

Brief communications

Macadamia nut anaphylaxis: Demonstration of specific IgE reactivity and partial cross-reactivity with hazelnut

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Anaphylaxis resulting from nut allergy is a serious and potentially fatal condition.¹ There have been no reported cases of anaphylaxis caused by macadamia, a prized table nut originating from the Australian tree species *Macadamia intergrifolia* and *Macadamia tetraphylla* and their hybrids and now grown extensively in the United States. Macadamia nut oil has previously been shown to possess some IgE-binding capacity in the pooled sera of food-allergic individuals,² but there have been no data regarding individual macadamia-allergic patients. We report a case of anaphylaxis caused by macadamia nut ingestion and confirmation of the causative allergen by immunoblotting.

CASE HISTORY

The patient was an 18-year-old woman with a history of infantile eczema and seasonal allergic rhinitis caused by grass pollen but no history of nut allergy, who had immediate oral itching when eating a slice of flourless orange cake made with macadamia meal. Within 5 minutes this had progressed to anaphylaxis. On arrival at hospital the patient was hypotensive (blood pressure 80 mm Hg systolic) and required treatment with epinephrine, oral prednisolone, diphenhydramine, and overnight observation before being discharged the next day fully recovered with advice to avoid all nuts and to carry self-injectable epinephrine. She denied prior ingestion of macadamia nut and eats only pistachio nuts because of taste aversion for other nuts, including peanuts.

METHODS

Serum-specific IgE was quantified with use of the Kallestad Allercoast EAST system (Sanofi-Pasteur Diagnostics) with normal range less than 0.18 Allercoast EAST units (AEU) per milliliter. Skin prick tests (SPTs) were performed with standardized extracts (Bayer) with 3 mm greater than sodium chloride solution negative control indicating positivity. Nut extracts were obtained by defatting 10 g of commercially available nuts (crushed raw and roasted macadamia, peanut, and hazelnut) in acetone, followed by 5 washes in diethyl ether. Once ground to

Abbreviations used

AEU:	Allercoast EAST units
CBB:	Coomassie brilliant blue
NC:	Nitrocellulose
SPT:	Skin prick test

a very fine powder under liquid nitrogen, nut proteins were extracted in 30 mL of PBS overnight at 4°C with gentle shaking. Macadamia nut oil proteins were extracted in PBS overnight at 4°C with gentle shaking. Rye grass pollen was used as a control allergen and proteins were extracted in PBS for 3 hours at 4°C with gentle shaking as described elsewhere.³ Protein estimation of nut and pollen extracts was performed with use of the Bradford protein assay, as described previously.³ Nut extracts were resolved by 16% "mini" SDS-PAGE (20 µg of each extract was loaded per lane), and 1 gel was stained with Coomassie brilliant blue (CBB) to visualize constituent proteins and the other 3 gels were transferred to nitrocellulose (NC) for immunoblotting as described previously.³ NC blots were incubated with a 1:5 dilution of serum from either the macadamia-allergic patient or a nonallergic negative control patient and a negative control membrane without the serum incubation. The inhibition immunoblot analysis involved individual NC strips containing roasted macadamia extract incubated in a series of patient serum dilutions (1:10, 1:15, 1:30, 1:60, and 1:120) to determine the ideal serum IgE concentration where IgE was neither in excess or limiting. Once the ideal serum dilution was determined (1:60), the NC strips were individually incubated with either the macadamia-positive serum only (positive control) or in the same serum that had been preincubated at room temperature for 1 hour with 25, 50, or 100 µg of potential inhibitors (macadamia nut, macadamia oil, hazelnut, peanut, and rye grass pollen extracts).

RESULTS

Serum specific IgE was weakly positive for peanut (0.42 AEU/mL), hazelnut (1.07 AEU/mL), and almond (0.57 AEU/mL). SPTs were negative to almond (0 mm) and hazelnut (0 mm) (peanut not tested) and positive to rye grass pollen (12 mm), house dust mite (9 mm), and histamine control (5 mm). Because no commercial macadamia-specific IgE assay or SPT reagent was available, the cut surface of a roasted macadamia nut was rubbed onto the volar aspect of the patient's forearm and onto a nonallergic control individual, together with a small amount of macadamia oil. The cut surface of the nut induced a skin wheal of 30-mm diameter in the patient but not the control individual; the oil did not elicit a reaction in either. The CBB gel (Fig 1, A) showed macadamia nut to have a different profile of constituent proteins from either hazelnut or peanut, with the bulk of the protein being of low molecular mass. The roasted and raw

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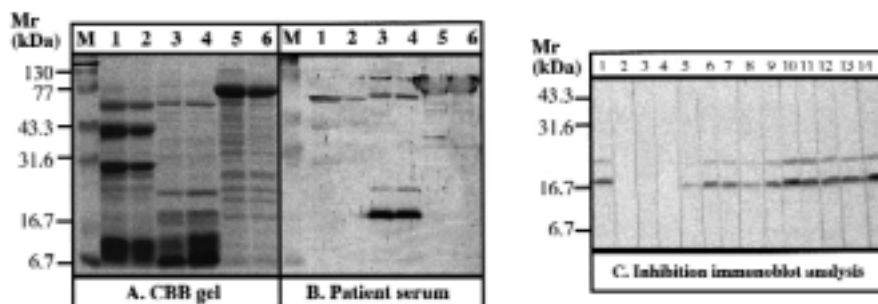


FIG 1. Crude nut extracts resolved by SDS-PAGE and total proteins visualized with CBB staining (**A**) or transferred onto NC membrane for IgE antibody detection with macadamia-allergic patient serum (**B**). Nut extracts included raw and roasted hazelnut (lanes 1 and 2, respectively), macadamia nut (lanes 3 and 4), and peanut (lanes 5 and 6). Lane M, Prestained molecular mass markers. SDS-PAGE-resolved roasted macadamia nut proteins were analyzed by inhibition immunoblot analysis (**C**). Lane 1, NC strips incubated in serum only (1:60 dilution); lanes 2 to 4, serum preincubated with macadamia nut extract (25, 50, and 100 μ g, respectively); lane 5, serum preincubated with macadamia oil extract (25 μ g); lanes 6 to 8, serum preincubated with hazelnut extracts (25, 50, and 100 μ g, respectively); lanes 9 to 11, serum preincubated with peanut extracts (25, 50, and 100 μ g, respectively); lanes 12 to 14, serum preincubated with rye grass pollen extract (25, 50, and 100 μ g, respectively). Mr, Molecular weight.

macadamia extracts had a similar protein profile. The immunoblot experiments (Fig 1, B) showed strong binding by IgE in the patient's serum to a protein of 17.4 kd in both raw and roasted extracts. This was not seen with a nonallergic control serum or with a no-serum control (data not shown). The patient's serum also exhibited moderate IgE binding to hazelnut proteins but no significant IgE binding to peanut. The inhibition immunoblot experiments (Fig 1, C) showed complete inhibition of IgE binding by the macadamia nut extracts with 25, 50, and 100 μ g; no inhibition was observed with use of the same amount of peanut or crude rye grass extracts. The hazelnut extract showed partial inhibition at 100 μ g, as did the macadamia oil extract at 25 μ g.

DISCUSSION

We have demonstrated that macadamia nut can be a cause of life-threatening anaphylaxis. The mechanism of the clinical allergy has been confirmed with Western blot

analysis indicating specific IgE binding to a low molecular weight protein of 17.4 kd that appears to be the major allergen in this patient, present in both raw and roasted extracts. This is confirmed by inhibition of binding by macadamia extract and the inhibition assays also show that macadamia oil produces moderate inhibition to IgE binding, indicating it should be avoided by macadamia-allergic individuals. We have found no evidence of cross-reactivity with peanut proteins in this patient, but hazelnut extract did provide weak inhibition, indicating that some proteins of macadamia and hazelnut may share similar IgE epitopes.

REFERENCES

1. Yunginger JW, Sweeney KG, Sturmer WQ, Giannandrea LA, Teigland JD, Bray M, et al. Fatal food-induced anaphylaxis. *JAMA* 1988;260:1450-2.
2. Teuber SS, Brown RL, Haapanen LAD. Allergenicity of gourmet nut oils processed by different methods. *J Allergy Clin Immunol* 1997;99: 502-7.
3. Suphioglu C, Singh MB, Simpson RJ, Ward LD, Knox RB. Identification of canary grass (*Phalaris aquatica*) pollen allergens by immunoblotting: IgE and IgG antibody binding studies. *Allergy* 1993;48:273-81.