Timing of allergenic food introduction to the infant diet and risk of allergic or autoimmune disease: a systematic review and meta-analysis

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Date of Revision: 19th August 2016

Word Count: 3486

Key Points

Question: Does the timing of allergenic food introduction to infants affect their risk of developing allergic or autoimmune disease?

Findings: There was moderate certainty evidence that early introduction of egg (from 4-6 months) or peanut (from 4-11 months) was associated with reduced risk of egg or peanut allergy respectively. There was low to very low certainty evidence that early fish introduction was associated with reduced allergic sensitization and rhinitis, and high certainty evidence that timing of gluten introduction was not associated with celiac disease risk.

Meaning: Early introduction of egg or peanut to infants was associated with a reduced risk of egg or peanut allergy.

Abstract

Importance: Timing of allergenic food introduction to the infant diet may influence allergic or autoimmune disease risk, but the evidence for this has not been comprehensively synthesized.

Objective: To systematically review and meta-analyze evidence that timing of allergenic food introduction during infancy influences risk of allergic or autoimmune disease.

Data Sources: MEDLINE, EMBASE, Web of Science, CENTRAL and LILACS, searched between January 1946 and March 2016.

Study Selection: Intervention trials and observational studies that evaluated timing of allergenic food introduction during the first year of life and reported allergic or autoimmune disease, or allergic sensitization were included.

Data Extraction and Synthesis: Data were extracted in duplicate and synthesized for meta-analysis using generic inverse variance or Mantel-Haenszel methods with a random effects model. GRADE was used to assess the certainty of evidence.

Main Outcome(s) and Measure(s): Wheeze, eczema, allergic rhinitis, food allergy, allergic sensitization, type 1 diabetes mellitus, celiac disease, inflammatory bowel disease, autoimmune thyroid disease and juvenile rheumatoid arthritis.

Results: Of 16,289 original titles screened, data were extracted from 204 titles reporting 146 studies. There was moderate certainty evidence from 5 trials (1915 participants) that early egg introduction was associated with reduced egg allergy (RR 0.56 95%CI 0.36, 0.87 I^2=36%; P=0.009). Absolute risk reduction for a population with 5.4% incidence of egg allergy was 24 cases per 1000 population (95% CI 7, 35). There was moderate certainty evidence from 2 trials (1550 participants) that early peanut introduction was associated with

reduced peanut allergy (RR 0.29 95%CI 0.11, 0.74 I^2=66%; P=0.009). Absolute risk reduction for a population with 2.5% incidence of peanut allergy was 18 cases per 1000 population (95% CI 6, 22). Certainty of evidence was downgraded due to imprecision of effect estimates and indirectness of the populations and interventions studied. Timing of egg or peanut introduction was not associated with risk of allergy to other foods. There was low to very low certainty evidence that early fish introduction was associated with reduced allergic sensitization and rhinitis. There was high certainty evidence that timing of gluten introduction was not associated with celiac disease risk, and timing of allergenic food introduction was not associated with other outcomes.

Conclusions and Relevance: In this systematic review, early egg or peanut introduction to the infant diet was associated with lower risk of developing egg or peanut allergy. These findings must be considered in the context of limitations in the primary studies.

Introduction

Increasing attention has focused on the role of timing of introduction of allergenic food into the infant diet and risk of allergic and autoimmune diseases. Infant feeding guidelines have moved away from advising parents to delay the introduction of allergenic food, but most guidelines do not yet advise early feeding of such foods.[1-3] Several professional organizations have responded to recent research findings by issuing interim guidance advising early peanut introduction in infants at high risk of peanut allergy, with some caveats. [4,5] However a randomized clinical trial of early introduction of multiple allergenic foods did not show efficacy for preventing food allergy[6], and a trial of early gluten introduction showed no effect on risk of celiac disease[7]. The implications for preventing food allergy or other immune-mediated health conditions in the general population are not clear.

To inform UK Infant Feeding Guidance, we undertook a systematic review and meta-analysis for the UK Food Standards Agency, evaluating whether timing of allergenic food introduction to the infant diet influences risk of allergic or autoimmune disease. This is one of a series of systematic reviews of dietary exposures in pregnancy or infancy and immune outcomes, the first of which reviewed hydrolyzed infant formula[8]. The immunological mechanisms underlying the different allergic and autoimmune diseases vary. For example, most food allergy is characterized by IgE-mediated inflammation, whereas type 1 diabetes mellitus is caused by T-cell mediated islet cell destruction.[9,10] However, these diseases share a common feature of impaired immune tolerance, and immune function in infancy may be modified by dietary exposures. Therefore a comprehensive range of allergic and autoimmune outcomes were included.

Methods and Literature Search

Methods are described in the Supplemental Material. This systematic review is reported according to PRISMA guidance[11]. We searched The Cochrane Library, EMBASE, LILACS, MEDLINE, Web of Science and http://apps.who.int/trialsearch from 1st January 1946 to 8th March 2016. Intervention trials and observational studies evaluating age at allergenic food introduction (milk, egg, fish, shellfish, tree nuts, wheat, peanuts, soya)[12] during the first year, and allergic or autoimmune disease at any age were included. Other systematic reviews rated as high quality using published criteria[13] were also included, as per the study protocol, to avoid duplicating existing work. Where other systematic reviews were included, original studies which were not captured by the other reviews were also summarized. Outcomes evaluated were wheeze, eczema, allergic rhinitis, food allergy (a reproducible hypersensitivity reaction to a food), allergic sensitization (the presence of specific IgE to an allergen), type 1 diabetes mellitus (TIDM), celiac disease, inflammatory bowel disease, juvenile rheumatoid arthritis, psoriasis and vitiligo.

Data were extracted in duplicate, and risk of bias assessed using the Cochrane Risk of Bias tool and the National Institute for Clinical Excellence methodological checklists for intervention and observational studies, respectively. Publication bias was assessed using funnel plots and Egger's test, where meta-analyses included ≥10 studies. Random effects meta-analyses used generic inverse variance and Mantel-Haenszel methods for observational and intervention studies, respectively. Heterogeneity was quantified using I^2. Meta-analyses with I^2 >80% were not pooled. For meta-analyses with >5 studies we explored heterogeneity in pre-specified subgroup analyses of study design, risk of bias, risk of conflict of interest, and features of the population, intervention and outcome assessment. For meta-analyses with

≤5 studies we explored statistical heterogeneity descriptively, and also conducted sensitivity analyses by study design and risk of bias for the key review findings.

Post hoc trial sequential analysis (TSA) was used to quantify statistical reliability of moderate or high certainty review findings using 2-sided 5% significance, 80% power and control event rates from included studies to estimate optimal heterogeneity-adjusted and non-adjusted information sizes needed to identify relative risk reductions of 10, 20 and 30%. TSA quantifies statistical reliability of data in a cumulative meta-analysis in a similar way to an interim analysis in a single randomized clinical trial. GRADE was used to assess certainty of evidence, and the protocol was registered on PROSPERO.[14] Ethical approval was not required by the Imperial College Joint Research Office. Dataset and statistical code are available from the corresponding author.

Results

Search results are summarized in eFigure 1 in the Supplement (existing systematic reviews) and eFigure 2 (original studies). A summary of the findings of the 2 included systematic reviews is shown in eTables 1 and 2.

Title, abstract and full text screening of original studies yielded 146 eligible studies (204 separate titles). Overall, 24 intervention trials (39 titles) evaluated allergic outcomes in 13,298 participants, and 5 intervention trials (6 titles) evaluated autoimmune diseases in 5,623 participants. Sixty-nine observational studies (90 titles) reported allergic outcomes in 142,103 participants, and 48 observational studies (69 titles) evaluated autoimmune diseases in 63,576 participants. No study reported psoriasis or vitiligo. For allergic outcomes, these included 55 cohort studies (one retrospective), 2 nested case-control studies, and 12 case-control or cross-sectional studies. For autoimmune diseases, there were 7 cohort studies, 4 nested case-control studies, and 37 case-control studies. Characteristics of included studies are summarized in eTables 3, 4 (allergic outcomes), 5 and 6 (autoimmune outcomes). More detailed characteristics of the intervention studies of egg or peanut introduction that reported egg or peanut allergy are shown in Tables 1 and 2.

Risk of bias was low in 4/24 (17%) intervention trials and 29/69 (42%) observational studies for allergic outcomes (eTables 7 and 8), and in 1/5 (20%) intervention trials and 10/48 (21%) observational studies for autoimmune outcomes (eTables 9 and 10). The main issues identified were attrition bias in intervention trials and lack of adjustment for potential confounders in observational studies.

The key findings of the systematic review are summarized in Table 3, with GRADE evidence assessment summarized in Table 4, and specific analyses for all positive or high certainty findings in Figures 1-3. More detailed methods and a summary of all findings (eTable 11) are

in the Supplement. The full report with a detailed description of all findings including meta-analyses and detailed methodology is available on the UK Food Standards Agency website http://www.food.gov.uk/science/research/allergy-research/fs305005, together with an associated statement by the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment http://cot.food.gov.uk/cotstatements.

Risk of Food Allergy and Allergic Sensitization

Fifteen intervention trials reported food allergy to any food, or to milk, egg or peanut separately, in 10,304 participants. Seventeen trials reported allergic sensitization to any allergen, aeroallergen, food allergen, egg, peanut or milk in 7,310 participants. A summary of findings is shown in the Supplement (eTable 11). Key findings for food allergy and allergic sensitization to egg, peanut or milk are summarized in Figures 1A and 1B.

Meta-analysis of 5 trials (1,915 participants) showed evidence that egg introduction at 4-6 months was associated with lower risk of egg allergy compared with later egg introduction (RR 0.56 95%CI 0.36, 0.87; P=0.009; moderate heterogeneity I^2=36%).[6,15-18] Absolute risk reduction for a population with 5.4% incidence of egg allergy was 24 cases per 1000 population (95% CI 7, 35). Meta-analysis of 4 trials (1,786 participants) showed no association between timing of egg introduction and egg sensitization.

Meta-analysis of 2 trials (1,550 participants) showed evidence that peanut introduction at 4-11 months was associated with lower risk of peanut allergy (RR 0.29 95%CI 0.11, 0.74; P=0.009; high heterogeneity I^2=66%).[4-6] Absolute risk reduction for a population with 2.5% incidence of peanut allergy was 18 cases per 1000 population (95% CI 6, 22). One trial (640 participants) reported significantly reduced allergic sensitization to peanut with early peanut introduction, but numerical data were not reported; a second trial (1,168 participants) found no significant association (Figure 1B).[4,6]

For several key findings there was moderate to high statistical heterogeneity. For the egg introduction and egg allergy analysis, heterogeneity was due to the abstract publication of Natsume and colleagues [17] – the authors declined to share further information about their study. The study of Perkin and colleagues[6], which used multiple allergenic food introduction, had findings that were consistent with other studies[15,16,18], where egg was the only allergenic food used. For the peanut introduction and peanut allergy analysis, the high heterogeneity was attributed to the high treatment adherence in the study by Du Toit and colleagues[4], compared with more variable treatment adherence in the study by Perkin and colleagues[6]. For the egg introduction and egg sensitization analysis, heterogeneity was due to the abstract publication of Bellach and colleagues[16], which used specific IgE rather than skin prick testing to determine egg sensitization.

In interventional studies, there was no association between timing of introduction of cow's milk [19, 20](Figure 1) or other allergenic food and food allergy or allergic sensitization, and no association between timing of introduction of one allergenic food and risk of food allergy or allergic sensitization to a different food (eTable 11 in Supplement).

Abstract publications made a significant contribution to the analysis of egg introduction and egg allergy. However the findings were similar in sensitivity analyses excluding those abstract publications whose authors were unable to share full trial findings (eFigure 3A), or excluding studies at high or unclear risk of bias (eFigure 3B). In sensitivity analyses of allergic sensitization that excluded abstracts (eFigure 4A) or studies at high or unclear risk of bias (eFigure 4B), early egg introduction was associated with significantly reduced risk of allergic sensitization to egg.

Eighteen observational studies reported food allergy in 40,194 participants; and 20 studies reported allergic sensitization in 23,466 participants. One prospective cohort study (699

participants) found an association between early egg introduction and decreased egg allergy (OR 0.29 95%CI 0.15, 0.56) and adjusted for possible reverse causation.[21] Three cohort studies (13,472 participants), which could not be meta-analyzed due to statistical heterogeneity and heterogeneity of analysis method (Figure 2A) found early fish introduction (before 6-9 months) was associated with reduced allergic sensitization to any allergen or food allergens.[22-24] There was no association between timing of introduction of other allergenic foods and risk of food allergy or allergic sensitization. Assessment for publication bias in analyses of food allergy and allergic sensitization was not possible, due to the limited number of studies in each meta-analysis.

Risk of Allergic Rhinitis

Thirteen intervention trials (6,333 participants) and 12 observational studies (25,147 participants) reported allergic rhinitis. A summary of findings is shown in the Supplement (eTable 11). Four cohort studies (12,781 participants) (Figure 2B) found fish introduction before 6-12 months was associated with reduced allergic rhinitis at age ≤ 4 years (OR 0.59 95% CI 0.40, 0.87; high heterogeneity I^2=59%) or 5-14 years (OR 0.68 95% CI 0.47, 0.98).[22,23,25,26] In a sensitivity analysis excluding studies at high or unclear risk of bias (eFigure 5), the association between early fish introduction and reduced allergic rhinitis at age ≤4 years was not statistically significant. It was not possible to explain the heterogeneity in the fish introduction and allergic rhinitis analysis. In other intervention and observational studies, timing of allergenic food introduction was not associated with risk of allergic rhinitis. Assessment for publication bias in analyses of allergic rhinitis was not possible, due to the limited number of studies in each meta-analysis.

Risk of Wheeze

Sixteen intervention trials (8,433 participants) and 30 observational studies (65,601 participants) reported wheeze. A summary of findings is shown in the Supplement (eTable 11). Three cohort studies (11,155 participants) found fish introduction before 8-12 months was associated with reduced recurrent wheeze at age ≤ 4 years (OR 0.72 95% CI 0.59, 0.87; no heterogeneity I^2=0%).[23,25,27] However, 5 other studies (13,033 participants) found no association between timing of fish introduction and wheeze.[28-32] In other intervention and observational studies there was no association between timing of allergenic food introduction and risk of wheeze. Assessment for publication bias in analyses of wheeze was not possible, due to the limited number of studies in each meta-analysis.

Risk of Eczema

Seventeen intervention trials (6,798 participants) and 37 observational studies (59,120 participants) reported eczema. A summary of findings is shown in the Supplement (eTable 11). For most analyses of intervention trials, data were sparse; for several analyses of observational studies statistical heterogeneity was high. Overall there was no consistent association between timing of allergenic food introduction and risk of eczema, from either intervention or observational studies. Assessment for publication bias in analyses of eczema was not possible, due to the limited number of studies.

Risk of Autoimmune Diseases

Five intervention trials (5,623 participants) and 48 observational studies (63,576 participants) reported autoimmune disease, and 2 other systematic reviews of observational data were identified. A summary of findings is shown in the Supplement (eTable 11). The systematic reviews found no consistent evidence for an association between timing of gluten introduction and celiac disease. [33, 34] Intervention trials also found no association between timing of gluten introduction and celiac disease (Figure 3) or TIDM, or milk introduction and

TIDM.[7,35-38] In sensitivity analyses excluding studies at high or unclear (eFigure 6A) or only high risk of bias (eFigure 6B), there was no association between timing of gluten introduction and celiac disease. For the gluten introduction and celiac disease analysis, heterogeneity was due to the study of Sellitto and colleagues[37] – in this study the control group had not yet ingested gluten at the time of outcome assessment, so that celiac disease or serology could not manifest.

Observational studies found no association between timing of gluten introduction and risk of celiac disease or inflammatory bowel disease; milk introduction and celiac disease or juvenile idiopathic arthritis; or timing of allergenic food introduction and risk of TIDM. There was no evidence of publication bias in analyses of milk introduction and TIDM (Egger's test P=0.26 and P=0.59), and assessment for publication bias was not possible for other comparisons, due to the limited number of studies in each meta-analysis.

GRADE evaluation of certainty of findings

Key findings were affected by the study of select populations with either active allergic disease, absence of allergic sensitization to the intervention food, or both. There was also significant variation between the populations studied in each trial, and interventions varied from early short term (3-4 days) introduction of an allergenic food, through early sustained introduction of single or multiple allergenic foods to trials of delayed allergenic food introduction, and multifaceted studies that also included other dietary components - often together with environmental control measures such as tobacco smoke and house dust mite avoidance. GRADE of evidence was therefore reduced in several analyses due to indirectness of the population or intervention (Table 4). GRADE of evidence for the egg and peanut findings was also reduced due to imprecise effect estimates, but was increased for peanut due to the strong effect size seen in the trial of Du Toit and colleagues.[4]

Trial Sequential Analysis of moderate or high certainty findings

Peanut introduction and peanut allergy were not evaluated using TSA, due to insufficient data in the meta-analysis to estimate a sufficient number of points for the monitoring boundaries. There were also insufficient data to perform TSA for 10% or 20% relative risk reduction for other findings. Whether early egg introduction was associated with a 30% reduction in risk of egg allergy using TSA was assessed. The heterogeneity-adjusted and non-adjusted optimal information sizes for detection of a 30% relative risk reduction for egg allergy were 8,643 and 5,239 study participants respectively. TSA for this outcome is shown in eFigures 7A and 7B. Although the conventional line of statistical significance was crossed (z=1.96) in both analyses, the optimal information size was not reached in either case. The cumulative z-score did not cross the monitoring boundary, although it is close in non-adjusted TSA. It cannot be confidently concluded that early egg introduction reduces egg allergy by at least 30%, and further trials are required to quantify the treatment effect.

TSA was also used to evaluate whether early gluten introduction increases celiac disease risk by 30%. The heterogeneity-adjusted and non-adjusted optimal information sizes for detection of a 30% increase in relative risk for celiac disease were 3,599 and 9,497 study participants respectively. TSA for this outcome is shown in eFigures 7C and 7D. The conventional line of statistical significance was not crossed and the optimal information size was not reached. The cumulative z-score was close to the line of futility in non-adjusted TSA. It cannot be confidently concluded that further studies of timing of gluten introduction and risk of celiac disease are futile.

Discussion

This systematic review found evidence that timing of introduction of certain allergenic foods to the infant diet was associated with the risk of allergic disease, but not autoimmune disease. There was moderate certainty evidence that introduction of egg to the infant diet at 4-6 months was associated with reduced egg allergy, and introduction of peanut at 4-11 months was associated with reduced peanut allergy, compared with later introduction of these foods. There was low certainty evidence that fish introduction before 6-12 months was associated with reduced allergic rhinitis, and very low certainty evidence that fish introduction before 6-9 months was associated with reduced allergic sensitization.

The evidence base for a relationship between early allergenic food introduction and food allergy to the same food was limited to a relatively small number of studies and events and was only statistically significant for egg and peanut. Heterogeneity-adjusted TSA of early egg introduction for egg allergy suggests that further trials are warranted to confirm the findings and quantify the magnitude of the treatment effect. Heterogeneity for egg introduction was attributable to one small study presented in abstract form only.[13] TSA without adjustment for heterogeneity showed stronger evidence that early egg introduction reduced risk of egg allergy by ≥30%, but without crossing the trial sequential monitoring boundary. TSA of early peanut introduction for peanut allergy was not possible, due to the small number of studies and events in this analysis. The inability to undertake TSA for this outcome emphasizes the value of further intervention studies of peanut introduction and peanut allergy. [39]

These findings are consistent with a large body of experimental data in various animal models, where early enteral antigen exposure is established as effective for preventing allergic sensitization to the same antigen.[40] This phenomenon of oral tolerance has not been directly shown to occur in humans until recently.[4,41] Oral tolerance in humans appears to be

antigen-specific, with no data showing early introduction of one allergenic food influences the development of allergy to a different allergenic food.

In contrast to egg and peanut allergy, this review found that oral tolerance was not relevant to celiac disease, suggesting the findings may not be generalizable beyond food allergy mediated by IgE antibodies. TSA of gluten introduction and celiac disease risk found that further trials would not be futile; however available data show no evidence of an association. Ongoing work is evaluating a potential role for oral tolerance in other autoimmune diseases – for example the induction of immune tolerance to insulin for preventing TIDM.[42] There was also no consistent evidence that early cow's milk introduction influences risk of TIDM, which is consistent with recent literature – for example a trial of extensively hydrolyzed versus intact infant formula showed no effect on TIDM risk.[43]

There was lower certainty evidence that early fish introduction was associated with reduced allergic sensitization or rhinitis. Sensitivity analysis of low risk of bias studies found the association with allergic rhinitis at age ≤4 years was not statistically significant. One plausible biological mechanism is that early exposure to the anti-inflammatory effects of omega-3 polyunsaturated fatty acids, present in fish, might influence the development or expression of allergic sensitization and associated inflammatory disease.[44]

These data conflict with previous recommendations to delay introduction of allergenic foods to the infant diet and suggest that current guidelines that do not advise early introduction of allergenic foods may need to be revised.[1-3] They are however consistent with one recent intervention trial and a consensus statement regarding introduction of peanut to the infant diet,[4,5] and any differences in conclusions from other trials can be explained by the increased statistical power derived from meta-analysis.

Despite the comprehensive approach used in this review, it was not possible to exclude clinically important effects in most analyses because there were few studies. Certainty of evidence was downgraded due to imprecision, and indirectness and variation in interventions used and populations studied. However, there was not a clear difference in outcome between studies of different populations in our analyses – for example in meta-analysis of egg introduction and egg allergy 3 studies undertaken in normal risk, high risk and very high risk populations had similar findings. Risk of bias assessment used different instruments for intervention and observational studies, which may not be directly comparable.

These systematic review findings should not automatically lead to new recommendations to feed egg and peanut to all infants. The imprecise effect estimates, issues around indirectness and inconclusive TSA findings all need to be considered, together with a careful assessment of the safety and acceptability of early egg and peanut introduction in different populations.

Conclusion

In this systematic review, early introduction of egg or peanut to the infant diet was associated with lower risk of developing egg or peanut allergy. These findings must be considered in the context of limitations in the primary studies.

Acknowledgments

Dr Boyle had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Independent peer review of study protocol and report: Professor Graham Devereux PhD, University of Aberdeen (compensation received) and Carina Venter PhD, Cincinnati Children's Hospital (compensation received).Members of the UK Food Standards Agency, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), and the UK Scientific Advisory Committee on Nutrition (SACN) (no compensation received). Advice on statistical analysis: Professor Peter Burney PhD, Imperial College London (no compensation received), Professor Doug Altman PhD, Oxford University (no compensation received).

Translation of foreign language reports: Yujie Zhao PhD, Szymon Mikolajewski BSc, Andre Amaral PhD, Mari Kihara PhD, Christian Nielsen PhD, Radoslav Latinovic BSc, Stephanie MacNeill PhD, Andreas Forsters PhD, Daniel Munblit MD, Sze-Chin Tan MD, and Claudia Gore MD, all from Imperial College London (no compensation received).

Literature search training: Jackie Cousins BSc, Imperial College London (no compensation received).

Collation of data for risk of bias analysis and characteristics of included studies tables in the full Food Standards Agency report: Evangelia Andreou PhD, Cambridge University (compensation received).

Production of graphics for statistical Figures: Jamie Reeves BSc, Imperial College London (no compensation received).

375 Comments on a previous version of the manuscript: Michael Perkin PhD, St George's
376 University London (no compensation received).
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Author contributions: RJB, VG-L and JL-B designed the study and wrote the protocol. TR wrote the search strategy and undertook the literature search. AL, AG, SC, JC, ZR, NG, KJ, NT-M and UN undertook title screening and data extraction, supervised by RJB, VG-L and DI. DI, VG-L and RJB undertook all data cleaning, and DI undertook all data analysis. MT and JL-B advised on methodology, statistical analyses and interpretation of findings including GRADE evaluation of the certainty of evidence. RJB wrote the study report. DI, VG-L and RJB wrote the manuscript. All authors contributed to the final manuscript. RJB acts as guarantor of the manuscript.

Funding/Support: This work was funded by the Food Standards Agency, and supported by a National Institute for Health Research Biomedical Research Centre and the MRC-Asthma UK Centre in Allergic Mechanisms of Asthma. Marialena Trivella was supported by Cancer Research UK.

Role of the Funder/Sponsor: The Food Standards Agency commissioned this work, commissioned external peer review of the study protocol, statistical methods and study report, and thereby contributed to design and conduct of the study, and interpretation of data. Food Standards Agency contributed to review and approval of the manuscript. Food Standards Agency had no role in the collection, management or analysis of data, preparation of the manuscript or decision to submit the manuscript for publication. The National Institute for Health Research and Cancer Research UK had no role in the design and conduct of the study; collection, management, analysis and interpretation of data; preparation, review or approval of the manuscript; and decision to submit the manuscript for publication.

Competing interest statement of authors: All authors have completed the ICMJE uniform disclosure form at http://www.icmje.org/coi_disclosure.pdf and declare: RJB, VG-L, JL-B, DI, SC, UN and NT-M received support from the UK Food Standards Agency for the

submitted work. No other support was received from any organization for the submitted work. The authors have no financial relationships with any organizations that might have an interest in the submitted work in the previous three years. The authors report no other relationships or activities that could appear to have influenced the submitted work.

Transparency declaration: Dr Boyle affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned and registered have been explained.

Information on previous presentation: A small part of the information reported in this manuscript have been presented by NG at the MEDELA 10th International Breastfeeding and Lactation Symposium, Warsaw, Poland, April 2015 with Poster title “Breastfeeding duration, timing of cow’s milk introduction and risk of Type 1 Diabetes Mellitus: a systematic review and meta-analysis”.

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Figure legends

Figure 1. Early allergenic food introduction and risk of food allergy or food sensitization.

Effect of early versus late dietary introduction of allergenic food (egg, milk or peanut) on risk of food allergy (A) or allergic sensitization (B) to the same food. Data are from randomized clinical trials. 'Event' refers to food allergy (A) or allergic sensitization (B) to the same food. The size of the data markers relates to study weights in the meta-analysis.

Figure 2. Early fish introduction and risk of allergic sensitization or rhinitis.

Association between early dietary introduction of fish and different forms of allergic sensitization (A) or allergic rhinitis (B). Data are from observational studies. The size of the data markers relates to study weights in the allergic rhinitis meta-analysis. Age represents age at outcome assessment.

Figure 3. Early gluten introduction and risk of celiac disease.

Effect of early versus late dietary introduction of gluten on risk of celiac disease. Data are from randomized clinical trials. 'Event' refers to celiac disease. The size of the data markers relates to study weights in the meta-analysis.

Table 1 Characteristics of randomized clinical trials of early versus late egg or peanut introduction and risk of egg or peanut allergy

Study	Country	Population	Intervention	No participants in early introduction group	No participants in late introduction group	Outcomes reported	Age at outcome assessment
[a]Bellach, 2016 [16] HEAP Study	Germany	'Normal risk' infants aged 4-6 months with specific IgE to egg <0.35 kU/L	Pasteurised egg white powder (2.5g protein) versus rice powder 3 times per week from 4-6 months to 12 months	184	199	Egg allergy diagnosed by oral food challenge, plus specific IgE to egg ≥0.35 kU/L	1 year
Du Toit, 2015 and 2016 [4,41] LEAP Study	UK	'High risk'4-11 month old infants with moderate or severe eczema or egg allergy, and peanut SPT <4mm	Six grams peanut protein per week as Bamba or peanut butter divided between ≥3 meals versus peanut avoidance, from randomization to 5 years	319	321	Peanut allergy diagnosed by oral food challenge	5 and 6 years
[b]Halpern, 1973 [45]	USA	'Normal risk' Caucasian infants seen at birth by one of 11 private paediatricians	Egg yolk given before 3 weeks, versus after 6 months. No further details on dosing, form or frequency available	~875	~875	Allergy to egg yolk defined as reproducible characteristic symptoms on ≥3 separate challenge feeds	7 months
[a]Natsume, 2016 [17]	Japan	'High risk' infants with eczema by 4-5 months	Heated egg powder 50mg daily from 6-9 months, 250mg daily from 9-12 months versus placebo from 6 to 12 months	60	61	Egg allergy diagnosed by oral food challenge	1 year
Palmer, 2013 [15] STAR Study	Australia	'High risk' singleton term infants with moderate or severe eczema (SCORAD ≥15) and no prior egg or solid food intake	One teaspoon of pasteurized whole egg powder daily (0.9g protein), versus rice flour powder from 4 months to 8 months age	49	37	Egg allergy diagnosed by oral food challenge to pasteurized egg, plus positive SPT	1 year

Study	Country	Population	Intervention	No participants in early introduction group	No participants in late introduction group	Outcomes reported	Age at outcome assessment
[c]Palmer, 2016 [46] STEP Study	Australia	'High risk' infants with an atopic mother, no prior egg ingestion and no prior allergic disease	Pasteurized whole egg powder daily (0.9g protein) versus rice powder daily from 4-6 months to 10 months	407	413	Egg allergy diagnosed by oral food challenge to pasteurized egg, plus a positive SPT	1 year
Perkin, 2016 [6] EAT Study	UK	'Normal risk' singleton term infants exclusively breastfed for ≥3 months	Sequential introduction of six allergenic foods aiming for 4g protein per week for each food - cow's milk (yogurt), then peanut, boiled egg, sesame, fish and wheat from age 3 months, versus avoidance to ≥6 months	652	651	Egg allergy and peanut allergy diagnosed by oral food challenge	1 and 3 years
Tan, 2016 [18] BEAT Study	Australia	'High risk' infants with a first degree relative with allergic disease, and egg SPT <2mm at age 4 months	Pasteurised whole egg powder daily (350mg egg protein) versus rice powder daily from the time of solid food introduction until 8 months	165	154	Egg allergy diagnosed by oral food challenge to lightly cooked whole egg	1 year

559 BEAT Beating Egg Allergy Trial; EAT Enquiring About Tolerance; HEAP Hen's Egg Allergy Prevention; LEAP Learning Early
560 about Peanut Allergy; SCORAD Scoring Atopic Dermatitis, score ranges from 0 to 103, with higher scores indicating more severe
561 eczema. SCORAD can be classified as mild (<15) moderate (15 to 40) and severe (>40) eczema; SPT Skin Prick Test; STAR Solids
562 Timing for Allergy Reduction; STEP Starting Time of Egg Protein. [a]Abstract publication, authors unable to share further details. [b]Data
563 not reported in a form that could be included meta-analysis. [c]Study completed but not published at the time of the systematic review
564 search, so not included in primary analyses, but included in the *post hoc* Trial Sequential Analysis.

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Table 2 Risk of bias and directness of evidence from randomized clinical trials of early versus late egg or peanut introduction and risk of egg or peanut allergy

Study	Breastfeeding status at randomization	Age at randomization	Eczema status at enrolment	Risk of Bias	Conflict of interest	Indirectness of evidence
[a]Bellach, 2016 [16] HEAP Study	250 (65%) breastfed	Mean 4.7 months	33 (8.5%)	Unclear	Unclear	Population with no egg sensitization
Du Toit, 2015 and 2016 [4,41] LEAP Study	268 (42%) breastfed	Mean 7.8 months	571 (89%) severe eczema, mean SCORAD 34	Unclear, related to blinding of outcome assessment	Low	Population with severe eczema but no high level peanut sensitization, and high level of treatment adherence
[b]Halpern, 1973 [45]	Enrolled at birth. 459 (26%) infants were breastfed	Birth	Nil, enrolled at birth	Unclear risk of selection, assessment and attrition bias	Low	Specific form of egg and timing of introduction
[a]Natsume, 2016 [17]	Unclear	Unclear	121 (100%)	Unclear	Unclear	Population with eczema
Palmer, 2013 [15] STAR Study	71 breastfed (83%)	4 months	86 (100%) eczema, median SCORAD 33	Low	Unclear, due to support of authors by formula milk and egg industries	Population with moderate or severe eczema
[c]Palmer, 2016 [46] STEP Study	541 (66%) breastfed	Median 5.8 months in early, and 5.9 months in late group	Nil (0%)	Low	Low	Population with no eczema

Study	Breastfeeding status at randomization	Age at randomization	Eczema status at enrolment	Risk of Bias	Conflict of interest	Indirectness of evidence
Perkin, 2016 [6] EAT Study	1,303 (100%) exclusively breastfed	Median 3.4 months	317 (24%) Median SCORAD 7.5	Unclear, related to blinding of outcome assessment	Low	Specific multiple allergenic food introduction schedule which was difficult to adhere to. Intervention group underwent baseline SPT to exclude egg or peanut sensitization
Tan, 2016 [18] BEAT Study	142 (45%) exclusively breastfed	Median 3.8 months	82 (26%)	Low	Low	Population with no egg sensitization

BEAT Beating Egg Allergy Trial; EAT Enquiring About Tolerance; HEAP Hen's Egg Allergy Prevention; LEAP Learning Early about Peanut Allergy; SCORAD Scoring Atopic Dermatitis, score ranges from 0 to 103, with higher scores indicating more severe eczema. SCORAD can be classified as mild (<15) moderate (15 to 40) and severe (>40) eczema; SPT Skin Prick Test; STAR Solids Timing for Allergy Reduction; STEP Starting Time of Egg Protein. [a]Abstract publication, authors unable to share further details. [b]Data not reported in a form that could be included meta-analysis. [c]Study completed but not published at the time of the systematic review search, so not included in primary analyses, but included in the *post hoc* Trial Sequential Analysis.

575 **Table 3 Summary of key review findings for early versus late introduction of allergenic food to the infant diet**

Dietary Exposure	Outcome	Study Design	Effect estimate (95% CI)	Evidence GRADE	Control Risk Cases per 1000 population	Risk Difference Cases per 1000 population (95% CI)	Number needed to treat (95% CI)
Egg Introduction	Egg Allergy	6 RCT N=3,665	RR = 0.56 (0.36 to 0.87)	Moderate	54 (normal risk) 100 (high risk) 500 (very high risk)	24 cases less (7 less to 35 less) 44 cases less (13 to 64) 220 cases less (65 less to 320 less)	42 (29 to 143) 23 (16 to 77) 5 (3 to 15)
Peanut Introduction	Peanut Allergy	2 RCT N=1,550	RR = 0.29 (0.11 to 0.74)	[a]Moderate	25 (normal risk) 170 (high risk)	18 cases less (6 less to 22 less) 121 cases less (44 less to 151 less)	56 (45 to 167) 8 (7 to 23)
Fish Introduction	Allergic Rhinitis	4 PC N=12,781	*Rhinitis at age ≤ 4* OR = 0.59 (0.40 to 0.87) *Rhinitis at age 5-14* HR = 0.68 (0.47 to 0.98)	Low	*Rhinitis at age ≤ 4* 50 (normal risk) 100 (high risk) *Rhinitis at age 5-14* 100 (normal risk) 200 (high risk)	18 cases less (6 less to 30 less) 38 cases less (12 less to 57 less) 32 cases less (2 less to 53 less) 64 cases less (4 less to 106 less)	56 (33 to 167) 26 (18 to 83) 31 (19 to 500) 16 (9 to 250)
Fish Introduction	Allergic Sensitization	5 PC N=14,193	*Any allergen* OR= 0.75 (0.64 to 0.88) *Any food* OR= 0.52 (0.37 to 0.73)	Very Low	*Sensitization to any allergen* 200 (normal risk) 400 (high risk) *Sensitization to any food* 100 (normal risk) 200 (high risk)	42 cases less (20 less to 62 less) 67 cases less (30 less to 101 less) 45 cases less (25 less to 61 less) 85 cases less (46 less to 115 less)	24 (16 to 50) 15 (10 to 33) 22 (16 to 40) 12 (9 to 22)
Gluten Introduction	Celiac Disease	4 RCT N=1,822	RR = 1.22 (0.81 to 1.83)	High	10 (normal risk) 100 (high risk)	2.2 cases more (1.9 less to 8.3 more) 22 cases more (19 less to 83 more)	- -

Dietary Exposure	Outcome	Study Design	Effect estimate (95% CI)	Evidence GRADE	Control Risk Cases per 1000 population	Risk Difference Cases per 1000 population (95% CI)	Number needed to treat (95% CI)
Cow's Milk Introduction	TIDM	7 PC 1 NCC 25 CC N=42,858	*CM at ≤ 0-2 months* OR = 1.20 (0.53 to 2.71) *CM at ≤ 3-4 months* OR = 0.92 (0.75 to 1.13) *CM at ≤ 5-7 months* OR = 1.88 (1.05 to 3.39)	Very Low	*CM at ≤ 0-2 months* 1 (normal risk) 10 (high risk) *CM at ≤ 3-4 months* 1 (normal risk) 10 (high risk) *CM at ≤ 5-7 months* 1 (normal risk) 10 (high risk)	0.2 cases more (0.5 less to 1.7 more) 2 cases more (4.7 less to 16.6 more) 0.1 cases less (0.2 less to 0.1 more) 0.8 cases less (2.5 less to 1.3 more) 0.9 cases more (0.0 to 2.4 more) 8.6 cases more (0.5 more to 23.1 more)	- - - - - -
Cow's Milk Introduction	Eczema	12 RCT 1 qRCT 3 CCT N = 6,752	*Eczema at age ≤ 4* RR = 1.14 (0.87 to 1.49) *Eczema at age 5to14* RR = 1.05 (0.90 to 1.23)	Low	*Eczema at age ≤ 4* 200 (normal risk) 300 (high risk) *Eczema at age 5to14* 50 (normal risk) 100 (high risk)	28 cases more (26 less to 98 more) 42 cases more (39 less to 147 more) 3 cases more (5 less to 12 more) 5 cases more (10 less to 23 more)	- - - -
Cow's Milk Introduction	Wheeze	11 RCT 1 qRCT 3 CCT N = 7,793	*Wheeze at age ≤ 4* RR = 1.12 (0.77 to 1.62) *Recurrent wheeze at age ≤ 4* RR = 1.18 (0.77 to 1.81)	Low	*Wheeze at age ≤ 4* 200 (normal risk) 300 (high risk) *Recurrent wheeze at age ≤ 4* 100 (normal risk) 200 (high risk)	24 cases more (46 less to 124 more) 36 cases more (69 less to 186 more) 18 cases more (23 less to 81 more) 36 cases more (46 less to 162 more)	- - - -

576 95% CI, 95% Confidence Interval; OR, Odds Ratio; RR, Risk Ratio; HR, Hazard Ratio; CM, Cow's Milk. Data are shown for all

577 positive findings, and for other findings where meta-analysis of a significant number of studies and participants was possible. Number

needed to treat is only given for outcomes where we found evidence of association. Risk Difference is the absolute risk reduction for different control risks. [a]GRADE of evidence increased due to strong effect size. Control risks are estimated from included studies, or where relevant from other large population-based studies, for populations at different risk of the outcome. Specifically the risks for egg allergy refer to an unselected population of infants (normal risk), infants at high hereditary risk of allergic disease (high risk) and infants with moderate-severe eczema (very high risk); risks for peanut allergy refer to an unselected population of infants (normal risk) and infants with moderate-severe eczema (high risk); risks for allergic rhinitis, allergic sensitization, eczema and wheeze refer to an unselected population of infants (normal risk) and infants at high risk of allergic disease due to having an affected first-degree relative (high risk); risks for celiac disease and TIDM refer to an unselected population of infants (normal risk) and infants at high risk of disease due to either having an affected first-degree relative, or due to having a high risk genotype (high risk).

588 **Table 4 GRADE of evidence assessment for key review findings for early versus late introduction of allergenic food to the**
589 **infant diet**

Dietary Exposure	Outcome	[a]Study Design	Risk of bias	Inconsistency	Indirectness	Imprecision
Egg Introduction	Egg Allergy	6 RCT N=3,665	Not Serious 1 study at high risk of bias, no studies at high risk of conflict of interest	Not serious I^2=36% (P=0.18). Study estimates vary from 0.22 to 0.69 for the studies at low risk of bias	Serious 3 studies only recruited infants without egg sensitization; 1 study only infants with eczema; 1 study used multiple allergenic foods	Not serious 95% CI for RR is wide. Trial sequential analysis suggests that optimum information size has not yet been reached
Peanut Introduction	Peanut Allergy	2 RCT N=1,550	Not Serious Neither study had a high risk of bias or conflict of interest	Not Serious I^2=66% (P=0.09), study estimates vary from 0.49 to 0.19 but heterogeneity is likely to be explained by differences in participant adherence to the intervention	Serious 1 study only recruited infants with egg allergy or eczema, and without high-level peanut sensitization; 1 study used multiple allergenic foods	Serious 95% CI for RR is wide
Fish Introduction	Allergic Rhinitis	4 PC N=12,781	Not serious 1 study at high risk of bias; all studies with low risk of conflict of interest	Not Serious I^2=59% (P=0.086), study estimates varying from 0.45 to 0.77 but all 4 studies statistically significant, and heterogeneity was reduced when early onset eczema cases were excluded from analysis due to potential reverse	Not serious Studies all undertaken in Scandinavia. 3 studies were in representative birth cohorts, 1 in a birth cohort selected for high risk of TIDM	Not serious 95% CI are wide, but not close to 1, and together the 4 studies include over 12,000 participants

Dietary Exposure	Outcome	[a]Study Design	Risk of bias	Inconsistency	Indirectness	Imprecision
				causation		
Fish Introduction	Allergic Sensitization	5 PC N=14,193	Not serious 2 studies ~700 participants high risk of bias; 3 studies (~ 13,000) low risk of bias; no conflict of interest	Not serious Extreme heterogeneity for meta-analysis of inhalant sensitization; consistent findings for other sensitizations	Serious Allergic sensitization is an indirect measure of disease	Not serious 3 studies at low risk of bias were consistent – OR or HR from 0.41 to 0.78, and included over 13,000 participants
Gluten Introduction	Celiac Disease	4 RCT N=1,822	Not serious 1 study with high risk of bias; all studies with low or unclear risk of conflict of interest	Not Serious I^2=46% (P=0.13), due to 1 small study with high risk of bias; other estimates from 0.96 to 1.66	Not Serious Two studies used only serology, but this surrogate is highly correlated with clinical disease. All participants had high risk genotype or family history	Not Serious significant benefit unlikely – lower bound RR 0.81, or 0.85 with high risk of bias study excluded
Cow's Milk Introduction	TIDM	7 PC 1 NCC 25 CC N=42,858	Not Serious 12 studies with high overall risk of bias; all studies with low risk of conflict of interest	Serious High or extreme statistical heterogeneity in several analyses. In some meta-analyses significant associations were seen for retrospective, but not for prospective studies	Not Serious All but one prospective reported islet autoimmunity as a surrogate for TIDM. Retrospective studies used clinical diagnosis	Not Serious Studies included over 40,000 participants. 95% CI for meta-analyses of prospective studies were wide

Dietary Exposure	Outcome	[a]Study Design	Risk of bias	Inconsistency	Indirectness	Imprecision
Cow's Milk Introduction	Eczema	12 RCT 1 qRCT 3 CCT N=6,752	Serious 8 studies with high risk of bias; 2 studies had high risk of conflict of interest	Not Serious I^2= 0 to 54% for different analyses, with estimates ranging from 0.32 to 3.63	Serious Four studies had a multifaceted intervention; 5 others compared cow's milk introduction with soya, another allergenic food	Not Serious 95% CI were wide for some comparisons; but overall over 6000 participants were included
Cow's Milk Introduction	Wheeze	11 RCT 1 qRCT 3 CCT N=7,793	Serious 7 studies with high overall risk of bias; 2 studies with high risk of conflict of interest	Serious I^2 = 0 to 59% for different analyses, with estimates ranging from 0.13 to 2.98. Two meta-analyses showed significant effects, not supported by other analyses	Not Serious Four studies had a multifaceted intervention; 6 others compared cow's milk introduction with soya; however findings were consistent with a study of cow's milk introduction without soya	Not Serious 95% CI were wide for some comparisons; but overall over 6000 participants were included

590 RCT, Randomized Clinical Trial; qRCT, quasi-Randomized Clinical Trial; CCT, Controlled Clinical Trial; PC Prospective Cohort
591 study; NCC, Nested Case-Control Study; CC, Case-Control study; TIDM, Type 1 Diabetes Mellitus. GRADE assessments are shown
592 for all positive findings, and for other findings where meta-analysis of a significant number of studies and participants was possible.
593 Evaluation of publication bias was only possible for one outcome – timing of cow's milk introduction and TIDM. Here Funnel plots
594 were not asymmetrical, and Egger's test was not significant - P=0.26 for milk introduction ≤0-2 months; P=0.59 for milk introduction
595 ≤3-4 months – suggesting no evidence of publication bias. For other analyses, there were insufficient studies to undertake formal

testing of publication bias. [a]Note that the number of studies included is not always the same as the number of studies included in meta-analysis, since some studies did not report data in a form that could be included in meta-analysis. All data were considered when making GRADE assessments.