |

tsp, Teaspoon (5 mL).

The cakes were divided into 3 portions administered at 30-min intervals (1/8 at challenge start, 3/8 after 30 min, and the last half 60 min after challenge start).

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