Tolerance to extensively heated milk in children with cow's milk allergy

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Background: Cow's milk allergy is the most common childhood food allergy. Previously we noted that children who outgrew their milk allergy had milk-specific IgE antibodies primarily directed against conformational epitopes; those with persistent milk allergy also had IgE antibodies directed against specific sequential epitopes.

Objective: Because high temperature largely destroys conformational epitopes, we hypothesized that some children with milk allergy would tolerate extensively heated (baked) milk products.

Methods: Children with milk allergy were challenged with heated milk products; heated milk–tolerant subjects were subsequently challenged with unheated milk. Heated milk–tolerant, unheated milk–reactive subjects ingested heated milk products for 3 months and were then re-evaluated. Immune responses were assessed in all subjects; growth and intestinal permeability were followed in heated milk–tolerant subjects.

Results: One hundred children (mean age, 7.5 years; range, 2.1-17.3 years) underwent heated milk challenges. Sixty-eight subjects tolerated extensively heated milk only, 23 reacted to heated milk, and 9 tolerated both heated and unheated milk. Heated milk–reactive subjects had significantly larger skin prick test wheals and higher milk-specific and casein-specific IgE levels than other groups. At 3 months, subjects ingesting heated milk products had significantly smaller skin prick test wheals and higher casein-IgG_4 compared with baseline; other immunologic parameters, growth, and intestinal permeability were not significantly different. Heated milk–reactive subjects had more severe symptoms during heated milk challenge than heated milk–tolerant subjects experienced during their unheated milk challenge.

Conclusion: The majority (75%) of children with milk allergy tolerate heated milk. (J Allergy Clin Immunol 2008;122:342-7.)

Key words: *Milk allergy, cow's milk allergy, baked milk, heated milk, food allergy, intestinal permeability, oral food challenge*

Cow's milk allergy is the most common childhood food allergy.[1,2] The majority of children with milk allergy become tolerant by school age[1,3-5]; however, the mechanism of tolerance induction remains unknown, and recent reports suggest that a subset may not lose milk allergy until considerably later in life.[6,7] Current management relies on complete milk elimination, which has been postulated to expedite the development of natural tolerance and to prevent subclinical gastrointestinal inflammation that could inhibit normal growth. Adhering to such a diet is very difficult because of the presence of milk in many processed foods.

Individuals with food allergy may generate specific IgE antibodies against conformational (dependent on tertiary structure) and sequential epitopes. We previously noted that children who outgrew milk allergy had milk-specific IgE antibodies primarily directed at conformational epitopes, whereas children with persistent milk allergy had a significant portion of their IgE antibodies directed at specific sequential epitopes.[8-11]

Previous reports in children with egg allergy suggested that some can tolerate egg in the heated but not in the nonheated form.[12,13] In this study, we evaluated whether patients with milk allergy could tolerate extensively heated milk products.

METHODS

Participants

Subjects with milk allergy were recruited from the Mount Sinai Pediatric Allergy clinics. The study was approved by the Mount Sinai Institutional Review Board, and informed consent was obtained before enrollment.

The eligibility criteria included individuals between the ages 0.5 and 21 years, a positive skin prick test (SPT) or detectable serum milk-specific IgE, and a history of an allergic reaction to milk within 6 months before study entry; or milk-specific IgE levels or SPT highly predictive for clinical reactivity (if $\leq$2 years old, a level >5 kU_A/L; if >2 years old, a level >15 kU_A/L[14,15]; SPT wheal diameter $\geq$8 mm[16]). Exclusion criteria included negative SPT and undetectable milk-specific IgE; unstable asthma, allergic rhinitis, or atopic dermatitis; milk-induced eosinophilic gastroenteropathy; a recent reaction to a heated milk-product; or pregnancy.

Design

On the basis of the outcome of the heated milk challenge, subjects were categorized as heated milk–reactive or heated milk–tolerant. Heated

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Abbreviation used
SPT: Skin prick test

milk–tolerant subjects were further challenged with unheated milk; if tolerant, they were considered to have developed tolerance to milk. Heated milk–reactive subjects were instructed strictly to avoid all forms of milk (Fig 1). Heated milk–tolerant subjects were instructed to introduce heated milk products into their diet and were contacted monthly to monitor reactions and intake of heated milk products; they returned for re-evaluation at 3 months (Fig 1). Milk-tolerant subjects were instructed to add milk into the diet. Age, sex, milk-specific IgE, and SPT–matched subjects who fulfilled the inclusion criteria but declined participation served as a comparison group for the rate of natural tolerance development, defined as ingestion of all forms of milk at the end of the study—that is, tolerance to milk.

Allergy evaluation

Skin prick tests were performed as previously described.[17] A serum sample was collected for the measurement of specific IgE and IgG_4 to milk, casein, and β-lactoglobulin by using the UniCAP (Phadia, Portage, Mich).

Heated milk challenges were performed openly under physician supervision in the Mount Sinai General Clinical Research Center. Each muffin and waffle contained 1.3 g milk protein (nonfat dry milk powder; Nestle Carnation, Glendale, Calif). The muffin was baked at 350°F for 30 minutes in an oven, and the waffle (<0.625 inches thick to ensure thorough heating) was cooked in a waffle maker at approximately 500°F for 3 minutes. Each food was administered in 4 equal portions over 1 hour. The muffin was served first; if no symptoms were observed, 2 hours later, the waffle was served. Subjects were monitored throughout and for 2 to 4 hours after the completion of the final challenge. Challenges were discontinued at the first objective sign of reaction, and treatment was initiated immediately.

Children who tolerated both a muffin and a waffle were considered heated milk–tolerant. Heated milk–tolerant children with laboratory test results less than 95% predictive of clinical reactivity (milk-specific IgE levels ≤ 15 kU_A/L or SPT mean wheal diameter ≤ 8 mm)[14-16] were further challenged to regular unheated milk. Increasing doses of skim unheated milk totaling 240 mL (or other unheated milk products containing 8-10 g milk protein, eg, yogurt) were administered as previously described.[18-20]

Heated milk–tolerant subjects were instructed to ingest at least 1 to 3 servings per day of store-bought products with milk listed as a minor ingredient or home-baked products containing 1 cup of milk per 1 cup of flour, baked at ≥350°F for at least 30 minutes. Waffles <0.6 in thick were cooked as per the waffle maker's instructions.

Anthropometrics

To monitor effects of heated milk ingestion on growth, anthropometric parameters were followed. Weight and height percentiles for age and *z* scores were calculated with Nutchildren (Epi Info 3.4.1; Centers for Disease Control and Prevention, Atlanta, Ga).

Intestinal permeability

To monitor for subclinical gastrointestinal hypersensitivity responses, intestinal permeability was assessed by a measurement of urinary clearance of nonmetabolized sugars, as published.[21] A lactulose to mannitol ratio ≥0.025 was considered abnormal on the basis of the internal laboratory reference (Dr Jon Meddings, University of Alberta, Edmonton, Canada).

Statistics

t Tests or Mann-Whitney rank-sum tests were used for determining statistical significance ($P < .05$) between continuous variables; a paired *t* test or Wilcoxon signed-rank test was used when comparing different time points. Dichotomous variables were analyzed by using the χ^2 test. Predictive probabilities of the heated milk challenge outcome compared with milk-specific IgE and SPT results were calculated with logistic regression as published.[15,22] For multiple comparisons, ANOVA was performed using SPSS 13.0 (SPSS Inc, Chicago, Ill). The cut-points for milk-specific IgE level compared with the outcome of heated milk challenges were calculated as previously published.[23]

RESULTS

Oral food challenge to milk

One hundred children (mean age, 7.5 years; range, 2.1-17.3 years) underwent heated milk challenges. Sixty-eight children (68%) tolerated heated milk only (heated milk–tolerant), 23 (23%) reacted to heated milk (heated milk–reactive), and 9 (9%) tolerated heated milk and unheated milk challenges (tolerance to milk; Fig 1). Baseline clinical characteristics were not different among the groups (see this article's Table E1 in the Online Repository at www.jacionline.org).

Immunologic responses to milk at baseline

At baseline, heated milk–reactive subjects had significantly larger SPT mean wheal diameters and greater milk-specific, β-lactoglobulin–specific, and casein-specific IgE concentrations than heated and unheated milk–tolerant subjects (Table I). Casein-specific and β-lactoglobulin–specific IgG_4 levels were not significantly different among the 3 groups. None of the parameters evaluated were significantly different between heated milk–tolerant and milk-tolerant subjects.

Immunologic responses to milk in heated milk–tolerant subjects at 3 months

Heated milk–tolerant subjects had significantly smaller SPT mean wheal diameters and significantly greater casein-IgG_4 concentrations at 3 months compared with baseline. Median casein-specific and β-lactoglobulin–specific IgE, β-lactoglobulin–specific IgG_4, and milk-specific IgE levels were not significantly different (Table II).

Growth and intestinal permeability in heated milk–tolerant subjects

There were no significant differences in weight percentiles for age (mean, 40.5 vs 39.9; $P = .634$) and weight *z* score (mean, −0.313 vs −0.326; $P = .773$), height percentiles for age (median, 29.650 vs 30.25; $P = .996$), height *z* score (mean, −0.445 vs −0.229; $P = .142$), and body mass index (median, 16.311 vs 15.775; $P = .652$) between baseline and 3 months. The initial 29 subjects had intestinal permeability testing that showed no significant differences between baseline (median, 0.025; range, 0.01-0.080) and 3-month lactulose to mannitol ratio determinations (median, 0.028; range, 0.008-0.088; $P = .712$).

Withdrawals

Ten subjects did not return for a 3-month visit. The parents of 9 subjects were contacted by telephone; 1 family moved out of the country. Four of the 9 subjects for whom information was available continued to ingest heated milk products outside the study with no reported adverse effects. Five subjects chose not to introduce heated milk because of anxiety.

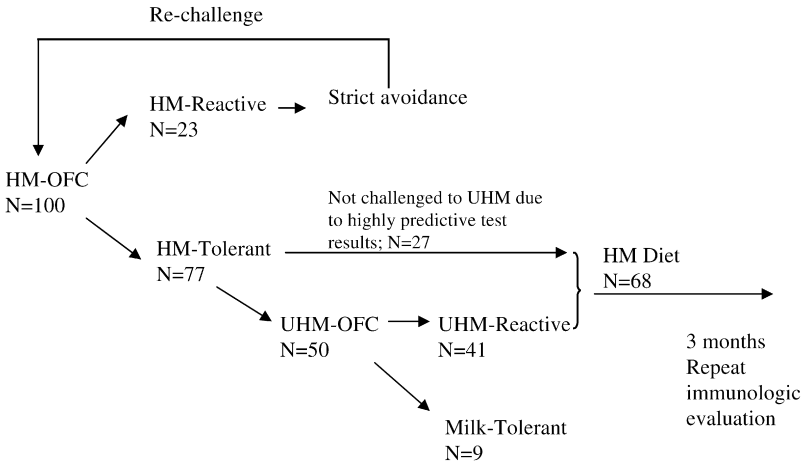

FIG 1. Study design and enrollment. *HM,* Heated milk; *OFC,* oral food challenge; *UHM,* unheated milk.

TABLE I. Baseline immunologic responses to milk proteins

	Heated milk–reactive (I)	Heated milk–tolerant (II)	Milk-tolerant (III)	*P* value* (I vs II)	*P* value* (I vs III)	*P* value* (II vs III)
Milk SPT, wheal size (mm), median (range)	9.5 (5-24)	7 (2.5-19)	6 (0-8)	**.009**	**.001**	.083
Milk IgE (kU_A/L), median (range)	11.6 (0.69-101)	2.43 (0-79.1)	0.925 (0.001-6.06)	**<.001**	**.003**	.854
Casein IgE (mg_A/L), median (range)	14.15 (0.71-101)	1.41 (0-101)	1.475 (0.48-3.69)	**<.001**	**.013**	.850
β-Lactoglobulin IgE (mg_A/L), median (range)	4.48 (0-101)	0.43 (0-63.7)	0.001 (0.001-2.32)	**.002**	.057	.960
Casein IgG_4 (mg_A/L), median (range)	1.53 (0.04-6.73)	0.64 (0-23.8)	1.35 (0.09-31)	.999	.173	.113
β-Lactoglobulin IgG_4 (mg_A/L), median (range)	0.57 (0-8.38)	0.36 (0-31)	1.23 (0.06-31)	.896	.326	.146
Casein IgE/IgG_4 ratio, median (range)	10.58 (0-69.25)	1.69 (0-131.2)	1.327 (0-3.667)	.079	.068	.588
β-Lactoglobulin IgE/IgG_4 ratio, median (range)	4.259 (0-54.74)	0.496 (0-1120)	0.0008 (0-2.417)	.868	.971	.82

Statistically significant *P* values are in boldface text.
*Determined by using ANOVA. *P* value less than .05 was considered statistically significant.

TABLE II. Comparison of baseline and 3-month immunologic parameters in heated milk–tolerant subjects

	Baseline median (range)	3-Month median (range)	*P* value*
Milk SPT, wheal size (mm)	8 (2.5-19)	7 (2-10.5)	**.001**
Milk IgE (kU_A/L)	2.5 (0-79.1)	1.99 (0-76)	.493
Casein IgE (mg_A/L)	1.29 (0-101)	1.6 (0-84)	.769
β-Lactoglobulin IgE (mg_A/L)	0.15 (0-63.7)	0.49 (0-18.7)	.758
Casein IgG_4 (mg_A/L)	0.54 (0-8.1)	1.02 (0.05-14.7)	**.005**
β-Lactoglobulin IgG_4 (mg_A/L)	0.29 (0-11.3)	0.49 (0-31)	.328
Undetectable casein IgG_4 (%)	6 (12)	0 (0)	**.027**†
Undetectable β-lactoglobulin IgG_4 (%)	7 (14.3)	6 (12)	1.0‡
Casein IgE/IgG_4 ratio	1.43 (0-131.2)	1.38 (0-55.4)	.148
β-Lactoglobulin IgE/IgG_4 ratio	0.23 (0-235.9)	0.49 (0-17.4)	.319

Statistically significant *P* values are in boldface text.
Determined by using *the paired *t* test or Wilcoxon signed-rank test, †Fisher exact test, and ‡χ^2 test. *P* value less than .05 was considered statistically significant.

Reactions in heated milk–tolerant subjects at large

No subjects reported serious acute reactions to heated milk, but 1 returned for a repeat heated milk challenge because of oral pruritus with homemade bread and Belgian waffles. She tolerated a repeat heated milk challenge without symptoms. It was determined that the homemade products were not sufficiently baked, and she resumed ingesting heated milk products prepared with strict adherence to our instructions.

Predictive value of allergy tests for the outcome of heated milk challenge

All subjects with undetectable serum milk-specific IgE (6/6) or small SPT (wheal <5 mm; 9/9) tolerated heated milk challenge, and thus both those negative tests had excellent sensitivity (100%) and negative predictive value (100%). However, specificity and positive predictive values were poor. Probability curves for passing the heated milk challenge based on milk-specific IgE and SPT results were generated (Fig 2). When different values of milk IgE were analyzed, the highest reaction rates to heated milk challenge were seen with milk IgE $\geq$35 kU_A/L; 6 of 7 subjects reacted (Table III). On the basis of a statistical model,[23] 5 kU_A/L milk-specific IgE was chosen as the optimal cut-point for identifying those who would tolerate a heated milk challenge.

Severity of symptoms during failed oral food challenges

Symptoms and treatments during challenges are shown in Table E2 (in the Online Repository available at www.jacionline.org). Eight of 23 heated milk–reactive subjects (35%) experienced anaphylaxis[24] and received epinephrine. The subjects reacted to a median dose of 0.5 g heated milk. No significant differences were found between subjects who had anaphylaxis at the time of heated milk challenge and those that did not for age, history of asthma, respiratory symptoms at first reaction to milk or milk-induced anaphylaxis, elapsed time since last anaphylaxis, SPT size, or lifetime peak milk-specific IgE levels (data not shown). None of the 41 subjects tolerating heated milk but

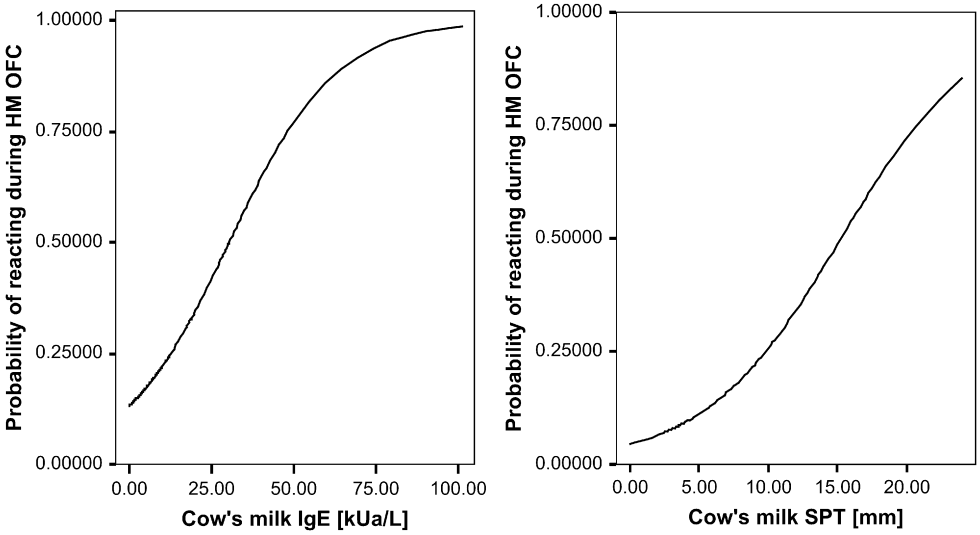

FIG 2. Predicted probabilities of the heated milk challenge outcome in regard to milk-specific IgE and SPT. Logistic regression was used to calculate the probability of reacting during heated milk *(HM)* oral food challenge *(OFC)* in regard to serum milk-specific IgE antibody concentration and SPT wheal size.

TABLE III. Percent tolerating heated milk oral food challenge in comparison with milk-specific IgE and SPT

Milk IgE (kU_A/L)	<0.35	0.35 to <5	5 to <20	20-100	<35	>15	>35
Tolerant	6/6 (100%)	51/57 (89.5%)	12/18 (66.7%)	4/14 (28.6%)	72/89 (80.9%)	6/17 (35.3%)	1/7 (14.3%)
Milk SPT wheal (mm)	0 to <3	3 to <5	5 to <8	<10	≥8	≥10	>14
Tolerant	2/2 (100%)	7/7 (100%)	32/39 (82.1%)	58/68 (85.3%)	31/45 (68.9%)	15/25 (60%)	2/6 (33.3)

Milk-specific IgE ≥15 kU_A/L and milk SPT wheal ≥8 mm have a 95% predictive value for acute reactions during an oral challenge with nonheated milk.[15,16]

reacting to unheated milk required epinephrine (Fig 1). The subjects reacted to a median dose of 0.4 g unheated milk.

DISCUSSION

The current standard of care for food allergy includes strict food avoidance.[25] It has been suggested that "outgrowing food allergy" may be expedited by strict avoidance, in that repeated exposures to even small allergen quantities may "boost" the IgE response and delay the development of tolerance in some patients.[5] However, the evidence to support this recommendation is limited. In children with atopic dermatitis and milk allergy, 20% of those adhering to a milk elimination diet for 1 to 2 years and 38% for 3 years had resolved milk allergy.[26,27] Anecdotal evidence suggests that some children with food allergy achieve tolerance despite continued or intermittent food exposures. Our group has reported that individuals with persistent milk allergy possessed higher levels of IgE antibodies directed at specific sequential casein epitopes compared with individuals who achieved tolerance.[8-11,28] On the basis of these observations and on the fact that extensive heating alters the conformation of milk proteins, we hypothesized that a majority of subjects with milk allergy (presumably those that do not generate significant IgE antibodies against sequential epitopes) would tolerate heated milk products. We further hypothesized that heated milk–tolerant children would be more likely to outgrow milk allergy, and in general, they would exhibit a less severe milk-allergic phenotype.

We found that the majority (75%) of children with a recent diagnosis of milk allergy tolerated extensively heated milk during an initial oral food challenge. Heated milk–tolerant subjects had significantly smaller SPT wheals, lower milk-specific and casein-specific IgE, and lower IgE/IgG_4 ratios to casein and β-lactoglobulin compared with heated milk–reactive subjects. These findings further support the notion that children with milk allergy are clinically and immunologically heterogeneous, and that reactivity to heated milk proteins is a marker of this heterogeneity.

The allergenicity of food proteins may be altered by processing. High heat—baking, for example—was found to reduce allergenicity of many food proteins, presumably by altering the conformation of heat-labile proteins that results in loss of conformational epitopes.[29] The classic examples are birch tree pollen allergen Bet v 1 cross-reactive proteins in apple (Mal d 1) and carrot (Dau c) that in the uncooked form cause oral symptoms (pollen–food allergy syndrome) but after heating are readily tolerated. In contrast, Bet v 1 cross-reactive protein in soybean, Gly m 4 retains allergenicity in heat-processed foods, suggesting that thermostability is highly variable and food-specific, even for the food allergens from the same protein family.[30] Alternatively, in the case of peanut proteins, high temperature may enhance allergenicity as a result of glycation (the Maillard reaction) that induces the formation of Ara h 2 aggregates that are more resistant to gastric digestion and bind IgE antibody more effectively than unheated Ara h 2.[31,32] Behavior under heating conditions is one of the determinants of allergenicity and may explain the different sensitizing potential of related foods, such as peanut and soybean. In addition to an individual susceptibility to heat and digestion, interactions with other substances present in a complex food, collectively referred to as the *food matrix effect*, may be crucial.[33] Published data indicate that heating decreases but does not completely eliminate milk allergenicity.[34] The caseins and α-lactalbumin have a higher heat stability than the other whey proteins, β-lactoglobulin and serum albumin.[35] After heating milk at 100°C for 10 minutes,

a substantial reduction of allergenicity was noted. Our studies evaluating the effects of heating (time and temperature) and food matrix on allergenicity of casein and whey proteins are ongoing.

Ingestion of heated milk products was associated with a statistically significant decrease in SPT wheals and an increase in casein-IgG_4 levels at 3 months compared with baseline. Decreasing levels of milk-specific IgE, increasing levels of β-lactoglobulin–IgG_4, and decreasing casein IgE/IgG_4 ratios were also noted, although these did not reach statistical significance. We hypothesize that these differences will become more significant as the subjects continue to ingest heated milk products. In view of earlier observations[27,36,37] in children who outgrew milk allergy, that casein and β-lactoglobulin IgE, IgG_1, IgG_4, and IgE/IgG_4 ratios were initially lower and further decreased over time, our findings suggest that ingestion of heated milk is associated with responses to casein and β-lactoglobulin that favor development of tolerance. In addition, the humoral changes observed in our study are in line with reports of the effects of oral desensitization to milk and egg white.[38,39] At this time, we do not know whether the ingestion of extensively heated milk products will result in permanent tolerance to unheated milk. Our follow-up study will address this issue. We hypothesize that the heated milk–tolerant subjects are the ones who respond favorably to oral milk desensitization. Preliminary mechanistic studies to determine the humoral and cellular mechanisms associated with nonreactivity to heated milk and the impact of heated milk ingestion on the development of milk tolerance reveal that heated milk–tolerant subjects have higher frequencies of allergen-specific forkhead box protein 3^+ ($FoxP3^+$) regulatory T cells[40] and lower basophil reactivity to milk allergens,[41] consistent with the hypothesis that patients with milk allergy who are tolerant to heated milk products define a distinct subset of children with milk allergy with more intact immune regulation.

We attempted to define diagnostic criteria for selecting subjects for heated milk challenge. We found that no subject with undetectable serum milk-specific IgE antibodies (<0.35 kU_A/L) or SPT mean wheal diameters <5 mm reacted to heated milk, whereas those with milk-specific IgE >35 kU_A/L had about an 85% chance of reacting during heated milk challenge. A decision point of 5 kU_A/L was proposed for identifying those who would tolerate heated milk challenge (approximately 90% rate of tolerating heated milk challenge). Children with milk allergy reacting to heated milk products were at higher risk for systemic reactions than those reacting only to unheated milk; the rate of epinephrine treatment was 35% in heated milk challenges compared with 0% in the nonheated milk challenges. This suggests that heated milk–reactive children have a more severe phenotype of milk allergy. A potential weakness of the current study is that children who tolerated heated milk and had milk-specific IgE or SPTs greater than the 95% predictive values were not challenged with unheated milk, for ethical reasons. However, because no child tolerating heated milk experienced a severe reaction to unheated milk in this trial, all children in our follow-up trial who do not react to heated milk will be challenged with unheated milk to confirm reactivity to unheated milk. Mechanistic studies are underway to characterize further the factors associated with nonreactivity to heated milk and predictors of the severity of clinical reactions.

Although our findings suggest that the majority of children with milk allergy will tolerate heated milk products, several limitations still preclude their application to clinical practice. We did not test a sufficient number of infants and young children to extend our conclusions to these younger age groups. Diagnostic criteria for selecting subjects who are likely to tolerate heated milk challenge and long-term effects of heated milk ingestion on growth and atopic diseases have yet to be established. We cannot exclude the possibility that the lower dose of heated milk (1.3 g per each food; total, 2.6 g) administered during the heated milk challenges, compared with the higher doses of milk potentially administered during the unheated milk challenges (8 g), confounded our observations in some individuals. However, the median eliciting dose of unheated milk protein was 0.4 g, and >80% of the subjects reacted to less than the amount served during the heated milk challenges (1.3 g). Furthermore, the study design aimed to reproduce the typical forms and quantities of milk protein in the heated (baked) products that are being ingested by children in real-life situations. In addition, practical limitations exclude the possibility of creating baked products of reasonable texture and quantity if milk protein amounts equivalent to the unheated milk challenges are used. Finally, heated milk challenges must be approached with caution and with all safety measures used in performing challenges to unheated milk.

Despite these reservations, our findings strongly suggest that there are at least 2 different phenotypes of IgE-mediated milk allergy in children. *Type I* individuals are eventually able to terminate T_H2 responsiveness and clinical reactivity, resulting in "transient" food allergy. *Type II* individuals are not able to downregulate T_H2 responsiveness and have persistent food allergy. If confirmed, this should change our approach to the diagnosis and management of milk allergy. Allowing ingestion of heated milk products will dramatically improve the quality of life for the majority of subjects with milk allergy by vastly increasing the variety of food products they are able to consume. This dietary change would facilitate fulfilling nutritional requirements, reduce parental anxiety, lessen discomfort in social situations, and potentially provide an effective strategy to shorten the time to achieve natural tolerance.

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Clinical implications: Our findings suggest that there are at least 2 different phenotypes of IgE-mediated milk allergy in children, which may change our approach to the diagnosis and management of milk allergy.

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TABLE E1. Baseline clinical characteristics of study participants

Characteristics*	All groups	Heated milk–reactive	Heated milk–tolerant	Milk-tolerant
Total subjects (%)	100 (100)	23 (23)	68 (68)	9 (9)
Male sex (%)	63 (63)	14 (61)	45 (66)	4 (44)
Age (y), median (range)	6.6 (2.1-17.3)	7.3 (3.4-14.9)	6.6 (2.1-17.3)	6.6 (2.6-14.7)
Asthma (%)	67 (67)	15 (65)	48 (71)	4 (44)
Allergic rhinitis (%)	80 (80)	17 (74)	57 (84)	6 (67)
Atopic dermatitis				
Current (%)	44 (44)	9 (39)	29 (43)	6 (67)
Resolved (%)	40 (40)	12 (52)	25 (37)	3 (33)
Additional food allergies* (%)	83 (83)	21 (91)	54 (79)	8 (89)
Family history of atopy (%)	94 (94))	22 (100)	63 (93)	9 (100)
Family history of milk allergy in a sibling (%)	23 (23)	6 (27)	17 (25)	0 (0)
Exclusively breast-fed (%)	70 (70)	16 (70)	47 (69)	7 (78)
Time exclusively breast-fed (mo), median (range)	5.8 (0.25-22)	5 (1-13.5)	6 (0.25-22)	3 (0.25-6)
Reaction while exclusively breast-fed (%)	42 (42)	9 (56)	30 (64)	3 (43)
Atopic dermatitis (%)	34 (34)	9 (100)	23 (77)	2 (67)
Gastrointestinal symptoms (%)	14 (33)	3 (33)	10 (33)	1 (33)
Lifetime history of allergic reaction to milk	97 (97)	22 (96)	67 (99)	8 (89)
Lifetime history of milk-induced anaphylaxis (%)	39 (39)	11 (48)	27 (40)	1 (11)
Age at first reported milk-induced reaction (mo), median (range)	4 (0.1-48)	4 (0.1-48)	4 (0.1-43)	3.5 (0.1-8)
Symptoms at first reported milk-induced reaction				
Skin† (%)	75 (75)	13 (59)	56 (84)	6 (75)
Upper respiratory‡ (%)	15 (15)	2 (9)	12 (18)	1 (13)
Lower respiratory§ (%)	8 (8)	1 (5)	6 (9)	1 (13)
Shortness of breath/respiratory distress‖ (%)	4 (4)	3 (14)	1 (1)	0 (0)
Gastrointestinal¶ (%)	39 (39)	11 (50)	24 (36)	4 (50)
Cardiovascular# (%)	0 (0)	0 (0)	0 (0)	0 (0)
Anaphylaxis** (%)	12 (12)	4 (17)	7 (11)	1 (13)

No statistically significant differences among the 3 groups unless noted; χ^2 test (dichotomous variables), *t* test (continuous variables).

*Limited to the major food allergens (egg, soy, wheat, peanut, tree nuts, fish, and shellfish).

†Atopic dermatitis, pruritus, rash, hives, or angioedema.

‡Sneezing, rhinoconjunctivitis, throat symptoms (grabbing or scratching neck; throat pruritus, pain, or tingling) or cough.

§Wheezing, shortness of breath, or respiratory distress (gasping, cyanosis, decreased oxygen saturation).

‖$P = .04$, χ^2 test.

¶Abdominal pain, vomiting, or diarrhea.

#Dizziness, loss of consciousness, or hypotension.

**Defined by the clinical criteria from the Second Symposium on Anaphylaxis.[24]

TABLE E2. Symptoms and treatments administered during oral food challenges

	Heated milk–reactive	Heated milk–tolerant, unheated milk–reactive
Total subjects (%)	23 (23)	41 (41)
Severity of symptoms, median anaphylaxis grade (range)*	2 (1-5)	2 (1-3)
Symptoms observed		
Skin‡ (%)	21 (91)	35 (85)
Upper respiratory§ (%)	17 (74)	26 (63)
Lower respiratory‖ (%)	4 (17)	2 (5)
Gastrointestinal¶ (%)	8 (35)	9 (22)
Cardiovascular# (%)	1 (4)	1 (2)
Treatments received		
Diphenhydramine (%)	22 (96)	41 (100)
Epinephrine (%)†	8 (35)	0 (0)
Methylprednisolone, intravenous (%)	6 (26)	2 (5)
Albuterol, nebulized (%)	2 (9)	0 (0)
Intravenous fluids (%)	2 (9)	0 (0)
Cetirizine (%)	1 (4)	0 (0)
Ranitidine (%)	1 (4)	0 (0)

No statistically significant differences seen between the 2 groups, except for *anaphylaxis grade, $P = .006$; and †treatment with epinephrine, $P < .001$. P value less than .05 was considered statistically significant.

‡Atopic dermatitis flare, pruritus, rash, hives or angioedema.

§Sneezing, rhinoconjunctivitis, throat symptoms (hoarseness; throat pruritus, pain or tingling; "throat feels weird/funny") or cough.

‖Wheezing, shortness of breath or respiratory distress (gasping, cyanosis, decreased oxygen saturation).

¶Abdominal pain, nausea, vomiting or diarrhea.

#Dizziness, loss of consciousness or hypotension.