

Skin testing with different food formulations in pediatric patients with eosinophilic esophagitis

To the Editor,

Eosinophilic esophagitis (EoE) is an immune-mediated inflammatory condition characterized clinically by esophageal dysfunction and evidence of ≥ 15 eosinophils per high-power field on esophageal biopsy.¹ EoE is reportedly triggered by food and/or environmental allergens. Treatments include topical corticosteroids, proton-pump inhibitors, or dietary avoidance measures. Currently, non-invasive tests such as skin prick test (SPT), atopy patch test (APT), and specific serum IgE (sIgE) are utilized by clinicians to identify potential offending allergens in their EoE patients.¹

While APTs are used in the workup for non-IgE-mediated allergies, recent data argue their utility may not be as useful as previously believed.² A major drawback of APT in food allergy is lack of standardization of allergenic extracts and variability in the sensitivity and specificity of testing results. In fact, Eckman et al showed the sensitivity of APT in adults was only 5.9% using histological confirmation with food reintroduction.² While evidence does not necessarily support the use of SPT, APT, and sIgE, these tests are still used by clinicians in EoE workup. A significant issue concerning food-specific assay standardization is the variety of formulations available. There are currently no set SPT or APT guidelines available for testing in non-IgE-mediated food allergies; however, SPT guidelines exist for IgE-mediated food allergies.³ Physicians typically use fresh foods for APT testing which can limit test convenience, as perishable fresh foods must be frequently replaced. The potential for use of powder or extract testing for EoE would greatly increase feasibility of these testing modalities.

Clinicians may choose between fresh, powder, and extract forms of food antigens for SPT and APT. There has been little research exploring the differences among fresh, powder, and extract for SPT or APT in EoE patients. Previous SPT research suggests that fresh food may be more sensitive for IgE food allergy testing.^{4,5} For APT, Canani et al⁶ found that fresh cow's milk and egg had both a higher sensitivity and negative predictive value (NPV), but a similar positive predictive value (PPV) as commercial extract for food allergy-related testing. Differences between extracts have also been reported with lyophilized extracts having a higher sensitivity and NPV than commercial extract for food allergy-related gastrointestinal symptoms.⁷ In order to be of true clinical value, consensus must exist among

experts regarding appropriate formulations, testing methodologies, and interpretation of APTs.

In EoE, SPT and APT can be used to detect potential food triggers, but generalizability is limited by the different requirements for each test: fresh foods for APT and generally the use of extract for SPT. If powder form could be employed for SPT and APT, this would increase confidence in the results, as equivalent forms of the allergen can be used in both tests. From a practical standpoint, powder also has the added benefit of a longer shelf life. Conversely, if extract could be employed for both tests, the same confidence and practicality could also be achieved. Therefore, we hypothesized that powder would yield the same standard as the common practice of using extract in SPT and fresh in APT for EoE testing. To test this hypothesis, we compared fresh, powder, and extract forms of milk, egg, soy, and wheat in SPT and APT testing, in pediatric patients with EoE, in order to compare skin reactivity with different formulations of each food and determine if different forms are equivalent.

This single-center pilot study was approved by the Baylor College of Medicine IRB. Subjects 1-18 years of age with biopsy-proven EoE were recruited from the Texas Children's Hospital Eosinophilic Gastrointestinal Disorders Clinic. Written informed consent was obtained prior to participation. SPTs were performed using a QUINTIP Skin Test Device. Food preparations for SPT and APT consisted of fresh (F), powder (P), and extract (E) forms of milk, egg, soy, and wheat. The food preparations were prepared by placing either 0.5g of fresh food, 1g dry food mixed with 1mL of isotonic saline until paste, or using commercial liquid food extract (please see Appendix S1 for information on where allergens and extracts were obtained from). SPTs were performed on the volar forearm and APTs on the back with fresh (F), powder (P), and extract (E) forms of milk, egg, soy, and wheat. For SPT, the food preparations were directly pricked through the skin, and for APTs, the measured food preparations were placed separately on a paper disk, or for extract, the paper disk was saturated with 3-4 drops of commercial extract and adhered to a Finn chamber and placed on the back. Histamine (positive control) and saline (negative control) were applied for comparison measures for SPT and saline (negative control) for APT. Of note, the wheal for an SPT (food) was considered positive if it was 3mm larger than the SPT (saline) wheal. Therefore, the SPT (food) was calculated as follows: SPT (food)-SPT (saline). To avoid negative values when calculating for the SPT (food), the final value was recorded as the maximum of the calculated difference or zero (see Table S1 for summary statistics). APTs applied to the skin

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were removed at 48 hours and read at 72 hours. APT results were classified according to International Contact Dermatitis Research Group on a scale of 0-3.⁸ Pairwise differences (fresh-powder; extract-fresh; and extract-powder) were computed for each food-test combination. Wilcoxon signed-rank test was used for pairwise comparisons for the different formulations on SPT and APT. *P*-values were adjusted for multiple hypotheses with the Holm stepdown Bonferroni method. *P* < .05 was considered significant.

Twenty-eight patients were enrolled and underwent SPT and APT for milk, egg, soy, and wheat. Each food was tested with three different food preparations: fresh, powder, and extract. Of the 28 patients, 1 subject was removed from study due to intolerance of patch adhesives and 2 subjects were removed from analysis as they did not receive all patches. A pairwise comparison of SPT wheal size between fresh-powder, fresh-extract, and powder-extract forms of food allergens showed no significant difference (*P* > .05) (Table 1). Significant differences were observed in APT pairwise comparisons for different food preparations (Table 2). A significant difference in APT skin reactivity (*P* < .05) was seen between extract-fresh and extract-powder for all food allergens, where skin reactivity was more pronounced in the fresh and powder forms compared to the extract. No significant difference was observed between fresh-powder forms for any food allergens. Adjusting for multiple hypotheses, there remained no significant difference between fresh-powder forms of food allergens. No significant differences in APT skin reactivity were observed between different egg preparations. APT skin reactivity was more pronounced in the fresh forms for milk (*P* = .005) and soy (*P* = .010) compared to extract. APT skin reactivity was more positive in the fresh (*P* = .011) and powder (*P* = .021) forms of wheat compared to wheat extract.

In summary, skin reactivity for three different formulations (fresh, powder, and extract) of common EoE food allergens (milk, egg, soy, and wheat) were compared using SPT and APT. Pairwise comparison of SPT formulations for milk, egg, soy, and wheat (fresh-powder,

fresh-extract, and powder-extract) showed no significant difference. However, a statistical difference in APT skin reactivity grade between fresh-extract and powder-extract was found for all foods. Using APT, skin reactivity for extract was significantly less compared with fresh milk (adj *P*-value = .005), soy (adj *P*-value = .010), and wheat (adj *P*-value = .011). APT skin reactivity for wheat extract was also significantly reduced from powder (adj *P*-value = .021). The comparison between powder and extract was only significant for wheat. After adjusting for multiple hypotheses, fresh and powder formulations displayed similar APT responses, while use of extracts displayed significantly less inflammation.

Best practice management of EoE includes topical swallowed corticosteroids, proton-pump inhibitors, and/or dietary elimination. Standard food elimination diets require allergen avoidance followed by repeat endoscopy with biopsy to reassess disease activity. This approach can be costly, time-consuming, inconvenient, and not without adverse effects such as side effects from anesthesia or the rare instance of esophageal perforation from multiple biopsies. Utilizing SPT and APT as part of a test-based elimination diet with allergen avoidance may lead to disease remission in up to 65%-77% patients.⁹⁻¹¹ Studies have shown SPT to have an NPV of >90% (except for egg, wheat, soy 80%-90%, and milk 30%) and PPV of 50%. APT has NPV of >90% (except for milk 31%) and PPV of 44%. Using combined APT and SPT to form an elimination diet, NPV averaged 92% and PPV was 44%, with the exception of milk which had an NPV and PPV at 44%.⁹ Although test-based elimination diet studies show variable sensitivities, testing may offer useful guidance for clinicians and families, especially for patients who have difficulty or low compliance with empiric elimination diets. If shown to induce equivalent measurable reaction as fresh food for APT or extract for SPT, powder could replace fresh in SPT or APT testing.

Our research work explores the hypothesis that powdered allergen could reflect immune skin test response as precisely as fresh allergen. It demonstrates the potential for use of non-perishable

Food	Comparison	N	Median difference (mm) (min, max)	P-Value	Stepdown Bonferroni P-value
Milk	Fresh-powder	15	0 (-2, 1)	1.000	1.000
	Extract-fresh	23	0 (-5, 1)	.250	1.000
	Extract-powder	15	0 (-2, 1)	1.000	1.000
Egg	Fresh-powder	15	0 (-2, 2)	.750	1.000
	Extract-fresh	23	0 (-12, 4)	.082	1.000
	Extract-powder	15	0 (-5, 2)	.687	1.000
Soy	Fresh-powder	15	0 (-3, 4)	.562	1.000
	Extract-fresh	22	0 (-3, 5)	1.000	1.000
	Extract-powder	15	0 (-4, 3)	1.000	1.000
Wheat	Fresh-powder	15	0 (-5, 0)	.125	1.000
	Extract-fresh	22	0 (-1, 3)	.500	1.000
	Extract-powder	15	0 (-5, 0)	.250	1.000

Note: There were no significant differences seen in these testing conditions.

TABLE 1 Wilcoxon signed-rank test *P*-values testing for significant differences in comparison between food formulations on skin prick testing (SPT)

TABLE 2 Wilcoxon signed-rank test *P*-values testing for significant differences in comparison between food formulations on atopy patch testing (APT)

Food	Comparison	N	Median difference (mm) (min, max)	<i>P</i> -Value	Stepdown Bonferroni <i>P</i> -value
Milk	Fresh-powder	25	0 (−1, 1)	.491	1.000
	Extract-fresh	25	−0.5 (−2, 0.5)	<.001*	.005*
	Extract-powder	25	0 (−2, 0.5)	.008*	.156
Egg	Fresh-powder	22	0 (−2, 1)	1.000	1.000
	Extract-fresh	22	0 (−2.5, 0)	.008*	.156
	Extract-powder	22	0 (−2, 0.5)	.016*	.269
Soy	Fresh-powder	24	0 (−1, 2)	.433	1.000
	Extract-fresh	24	−0.5 (−2, 0.5)	<.001*	.010*
	Extract-powder	24	−0.5 (−2, 2)	.015*	.269
Wheat	Fresh-powder	24	0 (−1, 1)	.508	1.000
	Extract-fresh	24	−0.3 (−2, 0)	<.001*	.011*
	Extract-powder	24	0 (−2, 0)	.001*	.021*

Note: The (*) denotes significant *P*-values (*P* < .05).

powdered food APT over fresh formulations in EoE patients. There are few studies exploring different food formulations in APT and SPT, with fresh foods used to study efficacy of APT and SPT testing. Fresh food is shown to be more sensitive than extract when testing for IgE food allergies and is closest to what patients consume.¹¹ We have demonstrated that all food allergens had lesser APT responses to extract than fresh or powder forms. Most interestingly, APT for fresh and powder forms were not significantly different in our study, indicating that they may be used interchangeably. Further studies correlating esophageal inflammation and positive SPT and APT tests are necessary to validate these results.

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CONFLICT OF INTEREST

Carla M. Davis, MD, FAACAP, has a conflict of interest with research contract funding from Regeneron and Allakos. All other authors report no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

	N	NMiss	Median	Min	Max
Milk SPT-E	24	1	0.00	0.00	4.00
Milk SPT-F	23	2	0.00	0.00	5.00
Milk SPT-P	15	10	0.00	0.00	2.00
Milk APT-E	25	0	0.00	0.00	1.00
Milk APT-F	25	0	1.00	0.00	2.00
Milk APT-P	25	0	0.00	0.00	2.00
Egg SPT-E	24	1	0.00	0.00	6.00
Egg SPT-F	23	2	0.00	0.00	12.00
Egg SPT-P	15	10	0.00	0.00	5.00
Egg APT-E	22	3	0.00	0.00	0.50
Egg APT-F	22	3	0.00	0.00	3.00
Egg APT-P	22	3	0.00	0.00	2.00
Soy SPT-E	23	2	0.00	0.00	5.00
Soy SPT-F	22	3	0.00	0.00	4.00
Soy SPT-P	15	10	0.00	0.00	5.00
Soy APT-E	24	1	0.00	0.00	2.00
Soy APT-F	24	1	1.00	0.00	2.00
Soy APT-P	24	1	1.00	0.00	3.00
Wheat SPT-E	23	2	0.00	0.00	3.00
Wheat SPT-F	22	3	0.00	0.00	1.00
Wheat SPT -P	15	10	0.00	0.00	5.00
Wheat APT-E	24	1	0.00	0.00	1.00
Wheat APT-F	24	1	0.75	0.00	2.00
Wheat APT-P	24	1	0.25	0.00	2.00

3 Table E1: Summary statistics for SPTs and APTs. An SPT(food) was considered
4 positive if the wheal was ≥ 3 mm than the SPT(saline) wheal. The SPT(food) was
5 calculated as follows: SPT(food)-SPT(saline). To avoid negative values when
6 calculating SPT(food), the final value was recorded as the maximum of the calculated
7 difference or zero. No changes were made to the APT observations. APT scores were
8 based on the grading system defined by Wilkinson et al⁷ and minimum and maximum
9 values were defined as: 0=negative; 0.5=doubtful reaction; 1=weak reaction; 2=strong

- 10 reaction; 3=extreme reaction. (SPT=skin prick test; APT=atopy patch test; E=Extract;
11 P=Powdered; F=Fresh; N=Number; NMiss= number missing)

1 Supplementary Material:

2

3 Methods:

4

5 The food preparations were prepared by placing either 0.5g of fresh food (Kroger brand
6 Simple Truth Organic Plain Soymilk, Kroger brand Whole Milk, Nature's Own Whole
7 Wheat bread and Kroger brand small fresh eggs), 1g dry food (Bob's Red Mill 100%
8 stone ground whole wheat flour, Bob's Red Mill soy flour and Bob's Red Mill milk
9 powder; Deb-El Just Whites egg white powder) mixed with 1mL of isotonic saline until
10 paste, or using commercial liquid food extract (Greer 1:20 w/v milk, egg, and wheat;
11 Greer 1:40 w/v soy).