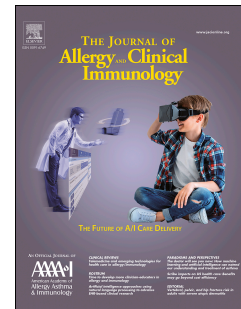


Journal Pre-proof

Reproducibility of food challenge to cow's milk: a systematic review with individual participant data meta-analysis

Paul J. Turner, FRCPCH PhD, Nandinee Patel, MD, Dianne E. Campbell, MBBS PhD, Hugh A. Sampson, MD, Mayu Maeda, MD PhD, Toshio Katsunuma, MD PhD, Joost Westerhout, PhD, W. Marty Blom, PhD, Joseph L. Baumert, PhD, Geert F. Houben, PhD, Benjamin C. Remington, PhD



PII: S0091-6749(22)00764-3

DOI: <https://doi.org/10.1016/j.jaci.2022.04.035>

Reference: YMAI 15587

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 15 February 2022

Revised Date: 1 April 2022

Accepted Date: 29 April 2022

Please cite this article as: Turner PJ, Patel N, Campbell DE, Sampson HA, Maeda M, Katsunuma T, Westerhout J, Blom WM, Baumert JL, Houben GF, Remington BC, Reproducibility of food challenge to cow's milk: a systematic review with individual participant data meta-analysis, *Journal of Allergy and Clinical Immunology* (2022), doi: <https://doi.org/10.1016/j.jaci.2022.04.035>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2022 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology.



Reproducibility of eliciting doses (ED) to cow's milk

- Individual participant data meta-analysis (21 studies, 1389 participants)

Cow's Milk ED₀₅

(dose causing objective symptoms in 5% of allergic individuals)



The ED₀₅ for cow's milk
(dose causing objective symptoms
in 5% of allergic individuals)
is $\leq 3\text{mg}$ cow's milk protein,
about 0.1ml of fresh milk



2.4 per 1000
(95% CI 1.0-5.5)
react with
anaphylaxis

95%: no objective
symptoms

1.8% (upper 95%CI 6.4%) of those
with no objective symptoms might
react on a subsequent occasion
(and vice versa)

5% will have objective symptoms

TITLE PAGE**Reproducibility of food challenge to cow's milk: a systematic review with individual participant data meta-analysis**

Paul J. Turner FRCPCH PhD^{a*}, Nandinee Patel MD,^a Dianne E. Campbell MBBS PhD,^{b,c} Hugh A. Sampson MD,^{c,d} Mayu Maeda MD PhD,^e Toshio Katsunuma MD PhD,^f Joost Westerhout PhD,^g W. Marty Blom PhD,^g Joseph L. Baumert PhD,^h Geert F. Houben PhD,^g Benjamin C Remington PhD^h

Affiliations:

^aNational Heart & Lung Institute, Imperial College London, London, United Kingdom;

^bDepartment of Allergy and Immunology, The Children's Hospital at Westmead, Sydney, Australia;

^cDBV Technologies, Montrouge, France;

^dDivision of Pediatric Allergy and Immunology, Icahn School of Medicine at Mount Sinai, New York, USA;

^eDepartment of Pediatrics, Showa University School of Medicine, Tokyo, Japan;

^fDepartment of Pediatrics, Daisan Hospital, The Jikei University School of Medicine, Tokyo, Japan;

^gTNO, The Netherlands Organisation of Applied Scientific Research, Utrecht, The Netherlands;

^hFood Allergy Research and Resource Program, University of Nebraska, Lincoln, Nebraska, USA.

***Corresponding author:**

Dr Paul Turner

National Heart & Lung Institute,

Imperial College London,

Norfolk Place

London, W2 1PG

Tel: +44 (0)20 3312 7754

Email: p.turner@imperial.ac.uk

Word Count: 2971 words

Funding:

This research was funded in part by a UK Medical Research Council Clinician Scientist award to PJT (reference MR/K010468/1). NP and PJT are supported through the NIHR Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London. The views expressed in this article are those of the author(s) and do not necessarily reflect those of the NHS, NIHR, or the Department of Health.

Competing interests

PJ Turner reports grants from UK Medical Research Council, NIHR/Imperial BRC and JM Charitable Foundation during the conduct of the study; personal fees from UK Food Standards Agency, Aimmune Therapeutics, Allergenis, Aquestive Therapeutics, outside the submitted

47 work. DE Campbell is a part-time employee of DBV Technologies and reported receiving grant
48 support from National Health and Medical Research Council of Australia and personal fees from
49 Allergenis, Westmead Fertility Centre, and Financial Markets Foundation for Children. HA
50 Sampson reported receiving consultancy fees from N-Fold, LLC, DBV Technologies, and Siolta
51 Therapeutics, grant funding from the National Institutes of Health (NIH)/National Institute of
52 Allergy and Infectious Diseases (NIAID), and royalties from Elsevier. T Katsunuma reports
53 lecture fees from Kyorin Pharmaceutical, Maruho, and Torii Pharmaceutical, outside the
54 submitted work. J Westerhout, WM Blom and GF Houben report financial support from Dutch
55 Governmental TNO Research Cooperation Funds, Netherlands, and the Food Allergy Research
56 and Resource Program (FARRP) of the University of Nebraska, USA. BC Remington reports
57 grants from FARRP, grants and personal fees from DBV Technologies, and travel support from
58 ILSI Europe. The other authors declare no competing interests.

ABSTRACT:

Background: Cow's milk (CM) is an increasingly common cause of severe allergic reactions, but there is uncertainty with respect to severity of reactions at low level CM exposure, as well as the reproducibility of reaction thresholds.

Objective: To undertake an individual participant data (IPD) meta-analysis of studies reporting double-blind, placebo-controlled food challenges (DBPCFC) in CM, to determine the rate of anaphylaxis to low level exposures and the reproducibility of reaction thresholds.

Methods: Systematic review and individual participant data (IPD) meta-analysis of studies reporting relevant data. Authors were contacted to provide additional data and/or clarifications as needed. Risk of bias was assessed using the National Institute for Clinical Excellence methodological checklists.

Results: 34 studies were included, representing data from over 1000 participants. The cumulative ED₀₁ and ED₀₅ (cumulative doses causing objective symptoms in 1% and 5% of the at-risk allergic population) were 0.3 (95%CI 0.2-0.5) and 2.9 (95%CI 1.6-5.4) mg, respectively. At meta-analysis, 4.8% (95%CI 2.0-10.9%) and 4.8% (95%CI 0.7-27.1%) of individuals reacting to ≤5mg and ≤0.5mg of CM protein (respectively) had anaphylaxis (minimal heterogeneity, I²=0%). 110 individuals subsequently underwent a repeat DBPCFC: the intra-individual variation in reaction threshold was limited to a ½-log change in 80% (95%CI 65-89%) of participants. Two individuals initially tolerated 5mg CM protein but then reacted to this dose at a subsequent challenge, although neither had anaphylaxis.

Conclusions: Around 5% of CM-allergic individuals reacting to an ED₀₁ or ED₀₅ exposure might have anaphylaxis to that dose. This equates to 5 and 24 anaphylaxis events per 10,000 patients exposed to an ED₀₁ or ED₀₅ dose respectively, in the broader CM-allergic population. The vast majority of these anaphylactic reactions would be at the more mild end of the spectrum of anaphylaxis severity, responding to single dose of epinephrine.

Clinical Implication:

Around 5% of people with cow's milk allergy react to ≤ 2.9 mg of protein (about 0.1ml of fresh milk) and 5% of those will have anaphylaxis. This equates to 24 per 10,000 milk-allergic individuals reacting to an ED₀₅ exposure with anaphylaxis.

Capsule Summary

This meta-analysis reporting over 1000 challenges to cow's milk provides further evidence as to the estimated proportion of individuals reacting with anaphylaxis to low-level CM exposure, and the reproducibility of reaction thresholds at food challenge.

Keywords

Allergy, Anaphylaxis, Cow's milk, Eliciting dose, Food challenge, Thresholds, Precautionary Allergen Labelling

Abbreviations:

95%CI	95% confidence interval
DBPCFC	Double-blind placebo-controlled food challenge
DLS	Dose limiting symptoms
ED	Eliciting dose
FC	Food challenge
LOAEL	Lowest-observed adverse effect level
PAL	Precautionary allergen labelling
VITAL	Voluntary Incidental Trace Allergen Labelling

INTRODUCTION

The gold standard for diagnosis of food allergy is the double-blind, placebo-controlled food challenge (DBPCFC),¹ although in practice most centers perform “open” unblinded food challenges (FC) for pragmatic reasons. Increasingly, reaction threshold data from FC are being used to inform clinical management, either to help inform the starting dose for patients undergoing food allergen immunotherapy, or to guide dietary liberalization in those with higher reaction thresholds (either de novo or following immunotherapy).² Separately, the use of challenge thresholds (modelled to reflect population dose-distributions) is now guiding allergen risk assessment in industry and potentially, the need for “may contain” precautionary allergen labelling (PAL).^{3,4}

However, while dose-distribution data for most “priority” allergens have been published,⁵ there is less certainty over how reaction thresholds may change over time – i.e. the reproducibility of the precise dose causing allergic symptoms in a given individual. We recently published an analysis of the reproducibility of reaction thresholds and anaphylaxis in peanut-allergic individuals,⁶ and have proposed that with the larger datasets now available pertaining to peanut, it might be possible to use peanut as a reference allergen in terms of the occurrence of anaphylaxis following low dose exposures.⁷ However, it is important to generate further evidence that this is a reasonable assumption to make, particularly for those allergens more frequently associated with fatal reactions.

Cow's milk (CM) allergy is the one of the most common food allergies but often resolves in early childhood. In Europe, the prevalence of challenge-proven CM allergy is <1% in children up to age 2,⁸ with similar estimates for North America.^{9,10} However, CM allergy has a number of different phenotypes, and children with persisting CM allergy may be more at risk of severe reactions.¹¹ CM allergy may persist in up to 30% of CM-allergic children.¹² Over the last 25

years, CM has become the most common single cause of fatal anaphylaxis in children in the United Kingdom.¹³ Epidemiological data also show CM is a common cause of fatal and near-fatal reactions in USA and Canada,¹⁴⁻¹⁶ France,¹⁷⁻¹⁸ Australia¹⁹ and Israel.²⁰ Given these data and the ubiquitous nature of dairy in the human diet, it is important to have more robust data assessing whether CM is of greater concern in terms of risk of severe reactions compared to peanut.

In this analysis, we undertook a systematic review and meta-analysis of individual participant data (IPD)²¹ to (i) update previous dose-distribution modelling for CM and provide more robust estimates for population-based eliciting doses, (ii) evaluate the proportion of reactions at low levels of allergen exposure that might be classified as anaphylaxis, and (iii) determine the reproducibility of individual reaction thresholds over time.

METHODS

We undertook a systematic review of the literature to identify studies which have undertaken double-blind, placebo-controlled food challenges (DBPCFC) in CM-allergic individuals (adults and children). Study sponsors and/or authors were contacted and asked to provide both aggregate and (in the case of individuals who underwent repeat challenges to CM) anonymized individual participant data which could then be included for meta-analysis. This review was undertaken and reported in accordance with the PRISMA-IPD Statement.²¹

Search strategy

We searched Medline and Embase for articles published between January 1984 and December 2021 which described DBPCFC to CM, using the search terms “double-blind”, “allergy”, “challenge” and “cow’s milk”. There was no registered protocol for this review, but the methods and analyses were planned *a priori*. No language restrictions were made, and we planned to include non-English papers if they met our inclusion criteria. Abstracts were independently screened by 2 authors, and disagreements were resolved by discussion. We also reviewed reference lists of included studies and review articles to identify other relevant studies.

Study selection

Inclusion criteria were as follows: (i) Participants: adults and children with suspected CM allergy; (ii) Intervention: DBPCFC, conducted in a manner consistent with PRACTALL consensus,¹ with a minimum of ≥ 8 subjects with objective symptoms at FC; (iii) Outcomes: study-defined cumulative eliciting dose (either maximum tolerated dose or reaction threshold dose) or lowest-observed adverse effect level (LOAEL); and the occurrence of anaphylaxis. Studies needed to satisfy all 3 inclusion criteria to be included. We also included historical studies incorporated into the TNO-FARRP database of reaction thresholds that were otherwise eligible for inclusion, since these had already been quality-checked.⁵ Where more than one data series included the

same individuals during an overlapping time period, we included the data series with the largest number of individuals where we could be certain that no duplication was present. Excluded studies are reported in Table E1, together with the reason for exclusion.

Data extraction and analyses

Analyses were planned prospectively. Data relating to the No Observed Adverse Effect Level (NOAEL, highest dose with no objective symptoms) and Lowest Observed Adverse Effect Level (LOAEL, first dose with objective symptoms) or dose causing study-defined dose-limiting symptoms (DLS) were extracted in duplicate;²² any discrepancies identified between extracted data and published data were resolved by discussion and/or by contacting authors or study sponsors for clarification. Population dose distributions were determined as previously described, using Bayesian Stacked Parametric Survival methods with Frailty Components and Interval Censored Failure Times, also referred to as “Stacked Model Averaging”.²³

Previous published estimates for discrete ED₀₅ for CM is 2.4 (95% CI 1.3-5.0) mg CM protein.⁵ On this basis, we then extracted for each study the number of participants experiencing objective symptoms and/or meeting study-defined challenge stopping criteria to a discrete dose of ≤ 5 mg CM protein, and noted whether the individual had symptoms consistent with anaphylaxis (as defined by the study authors). The different anaphylaxis definitions used are included in Table E2. Where individual patient symptom data was available, anaphylaxis was determined according to the WAO 2020 consensus criteria, by two independent investigators (PJT, NP).²⁴ Risk of bias was assessed using the National Institute for Clinical Excellence methodological checklists for cohort studies.²⁵ Rates were pooled across studies using a generalized linear mixed model in R (metaprop function, metafor package, logit transformation with a random intercept logistic regression model for the summary estimate, with a continuity correction of 0.5). This approach avoids many of the issues surrounding the use of

transformations when undertaking meta-analyses of proportions.^{26,27} Heterogeneity was quantified using the I^2 statistic. We conducted meta-analysis even if significant heterogeneity was seen between study estimates, as is the norm when conducting meta-analysis of proportions. The statistical program used for meta-analysis was R, version 4.0.3 (R Project). Binomial confidence intervals were calculated using the Clopper–Pearson interval. Statistical significance was set at 2-sided $P < .05$. Sensitivity analyses were performed to assess for any difference between those studies reporting LOAELs (defined according to Westerhout et al²²) and those which used DLS, on the overall pooled estimate.

In order to assess the reproducibility of challenge thresholds over time within individuals, we extracted individual participant data of individuals who underwent a further food challenge following the initial DBPCFC (conducted according to the same protocol), from relevant interventional studies (for example, participants who were randomized to a placebo control arm in studies of food allergy desensitization). The log-fold change in reaction threshold for each subject was calculated. Normality of distribution was assessed using the D'Agostino-Pearson test, and the distributions then used in IPD-meta-analysis. We included a sensitivity analysis to assess the degree of stability in reaction threshold in CM-allergic individuals reacting to lower eliciting doses (≤ 50 mg CM protein, approximately 1.5ml of fresh milk). Separately, we evaluated the reproducibility in the occurrence of anaphylaxis after a repeat exposure.

Ethical approval

Ethical approval was not required as this was a post-hoc analysis of anonymized participant data from multiple clinical trials, all of which had their own individual ethics approval.

RESULTS:

Twenty-seven studies were identified as eligible for inclusion together with 7 studies already included in the TNO-FARRP dataset, resulting in a total of 34 studies (Figure 1).^{5,8,28-58} Details of the individual studies appear in Table E2, and risk of bias assessment in Table E3. No study had a high risk of bias or poor external validity.

Dose-distribution modelling

Data from 1002 challenges were included in the analysis. The estimated discrete and cumulative eliciting doses for the amount of CM protein predicting to cause objective symptoms in 1%, 5% and 10% of the CM-allergic population (ED₀₁, ED₀₅ and ED₁₀, respectively) are shown in Table 1 and Figure E1. The cumulative ED₀₁ and ED₀₅ (95% confidence intervals) were 0.3 (0.2 - 0.5) and 2.9 (1.6 - 5.4) mg, respectively. We therefore then assessed the rate of anaphylaxis to low level exposures on the basis of the calculated upper 95% CIs for ED₀₁ and ED₀₅, i.e. 0.5mg and 5mg respectively.

Anaphylaxis at low levels of allergen exposure

Overall, data from 1389 participants was available across 23 studies, of whom 105 individuals (across 14 studies) reacted (according to individual study-defined criteria) to ≤ 5 mg CM protein. Using the estimated dose-distribution modelling (Table 1), we then determined the number of individuals in each study reacting to ≤ 5 mg and ≤ 0.5 mg CM protein, and the proportion who had anaphylaxis. There was no evidence of publication bias in terms of the rate of anaphylaxis in low-dose reactors by study size (funnel plot shown in Figure E2). At meta-analysis, 4.8% (95%CI 2.0-10.9%) of those reacting to an exposure of ≤ 5 mg CM protein (discrete dose) had anaphylaxis (minimal heterogeneity [$I^2=0\%$], Fig 2A). In a sensitivity analysis, we did not identify any significant differences in estimates when comparing studies which used LOAEL and those

using DLS ($P=1.0$, Figure E3). There were no anaphylaxis reactions reported as refractory anaphylaxis (i.e. reactions which did not respond to 1-2 doses of adrenaline).

Seven studies included an initial challenge dose of ≤ 0.5 mg CM protein, of which 3 reported a total of 21 individuals who reacted to ≤ 0.5 mg. At meta-analysis, 4.8% (95%CI 0.7-27.1%) of reactions to ≤ 0.5 mg CM protein were anaphylaxis (minimal heterogeneity [$I^2 = 0\%$], Fig 2B). Due to fewer reactions at this level of allergen exposure, no sensitivity analyses were undertaken.

Reproducibility of reaction thresholds

Five interventional studies (clinical trials of food allergen desensitization) included participants allocated to placebo treatment and who underwent repeat food challenge to CM, 5-12 months after initial baseline challenge.^{28,31,33,41,46} Data from these participants were included in the IPD-meta-analysis. The dose distributions for baseline DBPCFC in these participants are shown in Figure 3, together with the proportion reacting at each dosing level with anaphylaxis (defined according to WAO 2020 criteria²⁴). The median cumulative reaction threshold for this combined cohort was 144mg CM protein (IQR 44-444 mg); the overall rate of anaphylaxis in this sub-cohort was 14.5%. Compared to published dose-distributions for CM-allergic individuals,⁵⁹ there was no evidence of skewing towards a more sensitive population (Figure 3).

The distributions of log-change in reaction thresholds for study participants within each included cohort are shown in Figure 4. These distributions were analyzed by IPD-meta-analysis to determine the proportion of participants with a change in reaction threshold at repeat challenge, and whether this differed in patients who reacted to lower levels of CM exposure (see Table 2). Overall, 80% (95%CI 65-89%) of participants reacted at repeat challenge to within $\pm \frac{1}{2}$ -log

increment compared to initial challenge, equivalent to within one dosing interval using a PRACTALL-based semi-log dosing regimen (e.g. a change in threshold from 100mg to 300mg CM protein). Individuals who reacted to lower doses at first DBPCFC were more likely to react to higher doses at repeat challenge with a $\geq \frac{1}{2}$ -log increase in reaction threshold. Four individuals reacted to ≤ 5 mg at initial challenge; all reacted to ≥ 5 mg at subsequent challenge. Two individuals reacted to ≤ 5 mg CM protein at subsequent challenge, having initially tolerated this dose; none had anaphylaxis. Reassuringly, no individuals who initially tolerated an ED_{05} level of milk exposure had anaphylaxis to a $\leq ED_{05}$ exposure at subsequent challenge.

Recurrence of anaphylaxis

Lastly, we analyzed data from participants who underwent repeat DBPCFC, and had anaphylaxis (according to WAO 2020 consensus criteria²⁴) on at least one occasion. Data were available from 3 studies, yielding a total of 33 of 89 (37%) participants who had at least one anaphylaxis reaction. For the pooled analysis, the change in eliciting dose is shown in Figure 5. As with the above data relating to reproducibility of eliciting dose, the dose at which participants experienced anaphylaxis also varied: 4/33 had anaphylaxis at both FC, reacting to the same threshold on both occasions. The occurrence of anaphylaxis was not predictable, with 17/33 (52%, 95%CI 34-69%) participants having anaphylaxis at one FC but not at the second FC to the same or a higher level of CM exposure.

Discussion

In this IPD-meta-analysis of threshold and symptom data from over 1000 DBPCFC to CM, around 5% of CM-allergic individuals reacting to ED₀₁/ED₀₅ levels of CM with objective symptoms had anaphylaxis. Within the CM-allergic population, this equates to an ED₀₅ exposure of CM (2.9mg CM protein, around 0.1ml of fresh milk) causing anaphylaxis in 24 (95%CI 10-54.5) per 10,000 CM-allergic individuals, and an ED₀₁ exposure causing anaphylaxis in 4.8 (95%CI 0.7-27.1) per 10,000 individuals. These data are reassuringly consistent with a similar analysis for peanut using a more extensive dataset of over 3000 DBPCFC,⁶ and provide further evidence to the contention that these data are sufficiently robust to be used for hazard characterization at a low-level allergen exposure for other priority allergens.⁷

We have previously reported that around 70% of peanut-allergic individuals have a degree of “shift” of up to ½-log in clinical reactivity (equivalent to up to 1 dosing increment when using a semi-log based dosing regimen, such as that recommended by PRACTALL consensus, e.g. from 100mg to 300mg protein).⁶ In the current analysis, we found a similar degree of reproducibility in reaction thresholds in CM-allergic patients. These data therefore imply that the variability in reaction thresholds to peanut previously reported may well apply to other food allergens.

Despite variations in the inclusion characteristics of the included studies and specific challenge protocols, there was only minimal heterogeneity observed at meta-analysis, providing reassurance as to the low level of uncertainty of the resulting pooled estimates. Heterogeneity was also minimal when comparing clearly-defined criteria for LOAEL to DLS, with little impact on the overall pooled estimate. We have previously reported that the use of different anaphylaxis definitions across studies does not significantly affect the overall pooled estimates in sensitivity analyses for peanut challenges,⁶ but were unable to confirm this by undertaking

sensitivity analysis in the dataset for CM due to some individual studies included not describing which anaphylaxis definition was used.

These data provide further support for the use of eliciting doses (either ED₀₅ or ED₀₁) to inform the need for PAL following a formal allergen risk assessment by food businesses.³⁻⁵ While there will always been a very limited number of CM-allergic individuals who would still react to these levels of CM exposure, and around 5% of those with anaphylaxis, these data imply that the vast majority of reactions would be at the more mild end of the spectrum of anaphylaxis severity, responding to single dose of epinephrine.⁶⁰ Whether this residual risk is acceptable to patients with CM-allergy needs to be determined: an ED₀₁ level (rather than ED₀₅) would result in a fewer individuals reacting compared to an ED₀₁ level (1% of the at-risk allergic population rather than 5%). However, the “trade-off” might be more indiscriminate use of PAL, as ED₀₁ levels are frequently below the limit of allergen analytical detection,⁶¹ thus food businesses might take a risk-averse approach and apply more PAL. This would therefore have a paradoxical effect of increasing PAL use. In contrast, use of ED₀₅ (which is achievable with existing analytics) is likely to result in less indiscriminate use of PAL, and thus significant benefits of increased food choice and consumer confidence that allergen labeling is based on a proper risk assessment procedure with adequate analytical capabilities to verify the risk management.^{62,63} Given the reproducibility of challenge thresholds (particularly in low-dose reactors), it is possible to identify individuals who might react to an ED₀₅ level of exposure using single-dose challenges (in much the same way as has been proposed for peanut).²⁹ Reassuringly, there were no CM-allergic patients who tolerated an ED₀₅ level of milk exposure, but then had anaphylaxis to an exposure lower than this at subsequent challenge. Identifying such “very low-dose” reactors could therefore allow for these individuals to receive targeted advice to maintain strict CM avoidance, while allowing the majority to have a greater level of dietary choice.⁶²

Finally, all the food challenges included would have been undertaken when the participants were well and without obvious cofactors, which can affect reaction thresholds. We have previously reported that while cofactors can affect threshold, the magnitude of this impact at a population level is well within the day-to-day variation in thresholds for peanut⁶ in the absence of cofactors, and now CM. However, at an individual patient level, such an impact needs to be considered if using threshold data to inform personalized allergy management advice to any given individual.

Strengths and Limitations of this study

Although the analysis set included over 1000 challenges to CM conducted in 27 countries globally encompassing 5 continents, this is less than an equivalent dataset for peanut previously published,⁶ resulting in slightly less certainty over the estimates reported. We were unable to undertake sensitivity analyses based on participant age (as these data were not always available due to data confidentiality regulations), but note that the data includes a significant proportion (at least 115, 7.3%) of teenagers and adults and are therefore not limited to younger children who may be more likely to outgrow their allergy.

Of the five studies included in the IPD-meta-analysis, at least half of the participants included were under age 10 years, and therefore it is possible that in some individuals with a higher eliciting dose at the second DBPCFC, this might have been due to progression towards natural resolution. However, we did not observe a trend towards an increase in reaction threshold, with only 8 (of 110, 7.3%) participants demonstrating a greater than 1-log increase in reaction threshold. These data therefore provide reassurance that under typical conditions for performing DBPCFC, the clinical reaction threshold is reproducible and thus food challenge remains an accurate tool to assess response to interventions for the treatment of food allergy.

Conclusions

We have reported that around 4% of individuals reacting to an ED₀₁ or ED₀₅ level of exposure to CM will have anaphylaxis to that dose; in the dataset reported here,, there were no cases of refractory anaphylaxis. This equates to a risk of anaphylaxis in the broader CM-allergic population of 5 and 24 anaphylaxis events per 10,000 patients exposed to an ED₀₁ or ED₀₅ dose, respectively. The vast majority of these anaphylaxis reactions would be at the milder end of the spectrum of anaphylaxis severity, responding to single dose of epinephrine. Although the reproducibility of reaction thresholds can vary (as for peanut), less than 5% will react to a sub-ED₀₅ level having tolerated it previously and those that do are very unlikely to have anaphylaxis. These data will help inform future strategies to establish evidence-based approaches to allergen management, to help protect the food-allergic consumer from unintended allergen exposure.

ACKNOWLEDGEMENTS

We thank the authors of the included studies and the study sponsors for their assistance in providing data for this analysis.

Author Contributions:

P.J. Turner conceived the study design and led the data analysis. J. Westerhout and B.C. Remington undertook the analysis to estimate population dose distributions. All authors contributed data to the analysis and were involved in data interpretation. N. Patel and P.J. Turner co-wrote the first draft of the manuscript, and all authors reviewed the manuscript and amended or approved the final version.

391 REFERENCES

- 392 1. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, et
393 al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy
394 of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology
395 PRACTALL consensus report. J Allergy Clin Immunol. 2012 Dec;130(6):1260-74.
- 396 2. Graham F, Caubet JC, Eigenmann PA. Can my child with IgE-mediated peanut allergy
397 introduce foods labeled with "may contain traces"? Pediatr Allergy Immunol 2020;31:601-7.
- 398 3. Allen KJ, Remington BC, Baumert JL, Crevel RW, Houben GF, Brooke-Taylor S, et al.
399 Allergen reference doses for precautionary labeling (VITAL 2.0): clinical implications. J
400 Allergy Clin Immunol. 2014;133(1):156-64.
- 401 4. Madsen CB, van den Dungen MW, Cochrane S, Houben GF, Knibb RC, Knulst AC, et al.
402 Can we define a level of protection for allergic consumers that everyone can accept? Regul
403 Toxicol Pharmacol 2020;117:104751.
- 404 5. Remington BC, Westerhout J, Meima MY, Blom WM, Kruizinga AG, Wheeler MW, et al.
405 Updated population minimal eliciting dose distributions for use in risk assessment of 14
406 priority food allergens. Food Chem Toxicol. 2020;139:111259.
- 407 6. Patel N, Adelman DC, Anagnostou K, Baumert JL, Blom WM, Campbell DE, et al. Using
408 data from food challenges to inform management of consumers with food allergy: A
409 systematic review with individual participant data meta-analysis. J Allergy Clin Immunol.
410 2021 Jun;147(6):2249-2262.e7.
- 411 7. Turner PJ, Patel N, Ballmer-Weber BK, Baumert JL, Blom WM, Brooke-Taylor S, et al.
412 Peanut Can Be Used as a Reference Allergen for Hazard Characterization in Food Allergen
413 Risk Management: A Rapid Evidence Assessment and Meta-Analysis. J Allergy Clin
414 Immunol Pract. 2022 Jan;10(1):59-70.

8. Schoemaker AA, Sprickelman AB, Grimshaw KE, Roberts G, Grabenhenrich L, Rosenfeld L, et al. Incidence and natural history of challenge-proven cow's milk allergy in European children - EuroPrevall birth cohort. *Allergy*. 2015 Aug;70(8):963-72.
9. Clarke AE, Elliott SJ, St Pierre Y, Soller L, La Vieille S, Ben-Shoshan M. Temporal trends in prevalence of food allergy in Canada. *J Allergy Clin Immunol Pract*. 2020;8(4):1428-1430.e5.
10. Gupta RS, Warren CM, Smith BM, Blumenstock JA, Jiang J, Davis MM, et al. The Public Health Impact of Parent-Reported Childhood Food Allergies in the United States. *Pediatrics* 2018;142(6):e20181235.
11. Turner PJ, Baumert JL, Beyer K, Boyle RJ, Chan CH, Clark AT, et al. Can we identify patients at risk of life-threatening allergic reactions to food? *Allergy*. 2016 Sep;71(9):1241-55.
12. Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. *J Allergy Clin Immunol*. 2007 Nov;120(5):1172-7.
13. Baseggio Conrado A, Ierodiakonou D, Gowland MH, Boyle RJ, Turner PJ. Food anaphylaxis in the United Kingdom: analysis of national data, 1998-2018. *BMJ*. 2021 Feb 17;372:n251.
14. Poirot E, He F, Gould LH, Hadler JL. Deaths, hospitalizations, and emergency department visits from food-related anaphylaxis, New York City, 2000-2014: implications for fatality prevention. *J Public Health Manag Pract*. 2020;26:548-56.
15. Xu YS, Kastner M, Harada L, Xu A, Salter J, Wasserman S. Anaphylaxis-related deaths in Ontario: a retrospective review of cases from 1986 to 2011. *Allergy Asthma Clin Immunol* 2014;10:38.
16. Ramsey NB, Guffey D, Anagnostou K, Coleman NE, Davis CM. Epidemiology of anaphylaxis in critically ill children in the United States and Canada. *J Allergy Clin Immunol Pract* 2019;7:2241-9.
17. Pouessel G, Beaudouin E, Tanno LK, Drouet M, Deschildre A, Labreuche J, et al. Food-related anaphylaxis fatalities: analysis of the Allergy Vigilance Network

database. Allergy 2019;74:1193-6.

18. Pouessel G, Chagnon F, Trochu C, Labreuche J, Lejeune S, Recher M, et al. Anaphylaxis admissions to pediatric intensive care units in France. Allergy 2018;73:1902-5.

19. Mullins RJ, Wainstein BK, Barnes EH, Liew WK, Campbell DE. Increases in anaphylaxis fatalities in Australia from 1997 to 2013. Clin Exp Allergy 2016;46: 1099-110.

20. Levy MB, Goldberg MR, Nachshon L, Tabachnik E, Katz Y. Lessons from cases of mortality due to food allergy in Israel: cow's milk protein should be considered a potentially fatal allergen. Isr Med Assoc J. 2012 Jan;14(1):29-33.

21. Stewart LA, Clarke M, Rovers M, Riley RD, Simmonds M, Stewart G, et al; PRISMA-IPD Development Group. Preferred Reporting Items for Systematic Review and Meta-Analyses of individual participant data: the PRISMA-IPD Statement. JAMA. 2015;313(16):1657-1665.

22. Westerhout J, Baumert JL, Blom WM, Allen KJ, Ballmer-Weber B, Crevel RWR, et al. Deriving individual threshold doses from clinical food challenge data for population risk assessment of food allergens. J Allergy Clin Immunol. 2019;144(5):1290-1309.

23. Wheeler MW, Westerhout J, Baumert JL, Remington BC. Bayesian Stacked Parametric Survival with Frailty Components and Interval-Censored Failure Times: An Application to Food Allergy Risk. Risk Anal. 2021 Jan;41(1):56-66.

24. Cardona V, Ansotegui IJ, Ebisawa M, El-Gamal Y, Fernandez Rivas M, Fineman S, et al.; World Allergy Organisation Anaphylaxis Committee. Anaphylaxis guidance 2020. WAO Journal 2020;13(10):100472.

25. National Institute for Health and Care Excellence. The social care guidance manual - Process and methods. Published: 30 April 2013, updated July 2016. Available at [nice.org.uk/process/pmg10](https://www.nice.org.uk/process/pmg10).

26. Lin L, Xu C. Arcsine-based transformations for meta-analysis of proportions: Pros, cons, and alternatives. Health Science Reports. 2020;3(3):e178. doi:10.1002/hsr2.178.

27. Schwarzer G, Chemaitelly H, Abu-Raddad LJ, Rücker G. Seriously misleading results using inverse of Freeman-Tukey double arcsine transformation in meta-analysis of single proportions. *Res Synth Methods*. 2019;10(3):476-483.
28. Turner PJ, Duca B, Chastell SA, Alvarez O, Bazire R, Vazquez-Ortiz M, et al. IgE-sensitization predicts threshold but not anaphylaxis during oral food challenges to cow's milk. *Allergy*. 2021 Dec 7. doi: 10.1111/all.15195. Epub ahead of print.
29. Turner PJ, d'Art YM, Duca B, Chastell SA, Marco-Martin G, Vera-Berrios RN, et al. Single-dose oral challenges to validate eliciting doses in children with cow's milk allergy. *Pediatr Allergy Immunol*. 2021 Jul;32(5):1056-1065.
30. Inuo C, Tanaka K, Nakajima Y, Yamawaki K, Matsubara T, Iwamoto H, et al. Tolerability of partially and extensively hydrolysed milk formulas in children with cow's milk allergy. *Asia Pac J Clin Nutr*. 2019;28(1):49-56.
31. Maeda M, Imai T, Ishikawa R, Nakamura T, Kamiya T, Kimura A, Fujita S, Akashi K, Tada H, Morita H, Matsumoto K, Katsunuma T. Effect of oral immunotherapy in children with milk allergy: The ORIMA study. *Allergol Int*. 2021 Apr;70(2):223-228.
32. Purington N, Chinthrajah RS, Long A, Sindher S, Andorf S, O'Laughlin K, et al. Eliciting Dose and Safety Outcomes From a Large Dataset of Standardized Multiple Food Challenges. *Front Immunol*. 2018 21;9:2057.
33. DBV Technologies Announces Results from Phase II Study of Viaskin Milk in Milk-Allergic Patients. February 26, 2018. Available at: https://www.dbv-technologies.com/wp-content/uploads/2018/02/4511-pr-miles-final_eng-pdf.pdf
34. Yanagida N, Sato S, Asaumi T, Ogura K, Ebisawa M. Risk Factors for Severe Reactions during Double-Blind Placebo-Controlled Food Challenges. *Int Arch Allergy Immunol*. 2017;172(3):173-182.

35. Gushken AK, Castro AP, Yonamine GH, Corradi GA, Pastorino AC, Jacob CM. Double-blind, placebo-controlled food challenges in Brazilian children: adaptation to clinical practice. *Allergol Immunopathol (Madr)*. 2013 Mar-Apr;41(2):94-101.
36. Blom WM, Vlieg-Boerstra BJ, Kruizinga AG, van der Heide S, Houben GF, Dubois AE. Threshold dose distributions for 5 major allergenic foods in children. *J Allergy Clin Immunol*. 2013;131(1):172-9.
37. Dambacher WM, de Kort EH, Blom WM, Houben GF, de Vries E. Double-blind placebo-controlled food challenges in children with alleged cow's milk allergy: prevention of unnecessary elimination diets and determination of eliciting doses. *Nutr J*. 2013 Feb 8;12:22.
38. Lee JH, Kim WS, Kim H, Hahn YS. Increased cow's milk protein-specific IgG4 levels after oral desensitization in 7- to 12-month-old infants. *Ann Allergy Asthma Immunol*. 2013;111(6):523-8.
39. Keet CA, Frischmeyer-Guerrerio PA, Thyagarajan A, Schroeder JT, Hamilton RG, Boden S, et al. The safety and efficacy of sublingual and oral immunotherapy for milk allergy. *J Allergy Clin Immunol*. 2012;129(2):448-55, 455.e1-5.
40. Rolinck-Werninghaus C, Niggemann B, Grabenhenrich L, Wahn U, Beyer K. Outcome of oral food challenges in children in relation to symptom-eliciting allergen dose and allergen-specific IgE. *Allergy* 2012;67(7):951-7.
41. Pajno GB, Caminiti L, Ruggeri P, De Luca R, Vita D, La Rosa M, et al. Oral immunotherapy for cow's milk allergy with a weekly up-dosing regimen: a randomized single-blind controlled study. *Ann Allergy Asthma Immunol*. 2010;105(5):376-81.
42. Caminiti L, Passalacqua G, Barberi S, Vita D, Barberio G, De Luca R, et al. A new protocol for specific oral tolerance induction in children with IgE-mediated cow's milk allergy. *Allergy Asthma Proc*. 2009;30(4):443-8.

43. Orhan F, Karakas T, Cakir M, Aksoy A, Baki A, Gedik Y. Prevalence of immunoglobulin E-mediated food allergy in 6-9-year-old urban schoolchildren in the eastern Black Sea region of Turkey. *Clin Exp Allergy*. 2009 Jul;39(7):1027-35.
44. Longo G, Barbi E, Berti I, Meneghetti R, Pittalis A, Ronfani L, et al. Specific oral tolerance induction in children with very severe cow's milk-induced reactions. *J Allergy Clin Immunol*. 2008 Feb;121(2):343-7.
45. Lam HY, van Hoffen E, Michelsen A, Guikers K, van der Tas CH, Bruijnzeel-Koomen CA, et al. Cow's milk allergy in adults is rare but severe: both casein and whey proteins are involved. *Clin Exp Allergy*. 2008;38(6):995-1002.
46. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, Matsui EC, Burks AW, Wood RA. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol*. 2008 Dec;122(6):1154-60.
47. Staden U, Rolinck-Werninghaus C, Brewe F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. *Allergy*. 2007 Nov;62(11):1261-9.
48. Morisset M, Moneret-Vautrin DA, Guenard L, Cuny JM, Frentz P, Hatahet R, et al. Oral desensitization in children with milk and egg allergies obtains recovery in a significant proportion of cases. A randomized study in 60 children with cow's milk allergy and 90 children with egg allergy. *Eur Ann Allergy Clin Immunol*. 2007;39(1):12-9.
49. Patriarca G, Nucera E, Pollastrini E, Roncallo C, De Pasquale T, Lombardo C, et al. Oral specific desensitization in food-allergic children. *Dig Dis Sci*. 2007;52(7):1662-72.
50. Devenney I, Norrman G, Oldaeus G, Strömberg L, Fälth-Magnusson K. A new model for low-dose food challenge in children with allergy to milk or egg. *Acta Paediatr*. 2006 Sep;95(9):1133-9.

51. Flinterman AE, Knulst AC, Meijer Y, Bruijnzeel-Koomen CA, Pasmans SG. Acute allergic reactions in children with AEDS after prolonged cow's milk elimination diets. *Allergy*. 2006 Mar;61(3):370-4.
52. Fiocchi A, Travaini M, D'Auria E, Banderali G, Bernardo L, Riva E. Tolerance to a rice hydrolysate formula in children allergic to cow's milk and soy. *Clin Exp Allergy*. 2003 Nov;33(11):1576-80.
53. Morisset M, Moneret-Vautrin DA, Kanny G, Guénard L, Beaudouin E, Flabbée J, Hatahet R. Thresholds of clinical reactivity to milk, egg, peanut and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. *Clin Exp Allergy* 2003;33(8):1046-51.
54. Patriarca G, Buonomo A, Roncallo C, Del Ninno M, Pollastrini E, Milani A, et al. Oral desensitisation in cow milk allergy: immunological findings. *Int J Immunopathol Pharmacol*. 2002 Jan-Apr;15(1):53-58.
55. Baehler P, Chad Z, Gurbindo C, Bonin AP, Bouthillier L, Seidman EG. Distinct patterns of cow's milk allergy in infancy defined by prolonged, two-stage double-blind, placebo-controlled food challenges. *Clin Exp Allergy* 1996;26(3):254-61.
56. Nørgaard A, Bindslev-Jensen C. Egg and milk allergy in adults. Diagnosis and characterization. *Allergy*. 1992 Oct;47(5):503-9.
57. Høst A, Samuelsson EG. Allergic reactions to raw, pasteurized, and homogenized/pasteurized cow milk: a comparison. A double-blind placebo-controlled study in milk allergic children. *Allergy*. 1988 Feb;43(2):113-8.
58. Hill DJ, Ford RP, Shelton MJ, Hosking CS. A study of 100 infants and young children with cow's milk allergy. *Clin Rev Allergy*. 1984 May;2(2):125-42.
59. Houben GF, Baumert JL, Blom WM, Kruizinga AG, Meima MY, Remington BC, et al. Full range of population eliciting dose values for 14 priority allergenic foods and recommendations for use in risk characterization. *Food Chem Toxicol* 2020; 146:111831.

- 564 60. Patel N, Chong KW, Yip AYG, Ierodiakonou D, Bartra J, Boyle RJ, Turner PJ. Use of
565 multiple epinephrine doses in anaphylaxis: A systematic review and meta-analysis. *J Allergy*
566 *Clin Immunol.* 2021 Nov;148(5):1307-1315.
- 567 61. Holzhauser T, Johnson P, Hindley JP, O'Connor G, Chan CH, Costa J, et al. Are current
568 analytical methods suitable to verify VITAL® 2.0/3.0 allergen reference doses for EU
569 allergens in foods? *Food Chem Toxicol.* 2020 Nov;145:111709.
- 570 62. Turner PJ, Kemp AS, Campbell DE. Advisory food labels: consumers with allergies need
571 more than "traces" of information. *BMJ.* 2011 Oct 13;343:d6180.
- 572 63. DunnGalvin A, Roberts G, Schnadt S, Astley S, Austin M, Blom WM, et al. Evidence-based
573 approaches to the application of precautionary allergen labelling: Report from two iFAAM
574 workshops. *Clin Exp Allergy.* 2019;49(9):1191-1200.
- 575

FIGURE LEGENDS

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram

Figure 2: Meta-analysis of aggregate data from 19 studies assessing the proportion of CM-allergic individuals reacting with objective symptoms to $\leq 5\text{mg}$ (**Fig 2A**) and $\leq 0.5\text{mg}$ CM protein (**Fig 2B**) who experienced anaphylaxis at that dose.

Figure 3: Dose-distribution for reaction threshold at baseline DBPCFC in 110 participants included in the IPD-meta-analysis (pooled cohort of 5 studies) who underwent 2 challenges. The proportion of subjects reacting at each dosing level with anaphylaxis (defined according to WAO 2020 criteria²⁴) is also shown (grey bars). Population reference distribution derived from Houben et al.⁵⁹

Figure 4: Violin plot of the distributions of log-change in reaction thresholds (from initial to repeat DBPCFC) for study participants within each included cohort. A change of $\frac{1}{2}$ -log in eliciting dose is equivalent to a shift in reaction threshold by one dosing increment using a PRACTALL-based semi-log regimen. Red dashed line represents the median, red dotted lines the interquartile range.

Figure 5: Change in reaction threshold in the 33 participants who underwent two CM challenges, and experienced anaphylaxis on at least one occasion. **Fig 5A**, absolute change in threshold. **Fig 5B**, violin plot of the distributions of log-fold change in reaction thresholds between first and second challenge, unless otherwise stated. Red dashed line represents the median, red dotted lines the interquartile range.

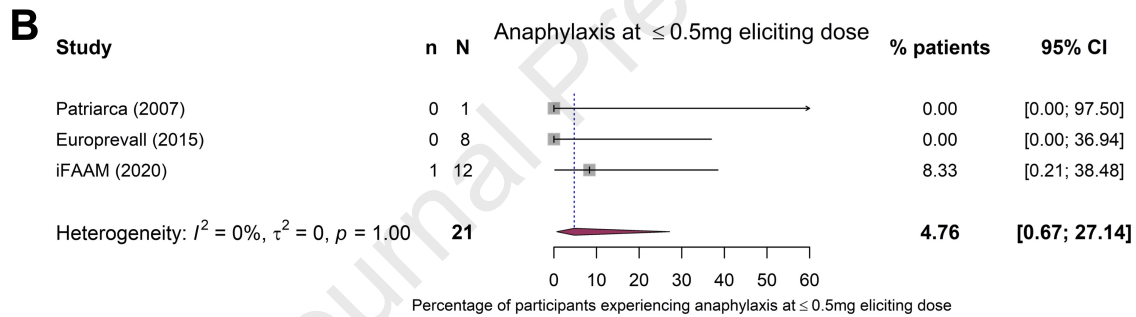
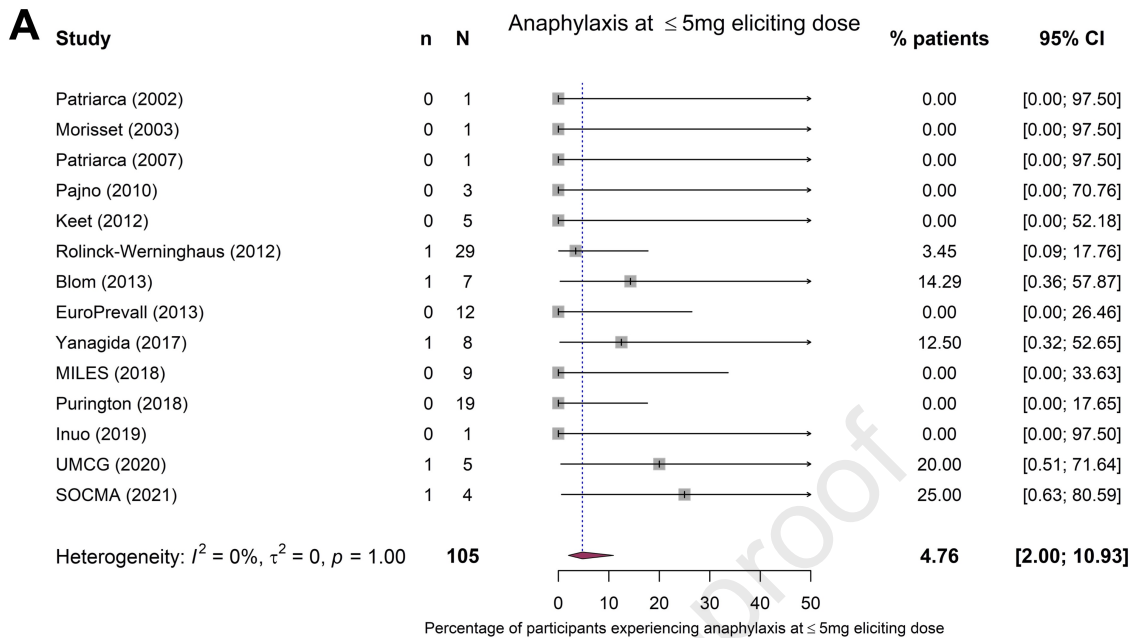
608 **Table 1:** Predicted ED₀₁, ED₀₅ and ED₁₀ values for cow's milk

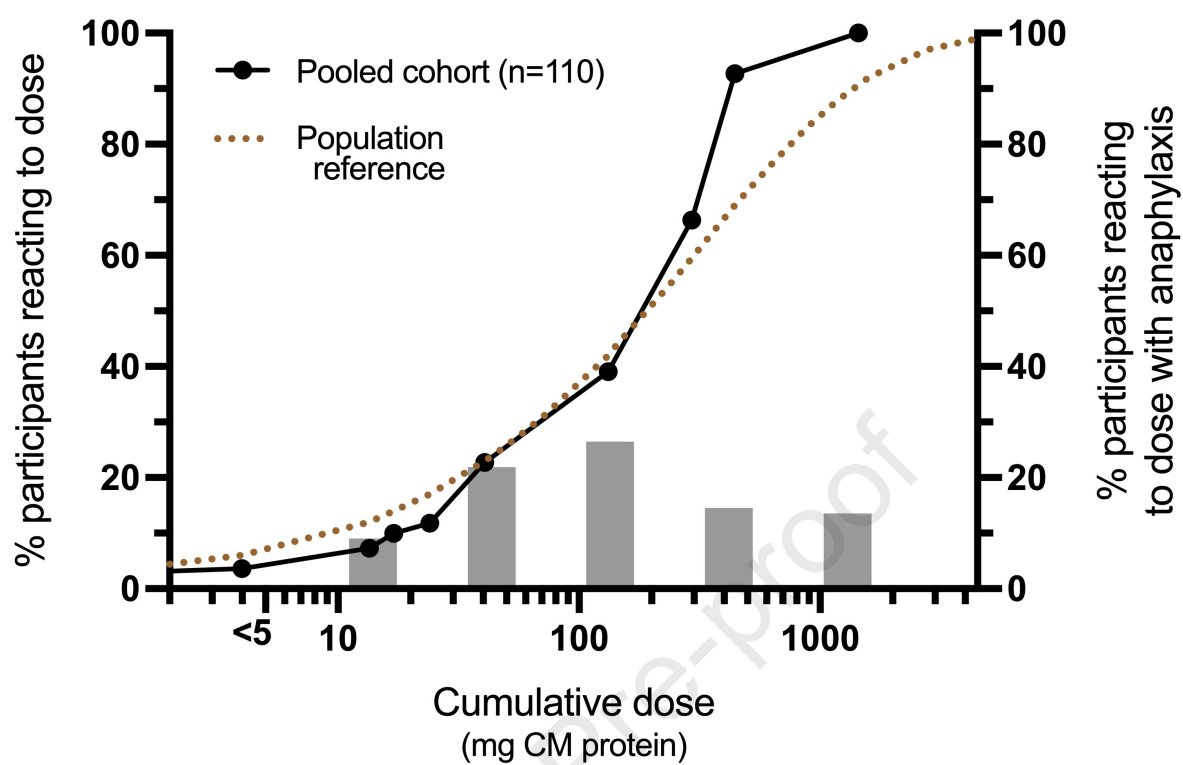
	Discrete dose mg CM protein (95% CI)	Cumulative dose mg CM protein (95% CI)
ED ₀₁	0.2 (0.1 - 0.3)	0.3 (0.2 - 0.5)
ED ₀₅	2.1 (1.2 - 3.8)	2.9 (1.6 - 5.4)
ED ₁₀	6.5 (3.9 - 11.6)	9.3 (5.4 - 17.0)

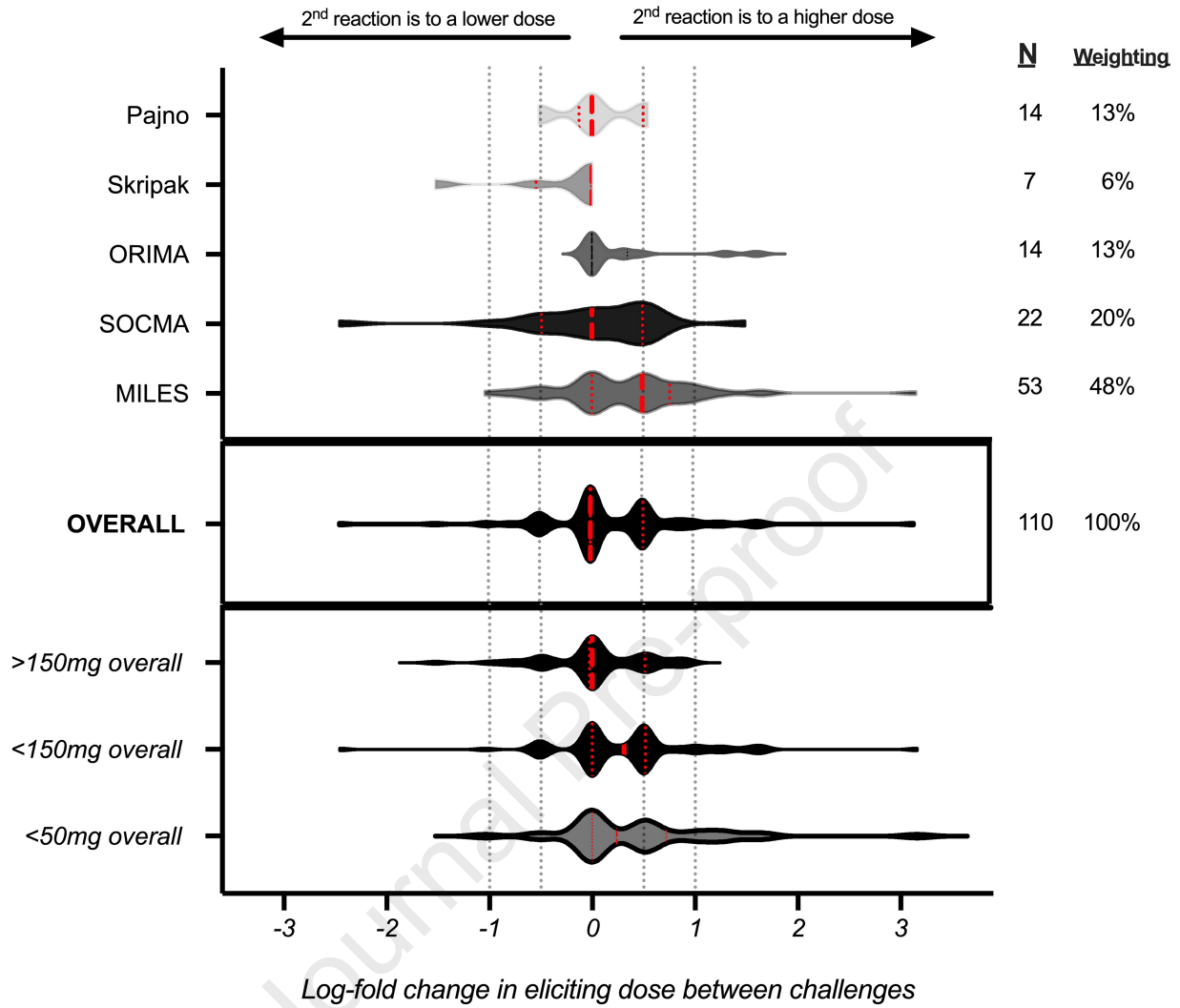
609

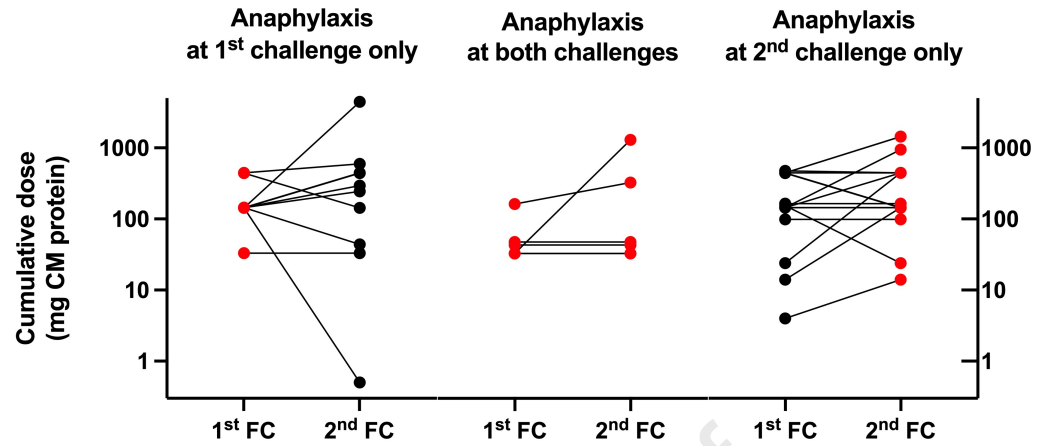
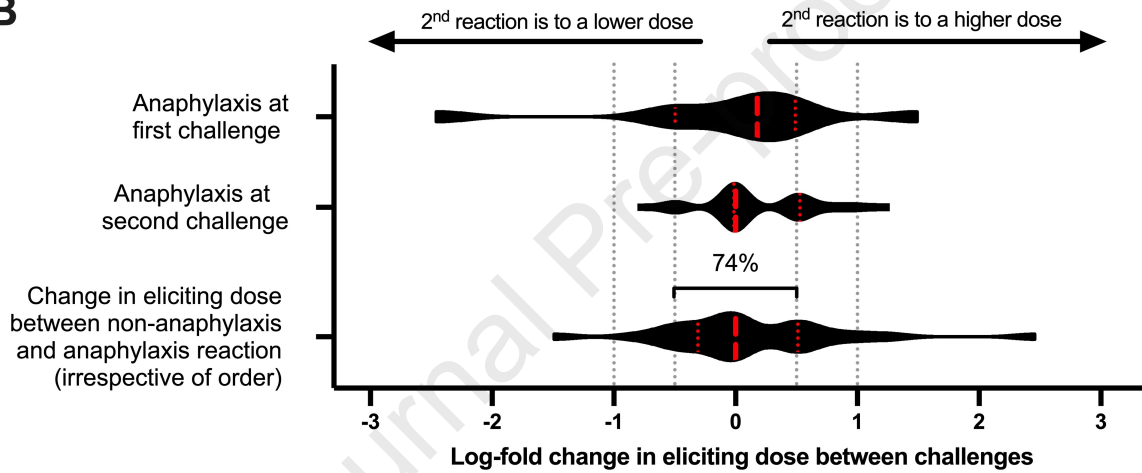
Cumulative reaction threshold at initial challenge (mg CM protein)	At IPD-meta-analysis, proportion of participants (and 95% CI) with:						
	Increase in threshold		No change in threshold	Decrease in threshold		$\pm$ max $\frac{1}{2}$-log change	$\pm$ max 1-log change
	$>\frac{1}{2}$ -log	any		any	$>\frac{1}{2}$ -log		
Any n=110	11% (3.8, 28)	38% (22, 57)	43% (27, 61)	17% (11, 26)	6.4% (3.1, 13)	80% (65, 89)	91% (84, 95)
>150 mg i.e. no objective symptoms to 5ml CM n=43	3.5% (0.05, 71)	23% (6.9, 54)	46% (27, 65)	19% (9.6, 33)	7.0% (2.3, 20)	83% (55, 95)	100% (75, 100)
<150 mg n=67	17% (6.8, 38)	47% (25, 69)	38% (19, 63)	13% (7.1, 24)	4.5% (1.5, 13)	72% (57, 84)	87% (76, 93)
<50 mg n=36	21% (5.4, 55)	47% (26, 69)	47% (21, 75)	8.3% (2.7, 23)	2.8% (0.4, 17)	84% (45, 97)	88% (58, 97)

Table 2: Proportions of participants with a change (or no change) in threshold, (overall and cohorted into those with lower reaction thresholds to CM), by IPD-meta-analysis. A $\frac{1}{2}$ -log and 1-log change in threshold is equivalent to a shift in reaction threshold by 1 or 2 dosing increments when using a challenge protocol based on PRACTALL.







A**B**

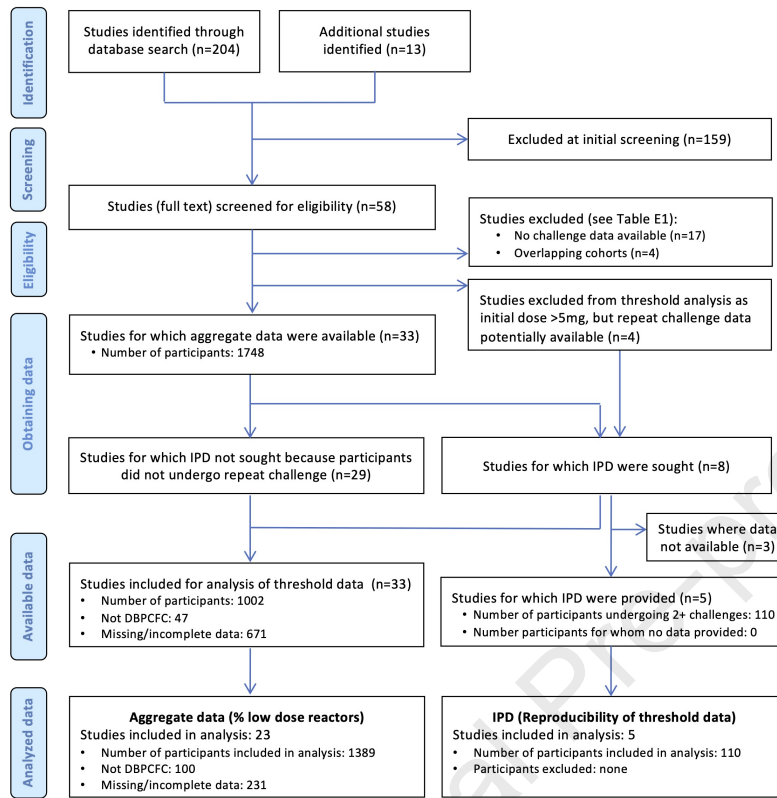


Table E1: Excluded studies which otherwise met the study inclusion criteria

Study	Reason for exclusion
Ruinemans-Koerts 2019. Clin Exp Allergy 49(3):350-6. doi: 10.1111/cea.13307	Required data not in paper and no response from authors
De Schryver 2019. JACI In Practice 7:1912-19. doi: 10.1016/j.jaip.2019.02.007.	Required data not in paper and no response from authors
Capucilli 2018. Ann Allergy Asthma Immunol. 2018;121:580-7. doi 10.1016/j.anai.2018.07.018.	Required data not available.
Pettersson 2018. Allergy 73:1532-40 doi: 10.1111/all.13423	Unable to clarify overlap with other datasets e.g. Blom et al 2013.
ElBadawy 2017 Egypt J Immunol. 24(2):109-125	Required data not in paper and no response from authors
Ebrahimi 2017. Iran J Allergy Asthma Immunol. 16(3):183-192	Required data not available: no conversion factor to mg protein
Andorf 2017. JACI Pract 5:1325-34 doi: 10.1016/j.jaip.2017.01.016	Required data not in paper and no response from authors
Takahashi 2016. Allergy Asthma Clin Immunol. 26;12(1):44. doi 10.1186/s13223-016-0150-0.	Required data not in paper and unable to contact authors
Wood 2016. JACI 137(4):1103-1110.e11. doi: 10.1016/j.jaci.2015.10.005	Required data not in paper and no response from authors
Okada 2015. Allergol Int. 2015;64(3):272-6. doi: 10.1016/j.alit.2015.04.002.	Unable to clarify overlap with Yanagida 2017.
Vázquez-Ortiz 2013. Clin Exp Allergy 43(1):92-102. doi: 10.1111/cea.12012	Required data not in paper and no longer available.
Alessandri 2012. PLoS ONE 7(7):e40945 doi: 10.1371/journal.pone.0040945	Required data not in paper and no longer available.
Alvaro 2012. Eur J Pediatr. 171(9):1389-95 doi: 10.1007/s00431-012-1739-z	Required data not in paper and no longer available.
Eller 2012. Ann Allergy Asthma Immunol. 108:332-6. doi: 10.1016/j.anai.2012.03.010	Required data not in paper and no response from authors
Longo 2012. Eur Ann Allergy Clin Immunol. 44(2):54-60.	Required data not in paper and no response from authors.
Martorell 2011 Clin Exp Allergy 2011;41(9):1297-304. doi: 10.1111/j.1365-2222.2011.03749.x.	Challenge 1 st dose >5mg. Data re thresholds in participants undergoing repeat challenge not available.
Staden 2008. JACI 122(2):418-9. doi: 10.1016/j.jaci.2008.06.002.	Required data not in paper and no response from authors.
Fiocchi 2006. Clin Exp Allergy. 2006;36(3):311-6. doi: 10.1111/j.1365-2222.2006.02428.x.	Required data not in paper and no longer available
Celik-Bilgili 2005. Clin Exp Allergy 35(3):268-73. doi: 10.1111/j.1365-2222.2005.02150.x.	Required data not in paper and no response from authors. ?overlap with Rolinck-Werninghaus 2012
Niggemann 2004. J Investig Allergol Clin Immunol. 14(2):98-103.	Required data not in paper and no response from authors
Fiocchi 2003. Pediatrics 2003; 112 (2): 359–362. doi: 10.1542/peds.112.2.359	Overlap with Fiocchi 2003, Clinical Experimental Allergy.
Rancé 2002. Arch Pediatr. 9 S3:402s-407s. doi: 10.1016/s0929-693x(02)00151-3	Required data not in paper and no longer available
Giampietro 2001. PAI 12(2):83-6. doi: 10.1034/j.1399-3038.2001.012002083.x	Required data not in paper and no response from authors
Niggemann 2001. PAI 12(2):78-82. doi: 10.1034/j.1399-3038.2001.012002078.x	Required data not in paper and no response from authors

Table E2: Characteristics of included cohorts. All doses expressed in mg CM protein.

Study	N	Age of cohort	Study type	Challenge protocol (mg CM protein)	Threshold definition	Anaphylaxis definition used	Median cumulative dose	Number with symptoms to Objective symptoms	Study-defined Anaphylaxis No. Symptoms	N included in analysis for		ED data censored	
										ED	Ana	Right ^a	Left ^b
SOCMA study 2021 ²⁸ UK, Spain	83	6-18y Median 10y	Immunotherapy	DBPCFC, 20-30min intervals ½,3,10,30,100,300,1000,3000	DLS	WAO	143.5mg	4	1 Throat tightness, cough	83	83 [#]	4	1
iFAAM 2020 ²⁹ UK, Spain, Ireland	172	Median 1.0y (range 0.2–16 y)	Diagnostic	Single-dose FC, 0.5mg DBPCFC in 50%	LOAEL	WAO	433mg	12	1 Bilateral wheeze, erythema	##	172		
UMCG 2020 ⁵ Netherlands	53	Median 2.3y (range 0.3-17.6 y)	Diagnostic	DBPCFC. pre 2007: 1.75,3.5,14,70,130,350,570 DBPCFC, after 2007: 0.6,3,10,30,100,300,1000	LOAEL	CVS/Lower resp symptoms	437.5 mg	5	1 Vocal hoarseness with mild dyspnoea	53	53	12	5
UMCU 2020 ⁵ Netherlands	15	15 adults (≥18 y)	Diagnostic	DBPCFC, 30min intervals 0.3,3,30,90,300,900,3000,9000	LOAEL	CVS/Lower resp symptoms	1560 mg	1	Data not available	15		4	1
Inuo 2019 ³⁰ Japan	25	Median 4.3y (range 1-9y)	Diagnostic	DBPCFC, 30min intervals, 1st dose 5mg	DLS	CVS/Lower resp symptoms	61mg	1	0	§	25		
Maeda 2021 ³¹	28	Mean 5.4y (range 3-12y)	Immunotherapy	DBPCFC, 15min intervals 33,66,66,165,330,660,990,990	DLS	Sampson IV	66mg	0	0	§§	28		
Purington 2018 ³² USA	67	Children + adults	Diagnostic	DBPCFC, 15 min interval 1.7,5,20,50,100,100,100,125	DLS	NIAID	327mg	19	0	§	51		
MILES study 2018 ³³ USA	198	Children, 2-11y	Immunotherapy	DBPCFC, 20min intervals 1,3,10,30,100,300	DLS	Consistent with WAO*	144mg	9	0	§§	198		
Yanagida 2017 ³⁴ Japan	164	Children ≥5y Median 8.6 y	Diagnostic	DBPCFC, 30min intervals 71,142,283,425,779	LOAEL	Sampson IV	840mg	8	1 GI symptoms, cough, dyspnea	164	164	0	15
Gushken 2013 ³⁵ Brazil	58	Median 5.3y (range 1.1-15y)	Diagnostic	DBPCFC, 15min intervals 33,132,330,462,660,792,792	LOAEL	CVS/Lower resp symptoms	450mg	N/A	N/A	39	39	0	18
Blom 2013 ³⁶ Netherlands	93	Median 2.2y (range 0.6-13.9y)	Diagnostic	DBPCFC, 30min intervals 1.75,3.5,14,70,350,1750,2190	LOAEL	CVS/Lower resp symptoms	389mg	7	1 Vocal hoarseness, mild cough, pruritus, face angioedema	78	78	8	4
Dambacher 2013 ³⁷ Netherlands	21	Median 0.7y (range 0.3-11y)	Diagnostic	DBPCFC, 20min intervals 18,180,360,540,720,1080,1620	LOAEL	CVS/Lower resp symptoms	1620mg	0	0	21	21	3	3
Lee et al, 2013 ³⁸ South Korea	31	Mean 0.7y	Immunotherapy	DBPCFC, 30min intervals 3.3,16.7,33,67,167,333	DLS	CVS/Lower resp symptoms	165mg	0	0	§	31		
EuroPrevall 2013 ⁸ Europe (16 countries)	69	2 adults, 67 children Median 1.2y	Diagnostic	DBPCFC, 20min; 0.003,0.03, 0.3,3,30,90,300,900,3000	LOAEL	CVS/Lower resp symptoms	123mg	12	0	§	69		
Keet 2012 ³⁹ USA	30	Median 8y (range 6-15y)	Immunotherapy	DBPCFC, 15min intervals 0.1,1 10,40,100,400,800,1200	DLS	Not stated	51mg	5	0	30	30	0	0
Rolinck-Werninghaus 2012 ⁴⁰ Germany	305	Median 1y (range 0.2-16y)	Diagnostic	DBPCFC (73%), open FC (27%) 3,10,30,100,300,1000,3000	LOAEL	Sampson IV	1443mg	29	1 Anaphylaxis (respiratory symptoms)	305	305	0	29
Pajno 2010 ⁴¹ Italy	30	Median 10y (range 4-13y)	Immunotherapy	DBPCFC 30min intervals 3,9,30,90,300,900,3000	DLS	CVS/Lower resp symptoms	100mg	3	0	§	30		
Caminiti 2009 ⁴² Italy	11	Mean ~8y (range 5-10y)	Immunotherapy	DBPCFC, 20min intervals 3,9,30,90,300,900,3000	DLS	CVS/Lower resp symptoms	145mg	0	0	11	11	0	0
Orhan 2009 ⁴³ Turkey	4	Median 8.5y (range 7-9y)	Diagnostic	DBPCFC, 15min intervals 165,330,1320,2475,4950	LOAEL	Not stated	3050mg	N/A	N/A	4		0	0
Longo 2008 ⁴⁴ Italy	60	Mean 8y (range 5-17y)	Immunotherapy	DBPCFC, 20min intervals 0.75,1.5,3,6,12,24	LOAEL	Not stated	12mg	9	Data not available	60		0	9

Study	N	Age of cohort	Study type	Challenge protocol (mg CM protein)	Threshold definition	Anaphylaxis definition used	Median cumulative dose	Number with symptoms to ≤5mg discrete dose		N included in analysis for ED	Ana	ED data censored	
								Objective symptoms	Study-defined Anaphylaxis No. Symptoms			Right ^a	Left ^b
Lam 2008 ⁴⁵ Netherlands	10	Median 40y (range 17-68y)	Diagnostic	DBPCFC, 30min intervals 0.3,3,30,90,300,900,3000,9000	LOAEL	CVS/Lower resp symptoms	900mg	0	0	§§§	10		
Skripak 2008 ⁴⁶ USA	20	Median 9.5y (range 6-15y)	Immunotherapy	DBPCFC, interval not stated 40,100,400,800,1200	LOAEL	Not stated	40mg	N/A	N/A	20		0	14
Staden 2007 ⁴⁷ Germany	24	2.9y, 3.6y, 5.8y	Immunotherapy	DBPCFC, 30min intervals 3,10,33,100,330,1000,3300	LOAEL	Not stated	99mg	0	0	3		0	0
Morisset 2007 ⁴⁸ France	60	Mean 2.2y (range 1.1-6.5y)	Immunotherapy	Single blinded, 20min interval 3.3,6.6,16.5,50,165,495,1320	LOAEL	CVS/Lower resp symptoms	1650mg	0	0	11	11	0	1
Patriarca 2007 ⁴⁹ Italy	11	Median 5.5y (range 3-15y)	Immunotherapy	DBPCFC, 30min intervals 0.033,0.165,0.33,0.66,1.65,3.3,6.6, 16.5,33,132,264,528,1056,1980	DLS	CVS/Lower resp symptoms	102mg	1	0	§	11		
Devenney 2006 ⁵⁰ Sweden	4	Median 3.3y (range 1.8-5.5y)	Diagnostic	DBPCFC, 20min intervals 3.3,16.5,165,500,1000	LOAEL	Not stated	DNA	At least 1	Data not available	2		0	1
Flinterman 2006 ⁵¹ Netherlands	11	Median 4.0y (range 1.8-10.3y)	Diagnostic	DBPCFC, 20-30min intervals 180,360,540,720,1080,1620	LOAEL	Not stated	540mg	N/A	N/A	11		0	3
Fiocchi 2003 ⁵² Italy	18	Median 5y (range 0.5-9.8y)	Diagnostic	DBPCFC, 30min intervals 198,396,792,1584	LOAEL	Not stated	504mg	N/A	N/A	12		0	5
Morisset 2003 ⁵³ France	59	52 children, 7 adults	Diagnostic	Blinded FC, 20min interval 3.3,6.6,16.5,50,165,495,1320	LOAEL	CVS/Lower resp symptoms	DNA	1	0	3	59	0	1
Patriarca 2002 ⁵⁴ Italy	8	Median 10.5y (range 5-15y)	Immunotherapy	DBPCFC, 30min intervals 0.33,3.3,16.5,33,165,333,1000	LOAEL	CVS/Lower resp symptoms	218mg	1	0	8	8	0	0
Baehler 1996 ⁵⁵ Canada	69	Mean 3y	Diagnostic	DBPCFC, 15min intervals; 1.8,16.5,33,83,165,330,660,1000	LOAEL	CVS/Lower resp symptoms	180mg	0	0	10	10	0	0
Norgaard 1992 ⁵⁶ Denmark	4	Adults (range 29-44y)	Diagnostic	DBPCPFC, 30min intervals First dose 16.5mg	LOAEL	Not stated	1700mg	0	0	3	3	0	1
Høst & Samuelsson 1988 ⁵⁷ Denmark	5	Median 1.2y (range 1.0-3.3y)	Diagnostic	DBPCPFC, 120min intervals First dose 165mg	LOAEL	Not stated	165mg	N/A	N/A	3		0	2
Hill 1984 ⁵⁸ Australia	100	Mean 1.4y 95% ≤3 years	Diagnostic	Open FC, 30min intervals 330,660,990,2000,4000,8000	LOAEL	Not stated	594 mg	N/A	N/A	53		0	28

CVS, cardiovascular; DBPCFC, double-blind, placebo-controlled food challenge; DLS, dose-limiting symptoms; DNA, data not available; ED, eliciting dose; FC, food challenge; LOAEL, Lowest-observed adverse effect level; N/A, not applicable.

#Overlap with iFAAM 2020 – so data only used to inform dose-distribution for eliciting dose, severity analysis for ≤5mg exposure and reproducibility analyses

##Overlap with SOCMA study 2021 – so data used to inform severity analysis for ≤1mg CMP exposure only

*individual participant symptom data was available in these studies, and used to re-assign the occurrence of anaphylaxis (or not) using WAO 2020 criteria

§Detailed symptom/threshold data not available so data used to inform severity analysis only

§§Data used to inform severity and reproducibility analyses only

§§§Data included in UMCU 2020 adult data, thus only used for severity analysis

Table E3: Risk of bias in the included studies

Study	Design	Interval in between FC	Selection bias*	Attrition bias	Detection bias	Internal validity	External validity**	Comments
SOCMA study 2021 ²⁸	Interventional RCT	6 months	Low	Low	Low	++	+	Inclusion cED ≤1443mg so potential skewing of population
iFAAM 2020 ²⁹	Diagnostic	n/a	n/a	n/a	Low	++	++	Min 1 hour interval to assess tolerance to 0.5mg CMP
UMCG 2020 ⁵	Diagnostic	n/a	n/a	n/a	Low	+	++	
UMCU 2020 ⁵	Diagnostic	n/a	n/a	n/a	Low	+	+	
Inuo 2019 ³⁰	Dose-ranging study	n/a	n/a	Low	Low	+	+	
Maeda 2021 ³¹	Interventional RCT	12 months	Low	Low	Moderate	+	+	15min intervals in between challenge doses
Purington 2018 ³²	Diagnostic	n/a	n/a	n/a	Moderate	+	-	Participants tolerating ≥500mg excluded from analysis. 15min intervals in between challenge doses
MILES study 2018 ³³	Interventional RCT	12 months	Low	Low	Low	++	+	Inclusion cED ≤444mg so potential skewing of population.
Yanagida 2017 ³⁴	Diagnostic	n/a	n/a	n/a	Moderate	+	++	
Gushken 2013 ³⁵	Diagnostic	n/a	n/a		Moderate	+	±	15min intervals in between challenge doses
Blom 2013 ³⁶	Diagnostic	n/a	n/a	n/a	Low	++	++	No information on self-selection due to subjects declining to participate
Dambacher 2013 ³⁷	Diagnostic	n/a	n/a	n/a	Low	+	±	
Lee et al, 2013 ³⁸	Interventional	n/a	n/a	n/a	Low	+	±	
EuroPrevall 2013 ⁸	Diagnostic	n/a	n/a	n/a	Low	++	+	80 challenges eligible, data available for 67 subjects.
Keet 2012 ³⁹	Interventional RCT	n/a	n/a	n/a	Moderate	++	±	15min intervals in between challenge doses
Rolinck-Werninghaus 2012 ⁴⁰	Diagnostic	n/a	n/a	n/a	Low	++	++	
Pajno 2010 ⁴¹	Interventional RCT	18 weeks	Low	Low	Low	+	±	
Caminiti 2009 ⁴²	Interventional	n/a	n/a	n/a	Low	+	±	
Orhan 2009 ⁴³	Diagnostic	n/a	n/a	n/a	Moderate	+	±	15min intervals in between challenge doses
Longo 2008 ⁴⁴	Interventional RCT	n/a	n/a	Low	Moderate	++	+	
Lam 2008 ⁴⁵	Diagnostic	n/a	n/a	n/a	Low	+	±	
Skripak 2008 ⁴⁶	Interventional RCT	23 weeks	Low	Low	Unclear	±	±	Dosing interval not stated.
Staden 2007 ⁴⁷	Interventional	n/a	n/a	n/a	Low	+	±	
Morisset 2007 ⁴⁸	Interventional	n/a	n/a	n/a	Moderate	+	±	
Patriarca 2007 ⁴⁹	Interventional	n/a	n/a	n/a	Low	+	±	
Devenney 2006 ⁵⁰	Diagnostic	n/a	n/a	n/a	Moderate	+	±	
Flinterman 2006 ⁵¹	Diagnostic	n/a	n/a	n/a	Low	+	±	
Fiocchi 2003 ⁵²	Dose-ranging study	n/a	n/a	n/a	Low	+	±	
Morisset 2003 ⁵³	Diagnostic	n/a	n/a	n/a	Moderate	+	+	
Patriarca 2002 ⁵⁴	Interventional	n/a	n/a	n/a	Low	+	±	
Baehler 1996 ⁵⁵	Diagnostic	n/a	n/a	n/a	Moderate	+	+	15min intervals in between challenge doses
Norgaard 1992 ⁵⁶	Diagnostic	n/a	n/a	n/a	Low	+	±	
Høst & Samuelsson 1988 ⁵⁷	Diagnostic	n/a	n/a	n/a	Low	+	±	
Hill 1984 ⁵⁸	Diagnostic	n/a	n/a	n/a	Unclear	+	±	

*Selection bias refers to possible differences in subject allocation between intervention and control groups. This was not relevant for studies which were not used for the IPD meta-analysis to assess reproducibility of reaction thresholds at DBPCFC.

**External validity assesses for whether selection bias impacts on whether the study data are generalizable to the overall CM-allergic population, and described as ++ (all or most of the criteria have been fulfilled, and where not the conclusions are very unlikely to alter), + (some criteria have been fulfilled, and where not fulfilled or adequately described, the conclusions are unlikely to alter), - (few or no checklist criteria fulfilled).

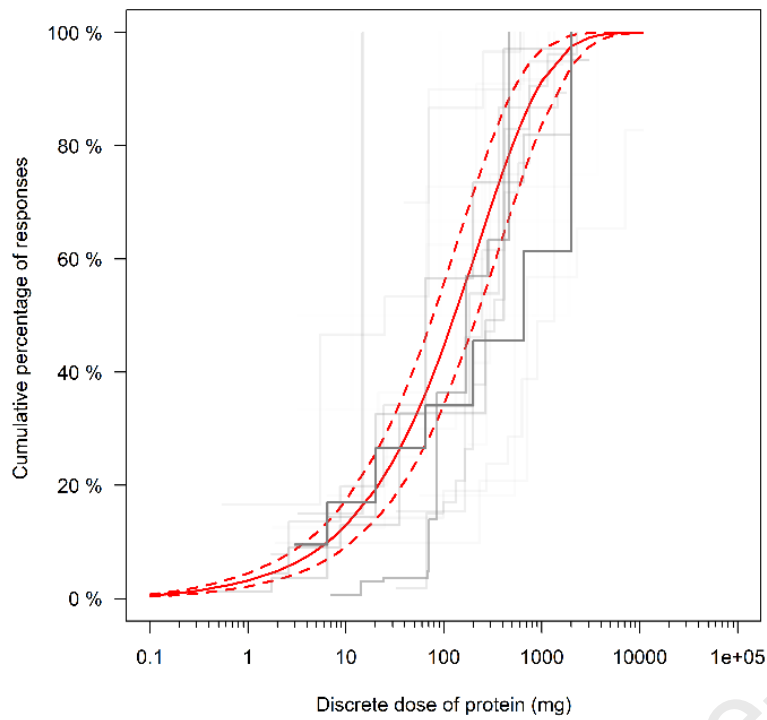
ONLINE REPOSITORY

FIGURE LEGENDS

Figure E1: Eliciting dose curves from the stacked model averaged population threshold dose distributions for cow's milk, based on discrete (A) and cumulative (B) dose datasets. Doses are expressed in mg protein.

Figure E2: Funnel plot of the included studies. There was no evidence of publication bias in terms of the rate of anaphylaxis in low-dose reactors, by size of study.

Figure E3: Meta-analysis of aggregate data from 13 studies assessing the proportion of individuals with cow's milk allergy reacting to 5mg or less with anaphylaxis, divided by the criteria used to define threshold (1) LOAEL and (2) study-defined dose-limiting symptoms.

A**B**