

Food Proteins in Human Breast Milk and Probability of IgE-Mediated Allergic Reaction in Children During Breastfeeding: A Systematic Review



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What is already known about this topic? Data from some case series and observational studies have suggested links between maternal diet during breastfeeding and potential symptom development in infants. However, data from double-blinded, placebo-controlled randomized trials are lacking.

What does this article add to our knowledge? In this systematic review, we found that levels of food proteins in human milk were much lower than the eliciting dose for 1% of allergic individuals across the samples and studies.

How does this study impact current management guidelines? The probability of an IgE-mediated allergic reaction in a food-allergic infant breastfed by a woman consuming the relevant food can be estimated as $\leq 1:1000$, which may impact approaches to management of children during breastfeeding.

BACKGROUND: Previous reports suggested that food proteins present in human milk (HM) may trigger symptoms in allergic children during breastfeeding, but existing evidence has never been reviewed systematically.

OBJECTIVE: To assess the probability of food proteins in HM to trigger allergic reactions in infants with IgE-mediated food allergy.

METHODS: Electronic bibliographic databases (MEDLINE, EMBASE) were systematically searched from inception to November 3, 2021. The data regarding the levels of food proteins detected in HM were extracted and compared with data from the Voluntary Incidental Trace Allergen Labelling (VITAL 3.0) guide to assess the probability of food-allergic individuals to experience immediate type allergic reactions on ingesting HM.

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Abbreviations used

BLG- β -Lactoglobulin

ED- Eliciting dose

ELISA- Enzyme-linked immunosorbent assay

HM- Human milk

LLOD- Lower limit of detection

OM- Ovomucoid

OVA- Ovalbumin

PRISMA- Preferred Reporting Items for Systematic Reviews and Meta-Analyses

VITAL- Voluntary Incidental Trace Allergen Labelling

RESULTS: A total of 32 studies were identified. Fourteen studies assessed excretion of cow's milk proteins into HM, 9 egg, 4 peanut, and 2 wheat; 3 measured levels of cow's milk and egg proteins simultaneously. We found that levels of all food proteins across the studies were much lower than the eliciting dose for 1% of allergic individuals (ED01) in most of the samples. The probability of an IgE-mediated allergic reaction in a food-allergic infant breastfed by a woman consuming the relevant food can be estimated as $\leq 1:1000$ for cow's milk, egg, peanut, and wheat.

CONCLUSIONS: To our knowledge, this is the first systematic review that assesses and summarizes evidence on food proteins in HM and potential for IgE-mediated allergic reactions. Our data suggest that the probability of IgE-mediated allergic reactions to food proteins in HM is low. © 2022 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2022;10:1312-24)

Key words: Human milk; Breast milk; Food proteins; Allergens; Cow's milk; Egg; Peanut; Gliadin; Food allergy; Allergy; Breastfeeding; Infant; Child

The first reports of food protein excretion into human milk (HM) appeared almost a century ago.¹ Since then, a range of nonhuman proteins have been measured in HM, with cow's milk proteins being most frequently assessed, followed by egg, peanut,

and wheat. These food proteins were studied because of their potential roles as common food allergens.²

The presence of food proteins in HM and consequences to child health have generated great interest in the allergy field, and the presence of food proteins in HM has been linked with the exacerbation of symptoms in allergic children,³ although systematic reviews of randomized controlled studies on colic⁴ and eczema⁵ did not show a benefit of maternal dietary exclusions. Parents often link unspecific symptoms (eg, crying, vomiting, or rashes) in infants during breastfeeding with IgE-mediated food allergy, but the link is very rarely confirmed by an oral food challenge.⁶ In the presence of insufficient data, development of evidence-based clinical guidelines on the treatment of children with food allergy during breastfeeding is not possible. The consequence could be inappropriate restricted maternal diets or breastfeeding cessation in routine clinical practice.⁷

High-quality investigation of existing data into the links between maternal diet, the presence of food proteins in HM, and child food allergy is needed. The aim of this systematic review is to summarize the available evidence regarding the presence of food proteins in HM and assess the likelihood that food protein passage in human breast milk provokes IgE-mediated food-allergic reactions.

METHODS

This systematic review is reported in accordance with the recommendations set forth by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.⁸ The review was registered with the National Institute for Health Research's PROSPERO (PROSPERO 2019 CRD42019126901. Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019126901) on April 12, 2019.

Search strategy

An extensive electronic search of the MEDLINE and EMBASE databases via OVID was performed on January 23, 2019, using both free text and MESH terms. Additional searches were performed on July 17, 2020, and November 3, 2021, to screen for recent papers

Institute of Allergy and Infectious Diseases—sponsored Guidelines for Peanut Allergy Prevention; has served as a consultant for the Canadian Transportation Agency, Thermo Fisher, Intrimmune, and Aimmune Therapeutics; is a member of physician/medical advisory board attendance at boards for Aimmune Therapeutics, DBV Technologies and Sanofi/Genzyme, Genentech, GlaxoSmithKline, Novartis, Nutricia, Kaléo Pharmaceutical, Nestlé, Aquestive Therapeutics, Allergy Therapeutics, AllerGenis, Aravax, Prota Therapeutics and has been paid for legal testimony in cases related to Therapeutics, Pfizer, US World Meds, and Monsanto; is a member of the scientific advisory council for the National Peanut Board; has received honorarium for lectures from Thermo Fisher, Aimmune Therapeutics, DBV Technologies, BEFORE Brands, multiple state allergy and food anaphylaxis societies, the American College of Allergy, Asthma and Immunology, the European Academy of Allergy, and Clinical Immunology; is an associate editor for the *Annals of Allergy, Asthma & Immunology*; and is a member of the Joint Taskforce on Allergy Practice Parameters. J. Genuneit has been the project manager of research grants from Danone to both Ulm University and Leipzig University in relation to studies of the composition of breast milk. H. A. Brough declares speaker honoraria from DBV Technologies and Sanofi. M. B. Azad holds a Tier 2 Canada Research Chair in the Developmental Origins of Chronic Disease and is a fellow in the Canadian Institutes for Advanced Research (CIFAR) Humans and the Microbiome Program. She regularly speaks at conferences and workshops on infant nutrition, some sponsored by Medela, the Institute for the Advancement of

Breastfeeding & Lactation Education, and Prolacta Biosciences. She has contributed to online courses on breast milk and the infant microbiome produced by Microbiome Courses. She serves in a volunteer capacity as Secretary to the International Society for Research on Human Milk and Lactation and as a member of the National Academy of Sciences, Engineering and Medicine Committee on Scanning New Evidence on the Nutrient Content of Human Milk. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. K. M. Järvinen reports research funding for related work from the NIH and unrelated funding from Aimmune Therapeutics, Food Allergy Research and Education, and Janssen Research and Development; consulting fees from Merck and DBV Technologies; and royalties from UpToDate. The rest of the authors declare that they have no relevant conflicts of interest.

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that may have been published during the work on this systematic review. The search strategies are provided as [Appendix E1](#), available in this article's Online Repository at www.jaci-inpractice.org. At the screening stage, further studies were traced through reference lists of included studies to ensure that no original published data had been missed. Authors of identified papers and experts in the field were contacted for missing data.

The relationships between maternal diet, food protein concentrations in HM, and probability of an allergic reaction were studied. The foods included the 9 common food allergens, including cow's milk, hen's egg, peanut, sesame, wheat, fish, shellfish, soya, tree nuts (hazelnut, almond, brazil nut, cashew, pecan, pistachio, pine nut, and walnut), and their proteins.

Eligibility criteria and selection of articles

Types of studies. Observational studies (prospective/retrospective cohort, case-control, and cross-sectional), randomized controlled trials as well as cluster randomized trials, and quasi-experimental trials were included. Therefore, we included original studies if they (1) reported any presence of food proteins in HM; (2) assessed the association between food consumption and levels of food proteins in HM; and (3) were published in English. Studies that did not perform a quantitative assessment of food protein level in HM were excluded.

Types of participants. Lactating women, regardless of their age, country of origin, health status, and their offspring's risk for food allergy.

Types of interventions or exposures. Studies of all designs were included if they reported original data and included a quantitative assessment of food proteins in HM and details of maternal diet and/or exposure to food. Thus, we included (a) observational studies reporting data on the levels of food protein in HM regardless of known amounts of food ingestion by the lactating mother and (b) interventional studies where authors provided details on the dose and type of food proteins consumed and/or maternal diet at the time of HM sample collection.

Definitions of interventional and observational studies for the purpose of this article is presented in the Supplementary methods available in this article's Online Repository at www.jaci-inpractice.org.

Comparator. The data on eliciting doses (ED) of food proteins from Voluntary Incidental Trace Allergen Labelling (VITAL 3.0) guide.⁹ Eliciting doses for food proteins found in HM based on VITAL 3.0 data are outlined in [Table E1](#), available in this article's Online Repository at www.jaci-inpractice.org.

Outcomes of interest. Levels of food proteins in HM and probability of allergic reaction.

Details of the data extraction and approach to the probability of allergic reaction estimation are presented in the Supplementary methods available in this article's Online Repository at www.jaci-inpractice.org.

RESULTS

Synthesis

Based on the search up to November 3, 2021, a total of 8341 titles were identified and 5110 relevant titles/abstracts were screened for eligibility after duplicate removal ([Figure 1](#)). Of these, 50 were selected for full-text assessment, 16 studies did not meet inclusion criteria, and 2 papers analyzing overlapping

datasets^{10,11} were further excluded. Thirty-two manuscripts were included in our systematic review.

Description of the studies

Thirty-two studies included in this systematic review were published between 1983 and 2020 and can be grouped into 2 main categories in accordance with the study design: (a) interventional¹²⁻³³ and (b) observational.^{16,17,19,34-43} All included interventional studies administered either single^{12-15,18-21,23-43} or multiple^{16,17,22} foods for various durations during lactation.

Fourteen studies investigated cow's milk proteins (β -lactoglobulin [BLG] and casein),^{13,18-20,23,24,26,31,34,36-38,41,42} 9 measured egg proteins (ovalbumin [OVA]^{14,21,25,27,28,39,40,43} and ovomucoid [OM]³⁵), 4 assessed peanut proteins (Ara h 2 and Ara h 6),^{12,29,30,33} and 2 looked at wheat protein (gliadin).^{15,32} Three studies measured levels of cow's milk (BLG) and egg (OVA) proteins simultaneously.^{16,17,22}

Participant characteristics. Study populations included participants from 14 countries, with most research conducted in the United Kingdom (5)^{14,22,37,42,43} and Australia (5),^{25,27,28,39,40} followed by Finland (4),^{20,23,31,41} Japan (4),^{16,17,21,35} Denmark (2),^{18,19} Italy (2),^{13,32} Germany (2),^{29,30} Sweden (2),^{34,36} France (1),¹² USA (1),³⁸ Argentina (1),¹⁵ Canada (1),³³ Switzerland (1),²⁶ and Thailand (1).²⁴

Sample size ranged from 2¹² to 251 participants³⁹ while the maximum number of HM samples reached 820.²⁷

Measurement of food proteins in human milk. The time of sample collection (time of the day and pre- or post-breastfeed) differed between the studies and details were rarely reported. Colostrum was collected in 3 studies,^{15,38,41} but information on the days postpartum for colostrum collection was presented in only 1 study.¹⁵ Mature milk was sought in all the studies reviewed, with time of collection varying between 1 week³⁴ and 12 months²² since birth. Thirteen studies did not provide any information on the timing of HM collection.^{14,19,21,23-26,28,30-33,35,36}

Two methods were used for food protein assessment in HM, with enzyme-linked immunosorbent assay (ELISA)^{12,13,15-19,23-25,27-33,35,37-43} being the most prevalent, followed by radioimmunoassay.^{14,21,22,26,34,36} Lower limit of detection (LLOD) varied between 0.002^{20,23,24,31} and 5 ng/mL^{34,36} for BLG; 0.05^{39,40} and 1.0 ng/mL²¹ for OVA; 0.1²² and 0.3 ng/mL³⁵ for OM; 0.015³³ and 0.03 ng/mL³³ for Ara h 1; 0.03³⁵ and 40 ng/mL²⁹ for Ara h 2; 0.006¹² and 0.7 ng/mL³⁰ for Ara h 6; and 1¹⁵ and approximately 5 ng/mL³² for gliadin. No data on LLOD were available for α -S1-casein and BLG immuno-like protein. LLOD was lower than ED01 in all the studies.

Cow's milk proteins

Cow's milk proteins were the most frequently assessed food protein in HM ([Table 1](#)) with 10 interventional^{16-20,22-24,26,31} and 7 observational studies looking at BLG.^{19,34,36-38,41,42} BLG immuno-like protein¹³ and α -S1-casein⁴² levels were investigated in 1 study each. Levels of BLG differed largely within and between the studies with lowest levels measured 0.002 ng/mL and highest reaching 415 ng/mL in interventional²⁶ and 800 ng/mL in observational³⁴ studies.

BLG was detected in 41.5% of samples across all observational studies. In the only study investigating α -S1-casein in HM, the

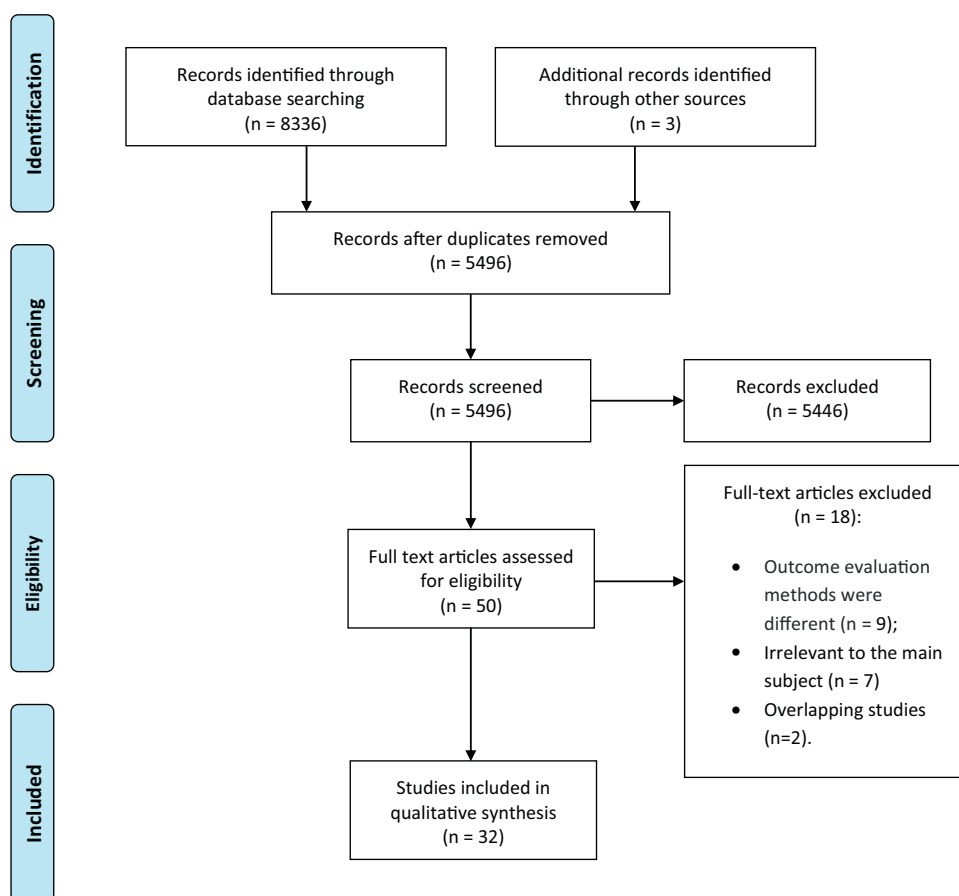


FIGURE 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram of the systematic search and included studies.

levels above the LLOD were found in the 46.4% of samples. One of 7 studies detected levels of BLG above ED01,³⁴ whereas no studies found levels above ED05. The overall probability for BLG is 1:5712 to elicit a reaction in what are considered the most highly sensitive group of individuals allergic to this protein.

In the interventional studies, pasteurised cow's milk as an unmodified drink was the most commonly used product (Table E2, available in this article's Online Repository at www.jaci-inpractice.org). The volumes ranged from 200^{13,16,17} to 500 mL^{18,19} as a single ingestion. One study reported varying volumes (from 100 to 2300 mL) of cow's milk provided to the mothers in a single study.²⁰ The proportion of samples with detectable BLG ranged between 16.7%¹⁹ and 54.2%¹⁶ of samples. On average, BLG was found in 52% of the samples across the studies. Median levels varied between 1.23²⁴ and 4.2 ng/mL.¹⁸ In 4^{19,23,26,31} of 6 studies, the impact of the intervention on the levels of BLG was unclear as no formal statistical comparison was provided (Table E3, available in this article's Online Repository at www.jaci-inpractice.org). The difference between the medians in the only study reporting a significant change after intervention was 0.65 ng/mL.²⁴

Two^{18,26} of 10 interventional studies reported levels of BLG over ED01, whereas no samples contained BLG in concentrations equal to ED05 or above. Overall, up to 5 of 430 samples contained the levels of BLG between ED01 and ED05. Thus, the probability of having sufficient quantities within a single

breastfeed of BLG to elicit allergic reactions in an individual allergic to this protein was estimated at 1:2893.

Egg proteins

Egg proteins were frequently assessed in HM, with OVA investigated in 6 interventional^{14,21,22,25,27,28} and 5 observational^{16,17,39,40,43} studies, and OM measured in 1 interventional²² and 1 observational³⁵ study. No observational studies recorded maternal dietary intake details. OVA was detected in 37.3% of samples across the observational studies, whereas levels of OM above the LLOD were found in 32.4% of samples (Table II).

In the interventional studies reviewed, raw eggs^{14,21,22,27,39} and baked egg products^{25,27,28} were used. Studies using raw eggs fed lactating women 1^{14,22,27,39} or 2 raw eggs.²¹ Three studies^{25,27,28} used baked egg products and egg containing foods, such as omelet, quiche, egg in baked goods, and egg in meatballs with the amount of egg used in food preparation ranging from 1 to 4 eggs. Three of 4 studies comparing OVA level changes before and after intervention found a statistically significant increase, with the difference between the medians varying from 0.15²⁵ to 1.18 ng/mL²⁷ after cooked/baked egg ingestion and 0.28 ng/mL after raw egg²⁷ ingestion, respectively (Table E4, available in this article's Online Repository at www.jaci-inpractice.org).

TABLE I. Milk proteins (β -lactoglobulin, α -S1-casein) levels in human milk

Study	No. of women/samples	Approach to HM sampling	Lower detection limit (ng/mL)	Proportion (%) of samples with detectable protein level, if reported	Median/mean protein level (ng/mL), if reported	Highest protein level ng/mL (range), if reported	Proportion (%) of samples or women with protein $\geq$ ED01	Proportion (%) of samples or women with protein $\geq$ ED05
Interventional								
Breast milk samples from women after cow's milk (CM) ingestion								
β -Lactoglobulin immuno-like protein								
Bertino et al, ¹³ 1996	14/282	Collected in the afternoon in mothers on the high (500 mL per day), medium (200 mL per day) and free intake CM diet for at least 10 d before sampling	NR	149/282 (52.8) samples	27.1 (mean)	304 (0.2-304)	NR	0/282 (0) samples
β -Lactoglobulin								
Fukushima et al, ¹⁶ 1997	24/72	Morning (noon) and evening samples after ingestion of 200 mL of CM considering regular ingestion of milk a week before sampling day	0.1	39/72 (54.2) samples	NR	16.5	0/72 (0) samples	0/72 (0) samples
Høst et al, ¹⁹ 1988	19/19	4 h after ingestion of 500 mL of CM	0.3	4/19 (21) samples	NR	45 (0.5-45)	0/19 (0) samples	0/19 (0) samples
Høst et al, ¹⁸ 1990	20/300	7 d of every 3 wk before and 4, 8, 12, 24 h after a single ingestion of 500 mL of CM	0.3	19/20 (95) women	4.2	150 (0.9-150)	2/20 (10) women	0/20 (0) women
Järvinen et al, ²⁰ 1999	26/36	Collected before and at 1, 2, 3, and 4 h after CM ingestion	0.002	13/26 (50) women	NR	11.54 (0.01-11.54)	0/36 (0) samples	0/36 (0) samples
Kilshaw et al, ²² 1984	19/approx. 57*	Before and 2, 4, 6 h after ingestion of half a pint of CM	0.1	10/19 (52.6) women had at least 1 sample with detectable	0.11 [†] (0.05-0.72)	6.4 (0.05-6.4)	0/19 (0) women	0/19 (0) women
Makinen-Kiljunen et al, ²³ 1992	3/approx. 10	Samples collected from 2 mothers 0, 1, 2 h after ingestion of 400 mL CM and from 1 mother in addition after 4, 6, 8, and 20 h	0.002	3/3 (100) women	4.4 (0.36) (mean)	NR	0/3 (0) women	0/3 (0) women
Matangkasombut et al, ²⁴ 2017	19/85	Before and 3, 6, 24 h, and 3, 7 d after ingestion of 240 mL of CM	0.002	NR	1.23 (1.03-2.29)	3.91	0/85 (0) samples	0/85 (0) samples
Monti et al, ²⁶ 1989	4/NR	Collected on the third day and then on the fourth day 4 h after ingestion of CM solution	NR	NR	NR	415 (0.6-415)	Between 1 and 4/4 (25-100) women	0/4 (0) women

Fukushima et al, ¹⁷ 1997	25/between 50 and 150	1-3 h, 4-8 h, 9-15 h after the ingestion of 200 mL of hydrolyzed formula or CM	0.1	Formula group: 2/12 (16.7) women (first sampling); 3/12 (25) women (second sampling); CM group: 11/13 (84.6) women (first sampling); 8/13 (61.5) women (second sampling)	NR	17	0/25 (0) women	0/25 (0) women
Sorva et al, ³¹ 1994	53/151	Before and 1 and 2 h after a CM ingestion after a 24-h CM-free diet	0.002	75/151 (50) samples	0.01 (range 0.0-3.5) basal level; 0.12 (0.01-7.84) 0 to 1 h increase	7.84 (0.01-7.84)	0/151 (0) samples	0/151 (0) samples
Total	212/between 780 and 880		0.002-0.3	118/242 (48.8) samples Approx. 69/118 (58.5) women			0/363 (0) samples Between 3 and 6/71 (4.2-8.5) women	0/363 (0) samples 0/71 women
Observational								
Breast milk samples from women with no special dietary intervention								
β -Lactoglobulin								
Axelsson et al, ³⁴ 1986 ³⁴ 1986	25/232	Every 2 wk from the first week of infant's life up to 31 wk after delivery at mothers who had a CM containing diet (from 0.2 to 1.5 l per day)	5	93/232 (40) samples	NR	800 (5-800)	Approx. 5/232 (2.2) samples	0/232 (0) samples
Høst et al, ¹⁹ 1988	6/6	Collected after 4 wk with no dietary intervention	0.3	1/6 (16.7) samples	NR	10.5	0/6 (0) samples	0/6 (0) samples
Jakobsson et al, ³⁶ 1985	38/38	No dietary intervention	5	18/38 (47) samples	Approx. 2.5 $\ddagger$	33 (5-33)	0/38 (0) samples	0/38 (0) samples
Lovegrove et al, ³⁷ 1996	24/approx. 312 (24 of 34 on unrestricted diet/approx. 312)	Day 1 postnatally and daily until days 14, 21, and 28 with no dietary intervention	0.08	Raw data not reported	NR	0.62-5.9 (atopic), 0.08-3.01 (nonatopic) 1.45-3.66 (atopic diet) individual variation	0/312 (0) samples	0/312 (0) samples
Machtlinger et al, ³⁸ 1986	57/54	Around 2 wk of lactation with no dietary intervention	0.1	24/54 (45) samples	NR	6.4	0/54 (0) samples	0/54 (0) samples
Savilahti et al, ⁴¹ 1991	109/44§	Samples at 3 d and then 2, 4, 6, 9, 12 mo from mothers with no dietary intervention	0.1	26/44 (59) samples	NR	33	0/44 (0) samples	0/44 (0) samples

(continued)

TABLE I. (Continued)

Study	No. of women/samples	Approach to HM sampling	Lower detection limit (ng/mL)	Proportion (%) of samples with detectable protein level, if reported	Median/mean protein level (ng/mL), if reported	Highest protein level ng/mL (range), if reported	Proportion (%) of samples or women with protein $\geq$ ED01	Proportion (%) of samples or women with protein $\geq$ ED05
Stuart et al, ⁴² 1984	28/28	7-8 d postpartum reported	NR	5/28 (18) samples	NR	Approx. 19	0/28 (0) samples	0/28 (0) samples
Total	287/714		0.08-5	Approx. 167/402 (41.5) samples			5/714 (0.7) samples	0/714 (0) samples
α -S1-Casein								
Stuart et al, ⁴² 1984	28/28	Samples taken 7 or 8 d after giving birth	NR	13/28 (46.4) samples	NR	Approx. 16	0/28 (0) samples	0/28 (0) samples

ED, Eliciting dose; HM, human milk; NR, not reported.

*Samples were calculated only after intervention.

†Calculated from data presented in table 1 of the Kilshaw et al, 1984 manuscript—based on maximum concentrations from individual participants.

‡Calculated from data presented in figure 3 of the Jakobsson et al, 1985 manuscript.

§Calculated from data presented in figure 1 of the Savilahti et al, 1991 manuscript.

Across all the studies, 25.3% of samples had detectable levels of OVA ranging between 0.1 ng/mL and 29.82 ng/mL.²⁷ Concentration of OM largely varied from 0.1 to 2.88 ng/mL in interventional²² and 0.3 to 37 ng/mL in observational³⁵ studies, respectively. None of the interventional studies detected OVA or OM levels above ED01; hence we were unable to estimate the probability of having sufficient quantities consumed within a single breastfeed to elicit an allergic reaction in the individual allergic to this protein.

A single observational study reported levels of OVA above ED01 with an approximate of 6 samples surpassing the threshold. Thus, the probability of having sufficient quantities within a single breastfeed of OVA to elicit allergic reactions in an individual allergic to this protein was estimated at 1:3846.

Peanut proteins

All studies assessing the levels of peanut proteins (Ara h 1, Ara h 2, and Ara h 6) in HM were interventional. Roasted peanuts were used as the intervention in all the studies reviewed, with the amount of peanut ingestion varying between 30 g¹² and 100 g.^{29,30} Two^{30,33} of 3 studies found statistically significant changes in the levels of peanut proteins after intervention (Table E5, available in this article's Online Repository at www.jaci-inpractice.org).

Levels of Ara h 2 ranged between 0.1 ng/mL and 2602 ng/mL.²⁹ Concentration of Ara h 6 varied between 0.1 ng/mL and 79 ng/mL.³⁰ One study³³ assessed Ara h 1 and Ara h 2 concentrations simultaneously and the highest protein level was 430 ng/mL.

The number of samples with detectable peanut protein was 12.5%^{29,30} for Ara h 2 and 28.3%^{12,30} for Ara h 6, whereas levels of Ara h 2 above ED01 were found in 4%^{29,30} of samples and levels of Ara h 6 in 0.5%^{12,30} of samples. Only 1 study²⁹ reported a single sample with the Ara h 2 level above ED05. Overall, 12 of 325 samples contained levels of peanut proteins above ED01, and levels above ED05 were detected in a single sample across all the interventional studies (Table III). The probability of IgE-mediated allergic reaction in an individual allergic to this protein is 1 in 1000.

Wheat protein

Gladin levels were measured in 2 studies only. In the first study,¹⁵ mothers were on an unrestricted diet for 3 days, followed by 3 days of a gluten-free diet and then an unrestricted diet for the last 3 days. The second study³² collected HM samples after ingestion of 20 g of gluten. Levels of gliadin above ED01 were not detected in 1 study,³² whereas 6.1% of HM samples in another¹⁵ contained levels above ED01 (Table IV). The estimated probability of an allergic reaction in an individual allergic to this protein, due to the presence of gliadin in HM, can be estimated as 1 in 1720.

DISCUSSION

IgE-mediated food allergy is a common problem in infancy and early childhood, but risks of allergic reactions in children during breastfeeding remain unclear. The evidence of associations between maternal diet, presence of food proteins in HM, and allergic reactions in children during breastfeeding is still a matter of controversy. In this work, we reviewed existing evidence and found that levels of food proteins in HM were much lower than the dose required for eliciting an IgE-mediated allergic reaction in most of the HM samples. Although the

TABLE II. Egg proteins (ovalbumin and ovomucoid) levels in human milk

Study	No. of women/ samples	Approach to HM sampling	Lower detection limit (ng/mL)	Proportion (%) of samples with detectable protein level, if reported	Median/mean protein level (ng/ mL), if reported	Highest protein level and range (ng/mL) reported	Proportion (%) of samples or women with protein ≥ED01	Proportion (%) of samples or women with protein ≥ED05
Interventional								
Breast milk samples from women after egg ingestion								
Ovalbumin								
Cant et al, ¹⁴ 1985	19/between 19 and 76	Before and 2, 4, and 6 h after ingestion of 1 raw egg	0.1	14/19 (73.4) women	1.6 (eczema and “+” SPT); 2.4 (no eczema and “–” SPT) (mean)	4.0 (0.2-4.0)	0/19 (0) women	0/19 (0) women
Kageyama et al, ²¹ 1983	3/8	Before, 2 and 8 h after the ingestion of 2 raw eggs	1.0	Approx. 4/8 (50) samples	NR	Approx. 4	0/8 (0) samples	0/8 (0) samples
Kilshaw et al, ²² 1984	22/between 22 and 88	Before, 2, 4, and 6 h after the ingestion of half a pint of pasteurized cow’s milk mixed with 1 raw egg	0.1	13/22 (59) women	0.39* (0.05-2.16)	6.17	0/22 (0) women	0/22 (0) women
Metcalf et al, ²⁵ 2016	109/307	2, 4, and 6 wk of lactation during the dietary intervention with different quantities of protein: HE, LE, EF	0.1	39/85 (46) samples in EF; 58/118 (49.1) samples in LE; 54/ 104 (52) samples in HE	0.05† (0.05-0.20) (6 wk—EF); 0.05† (0.05-0.41) (6 wk— LE); 0.20† (0.05- 0.96) (6 wk—HE)	46.0‡	0/307 (0) samples	0/307 (0) samples
Palmer et al, ²⁷ 2005	41/818	Before and 2, 4, 6, and 8 h after ingestion of the test muffin and milkshake containing different quantities of egg protein vs placebo during 4 clinical visits	0.1	7/204 (3.4) samples—no egg; 41/205 (20) samples—1 raw egg; 38/205 (18.5) samples—1/2 cooked egg; 69/204 (33.8) samples—1 cooked egg	0.00 (–0.01 to 0.11) (no egg); 0.28 (0.43- 1.74) (1 raw egg); 0.00 (0.57-3.91) (1/ 2 cooked egg); 1.18 (1.41-4.91) (1 cooked egg).	29.82§	0/818 (0) samples	0/818 (0) samples
Palmer et al, ²⁸ 2008	32/376	Before and at 2, 4, and 6 h after eating a muffin containing 1 egg	0.1	49/188 (26.1) samples— egg group; 6/188 (3.2) samples— control group	0.89 (0.38-2.35) (egg group); 0.00 (–0.25 to 0.68) (control group)	14.21‡	0/376 (0) samples	0/376 (0) samples
Total	226/approx. 1550-1673		0.1-1.0	27/41 (65.9) women Approx. 313/1032 (30.3) samples			0/1509 (0) samples 0/41(0) women	0/1509 (0) samples 0/41 (0) women
Observational								
Breast milk samples from women with no dietary intervention								
Ovalbumin								
Fukushima et al, ¹⁶ 1997	24/72	Samples taken in the morning, at noon, and in the evening with no dietary intervention	0.1	3/72 (4.2) samples	NR	0.7	0/72 (0) samples	0/72 samples

(continued)

TABLE II. (Continued)

Study	No. of women/ samples	Approach to HM sampling	Lower detection limit (ng/mL)	Proportion (%) of samples with detectable protein level, if reported	Median/mean protein level (ng/ mL), if reported	Highest protein level and range (ng/mL) reported	Proportion (%) of samples or women with protein ≥ED01	Proportion (%) of samples or women with protein ≥ED05
Fukushima et al, ¹⁷ 1997	25/between 50 and 150	Samples taken at 1-3 h, 4-8 h, 9- 15 h with no dietary intervention	0.1	11/50 (22) samples	NR	Approx. 25¶	0/25 (0) women	0/25 (0) women
Rekima et al, ⁴⁰ 2016	251/248	Between day 3 and day 7 postpartum in allergic and nonallergic women with no dietary intervention	0.05	124/251 (49) samples	All the samples 0.0 (0.0-0.13) Allergic 0.05 (0.0-0.15)	3.94	0/248 (0) samples	0/248 (0) samples
Rekima et al, ³⁹ 2020	102/102	Samples collected at 6 mo postpartum from allergic mothers with no dietary intervention	0.05	45/100 (45) samples	0.171 (0.05-3.73)	3.73	0/102 (0) samples	0/102 (0) samples
Vance et al, ⁴³ 2005	68/125	At 3 mo postpartum with no dietary intervention	0.15-0.3	40/125 (32) samples	0.70	1258 (0.12-1258)	6/125 (5)*,‡	0/125 (0) samples
Total	368/595-695		0.05-0.1	223/598 (37.3) samples			6/545 (1.1) samples 0/25 (0) women	0/545 (0) samples 0/25 (0) women
Interventional Breast milk samples from women after egg ingestion								
Ovomucoid								
Kilshaw et al, ²² 1984	9/between 9 and 36	Before and 2, 4, and 6 h after the ingestion of 1/2 pint of pasteurized cow's milk mixed with 1 raw egg	0.1	7/9 (77.8) women	1.0 (0.44-1.41)	2.88	0/9 (0) samples	0/9 (0) samples
Observational Breast milk samples from women with no dietary intervention								
Ovomucoid								
Hirose et al, ³⁵ 2001	37/37	No dietary intervention	0.3	12/37 (32.4) women	0.15 (IQR: 0.00- 0.18)	37	0/37 (0) samples	0/37 (0) samples

ED, Eliciting dose; EF, egg-free diet; HE, high-egg (>4 eggs/wk); HM, human milk; IQR, interquartile range; LE, low-egg (1-3 eggs/wk); NR, not reported; SPT, skin prick test.

*Calculated using data from tables 1 and 2 of the Kilshaw et al, 1984 manuscript, based on maximum concentrations from individual participants.

†Highest values across collection times reported.

‡In Metcalfe et al, 2016, all values above the upper limit of assay detection were assigned the value of 4.0 ng/mL according to figure 2. The OVA levels found in samples that were all >4 ng/mL: 19.8 ng/mL, 21.66 ng/mL, 38.38 ng/mL, and 46.0 ng/mL.

§Reported by the author.

||Calculation is based on the intervention group only.

¶Approximated from figure 2 of the Fukushima et al, 1997 manuscript.

#As number of samples above ED01 in the Vance et al study⁴³ could not be reliably calculated, we used method suggested by Hozo and colleagues^{E11} to estimate the variance. Per authors recommendations we used the formula range/6 as it provides the best estimator for the standard deviation (variance) for studies with a sample size over 70.

TABLE III. Peanut proteins (Ara h 1, Ara h 2, and Ara h6) levels in human milk

Study	No. of women/samples	Approach to HM sampling	Lower detection limit (ng/mL)	Proportion (%) of samples with detectable protein level, if reported	Median/mean protein level (ng/mL), if reported	Highest protein level (range) (ng/mL) reported	Proportion (%) of samples or women with protein $\geq$ ED01#	Proportion (%) of samples or women with protein $\geq$ ED05#
Ara h 1 and Ara h 2*								
Vadas et al, ³³ 2001	23/approx. 184	Samples taken at hourly intervals after ingestion of 50 g of dry roasted peanuts	0.015-0.03	11/23 (48) women	200 median peak 222.3 (120-430) mean peak	430	4/23 (17) women	0/23 (0) women
Ara h 2								
Schocker et al, ²⁹ 2016	32/106	Samples taken at 0, 1, 2, 3, 4, 8, and 12 h after consumption of 100 g of dry roasted peanuts	40	12/106 (11.3) samples	20 (20-20)	2602 (20-2602)	9/106 (8.5) samples	1/106 (0.9) samples
Schocker et al, ³⁰ 2017	40/175	Samples taken before and at different times after ingestion of 100 g of dry roasted peanuts	1.3	23/175 (13) samples	0.65 (0.65-1.3)	184 (0.1-184)	2/175 (1.1) samples	0/175 (0) samples
Ara h 6								
Bernard et al, ¹² 2014	2/44†	Twelve-week breast milk samples taken before and over the period of 26 h after ingestion of 30 g of roasted peanuts	0.006	2/2(100) women 42/44† (95) samples	—	3.37	0/2 (0) women 0/44† (0) samples	0/2 (0) women 0/44† (0) samples
Schocker et al, ³⁰ 2017	40/175	Samples taken before and at different times after ingestion of 100 g of dry roasted peanuts	0.7	20/175 (11.4) samples	0.35 (0.35-0.35)	79 (0.1-79)	1/175 (0.6) samples	0/175 (0) samples
Total	42/219		0.006-40	97/500 (19.4) samples 13/25 (52) women			12/325 (3.7) samples 4/25 (16) women	1/325 (0.3) samples 0/25 (0) women

All studies were interventional.

ED, Eliciting dose; HM, human milk.

*Authors reported total peanut protein levels (no separate values for Ara h 1 and Ara h 2 were presented).

†The number has been confirmed after communication with the authors.

TABLE IV. Gliadin levels in human milk

Study	No. of women/samples	Approach to HM sampling	Lower detection limit (ng/mL)	Proportion (%) of samples with detectable protein level, if reported	Median/mean protein level (ng/mL), if reported	Highest protein level (range) (ng/mL) reported	Proportion (%) of samples or women with protein $\geq$ ED01	Proportion (%) of samples or women with protein $\geq$ ED05
Interventional								
Breast milk samples from women after gluten ingestion								
Gliadin								
Troncone et al, ³² 1987	53/80	Samples* taken 2-4 h after ingestion of 20 g of gluten	Approx. 5	54/80 (67.5) samples	NR	95 (5-95)	0/80 (0) samples	0/80 (0) samples
Chirido et al, ¹⁵ 1998	6/49MM	Two weeks postpartum, samples taken during 9-d diet: 3 d of unrestricted diet, 3 d of gluten-free diet and unrestricted diet for the last 3 d	1	49/49 (100) samples	178 (mean)	1200 (5-1200)	3/49 (6.1) samples	0/49 (0) samples
Total			1 to approx. 5	103/129 (80) samples			3/129 (2) samples	0/129 (0) samples

All studies were interventional.

C, Colostrum; ED, eliciting dose; HM, human milk; MM, mature milk.

*Human milk was collected at 1 week, 6 weeks, 3 months, and 5 months.

probability of an IgE-mediated reaction in allergic children is low, the evidence remains limited due to high heterogeneity between studies. Available data are sparse and inconclusive; well-controlled trials are required to investigate the effects of food proteins in HM and allergic reactions in children.

Many studies were conducted 20 or more years ago and lack methodological rigor and precise result reporting. Important aspects such as time of sample collection, laboratory methods used, and details regarding food protein levels varied significantly between the studies with some data being unavailable. Most studies do not report ELISA and/or radioimmunoassay coefficient of intra-assay, inter-assay variation. A wide range of sampling methodologies and the collection methods used in studies reporting HM composition, and lack of "gold standard" methodology have been recently demonstrated in a systematic review.⁴⁴ Significant variations in methodology make direct comparison of the food protein concentrations across the studies impossible. Detailed information about participant characteristics was often lacking, with less than a half of the reviewed studies providing data on maternal health and particularly maternal allergic status. This should be further investigated as it is still unclear if the mechanisms of food protein passage into HM differ between women of variable health status and particularly between allergic and nonallergic women.⁴⁵

Large variations in the concentrations of food proteins detected in HM were found between studies and also even within specific food proteins within the same study between women after ingestion of the same test meal/drink. It is unclear why the concentration of peanut proteins and gliadin reached hundreds of ng/mL whereas OVA were mostly reported at the levels below 1 ng/mL. Available data do not provide definitive answers on whether methods of detection or physiological differences in antigen absorption in the maternal gut and secretion into HM were main contributors to these differences.

Most studies measured a single food protein in HM, with only 3 concurrently assessing both milk and egg proteins. As such, there are insufficient data to establish if there are intrawoman differences in food protein excretion into HM between different food proteins, and if some mothers consistently excrete larger quantities of all food proteins into their HM. This aspect is of physiological importance and remains one of the main unanswered questions.

There were different approaches undertaken to the interventions used across the various studies, some using raw foods^{14,21,22} as well as baked/cooked products.^{25,28} The levels of BLG remained unchanged in 5 of 6 studies, whereas egg and peanut protein levels significantly increased in most of the studies after intervention. It is important to note that although the increase was statistically significant, it is unknown whether such a small change in concentration has clinical significance. Cooked egg intake resulted in higher HM concentrations than for raw egg in one of the studies, but none of the studies have measured OVA HM levels after 2 cooked eggs were eaten, which is a common quantity for some breastfeeding mothers to eat in a single meal.²⁷ Unfortunately, different modalities of food protein processing and cooking were not investigated in other studies and their impact on the levels of protein excretion should be assessed in future research. Interindividual variation in food protein levels in HM detected (or not detected) even after the same test dose of food protein is consumed by women at the same stage of lactation requires further investigation.

Limitations

Our systematic review has several limitations. First, this review has only focused on IgE-mediated (immediate hypersensitivity) and not non-IgE-mediated food allergy reactions. Non-IgE-mediated responses in young children to egg and peanut are however far less common than IgE-mediated reactions. The VITAL data used as a comparator allowed us to estimate the likelihood that the amount of food protein in a single HM feed could result in an IgE-mediated reaction. However, these findings cannot be extrapolated to non-IgE-mediated food allergy reactions, and separate investigations are required for the diverse enteropathies associated with food allergy. ED have been established in children but not for infants during breastfeeding, which may have very distinct immune reactivity than adults. It is important to note, however, that we used the most conservative approach possible and many studies, informing VITAL 3.0 used 0 mg cutoff as the “no observed adverse effect level,” which leads to a much lower ED01 and ED05 than if the patients who reacted to the first dose would be removed from the analysis.⁴⁶ Our findings also only relate to single-dose exposure and do not take into account repeated low-dose ingestion after multiple HM feeds each day. In addition, the comparisons with ED from single food allergen oral challenge doses do not reflect the potential complex mixture of food proteins and other HM components within an HM feed that may modify responses. Other HM constituents, such as lipids and immunological components, may result in changes to food allergen thresholds and may have a bias on our findings in ways that we currently do not yet understand. The food proteins expressed in HM most probably are dependent on maternal diet, and after gastrointestinal tract absorption they transported and then secreted into HM. It is possible that proteins can be partially digested before absorption, whereas many immunoassays only detect intact proteins. Partially digested proteins may be missed by the sandwich ELISAs. We can conclude from immune assays that proteins are detected by antibody-based detection methods, but we cannot definitively conclude whether those antigens will be recognized by the infant immune system as allergenic or tolerogenic.

This review was restricted to studies analyzing levels of food proteins in HM and studies using HM for skin prick testing, and then corresponding food challenges were not included. We focused on studies measuring food proteins in HM and not directly assessing clinical outcomes, which is also a potential limitation. Although search incorporated terms for all the most common allergens, no studies were published for some of them (eg, tree nuts, sesame, fish, etc), which did not allow us to estimate the probability of an allergic reaction for these foods. BLG was measured in many studies, but it is not a major cow's milk allergen. However, the only study assessing casein⁴² confirmed our hypothesis that the levels are too low to elicit reaction in most children. We also acknowledge that between study assay differences might contribute to some of the differences, but it seems extraordinarily unlikely that they would lead to very significant changes in the results.

CONCLUSIONS

Our systematic review suggests that the probability of an IgE-mediated allergic reaction to food proteins transmitted via HM is low, although it may occur in a small number of children. However, existing evidence is sparse, methodology is diverse, and

no definitive conclusions can be drawn because of complexities around HM composition and inter-women variations. This review should not be seen as a direct recommendation for clinical practice, but rather as an exercise in revealing “gray areas” and unmet evidence in the field. Future research should investigate the standardized methods of quantification and combine measurements of food proteins with other HM constituents. Research identifying women able to excrete high quantities of food proteins into their milk is required. Studies aiming at the assessment of both IgE-mediated and non-IgE-mediated allergic reactions in children during breastfeeding coupled with oral HM challenges are also urgently needed.

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ONLINE REPOSITORY

Appendix E1 Search strategies

Database: Embase Classic+Embase <1947 to 2019 January 23> (via Ovid)

- 1 (Cow* milk or Bos d or Bos d 4 Bos d 5 or Bos d 6 Bos d 7 or Bos d 8 or Bos d 9 or Bos d 10 or Bos d 11 or Bos d 12 or casein* or lactalbumin or Alpha-lactalbumin or Beta-lactoglobulin or alphaS1-casein or alphaS2-casein or beta-casein or kappa-casein or lactoglobulin or lactoferrin or cow* milk protein* or dairy).ab,kw,ti.
- 2 (Egg or hen* egg or Gal d or Gal d 1 or Gal d 2 or Gal d 3 or Gal d 4 or Gal d 5 or Gal d 8 or Gal d 9 or ovomucoid or ovalbumin or ovotransferrin or conalbumin or egg lysozyme or ovomucin or phosvitin or alpha-livetin or apovitellenin* or egg protein*).ab,kw,ti.
- 3 (Peanut or arachis or Ara h or Ara h 1 or Ara h 2 or Ara h 3 or Ara h 5 or Ara h 6 or Ara h 7 or Ara h 8 or Ara h 9 or Ara h 10 or Ara h 11 or Ara h 12 or Ara h 13 or Ara h 14 or Ara h 15 or Ara h 16 or Ara h 17 or Cupin or Conglutin or Profilin or oleosin or non-specific Lipid Transfer Protein or peanut protein*).ab,kw,ti.
- 4 (Sesame or Ses i or Ses i 1 or Ses i 2 or Ses i 3 or Ses i 4 or Ses i 5 or Ses i 6 or Ses i 7 or 2S albumin or 7S vicilin-like globulin or 11S globulin or sesame protein*).ab,kw,ti.
- 5 (Wheat or Triticum or Tri a or Tri a 12 or Tri a 14 or Tri a 15 or Tri a 17 or Tri a 18 or Tri a 19 or Tri a 20 or Tri a 21 or Tri a 25 or Tri a 26 or Tri a 27 or Tri a 28 or Tri a 29 or Tri a 30 or Tri a 31 or Tri a 32 or Tri a 33 or Tri a 34 or Tri a 35 or Tri a 36 or Tri a 37 or Tri a 39 or Tri a 40 or Tri a 41 or Tri a 42 or Tri a 43 or Tri a 44 or Tri a 45 or Tri a 12 or Tri a 12 or Monomeric alpha-amylase inhibitor or beta-amylase or Agglutinin isolectin 1 or seed storage protein or Gamma gliadin or Omega-5 gliadin or Alpha-beta-gliadin or Thioredoxin or High molecular weight glutenin or Thiol reductase homologue or Dimeric alpha-amylase inhibitor or Tetrameric alpha-amylase inhibitor or Triosephosphate-isomerase or 1-cys-peroxiredoxin or serpin or Glyceraldehyde-3-phosphate-dehydrogenase or Dehydrin or Low molecular weight glutenin or Alpha purothionin or Serine protease inhibitor-like protein or Mitochondrial ubiquitin ligase activator of NFKB 1 or Hypothetical protein from cDNA or Endosperm transfer cell specific PR60 precursor or Elongation factor 1 or EIF1 or wheat protein*).ab,kw,ti.
- 6 (Fish or Cod or Gadus or Gad c or Gad m or Sal s or Gad c 1 or Gad m 1 or Gad m 2 or Gad m 3 or Beta-parvalbumin or Beta-enolase or Aldolase A or Sal s 1 or Sal s 2 or Sal s 3 or cod protein* or salmon protein* or fish protein* or shell fish or shellfish or shrimp* or crustacean* or sea food or seafood).ab,kw,ti.
- 7 (Soya or soy or soybean or Gly m 1 or Gly m 2 or Gly m 3 or Gly m 4 or Gly m 5 or Gly m 6 or Gly m 7 or Gly m 8 or beta-conglycinin or defensin or glycinin or legumin or seed biotinylated protein or soy* protein).ab,kw,ti.
- 8 (Hazelnut or filbert* or almond or brazil nut or cashew or chestnut or pecan or pistachio or pine nut or walnut or Cor a 1 or Cor a 2 or Cor a 6 or Cor a 8 or Cor a 9 or Cor a 10 or Cor a 11 or Cor a 12 or Cor a 13 or Cor a 14 or Pru du 3 or Pru du 4 or Pru du 5 or Pru du 6 or Pru du 8 or Ber e 1 or

Ber e 2 or Ana o 1 or Ana o 2 or Ana o 3 or Cas s 1 or Cas s 5 or Cas s 8 or Cas s 9 or Car i 1 or Car i 2 or Car i 4 or Pis v 1 or Pis v 2 or Pis v 3 or Pis v 4 or Pis v 5 or Pin p 1 or Jug r 1 or Jug r 2 or Jug r 3 or Jug r 4 or Jug r 5 or Jug r 6 or Jug r 7 or Jug r 8 or Jug n 1 or Jug n 2 or Jug n 4 or nut protein or tree nut protein).ab,kw,ti.

- 9 (Allergen-transfer or allergen transfer or protein transfer or peanut transfer or egg transfer or cow* milk transfer or wheat transfer or sesame transfer or cod transfer or salmon transfer or shell fish transfer or shellfish transfer or sea food transfer or seafood transfer or wheat transfer or sesame transfer or soy* transfer or hazelnut transfer or almond transfer or brazil nut transfer or cashew transfer or chestnut transfer or pecan transfer or pistachio transfer or pine nut transfer or walnut transfer or tree nut transfer).ab,kw,ti.
- 10 (Maternal food consumption or maternal peanut consumption or maternal cow* milk consumption or maternal egg consumption or maternal fish consumption or maternal cod consumption or maternal salmon consumption or maternal shell fish consumption or maternal shellfish consumption or maternal sea food consumption or maternal seafood consumption or maternal wheat consumption or maternal sesame consumption or maternal soy* consumption or maternal hazelnut consumption or maternal almond consumption or maternal brazil nut consumption or maternal cashew consumption or maternal chestnut consumption or maternal pecan consumption or maternal pistachio consumption or maternal pine nut consumption or maternal walnut consumption or maternal tree nut consumption).ab,kw,ti.
- 11 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
- 12 (breast milk or breast milk or breast milks or human milk or human breast milk or breast milk human or mature milk or colostrum or early milk or transitional milk).ab,kw,ti.
- 13 (breastfeeding or breast feeding or breast-feeding).ab,kw,ti.
- 14 breast milk/
- 15 breast feeding/
- 16 lactation/
- 17 12 or 13 or 14 or 15 or 16
- 18 11 and 17
- 19 **limit 18 to human**

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- 1 (Cow* milk or Bos d or Bos d 4 Bos d 5 or Bos d 6 Bos d 7 or Bos d 8 or Bos d 9 or Bos d 10 or Bos d 11 or Bos d 12 or casein* or lactalbumin or Alpha-lactalbumin or Beta-lactoglobulin or alphaS1-casein or alphaS2-casein or beta-casein or kappa-casein or lactoglobulin or lactoferrin or cow* milk protein* or dairy).ab,kf,ti.
- 2 (Egg or hen* egg or Gal d or Gal d 1 or Gal d 2 or Gal d 3 or Gal d 4 or Gal d 5 or Gal d 8 or Gal d 9 or ovomucoid or ovalbumin or ovotransferrin or conalbumin or egg lysozyme or ovomucin or phosvitin or alpha-livetin or apovitellenin* or egg protein*).ab,kf,ti.
- 3 (Peanut or arachis or Ara h or Ara h 1 or Ara h 2 or Ara h 3 or Ara h 5 or Ara h 6 or Ara h 7 or Ara h 8 or Ara h 9 or Ara h 10 or Ara h 11 or Ara h 12 or Ara h 13 or Ara h 14 or Ara h 15 or Ara h 16 or

- Ara h 17 or Cupin or Conglutin or Profilin or oleosin or non-specific Lipid Transfer Protein or peanut protein*).ab,kf,ti.
- 4 (Sesame or Ses i or Ses i 1 or Ses i 2 or Ses i 3 or Ses i 4 or Ses i 5 or Ses i 6 or Ses i 7 or 2S albumin or 7S vicilin-like globulin or 11S globulin or sesame protein*).ab,kf,ti.
 - 5 (Wheat or Triticum or Tri a or Tri a 12 or Tri a 14 or Tri a 15 or Tri a 17 or Tri a 18 or Tri a 19 or Tri a 20 or Tri a 21 or Tri a 25 or Tri a 26 or Tri a 27 or Tri a 28 or Tri a 29 or Tri a 30 or Tri a 31 or Tri a 32 or Tri a 33 or Tri a 34 or Tri a 35 or Tri a 36 or Tri a 37 or Tri a 39 or Tri a 40 or Tri a 41 or Tri a 42 or Tri a 43 or Tri a 44 or Tri a 45 or Tri a 12 or Tri a 12 or Monomeric alpha-amylase inhibitor or beta-amylase or Agglutinin isolectin 1 or seed storage protein or Gamma gliadin or Omega-5 gliadin or Alpha-beta-gliadin or Thioredoxin or High molecular weight glutenin or Thiol reductase homologue or Dimeric alpha-amylase inhibitor or Tetrameric alpha-amylase inhibitor or Triosephosphate-isomerase or 1-cys-peroxiredoxin or serpin or Glyceraldehyde-3-phosphate-dehydrogenase or Dehydrin or Low molecular weight glutenin or Alpha purothionin or Serine protease inhibitor-like protein or Mitochondrial ubiquitin ligase activator of NFKB 1 or Hypothetical protein from cDNA or Endosperm transfer cell specific PR60 precursor or Elongation factor 1 or EIF1 or wheat protein*).ab,kf,ti.
 - 6 (Fish or Cod or Gadus or Gad c or Gad m or Sal s or Gad c 1 or Gad m 1 or Gad m 2 or Gad m 3 or Beta-parvalbumin or Beta-enolase or Aldolase A or Sal s 1 or Sal s 2 or Sal s 3 or cod protein* or salmon protein* or fish protein* or shell fish or shellfish or shrimp* or crustacean* or sea food or seafood).ab,kf,ti.
 - 7 (Soya or soy or soybean or Gly m 1 or Gly m 2 or Gly m 3 or Gly m 4 or Gly m 5 or Gly m 6 or Gly m 7 or Gly m 8 or beta-conglycinin or defensin or glycinin or legumin or seed biotinylated protein or soy* protein).ab,kf,ti.
 - 8 (Hazelnut or filbert* or almond or brazil nut or cashew or chestnut or pecan or pistachio or pine nut or walnut or Cor a 1 or Cor a 2 or Cor a 6 or Cor a 8 or Cor a 9 or Cor a 10 or Cor a 11 or Cor a 12 or Cor a 13 or Cor a 14 or Pru du 3 or Pru du 4 or Pru du 5 or Pru du 6 or Pru du 8 or Ber e 1 or Ber e 2 or Ana o 1 or Ana o 2 or Ana o 3 or Cas s 1 or Cas s 5 or Cas s 8 or Cas s 9 or Car i 1 or Car i 2 or Car i 4 or Pis v 1 or Pis v 2 or Pis v 3 or Pis v 4 or Pis v 5 or Pin p 1 or Jug r 1 or Jug r 2 or Jug r 3 or Jug r 4 or Jug r 5 or Jug r 6 or Jug r 7 or Jug r 8 or Jug n 1 or Jug n 2 or Jug n 4 or nut protein or tree nut protein).ab,kf,ti.
 - 9 (Allergen-transfer or allergen transfer or protein transfer or peanut transfer or egg transfer or cow* milk transfer or wheat transfer or sesame transfer or cod transfer or salmon transfer or shell fish transfer or shellfish transfer or sea food transfer or seafood transfer or wheat transfer or sesame transfer or soy* transfer or hazelnut transfer or almond transfer or brazil nut transfer or cashew transfer or chestnut transfer or pecan transfer or pistachio transfer or pine nut transfer or walnut transfer or tree nut transfer).ab,kf,ti.
 - 10 (Maternal food consumption or maternal peanut consumption or maternal cow* milk consumption or maternal egg consumption or maternal fish consumption or maternal cod consumption or maternal salmon consumption or maternal shell fish consumption or maternal shellfish consumption or maternal sea food consumption or maternal seafood consumption or maternal wheat consumption or maternal sesame consumption or maternal soy* consumption or maternal hazelnut consumption or maternal almond consumption or maternal brazil nut consumption or maternal cashew consumption or maternal chestnut consumption or maternal pecan consumption or maternal pistachio consumption or maternal pine nut consumption or maternal walnut consumption or maternal tree nut consumption).ab,kf,ti.
 - 11 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
 - 12 (breast milk or breastmilk or breast milks or human milk or human breast milk or breast milk human or mature milk or colostrum or early milk or transitional milk).ab,kf,ti.
 - 13 (breastfeeding or breast feeding or breast-feeding).ab,kf,ti.
 - 14 Milk, Human/
 - 15 Breast Feeding/
 - 16 LACTATION/
 - 17 12 or 13 or 14 or 15 or 16
 - 18 11 and 17
 - 19 limit 18 to humans

Supplementary Methods

Data extraction. All included papers were transferred into EndNote version X9 (Thomas Reuters, New York, NY) reference manager. To reduce potential selection bias 3 independent investigators (AG, AB, and DL) reviewed all titles and abstracts identified by the search for inclusion. Then, AG, AB, and DL independently reviewed full texts of all publications selected for data extraction. Any disagreements were resolved through discussion involving an additional reviewer (DM) and other co-authors if needed, until consensus was reached. All the included studies were discussed and approved by the review team.

The data from each study were extracted in duplicate, tabulated, and included author and year of publication, descriptive information concerning the study design, country and setting, baseline characteristics of the study population, methodology of milk sampling, timing of sample collection, number of participants, number of samples collected, maternal diet/intervention, protein(-s) measured, levels of protein(-s) in HM, and method of food protein concentration assessment.

Study type definitions. In this systematic review, the studies were divided into “observational” and “interventional” for illustrative purposes and simplicity of understanding.

Observational studies included research when the study team had no influence on exposures (eg, researchers did not give certain food in a predefined quantity to the mothers) and simply acted as “observers.”

Interventional studies were defined as those where investigator “assigned” exposure (eg, certain food in a known quantity to the breastfeeding women) to study participants to address how this influences the outcome. Thus, the exposure was under the investigator control, but not the study participant. All interventional studies, regardless of whether they were randomized or not, were included in this systematic review.

Probability of allergic reaction estimation. The probability of allergic reaction was estimated using data of VITAL 3.0 project that identifies eliciting doses (ED) for common food allergens.^{E1} The ED represents the amount of total protein that is predicted to cause an allergic reaction in a certain proportion of

individuals. Threshold values for ED01 (eliciting dose for 1% of allergic individuals) and ED05 (eliciting dose for 5% of allergic individuals) are based on data from oral food challenges.^{E1} We compared concentrations of food proteins in human milk reported in the reviewed studies with the eliciting doses outlined in VITAL 3.0 to estimate the probability of a single breastfeed to trigger an allergic reaction in a food-allergic child.

To estimate the total amount of food protein consumed during a single breastfeed, we used existing data on breastfeeding patterns from 1 to 6 months of lactation.^{E2} The estimation was calculated based on the levels of food proteins reported in the reviewed papers multiplied by the maximum single breastfeed volume (216

mL).^{E2} Percentages of food proteins out of the total protein content of the food product were based on values previously reported in the scientific literature (Table E1). Levels of food protein in a single feed were then compared with ED01 and ED05 data from VITAL 3.0 to calculate the number of samples containing food protein in the levels above ED01 and ED05 to assess the probability of an acute allergic reaction in an individual. If no data on the number of samples were available, but the number of mothers was known, each woman was equated to a single sample as a conservative estimation (the highest level available during the sampling period for each woman was used, where available). Values for ED01 and ED05 are presented in Table E1.

TABLE E1. Eliciting doses for food proteins found in human milk based on VITAL 3.0 data

Protein	Percentage of total food protein*	ED01 (mg protein)†	Protein isolate for ED01 (mg)	ED01 (ng/mL)‡	ED05 (mg protein)†	Protein isolate for ED05 (mg)	ED05 (ng/mL)‡
β-Lactoglobulin (BLG)	12% ^{E3}	0.2	0.024	111.11	2.4	0.288	1333.33
α-S1-Casein	26% ^{E4}	0.2	0.052	240.74	2.4	0.624	2888.89
Ovalbumin	54% ^{E5,E6}	0.2	0.108	500	2.3	1.242	5750
Ovomucoid	11% ^{E5,E6}	0.2	0.022	101.85	2.3	0.253	1171.3
Ara h 1	17.1% ^{E7}	0.2	0.0342	158.33	2.1	0.3591	1662.5
Ara h 2	9.3% ^{E8}	0.2	0.0186	86.11	2.1	0.1953	904.17
Ara h 6	5.8% ^{E7}	0.2	0.0116	53.7	2.1	0.1218	563.89
Gliadin	27.8% ^{E9}	0.7	0.1946	900.93	6.1	1.6958	7850.93

ED, Eliciting dose; VITAL, Voluntary Incidental Trace Allergen Labelling.

As the number of samples above ED01 in the Vance et al study^{E10} could not be reliably calculated, we used method suggested by Hozo et al^{E11} to estimate the variance. Per the authors' recommendations, we used the formula range/6 as it provides the best estimator for the standard deviation (variance) for studies with a sample size over 70.

*Percentage of a given food out of the total protein content of the food product.

†Eliciting doses in accordance to VITAL 3.0.

‡Eliciting doses were calculated for a single breastfeed of 216 mL.

TABLE E2. Interventions used in the studies reviewed

Study	The amount of intervention	Type of intervention
β-Lactoglobulin immuno-like protein		
Bertino et al, ^{E12} 1996	The high (500 mL per day), medium (200 mL per day), and free intake CM diet	Not clarified
β-Lactoglobulin (BLG)		
Fukushima et al, ^{E13} 1997	200 mL	Not clarified
Høst et al, ^{E14} 1988	500 mL	Pasteurized and homogenized CM
Høst et al, ^{E15} 1990	500 mL	Pasteurized and homogenized CM
Järvinen et al, ^{E16} 1999	From 100 to 2300 mL	Not clarified
Kilshaw et al, ^{E17} 1984	Half a pint	Pasteurized milk
Makinen-Kiljunen et al, ^{E18} 1992	400 mL	Pasteurized and homogenized CM
Matangkasombut et al, ^{E19} 2017	400 mL	Pasteurized CM
Monti et al, ^{E20} 1989	Concentrate with 4 g of BLG	CM protein solution
Fukushima et al, ^{E21} 1997	200 mL	Not clarified
Sorva et al, ^{E22} 1994	400 mL	Fat-free CM load
Ovalbumin (OVA)		
Cant et al, ^{E23} 1985	One	Raw egg
Kageyama et al, ^{E24} 1983	Two	Raw egg
Metcalf et al, ^{E25} 2016	No egg	Egg-free diet
	1-3 eggs a week	Low-egg diet
	>4 eggs a week	High-egg diet
Palmer et al, ^{E26} 2005	No egg	—
	One	Raw egg
	Half an egg	Cooked egg
Palmer et al, ^{E27} 2008	One	Cooked egg
	Egg-free muffin Muffin containing 1 whole egg (55 g)	
Ovomucoid (OM)		
Kilshaw et al, ^{E17} 1984	One	Raw egg
Ara h 1 and Ara h 2		
Vadas et al, ^{E28} 2001	50 g	Dry roasted peanuts
Ara h 2		
Schocker et al, ^{E29} 2016	100 g	Dry roasted peanuts
Schocker et al, ^{E30} 2017	100 g	Dry roasted peanuts
Ara h 6		
Schocker et al, ^{E30} 2017	100 g	Dry roasted peanuts
Gliadin		
Troncone et al, ^{E31} 1987	20 g	Gluten
Chirido et al, ^{E32} 1998	3 d of unrestricted diet, 3 d of gluten-free diet and unrestricted diet for the last 3 d	

CM, Cow's milk.

TABLE E3. Median/mean milk protein (β -lactoglobulin) level at a baseline and after intervention

Author	Type of intervention	Timing of ingestion	Median/mean baseline protein level (range) (ng/mL), if reported	Median/mean protein level after intervention (range) (ng/mL), if reported	P value	Effect
Høst et al, ^{E14} 1988	500 mL of pasteurized and homogenized CM	4 h after ingestion	0.00* (0.0-10.5)	0.00* (0.0-45)	NR	Unclear
Järvinen et al, ^{E16} 1999	From 100 to 2300 mL of CM	Before and 1, 2, 3, and 4 h after ingestion	0.01† (<0.01-0.62)	0.04‡ (<0.1-11.54)	P = .008	↑
Makinen-Kiljunen et al, ^{E18} 1992	400 mL of pasteurized and homogenized CM	0, 1, and 2 h after ingestion	0.002‡ (0.0-0.2)	0.3‡ (0.0-2.0)	NR	Unclear
Matangkasombut et al, ^{E19} 2017	240 mL of pasteurized CM	Before and at 3, 6, and 24 h, and 3 and 7 days after ingestion	0.58 (0.38-0.88)	1.23 (1.03-2.29)	P < .001	↑
Monti et al, ^{E20} 1989	A solution of CM concentrate with 4 g of BLG	Before and 4 h after ingestion	NR (0.6-1)	NR (6-415)	NR	Unclear
Sorva et al, ^{E22} 1994	400 mL of fat-free CM	Before and 1, 2 h after ingestion	0.01 (0.0-3.5)	0.12 (0.01-7.84)—0 to 1 h increase 0.07 (0.01-2.34)—1 to 2 h increase	NR	Unclear

CM, Cow's milk; NR, not reported.

*Numbers were calculated using table 4 of the Høst et al, 1988 manuscript.

†Numbers were calculated using table 1 of the Järvinen et al, 1999 manuscript.

‡Numbers were calculated using figure 4 of the Makinen-Kiljunen et al, 1992 manuscript.

TABLE E4. Median/mean egg protein (ovalbumin) level at a baseline and after intervention

Author	Type of intervention	Timing of ingestion	Median/mean baseline protein level (range) (ng/mL), if reported	Median/mean protein level after intervention (range) (ng/mL), if reported	P value	Effect
Cant et al, ^{E23} 1985	One raw egg	Before and 2, 4, and 6 h after ingestion	0.00 (0.0-0.00)	NR (0.2-4.0)	NR	Unclear
Metcalfe et al, ^{E25} 2016	Egg-free diet (no egg) Low-egg diet (1-3 eggs a week) High-egg diet (>4 eggs a week)	At 2, 4, and 6 wk of lactation 2-6 h before HM collection	0.05 (0.05-0.20) 6 wk of lactation	0.05 (0.05-0.41)—low-egg; 0.20 (0.05-0.96)—high egg 6 wk of lactation	P = .04	= (in comparison to low-egg group) ↑ (in comparison to high-egg group)
Palmer et al, ^{E26} 2005	No egg One raw egg Half a cooked egg One cooked egg	Before and at 2, 4, 6, and 8 h after ingestion	0.00	0.28—1 raw egg 0.00—half a cooked egg 1.18—1 cooked egg	P = .05	↑ (in comparison to 1 raw and cooked egg) = (in comparison to half a cooked egg)
Palmer et al, ^{E27} 2008	Egg-free muffin Muffin containing 1 whole egg (55 g)	Before and at 2, 4, and 6 h after ingestion	0.00 (days 3, 12, 23)	0.63 (day 3) 0.81 (day 12) 0.89 (day 23)	P < .05	↑

HM, Human milk; NR, not reported.

TABLE E5. Median/mean peanut proteins (Ara h 1, Ara h 2, and Ara h6) level at a baseline and after intervention

Author	Type of intervention	Timing of ingestion	Median/mean baseline protein level (range) (ng/mL), if reported	Median/mean protein level after intervention (range) (ng/mL), if reported	P value	Effect
Ara h 1 and Ara h 2						
Vadas et al, ^{E28} 2001	50 g of dry roasted peanuts	Before and 1, 2, 3, 4, 6, 8, and 12 h after ingestion	0.00*	200 (120-430) median peak	NR	↑
Ara h 2						
Schocker et al, ^{E29} 2016	100 g of dry roasted peanuts	Before and 1, 2, 3, 4, 8, and 12 h after ingestion	20† (0-40)	20‡ (20-2602)	NR	Unclear
Schocker et al, ^{E30} 2017	100 g of dry roasted peanuts	Before and 1, 2, 3, 4, 8, and 12 h after ingestion	0.65‡ (0.65-2.5)	0.7‡ (0.65-184)	$P < .05$ §	↑
Ara h 6						
Schocker et al, ^{E30} 2017	100 g of dry roasted peanuts	Before and 1, 2, 3, 4, 8, and 12 h after ingestion	0.35 (0.35-1.5)	0.35 (0.35-79)	$P < .05$ ¶	↑

NR, not reported.

*Median was calculated using figure 1 of the Vadas et al, 2001 manuscript.

†Numbers were calculated using table 2 of the Schocker et al, 2016 manuscript.

‡Numbers were calculated using supplementary table 1 of the Schocker et al, 2017 manuscript.

§Ara h 2 concentrations were not significantly higher after 1 h ($P = .051$), significantly higher after 2 h ($P = .002$), and after 4 h ($P = .029$), respectively, compared with the basal samples ($t = 0$).

||Numbers were calculated using supplementary table 1 of the Schocker et al, 2017 manuscript.

¶Ara h 6 were significantly higher after 1 h ($P = .006$) and 2 h ($P = .001$) compared with the basal samples ($t = 0$).

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