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The baked side: Cow's milk and egg protein threshold dose distributions in children reacting to baked milk and baked egg

Rocco Luigi Valluzzi, MD[a]*, Carla Riccardi, MD[b], Sara Urbani, MD[b], Davide Ursi, MD[c], Deborah Zavettieri, MD[d], Francesco Di Girolamo, PhD[e], Lamia Dahdah, MD[b], Veronica Calandrelli, PharmD[b], Vincenzo Fierro, MD[b] and Alessandro Fiocchi, MD[b]

ABSTRACT

Background: Children allergic to milk and egg, but tolerant to baked products, display higher reactivity thresholds than the general population of children allergic to milk and egg. We sought to verify the reactivity thresholds of milk- and egg-allergic children who also react to baked milk and baked egg, respectively.

Methods: We retrospectively assessed consecutive oral food challenge (OFC) for baked milk and egg between January 2018 and March 2022 in a population of baked milk- and baked-egg allergic children.

Results: Among 407 children included (median age 56 - IQR 31.1–103.7 months, 67.1% male), 93 (23.6%) returned positive OFC results, 41 with baked milk, and 52 with baked egg. The most conservative ED01 was 0.4 mg total protein (IQR 0.1–2.7) for milk and 2.2 mg total protein (IQR 0.6–7.3) for egg. The respective ED05 was 3.9 (IQR 1.1–14) mg for milk and 11.7 (IQR 5–27.2) mg for egg. Such thresholds are consistent to those found for fresh milk (0.8 times for ED01, 1.1 times for ED05). For egg, they are 6.5 (egg ED01), and 7.5 (egg ED05) times lower than for native form.Compared to the currently used thresholds, they are 1.3 (milk ED01), 1.3 (milk ED05), 11 (egg ED01), and 4.9 (egg ED05) times higher.

Conclusions: Milk thresholds are similar to those already observed in baked allergic versus baked tolerant children, while EDs for egg are at least 1.6 times higher than those currently indicated.Egg-allergic patients could be exempt from the recommendations of absolute avoidance of foods when present in infinitesimal quantities, represented by precautionary allergen labelling based on current EDs.

Keywords: Challenge tests, Egg hypersensitivity, Food hypersensitivity, Milk hypersensitivity, Pediatrics

[a]Allergy Unit, Piazza S. Onofrio Bambino Gesù Children's Hospital, IRCCS, 00165, Rome, Holy See, Italy
*Corresponding author. E-mail: roccoluigi.valluzzi@opbg.net
Full list of author information is available at the end of the article

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INTRODUCTION

The clinical interest of the eliciting doses (EDs) in food allergy is growing.[1] In these patients, the probability of reacting to a specific food depends on the EDs. OIT (oral Immunotherapy) studies indicate that increasing the ED reduces the risk of an allergic reaction from accidental exposure,[2] and the use of anti-IgE reduces the likelihood and the severity of the reactions by increasing the threshold doses for different foods.[3] It is, therefore, not surprising that the most recent and authoritative assessments confirm relying on the EDs to establish the precautionary labeling (PAL) limit, ie, the quantity of accidently contaminating food below which it is not necessary to issue an alarm.[4]

The Voluntary Incidental Trace Allergen Labelling (VITAL) established doses to which one percent of the allergy patient population reacts (ED01) of 0.3 mg total protein for milk and 0.2 mg for egg.[5,6] An ED05 of 3.1 mg for milk and 2.4 mg for egg has been calculated.[5–8] Under this amount, the manufacturing companies are suggested to renounce to affix PAL alluding to the risk of allergic reactions. Such thresholds have been elaborated on published diagnostic challenges. For milk and egg in particular, none of the included studies distinguished patients tolerant or reactive to baked foods.[9-32] Yet, due to structural changes of food proteins upon heating,[33] 69%–73% of children allergic to milk and egg seen at tertiary centers are tolerant to the baked forms,[34–41] and in an open population 80.3% of children with challenge-proven egg allergy tolerated a baked egg challenge.[42] While retrospective studies suggests that children who tolerate baked egg are less sensitive to native egg compared with children with unknown baked reactivity,[43] the only published study comparing thresholds found similar ED10 and ED50 values in baked tolerant and baked-allergic children.[44] Based on it, it was argued that extensive heating of hen's egg and cow's milk in a food matrix impacts only marginally the threshold dose-distribution curves.

Recently, we found that the subpopulation of children allergic to milk/egg, but tolerant to baked proteins, displays higher reactivity thresholds than the population of children who are allergic to milk/egg regardless of thermal processing.[45] This raises the question of whether children reactive to baked foods have lower thresholds. If so, they should be considered as a subpopulation of particular risk for clinical advice regarding the possible presence of allergenic contaminants in tiny quantities in commercial foods. To clarify this, we present here the EDs of children allergic to milk and egg who also react to baked proteins.

Caseload and methods study design

Bambino Gesù Children's Hospital is to a third-level hospital with a catchment area corresponding to the entire Italian nation. Our center accommodates various risk levels associated with food allergies. In it, patients with a history of immediate reactions to milk and/or egg sensitized to such foods are subjected to diagnostic oral food challenges (OFCs) on a scheduled basis in a programmed way, until they get tolerance.[46] OFCs are performed in an office setting with facilities for preparation, administration, and monitoring. Patients' reactions are assessed until objective symptoms appear. Those already consuming baked milk and/or egg get OFCs directly with raw milk and/or egg. Otherwise, patients are preliminarily evaluated for tolerance to baked milk and/or baked egg.[47] OFCs are not performed only in those patients with a recent anaphylactic reaction.[48]

In this study, we retrospectively evaluated the thresholds of reactivity of consecutive children who underwent OFC for baked milk and/or baked egg between January 2019 and March 2022. For those who have repeatedly undergone OFC for the same food, we have included the first procedure for each type of food. Patients with a personal history of allergic reaction to both baked milk and baked egg performed 2 different OFCs.

Clinical suspicion was based on medical history, with admission symptoms grouped in patient medical records based on parental reports. We only considered children with a personal history of immediate mild to severe systemic reactions, developed up to 2 h after food consumption. Parents granted their written agreement after being fully aware of the risks of the procedure prior to the OFC. Sensitivity to the relevant foods was assessed on the day we delivered OFC using a skin prick test (SPT) and the determination of specific

IgE (sIgE). Patients were excluded if both their sIgE determination (cut-off 0.35 kU/L; ImmunoCAP Thermo Fisher, Uppsala, Sweden) and skin prick test (wheal Ø cut-off 3 mm; Lofarma, Milan, Italy) were below the cut-offs.

We performed a seven-step OFC, open or blinded double-blind, placebo-controlled food challenges (DBPCFC). We administered milk-free, egg-containing biscuits ("Novellino", Camporelli S.p.A., Novara, Italy; 2.09 g egg protein per 100 g product) for confirmation of baked egg allergy, and egg-free, milk-containing biscuits ("Biscottino Primi Mesi", Plasmon, Milano, Italy; 1.30 g milk protein per 100 g product) for confirmation of baked milk allergy.

Specifically, for baked milk, we set the lowest dose to 1.3 mg of protein and the total amount of protein to 837.2 mg, corresponding to 100 mg and 64.4 g of biscuits, respectively; for egg, we set the lowest dose to 2.1 mg and the total amount of protein at 1115 mg of proteins (about a 53.4 g of biscuits). Equivalent amount of baked milk and baked egg used in OFCs are reported in Supplemental Table 1 in the Supplemental Materials. We derived the protein content of biscuits according to the product data sheets. Patients remained under observation for at least 2 h after the last dose.

All parents or legal tutors of the patients received information about the risks of OFCs, and written informed consent was obtained for each patient.

The institutional review board approved the protocol. We got approval from our local Ethic Committees to the use of such clinical data.[40]

Symptom grading and threshold data

As suggested by the main guidelines, we performed OFCs in hospital with close supervision and immediate availability of emergency treatment. Our center accommodates various risk levels associated with food allergies. Low-risk OFCs are performed in an office setting with facilities for preparation, administration, and monitoring. The same clinicians observed and assessed the outcome of the oral food challenges, and doses were administered until objective symptoms appeared. For higher-risk challenges, we ensure necessary materials and support for treating severe reactions, including immediate access to emergency services. The expected severity of symptoms and the need for equipment influences decisions regarding the location of an OFC. Where there is a significantly high risk of a severe reaction, it is preferable to conduct these challenges within the intensive care unit.[49,50] We classified patient's reactive symptoms into 5 groups, from subjective symptoms (nausea, abdominal pain, pruritus, oral allergy syndrome) to systemic reactions.[51] We designated the starting point at which objective symptoms appear at a dose as the Lowest Observed Adverse Effect Level (LOAEL) and the highest dose that does not produce any objective symptoms as the No Observed Adverse Effect Level (NOAEL).

According to previous studies, we choose an Interval-Censoring Survival Analysis (ICSA) approach to our data.[45,52] To summarize, if a patient developed an objective reaction after the first dose, the NOAEL was considered being 0 and the LOAEL equal to the first dose (left censored). If a patient developed no objective reaction after the seventh dose, the entire cumulative dose was considered as NOAEL and the LOAEL set to infinity (right censored). If the patient discontinued the test because of subjective symptoms, we considered the final administered dose as right censored. In all other cases, interval-censoring occurs bounded by the NOAEL and LOAEL.

Statistical analysis

We used multivariate logistic regression analysis to assess independent predictors (age, sex, history of clinical manifestations, sIgE level results or SPTs wheal size) of patients' individual LOAEL. A p-value less than 0.05 was considered statistically significant; sIgE values $>$ 100 kU/L received a designated value of 101 kU/L (JMP®, Version 15. SAS Institute Inc., Cary, NC, 1989–2023).

We used SAS 15.1 (SAS Institute, Cary, NC, U.S.A.) and LIFEREG procedure to fit parametric models to the interval-censored data. We considered Log-normal, log-logistic, and Weibull models to fit these data and to extrapolate EDs (ED01, ED05, ED10, ED25 and ED50), which are the doses predicted to elicit allergic reactions in 1%, 5%, 10%, 25%, and 50% of milk and egg-allergic patient, respectively.[53] Confidence intervals (CI) were calculated and added.

RESULTS

Patient characteristics

Of 442 consecutive patients evaluated in the period considered, excluding repeat testing, 229 completed baked milk, and 197 baked egg OFC. Nineteen patients, who referred a personal history of allergic reactions to both baked milk and baked egg, underwent both OFCs.

The mean age of the 407 patients was 74.8 months (SD 55.5). The median age is listed in Table 1, along with their clinical characteristics. Allergy tests results of 426 patients are reported in Table 2.

Of all OFCs assessed, 416 (97.7%) were open and 10 (2.3%) DBPCFC. None of the patients who underwent DBPCFC reacted to the placebo, and they were considered homogeneous with those who underwent open OFC.

One hundred sixty-nine patients reported possible allergic reactions both to milk and egg.

Forty-one (17.9%) of the 229 patients who completed OFC with baked milk had a positive result. Eleven of them had reported with a history of a severe systemic reaction,[51] 15 reported allergic reactions to other foods (12 to egg), 15 had a history of atopic dermatitis, and 20 had a history of respiratory disease, such as asthma and/or rhinitis, whether allergic or not fully diagnosed.

After challenge with baked milk, the most common symptoms were respiratory (28 patients, 68.3%). Nine patients (21.9%) developed only respiratory reactions: of them 3 were classified in Group 2, 4 in Group 3 (1 with throat pruritus or tightness), and 2 in Group 4. Three patients developed only skin reactions (2 with generalized urticaria) and 5 only gastrointestinal symptoms (4 with emesis). Twenty-four patients developed a systemic reaction (58.5%): 8 were included in Group 2, 4 in Group 3, and twelve in Group 4. Of the last patients, 4 developed localized urticaria and dyspnea, 3 generalized urticaria and dyspnea, 1 dyspnea, nausea, emesis and tachycardia, 1 generalized urticaria, dyspnea and tachycardia, 1 dyspnea and mild lip swelling, 1 dyspnea, nausea and emesis, and the last 1 localized urticaria, dyspnea, repetitive vomiting, mild hypotension and change in activity (this was the only patient of both groups that needed hospitalization).

Of the 197 patients who completed OFC with baked egg, 52 (26.4%) were positive. Forty-one patients reported allergic reactions to other foods (33 to milk), 21 a history of atopic dermatitis, and 29 a personal history of respiratory diseases;

	Baked Milk	Baked Egg	Total
Age (months), median (25th,75th percentile)	56 (30, 112)	56 (35, 99)	56 (31.2, 103.7)
Male, n (%)	156 (68.1)	130 (66)	286 (67.1)
Atopic dermatitis, n (%)	67 (29.2)	88 (44.7)	155 (36.4)
Respiratory disease, n (%)	73 (31.9)	91 (46.2)	164 (38.5)
Anaphylaxis, n (%) ER visits in the clinical history, n (%) User epinephrine in last year, n (%)	44 (19.2) 18 (4.2) 23 (5.4)	39 (19.8) 18 (4.2) 19 (4.5)	83 (19.5) 36 (8.5) 42 (9.8)
Challenges performed, n OFC, n (%) DBPCFC, n (%)	229 225 (98.3) 4 (1.7)	197 191 (97) 6 (3)	426 416 (97.6) 10 (2.3)
Positive outcome, n (% of performed challenges)	41 (17.9)	52 (26.4)	93 (20.4)
First dose responders, n (% of performed challenges)	1 (0.1)	–	1 (0.3)
LOAEL, mg	1.3	8.4	–

Table 1. Characteristics of 426 patients who completed OFC protocol. *DBPCFC, double-blinded placebo-controlled food challenge; ER, Emergency room; LOAEL, lower observed adverse effect level; OFC, open food challenge.*

	Positive skin prick test results, n (%) [Median; 25th-75th percentile]	Positive specific IgE assay results, n (%) [Median; 25th-75th percentile]
Baked milk (n = 229)		
Milk (PTP)	127 (55.4) [9;6–13]	138 (60.3) [6.79; 1.84–39.12]
α-lactalbumin	91 (39.7) [6; 5–9.5]	93 (40.6) [2.55; 1.17–11.12]
β-lactoglobulin	105 (45.8) [6;4–9]	89 (38.9) [2.34; 0.70–8.71]
Casein	55 (24) [4;3–6]	90 (39.3) [8.46; 0.78–25.23]
Baked egg (n = 197)		
Egg white	121 (61.4) [6;5–10]	127 (64.5) [3.23; 1.29–14.41]
Egg yolk	95 (48.2) [4;3–6]	90 (45.7) [2.13; 0.85–5.74]
Egg white (PTP)	96 (49.7) [8;5–11]	–
Egg yolk (PTP)	57 (28.9) [5;4–7]	–
Ovalbumin	–	78 (39.6) [2.29; 1.21–10.24]
Ovomucoid	–	61 (31) [2.97; 0.66–11]

Table 2. Allergy test results of the patients. *PTP, prick to prick test.*

only 12 reported an anaphylactic reaction in their medical history.

After baked egg food challenge, the most frequent symptoms were skin reactions (31 patients, 59.6%). Fifteen patients (28.8%) developed only skin reactions (11 classified in Group 1 and 4 with urticaria and/or angioedema in Group 2). Sixteen patients developed only gastrointestinal reactions, 10 with emesis (Group 2), 3 with repetitive vomiting (Group 3), and 3 with oral pruritus and mild lip swelling (Group 1). Only 2 patients developed a respiratory reaction, 1 with rhinorrhea and nasal congestion (Group 3) and 1 with dyspnea and wheezing (Group 4). Nineteen patients developed a systemic reaction during baked egg challenge (36.5%); 8 developed skin symptoms (mainly localized pruritus and urticaria) followed by emesis or repetitive vomiting: 5 were included in Group 2 and 3 in Group 3. Of the remaining patients with systemic reactions, 8 were classified in Group 2, 1 in Group 3, and 2 (with urticaria and dyspnea) in Group 4.

Of 19 patients with a personal history of allergic reaction to both baked foods, 15 tolerated both baked foods. Two patients reacted only to baked milk (1 with nasal congestion and sneezing and 1 with rhinorrhea and marked congestion) and 1 patient only to baked egg (urticaria). One patient reacted to both baked foods, developing dyspnea during the baked milk OFC and urticaria during the baked egg OFC.

Only 1 patient challenged with baked milk developed objective symptoms at the first dose (left-censored) with a LOAEL of 1.3 mg milk. We observed 10 (24.3%) and 7 (13.4%) reactions after consuming the last dose of baked milk and baked egg, respectively (Fig. 1).

After logistic regression analysis, we found a significant correlation between the individual threshold dose and milk sIgE value only ($p < 0.001$). As reported in Fig. 3, higher levels of milk sIgE had a high probability of reacting at lower LOAEL, as well as the opposite (OR = 0.82; CI95% = 0.96–0.99). No significant correlation was found for other variables, such as age, sex, atopic diseases, referred manifestation, SPT wheal size, or type of symptoms developed at OFC.

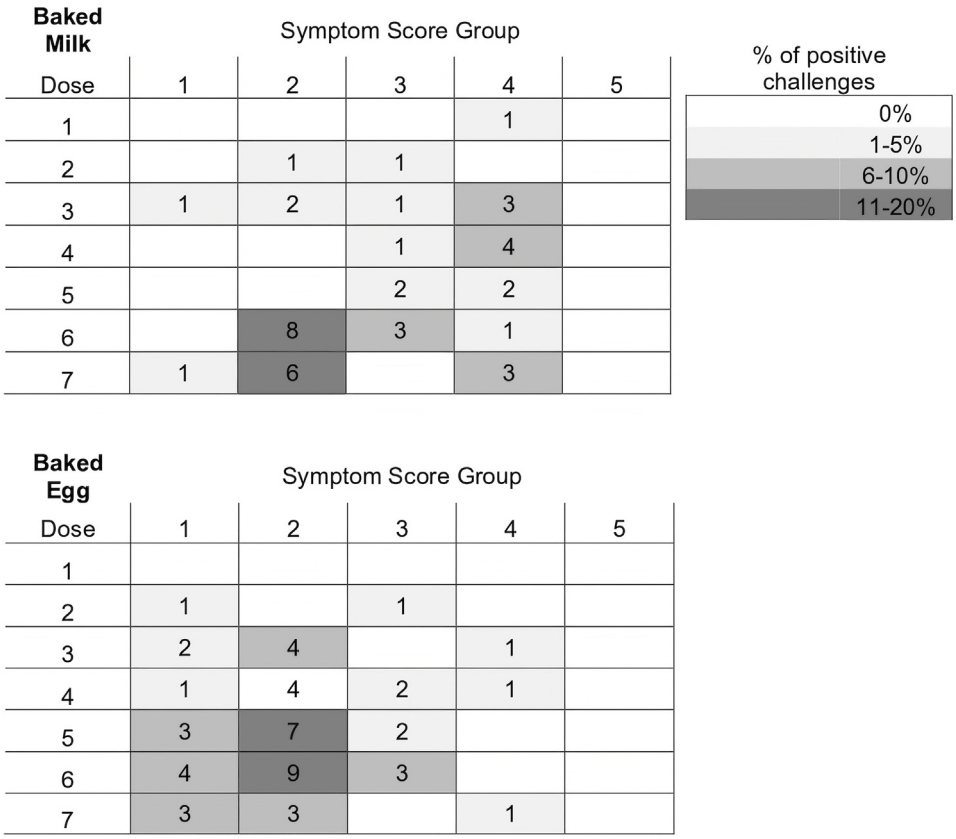

Fig. 1 Symptom Score Group observed during baked milk and egg positive food challenges. The number of patients given to a particular dose is indicated in the plots. The intensity of gray shading denotes the percentage of challenged patients responding with a given symptom score.[51]

Eliciting doses

We fitted the data with log-normal, log-logistic, and Weibull distribution models (Fig. 2a and b) and extrapolated EDs from these models. The milk EDs ranged from 0.4 to 1.3 mg proteins (ED01), from 3.9 to 4.8 mg (ED05), from 8.9 to 10.4 (ED10), from 27.1 to 40.4 (ED25), and from 93.9 to 132.1 (ED50). The egg EDs ranged from 2.2 to 4.4 mg (ED01), from 11.7 to 15.9 (ED05), from 24.3 to 28.3 mg (ED10), from 54.9 to 69.5 (ED25) and from 135.7 to 172.5 mg (ED50) (Table 3).

DISCUSSION

Although the practice of performing OFCs with baked products is widespread,[54] few data are available specifically on the reactivity thresholds in baked milk/egg allergic children.[44] In this study, we confirm that children allergic to baked milk display threshold dose levels close to those observed for fresh milk. We found a prevalence of positive challenges to baked milk of 17.9%, inferior to the 26.1% found for fresh milk, but consistent with the one discovered in previous investigations.[36–38,47–61] This result is in line with previous research,[44,55] although the similarity with the fresh milk EDs is more significant for the ED01 and ED05 (Table 3).

For baked egg, we found a prevalence of positive challenges of 26.4%, similar to some studies cited above.[34,35] The thresholds we found for baked egg were consistently higher than for baked milk: our most conservative ED01 was 2.2 mg total protein (IQR 0.6–7.3), with an ED05 of 11.7 (IQR 5–27.2) mg. Such thresholds are 6.5 (egg ED01), and 7.5 (egg ED05) times lower than for native form. This contrasts with currently available data, which designates baked egg thresholds at levels similar to those of native egg.[6,44] Recently, an ED01 of 1.4 mg for egg was identified in a population of children allergic to egg, but no data were collected for baked

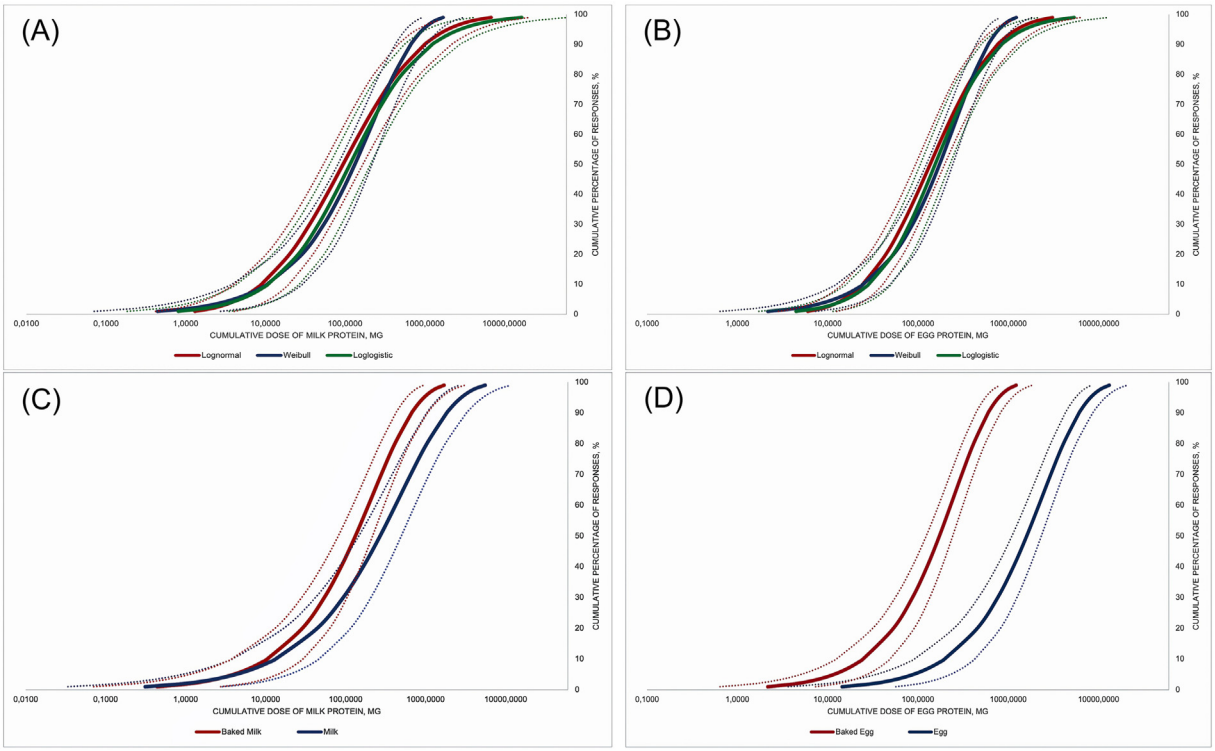

Fig. 2 Comparison between the distribution curves of thresholds for baked cow's milk (A) and baked hen's egg (B) proteins, and the previously calculated distribution curves for these allergens in their pasteurized form (C–D), based on Log-normal, Log-logistic, and Weibull distribution models. The dashed lines indicate the 95% confidence interval

OFCs, due to the lack of standardized criteria for processing.[62] However, a retrospective study suggested that children tolerant of baked egg react at higher EDs of native egg compared with those avoiding it.[43]

Our prevalence of positive baked egg OFCs is lower than Turner et al (36%), who administered a total amount of 1 g protein.[63] In that study, 38 of the 86 reactive patients (44.2%) developed objective symptoms after the third of 4 doses. We found a similar result for raw egg (48.9% after the fifth of 7 doses), while of 52 patients positive to baked egg oral food challenge, 35 reacted after the 5th dose (67.3%). Indeed, only 1 of our 98 reactive patients (1%, including raw and baked egg OFCs) developed objective symptoms after the first dose of OFC with raw egg (LOAEL 12.36 mg).[45] These findings indicate that reactivity is not directly dependent on the amount of protein administered, and that there is no concordance between the meal (pasteurized or baked egg) administered in oral food challenge and the most conservative ED01 theorized in VITAL protocol (0.03 mg, raw egg white). Of note, the only patient who reacted after the first dose of baked milk developed a more severe reaction (oral pruritus and dyspnea) with a lower amount of protein (1.3 mg) compared to the analogous first-dose responder to fresh milk OFC of our previous study (urticarial rash after 0.1 ml administration equivalent to 3.43 mg milk protein).

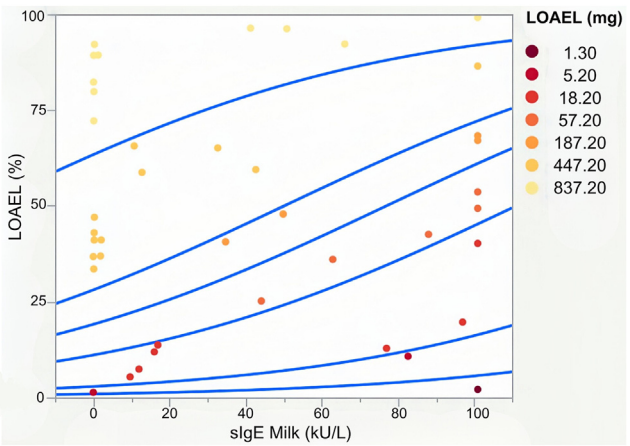

Fig. 3 Correlation between Individual threshold doses and milk sIgE levels. The axes measure sIgE levels and LOAEL percentages. Color-coded data points indicating different LOAEL values. Blue lines represent the correlation model and its 95% confidence intervals

	Model	ED01 mg protein (95% CI)	ED05 mg protein (95% CI)	ED10 mg protein (95% CI)	ED25 mg protein (95% CI)	ED50 mg protein (95% CI)
Milk	Log-normal	1.3 (0.4–4)	4.5 (1.8–11.2)	8.9 (4–19.6)	27.1 (14.3–51.5)	93.9 (53–166.1)
	Log-logistic	0.8 (0.2–3.5)	4.8 (1.6–13.9)	10.7 (4.3–26.4)	35.1 (17.4–70.7)	115 (63.5–208.2)
	Weibull	0.4 (0.1–2.7)	3.9 (1.1–14)	10.4 (3.7–29.1)	40.4 (19.8–82.4)	132.1 (80.6–216.6)
Egg	Log-normal	6 (2.8–12.6)	14.9 (8.3–26.9)	24.3 (14.5–40.7)	54.9 (36.1–83.5)	135.7 (93.5–197)
	Log-logistic	4.4 (1.7–11.4)	15.9 (8–31.4)	28.3 (15.9–50.2)	66.1 (42.4–102.9)	154.2 (105.8–224.9)
	Weibull	2.2 (0.6–7.3)	11.7 (5–27.2)	24.6 (12.4–48.9)	69.5 (43–112.4)	172.5 (122.9–242.1)

Table 3. ED1. ED5. ED10. ED25 and ED50 (mg protein) of baked milk and baked egg. *CI, Confidence interval; ED, eliciting dose; ED01, ED05, ED10, ED25, ED50: cumulative amount of food protein predicted to cause an allergic reaction in 1%, 5%, 10%, 25% or 50% of the food allergic population, respectively.*

The prevalence of patients who reacted to baked milk with systemic reaction (Grade 4 Sampson) was higher than after fresh milk OFC (34.2% vs 18.4%): this indicates that lower threshold could entail higher probability for severe reactions, running again the contrary common wisdom that there is no correlation between the eliciting dose and the severity of the subsequent reaction.[56]

In our group, cutaneous reactions were the most frequent manifestations: 50 of 87 positive patients (57.4%) developed a skin symptom (mainly urticarial eruptions) during OFC, without appreciable differences between those allergic to milk and those allergic to egg. However, we discovered a food-related variation in the number of kids who had respiratory vs gastrointestinal symptoms at OFC. Gastrointestinal symptoms were seen more frequently in the baked egg group, with 46.9% experiencing vomiting, compared with 34.2% ($p < 0.05$) in the baked milk group. Children in the baked milk group experienced dyspnea and other respiratory symptoms at a rate of 31.5% and 36.8%, respectively, compared to only 7.9% ($p < 0.005$) and 13.1% ($p < 0.005$) for the baked egg group. This underlines the different behaviors between the 2 types of allergies, with milk-allergic children more prone to respiratory symptoms.

The rate of epinephrine-requiring anaphylaxis following OFC was similar in both groups; 4.9% (baked milk, 2 patients) versus 1.9% (baked egg, 1 patient); none required a second epinephrine dose. The use of bronchodilators differed significantly between the 2 groups, with 16 patients (39%) in the baked milk group compared to only 1 patient in the baked egg group ($p < 0.001$). Seven of the anaphylactic patients to baked milk, vs just 2 of those developing anaphylaxis to baked egg, had a history of asthma ($p = 0.13$). Again, this indicates that baked milk allergy is more likely to involve the respiratory tract. In the past, several suggestions linked cow's milk allergy to asthma and recurrent respiratory infections.[57-59]

These results are in line with earlier research, which found a greater prevalence of gastrointestinal symptoms in baked vs non-baked egg OFCs[34,40,60,61] as well as a higher incidence of

respiratory symptoms in baked versus non-baked milk challenges.[61] In our group, no patient experienced a Group 5 systemic reaction, most likely because of the skilled staff's quick response at the first appearance of objective symptoms.

Apart from the obvious limitation due to the single-center study design, our study presents methodological characteristics that could limit its clinical meaning. For feasibility reasons, OFCs performed with baked products contain a lower quantity of allergenic proteins than those possible with fresh products. In our 'baked' OFCs, every step-dose and total amount of milk or egg protein are lower in baked than in fresh OFC protocol: we administered a total amount of 0.8 g of milk protein compared with the 4.9 g used in our fresh milk OFC[45] and in similar studies.[36,37,63] The total amount of egg protein we administered as egg biscuits was also lower (1.1 g) than for raw egg OFC (8.6 g). Other studies administered a total egg protein ranging from 1 g[64] to 2.2 g.[34,35,63,64] If from a theoretical point of view this can be considered a limitation, from a practical point of view it reflects the impossibility of administering during OFCs quantities of baked products significantly higher than those possibly consumed during real life: our OFCs reflect reference doses.

This study suggests additional considerations regarding the clinical presentation of milk/egg allergy. First, compared with the OFC outcomes of children tolerant to baked, the number of positive results is lower: 17.9% and 26.4%, for baked egg and milk respectively, compared with 26.1% and 34.5% of OFC with fresh milk and egg. This indicates that when a child is allergic to milk or eggs, pediatricians and families tend to adopt prudential avoidance behaviors for baked foods, which does not correspond to reality. Therefore, carrying out tests with baked foods can help a good number of children free themselves from unnecessary dietary constraints.

Second, when a milk/egg allergic child reacts to baked forms, her clinical characteristics show more severity. Compared with children baked-tolerant recruited in the same setting and in the same timeframe,[45] they display a greater risk of severe reactions (19.5% vs. 12.1%), the need to resort to the emergency room (8.5% vs. 5.2%), and the use of adrenaline (9.8% vs. 5.8%). This difference in severity of the allergy also explains the different performance of diagnostic tests in the populations of children who react to baked egg compared to those who do not react to it.[65] These individuals are likely to exhibit more psychosocial burden than their counterparts with CMA who are not allergic to baked forms.[66]

Third, as in our caseload, the mean age was superior to the median, we confirm that baked food allergy is more frequent in younger ages and that milk and egg allergies fade over time.[47,67]

Taken together, the data suggest that milk is a more dangerous food allergen than egg, in line with epidemiological studies assessing the severity of food reactions, where milk predominates over egg.[68]

The significant correlation between milk sIgE level and individual threshold agrees with previous studies, which evaluated it as a positive predictive value for oral food challenge outcome[69–71] and specifically for individual threshold dose.[72] We found no statistical correlation between patients' individual threshold and their age, gender and/or SPT levels in both our studies. However, we evaluated a sufficient sample size to allow for accurate estimations of population dose-threshold distributions, but this may not assess a predictive value for demographic and/or diagnostic variables as a predictor of individual threshold and/or positive outcome of challenge.

As we had previously reported, it will be ideal to validate the data within the subjects by comparison of the reproducible doses elicited at least among the patients who have undergone repeated OFCs and/or among those who have tolerated baked milk and had reacted to the OFC of fresh milk.[73]

A limitation of our study is the insufficient number of patients with simultaneous allergies to both milk and egg who had positive OFCs for both allergens, which prevented us from analyzing the eliciting doses for these patients. Further studies with larger sample sizes are needed to adequately determine the eliciting doses for individuals with concurrent egg and milk allergies.

In conclusion, while fresh and baked milk EDs were equivalent in our evaluations, egg EDs were

greater than those previously reported (Fig. 2c and d). Establishing the ED01 for patients allergic to baked egg between 2.2 and 6 mg, our data document that this reactivity is significantly greater than that of patients tolerant to baked egg, whose ED01 is 14.4–29.7 mg. However, the more sensitive threshold of baked egg-allergic children remains higher than the thresholds used as the limit for PAL.[4,6] Should these data be confirmed by direct comparison of reactivity to raw versus baked forms within the same patients, we hypothesize that egg-allergic children who tolerate baked egg, and possibly even some who react to it, may not need to avoid all foods that may contain egg. However, this recommendation should be made with caution, pending further studies to verify the safety and tolerance levels for these children. In addition, we open the possibility of adopting a less conservative threshold for egg PAL when the contamination happens in baked products. Should our results be confirmed, this change might exempt food producers from the need for precautionary labelling of egg traces in baked foods, aligning more closely with the actual tolerance possibilities. This could benefit both consumers with egg allergies and food producers.

Authors' consent for publication
All authors have read and consent to approval of the final manuscript.

Abbreviations
ED01, dose predicted to elicit allergic reactions in 1% of patients; ED05, dose predicted to elicit allergic reactions in 5% of patients; ICSA, Interval-Censoring Survival Analysis; LOAEL, Low Observed Adverse Effect Level; NOAEL, No Observed Adverse Effect Level; OFC, Oral Food Challenge; sIgE, specific IgE; SPT, Skin Prick Test.

Availability of data and materials
All data is available to the readers in the tables of the manuscript and references.

Author contributions
RLV developed the concept, prepared the infrastructure of the study, elaborated the data, performed the statistical analysis, and contributed made the draft. AF initiated the concept and contributed made the first draft. CR, SU, LD, VC, and VF administered the OFCs, evaluated the clinical data, included them in the database and contributed to the discussion. DU, DZ and FDG collaborated to the inclusion of data in the database, collaborated to the statistical analysis, and contributed to the discussion.

Ethics approval
This study protocol was reviewed and approved by the Ethics Committee.

Submission declaration
This manuscript is original and has not been published, nor is it currently under consideration for publication elsewhere.

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Declaration of competing interest
The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2024.101012.

Author details
[a]Allergy Unit, Piazza S. Onofrio Bambino Gesù Children's Hospital, IRCCS, 00165, Rome, Holy See, Italy. [b]Allergy Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy. [c]Department of the Woman, the Child, of General and Specialized Surgery, University of Campania Luigi Vanvitelli, Naples, Italy. [d]Department of Systems Medicine, The School of Pediatrics, University of Rome "Tor Vergata", Rome, Italy. [e]ARPA Lazio, Regional Agency for Environmental Protection, Rome, Italy.

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