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The role of Milk Ladder in cow's milk allergy

Edited by the SIAIP Food Allergy Committee Commission
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SUMMARY

Cow's milk allergy (CMA) is one of the most frequent causes of food allergy (FA) in children. Dietary avoidance is considered the main treatment for CMA, but it does not promote the development of tolerance. In contrast, milk oral immunotherapy (OIT) and the introduction of baked milk into the diet are two treatment options that may lead to tolerance development. Recently, the "milk ladder" (ML) has emerged as a third therapeutic option. ML can be defined as a gradual home reintroduction of foods containing milk starting from the most easily tolerated forms and progressing to whole cow's milk. Herein, we assess the impact of different processes on milk protein allergenicity, alongside studies evaluating the efficacy and safety of ML in children with non IgE-mediated and IgE-mediated CMPA. In non-IgE Mediated CMA, excluding FPIES, ML seems to be reasonable since it is not burdened with relevant side effects and is able to promote the early reintroduction of cow's milk. In IgE mediated CMA, the efficacy of ML seems to be like OIT although it is burdened with similar side effects, even serious as anaphylaxis. If used to perform the first step with baked milk oral challenge seems advisable. The next steps can be done at home. Only children without a clinical history of severe reactions or recurrent wheeziness can be candidates for ML, starting as soon as possible and in any case not in children over 6 years of age.

KEYWORD: Milk ladder, Cow's milk allergy, food allergy, tolerance development, oral immunotherapy

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Cow's milk protein allergy (CMPA) is one of the most frequent causes of food allergy (FA), described in 0.5-3% of children [1]. Cow's milk is the first food to be introduced into the diet, in the absence of maternal milk, and at weaning also, in the form of milk derivatives (parmesan or other cheese or other foods, pasta or biscuits containing it). Its prognosis is not always-favorable because, although tolerance develops over the years in most children, only about 50% of children develop tolerance before 5 years of age.[2] This causes prolonged diets and fear of reactions, which generally become more serious with increasing age.

CMPA is also the most frequent cause of death due to FA in children in Italy [3].

Treatment of CMPA consists of avoidance and the administration of drugs in case of allergic reaction. For many years it has been known that avoidance, however, does not promote the development of tolerance in everyone [4] and that, on the contrary, reintroducing milk with an oral immunotherapy or with the introduction of baked milk into the diet of cow's milk allergy in children can promote the development of tolerance even for raw milk [5,6]. Beyond these two strategies, a third dietetic advancement therapy was proposed: the milk ladder.

THE MILK LADDER: BIRTH AND EVOLUTION

Approximately a decade ago, the UK Ministry of Health, prompted by the escalating prevalence and frequent misdiagnosis of food allergies, entrusted the National Institute for Health and Clinical Excellence (NICE) with the task of crafting a pragmatic document refining the diagnosis and management of these conditions. This seminal document delineated the diverse spectrum of food allergies and furnished guidelines for accurate diagnosis and appropriate dietary management, alongside delineating criteria for specialist referral. Primary care, comprising General Pediatricians (GPs), Dietitians, Nurses, etc., was assigned the responsibility of managing milder presentations [7].

Subsequently, a more nuanced and detailed document was published in 2013. This document underscored the divergent management approaches for clinical presentations with immediate onset, likely IgE-mediated, compared to those with delayed onset, likely non-IgE-mediated [8].

While the former necessitated diagnostic tests (SPT), elimination diets, and, if warranted, oral food challenges (OFC) followed by allergist referral, the latter, comprising milder or moderate forms of delayed-onset, non-IgE-mediated CMPA, can be managed within primary care settings. The objective of this practical guide was to delineate the canonical algorithm requisite for CMPA diagnosis, implementation of elimination diets, and, predicated on clinical response, home-based food reintroduction in negative cases, or continuation of dietary restriction followed by a gradual milk reintroduction to evaluate tolerance.

Particularly for non-IgE-mediated CMPA, the gradual reintroduction protocol, known as the milk ladder (ML), was advocated. This entails commencing with minute quantities of purportedly less allergenic foods (because they contain low amounts of milk protein cooked extensively (over 180°C) and for a long time (over 30 minutes), and progressively advancing to potentially more allergenic options (due to the higher protein content of milk cooked at lower temperatures and for less time), with the ultimate goal of assessing tolerance. Notably, evidence suggesting that the introduction of extensively cooked milk-containing foods has a potential tolerogenic effects on subsequent raw milk and informed the ladder's construction, comprising 12 steps ranging from the less allergenic form of milk, such as baked milk (e.g. biscuits) to pasteurized milk. An example of a Milk Ladder is shown in Figure 1.

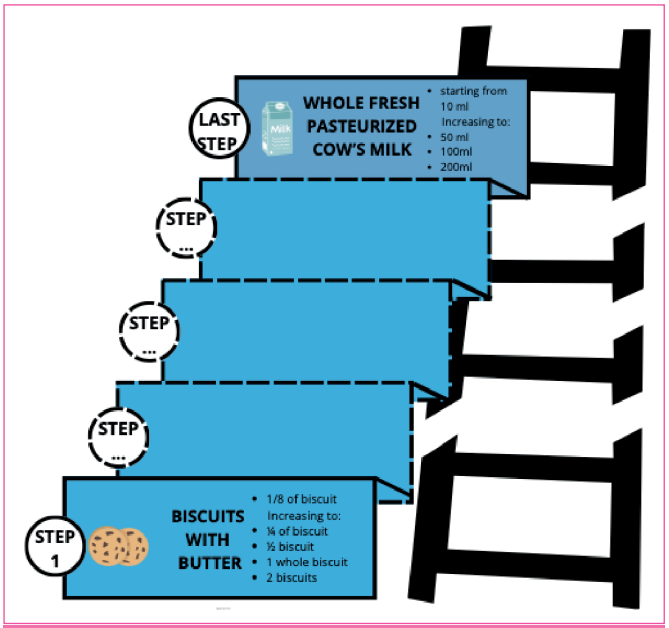

FIGURE 1. Example of Milk Ladder. Starting from the initial proposal of a food containing cow's milk in a less allergenic form, because it is in very low doses and in processed form, passing through other steps, variable in number and composition in the various proposals of literature, until reaching the gradual introduction of fresh whole cow's milk.

Over the ensuing years, these pragmatic directives gained traction beyond the confines of the United Kingdom, transcending non-IgE-mediated allergies to encompass other allergy types. Consequently, in 2017, the UK NICE guidelines for Milk Allergy in Primary Care (MAP) underwent revision, presenting an internationally applicable diagnostic and therapeutic algorithm (iMAP). This updated framework stratified clinical presentations into four categories, delineating criteria for allergen reintroduction tests, emphasizing their applicability solely to children with mild to moderate non-IgE-mediated CMPA, and excluding severe forms such as food protein-induced enterocolitis syndrome (FPIES) and IgE-mediated CMPA [9]. Additionally, this second iteration of the ML underwent simplification and modification, factoring in parameters such as cow's milk protein dosage, cooking duration, temperature, matrix effects, and dietary habits across diverse geographical regions. The updated ladder now comprises a streamlined sequence of six steps. Given the variegation in dietary habits globally, several nations tailored the iMAP to suit their specific dietary customs, as exemplified by adaptations observed in the work of Venter [10] and Hicks [11].

FACTORS AFFECTING MILK ALLERGENICITY

Influence of heating on allergenicity

Cow's milk contains approximately 30-35 grams of protein per liter. These mainly belong to two different categories based on their solubility at acidic pH: caseins (αS1-casein, αS2-casein, β-casein, and k-casein), which *account for 80%* of the *total,* and serum proteins (including the main β-lactoglobulin [β-LG], α-lactalbumin [α-LA]), which make up the remaining 20% [12].

The process of cooking can change protein allergenicity, mainly through alterations in protein folding, protein aggregation, or through chemical changes in molecular structure.

These modifications can change the form through which allergens are presented to the immune system during both the sensitization phase and the elicitation phase of the immune response [13].

Protein changes depend on several CMPA factors related to the cooking process, including cooking temperature and duration, as well as the intrinsic characteristics of the protein [14]. In CMPA, proteins with sequential epitopes, i.e., characterized by a specific linear amino acid sequence, are less sensitive to heat than conformational epitopes, i.e., formed by amino acid residues that are not contiguous but located in different areas of the polypeptide chain [15]. The cooking process initially results in a loss of the protein's tertiary structure and subsequently the secondary structure (55-70°C), disulfide bridge rupture (70-80°C), formation of new intra- and intermolecular interactions, rearrangement of disulfide bridges (80-90°C), and formation of aggregates (90-100°C) [16].

Heat treatment of cow's milk can change the tertiary structure of the serum proteins and make their conformational epitopes no longer detectable [17]. However, depending on the characteristics of the heat treatment to which cow's milk is exposed, a portion of serum proteins may still have a molecular structure capable of binding IgE, albeit with reduced affinity [13,18].

Caseins, on the other hand, are stable allergenic proteins that can withstand heat after 120 minutes of boiling at 90°C; caseins are also found to be present after matrix cooking at 350°F (176°C) [17] albeit reduced in quantity by about 30% [19]. This means that even if prolonged, heat exposition only partially reduces casein's ability to cause allergic reactions. In CMPA they have a structure with a predominance of linear epitopes that are little affected by heat [20] and therefore the IgE binding capacity of caseins is largely unaffected by the cooking process [21,22].

Influence of matrix on allergenicity: the Matrix effect

In addition to the cooking effect, related to the temperature reached by the food and the duration of the process, the presence of a matrix within which the food is cooked (e.g., wheat matrix) can further modify its allergenicity. The matrix, for example, may hinder protein digestion at the gastric level, promoting the preservation of allergenic epitopes and gut-level interaction with the immune system [23].

Furthermore, at the time of food preparation, allergenic proteins may interact with other macromolecules in the same food, including CMPA and carbohydrates, becoming less available for interaction with the immune system [23]. Among these interactions that occur during food processing, the Maillard reaction is one of the most significant: nonenzymatic glycation occurs between the free amino acid groups of the proteins involved and the aldehyde or ketone groups of the carbohydrates [13], which can lead to a reduction in the allergenicity of some foods [24,25].

The enzymatic effect of the fermentation

In addition to cooking, other steps in the processing of some foods, including the use of enzymes in the production of cheese, or the addition of bacteria involved in yogurt preparation and responsible for the fermentation process, can also reduce the allergenicity of proteins [26].

Milk Ladder: the rationale

ML works on the general principle of starting with the introduction of the smallest amount of the allergen, made less allergenic by cooking (e.g. biscuits, muffins, etc.) or fermentation (e.g. yoghurt, cheese, etc.) and, as the child tolerates it, progressing up the ladder to more allergenic forms and larger amounts, and finally intact milk proteins. In CMPA, it is believed that the ability of milk to induce allergic reactions in children with CMPA increases with the increase of both the allergenicity of these proteins and the amount of the milligrams of milk protein consumed. For these reasons, the first ML was built with a 12-step diet with incremental milk dose, starting with minute quantities of purportedly less allergenic foods such as malted milk biscuits and progressively advancing, with an up-built step process, through less and less heated preparations containing higher amounts of milk protein, such as muffins, scotch pancakes and lasagna, and finally fermented dairy products such as yogurt and uncooked milk, with the ultimate goal of assessing tolerance.

Cooking and the matrix effect on milk may also play an important role in the development of tolerance [27]; changes in protein structure and destruction of conformational epitopes may indeed result in reduced binding to IgE and prevent the onset of allergic symptoms [28].

In conclusion, it is believed that ML is essentially an oral immunotherapy, although not always labelled or recognized as such [29].

It was hypothesized that the baking process alters the stability of milk allergens and renders them less allergenic through decreased IgE binding. Regular consumption of these baked milk products decreases IgE levels and increases IgG4 levels, facilitating the acquisition of tolerance as the degree of allergenicity increases through the ML [30].

TABLE I. Tolerance of milk baked in wheat matrix in the form of muffins in children with CMPA according to grams of milk proteins.

Author	Reference	Children (n)	Grams of Milk proteins in the muffin	Tolerance (%)
Nowak-Wegrzyn, 2008	5	100	1.3	68
Kim, 2011	6	88	1.3	74
Weinbrand-Goichberg, 2017	35	85	1.3	82
Agyemang, 2018	36	84	1.3	72
Sirin Kose, 2019	34	91	1.3	75.5
Vilar, 2021	31	44	1.3	72.2
Kilic, 2021	38	80	2.6	62.5
Barbosa, 2017	37	30	2.8	46.7
Kiykim, 2019	39	15	3.2	60

CLINICAL STUDIES SUPPORTING MILK LADDER

The baked milk step

Baking degree and amount of milk: The rationale for the lower allergenicity of baked milk in vitro is supported by in vivo studies, which show that cow's milk in a baked form is tolerated by a relatively high proportion of milk-allergic children, with tolerance rates at about 70 to 80%. Although the recipe used to prepare the baked goods varied among the different studies, it is important to note that most of them employed milk baked in a wheat matrix in the form of muffins or cupcakes, containing a total amount of milk equivalent to 1.3 g of cow's milk protein per serving, with baking for at least 30 minutes at 180°C (350°F) [31,32,33,34,35,36]. Therefore, it is not surprising that tolerance rates to baked milk in fresh milk-allergic patients reported by the clinical trials are similar, ranging from 68 to 82%.

However, even with the same temperatures and matrix, as the milk content doubles, the rate of tolerance decreases, ranging from 46.7 to 62.5, as shown in the three studies that used > 2.6 gr or higher of milk protein [37,38,39] (Tab. I).

Some clinical trials have attempted to assess tolerance in vivo by performing successive OFCs with gradually more allergenic dairy products (both less cooked and containing increasing amounts of milk protein), starting with baked products. For instance, Nowak and colleagues challenged 136 children with IgE-mediated CMPA by performing sequential short-term OFCs with foods containing increasing amounts of milk and gradually less exposed to the effect of heat (muffins and pizza on the same day, the others all within 2 weeks in total). Thirty percent of the children reacted to muffins containing baked milk (containing 1.3 g CMP and baked at 180°C for at least 35 minutes), 23% tolerated muffins but later reacted to pizza (containing 4g CMP and baked at 218°C for at least 13 minutes), 8% tolerated both but reacted to rice pudding (containing 7.7 g CMP and baked at 160°C for at least 90 minutes), 32% reacted only to fresh milk (containing 8g CMP), and 7% also tolerated fresh milk [40]. However, it should be noted that in this study, in the various passages, the amounts of CM proteins administered also increased, which could represent a bias.

The baked + wheat matrix effect

An Italian trial [41], investigated the importance of the wheat matrix effect on reducing baked milk allergenicity, sequentially challenging 48 children with IgE-CMPA with a) ciambellone (a typical Italian baked cake) containing 3 g of cow's milk protein in wheat matrix, b) baked liquid cow's milk or c) partial hydrolyzed milk (HA milk) containing 6 g of protein or finally d) with Parmesan cheese containing 3 g of milk protein. In the study case series, 82% of children passed the challenge with HA formula, 81% with ciambellone, 78% with Parmigiano Reggiano and 56% with the OFC with baked liquid milk. A statistically significant difference ($p < 0.05$) was found among OFCs positivity rates, comparing ciambellone (39/48 = 81%) and baked liquid CM 18/32 = 56%). The authors concluded that the wheat matrix effect on tolerance of baked milk was relevant in slightly less than half of the cases.

THE MILK DERIVATIVE (DAIRY PRODUCTS) STEPS

Parmigiano cheese

In an Italian study [42], children with clinical reactivity to cow's milk (tested by DBPCFC) underwent OFC with Parmesan (Parmigiano Reggiano cheese aged 36 months), in an amount of 13.3 g, containing CMP equivalent to 200 ml of cow's milk. The study showed that 29 out of 50 children (58%) were able to tolerate that Parmesan. Also,

in another study 25 children with a history of IgE-mediated CMPA undergoing OFC with 36-month maturated Parmesan (10 g, equal to 3 g of proteins), 64% successfully passed the test [41].

Parmigiano Reggiano is a typical Italian cheese with a long aging process, varying between 12 and 36 months. During aging, proteases from rennet and lactic acid bacteria implement gradual and progressive digestion of milk proteins, particularly caseins. Therefore, the protein fraction constantly changes during cheese aging, shifting from whole proteins to gradually shorter peptides to single amino acids [42].

For this reason, longer aging can presumably reduce the allergenicity of the proteins contained in Parmigiano; however, to date, no studies comparing clinical tolerance between Parmigiano samples with different aging periods are available in the literature.

In addition, it should be noted that the Parmigiano Reggiano production process also includes a cooking phase at 55°C before aging, which could play an additional role in reducing the allergenicity of the final product.

The Butter step

Butter is a dairy product obtained by the separation of whole milk into cream and buttermilk, with a reduced milk protein content.

To our knowledge, only one trial has evaluated butter tolerance in patients allergic to cow's milk [43]. This study showed that most cow's milk allergic patients can consume raw butter without clinical reactions. 68 patients with IgE-mediated CMPA were enrolled; out of 44 patients with positive OFC for cow's milk (in the form of pumpkin pie, containing 25 ml of cow's milk, microwaved at 1,000 W for 90 seconds at a temperature of 89°C), 38 (86.4%) tolerated butter instead, in the amount of 10 g (with protein content equivalent to 2.9 ml of cow's milk).

In light of this result, the authors suggested that OFC with butter should be performed in patients who are expected to react to cow's milk, before performing OFC for milk, to be able to expand the range of foods allowed in their diet and thus improve their quality of life. It should be noted, however, that the amount of protein provided by OFC with butter was only a little more than one-tenth of that given during OFC with milk.

The Yogurt step

Yogurt is a dairy product made by bacterial fermentation of milk. Specifically, it is made by combining heated milk with bacteria, especially *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and letting it sit for several hours at a temperature of about 45°C. In addition, other Lactobacilli and Bifidobacteria are sometimes added in the preparation of yogurt. Yogurt cultures, which ferment and acidify milk, reduce the amount of intact protein in milk and may therefore facilitate CMP tolerance in individuals reactive to cow's milk. Changes in the allergenicity of different milk proteins depend on the species of lactic acid bacteria and specific fermentation conditions, as shown by several dairy technology studies [44,45,46].

Regarding studies on IgE-mediated CMPA case series, the results are conflicting. Monaco and colleagues report that 20/31 (64%) children with IgE-mediated CMPA with systemic symptoms could tolerate a total dose of 200 g of yogurt [47].

In the trial by Poza-Guedes [48],40 children with IgE-mediated CMPA with immediate gastrointestinal CMPA (having excluded children of extraintestinal manifestation of CMPA, lactose intolerance and celiac disease) underwent to OFC with CM. Patients with a positive OFC result was e offered a new OFC with yogurt under the same conditions. Patients who refused the OFC with yogurt were assigned to an elimination diet. All 25 patients with IgE-mediated CMPA diagnosed by positive OFC with fresh milk, passed OFC with yogurt. No reactions to yogurt were recorded at the 6 month follow-up and mean levels of serum IgE to beta-lactoglobulin (β-LG) and caseins decreased, but were increased in the 15 children that maintained CM diet. Since BLG is absent or very decreased in many yogurt [49] and BLG seems to have a role in the gastrointestinal phenotype of cow's milk allergy [50], the authors suggest trying with yogurt in children with immediate gastrointestinal CMA.

In contrast, other studies included samples of children with both IgE and non-IgE-mediated allergies. In Uncoglu's study [51], 15 out of 16 patients with IgE-mediated CMPA reacted to OFC with yogurt, while 11 out of 16 patients with non-IgE- mediated CMPA tolerated yogurt.

It should be noted that 60% of the subjects with IgE-mediated CMPA who reacted to yogurt had a concomitant egg allergy, which might play a role in the reduced tolerance rate.

In addition, the total amount of yogurt proposed was equivalent to 8-10 g of cow's milk protein (about 200-300 g of yogurt). Moreover, the authors hypothesized that, similarly to children with non-IgE-mediated CMPA who develop tolerance to fresh milk earlier than their counterparts with IgE-mediated CMPA, tolerance to fermented milk could also be achieved earlier in children with non-IgE-mediated CMPA [52].

A study by Kucukosmanoglu et al. [53] considered 34 children allergic to cow's milk protein (diagnosed by positive OFC for cow's milk) aged 24 +/- 13 months, but with both concomitant positive IgE (in 29 children) and negative IgE (in 5 children). The same children underwent a second OFC with yogurt 15 days later, and half (17/34) passed it without symptoms (Tab. II).

These results suggest that performing OFC with yogurt could be considered early, in children with non-IgE mediated CMPA, as yogurt can represent a valid dietary alternative to cow's milk. In addition, its reintroduction into the diet would allow only partial avoidance of cow's milk products, improving patients' quality of life and diet quality. Yogurt and cheeses could promote milk tolerance as they have beneficial probiotic-like health effects, including reinforcement of the epithelial barrier by increasing mucins, tight junction proteins, and Goblet and Paneth cells [54].

Evidence of efficacy and safety of Milk Ladder in literature

Since there is no accepted definition of ML, we have defined ML as "any process that includes the administration of milk or

TABLE II. Tolerance of Yogurt in Children with CMPA.

Author	Reference	Study population	Grams of yogurt (total intake)	Grams of Milk Protein Content (total intake)	Tolerance (%)
Monaco, 2019	47	IgE mediated with systemic symptoms	200	8	64%
Poza-Guedez, 2016	48	IgE mediated with gastrointestinal symptoms	NA	NA	100%
Uncuoglu, 2017	51	IgE and non- IgE CMPA	200-300	8-10	6.2% IgE 31.2% non-IgE
Küçükosmanoğlu, 2018	53	IgE (29) and non-IgE (5) CMPA children	133,4	4,3	50%

foods containing milk, progressively increasing both in terms of quantities and allergenicity in 3 or more steps, including the final administration of raw milk". A comprehensive search was conducted in Medline via PubMed and Scopus (from January 1, 2000, through May 10, 2024), using the keywords "milk allergy" or "food allergy" and ("baked milk" or "processed milk" or "cooked milk" or "heat modified milk" or "extensively heated milk" or "heated milk") AND ("ladder" or "tolerance" or "desensitization" or " immunotherapy" or "safety").

In non-IgE Mediated CMPA

Several studies have investigated the efficacy and safety in ML. However, to the best of our knowledge, even if ML was designed specifically for non-IgE-mediated CMPAs, only one study has evaluated its efficacy and safety in this kind of CMPA.

Mayer et al. [55] designed a prospective observational study to evaluate the mode of reintroduction of milk or other foods in subjects with gastrointestinal allergies excluding FPIES. The authors used a ladder-like allergen reintroduction, with a four-step scale to be climbed in 13-15 days. They found complete tolerance acquisition in 46% and partial tolerance in 36% of milk-allergic subjects. Of the 62 enrolled children, 42 had completed milk diet liberalization, and 33 had achieved partial liberalization, while 17 remained allergic at the end of the study. No adverse events were documented. In more than 50% of cases the first administration was sufficient to establish allergy status, but many required 2–5 attempts before the outcome was clear.

In IgE Mediated CMPA

In contrast, many studies investigated the efficacy and safety of ML in children with IgE-mediated CMPA. Seven were controlled studies (2 RCT, 2 prospective case-control, 3 retrospective case-control) (Tabs. III, IV).

The first RCT, by Amat et al. [56], enrolled children with cow's milk allergy confirmed with a positive DBPCFC. The aim of the study was to prospectively evaluate the efficacy and the safety of two OIT protocols in a cohort of children with persistent IgE-CMPA: a classic oral desensitization with fresh milk, called "high risk" vs a ML approach with a slow progression schedule starting with baked milk and then less and less heated milk over time (with a presumed lower risk of side-effects). The doses were administered daily at home and up dosing was performed under medical supervision up to a tolerance final dose of 2720 mg (around 200 ml) of cow's milk proteins in a 5 month period. b) the second group (n = 23), were invited to assume a ML with 3 steps, starting with baked cow's milk, such as biscuits, then less cooked such as cow's milk bars, and finally fresh milk. The quantity of cow's milk proteins was gradually increased each two weeks at home. The passage from baked milk to less cooked was realized after the dose of 210 mg of cow's milk proteins was achieved and from less cooked to fresh after reaching tolerance to 1970 mg of proteins. Even in this group, the goal was the achievement of tolerance at 2720 mg of fresh cow's milk proteins but over a longer period of 9 months. The study did not report differences between the two groups in quantity of cow's milk proteins reached or in the number and severity of reactions.

The second RCT by Esmaeilzadeh et al. [57], aimed at finding out whether baked milk products could accelerate the acquisition of tolerance of milk. 84 children with CMPA who tolerated baked milk in form of muffin OFC were randomized into two groups: the active group was asked to consume baked milk in the form of muffin for 6 months (with 1.3 g of milk's proteins) and then to consume baked cheese in the form of pizza in for 4 to 7 days per week for another 6 months. The control group was instructed to strictly avoid any milk products for one year. At the end of the study period, 88.1 % of the patients in the case group and 66.7 % of those in control

TABLE III. Milk Ladder Efficacy in IgE Mediated Cow's Milk Protein Allergy.

Authot/year Country	Reference	Design	Sample size	Diagnosis of food allergy	Control group	Ladder Protocol	Results
Amat 2017 France	56	Prospective RCT	41: AG: 23 CG: 18 Drop out: 4	Positive DBPCMC and sIgE ≥ 0.35 kU/L	Oral desensitization with raw milk. Schedule steps under medically controlled OFC every 5 weeks for 5 months	3 steps at home every 15 days	The study did not-report differences between the two groups in the quantity of cow's milk proteins reached: 15 patients resulted tolerant (36.6%), 11 were partially tolerant (26.8%) with a median threshold of tolerance of 697 mg (27.2-2550 mg), and 15 children (36.6%) were non-responders
Esmaeilzadeh 2018 Iran	57	Prospective RCT	84: AG: 42 CG: 42 Drop out: 0	Positive history of IgE mediated milk allergy plus positive SPT (more than > 8mm) or positive sIgE (> 5 kUA /L) in children < 2 years or >15 kUA /L for those > 2 years	Strict elimination diet for milk and dairy products	3 steps first OFC in hospital with BM followed by 2 steps at home every 6 months	Tolerant to raw milk: AG: 86%, CG: 52; p = .003 Time of tolerance: AG: 36 months (95% CI, 34.5-49.7) CG: 98 months (95% CI, 82.4-114.1); p < .001
Nowak-Węgrzyn 2018 USA	58	Prospective Case-control	170 AG: 136 CG: 34 Drop out: 32 AG 12 CG	Positive SPT or sIgE with convincing clinical history or elevated sIgE value over positive predictive values or DBPCMC in the previous two years	Children in strict elimination diet for milk and dairy products whose parents chose not to participate in the active intervention arm	4 steps under medical controlled OFC	The 48% (41 patients) of both active groups of 136 patients enrolled, became tolerant independently from the rapidity of progression of the ladder scale (6 months *vs* 12 months), whereas no subject in the control group resulted tolerant at the OFC performed at the end of the study
D'Art 2022 Ireland	59	Prospective Case-control	60: AG 40 CG 20 Drop out: AG 4 CG 1	A recent history of adverse reactions in association with positive allergy tests	Children who were submitted to ML without a previous supervised single-dose challenge	12 steps at home	No difference was seen between 6 *vs* 12 months escalations. Tolerant to raw milk: AG: 41 (48.2%; AG6: 61% and AG12: 73%) CG: none
Kim 2011 USA	60	Retrospective Case-control	149 AG: 89 CG: 60 Drop out: 11	Positive SPT or sIgE, and history of an allergic reaction to milk within 6 months; or sIgE levels or SPT >95% predictive for clinical reactivity (> 5 kUA /L in children < 2 years or >15 kUA /L for those > 2 years, SPT ≥ 8 mm)	Strict elimination diet for milk and dairy products	3 steps OFC under medical controlled every 6 months	Forty-one (59%) active subjects had unheated milk tolerance in contrast to 13 (22%) subjects in the comparison group. Subjects who underwent active treatment (the per-protocol group, which excludes those with persistent baked milk reactivity) were 16 times more likely than the comparison group to become unheated milk tolerant (p < 0.001)

(continues)

TABLE III. *(follows).* Milk Ladder Efficacy in IgE Mediated Cow's Milk Protein Allergy.

Authot/year Country	Reference	Design	Sample size	Diagnosis of food allergy	Control group	Ladder Protocol	Results
Efron 2018 Israel	61	Retrospective Case-control	110 AG:43 CG: 67 Drop out: 5 from AG (11.6%)	Positive OFC or positive SPT o sIgE with a value above 95% CI for age with convincing clinical history	Strict elimination diet for milk and dairy products	5 steps under medical controlled OFC every 3 months	The results showed a faster acquisition of tolerance in the ladder-treated group, 36 months compared to 98 months in the control
Dunlop 2018 USA	62	Retrospective, case control	206 children	86% of the patients included had had a prior reaction to milk and all were strictly avoiding all forms of milk at the time of challenge	Self-selected avoidance group of patients who failed the initial baked milk challenge	Indefinite steps (1 OFC with baked milk in the Hospital, then a ML like introduction at home without fixed steps until raw milk)	After a median of 49 months of follow-up, 43% of the 187 had progressed to direct milk, 20% to less-cooked forms of milk, 10% remained ingesting BM, and 28% were strictly avoiding milk

RCT: randomized controlled trial, AEs: adverse reactions, AG: active group, CG: control group, DBPCMC: double-blind placebo-controlled milk challenge, sIgE: specific IgE, OFC: oral food challenge, SPT: skin prick test, BM: baked milk, AG6 and AG12: active group with increase every 6 months or 12 months, CI: confidence interval, MP: milk proteins

group had developed tolerance to unheated milk (p-value: 0.018). In the control group, 11.9 % had a history of anaphylaxis to milk products; all of these patients remained reactive in unheated milk OFC at the end of the study. Within the case group, 40.5 % had a history of anaphylaxis; among these, 41.1 % (7/17) had developed milk tolerance by the end of the study. The authors did not present safety data but stated that considering the small number of patients who reacted to baked milk products during the study, baked milk exposure for patients with CMPA can be introduced as a safe therapeutic alternative method to OIT.

The third study by Nowak-Węgrzyn et al. [58] aimed to investigate the effect of more frequent versus less frequent introduction of a ML approach or higher doses of more allergenic (less heat denatured) forms of milk on progression to tolerance. The authors enrolled children with history of adverse reaction with cow's milk or with a positive DBPCMC associated with positive allergic tests or with specific IgE or skin prick tests predictive of adverse reaction. The patients underwent to a four step ML approach. In every step cow's milk was introduced with an OFC. The first step was baked CM (muffin), then pizza, rice pudding and finally non-cooked CM. During the first day, up to two foods were tested, muffin and pizza. If a child did not react to pizza, he/she would then return within 2 weeks and undergo rice pudding and non-baked liquid-milk-OFCs. When a reaction appeared, tests were interrupted, and patients were sent home with the indication of assuming the last tolerated food regularly. They returned after 6 months or 12 months to repeat the CMPA failed OFC and then to proceed towards the OFC with foods containing less cooked milk up to fresh milk. The principal goal was to evaluate the number of patients who underwent OFC every 6 months and those examined every 12 months who reached tolerance during the 36 months of the study. The results of both groups were confronted with a control group of children whose CMPA failed the initial OFC with muffins and who continued with the elimination diet. Altogether, 48% in both active groups became tolerant independently from the rapidity of progression of the ladder scale (6 months vs 12 months), whereas no subject in the control group resulted tolerant at the OFC performed end of the study. During baseline OFC and dose escalation 35% of adverse events occurred. Epinephrine was required for some reactions only during muffin and pizza OFC. No adverse reactions requiring intramuscular adrenaline occurred during the home reintroduction program, and no cases of eosinophilic esophagitis were reported.

In D'Art 's study [59], children < 12 months were enrolled and the diagnosis of CMPA was made based on a recent history of adverse reaction to the food in association with positive allergy tests. The aim of the study was to evaluate the impact of gradual home introduction of foods containing cows' milk. All subjects included in the study were subjected to the reintroduction of CMP using a ladder scale, but one group of these, obtained employing 2:1 randomization, had previously undergone the administration of a previously validated and normally tolerated dose of milk of 0.1 ml. The ladder scheme consisted of 12 steps, starting with the introduction of baked food with a malted biscuit, and continuing with increments performed in a protected environment of the protein dose of the less processed food, the muffin, pancake, lasagna, pizza, yogurt, cheese, up to an amount of 200ml fresh milk. Subjects who had initially undergone the OFC with 0.1 ml achieved partial liberalization of the diet more quickly (73%

TABLE IV. Milk Ladder Safety in IgE Mediated Cow's Milk Protein Allergy.

Author/year Country	Reference	Age (mean) Case *vs* controls	Safety	Reactions/Epinephrine	Reactions/Epinephrine at home	Eosinophilic esophagitis
Amat 2017 France	56	4 years *vs* 7 years (OIT)	There were not any differences even on the number and severity of reactions. One subject per group abandoned the study for severe adverse reactions	Adrenaline was necessary in five cases: two from the "high risk" group and three in the "low risk" group	Epinephrine use at home was similar in both arms: 11.8% (n = 2) in the OIT arm *vs* 14.3% (n = 3) in the ML like group (p = 1)	Not reported
esmaeilzadeh 2018 Iran	57	2.2 years *vs* 1.2 years (avoidance)	The Authors did not present safety data but stated that considering the small number of patients who reacted to BM products during the study, BM exposure for patients with CMPA can be introduced as a safe therapeutic alternative method to OIT	Not reported	Not reported	Not reported
nowak-Węgrzyn 2018 USA	58	7 years *vs* 6,6 (avoidance)	35% of adverse events occurred during the baseline OFC or escalation OFCs in the clinical center	16 children (39%) developed reactions during muffin baseline OFC and were treated with 20 administrations of epinephrine, 6 (19.4%) after the pizza challenge were treated with 10 administrations of epinephrine	No adverse reactions requiring intramuscular adrenaline	Not reported
D'Art 2022 Ireland	59	Less than 12 months old	There were no serious reactions progressing up the ladder. There were only 3 non-tolerated accidental exposures to milk during the study	Notreported	No reactions at home required adrenaline;	Not reported
kim 2011 USA	60	6.6 years *vs* not reported (avoidance)	6/89 (6.7%) had mild-to-moderate anaphylaxis during 8/172 (4.6%) challenges	Epinephrine was administered during 172 subsequent challenges at a higher rate among the BM-reactive group than among the BM-tolerant group (17% *vs* 3%), p = 0.04. 6/89 (6.7%) had mild-to-moderate anaphylaxis during 8/172 (4.6%) challenges, 2 subjects twice to the same food, one to muffin (54 months apart) and the other to pizza (9 months apart). 3 subjects had anaphylaxis after ingestion of 100% of the serving, which was pizza in all 3 cases	Not reported	Two (3.1%) male subjects in the AG had EoE. Five (8.3%) subjects in the comparison group reported EoE while strictly avoiding milk
Efron 2018 Israel	61	17 months started and followed to 40 months *vs* 70 months followed (avoidance)	Sixteen children (37%) in the intervention group had an allergic reaction recorded during the protocol (10 reactions occurred during OFC). One was hospitalized	Not reported	9 children (21%). Most reactions were mild such as minor rash around the mouth or tingling sensation of the tongue, with 2 children requiring an oral antihistamine. 2 children who reacted at home and were treated with epinephrine	Not reported

(continues)

TABLE IV. (*follows*). Milk Ladder Safety in IgE Mediated Cow's Milk Protein Allergy.

Author/year Country	Reference	Age (mean) Case *vs* controls	Safety	Reactions/Epinephrine	Reactions/Epinephrine at home	Eosinophilic esophagitis
Dunlop 2018 USA	62	6,8 years *vs* 8.8 years (avoidance)	There were 79 reported milk reactions involving 68 patients (33% of total) during follow-up. Of these, 78% were classified as mild, 14% severe	Of 11 severe reactions, 4 were accidental exposures, 3 were planned escalations, and 4 occurred with previously tolerated doses. None of the patients with severe reactions received epinephrine during their in-office challenge	While many patients gave no single reason for stopping the BM introduction 8 patients reported reactions to more concentrated forms of milk, often by accident, which led to discontinuation of all milk ingestion. Eight reactions were treated with epinephrine, including 2 accidental exposures, 2 with planned escalations, and 4 occurring with previously tolerated doses.	6 patients developed EoE

OIT: oral immunotherapy, BM: baked milk, CMPA: cow's milk protein allergy, EoE: Eosinophilic esophagitis,

vs. 50%) and more subjects were fully tolerant (30% *vs* 10% of controls) at 12 months. During the study, there were no severe or unexpected reactions, with only mild allergic symptoms that did not hinder the continuation of the reintroduction program. No subject received intramuscular adrenaline. Kim's study [60] was a controlled study that enrolled 89 subjects affected by CMPA. Starting by hypotheses that children with CMPA include two phenotypes, the milder one was tolerant of baked milk products but not unheated milk, whereas those with the more severe phenotype were baked milk reactive, they hypothesized that children with the milder phenotype of milk allergy would be able to ingest baked milk products daily without negative effects on the development of tolerance to unheated milk. The 3 step ML like introduction started with baked milk. Baked milk tolerant were instructed to incorporate baked milk products daily into their diets and after 6 or more months were offered challenges to baked cheese (pizza) products. After 6 or more months, baked cheese–tolerant children were offered challenges to unheated milk. Controls were 60 children retrospectively gathered and matched for age, sex, specific IgE value who fulfilled the inclusion criteria but were not initially challenged to baked milk products. Forty-one (59%) active subjects had unheated milk tolerance in contrast to 13 (22%) subjects in the comparator group. Subjects who underwent active treatment (the per-protocol group, which excludes those with persistent baked milk reactivity) were 16 times more likely than the comparison group to become unheated milk tolerant ($p < .001$). Epinephrine was administered at a higher rate among the baked milk–reactive group than among the baked milk–tolerant group (17% *vs* 3%, p 0.04). Two (3.1%) male subjects in the active group had eosinophilic esophagitis (EoE). Five (8.3%) subjects in the comparison group reported EoE, which developed while strictly avoiding milk.

The Efron's study [61] proposed a scale consisting of 5 steps from baked milk to fresh milk. It was a retrospective case-control study where children with CMPA who have passed an OFC with baked milk were treated with a ladder (n = 43) and were compared with a control group (n = 67) whose CMPA failed OFC with baked milk who were treated with strict avoidance until 4 years of age. The aim of the study was to evaluate the efficacy and safety of a ML approach on tolerance acquisition. The results showed a CMPA faster acquisition of tolerance in the ML treated group, 36 months compared to 98 months in the control. Sixteen children (37%) in the intervention group had an allergic reaction recorded during the ML protocol.

Finally, the work by Dunlop et al. [62] was designed to describe a long-term follow-up after baked milk introduction. The authors performed a retrospective chart review of consecutive BM OFCs performed in their clinic. Children had to pass challenge to a 2 gram (¼ cup) baked milk (usually a cake or muffin). The avoidance group was composed of patients whose CMPA failed the initial baked milk challenge. ML approach was without fixed steps, starting from baked milk and, after 2-3 months of this intake, gradually increasing quantities while

reducing the cooking degree, such as pancakes and waffles, then progressing to oven baked cheese, and eventually uncooked dairy products if there are no symptoms in prior steps. The study collected data on 107 children who tested positive for milk oral challenge, with 19 placed on an elimination diet due to the severity of clinical reaction to the provocation test, and 88 gradually invited to consume increasing quantities of milk, starting from well-cooked and then less processed until fresh milk. Of the latter group, 29% could tolerate fresh milk at the end of follow-up, and 33% could consume a certain quantity of cooked cheese, while 38% remained on an elimination diet. There were 79 reported milk reactions involving 68 patients (33% of total) during follow-up. Of these, 78% were classified as mild, 14% as severe, and 6 patients developed EoE.

DISCUSSION

ML has been designed to get gradual home reintroduction of CM into the diet of children affected by non-IgE mediated CMPA, except for FPIES, to confirm milk allergy or tolerance, and in this way avoid OFC in the hospital. The main advantages expected from ML *vs* immunotherapy are CMPA facilitating the early reintroduction of milk and dairy products into the diet, reducing the wait and costs for an OFC in the hospital, reducing dietary restrictions, indicating a list of foods containing milk starting from the most easily tolerated up to whole milk and thus reducing the risk of serious reactions and CMPA facilitate the development of tolerance [63]. Moreover, it was highlighted that it may reduce expenses and improve quality of life, reducing special foods and medical costs, anxiety and social limitations [64,65].

The only study performed in the literature in non-IgE mediated CMPA has confirmed that ML appears safe, having excluded IgE sensitization, even if the authors reveal that it was not easy to diagnose the development of tolerance or the persistence of the allergic state in all children [55]. This study was carried out by an experienced gastroenterologist and dietitian at the Gastroenterology Department of Great Ormond Street Foundation and required the instruction of parents in recognizing the symptoms and filling standardized questionnaires with a symptom score appropriate for the diagnosis of a non-IgE mediated CMPA. The results of the study need further confirmations and cannot be extrapolated to other settings.

Regarding IgE-mediated CMPA, even if the use of ML was excluded by the authors in both the first [8] and the second article [9] , the practice has spread and to date 7 controlled studies have been published. The studies are very different from each other both in terms of the objectives and of the ML schemes used: only one study [59] used 12 steps over 12 months starting from 0.1 ml of baked milk, whereas most studies used CMPA faster scales with only 3- 5 steps and one study [62] the first step with baked milk and later an ML approach without fixed steps. Only two studies used to upgrade steps at home [56,59], in the remaining five controlled studies, 3–made all upgrading steps as supervised oral challenges in the Hospital [58 60 61], 2 studies made only the first OFC with baked milk in Hospital and the next step at home [57,62]. In Italy, ML not been widely little followed, and most allergists reintroduce baked milk into the diet with an OFC or an early reintroduction of milk and dairy products into children's diets with oral immunotherapy [66,67].

As efficacy is concerned, the only study that controlled ML approach *vs* OIT [56] showed that the ML approach reaches similar tolerance levels. The other studies that have controlled the effectiveness of ML approach vs avoidance and showed that a significant higher proportion of ML patients reach tolerance earlier than controls who remain on strict diets. However, the great majority of these studies (4 out 5) enrolled children in which tolerance to baked milk had been demonstrated with an in hospital OFC, before starting ML [57,60,61,62]. This is worth of note as some authors suggest that BM tolerance could only highlight a less severe phenotype of CMPA[6,68]. On the other hand, in support of the effectiveness of the ML, other retrospective studies have described success rates higher than 90% [63,69,70].

In term of safety, 6/7 studies presented data on safety. The only study [57] that did not present data on safety suggests that ML is a safe therapeutic alternative method to OIT. However, it is necessary to emphasize that this study enrolled only children with CMPA who tolerated baked milk in the form of muffins.

All the other studies have shown that ML is burdened with side effects, which varies in different studies. Adrenaline was required in 5 out of 6 studies [56,58,60,61,62], with three reporting administration during home-based phases of the ML [56,61,62]. Two studies [58,59] stated that adrenaline was not necessary for the home phase. However, in the first study [58] the upgrading steps were performed in the hospital, where adrenaline was administered and in the second study [59], the mean age was the lower than all the other studies (< 12 months). This would be worth of note, as lower age was associated with success of ML in 2/7 studies [61,62] and the earliest reintroduction of milk it has been suggested to promote the development of tolerance in non-controlled-studies [71] while symptom severity does seem to increase with increasing age [72]. This emphasizes the importance of ensuring that families are well-trained in recognizing severe allergic reactions and know how to administer epinephrine. Additionally, clinicians should assess each child's risk before initiating home-based ML steps, especially in children with a history of anaphylaxis or asthma.

Onset of EoE was reported in 3/7 studies. In Kim's study [60] 3.1% of cases and 8.3% of controls had EoE while in Dunlop's study [62] it was referred only in 6/206 (0.03%) of cases. In the thirs, Nowak-Wegrzyn [58] observed that there were no cases of EoE among the participants in this study.

A systematic review with meta-analysis on baked egg and milk dietary advancement therapy (OIT plus Ladder or for milk and egg) has recently been published [73]. Inclusion criteria were randomized control trials (RCTs), cohort studies, case-control and case-series that include, among others, the words "ladder" and "milk" in their pre-specified PICO. Regarding the ML, it showed that while the meta-analyzed estimates do show high level of desensitization (OR 0.61 (0.33-0.86)) and tolerance (OR 0.69 (0.50-0.85)), compared to the amalgamated placebo estimates, the authors concluded that based on the available data, differences in protocols, patient populations,

study durations, lack of robust controls, and lack of clear study design, no conclusion could be reached if ML ingestion accelerates the development of tolerance. As for safety, based on the available safety outcomes including also single-arm meta-analyzed data, the authors suggest that the ML approach is generally safe. In their conclusions, caveated by very low certainty and high risk of bias, the authors underscore, however, that allergic reactions, including severe reactions at home requiring epinephrine, and unfortunately even two deaths attributable to the ML approach [74,75], do occur, and thus the ML is not without risk.

Some CMPA risk factors have been suggested to increase the risk of reactions during ML including increasing age (or age over 6 years), episodes of moderate-severe anaphylaxis, especially from baked milk, high skin prick test wheal or serum specific IgE levels, low previous reaction threshold, poor asthma control, psychosocial control, etc. [10,76]. Among these, only increasing age, as mentioned above, and milk specific IgE levels (in the Dunlop study [62], even if there was high interindividual variability in these associations) were inversely associated with the success of ML in the controlled studies selected in our review.

CONCLUSION

In non-IgE Mediated CMPAs, a prospective controlled study, in which FPIES and the presence of specific IgE were excluded, showed that the ML approach is not burdened with relevant side effects. Although this observation needs to be confirmed by other studies, considering the advantages of the ML approach, we believe it is advisable to follow it.

In IgE-mediated CMPA, considering that: a) several studies showed that ML approach is burdened, albeit infrequently, by adverse effects, even serious ones; b) there are no predictive CMPA factors to date capable of excluding serious reactions in the individual child; c) there is evidence that the introduction of extensively cooked milk can be tolerated in most children with APLV; d) there is evidence that the continuous introduction of cooked milk is followed by earlier tolerance for raw milk; e) in children with IgE-mediated CMA who do not tolerate unheated and baked milk, it is suggested do not use oral immunotherapy with baked cow's milk [77], we suggest that ML at home should not be routinely encouraged in children with IgE-mediated allergy. If used to perform the first step with baked milk as supervised, oral challenges in the hospital seems advisable. The next steps could be one of the following if BM is tolerated, and it is possible to continue BM administration (and eventually try to introduce other milk derivatives such as parmesan or yogurt with OFC) and subsequently verify the development of CM tolerance or to continue an ML approach, only in children without a clinical history of severe reactions or recurrent wheeziness starting as early as possible and anyway not in children > 6 years old, until the gradual introduction of raw cow's milk is obtained.

If BM is not tolerated, start an oral immunotherapy with milk.

In all three ways, allergic reactions, even serious ones, are possible and therefore must be performed with the same precautions as oral immunotherapy. Thus, parents should be informed regarding the risks of these procedures as well as the ongoing availability of emergency medications and prompt treatment of reactions, even while eating forms and doses of milk that have previously been tolerated. Further prospective controlled studies are needed to clarify which of the three is the best and/or is most suitable for the different phenotypes of CMA. Until then, the choice of which of the three paths to follow remains entrusted in the individual case to the allergist based on age, and clinical history, considering the preferences of the parents and, when possible, of the child.

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The authors declare that they have no competing interests.

Authors' contributions

Conceptualization, MC; CA, BC, ED; methodology, MC; CA, BC, ED; writing-original draft preparation, MC, CA, BC, ED, FD, GCI, EM, FM, VN, EP, GG, MUAS; writing-review and editing, MC, CA, BC, ED, FD, GCI, EM, FM, VN, EP, GG ,MUAS; All authors made substantial revision of the manuscript and approved the final version.

References

1 Schoemaker AA, Sprikkelman AB, Grimshaw KE, et al. Incidence and natural history of challenge-proven cow's milk allergy in European children -Euro Prevall birth cohort. Allergy 2015;70:963-972. https://doi.org/10.1111/all.12630

2 Wood R, Sicherer SH, Vickery BP, et al. The natural history of milk allergy in an observational cohort. J Allergy Clin Immunol 2013;131:805-812. https://doi.org/10.1016/j.jaci.2012.10.060

3 Novembre E, Gelsomino M, Liotti L, et al. Fatal food anaphylaxis in adults and children. Ital J Pediatr 2024;50:40. https://doi.org/10.1186/s13052-024-01608-x

4 Sicherer SH, Bock SA. An expanding evidence base provides food for thought to avoid indigestion in managing difficult dilemmas in food allergy. J Allergy Clin Immunol 2006;117:1419-1422. https://doi.org/10.1016/j.jaci.2006.01.051

5 Nowak-Wegrzyn A, Bloom KA, Sicherer SH, et al. Tolerance to extensively heated milk in children with cow's milk allergy. J Allergy Clin Immunol 2008;122:342-7, 347.e1-2. https://doi.org/10.1016/j.jaci.2008.05.043

6 Kim JS, Nowak-Wegrzyn A, Sicherer SH, et al. Dietary baked milk accelerates the resolution of cow's milk allergy in children. J Allergy Clin Immunol 2011;128:125-131.e2. https://doi.org/10.1016/j.jaci.2011.04.036

7 National Institute for Health and Clinical Excellence, NICE. Diagnosis and assessment of food allergy in children and young people in primary care and community settings. London 2011. www.nice.org.uk/CG116

8 Venter C, Brown T, Shah N, et al. Diagnosis and management of non-IgE-mediated cow's milk allergy in infancy - a UK primary care practical guide. Clin Transl Allergy 2013;3:23. https://doi.org/10.1186/2045-7022-3-23

9 Venter C, Brown T, Meyer R, et al. Better recognition, diagnosis and management of non-IgE-mediated cow's milk allergy in infancy: iMAP-an international interpretation of the MAP (Milk Allergy in Primary Care) guideline. Clin Transl Allergy 2017;7:26. https://doi.org/10.1186/s13601-017-0162-y

10 Venter C, Meyer R, Ebisawa M, et al. Food allergen ladders: a need for standardization. Pediatr Allergy Immunol 2022;33e13714. https://doi.org/10.1111/pai.13714

11 Hicks A, Fleisher D, Venter C. The future of cow's milk allergy – milk ladders in IgE-mediated food allergy. Front Nutr 2024;11:1371772. https://doi.org/10.3389/fnut.2024.1371772

12 Villa C, Costa J, Oliveira MBPP, et al. Bovine Milk Allergens: a Comprehensive Review. Compr Rev Food Sci Food Saf 2018;17:137-164. http//doi.org/10.1111/1541-4337.12318.

13 Mills EN, Sancho AI, Rigby NM, et al. Impact of food processing on the structural and allergenic properties of food allergens. Mol Nutr Food Res 2009;53:963-969. https://doi.org//10.1002/mnfr.200800236

14 Bloom KA, Huang FR, Bencharitiwong R, et al. Effect of heat treatment on milk and egg proteins allergenicity. Pediatr Allergy Immunol 2014;25:740-746. https://doi.org/10.1111/pai.12283

15 Dang TD, Peters RL, Allen KJ. Debates in allergy medicine: baked egg and milk do not accelerate tolerance to egg and milk. World Allergy Organ J 2016;9:2. https://doi.org/10.1186/s40413-015-0090-z

16 Davis PJ, Williams SC. Protein modification by thermal processing. Allergy 1998;53(46 Suppl):102-105. https://doi.org/10.1111/j.1398-9995.1998.tb04975.x

17 Bloom KA, Huang FR, Bencharitiwong R, et al. Effect of heat treatment on milk and egg proteins allergenicity. Pediatr Allergy Immunol 2014;25:740-746. https://doi.org/10.1111/pai.12283

18 Ehn BM, Allmere T, Telemo E, et al. Modification of IgE binding to beta-lactoglobulin by fermentation and proteolysis of cow's milk. J Agric Food Chem 2005;53:3743-3748. https://doi.org/10.1021/jf048121w

19 Hindley JP, Oliver MA, Thorpe C, et al. Bos d 11 in baked milk poses a risk for adverse reactions in milk-allergic patients. Clin Exp Allergy 2021;51:132-140. https://doi.org/10.1111/cea.13774

20 Wróblewska B, Jędrychowski L. Effect of technological modification on the immunoreactive properties of cow milk proteins. Alergia Astma Immunologia 2003;8:157-164.

21 Simonato B, Pasini G, Giannattasio M, et al. Food allergy to wheat products: the effect of bread baking and in vitro digestion on wheat allergenic proteins. A study with bread dough, crumb, and crust. J Agric Food Chem 2001;49:5668-5673. https://doi.org/10.1021/jf0104984

22 Nollet LML, Toldra F. Handbook of Dairy Foods Analysis. New York, USA: CRC Press 2009, p. 43.

23 Huang F, Nowak-Węgrzyn A. Extensively heated milk and egg as oral immunotherapy. Curr Opin Allergy Clin Immunol 2012;12:283-292. https://doi.org/10.1097/aci.0b013e3283535bc3

24 Gou J, Liang R, Huang H, et al. Maillard Reaction Induced Changes in Allergenicity of Food. Foods 2022;11:530. https://doi.org/10.3390/foods11040530

25 Benedé S, Martínez-Blanco M, López-CMP, et al. IgE-Binding and Immuno stimulating Properties of Enzymatic Crosslinked Milk Proteins as Influenced by Food Matrix and Digestibility. Nutrients 2022;14:4584. https://doi.org/10.3390/nu14214584

26 Venter C, Meyer R, Ebisawa M, et al. Food allergen ladders: a need for standardization. Pediatr Allergy Immunol 2022;33:e13714. https://doi.org/10.1111/pai.13714

27 Bavaro SL, De Angelis E, Barni S, et al. Modulation of Milk Allergenicity by Baking Milk in Foods: a Proteomic Investigation. Nutrients 2019;11:1536. https://doi.org/10.3390/nu11071536

28 Golkar A, Milani JM, Vasiljevic T. Altering allergenicity of cow's milk by food processing for applications in infant formula. Crit Rev Food Sci Nutr 2019;59:159-172. https://doi.org/10.1080/10408398.2017.1363156

29 Upton JEM, Wong D, Nowak-Wegrzyn A. Baked milk and egg diets revisited. Ann Allergy Asthma Immunol 2024;132:328-336. https://doi.org/10.1016/j.anai.2023.12.024

30 Cronin C, Ramesh Y, De Pieri C, et al. Early Introduction' of Cow's Milk for Children with IgE-Mediated Cow's Milk Protein Allergy: a Review of Current and Emerging Approaches for CMPA Management. Nutrients 2023;15:1397. https://doi.org/10.3390/nu15061397

31 Vilar LK, Araújo CMPA, Santos TP, et al. Baked Tolerance in Cow's Milk Allergy: Quite Frequent, Hard to Predict! Int Arch Allergy Immunol 2021;182:319-323. https://doi.org/10.1159/000511148

32 Kim JS, Nowak-Węgrzyn A, Sicherer SH, et al. Dietary baked milk accelerates the resolution of cow's milk allergy in children. J Allergy Clin Immunol 2011;128:125-131.e2. https://doi.org/10.1016/j.jaci.2011.04.036

33 Nowak-Wegrzyn A, Bloom KA, Sicherer SH, et al. Tolerance to extensively heated milk in children with cow's milk allergy. J Allergy Clin Immunol 2008;122:342-347, 347.e1-2. https://doi.org/10.1016/j.jaci.2008.05.043

34 Sirin Kose S, Asilsoy S, Uzuner N, et al. Outcomes of Baked Milk and Egg Challenge in Cow's Milk and Hen's Egg Allergy: Can Tolerance Be Predicted with Allergen-Specific IgE and Prick-to-Prick Test? Int Arch Allergy Immunol 2019;180:264-273. https://doi.org/10.1159/000502957

35 Weinbrand-Goichberg J, Benor S, Rottem M, et al. Long-term outcomes following baked milk-containing diet for IgE-mediated milk allergy. J Allergy Clin Immunol Pract 2017;5:1776-1778.e1. https://doi.org/10.1016/j.jaip.2017.04.018

36 Agyemang A, Feuille E, Tang J, et al. Outcomes of 84 consecutive open food challenges to extensively heated (baked) milk in the allergy office. J Allergy Clin Immunol Pract 2018;6:653-655.e2. https://doi.org/10.1016/j.jaip.2017.05.016.

37 Barbosa CPG, Castro APM, Yonamine GH, et al. Baked milk tolerant patient: Is there any special feature? Allergol Immunopathol (Madr) 2017;45:283-289. https://doi.org/10.1016/j.aller.2016.10.008

38 Kilic M, Çilkol L, Taşkın E. Evaluation of some predictive parameters for baked-milk tolerance in children with cow's milk allergy. Allergol Immunopathol (Madr) 2021;49:53-59. https://doi.org/10.15586/aei.v49i2.64

39 Kiykim A, Karakoc-Aydiner E, Gunes E, et al. Evaluation of a Standardized Bakery Product (SUTMEK) as a Potential Tool for Baked-Milk Tolerance and Immunotherapy Research Studies. Int Arch Allergy Immunol 2019;178:1-9. https://doi.org/10.1159/000492824

40 Nowak-Węgrzyn A, Lawson K, Masilamani M, et al. Increased Tolerance to Less Extensively Heat-Denatured (Baked) Milk Products in Milk-Allergic Children. J Allergy Clin Immunol Pract 2018;6:486-495.e5. https://doi.org/10.1016/j.jaip.2017.10.021

41 Sopo SM, Greco M, Monaco S, et al. Matrix effect on baked milk tolerance in children with IgE cow milk allergy. Allergol Immunopathol (Madr) 2016;44:517-523. https://doi.org/10.1016/j.aller.2016.03.005

42 Alessandri C, Sforza S, Palazzo P, et al. Tolerability of a fully maturated cheese in cow's milk allergic children: biochemical, immunochemical, and clinical aspects. PLoS One 2012;7:e40945. https://doi.org/10.1371/journal.pone.0040945

43 Yanagida N, Minoura T, Kitaoka S. Butter Tolerance in Children Allergic to Cow's Milk. Allergy Asthma Immunol Res 2015;7:186-189. https://doi.org/10.4168/aair.2015.7.2.186

44 Jedrychowski, L. (). Reduction of the antigenicity of whey proteins by lactic acid fermentation. Food Agr Immunol 1999;11:91-99. https://doi.org/10.1080/09540109999951

45 Kleber N, Weyrich U, Hinrichs J. Screening for lactic acid bacteria with potential to reduce antigenic response of β-lactoglobulin in bovine skim milk and sweet whey. Innov Food Sci Emerg Technol 2006;7:233-238. https://doi.org/10.1016/j.ifset.2005.12.005

46 Yao M, Xu Q, Luo Y, et al. Study on reducing antigenic response and IgE-binding inhibitions of four milk proteins of Lactobacillus casei 1134. J Sci Food Agric 2015;95:1303-1312. https://doi.org/10.1002/jsfa.6823

47 Monaco S, Russo G, Romano A, et al. Yogurt is tolerated by the majority of children with IgE-mediated cow's milk allergy. Allergol Immunopathol (Madr) 2019;47:322-327. https://doi.org/10.1016/j.aller.2018.10.005

48 Poza-Guedes P, Barrios Y, González Pérez R, et al. Yogurt in the Treatment of β-Lactoglobulin-Induced Gastrointestinal Cow's Milk Allergy. J Investig Allergol Clin Immunol 2016;26:327-329. https://doi.org/10.18176/jiaci.0083

49 Kontopidis G, Holt C, Sawyer L. Invited review: betalactoglobulin: binding properties, structure, and function. J Dairy Sci 2004;87:785-796. https://doi.org/10.3168/jds.s0022-0302(04)73222-1

50 Poza-Guedes P, Barrios Y, Sanchez-Machin I, et al. Role of specific IgE to β-lactoglobulin in the gastrointestinal phenotype of cow's milk allergy. Allergy Asthma Clin Immunol 2016;12:7. https://doi.org/10.1186/s13223-016-0111-7

51 Uncuoglu A, Yologlu N, Simsek IE, et al. Tolerance to baked and fermented cow's milk in children with IgE-mediated and non-IgE-mediated cow's milk allergy in patients under two years of age. Allergol Immunopathol (Madr) 2017;45:560-566. https://doi.org/10.1016/j.aller.2017.02.008

52 Boonyaviwat O, Pacharn P, Jirapongsananuruk O, et al. Role of atopy patch test for diagnosis of food allergy-related gastrointestinal symptoms in children. Pediatr Allergy Immunol 2015;26:737-741. https://doi.org/10.1111/pai.12382.

53 Küçükosmanoğlu E, Özen E, Eltan SB, et al. Most children who are allergic to cow's milk tolerate yogurt. J Int Med Res 2018;46:5099-5106. https://doi.org/10.1177/0300060518790430.

54 Maldonado Galdeano C, Cazorla SI, Lemme Dumit JM, et al. Beneficial effects of probiotic consumption on the immune system. Ann Nutr Metab 2019;74:115-124. https://doi.org/10.1159/000496426

55 Meyer R, De Koker C, Dziubak R, et al. The Challenge of Home Allergen Re-introductions Using the Ladder Approach in Children With Non-IgE Mediated Gastrointestinal Food Allergy. Front Allergy 2021;2:721686. https://doi.org/10.3389/CMPAlgy.2021.721686

56 Amat F, Kouche C, Gaspard W, et al. Is a slow-progression baked milk protocol of oral immunotherapy always a safe option for children with cow's milk allergy? A randomized controlled trial. Clin Exp Allergy 2017;47:1491-1496. https://doi.org/10.1111/cea.13022.

57 Esmaelizadeh H, Alyasin S, Haghagad M, et al. The effect of baked milk on accelerating unheated cow's milk tolerance: a control randomized clinical trial. Pediatr Allergy Immunol 2018;29:747-753. https://doi.org/10.1111/pai.12958

58 Nowak-Węgrzyn A, Lawson K, Masilamani M, et al. Increased Tolerance to Less Extensively Heat-Denatured (Baked) Milk Products in Milk-Allergic Children. J Allergy Clin Immunol Pract 2018;6:486-495.e5. https://doi.org/10.1016/j.jaip.2017.10.021

59 D'Art YM, Forristal L, Byrne AM, et al. Single low-dose exposure to cow's milk at diagnosis accelerates cow's milk allergic infants' progress on a milk ladder program. Allergy 2022;77:2760-2769. https://doi.org/10.1111/all.15312

60 Kim JS, Nowak-Wegrzyn A, Sicherer SH, et al. Dietary baked milk accelerates the resolution of cow's milk allergy in children. J Allergy Clin Immunol 2011;128:125-131. https://doi.org/10.1016/j.jaci.2011.04.036

61 Efron A, Zeldin Y, Gotesdyner L, et al. A Structured Gradual Exposure Protocol to Baked and Heated Milk in the Treatment of Milk Allergy. J Pediatr 2018;203:204-209.e2. https://doi.org/10.1016/j.jpeds.2018.07.091

62 Dunlop JH, Keet CA, Mudd K, et al. Long-Term Follow-Up After Baked Milk Introduction. J Allergy Clin Immunol Pract 2018;6:1699-1704. https://doi.org/10.1016/j.jaip.2018.01.024

63 Chomin A, Chan ES, Yeung J, et al. Safety and effectiveness of the Canadian food ladders for children with IgE-mediated food allergies to cow's milk and/or egg. Allergy Asthma Clin Immunol 2023;19:94. https://doi.org/10.1186/s13223-023-00847-7

64 Fong AT, Ahlstedt S, Golding MA, et al. The Economic Burden of Food Allergy: What We Know and What We Need to Learn. Curr Treat Options Allergy 2022;9:169-186 https://doi.org/10.1007/s40521-022-00306-5

65 Abrams EM, Kim H, Gerdts J, et al. Milk allergy most burdensome in multi-food allergic children. Pediatr Allergy Immunol 2020;31:827-834. https://doi.org/10.1111/pai.13274

66 Berti I, Badina L, Cozzi C, et al. Early oral immunotherapy in infants with cow's milk protein allergy. Pediatr Allergy Immunol 2019;30:572-574. https://doi.org/10.1111/pai.13057

67 Tosca MA, Olcese R, Marinelli G, et al. Oral Immunotherapy for Children with Cow's Milk Allergy: a Practical Approach. Children 2022;9:1872. https://doi.org/10.3390/children9121872

68 Wang J, Lin J, Bardina L, et al. Correlation of IgE/IgG4 milk epitopes and affinity of milk-specific IgE antibodies with different phenotypes of clinical milk allergy. J Allergy Clin Immunol 2010;125:695-702, 702.e1-702.e6. https://doi.org/10.1016/j.jaci.2009.12.017

69 Heng TA, Cronin C, O'Sullivan M, et al. Safety and effectiveness of a milk ladder for managing children with IgE-mediated milk allergy. Clin Exp Allergy 2024;54:61-63. https://doi.org/10.1111/cea.14419

70 Gallagher A, Cronin C, Ah Heng T, et al. Dietary Advancement Therapy using milk and egg ladders among children with a history of anaphylaxis. J Allergy Clin Immunol Pract 2024;12:2135-2143. https://doi.org/10.1016/j.jaip.2024.04.057

71 Ball HB, Luyt D. Home-based cow's milk reintroduction using a milk ladder in children less than 3 years old with IgE-mediated cow's milk allergy. Clin Exp Allergy 2019;49:911-920. https://doi.org/10.1111/cea.13366

72 Nachson L, Goldberg MR, Elizur A, et al. Food allergy in previously tolerated foods. Course and patient characteristics. Ann Allergy Asthma Immunol 2018;121:77-81. https://doi.org/10.1016/j.anai.2018.04.012

73 Anagnostou A, Mack DP, Johannes S, et al. The Safety and Efficacy of Baked Egg and Milk Dietary Advancement Therapy: a Systematic Review and Meta-analysis. J Allergy Clin Immunol Practice 2024;12:2468-2480. https://doi.org/10.1016/j.jaip.2024.06.016

74 https://wwwallergiclivingcom/2017/08/02/alabama-boy-3-dies-of-severe-reaction during-baked-milk-challenge-test/ (Accessed on: June 1, 2022).

75 https://wwwallergiclivingcom/2021/12/20/girl-with-milk-allergy-dies-of-severereaction-related-to-desensitization/ (Accessed on: December 25, 2021)

76 Chua GT, Chan ES, Yeung J, et al. Patient selection for milk and egg ladders using a food ladder safety checklist. Allergy Asthma Clin Immunol 2022;18:51. https://doi.org/10.1186/s13223-022-00696-w

77 Brozek JL, Firmino RT, Bognanni A, et al. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guideline update – XIV – Recommendations on CMA immunotherapy. World Allergy Organ J 2022;15:100646. http://doi.org/10.1016/j.waojou.2022.100646