

Sunflower oil is not allergenic to sunflower seed-sensitive patients

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The allergenicity of edible oils derived from sunflower seeds was investigated in two patients with anaphylactic sensitivity to sunflower seeds. Specific IgE-mediated hypersensitivity to sunflower seed was demonstrated by history, prick skin tests, positive passive transfer skin test, and RAST. Specific IgE directed toward sunflower oil, refined or cold pressed, could not be conclusively demonstrated. The Prausnitz-Küstner reaction with sunflower oils performed with one patient's serum was negative. Although the cold-pressed sunflower oil was found to contain a minute amount of protein, open challenge with the derivative oils resulted in no immediate or delayed reaction in the two patients studied. Sunflower oil ingestion in these patients who were highly sensitive to the parent material proved safe. Nonallergenicity of derivative products needs to be proven for each case. (J ALLERGY CLIN IMMUNOL 78:408-10, 1986.)

Anaphylactic reactions to foods can be explosive and dramatic life-threatening reactions.¹ Anaphylaxis to sunflower seed has been reported, and specific IgE was implicated in the mechanism of the reaction.² Derivative products manufactured from a parent material to which a patient is sensitive pose at least a theoretical risk if they are ingested. Peanut oil and soybean oil have been investigated for allergenicity in patients sensitive to the parent product, and it was demonstrated that these oils could be safely ingested by sensitive individuals.^{3, 4} Sunflower oils, to our knowledge, have not been similarly studied. Sunflower oils are ubiquitous as a constituent of margarine and salad and cooking oils. Additionally, the availability of a cold-pressed "natural" sunflower oil in health food stores further prompted our investigation into the allergenicity of these oils. The following are two case reports of anaphylaxis to sunflower seeds and our evaluation of the potential allergenicity of edible derivative oils in these patients.

Abbreviations used

P-K: Prausnitz-Küstner reaction
PBS: Phosphate-buffered saline

CASE REPORTS

Case 1

A 37-year-old woman experienced anaphylaxis manifested by diffuse pruritus, urticaria, angioedema, nausea and vomiting, chest tightness, and wheezing, followed by vascular collapse and loss of consciousness. This occurred within 20 minutes of ingestion of commercial sunflower seeds at home. She was successfully resuscitated with epinephrine, intravenous fluids, diphenhydramine, and steroids. After the anaphylactic reaction, she had several episodes of lip and facial swelling in the morning on awakening. These episodes resolved within 1 week. She then sought an allergy evaluation. By history it was determined she had eaten sunflower seeds in the past without incident. Her past history was negative for prior allergic reactions to foods. She had a history of anaphylactic reactions to fire ant venom. Her general health was good, and she was taking no medications of any kind. Physical examination was unremarkable.

Our evaluation included prick and intradermal skin testing, the P-K (Table I), RAST, and open challenge. Sunflower seed extract (1:20 w/v in 50% glycerine; Hollister-Stier, Spokane, Wash.) and an extract prepared from the causative seeds (extracted in diluent saline, 1 gm/100 ml, filtered) were used for prick testing and the P-K. Refined sunflower oil was obtained from the Kroger Co., Cincinnati, Ohio, and the cold-pressed sunflower oil was made by Hain

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Pure Food Co., Inc., Los Angeles, Calif. When the patient was tested, both sunflower seed extracts demonstrated strongly positive reactions by prick skin testing. Prick tests to the oils were negative, and intradermal testing with the oils was generally unsatisfactory due to their irritant properties.

For the P-K,⁵ serum was obtained from the patient and divided into two aliquots, one of which was heated at 56° C for 1 hour. The serum was screened for hepatitis B before transfer. Heated and unheated serum (0.07 ml) were each injected intradermally into three skin test sites of a nonatopic volunteer. After 24 hours, skin sites were challenged with intradermal injections of 0.02 ml of the commercial sunflower seed extracts (1:1000 w/v), refined sunflower oil (undiluted), and cold-pressed sunflower oil (undiluted). Saline and histamine (0.01 mg/ml) controls were used. The passive transfer recipient was strongly positive at the unheated serum site on challenge with sunflower seed extract. The sites challenged with derivative sunflower oils were negative. In addition, the heated serum site challenged with sunflower seed extract was negative.

The total IgE and RAST to sunflower seed in the patient were 58 IU/ml and class 4, respectively (IBT Reference Laboratory, a division of Immuno Biotech, Inc., Overland Park, Kan.).

Open challenge to the derivative oils was done under appropriate controlled conditions on 2 separate days, 48 hours apart. The individual oils (refined and cold-pressed sunflower oil) were first applied to the inner lip and oral mucosa with a swab. After 30 minutes the oil was ingested at intervals of 30 minutes in gelatin capsules (1 ml per capsule), in a sequential manner, 1, 2, 4, 8, and then 16 ml of the oil. No immediate or delayed reaction was observed on challenge with any derivative oil.

Case 2

A 29-year-old woman experienced diffuse pruritus, widespread urticaria, and facial angioedema within a few minutes of ingesting sunflower seeds. She was treated with epinephrine and antihistamines. Allergy evaluation demonstrated an 8 by 10 mm wheal with flare prick skin test to sunflower seed, negative prick tests to refined and cold-pressed sunflower oil, and a class 4 RAST (IBT Reference Laboratory) to sunflower seeds. The patient declined to have intradermal skin testing with derivative oils or the passive-transfer test but agreed to oral challenge with derivative oils. A progressive challenge with cold-pressed and refined sunflower oils was done on different days separated by 1 week. The patient ingested, at 30 minute intervals, 1, 2, 4, 8, and then 16 ml of each oil. No immediate or delayed reaction was observed.

Protein determination

Subsequent to the patient challenges, we were able to perform a protein determination on the cold-pressed sunflower oil. One volume (50 ml) of cold-pressed sunflower oil was extracted (separatory funnel) with two volumes (100

TABLE I. Skin test results in case 1

Test	Result (wheal size in mm)
Prick skin testing	
Histamine	7 by 7 With erythema
Saline	Negative
Sunflower seed extract (commercial)	12 by 16 With erythema
Sunflower seed extract (causative seeds)	16 by 16 With erythema
Refined sunflower oil	Negative
Cold-pressed sunflower oil	Negative
Intradermal (0.02 ml)	
Refined sunflower oil	6 by 6 With erythema
Cold-pressed sunflower oil	10 by 10 With ery- thema/pseudopods
Boiled cold-pressed sunflower oil	4 by 5 With erythema
Boiled refined sun- flower oil	4 by 4 With erythema
Control subjects (N = 7) sunflower oils	6 by 6 With erythema*
Passive transfer	
Histamine control	10 by 10 With ery- thema/pseudopods
Saline	1 by 2 No erythema
Unheated serum site/sunflower seed extract	12 by 13 With ery- thema/pseudopods
Heated serum site/sun- flower seed extract	2 by 3 No erythema
Sites challenged with oils	2 by 2 No erythema

*Varied from no reaction to 6 by 6 mm with erythema.

ml) of PBS at pH 7. The original volume of oil was extracted two more times with fresh aliquots of PBS. The PBS fractions were then combined. The residual oil-PBS emulsion was centrifuged, and the PBS phase was added to the previously combined PBS fractions. The PBS (300 ml) was concentrated by factors of 100 and 1000 by use of the Minicon-B15 clinical sample concentrator (Amicon Corp., Danvers, Mass.). The concentrated PBS fractions were assayed for protein content by use of the Lowry colorimetric procedure. No protein was detectable in the PBS concentrated 100 times. At 1000× concentration, the PBS yielded a protein concentration equivalent to 54 ng of protein per milliliter of oil. Spectrophotometric scans of 100× concentrated PBS failed to reveal any protein-characteristic peaks in the vicinity of 280 nm (aromatic amino acids) or 200 nm (peptide bond). The 1000× concentrated PBS could not be assayed by ultraviolet spectrophotometry because of volume constraints.

DISCUSSION

We have demonstrated sunflower-seed specific IgE antibody by at least two of three methods (skin testing, RAST, or passive transfer) in two patients with a history of anaphylaxis after sunflower seed ingestion. Because of the severity of these reactions and our subsequent confirmation of the presence of specific IgE, neither patient was challenged with sunflower seeds. Sunflower seeds in the raw form are avoidable, but sunflower seed oil may be unknowingly ingested in many forms. Although peanut and soybean oils were found to be safely ingested by peanut-allergic³ and soybean-allergic⁴ individuals, a report of anaphylaxis to sesame seed oil exists.⁶ The allergenicity of the sesame oil implicated in that case was not proven.

Both in vivo and in vitro tests to determine the allergenicity of oils are difficult to interpret. We noted that the use of oil for intradermal skin testing was associated with varying degrees of irritant responses in six of seven control subjects. However, our first patient's intradermal skin test to the cold-pressed sunflower oil appeared to demonstrate a greater response compared to the refined oil, leading to further speculation as to the possibility of minute protein contamination in the "natural" sunflower oil. A P-K test was therefore performed with one subject but failed to confirm the presence of IgE specific for protein in the cold-pressed oil (Table I).

In vitro methods to detect specific IgE to the protein in oils are limited by the physical characteristics of oils. RAST inhibition with oils is technically unsatisfactory and usually results in inhibition lines with negative slopes.⁷ This difficulty is due to coating of the paper discs with oil. Our attempts to perform RAST with the oils confirmed this problem.

The differential response to intradermal testing noted in our first patient prompted further investigation of the "natural" sunflower oil. The cold-pressed manufacturing process might reduce potential protein denaturation, thereby allowing a small amount of protein to remain that could theoretically be of sufficient quantity to cause anaphylaxis in a highly sensitive individual. The product label on the cold-pressed oil indicates a protein content of zero grams. Our assay for protein in this oil demonstrated detectable protein at a concentration of 54 ng/ml, which is just within the lowest useful limit of the Lowry assay.

Nevertheless, the results of open challenge with the sunflower oils indicated that they could be safely ingested by our two highly sensitive patients despite minute protein contamination in the cold-pressed sunflower oil. This was not surprising, since oral challenges in cases of food sensitivity often start with doses in the 100 to 250 mg range.⁸ The amount of

protein we detected in the cold-pressed oil was far less and was apparently a clinically insignificant quantity when it was ingested orally by our two patients. Our study therefore agrees with the findings of a recent study on soybean-allergic individuals in which a cold-pressed soybean oil was demonstrated not to be allergenic.⁴ However, since derivative oils may vary in comparison and degree of protein contamination, larger double-blind challenge studies are needed to demonstrate nonallergenicity of each analogous edible derivative oil. Until this is accomplished, we would recommend that nonallergenicity be proven individually in each instance.

ADDENDUM

Since submission of the manuscript, new data have been generated. Repeat protein determinations with fresh aliquots of cold-pressed and refined sunflower oil were performed, incorporating lyophilization and dialysis (with molecular weight cutoff of 3500). Lowry protein determinations on cold-pressed and refined sunflower oils demonstrated protein in both in the range of 2 to 8 µg/ml. Spectrophotometric scans of concentrated fractions (100X and 1000X) demonstrated strong absorbance at 200 nm, indicative of the presence of peptide bonds. Dialysis eliminated low molecular weight substances such as choline, known to cross-react in the Lowry method. Proteinaceous material found in microgram amounts will be used in skin testing as another test of allergenicity.

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