

Environmental and occupational disorders

Detection and quantitation of raw fish aeroallergens from an open-air fish market

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Background: IgE-mediated hypersensitivity to fish is a clinically relevant problem, particularly in several European countries. Although most allergic reactions to fish are caused by ingestion, occupational exposures to seafood allergens by inhalation have been correlated with respiratory symptoms. In Madrid, patients with fish allergy have exhibited respiratory symptoms after visits to an open-air fish market.

Objective: We sought to study the possibility of passively aerosolized fish allergen in an open-air fish market through air sampling and a competitive IgE immunoassay.

Methods: Air samples were collected on polytetrafluoroethylene filters by using air samplers. Samples were collected on 41 different days from both an open-air fish market and an outdoor residential area. Fish allergens were specifically quantified by competitive IgE immunoassay by using pooled sera from fish-sensitive individuals. A raw fish extract (10 mg of dry weight/mL) was used as the reference standard.

Results: Allergen was quantified in all 39 fish market air samples (2–25 ng/m³). The residential air samples contained no detectable allergen. The analytic limit of detection was 2 ng, allowing detection of 0.4 ng/m³ for the air volumes collected. A concentrated (30-fold) pool of fish market air samples was tested in serial dilutions and demonstrated an identical regression line to that of the raw fish standard.

Conclusion: By using air sampling and an immunochemical analytic technique, fish allergen is detectable in the air of an open-air fish market. Avoidance of a food allergen, such as fish, should include preventing exposure to aerosolized particles through inhalation in relevant environments. (*J Allergy Clin Immunol* 1999;166-9.)

Key words: Food allergy, fish, aeroallergen, air sampling, nonoccupational exposure, immunochemical detection

Fish is one of the most common sources of food allergy in adults.¹ In Norway the prevalence of fish allergy is

Abbreviations used

BWH: Boiled whiff and hake mixture

PTFE: Polytetrafluoroethylene

RWH: Raw whiff and hake mixture

estimated to be 1 of 1000 adults and may be higher in children.² Among children, published estimates of the prevalence of fish allergy range from 0.4% to 0.5% among Italian children³ to 3% of 3-year-old children in Finland.⁴ In Spain, fish has been identified as the second most common cause of allergic reactions to foods in children after eggs.⁵

Most allergic reactions to fish are caused by ingestion; however, inhalation of fish or seafood allergens has been implicated in adverse reactions, such as wheezing, asthma, urticaria, angioedema, and rhinitis.⁶ These reports most often involve occupational exposure during processing, handling, and cooking of fish.⁷⁻⁹ Although some nonoccupational exposures have been reported,^{6,10} these are mostly anecdotal reports. Inhalation is often cited as the probable exposure route, but the literature is scarce.

All of the previous studies of fish aeroallergens have examined the allergenicity of vapors from boiling fish. IgE-binding proteins have been identified in steam from boiling salmon and have been shown to be similar by inhibition immunoassay and immunoblotting to both raw and boiled salmon.¹¹ In Madrid, patients with IgE-mediated fish hypersensitivity have had reactions after exposure to fish odors in an open-air fish market.

The aims of this study were (1) to demonstrate that fish protein is aerosolized through passive evaporation of raw fish in an open-air fish market and (2) to quantify the aerosolized fish allergen (in nanograms per cubic meters) by using a competitive IgE immunoassay.

METHODS

Air sampling

Air samples were collected from an open-air fish market at a single location and a residential area not located near a fish market in Madrid, Spain. A volumetric air sampler (Quan-Tec-Air, Inc, Rochester, Minn) was used to collect air samples.¹² A flow rate of 2.5 L/sec through polytetrafluoroethylene (PTFE) filters (Quan-

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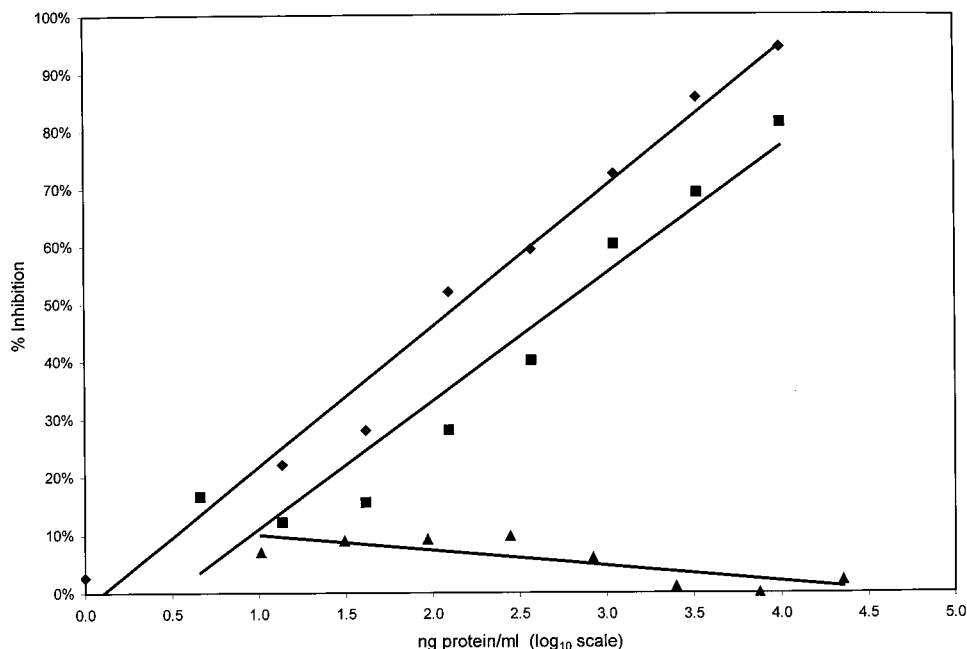


FIG 1. Inhibition of binding by RWH (diamonds), BWH (squares), and snow crab (triangles) of IgE anti-RWH antibodies to solid-phase RWH.

Tec-Air, Inc) allowed collections on 41 different days, Monday through Saturday, for varied sampling durations (4-37 hours).

Extraction of air filters

The allergens were directly extracted from the air samples by removing the PTFE from the woven backing and eluting it overnight in 1 mL of PBS containing 1% Tween-20. The extracts were manually expressed from the PTFE and centrifuged. The supernatants were then stored at -20°C .

Sera

Serum samples were obtained from 19 Spanish individuals allergic to fish with a history of symptoms after ingestion of cooked fish, exposure to raw fish, or both. The sera were first tested in Spain by using the Pharmacia CAP-FEIA System (Uppsala, Sweden) for fish-specific IgE antibodies to several types of fish, including whiff megrim (*Lepidorhombus whiffiagonis*), hake (*Merluccius merluccius*), tuna (*Thunnus thynnus*), swordfish (*Xiphias gladius*), and sardine (*Sardina pilchardus*). Sera were retested at the Mayo Clinic to confirm that all sera contained IgE antibodies to whiff, hake, or both by RIA, as previously described.¹³ Serum samples were pooled and stored at -20°C .

Extracts

Extracts of raw whiff and raw hake were prepared by extraction of antigen according to the method for fish extraction described elsewhere.¹⁴ Extracts of boiled whiff and hake were prepared by first boiling the fish in distilled water for 15 minutes before homogenization and extraction. Extracts were lyophilized and reconstituted (10 mg of dry weight/mL) in 50% glycerin. Snow crab (*Chionoecetes opilio*) extract was prepared as a control antigen, as described elsewhere.¹⁵ All extracts were stored at -20°C .

Inhibition RIAs

Raw fish allergens were quantified by using an equal mixture of raw whiff and hake (RWH) extracts as the immobilized allergen for

all the inhibition RIAs. We chose a raw extract because the fish market sold only raw fish, and all individual sera contained IgE antibodies to raw fish. An inhibition RIA was performed, as described elsewhere.¹³ A cross-inhibition study was performed to assess the cross-reactivity of raw and boiled fish. Patients who provided sera had a history of symptoms after ingestion of cooked fish, exposure to raw fish, or both. Snow crab was used as a negative control to demonstrate binding specificity. Various concentrations of RWH, boiled whiff and hake mixture (BWH), or snow crab were used as the aqueous competitive allergen. The assay was performed in Immulon IV microtiter plates. To prepare the immobilized allergen, RWH was diluted 1:500 in 200 mmol/L carbonate buffer (pH 9.2), and 100 μL was delivered to each well. After overnight incubation at room temperature, the plates were washed 3 times with assay diluent. Test extracts or RWH reference extract (50 μL) were then added at 8 decreasing concentrations (3-fold dilutions beginning at 0.2 mg/mL for RWH or BWH and 0.45 mg/mL for snow crab), and 50 μL of pooled sera (diluted 1:4 with assay diluent) was delivered to the plates. The plates were incubated overnight at room temperature and then washed 3 times. Iodine 125-labeled rabbit anti-IgE (20 ng/mL [100 μL]) was then delivered to each well. The plates were incubated overnight at room temperature, washed 3 times, and counted in a γ -scintillation counter. Air filter extracts from the fish market or a residential area competed with the immobilized RWH reference allergen for IgE-binding sites. Results were calculated and expressed as the percentage of inhibition. The results of the air sample inhibition immunoassay were calculated with linear regression and expressed as mass protein per cubic meter of air. The sensitivity of the assay was 2 ng, allowing detection of approximately 0.4 ng/m³.

Comparison of allergenic material in the fish market sample and RWH

The fish market samples were pooled by taking equal volumes of extract from each sample. The pooled extract was dialyzed by using a slide-A-lyzer dialysis cassette (Pierce Chemical Co), following

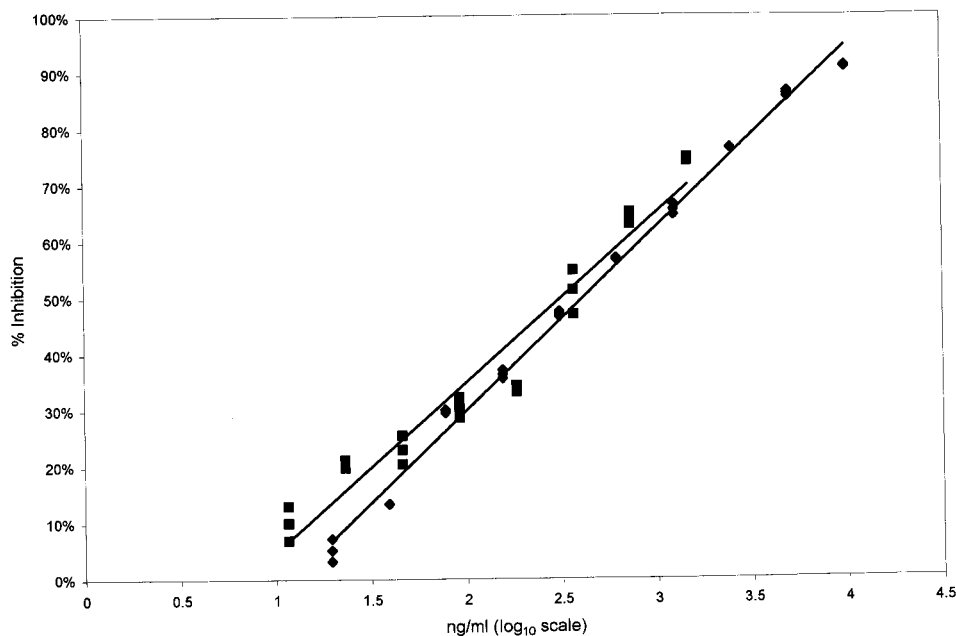


FIG 2. Inhibition of binding by RWH (diamonds) and fish market samples (squares) of IgE anti-RWH antibodies to solid-phase RWH.

TABLE I. Concentration of aerosolized fish allergen in individual air samples

Outdoor sampling location	No.	Mean (ng/m ³)	Range (ng/m ³)
Fish market	39	9.4	2-25
Residential area	8	<0.3	<0.3

the instructions provided with the kit. After dialysis, the fluid was lyophilized. The lyophilized material was reconstituted in distilled water, creating a 30-fold concentration. Serial 2-fold dilutions of the 30-fold concentration (beginning at 0.029 mg/mL) were used in an inhibition RIA to construct a dose-response curve.

RESULTS

Inhibition immunoassay of RWH, BWH, and snow crab

Varied concentrations of RWH, BWH, and snow crab were used in an inhibition RIA to establish a dose-response curve. Fig 1 presents the inhibition curves of each extract against RWH as the solid-phase reference allergen. Both RWH and BWH inhibited specific IgE binding in a dose-dependent manner, and no significant inhibition was produced by the snow crab extract.

Fish aeroallergens

Fish allergens were detected in each of the 39 different air samples from the fish market (Table I). No fish allergens were detected in the 8 air samples from the residential area. The amount of airborne allergens detected appeared to be independent of the duration of the sampling time. It is likely that variables such as weather, wind, amount of fish in the market, and day of the week

could play a role in the amount of allergens detected in the air. Air samples from the fish market were pooled, concentrated, and serially diluted, as described in the "Methods" section. Concentrated fish market samples demonstrated a dose-dependent reactivity similar to that of the RWH standard (Fig 2).

DISCUSSION

There are anecdotal reports of some patients with fish allergy who had immediate allergic symptoms after exposure to fish odors or fumes through inhalation. Although most of these episodes occurred when the patients were exposed to vapors from cooking fish, some were only exposed to the smell of raw fish.⁶

The air sampling methods and aeroallergen assays used here provide objective methods for assessment of airborne fish allergens. The same methodology could be used to investigate airborne concentrations of other potent food allergens, such as peanut, in confined areas, such as airplane passenger compartments.¹⁶ Intuitively, it is not surprising that fish aeroallergens are recoverable from an open-air fish market. It is interesting that the fish aeroallergen concentrations, although at the lower end of the scale, are of the same order of magnitude (10-1000 ng/m³) as many other occupational aeroallergens, such as latex¹⁷ and snow crab.¹¹ How the allergen becomes airborne is still unclear.

In our study the inhibition RIA showed the presence of IgE-binding components in all the air samples collected from the fish market and none of the residential samples. Moreover, the slopes of the dose-response curve for the pooled air sample from the fish market and the RWH

extract did not differ significantly, thereby demonstrating allergenic similarity and validating the quantification estimates.

Our findings demonstrate that avoiding the consumption of fish does not entirely eliminate the risk of contact with these allergenic proteins. Exposure to airborne fish allergen may result in an unexpected reaction in some patients with fish allergy. Whether inhaled exposure to the concentrations of fish allergen described in this study is sufficient to elicit symptoms in individuals with fish allergy is undetermined.

In conclusion, we have demonstrated that fish allergen is detectable in the air of an open-air fish market by using an RIA technique. Presumably these measurements would be possible in other fish-handling environments, such as fish-processing plants, businesses that sell raw fish, and restaurants. Avoidance of a food allergen, such as fish, should extend not only to elimination of the food from the diet but also to prevention of exposure to aerosolized particles through inhalation in certain environments.

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