

Letter to the Editor

Standard testing fails to identify patients who tolerate baked milk

To the Editor:

Cow's milk (CM) allergy (CMA) is one of the most common food allergies in children, with a prevalence of 0.5% to 3.8%.¹⁻³ Historically, patients were advised to avoid all forms of CM. However, recent studies have shown that about 75% of children with CMA can tolerate baked milk (BM).⁴ There is limited knowledge about the predictors of BM tolerance.¹

Previous studies have used open oral food challenges (OFCs) in populations felt to be at a lower risk of a reaction.^{1,4-7} Higher IgE levels to CM and the milk component casein, as well as larger CM skin prick tests (SPTs), have been suggested as predictors for BM reactivity, but cutoffs for recommending a BM OFC have varied.⁴⁻⁷

Identifying children who tolerate BM products is extremely important because incorporating BM into the diet may help to accelerate resolution of the allergy,⁸ while also improving nutrition and quality of life.¹

TABLE I. Baseline characteristics of children undergoing screening BM DBPCFC

Characteristic	Overall (n = 41)	BM tolerant (n = 11)	BM reactive (n = 30)	P value
Demographic				
Age at screening (y), median (range)	11 (3-18)	8 (4-16)	11 (3-18)	.86
Sex: male, n (%)	23 (56)	7 (64)	16 (53)	.56
Race, n (%)				
White	26 (63)	5 (45)	21 (70)	.49
Black	6 (15)	2 (18)	4 (13)	
Asian	5 (12)	2 (18)	3 (10)	
Multiple races	4 (10)	2 (18)	2 (7)	
Hispanic, n (%)	1 (2)	0	1 (3)	.54
Milk allergy history				
Age at diagnosis (mo), median (range)	9 (3-24)	9 (6-24)	9 (3-24)	.1
History of milk reaction, n (%)	41 (100)	11 (100)	30 (100)	—
Previous milk reaction form, n (%)				.55
Direct only	11 (27)	3 (27)	8 (27)	
Baked only	3 (7)	0 (0)	3 (10)	
Both direct and baked	27 (66)	8 (73)	19 (63)	
Exposed to BM before study, n (%)	31 (76)	9 (82)	22 (73)	.58
Months since most recent reaction, from screening, median (range)	15 (1-146)	15 (1-146)	15 (1-108)	.55
Milk exposure in most recent reaction, n (%)				.72
Direct	20 (49)	4 (36)	16 (53)	
Baked	15 (37)	5 (45)	10 (33)	
Cooked cheese	2 (5)	1 (9)	1 (3)	
Unsure	4 (10)	1 (9)	3 (10)	
No. of milk reactions, median(range)	3 (1-50)	3 (2-10)	4 (1-50)	.12
Screening OFC				
Maximum cumulative tolerated dose during DBPCFC (mg), n (%)				NA
4	3 (7)	0	3 (10)	
14	5 (12)	0	5 (17)	
44	6 (15)	0	6 (20)	
144	16 (39)	0	16 (53)	
444	11 (27)	11 (100)	0	
Required epinephrine during OFC, n (%)	18 (44)	0	18 (60)	.001
Other atopic history				
Current atopic dermatitis, n (%)	23 (56)	7 (64)	16 (53)	.56
Current asthma, n (%)	30 (73)	10 (91)	20 (67)	.12
Current allergic rhinitis, n (%)	29 (71)	8 (73)	21 (70)	.87
Nonmilk food allergies, n (%)	36 (88)	11 (100)	25 (83)	.15
No. of current food allergies, median (range)	5 (1-11)	5 (2-7)	4 (1-11)	.12
Laboratory and skin tests, median (range)				
Milk IgE (kU/L)	86.8 (7.2-1625)	42.7 (9.7-1007)	87.7 (7.2-1625)	.39
Alpha-lactalbumin IgE (kU/L)	19.1 (0.2-556)	9.9 (2.3-74.4)	20.1 (0.2-556)	.50
Beta-lactoglobulin IgE (kU/L)	20.6 (0.4-487)	14.1 (1.1-71.8)	24.5 (0.4-487)	.46
Casein-IgE (kU/L)	52.8 (4.2-1326)	22.7 (4.2-777)	64.8 (5.9-1326)	.12
Milk IgG ₄ (mg/L)	9.2 (6.9-19)	9.8 (7.6-14.5)	9.2 (6.9-19)	.53
Baseline milk SPT (wheal size in mm)	10 (5-26)	10 (6-26)	13 (5-25)	.44

Milk refers to CM.

NA, Not applicable.

TABLE II. Characteristics of BM-tolerant subjects

Patient	Age at DBPCFC (y)	Months since last milk reaction	Milk form at last reaction	Milk IgE (kU/L)	Casein-IgE (kU/L)	β -Lactoglobulin IgE (kU/L)	Milk IgG ₄ (mg/L)	SPT wheal (mm)	Follow-up-time since DBPCFC (mo)	Milk intake		
										Currently tolerated amount	Frequency	Symptoms
1	12	8	Cooked cheese	20.2	18.2	4.2	12.3	26	18	1-2 slices of cheese pizza	Daily to few times/wk	No symptoms with current amount. Oral pruritus with grilled cheese and ice cream. Hives, pruritus, periorbital swelling, and wheezing with 4 slices of pizza
2	16	146	Direct	1007	777	71.8	14.5	8	16	1/4-1/3 cup of goldfish crackers	1-2×/wk	No symptoms with current amount. Had occasional nausea with higher amounts
3	7	60	Unknown	96.4	9.7	5.3	9.8	10	16	Cooked cheese (up to 12 g)	4-5×/wk	Symptoms (oral pruritus) with unheated milk only. Fudge popsicle—oral pruritus and hives on lips
4	7	6	Baked	10.4	7.5	1.1	7.8	10	No follow-up	Unknown		
5	7	7	Baked	35.9	17.5	16.7	8.1	19	9	1/4 cup of goldfish crackers	5×/wk	No symptoms with current amount. With 1/2 cup of goldfish crackers, felt anxious, throat felt funny, had 1-2 hives
6	5	1	Baked	196	90.2	44	9.0	13	9	50 mg of BM	3×/wk	No symptoms with current dose. Oral pruritus and stomach pain with 250 mg (1/32 cup BM); accidental exposure to ice cream—hives on face and itchy throat
7	14	21	Baked	9.7	4.2	1.8	7.6	6	8	15-30 goldfish crackers	Daily	Mild oral pruritus with eating larger amounts of goldfish crackers
8	15	22	Baked	191	91.3	30.2	7.8	9	5	2000 mg (¼ cup) BM	Daily	Rarely has pruritus
9	14	24	Direct	42.7	22.7	13.1	14	8	5	2000 mg (¼ cup) BM	3-4×/wk	No symptoms with current dose. Accidental exposure to straight milk and had oral pruritus and abdominal pain
10	4	15	Direct	41.1	25.7	14.1	10.1	8	5	1000 mg (1/8 cup) of BM	Daily	No regular symptoms with current amount. Twice—active after eating BM and had cough, rhinorrhea, wheezing—treated at home with antihistamine and albuterol
11	8	2	Direct	333	344	49.2	10.6	10	2	10 goldfish crackers	Every other day	Stomachache with >10 crackers

Milk refers to CM.

Here, we report results of BM OFCs for patients undergoing screening for a BM oral immunotherapy randomized clinical trial (NCT03462030). This is the first report of double-blind placebo-controlled food challenges (DBPCFCs) to BM in a high-risk population that was challenged with a clear history of CM reactivity, most including BM, and no upper limit of IgE or SPT results.

Subjects with CMA were recruited from the Johns Hopkins Pediatric Allergy Clinic. The study was approved by the Johns Hopkins Institutional Review Board and informed consent was obtained. Eligibility criteria included age 3 to 18 years, positive SPT result to CM, CM-IgE more than 5 kU/L, and history of reactivity to CM (full inclusion/exclusion criteria in this article's Online Repository at www.jacionline.org). At the baseline visit, serum was collected for specific IgE to CM, casein, β -lactoglobulin, α -lactalbumin, and CM-IgG₄ using ImmunoCap (ThermoFisher, Uppsala, Sweden) and CM-SPTs were performed (Greer Laboratories, Lenoir, NC). Questionnaires were completed regarding demographic characteristics, previous milk reactions, other food allergies, and other atopic history.

To be eligible for enrollment in the randomized clinical trial, subjects had to react to less than or equal to 444 mg or approximately 3 teaspoons (1/20th cup) (cumulative) of BM (see additional details in this article's Online Repository at www.jacionline.org). BM was prepared as milk powder baked in a cake at 350°C for at least 30 minutes. Subjects tolerating a cumulative dose of 444 mg without dose-limiting symptoms were categorized as BM tolerant and excluded from the clinical trial. They were given detailed instructions for home BM introduction, starting at 250 mg (1/32nd of a cup), slightly lower than the maximum tolerated dose of 300 mg in the challenge to ensure safety. After screening, BM-tolerant subjects were seen in clinic or contacted via phone to assess current milk intake and symptoms.

t tests or Wilcoxon rank-sum tests were used when assessing between-group differences ($P < .05$) in continuous variables, and chi-square test was used to analyze categorical variables.

From February 2018 to November 2019, 41 subjects underwent BM DBPCFCs (Table I). All had a history of CM reactions and 31 had previous exposure to BM, with 30 of 31 reporting reactions. After the DBPCFC, 11 (27%) were categorized as BM tolerant (Tables I and II). There were no significant differences in age, sex, race/ethnicity, presence of atopic dermatitis, asthma, allergic rhinitis, or nonmilk food allergies between those who were BM reactive or tolerant (Table I). There were also no differences, or even a trend toward differences, in CM-IgE, casein-IgE, β -lactoglobulin-IgE, α -lactalbumin-IgE, CM-IgG₄, or CM-SPT between groups (Table I).

Of the 11 children who passed the BM OFC, 10 of 11 have had at least 1 clinic or phone follow-up visit and all 10 reported some milk in their diet (Table II). There was a wide range in milk intake, with 3 of 10 eating products with cooked cheese and 7 of 10 eating products with lesser amounts of BM, ranging from 10 goldfish crackers to 2000 mg (Table II). All were tolerating their current level of intake with no or infrequent symptoms, although 6 of 10 had symptoms (primarily abdominal pain or oral pruritus) with larger amounts, and 4 had symptoms with less heated forms of milk. One child reported exercise-related symptoms on 2 occasions. No reactions required epinephrine.

Thirty children had dose-limiting symptoms during the BM OFC. The median cumulative tolerated dose was 144 mg, with a

range of 4 to 444 mg. Symptoms included cutaneous (73%), gastrointestinal (73%), upper respiratory tract (37%), and lower respiratory tract (33%). Eighteen (60%) were treated with epinephrine.

Contrary to previous studies, we found no useful predictors of BM OFC outcome. Specifically, there was no difference in CM-IgE, casein-IgE, or SPT, as previously suggested.^{5-7,9} It has been proposed that casein-IgE is the best predictor of BM tolerance with a proposed 95% specificity of 20.2 kU/L for BM reactivity.⁵ In our cohort, there was no difference in casein-IgE between groups ($P = .12$) and the median casein-IgE in the BM-tolerant group was 22.7 kU/L. Previous studies have suggested optimal CM-IgE cutoffs for BM OFCs of 5 kU/L⁴ or 9.3 kU/L.⁷ In our study, 11 of 11 BM-tolerant children had a CM-IgE level of more than 9.3 (median, 42.7 kU/L). The remarkably poor predictive value of this measure is exemplified by a BM-tolerant subject with a CM-IgE level of 1007 kU/L (casein-IgE, 777 kU/L) and a BM-reactive subject with a CM-IgE level of 7.2 kU/L (casein-IgE, 5.91 kU/L) with a maximum tolerated dose of only 100 mg. This variability could in part be due to the differences in patient populations and preparation and dose of BM, but it nonetheless supports the real need for better biomarkers to phenotype patients allergic to milk.

In this study, we defined BM tolerant as those tolerating greater than or equal to 444 mg. We recognize that this is a smaller dose than that used in other studies to define tolerance (1.3, 2.6, 3.6 g)^{4,6,7}; however, all our BM-tolerant subjects have been able to keep some amount of milk in their diet and most have been able to gradually increase their dose at home with minimal symptoms. Similarly, from our study of BM OFCs done for clinical indications, we were often able to successfully introduce BM after failing OFCs with a goal dose of 2000 mg.⁹

In conclusion, this is the first report of DBPCFCs to BM in children with a history and/or laboratory testing suggesting severe milk allergy, all of whom we suspected would be BM reactive. We were surprised at the number of children found to be BM tolerant (27%) despite their reaction history, significantly elevated CM- and casein-IgE levels, and large SPT wheals. In the past, most of these children would not have been challenged in our traditional clinic setting, even recognizing the huge benefits of BM introduction. Although this suggests that all patients allergic to milk should be offered BM OFCs, irrespective of their IgE and SPT results, it is critically important to recognize that many patients in this study had significant challenge reactions, many at very low doses, with 18 of 30 (60%) requiring epinephrine. Challenges such as these need to be conducted with extreme caution, highlighting the need for improved biomarker testing and the importance of individualizing care and shared decision making for each and every patient.

Jennifer A. Dantzer, MD

Joan H. Dunlop, MD, MHS

Robert A. Wood, MD

From the Department of Pediatrics, Division of Allergy and Immunology, Johns Hopkins University School of Medicine, Baltimore, Md. E-mail: Jwrubel1@jhmi.edu.

This research was funded in part by the Myra Reinhard Family Foundation.

Disclosure of potential conflict of interest: J. A. Dantzer receives research funding from the National Institutes of Health/National Institute of Allergy and Infectious Disease (NIH/NIAID). J. H. Dunlop is supported by the Pearl M. Stetler Fund. R. A. Wood receives research funding from the NIH/NIAID, Aimmune, DBV, Sanofi, Regeneron, and Astellas.

REFERENCES

1. Leonard SA, Caubet J-C, Kim JS, Groetch M, Nowak-Węgrzyn A. Baked milk- and egg-containing diet in the management of milk and egg allergy. *J Allergy Clin Immunol Pract* 2015;3:13-23, quiz 24.
2. Flom JD, Sicherer SH. Epidemiology of cow's milk allergy. *Nutrients* 2019;11.
3. Dunlop JH, Keet CA. Epidemiology of food allergy. *Immunol Allergy Clin North Am* 2018;38:13-25.
4. Nowak-Węgrzyn A, Bloom KA, Sicherer SH, Shreffler WG, Noone S, Wanich N, 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.
5. Caubet J-C, Nowak-Węgrzyn A, Moshier E, Godbold J, Wang J, Sampson HA. Utility of casein-specific IgE levels in predicting reactivity to baked milk. *J Allergy Clin Immunol* 2013;131:222-4.e1-4.
6. Bartnikas LM, Sheehan WJ, Hoffman EB, Permaul P, Dioun AF, Friedlander J, et al. Predicting food challenge outcomes for baked milk: role of specific IgE and skin prick testing. *Ann Allergy Asthma Immunol* 2012;109:309-13.e1.
7. De Boer R, Cartledge N, Lazenby S, Tobias A, Chan S, Fox AT, et al. Specific IgE as the best predictor of the outcome of challenges to baked milk and baked egg. *J Allergy Clin Immunol Pract* 2020;8:1459-61.e5.
8. Kim JS, Nowak-Węgrzyn A, Sicherer SH, Noone S, Moshier EL, Sampson HA. Dietary baked milk accelerates the resolution of cow's milk allergy in children. *J Allergy Clin Immunol* 2011;128:125-31.e2.
9. Dunlop JH, Keet CA, Mudd K, Wood RA. Long-term follow-up after baked milk introduction. *J Allergy Clin Immunol Pract* 2018;6:1699-704.

<https://doi.org/10.1016/j.jaci.2020.03.030>

METHODS

Inclusion/exclusion criteria for the randomized clinical trial (NCT03462030)

Patients who meet *all* the following criteria are eligible for enrollment as study participants, including participants who

- are aged 3 to 18 years, either sex, any ethnicity or race.
- provide signed informed consent by parent or legal guardian and informed assent if applicable.
- have a history of symptomatic reactivity to CM (ie, eczema, urticarial, upper or lower respiratory tract symptoms, gastrointestinal disturbances, rash, oral symptoms).
- have a skin prick test positive to milk (diameter of wheal 3 mm $\geq$ negative control) and serum milk-specific IgE level more than 5 kU/L within the past 12 months.
- have a positive reaction to a cumulative dose of less than or equal to 444 mg of BM protein in the initial qualifying DBPCFC.
- use an effective method of contraception by females of childbearing potential to prevent pregnancy and agree to continue to practice an acceptable method of contraception for the duration of their participation in the study.
- have self-injectable epinephrine available at all times.

Patients who meet *any* of these criteria are not eligible for enrollment as study participants, including participants who

- have a history of severe anaphylaxis resulting in hypotension, neurological compromise, or mechanical ventilation.
- have a history of intubation related to asthma.
- tolerate more than 444 mg of BM protein at the initial qualifying DBPCFC.
- have allergy to placebo ingredients OR react to any dose of placebo during the qualifying OFC.
- have poor control of atopic dermatitis.
- are unable to tolerate at least 3 mg of BM protein on dose escalation day.
- are pregnant or lactating.
- have severe asthma defined by 2007 National Heart, Lung, and Blood Institute criteria steps 5 or 6.
- have severe or poorly controlled asthma defined by any of the following criteria:
 1. FEV₁ less than 80% of predicted,
 2. ICS dosing of more than 500 μ g daily of fluticasone (or equivalent inhaled corticosteroids based on National Heart, Lung, and Blood Institute dosing chart), or
 3. 1 or more hospitalization in the past year for asthma, or
 4. more than 1 emergency room visit in the past 6 months for asthma.
- use steroid medications (oral steroids, such as prednisone or Medrol, steroid injections, such as Kenalog, or intravenous or oral corticosteroid burst) in the following manner: History of daily oral steroid dosing within 4 weeks before baseline visit *or* for more than 1 month during the past year *or* more than 2 burst oral steroid courses in the past 6 months.
- are unable to discontinue antihistamines for 5 days for long-acting and 3 days for short-acting before skin testing or food challenges.
- are receiving omalizumab, mepolizumab, beta-blocker, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, calcium channel blockers, or tricyclic antidepressant therapy.
- have used immunomodulatory therapy (not including corticosteroids) or biologic therapy within the past year.

- have participated in any interventional study for treatment of a food allergy in the past 6 months.
- are on “build-up phase” of environmental allergen immunotherapy. Subjects tolerating maintenance allergen immunotherapy can be enrolled.
- have a history of eosinophilic esophagitis in the past 3 years.
- have a chronic disease (other than asthma, atopic dermatitis, rhinitis) requiring therapy (eg, heart disease and diabetes).
- have used an investigational drug within 90 days or plan to use an investigational drug during the study period.
- have severe reaction at initial DBPCFC, defined as
 - life-threatening anaphylaxis.
 - requiring overnight hospitalization.

Skin prick testing

Skin prick testing was performed with the commercial CM extract using the GREER Pick system according to Greer's instructions for “prick” method of skin testing. Participants were advised to stop antihistamines (5 days for long-acting and 3 days for short-acting) before skin testing. After 15 minutes, we measured the longest length of the wheal and flare. The size of the wheal was calculated as the CM wheal size (longest length) minus the saline wheal size (longest length).

Oral food challenges

The OFC cake was prepared by the Johns Hopkins Research Nutrition team. A milk, wheat, egg, soy, and nut-free cake batter was prepared. The active cake included organic, nonfat CM powder and the placebo cake included irradiated tapioca flour. The cake was baked at 350° for at least 30 minutes.

Food challenges were conducted per PRACTALL guidelines,^{E1} using the dosing table (Table E1) with doses given approximately 15 minutes apart.

Study data were collected and managed using Research Electronic Data Capture electronic data capture tools hosted at Johns Hopkins University.^{E2,E3} Research Electronic Data Capture is a secure, Web-based software platform designed to support data capture for research studies. Data were analyzed using Stata statistical software (version 14; StaCorp, College Station, Texas).^{E4}

REFERENCES

- E1. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology–European Academy of Allergy and Clinical Immunology PRACTALL consensus report. *J Allergy Clin Immunol* 2012;130:1260-74.
- E2. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377-81.
- E3. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.
- E4. StataCorp. *Stata Statistical Software: Release 14*. College Station, Texas: StataCorp LP; 2015.