tional IgE-binding bands were observed in the molecular range from 30 to 60 kDa.

Native parvalbumins were prepared from chicken leg, tuna, salmon, cod and carp muscle by ion-exchange and size-exclusion chromatography. Chicken (FM994924), turkey, cattle (FM178223), pig (FM994923) and horse (FM994925) α-parvalbumins were cloned using rapid amplification of cDNA ends (RACE) PCR technology and expressed in *Escherchia coli* M15. Recombinant α-parvalbumin from frog (Q8JIU2) was produced as described before (5). Protein sequence analysis of α-parvalbumins showed that chicken parvalbumin was 100% identical to turkey, 83% to cattle/horse, 82% to frog, 80% to pig, 79% to human (X63070) and 54% to cod β-parvalbumin.

Purified chicken and fish parvalbumins were analyzed in immunoblot for IgE reactivity using patient's serum. Chicken α-parvalbumin was strongly positive, fish β-parvalbumin was negative (Fig. 1A). IgE recognition of the 14 kDa band was abolished by preincubation of the serum with recombinant chicken α-parvalbumin.

IgE antibodies to α-parvalbumin from chicken, frog, pig, cattle and horse were quantified by *enzyme-linked immunosorbent assay* (*ELISA*) (Fig. 1B). Similar values were obtained for all parvalbumins analyzed, except for fish parvalbumins which were negative (data not shown). The SPT was clearly positive with purified, native chicken parvalbumin at a concentration of 10 μg/ml while the SPT was negative with native tuna β-parvalbumin.

In conclusion, we report a case of poultry meat allergy where we identified chicken α-parvalbumin as one of the implicated allergens. The patient was sensitized to α-parvalbumin from chicken with cross-reactivity to highly similar homologs from pig, horse and cattle. Remarkably, no IgE binding was found to the major fish allergen β-parvalbumin. The allergenic potential of α-parvalbumin is assumed to be very low in general, possibly because of their high identity to the human homolog (6). Even though expression levels of mammalian muscle α-parvalbumins are known to be minor, they could be potent food allergens because of the high thermal stability and digestion resistance of this protein family, which gives impact to the present report. The clinical relevance of α-parvalbumins as meat allergens might be reconsidered in food allergy diagnosis.

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Allergy for cheese: evidence for an IgE-mediated reaction from the natural dye annatto

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Key words: anaphylaxis; annatto; basophil; cheese.

A man with anaphylaxis from the natural dye annatto is presented. Diagnosis was established by history, skin tests and basophil activation test.

A 58-year-old atopic man was referred to our outpatients' clinic because of four episodes in 3 years of severe anaphylaxis with urticaria, angio-oedema and finally loss of consciousness within a few minutes after consumption of a sandwich or toast with Gouda cheese. Detailed history, quantification of specific IgE (sIgE) and skin tests confirmed allergy for house dust mite and cat epithelium. There was neither evidence of a classical IgE-mediated food allergy (in particular, wheat and diary products) nor of pollen or latex allergy that could have explained a secondary food allergy. Serum tryptase level was normal at the clinical visit. Careful revision of labels from different brands of Gouda cheese revealed that the reaction could potentially be elicited by the natural dye annatto (E160b).

Skin prick tests with an extract from Gouda cheese (Holland Delhaize, Belgium) and annatto (Ceska Annatto WS E160b) both tested in duplo and at a 10^{-3} dilution were positive with a wheal and flare reaction of 4/5 and 4/6 mm respectively.

Flow cytometric analysis (FACS-Calibur, BD, Immunocytometry Systems, San Jose, CA, USA) of activated basophils was performed using Alexa Fluor 448-coupled anti-IgE (Sigma-Aldrich, Chemic GmBH, Steinheim, Germany) and phycoerythrin-conjugated anti-CD63 (Pharmingen, BD Biosciences, Erembodegem, Belgium). The test included a negative control (stimulation buffer without any allergen), a positive control

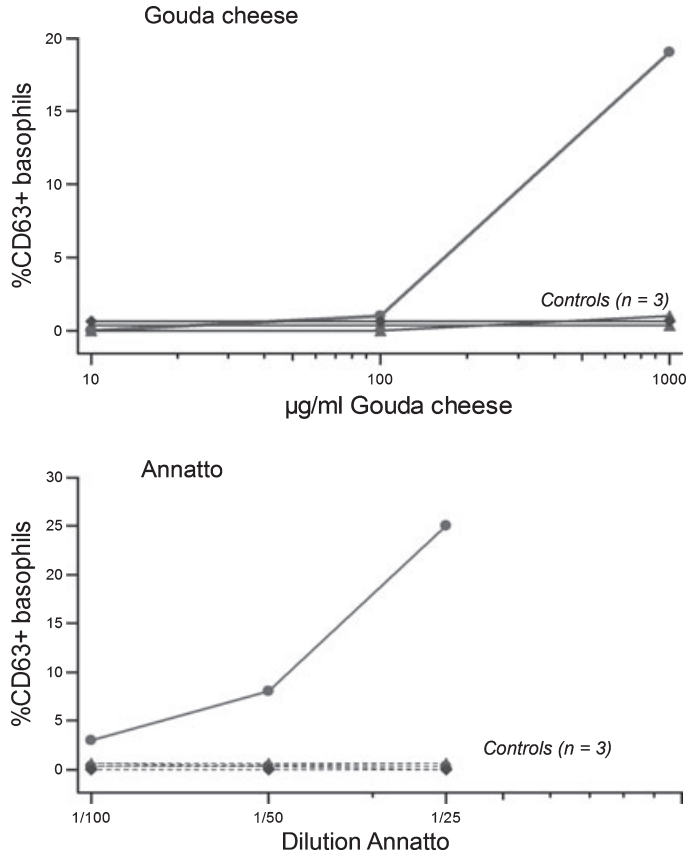

Figure 1. Top: upon challenge with the Gouda extract, basophils of the patient showed a manifest dose-dependent upregulation from 1% up to 19% (circles). In contrast, expression of CD63 on basophils from the 3 annatto-tolerant control individuals remained merely unchanged (diamonds). Bottom: challenge with annatto induced a dose-dependent activation of the basophils of the patient (circles), but not from the 3 annatto-tolerant control individuals (diamonds).

(anti-IgE), Gouda cheese extract (Holland Delhaize, serial dilution of 1 up to 1000 µg/ml) and annatto (serial dilution of 0.015 up to 0.6 µg/ml). To exclude nonspecific basophil activation, the test was also performed in three healthy control individuals who tolerated Gouda cheese and had entirely negative skin prick tests for Gouda cheese and annatto (both tested up to neat solution). Upon challenge with the cheese extract and annatto, basophils of the patient showed a manifest dose-dependent upregulation starting at 1% (spontaneous CD63 expression) up to 19% and 25% for the highest stimulation concentration, respectively (Fig. 1). In contrast, expression of CD63 on basophils from the three control individuals remained merely unchanged (0%) (Fig. 1).

Gouda cheese extract and annatto were spotted on nitrocellulose (Whatman Ltd, Maidstone, UK) and incubated with serum of the patient and the three control individuals. Bound specific IgE was detected with monoclonal anti-human IgE (Sigma) and anti-mouse IgG labelled with horseradish peroxidase (Sigma). Chemiluminescent staining was performed using Westernbreeze (PerBio Science, Aalst, Belgium). The intensity was photographed using a Chemidoc gel documentation system (BioRad Laboratories, Nazareth Eke, Belgium) and analysed with QUANTITY ONE software (BioRad). The patient showed an approximately twofold higher intensity for IgE as compared with the three control individuals.

Annatto is extracted from the reddish pulp that surrounds the seed of the achiote (*Bixa orellana* L.), a shrub from the tropical region of the American continent. The inedible fruit is harvested for its seeds, which contain annatto, also called *bixin/norbixin*. It is used to colour many food products, such as cheeses (e.g. Gouda, Cheddar, Red Leicester), margarine, butter, chips, rice, smoked fish and salad oils. Sold as a paste or powder for culinary use, mainly as a colorant, it is known as achiote, annatto or pimentão doce. Annatto is a main ingredient in the Mexican spice mixture recado rojo, or achiote paste. Annatto is growing in popularity as a natural alternative to synthetic food colouring compounds, and companies using annatto may label their products 'all natural' or 'no artificial colours'. Central and South American natives use the seeds to make a body paint, and lipstick. For this reason, the achiote is sometimes called the lipstick-tree.

The role of annatto (E160b, achiote, bixin, orlean, roucou) in food allergy has been reviewed comprehensively by Lucas et al. (1). From that study, it emerges that IgE-mediated allergy from this natural dye is rather anecdotal. It seems that only one well-documented case has been published so far (2). In their study, the authors describe a patient who presented generalized urticaria, angio-oedema and an anaphylactic shock within minutes after consumption of a cereal brand containing annatto extract. A possible asthmatic reaction associated with the ingestion of a pharmaceutical product containing annatto was described in a 53-year-old female (3). However, no tests were conducted to confirm the clinical suspicion of annatto allergy.

In our patient, without other apparent food allergies, the close relationship between ingestion of the annatto-containing cheeses and immediate anaphylactic reaction is highly indicative for an IgE-mediated reaction from the natural food colour additive. This presumption is endorsed by the positive skin tests, the basophil activation test (BAT) and IgE-immunoblot to annatto.

The principles and applications of the BAT have recently extensively been reviewed elsewhere (4). With respect to food allergy, the BAT has been validated in primary (e.g. *Anisakis simplex*, *Macrobrachium rosenbergii* and sesame) and pollen-related food allergies. In

individual patients, the BAT confirmed diagnosis of allergies related to coriander, lychee and mandarin.

From our case, it is clear that BAT can also help to diagnose allergy from food additives such as natural colouring agents. Although the BAT does not distinguish between IgE and non-IgE-mediated reactions, the IgE-immunoblot experiments point to an underlying IgE-mediated mechanism.

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Definition of an exercise intensity threshold in a challenge test to diagnose food-dependent exercise-induced anaphylaxis

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Key words: anaphylaxis; challenge test; exercise; food; urticaria.

Food-dependent exercise-induced anaphylaxis (FDEIA) is an IgE-mediated hypersensitivity requiring both intake of food and consecutive exercise to induce symptoms of anaphylaxis. Vigorous exercise in this case facilitates allergen absorption from the gastrointestinal tract. When food intake and exercise are exposed independently, patients will not experience allergic symptoms (1).

In order to confirm food-dependent exercise-induced anaphylaxis by provocation, a critical exercise intensity limit has to be exceeded.

We report on a 46-year-old woman with allergic rhinoconjunctivitis and a 3-year history of anaphylaxis during physical exercise after having taken a meal containing cakes and sugar. This patient had experienced four episodes with angioedema, urticaria, diarrhoea, vomiting, dyspnoea and cardiovascular collapse after ingesting desserts with sugar glaze and consecutive running. Skin prick test results were negative for powdered sugar; however, the results were positive for wheat and spelt flour, corn bran, hazel, alder, birch and grass. Detection of specific IgE antibodies by Immuno-CAP (Phadia, Freiburg, Germany) revealed a raised total IgE of 1037 IU/ml, and specific IgE class 4 to hazelnut, hazel, alder, birch and crab and class 3 to ω5-Gliadin. Provocation tests were performed with a combination of intake of powdered sugar and wheat sandwich and exercise. However, first provocation tests with 20 min of unstandardized bicycle ergometer exercise failed to induce any symptoms, as has been reported in the literature (2).

Thus, a defined supervised treadmill exercise in the sports medicine outpatient clinic was performed. On day 1, the maximal exercise intensity for the patient was identified by beginning a treadmill at a speed of 4 km/h and increasing the speed by 2 km/h for every 3 min. After 15 min and at a speed of 12 km/h, the maximal exercise intensity was reached with a heart rate of 170/min, a subjective Borg score of 17 (out of 20) and a maximal lactate level of 9.2 mmol/l. On day 2, directly after ingestion of a nutty wheat pastry containing ω5-Gliadin, the same procedure was followed. When a submaximal exercise intensity at a lactate level of 3.9 mmol/l was reached, the exercise was continued at a speed of 10 km/h until symptoms occurred. After 18 min and 3500 m (patient's heart rate 159/min), the patient reported pruritus and urticaria on her neck, décolleté and

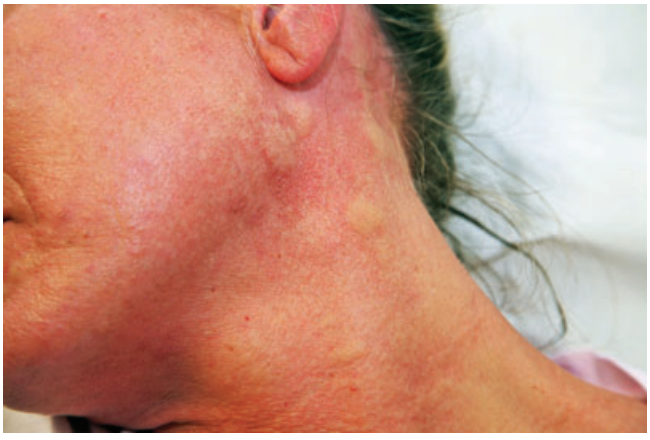

Figure 1. Wheals and erythema spreading from the patient's neck to the décolleté.

