father mentioned that on the placebo day he had eaten an omelet sandwich less than half an hour before the onset of the child's reaction; immediately afterwards he had played with and kissed his son without washing his hands or mouth. With this information, we suspected that the patient had experienced contact urticaria caused by egg allergen transferred by the father. The placebo challenge was repeated 1 week later and was completely negative.

This case clearly shows how a positive reaction during a placebo challenge can be caused by the inadvertent transfer of allergen from another party. It is therefore of great importance to advise the relatives of patients with food allergies not to ingest the food under study while the challenges are being performed to avoid false positive reactions. Furthermore, staff preparing challenge meals should be made aware of the risk of cross-contamination via hands, kitchen utensils, clothes, etc and advised to be extremely careful during the entire procedure. Children on the same ward can also inadvertently transfer allergens to each other during challenge observation periods. All these practical aspects of oral food challenges should be taken into account not only to improve test reliability but also to increase patient safety.

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Occupational Allergy to Fungal Lipase in the Pharmaceutical Industry

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Key words: Enzyme allergy. Fungal lipase. Occupational allergy. Occupational asthma. Occupational rhinoconjuntivitis.

Palabras clave: Alergia a enzimas. Lipasa fúngica. Alergia ocupacional. Asma ocupacional. Rinoconjuntivitis ocupacional.

Occupational allergy to lipase has been reported in the detergent industry [1-4]. While the main allergenic enzyme in the pharmaceutical industry is amylase, there have been reports of lipase sensitization, albeit without clinical relevance [5,6].

We report the case of a 46-year-old nonsmoking man with allergic rhinoconjunctivitis to grasses since the age of 34 years who had been working in the pharmaceutical manufacturing industry for 25 years. Five years prior to evaluation by our department, the patient started to exhibit rhinoconjunctivitis symptoms and dyspnea at the workplace while handling pancreatic enzyme preparation (PEP) tablets. The medication included fungal lipase (60 000 FIP units/g) derived from *Rhizopus oryzae* (American Laboratories Incorporated, Omaha, New England, USA), fungal amylase derived from *Aspergillus oryzae* (Amano Enzyme Incorporated, Nakaku, Nagoya, Japan), and pepsin. The symptoms started 3 hours after the patient first handled the tablets, worsened throughout the day, and improved after work. The patient did not experience symptoms out of work, during the weekend, during holidays, or at the workplace when PEP was not being manufactured.

Total serum immunoglobulin (Ig) E was 124 IU/mL; skin prick tests (SPTs) with commercial extracts of common aeroallergens, including molds and latex (ALK Abelló, Madrid, Spain) were positive for grass pollen but negative for Aspergillus oryzae amylase commercial extract (Leti, Madrid, Spain) and for substances handled during the manufacture of pharmaceutical products at the workplace, among them *Aspergillus oryzae amylase* and *Rhizopus oryzae* lipase (10% dilution in NaCL 0.9%). Serum specific IgE levels (ImmunoCAP; Phadia, Uppsala, Sweden) were 8.1 kU/L for *Dactylis glomerata*, 7.3 kU/L for *Festuca elatior*, 8.6 kU/L for *Lolium perenne*, 6.9 kU/L for *Phleum pratense*, 7.6 kU/L for *Poa pratensis*, and <0.35 kU/L for nAsp o 1 α-amylase. Skin patch tests with the European standard battery (Chemotechnique Diagnostics, Malmö, Sweden) were positive to neomycin sulphate and mercury ammonium chloride. Patch tests with occupational substances (10% in petrolatum) including PEP fungal enzymes were positive to fungal lipase. Baseline lung function tests showed reversible small

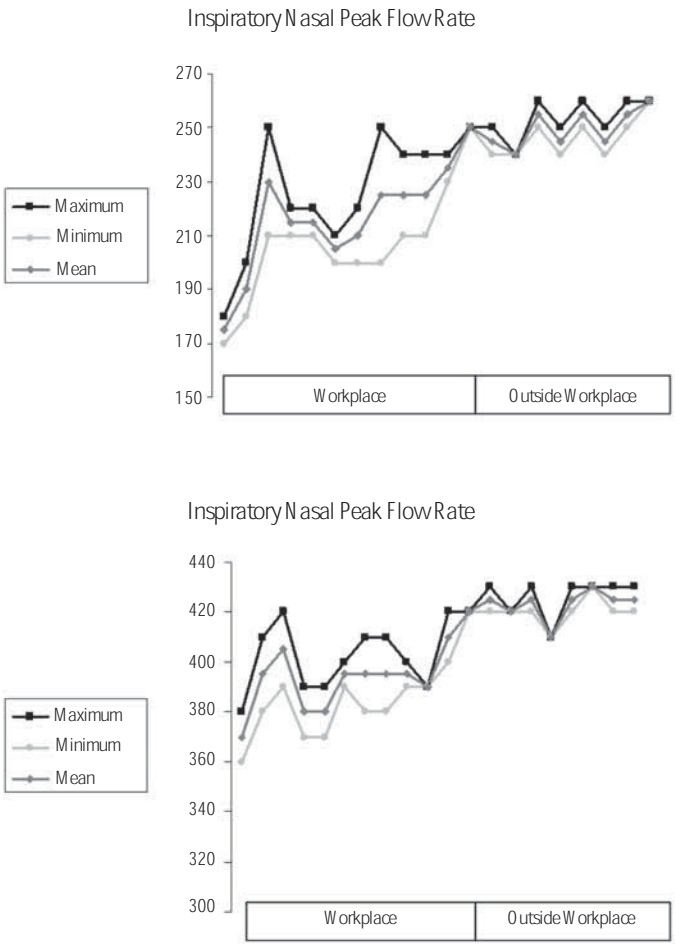

Figure. Inspiratory nasal peak flow rates and peak flow rate monitoring, 10 days at the workplace and 10 days outside the workplace.

airways obstruction (forced vital capacity, 4.27 L [110% of predicted]; forced expiratory volume in 1 second, 3.13L [97% of predicted], forced expiratory flow $[FEF]_{25\%-75\%}$, 2L/s [50% of predicted], 25% bronchodilator reversibility in $FEF_{25\%-75\%}$). A methacholine inhalation challenge test was positive (PC20 at 0.36 mg) when the patient had been at work for 2 weeks but negative when he had been off work for the same time. The 2 challenges were performed outside the grass pollen season.

Monitoring of nasal inspiratory peak flow (NIPF) and peak expiratory flow (PEF) in and outside the workplace showed a worsening of lung and nasal function at work, suggesting that the respiratory symptoms had an occupational origin [7]. The Figure shows the maximum, minimum, and median NIPF and PEF values. The daily variability in NIPF and PEF was greater when the patient was at work (10%-50% for NIPF and ≥20% for PEF) than when he was not (≤10% for NIPF and PEF). A specific nasal provocation test (SNPT) [8] yielded a positive symptom score.

Despite the negative SPT result for lipase, we decided to proceed with further investigation. Using an experimental ImmunoCAP test (Phadia), we detected serum specific IgE levels to fungal lipase of 4.5 KU/L.

A coworker who presented similar symptoms to those experienced by our patient during PEP handling tested positive to α-amylase and negative to lipase during skin prick and patch testing with the same series of occupational allergens as those used in our patient. The same tests carried out in 2 healthy subjects were negative to all extracts, as was an SNPT performed in a healthy worker.

The occupational origin of the respiratory symptoms experienced by our patient was evidenced by the worsening of respiratory function during exposure to PEP at the workplace. Sensitization to fungal lipase was confirmed on observing increased serum specific IgE levels and positive patch test and SNPT results. While occupational respiratory allergies caused by fungal enzymes are described in the literature [1-4], to the best of our knowledge, this is the first report of fungal lipase allergy in a patient not sensitized to amylase working in the pharmaceutical industry. The serum specific IgE and SNPT results and the delayed-type cutaneous reactivity pattern to lipase all suggest the involvement of IgE-mediated and cell-mediated mechanisms.

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Selective Allergy to Raw Pork

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Key words: Raw pork. Meat. Allergy.

Palabras clave: Carne cruda de cerdo. Carnes. Alergia.

Although meat allergies are rare, there are an increasing number of case reports, varying from oral allergy syndrome, skin involvement, bronchospasm, and even anaphylaxis, especially with regard to beef [1], to reactions following ingestion and inhalation of, or contact with, cattle, lamb, and horse meat. In many cases, an immunoglobulin (Ig) E–mediated immune mechanism was demonstrated. Pork allergy is less common, especially when it is not associated with allergy to meat from other mammals [2] or with the so-called pork-cat syndrome, where patients sensitized to cat dander develop symptoms after ingesting pork [3].

A 6-year-old child presented oral pruritus, perioral erythema, and mild labial angioedema every time he ate fresh and vacuum-packed cured ham. The reactions became increasingly severe (with onset a few minutes after ingestion), to the extent that he required antihistamines to control symptoms. He also presented symptoms when eating other cold meats such as pork loin, homemade chorizo, and *fuet* (cured sausage). He tolerated boiled ham, fried pork, beef, and lamb. The reactions occurred without exercise, and there was no history of reactions to food or drugs. Neither the patient nor his first-degree relatives had a history of atopic allergy.

Skin-prick-tests were performed with commercial extracts of pollen, profilin, Pru p 3, molds, dog, cat, horse and cow danders, mites, latex, and foods including milk, egg, meats, spices (ALK-Abelló, Madrid, Spain; LETI, Barcelona, Spain), and bovine serum albumin (Diater, Madrid, Spain). They were negative to all the allergens tested except commercial raw pork extract. Skin prick test results were positive to raw pork and negative to cooked pork, and raw and cooked beef.

Total-IgE (CAP system) was 87 kU_A/L and no specific IgE values above 0.35 kU_A/L to beef, cat dander, or bovine serum albumin were detected.

A boiled pork extract was prepared by boiling raw pork at 100ºC for 10 minutes and extracted with a magnetic stirrer at 10% (weight/volume) in a phosphate buffer. Afterwards, it was centrifuged and filtered through 0.8-, 0.45-, and 0.22-mm membranes and saved in aliquots at –20ºC. Likewise, a raw meat extract was analyzed (ALK-Abelló-EC-batch-U190). The two extracts and the molecular weight markers were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (16% acrylamide concentration) under nonreducing conditions. Proteins were then electrophoretically transferred onto NC papers [4], saturated with 0.2% Tween 20 in PBS, and incubated with the patient's serum diluted 1:5 for 18 hours. They were incubated with human anti-IgE monoclonal-antibody HE-2 (1:3000), and, after washing again, they were incubated with peroxidase-conjugated rabbit-antimouse-IgG diluted to 1:5000. Finally, proteins with IgE-binding capacity were detected by means of chemiluminescence.

As the Figure shows, the IgE in the patient's serum recognized a protein band of about 60 kDa in the raw pork extract, and this could coincide with the molecular weight of albumin. However, the patient's serum did not recognize any bands in the cooked meat extract. In the negative control, nonspecific binding was detected, but this did not coincide in intensity or in molecular weight with the band recognized by the patient's serum.

This is the first report of allergy to raw pork to demonstrate an IgE-mediated mechanism by identifying the allergenic

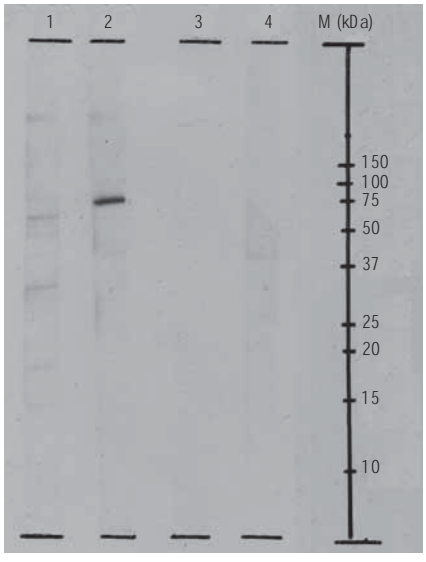

Figure. Results of IgE immunodetection. Lane 1, raw pork extract and negative control; lane 2, raw pork extract and the patient's serum; lane 3, cooked meat extract and negative control; lane 4, cooked meat extract and the patient's serum.