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Oral Immunotherapy for Food Allergy, Ready for Prime Time? Heated Egg and Milk

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Abstract Cow's milk and hen's egg allergies are two of the most common food allergies that affect children, with an estimated prevalence of 2–3 % each. Persistence of food allergy into late teen years and adulthood is being increasingly recognized, possibly due to strict avoidance practices. Heating has been known to alter food allergenicity, and the majority of milk- and egg-allergic patients tolerate heated forms of those foods. Heated milk and heated egg have been increasingly studied as oral immunotherapy (OIT) for treatment of milk and egg allergy. While heated milk and heated egg have been shown to be safe in selected milk- and egg-allergic cohorts, larger studies are needed to predict which patients are optimal candidates for this strategy and to further clarify whether ingestion of heated milk or heated egg truly accelerates the onset of clinical tolerance to unheated forms of these foods.

Keywords Egg allergy · Heated egg · Baked egg · Milk allergy · Heated milk · Baked milk · Recipe · OIT · OFC · Oral immunotherapy · Component testing · Ovomucoid · Casein · SPT · Specific IgE · IgG_4

Introduction

Cow's milk and hen's egg allergies are two of the most common food allergies that affect children, with an estimated prevalence of 2–3 % each [1, 2]. While tolerance to cow's milk and hen's egg has been thought to develop by school age in most children, persistence of food allergy is being increasingly recognized [3]. From 1997 to 2007, pediatric food allergy prevalence increased by 20 % in the US, partially due to persistence of allergy into later childhood years [2]. It has been theorized that strict avoidance may have contributed to this observed delay in tolerance development.

Heating of food proteins has been shown to alter food allergenicity [4]. However, in cow's milk and hen's egg allergy, extensive heating (i.e., baking at 350ºF for 30 min) may reduce allergenicity by denaturation of conformational epitopes [5•]. Altered gastrointestinal digestibility may also play a key role [6•]. Approximately 70 % of egg allergic and 75 % of milk allergic children may tolerate heated forms of these foods [7, 8].

Subcutaneous immunotherapy is unsafe as a food allergy therapy [9]. A landmark oral immunotherapy (OIT) study using egg powder (*not heated*) demonstrated effective desensitization for 75% of participants receiving treatment after 22 months of therapy, but 25% of all doses given were associated with adverse events [10••]. Despite these setbacks, participation in OIT trials has been shown to improve the quality of life (QOL) for participants and their families [11, 12]. Clinical trials have been published in recent years studying heated milk or heated egg OIT with the goal of hastening the onset of clinical tolerance to all forms of milk or egg via regular heated milk or heated egg ingestion.

The purpose of this review is to summarize the most recent heated milk and heated egg OIT immunologic and clinical data, and to comment on this treatment's readiness for broad clinical use.

Heated Egg and Heated Milk Allergenic Proteins: Epitopes and Components

Sequential epitopes are linear sequences of amino acids; conformational epitopes are comprised of non-sequential amino acids

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that are physically brought together by three-dimensional protein folding. Sequential epitopes are relatively heat stable, while conformational epitopes are heat labile. Both sequential and conformational epitopes may bind IgE and have been shown to be important in cow's milk and hen's egg allergy [4, 13]. The major allergenic components of cow's milk are whey proteins and casein proteins (Table 1). The whey proteins α-lactalbumin, β-lactoglobulin, and lactoferrin are more heat labile, while the most abundant whey protein, bovine serum albumin, along with the caseins are considered more heat stable [14, 15]. In hen's egg, the two major allergens have disparate allergenicity when heated: ovalbumin is heat labile, while ovomucoid is heat stable (see Table 1). Chatchatee *et al*. [16] reported that in cow's milk and hen's egg allergic patients, those who lack IgE antibodies to specific sequential epitopes are more likely to achieve clinical tolerance. Jarvinen *et al*. [17] note that children with more persistent egg allergy exhibit sensitization to a disproportionate number of sequential epitopes when compared to children who develop clinical tolerance more quickly.

Immunology of Food Allergen Tolerance

Food allergy results from a defect in developing or maintaining oral tolerance to food proteins. Many of the mechanisms underlying this lack of tolerance are unclear, and most of the known mechanisms are described in murine models. Food allergy sensitization can be initiated in murine models via inducing defects in the gastrointestinal epithelium's expression of tight junction proteins leading to barrier dysfunction. This observation has been supported by human data that report measurable alterations in gastrointestinal permeability in food allergic infants when compared to non-allergic healthy infants [18•].

Murine models also suggest appropriately timed and dosed allergen administration early in life may induce regulatory T cell (Treg) function and lessen the risk of clinical allergy developing. Some experts argue that delayed introduction of the most commonly allergenic foods, cow's milk, egg, wheat, soy, peanuts, tree nuts, fish and shellfish, (the prior dogma) should be reconsidered [19]. Robust prospective clinical data are needed to confirm the validity of an early allergenic food introduction strategy in infants.

Other cellular mechanisms of oral tolerance include CCR7-dependent migration of $CD103^+$ $CD11c^+$ dendritic cells to mesenteric lymph nodes, which in turn influence the generation of two inducible regulatory T cell populations called T_R1 cells and T_H3 cells [18•]. Tolerance to higher doses of antigen is thought to be mediated via alternative mechanisms such as T cell clonal deletion via FAS-mediated apoptosis and T cell anergy via T cell receptor ligation in the absence of various costimulatory molecules [18•]. The cytokine TGF-β also seems to play a central role in both T-cell anergy and T-cell clonal deletion [18•].

The immunologic mechanisms underlying heated egg and heated milk tolerance (and OIT) are less apparent. Wanich *et al*. [20] reported that heated milk-tolerant patients' basophils were less responsive to antigen stimulation than basophils from heated milk-allergic patients [20]. Martos *et al*. [6•] subsequently demonstrated that heated ovalbumin and ovomucoid were both markedly less effective in triggering basophil activation when compared to native (unheated) proteins. These investigators also noted that heating of ovalbumin and ovomucoid decreased intestinal absorption of these proteins across both the intestinal epithelium and Peyer's patches.

Thus, different subgroups of cow's milk- and hen's egg-allergic patients exist: those who tolerate heated forms of the

Table 1 Cow's milk and hen's egg allergenic components

		Protein	Allergen	Heating effects on allergenicity	Epitope
Cow's milk	Caseins	β-Casein	Bos d 8 beta	Heat stable	Sequential
		α_{s1}-Casein	Bos d 8 alpha-s1	Heat stable	Sequential
		α_{s2}-Casein	Bos d 8 alpha-s2	Heat stable	Sequential
		κ-Casein	Bos d 8 kappa	Heat stable	Sequential
	Whey	α-Lactalbumin	Bos d 4	Heat labile	Conformational
		β-Lactalbumin	Bos d 5	Heat labile	Conformational
		Lactoferrin	Bos d Lactoferrin	Heat labile	Conformational
		Bovine Serum Albumin	Bos d 6	Heat stable	Sequential
Hen's egg	Egg white	Ovomucoid	Gal d 1	Heat stable	Sequential
		Ovalbumin	Gal d 2	Heat labile	Conformational
		Ovotransferrin	Gal d 3	Heat labile	Conformational
		Lysozyme	Gal d 4	Heat labile	Conformational
	Egg yolk	α-Livetin	Gal d 5	Heat stable	Sequential
		Glycoprotein 42	Gal d 6	Heat stable	Sequential

Data from [14], [15]

food (possibly because these patients are sensitized to conformational epitopes that are denatured during the heating process) and those who react to both unheated and heated forms (possibly because of sensitization to sequential epitopes). These different allergic clinical phenotypes may partially be explained mechanistically by the variant basophil reactivity and intestinal absorption to heated allergen. The challenge however is predicting which patients who react to unheated milk or unheated egg will be able to tolerate heated forms of these foods.

Diagnosis

The gold standard for diagnosis of food allergy is a double-blind placebo-controlled food challenge (DBPCFC). Practically, DBPCFCs are challenging for clinicians to perform because of time and labor constraints. Instead, cow's milk and hen's egg allergies are typically diagnosed based on history and supportive evidence from either skin prick and/or specific IgE blood testing, or in the setting of a positive in-office open oral food challenge (OFC). Once the diagnosis of milk or egg allergy has been made, a heated milk or heated egg OFC may be required to diagnose heated milk or heated egg reactivity or tolerance.

Recipes

No universal agreement exists regarding the optimal recipe to use for heated milk or heated egg challenges. Nowak-Wegrzyn *et al*. use both baked muffins and waffles (each containing 1.3 g of milk protein) in their heated milk challenges [5•, 8]. The University of Wisconsin and Boston Children's Hospital have both released slightly different heated milk recipes [21, 22]. The milk protein content in these challenge protocols is comparable—the goal is for the subject to ingest one serving's worth of heated milk. For heated egg challenges, the range of egg protein content is more varied. Some have used a muffin recipe with only 1/3 of an egg in total content [23]; other investigators use a one-egg cake recipe [24] or a two-egg muffin recipe [25], while others have used up to five eggs [26, 27] in sponge cake. Proponents of the higher egg recipes suggest the increased egg content provides reassurance that the subject will be able to tolerate most forms of heated egg they may encounter outside of the clinical setting. This is particularly important in pediatric patients who may not physically be able to ingest a large muffin or a large slice of cake in one sitting. Refer to Table 2 for sample heated egg and heated milk recipes.

Challenge Protocols

The Adverse Reactions to Food Committee of the American Academy of Allergy, Asthma & Immunology has published an extensive guide to safely performing in-office food challenges for a variety of foods and additives [29]. The World Allergy Organization has published similar guidelines specifically for milk OFC [15]. These guidelines are helpful in regards to patient selection, patient preparation, and patient monitoring, but the guidelines do not focus specifically on heated egg or heated milk challenges. Various heated egg and heated milk protocols have been published elsewhere [5•, 7, 8, 21, 23, 27, 30]. Typically, these protocols involve dividing a serving of heated milk (or heated egg) product into 4–8 smaller pieces. These pieces are typically administered 15–30 min apart. After one serving has been cumulatively ingested, the patient is monitored for another 2 to 4 h post procedure to ensure no reaction develops.

Skin and Specific IgE Testing as Predictors of OFC

While OFCs are more practical than DBPCFCs, OFCs too can be time consuming and include the risk of anaphylaxis. Both whole allergen and allergen component testing have been studied extensively in recent years with one of the primary goals of allergen component testing in cow's milk and hen's egg allergy being the ability to accurately predict (without the need for an OFC) which patients will tolerate heated or unheated milk or egg. Table 3 summarizes pertinent heated milk and heated egg studies that evaluated the predictability of whole allergen and/or component testing.

Skin Testing

Nowak-Wegrzyn *et al*. [8] noted in a prospective cohort of milk allergic patients that an undetectable specific IgE to milk or a small (<5 mm) wheal size to unheated milk yielded both a negative predictive value (NPV) and sensitivity of 100 %, although the specificity and positive predictive value (PPV) of these same tests was quite poor. Bartinikas *et al*. [22] retrospectively found that children with a milk SPT wheal <12 mm were greater than 90 % likely to pass a heated milk challenge, and all children with a milk SPT wheal <7 mm passed a heated milk challenge.

Faraj *et al*. [33] retrospectively analyzed the predictability of SPT to both heated milk and heated egg in allergic subjects. Patients with a history of reactivity to heated milk or heated egg were excluded, and patients with negative unheated milk or unheated egg SPT underwent SPT to heated milk or heated egg. Negative SPT patients proceeded to an OFC. The NPV of heated product skin testing (egg and milk data were combined) demonstrated an NPV of 94.8 %. The small number of OFCs performed in this study likely influenced the data and is thus unlikely to be generalizable.

Table 2 Sample heated egg and heated milk recipes

Duke University Baked Egg Recipe [26] Heated egg 1 Serving sponge cake=approximately 5 g heated egg Ingredients: 5 eggs, separated 1 cup white sugar 4 tablespoons cold water 1 cup sifted cake flour 1 teaspoon baking powder	Directions: 1. Preheat oven to 350 degrees F. Grease a tube pan 2. Beat egg yolks and sugar together until very light. Add water. Sift together flour and baking powder. Add to batter. Beat egg whites until stiff. Fold into batter. Pour batter into prepared pan 3. Bake for 30–35 min until brown and pulls away from the edge of the pan 4. Cut into eight servings
Jaffe Food Allergy Institute [28••] Heated egg 1 Muffin=1/3 egg=approximately 2.2 g heated egg Ingredients: 1 cup all-purpose flour (or flour substitute) $^1/_4$ teaspoon salt 2 tablespoon cow's milk (or soy milk, rice milk, almond milk) 1 teaspoon baking powder $^1/_4$ teaspoon cinnamon 2 eggs $^1/_2$ cup sugar $^1/_4$ cup canola oil $^1/_2$ teaspoon vanilla 1 cup mashed ripe banana or applesauce	Directions: 1. Preheat oven to 350 degrees F 2. Line a muffin pan with 6 muffin liners 3. Mix together the liquid ingredients: milk or milk substitute, canola oil, vanilla extract, mashed ripe banana or applesauce and eggs. Set aside 4. In a separate mixing bowl, mix together the dry ingredients: flour or flour substitute, sugar, salt, cinnamon, baking powder 5. Add the liquid ingredients to the dry ingredients. Stir until combined. Some small lumps may remain 6. Divide the batter into the six prepared muffin liners. Depending on the size of your muffin tin, you may need to fill the muffin liners all the way to the top. If you make more than six muffins, please note how many muffins you make and bring at least two muffins with you on the day of the challenge 7. Bake for 30 to 35 min or until golden brown and firm to the touch
University of Wisconsin [21] Heated milk 1 $^1/_2$ pieces of cake=3 cupcakes=1 serving of heated milk Ingredients: Duncan Hines yellow cake mix OR Gluten free cake mix that calls for 1 c milk (or water) $^1/_3$ cup vegetable oil 3 large eggs OR egg replacer equivalent dried milk powder Pans/Baking Times: 13×9 inch13×9 inch-32–35 minutes OR 24 cupcake-18–21 minutes	Directions: 1. Preheat oven to 350 degrees F (light metal or glass pans); 325 for dark metal or nonstick pans. 2. GREASE sides & bottom of pan(s). Flour lightly or use baking cups for cupcakes. 3. Mix together 1 cup of milk and $^1/_3$ cup of milk powder. Some gluten-free mixes do not use milk. If the glutenfree recipe calls for 1 cup of water, rather than milk, replace the water with this milk mixture. 4. Blend the cake mix, milk + milk powder mixture, oil, and eggs (or egg replacer) in large bowl at low speed until moistened (about 30 seconds). Beat at medium speed for 2 minutes.Pour into baking pans and bake immediately 5. Bake at times listed above (add 3–5 minutes for dark or nonstick pans). Done when toothpick inserted in center comes out clean. COOL in pan on wire rack for 15 minutes. Cool completely before frosting (if doing). 6. CUT 13x9-inch cake into 12 equal pieces.
"Dose" is ¼ cup milk = 1 ½ pieces of cake or 3 cupcakes. Bring 6 pieces of cake or 6 cupcakes to the challenge.	

Specific IgE Testing

Caubet *et al*. [32•] reported that casein-specific IgE testing exhibited superior accuracy when compared to specific IgE to cow's milk or specific IgE to the more heat-labile milk component β-lactoglobulin. Patients with casein specific IgE >20.2 kU/l were likely (with 95 % sensitivity) to fail a heated milk challenge, and those with casein-specific IgE <0.94kU/l were likely (with 95 % specificity) to pass a heated milk challenge. The "optimal" cutoff point (74 % sensitivity, 77 % specificity) yielded a casein specific IgE of 4.95. These data are from one tertiary referral center, and the authors cautioned that component testing should not replace OFC since the data have not been validated in more age groups and at other centers.

Ford *et al*. [34••] subsequently showed that casein- and milk-specific IgEs were greater in heated milk reactors when compared to those who passed a heated milk challenge. These tests also did not simultaneously achieve high sensitivity and high specificity, and therefore are not recommended for clinical use in place of an OFC.

Combination Skin and Specific IgE Testing

Bartinikas *et al*. [22] also reported patients with a casein SPT wheal of ≤9 mm, milk-specific IgE of 1.0kU/l (similar to prior data [35] regarding unheated milk challenges), or casein-specific IgE of 0.9kU/l were all >90 % likely to pass a heated milk challenge. These data are weakened by the lack of an entry unheated milk challenge; thus, the utility of these cutoff values is unclear.

Table 3 Summary of predictive testing

Study	*N*	Whole protein-specific IgE	Components(s) blood testing	Whole protein skin prick testing	Component(s) skin prick testing	Comments	Limitations
Ando *et al*. [30]	108	• Egg white IgE (kU/l): 21.6 (0.64->100) in heated egg-reactive vs. 6.3 (<0.35–52.0) in raw egg-reactive but heated egg-tolerant patients ($p<0.05$)	• Ovalbumin IgE (kU/l): 26.2 (0.99–>100) in heated egg-reactive vs. 7.0 (<0.35–78.3) in raw egg-reactive but heated egg-tolerant patients ($p<0.01$) • Ovomucoid IgE (kU/l): 15.8 (<0.35–>100) in heated egg-reactive vs. 2.4 (<0.35–18.6) raw egg-reactive but heated egg-tolerant patients	N/A	N/A	• Ovomucoid IgE was superior to egg white or ovalbumin IgE via ROC analysis. Optimal cutoff point was 4.40 kU_A/l (76 % sensitivity and 81 % specificity)	• No SPT analysis • Low numbers of subjects with a history of anaphylaxis to egg
Lemon-Mulé [7]	117	• Egg white IgE (kU_A/l): 5.1 (1.9–11.1) in heated egg-reactive vs. 1.3 (0.6–4.3) in raw egg-reactive but heated egg-tolerant patients ($p=0.001^{\#}$)	• Ovalbumin IgE (KU_A/l): 7.0 (2.9–14.2) in heated egg-reactive vs. 1.6 (0.7–4.7) in raw-egg reactive but heated egg-tolerant patients ($p<0.001^{\#}$) • Ovomucoid IgE (KU_A/l): 3.3 (0.7–13.7) in heated egg-reactive vs. 1.0 (0–3.0) in raw egg-reactive but heated egg-tolerant patients ($p=0.019^{\#}$) • Ovalbumin IgE:IgG_4 ratio: 43.2 (19.1–130.9) in heated egg-reactive vs. 5.7 (1.7–81) in raw egg-reactive but heated egg-tolerant patients ($p=0.011^{\#}$) • Ovomucoid IgE:IgG_4 ratio: 46.2 (6.7–91.3) in heated egg-reactive vs. 7.4 (2–14.5) in raw egg-reactive but heated egg-tolerant patients ($p=0.012^{\#}$)	• Egg white: 8 (6.3–9) in heated egg-reactive vs . 6 (5–8) in raw egg-reactive but heated egg-tolerant patients	N/A	• Ovomucoid IgE was superior to ovalbumin IgE, egg white IgE, and egg white SPT in predicting heated egg challenge outcomes. • Ovomucoid IgE >50 kU_A/l>90 % predictive • Negative ovomucoid IgE still had a 10 % chance of reactivity to heated egg • Egg white SPT >15 mm was 60 % predictive of heated egg reactivity • Negative egg white SPT <5 % chance of heated egg reactivity	• Egg protein ingested during heated egg challenge significantly less than dose of heated egg during regular egg challenge, but similar to amount of heated egg ingested in a typical diet • Possible wheat matrix effect • Patients with a history of a reaction to heated egg 6 months prior were excluded
Leonard *et al.* [28••]	79	• Egg white IgE (kU_A/l): 13.5 (2.8–58.9) in heated egg-reactive vs. 1.9 (0.6–6.1) in regular egg-reactive but heated egg–tolerant patients ($p=0.002$)	N/A	• Egg white: 8 (7–15) in heated egg-reactive vs. 6 (0–19) in raw egg-reactive but heated egg-tolerant patients at baseline ($p=0.005$)	N/A	• Patients who initially tolerated heated egg were 3.3× more likely to develop tolerance to regular egg than those who initially reacted to heated egg ($p=0.017$) • Those who included heated egg in their diet were 14.6× more likely to develop tolerance when compared to subjects following strict avoidance; tolerance developed earlier (50 months vs. 78.7 months) ($p<0.0001$)	• Component IgE and IgG_4 data were limited to those who ingested heated egg, and baseline comparisons of heated egg reactors vs. non-reactors were not included
Cortot *et al.* [25]	52	• Egg white IgE (kU/l): 1.52 (median) in heated egg-reactive vs. 2.02 (median) in heated egg-tolerant but regular egg-reactive patients ($p=0.660$)	• Ovomucoid-specific IgE tested in only 7 subjects. Six did not react to heated egg with a range of <0.10–1.30 kU/l; one reacted with an ovomucoid-specific IgE of 4.85 kU/l	• Egg white SPT: 17 (median) in heated egg-reactive vs. 12 (median) in heated egg-tolerant but regular egg-reactive patients ($p=0.091$)	N/A	• 100 % NPV if egg white SPT <10 mm	• Retrospective • Not all patients had both baseline egg white IgE and SPT to egg white recorded • Ovomucoid-specific IgE tested in a minority of patients
Lieberman *et al.* [23]	100	• Egg white IgE (kUA/L): 2.81 (median) in heated egg-reactive vs. 5.85 (median) in heated egg-tolerant but regular egg reactive patients ($p=0.001$)	N/A	• Egg white SPT: 7 mm (median) identical in both groups	N/A	• Histories of prior reaction and SPT were not helpful predictors of heated egg challenge results	• Retrospective • Components not evaluated • Single center
Caubet *et al.* [31•]	107	• Egg white IgE higher in heated egg-reactive subjects when compared to heated egg-tolerant subjects ($p<0.05^{‡}$)	• Ovomucoid- and ovalbumin-specific IgE both higher in heated egg-reactive subjects when compared to heated egg-tolerant subjects ($p<0.05^{‡}$)	N/A	N/A	• Suggestion of a subset of patients who are ovalbumin reactive but not ovomucoid reactive in their component data	• Utility of IgG_4 data (and IgE/IgG4 ratios) debated—can be affected by egg exposure level

Table 3 (continued)

Study	N	Whole protein-specific IgE	Components(s) blood testing	Whole protein skin prick testing	Component(s) skin prick testing	Comments	Limitations
			• Ovalbumin- and ovomucoid- specific IgE/IgG$_4$ ratios were significantly higher in the heated egg-reactive subjects vs. heated egg-tolerant subjects (p=0.001 and p=0.003, respectively[‡])			• Egg white and ovalbumin specific IgE performed better than ovomucoid specific IgE in predictive models	• Complex statistical model and correlative statistics difficult to apply to clinical practice • Authors note their predictive model is not ready for broad clinical use
Nowak-Wegrzyn *et al.* [8]	100	• Milk IgE (kU$_A$/l): 11.6 (0.69–101) in heated milk-reactive vs. 2.43 (0–79.1) in heated milk-tolerant but regular milk-reactive patients (p<0.001)	• Casein IgE (mg$_A$/l): 14.15 (0.71–101) in heated milk-reactive vs. 1.41 (0–101) in heated milk-tolerant but regular milk-reactive patients (p<0.001) • β-Lactoglobulin IgE, (mg$_A$/l): 4.48 (0–101) in heated milk-reactive vs. 0.43 (0–63.7) in heated milk-tolerant but regular milk-reactive patients (p=0.002) • Casein IgG$_4$, β -lactoglobulin IgG$_4$, casein IgE:IgG$_4$ ratio, and β -lactoglobulin IgE/IgG$_4$ all no significant difference between heated milk-reactive and heated milk-tolerant but regular milk-reactive patients	• Milk: 9.5 (5–24) in heated milk-reactive vs. 6 (0–8) in heated milk-tolerant but regular milk-reactive patients (p=0.009)	N/A	• All patients with undetectable milk-specific IgE or small SPT to milk (wheal <5 mm) tolerated a heated milkchallenge yielding excellent sensitivity and NPV, but resulted in poor specificity and PPV of these tests • Patients with milk-specific IgE >35 kU$_A$/l had an 85 % chance of reaction to heated milk	• Milk protein ingested during heated milk challenge significantly less than dose of heated milk during unheated milk challenge • Children tolerating heated milk but with milk IgE >95 % PPV were not challenged to regular milk to prove clinical allergy
Caubet *et al.* [32•]	97 and 128[*]	• Cohort 1: Milk IgE (kU$_A$/l): 11.6 (0.7–101) in heated milk-reactive vs. 2.5 (0.2–79.1) in heated milk-tolerant but regular milk-reactive patients (p=0.00[†]) • Cohort 2: Milk IgE (kU$_A$/l): 11.9 (0.8–50.5) in heated milk-reactive vs. 4.0 (0.2–42.3) in heated milk-tolerant but regular milk-reactive patients. (p=0.00[†])	• Cohort 1: Casein-specific IgE (kU$_A$/l): 13.9 (0.7–101) in heated milk-reactive vs. 2.5 (0.2–79.6) in heated milk-tolerant but regular milk-reactive patients (p=0.00[†]) • Cohort 2: Casein-specific IgE (kU$_A$/l): 12.2 (0.5–67.0) in heated milk-reactive vs. 2.3 (0.2–30.5) in heated milk-tolerant but regular milk-reactive patients (p=0.00[†]) • Cohort 1: β-Lactoglobulin-specific IgE (kU$_A$/l): 4.6 (0.2–101) in heated milk-reactive vs. 0.5 (0.2–13.8) in heated milk-tolerant but regular milk-reactive patients (p=0.00[†]) • Cohort 2: β-Lactoglobulin-specific IgE (kU$_A$/l): 2.0 (0.2–15.4) in heated milk-reactive vs. 0.6 (0.2–19.8) in heated milk-tolerant but regular milk-reactive patients (p=0.00[†]) • Statistically significant IgE;IgG$_4$ ratios for both casein and β-lactoglobulin were also reported in both cohorts	N/A	N/A	• Overall best AUC for predicting heated milk reactivity was casein-specific IgE: 80.6 (cohort 1 and 2 data combined; both whole-allergen and component-specific IgE analyzed)	• Those who have outgrown all milk allergies were included in the comparison • Single center with limited age range of subjects
Bartnikas *et al.* [22]	35	• Milk IgE (kU/l): 2.39 (<0.35–31.0) in heated milk-reactive vs. 1.93 (<0.35–20.6) in heated milk-tolerant but regular milk-reactive patients (p=0.50)	• Casein specific IgE (kU/L): 1.07 (<0.35–31.5) in heated milk reactive vs 1.05 (<0.35–10.3) in heated milk-tolerant but regular milk-reactive patients. (p=0.69) • B-Lactoglobulin-specific IgE (kU/l): 0.63 (<0.35–2.07) in heated milk-reactive vs. <0.35 (<0.35–15.4) in heated milk-tolerant but regular milk-reactive patients (p=0.62)	• Milk SPT: 15 (7–20) in heated milk reactive vs 10 (0–20) in heated milk-tolerant but regular milk-reactive patients (p=0.12)	• Casein SPT: 9 (0–22) in heated milk-reactive vs. 5 (0–15) in heated milk-tolerant but regular milk-reactive patients (p=0.26)	• Patients with milk SPT <12 mm were >90 % likely to pass a heated milk challenge • No child with milk SPT <7 mm failed a heated milk challenge • >90 % predictive values for passing a heated milk challenge: casein SPT wheal of 9 mm,	• Retrospective • Diagnosis of heated milk allergy not confirmed by OFC at entry • Home reactions may have occurred because of inadequately heated milk product preparation

Table 3 (continued)

Study	N	Whole protein-specific IgE	Components(s) blood testing	Whole protein skin prick testing	Component(s) skin prick testing	Comments	Limitations
			• α-Lactalbumin-specific IgE (kU/l): 0.61 (<0.35–4.67) in heated milk-reactive vs. 0.73 (<0.35–8.63) in heated milk-tolerant but regular milk-reactive patients (p=0.50)			milk-specific IgE 1.0 kU/l, and casein-specific IgE of 0.9kU/l	

All specific IgE data displayed as median (range), and all SPT wheal diameter data displayed as median (range) in millimeters

[#] P value generated by comparing heated egg-reactive, heated egg-tolerant, and egg-tolerant subjects via ANOVA

*Data from two different cohorts. Cohort 1: n=97; cohort 2: n=128

[†] P values compare heated milk-reactive patients to a 0Agroup consisting of both heated milk=tolerant (but regular milk-reactive) patients and patients who have outgrown their milk allergy (both heated and regular forms of milk tolerant)

[‡] P values compare heated egg-reactive patients to a group consisting of both heated egg-tolerant (but regular egg-reactive) patients and patients who tolerate both heated and regular forms of egg

Cortot *et al*. [25] retrospectively analyzed their heated egg challenge data and assessed the predictability of specific IgE and SPT to unheated egg. No statistical difference in SPT wheal size or specific IgE to unheated egg white was reported between those who passed and failed a heated egg OFC. Swamy *et al*. [27] also retrospectively analyzed a cohort of 52 egg-allergic children who completed a heated egg OFC. Neither egg white-specific IgE nor ovomucoid-specific IgE levels reliably identified heated egg-tolerant subjects. Instead, egg white SPT was the best predictor of passing a heated egg OFC with an egg white SPT<10 mm, yielding a specificity of 74 %, sensitivity of 81 %, NPV of 85 % and PPV of 68 %.

IgE:IgG_4 ratios

Multiple investigators have explored the use of component IgE:IgG_4 ratios as predictors of OFC success. Casein- and β-lactoglobulin-specific IgE:IgG_4 ratios were shown to be significantly higher in heated milk-reactive versus heated milk-tolerant patients. However, multivariate analyses showed that casein- and β-lactoglobulin-specific IgE disproportionately influenced the IgE:IgG_4 data [32•]. In heated egg allergy, conflicting data exist as to which egg white component (ovomucoid or ovalbumin) is more predictive of heated egg reactivity [13, 30]. Ovomucoid is thought to be the more clinically relevant component because of its ability to withstand extensive heating and acid exposure [13]. In heated egg allergy, reactors exhibit higher ovalbumin- and ovomucoid-specific IgE:IgG_4 ratios [31•]. When investigators included these ratios into a preexisting logistical regression OFC prediction model, its AUC improved, but investigators cautioned that the model is not ready for clinical use [31•].

Taken together, these various single-center studies demonstrate that while both whole-allergen and component testing (both via SPT- and serum-specific IgE assays) have shown promise in predicting clinical reactivity to heated milk and heated egg, these tests remain investigational and cannot be recommended for broad clinical use as a surrogate for an OFC.

Clinical Trials

Two landmark trials have been published recently evaluating the introduction of dietary heated milk or heated egg in order to accelerate tolerance development to unheated milk or unheated egg [5•, 28••]. Patients who passed a heated milk OFC and incorporated heated milk regularly into their diet were 28 times more likely to become unheated milk tolerant when compared to heated milk-reactive subjects [5•]. Similarly, subjects who passed a heated egg challenge and incorporated heated egg regularly into their diet were 14.6 times as likely to develop regular egg tolerance versus heated egg reactive patients [28••]. Also, tolerance to egg in those incorporating heated egg into their diet was achieved more rapidly than in those who followed a strict avoidance strategy [28••]. Growth and other chronic atopic conditions (allergic rhinitis, asthma, and atopic dermatitis) were not negatively affected by regular heated milk ingestion, but eosinophilic esophagitis development continues to be a possible concern. It is important to note that 23 % of heated egg-tolerant patients required epinephrine during an unheated egg challenge in this landmark study [28••], and the authors cautioned that home introduction strategies advocated by others [24] have not been endorsed in the US.

Conclusions

Multiple challenges remain regarding effective management of cow's milk and hen's egg allergy. Various mechanisms of tolerance induction continue to be discovered in murine models, but translation to the clinic continues to be a challenge. Regular addition of heated milk or heated egg into the diet of unheated milk or egg allergic patients has been shown to hasten the onset of tolerance to unheated milk or egg [5•, 28••]. Physicians cannot however reliably predict which patients will tolerate heated forms of milk or egg using component testing—OFCs remain the practical standard for diagnosis. Larger prospective randomized controlled trials are needed to further clarify the degree to which addition of heated milk or heated egg accelerates the onset of clinical tolerance to unheated forms of these foods, the pertinent immunologic mechanisms at play in OIT, and whether long-lasting clinical tolerance can be confirmed once daily ingestion of the allergic food is ceased. Several challenges remain regarding studying the efficacy of heated milk or heated egg introduction in future clinical trials: (1.) heated food products generally are prepared at home by the family, (2.) quality control of heated products (*i.e.*, ensuring that heated products are effectively baked so that no unheated allergen is present), (3.) enrollment of younger children (who may receive increased benefit from early intervention via immunomodulatory strategies such as OIT), and (4.) the practicality of daily dietary compliance especially in younger patients.

Surrogates for food allergy testing such as component testing have shown some promise in small single-center studies, but no universal cutoff values exist. Basophil activation testing remains investigational but promising. More work is needed in the development of reliable testing that can be used in place of OFC, thus minimizing the real risk of OFC-related anaphylaxis.

Patient risk cannot be ignored, and while in selected circumstances heated milk and heated egg introduction at home (after a formal in office OFC) may be appropriate, risks and benefits must be thoroughly discussed prior to pursuing this strategy. Concern about the development of eosinophilic esophagitis while participating in OIT is real, but recent data suggest that heated products may not contribute to the development of eosinophilic esophagitis [36]. More studies are needed to clarify whether heated products should be introduced with the aim of *accelerating* the onset of tolerance. However, current evidence does not suggest that heated milk or heated egg introduction *delays* the onset of tolerance, and it is likely that the QOL of affected individuals and their families is improved with the addition of these heated products.

Compliance with Ethics Guidelines

Conflict of Interest Andrew Bird has received grant support from the Foundation of the American College of Allergy, Asthma and Immunology and has served on the Speaker's Bureau for Nutricia.

Matthew F. Feldman declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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