

## SUPPLEMENTARY MATERIAL

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

Food	Ingredients	Preparation, heating time and temperature	Dosing protocol
<b>Cake</b>	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
<b>Hard-boiled egg</b>	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
<b>Omelet</b>	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
<b>Soft-boiled egg</b>	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
<b>Egg mousse (raw egg)</b>	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<sup>+</sup> 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)
	<u>10 µg/mL</u>	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)
	<u>10 µg/mL</u>	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)
	<u>10 µg/mL</u>	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)
	<u>10 µg/mL</u>	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)
	<u>10 µg/mL</u>	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<sup>+</sup> basophils are corrected for spontaneous CD63-expression, by subtracting the %CD63<sup>+</sup> 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)
Newly defined optimal cutoffs				
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<sup>+</sup> basophils of egg allergic and egg tolerant children.

Extract	Egg white sIgE	Ovomucoid sIgE	Ovalbumin sIgE	Total IgE
<b>Cake</b>				
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
<b>Hard-boiled egg</b>				
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
<b>Omelet</b>				
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
<b>Soft-boiled egg</b>				
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
<b>Raw egg</b>				
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
<b>L1</b>		HE home: single episode of co-factor induced swelling of the lips	OM home: single episode of abdominal pain the day after intake		
<b>L2</b>			Sandwich, cake, cookie home: recurrent episodes of abdominal pain	SE OFC: co-factor* induced nausea, vomiting, conjunctivitis and sneezing at a cumulative dose of $\pm 17$ g egg white and $\pm 7$ g egg yellow, requiring antihistamines and betamethasone (drops)	
<b>L3</b>	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	
<b>L4</b>	Cake home: single episode of rash in face and eyes		OM home: single episode of peri-oral flush. Resolved spontaneously after 5 minutes		
<b>L5</b>					
<b>L6</b>					
<b>L7</b>	Cake home: flare-up of eczema		OM home: single episode of abdominal cramps		
<b>L8</b>	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 $\pm 17$ g egg white and $\pm 7$ g egg yellow, requiring antihistamines			
<b>L9</b>					
<b>L10</b>		HE home: two episodes of itchy lips			
<b>L11</b>	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		
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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.

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**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
<b>L1</b>	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
<b>L2</b>	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		
<b>L3<sup>s</sup></b>	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		
<b>L4</b>	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
<b>L5</b>	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
<b>L6</b>	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
<b>L7</b>	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
<b>L8</b>	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						
<b>L9</b>	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
<b>L10</b>	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
<b>L11</b>	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
<b>L12</b>	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. <sup>s</sup> 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.

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**Table S7.** Pearson correlation between egg sIgE levels and area under the BAT dose-response curve or maximal %CD63<sup>+</sup> 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
<b>Cake</b>								
Maximal % CD63	<b><i>r= 0.85</i></b>	r= 0.57	r= 0.41	r= 0.46	<b><i>r= 0.82</i></b>	r= 0.53,	r= -0.14	r= 0.63
AUC	<b><i>r= 0.85</i></b>	r= 0.44	r= 0.41	r= 0.27	<b><i>r= 0.82</i></b>	r= 0.56,	r= -0.14	r= 0.42
<b>Hard-boiled egg</b>								
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
<b>Omelet</b>								
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	<b><i>r= 0.68</i></b>	r= 0.64	r= -0.12	r= -0.15
<b>Soft-boiled egg</b>								
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	<b><i>r= 0.69</i></b>	r= 0.32	r= 0.46	r= 0.65	r= 0.65	r= -0.15	r= -0.07
<b>Raw egg</b>								
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,  $p \leq 0.05$