

SUPPLEMENTARY MATERIAL

Table S1: Oral food challenge protocols of the pre-TETI-II study.

| Food | Ingredients | Preparation, heating time and temperature | Dosing protocol |
|-----------------------------|--|---|---|
| Cake | 4 eggs, 200 g wheat flour*, 200 g plant-based butter and 200 g sugar | Oven: 35' at 165°C (329°F) | Sequential administration of 1, 2, 4, 8, 16 and 32 g <u>CUM dose:</u> 63 g cake |
| Hard-boiled egg | 1 egg Rusk to serve the portions | Boiled for 10' | Sequential administration of: 1) EW: 0.005, 0.01, 0.05, 0.5 g 2) EY: 0.005, 0.01, 0.05, 0.5 g 3) EW + EY: 1.5 + 1.5 g 5 + 5 g 4) EW: 10 g <u>CUM dose:</u> 17 g EW + 7 g EY |
| Omelet | 1 egg Rusk to serve the portions | Stove top: heated for 2' at 120°C (250°F) on both sides | Sequential administration of 0.005, 0.01, 0.05, 0.5 g, 1.5, 5 and 10 g <u>CUM dose:</u> 17 g omelet |
| Soft-boiled egg | 1 egg Brown bread to serve the portions | Boiled for 5-6' The egg yolk was drawn up with a syringe, to portion on bread | Sequential administration of: 1) EW: 0.005, 0.01, 0.05, 0.5 g 2) EY: 0.005, 0.01, 0.05, 0.5 g 3) EW + EY: 1.5 + 1.5 g 5 + 5 g 4) EW: 10 g <u>CUM dose:</u> 17 g EW + 7 g EY |
| Egg mousse (raw egg) | 3 eggs, 400 mL cream 35%** and 40 g sugar | The cream was whipped together with the sugar on medium-high speed. 150 mL of whipped cream was mixed with 50 mL of beaten egg white or egg yellow | Sequential administration of: 1) EW mousse: 0.005, 0.01, 0.05, 0.5 g 2) EY mousse: 0.005, 0.01, 0.05, 0.5 g 3) EW + EY mousse: 1.5 + 1.5 g 5 + 5 g <u>CUM dose:</u> 7 g EW + 7 g EY |

EW: egg white, EY: egg yellow.* In wheat allergic children or children with gluten intolerance, wheat flour was substituted by rice flour. ** In cow's milk allergic children, cream was substituted by soy cuisine. In case of concomitant soy allergy, dairy cream was replaced with rice cream. CUM dose: cumulative provocation dose.

Table S2: Mean %CD63⁺ basophils in response to the five egg extracts in egg allergic, egg tolerant and non-egg-sensitized non-allergic children.

| Extract | Concentration | Egg allergic | Egg tolerant | Non-egg allergic |
|-----------------|---------------|---------------------|--------------------|--------------------|
| Cake | 100 µg/mL | 58.37 (18.31-92.51) | 4.59 (0.17-10.48) | 2.13 (-0.08-5.83) |
| | 10 µg/mL | 47.08 (19.18-89.51) | 1.03 (-1.13-5.1) | 0.12 (-1.20-1.69) |
| | 1 µg/mL | 21.22 (2.23-77.91) | -0.13 (-1.09-0.79) | 0.31 (-0.83-2.04) |
| | 0.1 µg/mL | 2.74 (-1.75-14.31) | -0.21 (-1.39-0.66) | 0.35 (-0.88-1.38) |
| Hard-boiled egg | 100 µg/mL | 38.64 (9.96-82.11) | 2.0 (-0.93-13.48) | 0.61 (-0.95-4.58) |
| | 10 µg/mL | 40.34 (12.01-78.41) | 1.30 (-1.08-9.48) | 0.29 (-0.79-1.59) |
| | 1 µg/mL | 38.45 (12.71-80.61) | 0.79 (-1.02-6.46) | -0.10 (-1.03-1.08) |
| | 0.1 µg/mL | 34.98 (4.94-92.81) | 0.16 (-0.99-2.14) | 0.35 (-0.99-3.23) |
| Omelet | 100 µg/mL | 40.71 (13.51-82.71) | 1.41 (-0.97-10.68) | -0.03 (-1.69-0.88) |
| | 10 µg/mL | 46.55 (19.31-88.76) | 1.90 (-0.87-8.56) | 0.20 (-0.70-1.96) |
| | 1 µg/mL | 51.95 (19.51-90.61) | 0.82 (-0.59-3.52) | 1.10 (-0.89-9.00) |
| | 0.1 µg/mL | 28.64 (3.54-90.91) | -0.27 (-1.32-0.79) | 0.07 (-1.27-2.99) |
| Soft-boiled egg | 100 µg/mL | 42.69 (18.21-86.41) | 2.94 (-1.70-22.78) | 0.16 (-1.27-2.21) |
| | 10 µg/mL | 47.49 (19.41-82.56) | 1.35 (-1.5-8.35) | -0.08 (-1.20-1.78) |
| | 1 µg/mL | 52.15 (19.30-88.91) | -0.06 (-1.38-1.24) | -0.08 (-1.05-0.83) |
| | 0.1 µg/mL | 28.76 (2.51-88.31) | 0.69 (-1.30-7.88) | 0.35 (-1.32-4.28) |
| Raw egg | 100 µg/mL | 47.11 (21.10-85.31) | 1.76 (-1.01-11.78) | 0.88 (-0.74-5.17) |
| | 10 µg/mL | 49.82 (18.41-85.96) | 1.20 (-1.48-4.94) | -0.11 (-1.10-1.71) |
| | 1 µg/mL | 53.29 (22.01-92.61) | -0.11 (-1.04-1.84) | 0.02 (-0.92-1.27) |
| | 0.1 µg/mL | 23.54 (2.49-86.21) | -0.25 (-1.59-0.99) | 0.08 (-1.05-3.59) |

The %CD63⁺ basophils are corrected for spontaneous CD63-expression, by subtracting the %CD63⁺ basophils in the unstimulated control condition. The values are expressed as means (minimum-maximum).

Table S3: Discriminative capacity of the egg sIgE levels between true egg allergy and tolerance based on the standard cutoff 0.35 kUA/L, lower limit of detection of 0.10 kUA/L, and newly defined optimal cutoffs.

| IgE | AUC ROC | Cutoff (kUA/L) | Sensitivity (%) | Specificity (%) |
|----------------|---------------|---------------------------------|------------------|---------------------|
| Egg white sIgE | 0.95 (0.86-1) | 0.35 | 100 (72-100) | 50 (24-76) |
| Egg yolk sIgE | 0.94 (0.85-1) | | 90 (59.58-99.49) | 77.78 (45.26-96.05) |
| Ovomucoid sIgE | 1.00 (1-1) | | 100 (72.25-100) | 60 (31.27-83.18) |
| Ovalbumin sIgE | 0.89 (0.75-1) | | 100 (72.25-100) | 66.67 (35.42-87.94) |
| Egg white sIgE | | 0.10 = lower limit of detection | 100 (72-100) | 20 (3.6-51) |
| Egg yolk sIgE | | | 90 (59.58-99.49) | 44 (18.88-73.33) |
| Ovomucoid sIgE | | | 100 (72.25-100) | 30 (10.78-60.32) |
| Ovalbumin sIgE | | | 100 (72.25-100) | 33 (12.06-64.58) |
| | | | | |
| Egg white sIgE | | 0.99 | 100 (72-100) | 80 (49-96) |
| Egg yolk sIgE | | 1.05 | 80 (49.02-96.45) | 88.89 (56.50-99.43) |
| Ovomucoid sIgE | | 0.88 | 100 (72.25-100) | 100 (72.25-100) |
| Ovalbumin sIgE | | 1.01 | 80 (49.02-96.45) | 77.78 (45.26-96.05) |

Table S4: Pearson correlation between egg sIgE levels and area under the BAT dose-response curve or maximal %CD63⁺ basophils of egg allergic and egg tolerant children.

| Extract | Egg white sIgE | Ovomucoid sIgE | Ovalbumin sIgE | Total IgE |
|------------------------|----------------|----------------|----------------|-----------|
| Cake | | | | |
| Maximal % CD63 | <i>r= 0.52</i> | r= 0.43 | <i>r= 0.50</i> | r= 0.02 |
| AUC | <i>r= 0.56</i> | <i>r= 0.51</i> | <i>r= 0.55</i> | r= -0.0 |
| Hard-boiled egg | | | | |
| Maximal % CD63 | <i>r= 0.49</i> | r= 0.38 | <i>r= 0.54</i> | r= -0.02 |
| AUC | <i>r= 0.48</i> | r= 0.35 | <i>r= 0.55</i> | r= -0.08 |
| Omelet | | | | |
| Maximal % CD63 | <i>r= 0.49</i> | r= 0.37 | <i>r= 0.51</i> | r= 0.03 |
| AUC | <i>r= 0.48</i> | r= 0.34 | <i>r= 0.54</i> | r= 0.02 |
| Soft-boiled egg | | | | |
| Maximal % CD63 | r= 0.35 | r= 0.23 | r= 0.42 | r= -0.0 |
| AUC | r= 0.40 | r= 0.24 | <i>r= 0.50</i> | r= 0.01 |
| Raw egg | | | | |
| Maximal % CD63 | r= 0.50 | r= 0.39 | <i>r= 0.54</i> | r= 0.01 |
| AUC | r= 0.46 | r= 0.29 | <i>r= 0.56</i> | r= 0.06 |

AUC: area under the BAT CD63 dose-response curve. Bold values indicate significant correlations, $p < 0.05$.

Table S5: Allergic reactions during the pre-TETI-II study.

| | Visit 1: Cake | Visit 2: hard-boiled egg | Visit 3: omelet, pancakes or waffles | Visit 4: soft-boiled egg or sunny side-up | Visit 5: raw egg |
|-----|---|--|--|--|------------------|
| L1 | | HE home: single episode of co-factor induced swelling of the lips | OM home: single episode of abdominal pain the day after intake | | |
| L2 | | | Sandwich, cake, cookie home: recurrent episodes of abdominal pain | SE OFC: co-factor* induced nausea, vomiting, conjunctivitis and sneezing at a cumulative dose of ± 17 g egg white and ± 7 g egg yellow, requiring antihistamines and betamethasone (drops) | |
| L3 | Cake home: occasional itchy tongue *Accidental intake of raw dough resulted in urticaria on chin that resolved spontaneously | HE home: single episode of itchy tongue (egg was only boiled for 5 minutes) | | SE home: recurrent itching of the tongue after eating runny egg yolk | |
| L4 | Cake home: single episode of rash in face and eyes | | OM home: single episode of peri-oral flush. Resolved spontaneously after 5 minutes | | |
| L5 | | | | | |
| L6 | | | | | |
| L7 | Cake home: flare-up of eczema | | OM home: single episode of abdominal cramps | | |
| L8 | Cake OFC: lip tingling at a cumulative dose of 63 g cake. Disappeared quickly without medication | HE OFC: urticarial rash and coughing at a cumulative dose of ± 17 g egg white and ± 7 g egg yellow, requiring antihistamines | | | |
| L9 | | | | | |
| L10 | | HE home: two episodes of itchy lips | | | |
| L11 | Cake home: occasional abdominal cramps & flat stool (emotional factor) | HE home: occasional abdominal cramps & flat stool (emotional factor) | | SE home: sporadic itching of the mouth (emotional factor) | |
| L12 | | HE home: occasional nausea | OM home: once redness and itching of the back | | |

HE: hard-boiled egg, OM: omelet, SE: soft-boiled egg, OFC: oral food challenge. Red area: allergic reactions that led to discontinuation of the gradual reintroduction protocol.

* Clinical examination revealed a concurrent tonsillitis, which might have been a contributing factor to the allergic reaction.

Table S6: Evaluation of blood pressure, tryptase level and complement components before and after oral food challenges.

| | Parameters | Cake (V1) | | Hard-boiled egg (V2) | | Omelet (V3) | | Soft-boiled egg (V4) | | Raw egg (V5) | |
|-----------------------|------------------------|-----------|--------|----------------------|--------|-------------|--------|----------------------|--------|--------------|--------|
| | | Before | After | Before | After | Before | After | Before | After | Before | After |
| L1 | Tryptase (µg/L)* | 6 | 5.7 | 4.4 | 4.1 | 4.8 | 4.4 | 6.7 | 4.9 | 5.8 | 5 |
| | C3 (g/L) | 0.59 | 0.59 | 0.64 | 0.67 | 0.72 | 0.74 | 0.73 | 0.74 | 0.61 | 0.59 |
| | C4 (g/L) | 0.1 | 0.1 | 0.14 | 0.14 | 0.12 | 0.12 | 0.13 | 0.13 | 0.1 | 0.1 |
| | Blood pressure (mm Hg) | 105/76 | 95/57 | 127/63 | 98/57 | 113/71 | 115/74 | 96/70 | 107/89 | 127/65 | 123/58 |
| L2 | Tryptase (µg/L)* | 3.9 | 3.9 | 3.1 | 3.1 | 4.2 | 3.8 | 3.8 | 4.4 | | |
| | C3 (g/L) | 0.93 | 0.92 | 0.94 | 0.94 | 0.83 | 0.86 | 0.85 | 0.86 | | |
| | C4 (g/L) | 0.32 | 0.31 | 0.29 | 0.27 | 0.26 | 0.28 | 0.26 | 0.27 | | |
| | Blood pressure (mm Hg) | 104/58 | 100/50 | 95/52 | 92/52 | 101/67 | 107/58 | 106/59 | 115/80 | | |
| L3^s | Tryptase (µg/L)* | 3.3 | 2.9 | 3.7 | 3.3 | 3.1 | 3.1 | 3.2 | 3.3 | | |
| | C3 (g/L) | 1.05 | 1.04 | 0.96 | 0.85 | 0.95 | 0.95 | 0.75 | 0.80 | | |
| | C4 (g/L) | 0.22 | 0.21 | 0.18 | 0.16 | 0.19 | 0.19 | 0.13 | 0.14 | | |
| | Blood pressure (mm Hg) | 104/60 | 105/64 | 102/69 | 103/58 | 95/54 | 113/61 | 90/67 | 116/58 | | |
| L4 | Tryptase (µg/L)* | 3.4 | 3.8 | 5.2 | 4.3 | 5.1 | 5 | 5.3 | 5.1 | 4.9 | 4.7 |
| | C3 (g/L) | 1.05 | 1.03 | 0.93 | 0.89 | 0.82 | 0.81 | 0.91 | 0.89 | 0.93 | 0.90 |
| | C4 (g/L) | 0.22 | 0.22 | 0.19 | 0.18 | 0.15 | 0.15 | 0.17 | 0.18 | 0.21 | 0.2 |
| | Blood pressure (mm Hg) | 111/81 | 104/63 | 101/55 | 90/54 | 105/73 | 109/68 | 102/53 | 104/54 | 92/74 | 119/83 |
| L5 | Tryptase (µg/L)* | 5.3 | 5.9 | 3.8 | 4 | 6.7 | 6.8 | 6.8 | 5.2 | 6.6 | 5.8 |
| | C3 (g/L) | 1.05 | 1.09 | 0.98 | 1 | 0.84 | 0.80 | 0.82 | 0.78 | 0.90 | 0.86 |
| | C4 (g/L) | 0.25 | 0.26 | 0.16 | 0.17 | 0.12 | 0.12 | 0.13 | 0.13 | 0.13 | 0.13 |
| | Blood pressure (mm Hg) | 94/74 | 108/61 | 102/57 | 102/58 | 114/54 | 109/57 | 105/54 | 107/59 | 106/60 | 115/66 |
| L6 | Tryptase (µg/L) | 5.1 | 5.6 | 4.8 | 4.4 | 4.2 | 4.2 | 5.1 | 5.2 | 5.2 | 5.1 |
| | C3 (g/L) | 0.74 | 0.77 | 0.87 | 0.82 | 0.80 | 0.89 | 0.91 | 0.91 | 0.91 | 0.86 |
| | C4 (g/L) | 0.2 | 0.2 | 0.2 | 0.2 | 0.22 | 0.23 | 0.25 | 0.28 | 0.24 | 0.23 |
| | Blood pressure (mm Hg) | 96/68 | 101/86 | 100/62 | 95/62 | 103/67 | 103/58 | 115/75 | 96/55 | 101/67 | 110/65 |
| L7 | Tryptase (µg/L)* | 4.3 | 4.5 | 4.4 | 4.4 | 2.3 | - | 4.1 | 1 | 4.2 | 4 |
| | C3 (g/L) | 1.04 | 1.04 | 0.93 | 0.88 | 0.99 | - | 0.97 | 0.93 | 0.83 | 0.79 |
| | C4 (g/L) | 0.17 | 0.16 | 0.13 | 0.12 | 0.14 | - | 0.15 | 0.15 | 0.12 | 0.12 |
| | Blood pressure (mm Hg) | 95/65 | 95/54 | 107/54 | 114/56 | 104/73 | 103/56 | 98/57 | 107/50 | 89/40 | 94/55 |
| L8 | Tryptase (µg/L)* | 4.3 | 4.6 | 5.3 | 4.5 | | | | | | |
| | C3 (g/L) | 0.9 | 0.85 | 0.91 | 0.9 | | | | | | |
| | C4 (g/L) | 0.12 | 0.11 | 0.12 | 0.12 | | | | | | |
| | Blood pressure (mm Hg) | 111/64 | 102/51 | 113/82 | 114/84 | | | | | | |
| L9 | Tryptase (µg/L)* | 12.5 | 11.1 | 15.3 | 11.6 | 13 | 11.8 | 10.3 | 9.4 | - | 15.2 |
| | C3 (g/L) | 0.84 | 0.78 | 0.92 | 0.84 | 0.79 | 0.79 | 1.03 | 0.96 | 0.91 | 0.89 |
| | C4 (g/L) | 0.16 | 0.15 | 0.16 | 0.15 | 0.16 | 0.16 | 0.24 | 0.23 | 0.23 | 0.22 |
| | Blood pressure (mm Hg) | 102/70 | 108/57 | 99/62 | 105/72 | 105/75 | 111/68 | 105/65 | 112/67 | 109/68 | 107/65 |
| L10 | Tryptase (µg/L)* | 4.4 | 4.1 | 4.4 | 4.7 | 4 | 4.1 | 3.9 | 3.6 | 4.3 | 4.1 |
| | C3 (g/L) | 0.74 | 0.72 | 0.76 | 0.78 | 0.61 | 0.64 | 0.73 | 0.66 | 0.68 | 0.66 |
| | C4 (g/L) | 0.13 | 0.12 | 0.11 | 0.11 | 0.10 | 0.10 | 0.12 | 0.11 | 0.11 | 0.11 |
| | Blood pressure (mm Hg) | 100/65 | 104/72 | 100/76 | 115/64 | 114/51 | 117/57 | 102/70 | 110/53 | 108/57 | 111/52 |
| L11 | Tryptase (µg/L)* | 12.8 | 13.8 | 11.1 | 10.4 | 11.5 | 11.4 | 11.8 | 11.1 | 10.7 | 10.4 |
| | C3 (g/L) | 1.2 | 1.24 | 0.91 | 1.02 | 0.81 | 0.78 | 1.01 | 0.96 | 0.92 | 0.92 |
| | C4 (g/L) | 0.35 | 0.34 | 0.24 | 0.27 | 0.17 | 0.16 | 0.28 | 0.27 | 0.21 | 0.21 |
| | Blood pressure (mm Hg) | 104/77 | 99/58 | 102/61 | 104/61 | 119/64 | 105/55 | 95/50 | 103/64 | 100/62 | 110/61 |
| L12 | Tryptase (µg/L)* | 4.5 | 4.3 | 3.7 | 3.7 | 3.6 | 3.8 | 3.6 | 3.9 | 3.5 | 3.5 |
| | C3 (g/L) | 0.86 | 0.81 | 0.78 | 0.83 | 0.89 | 0.91 | 0.74 | 0.77 | 0.95 | 0.98 |
| | C4 (g/L) | 0.14 | 0.13 | 0.13 | 0.14 | 0.21 | 0.22 | 0.14 | 0.14 | 0.22 | 0.24 |
| | Blood pressure (mm Hg) | 94/52 | 99/58 | 106/65 | 101/57 | 102/55 | 101/57 | 110/70 | 98/61 | 99/56 | 101/65 |

* We could not find a significant increase in the serum tryptase level after any of the performed oral food challenge tests, based on the tryptase formula “> 2 µg/L+1.2 x over the individuals baseline”. Red boxes: children who experienced an allergic reaction during an oral food challenge (OFC). Blue highlighted area: represents the steps in the protocol that children could not undergo due to an allergic reaction during one of the previous steps. [§] L3 experienced a recurring itchy tongue upon exposure to runny egg yolk at home, but did not react during the in-hospital OFC with soft-boiled egg. Normal reference values for C3 and C4 are 0.79-1.52 and 0.16-0.38, respectively.

Table S7: Pearson correlation between egg sIgE levels and area under the BAT dose-response curve or maximal %CD63⁺ basophils during the first and last study visit of the pre-TETI-II study.

| Extract | Egg white sIgE | | Ovomucoid sIgE | | Ovalbumin sIgE | | Total IgE | |
|------------------------|-----------------------|-----------------------|----------------|---------|-----------------------|----------|-----------|----------|
| | V1 | V5 | V1 | V5 | V1 | V5 | V1 | V5 |
| Cake | | | | | | | | |
| Maximal % CD63 | <i>r= 0.85</i> | r= 0.57 | r= 0.41 | r= 0.46 | <i>r= 0.82</i> | r= 0.53, | r= -0.14 | r= 0.63 |
| AUC | <i>r= 0.85</i> | r= 0.44 | r= 0.41 | r= 0.27 | <i>r= 0.82</i> | r= 0.56, | r= -0.14 | r= 0.42 |
| Hard-boiled egg | | | | | | | | |
| Maximal % CD63 | r= 0.22 | r= 0.16 | r= 0.44 | r= 0.32 | r= 0.28 | r= 0.18 | r= 0.03 | r= -0.37 |
| AUC | r= 0.26 | r= 0.23 | r= 0.32 | r= 0.32 | r= 0.42 | r= 0.23 | r= -0.13 | r= -0.40 |
| Omelet | | | | | | | | |
| Maximal % CD63 | r= 0.41 | r= 0.60 | r= 0.47 | r= 0.25 | r= 0.62 | r= 0.64 | r= -0.12 | r= -0.15 |
| AUC | r= 0.44 | r= 0.60 | r= 0.50 | r= 0.25 | <i>r= 0.68</i> | r= 0.64 | r= -0.12 | r= -0.15 |
| Soft-boiled egg | | | | | | | | |
| Maximal % CD63 | r= 0.33 | r= 0.53 | r= 0.38 | r= 0.34 | r= 0.53 | r= 0.54 | r= -0.08 | r= -0.22 |
| AUC | r= 0.48 | <i>r= 0.69</i> | r= 0.32 | r= 0.46 | r= 0.65 | r= 0.65 | r= -0.15 | r= -0.07 |
| Raw egg | | | | | | | | |
| Maximal % CD63 | r= 0.41 | r= 0.53 | r= 0.23 | r= 0.25 | r= 0.57 | r= 0.59 | r= -0.18 | r= -0.40 |
| AUC | r= 0.51 | r= 0.57 | r= 0.32 | r= 0.46 | r= 0.67 | r= 0.57 | r= -0.19 | r= -0.20 |

AUC: area under the CD63 dose-response curve. Bold values indicate significant correlations, $p \leq 0.05$