

# Oral Challenge with Pasteurized Egg White from *Gallus domesticus*

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## Key Words

Allergy · Egg allergy · Food allergy · Oral provocation · Oral provocation test · Pasteurization · Salmonellosis · Avian flu

## Abstract

**Background:** Since raw egg may cause digestive toxic infections, we assessed whether pasteurized raw egg white is as reliable as fresh raw egg white in the diagnosis of egg allergy. **Methods:** Thirty-two egg-allergic children were challenged with both pasteurized and fresh raw egg white. Open challenges were carried out with increasing doses of pasteurized raw egg white and fresh raw egg white administered every 60 min. **Results:** Eleven children (34.4%) had positive challenges with pasteurized raw egg white. Twenty-one children (65.62%) who tolerated pasteurized raw egg white also had a negative challenge with fresh raw egg white. If the challenge with pasteurized raw egg white resulted positive, the study was stopped. The protein profile and IgE-binding capacity of both pasteurized and fresh egg white were almost identical as observed by SDS-PAGE and IgE immunoblotting. In the IgE immunoblotting-inhibition and ImmunoCAP-inhibition assays, both extracts behaved in a similar way. **Conclusions:** We did not find any relevant allergenic differences between fresh and pasteurized egg white. This study supports the use of pasteurized egg white in the diagnosis of allergy to fresh raw egg proteins.

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## Introduction

Since *Gallus domesticus* egg's main allergens are partially thermostable, the diagnosis of allergy to egg has to be made by challenge with raw egg white. However, fresh raw egg is potentially dangerous because it can cause digestive toxic infections (*Salmonella*, avian flu). There have been many outbreaks of food poisoning caused by *Salmonella* [1–5]. After a number of cases had been published in Spain, new laws were passed establishing minimum temperatures for preparation or cooking and storage requirements for eggs and egg products. In 1991, the Spanish Health Ministry (Royal Decree 1254) published some guidelines on the preparation and storage of foods that contain egg as an ingredient, along with the requirement that they be replaced with pasteurized egg products [6], except when those foods are heated to no less than 75°C. In addition, the pH cannot be greater than 4.2 in the finished product and the maximum storage temperature 8°C until the moment of consumption, with a maximum of 24 h from production to consumption.

The use of pasteurized raw egg white avoids the risk of food poisoning (*Salmonella*, avian flu) and complies with current legislation. However, in the process of pasteurization, heat is used. The effect of pH, processing temperature and preheating steps in 2 commercial egg white pasteurization procedures (Armour and Standard Brands methods) have been previously evaluated using a 5-strain cocktail of *Salmonella* [7].

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The objective of this study was to assess if pasteurized raw egg white is as reliable as fresh raw egg white in the diagnosis of egg allergy. We also investigated whether the recognition of allergens in pasteurized raw egg is the same as that in fresh raw egg, using serum from patients allergic to egg.

## Materials and Methods

### Patients

The in vivo study group included 32 children (mean age  $4.7 \pm 0.6$  years; interquartile range 3.01–5.13) previously diagnosed with raw egg allergy, who tolerated cooked egg whites and who were consecutively challenged with both pasteurized and fresh raw egg white during 2007. Open challenge was carried out with increasing doses (4, 8 and 16 ml) of pasteurized raw egg white, administered every 60 min. If this challenge was negative, on the second day, increasing doses of fresh raw egg white (1/8, 1/4 and 1/2 egg white) were given at 60-min intervals. The challenge was immediately stopped if objective clinical symptoms arose.

To avoid the rejection of the food, egg white was mixed with a cold dessert. Pasteurized egg offers certain culinary advantages. The product is fluid and has lost the natural elastic consistency of fresh raw egg white; therefore, it can be administered easily with a syringe and can be mixed with any other food without having to whip the whites. Beating the whites requires more time and increases the possibility of contamination.

### Serum Pool of Egg-Allergic Subjects

The in vitro study was performed with sera from our serum bank. Sera of 11 children (mean age  $4.2 \pm 0.9$  years), diagnosed with egg allergy confirmed by oral challenge during the last 3 years, were pooled and used in the in vitro study.

### Pasteurized Egg

An ultrapasteurized liquid egg product commercially available (Clara líquida pasteurizada Pascual Brik 1 kg; Leche Pascual SA, Aranda de Duero, Spain) was purchased at a local food store. Pasteurization is performed in such a manner that it assures the absence of *Salmonella*, due to heat treatment of  $57^{\circ}\text{C}$  for 180 s which eliminates pathogenic bacteria [8] and avian flu virus [9]. The shelf life of the product is 6 weeks if the package is kept closed and refrigerated at  $0\text{--}3^{\circ}\text{C}$ . Once opened, it should be consumed within 72 h. One liter is equivalent to 30 fresh raw egg whites (with an average weight of 65–70 g/egg). One egg white is equivalent to 33 ml.

### SDS-PAGE

This technique was performed with different stains (Coomassie blue and methenamine silver) using fresh and pasteurized raw egg white. The electrophoresis procedure was performed according to the method of Sutton et al. [10] with some modifications. SDS-PAGE was performed with a 12% polyacrylamide separating gel and a 4% stacking gel.

### IgE Immunoblotting

After SDS-PAGE, the proteins were transferred to a nitrocellulose membrane (pore size =  $0.2\text{ }\mu\text{m}$ ; Schleicher & Schüll, Dassel,

Germany) according to the method of Towbin and Gordon [11], and the allergens were identified by immunostaining using patients' sera [12].

### ImmunoCAP Inhibition

The inhibition assays of IgE-binding to egg white were performed against the 2 types of raw egg white (fresh and pasteurized) [13].

### IgE Immunoblotting Inhibition

This was carried out with both types of egg white as inhibitors in the liquid phase: fresh and pasteurized [14].

## Results

### IgE Determinations by ImmunoCAP

Total serum IgE (Immulate<sup>®</sup>, DPC 1000; DPC, Los Angeles, Calif., USA) and allergen-specific IgE (ImmunoCAP 250<sup>®</sup>; Phadia, Uppsala, Sweden) were determined against chicken egg white and egg yolk as well as to their allergenic proteins. Results are shown in table 1.

### ImmunoCAP Inhibition

ImmunoCAP inhibition was performed using fresh raw egg white as solid phase and with the different inhibitor extracts at the same protein concentration. The results were very similar for the 2 extracts (table 2).

### SDS-PAGE

Figure 1 shows the comparison of fresh and pasteurized egg whites, which correspond to lanes 1 and 2 of the methanamine silver and Coomassie blue stains. With both techniques, the bands appear to be the same for both extracts.

### IgE Immunoblotting and IgE Immunoblotting Inhibition

Figure 2 shows the IgE binding of the various extracts. Again, we observed that both extracts (fresh and pasteurized raw egg white) behave identically (fig. 2a). In addition, we performed an immunoblotting inhibition (fig. 2b and c) with the various extracts of egg whites, and we verified that the behavior of fresh and pasteurized raw egg white was always identical.

### Challenge Tests with Egg White

Open food challenges with both types of raw egg white were carried out. From July to October 2007, 32 egg-allergic children were consecutively challenged with both pasteurized and fresh raw egg whites; 11 children (34.38%) had a positive result with pasteurized raw egg white. Sev-

**Table 1.** Total serum IgE (kU/l) and allergen-specific IgE to different egg fractions (kU<sub>A</sub>/l) in the serum pool

|                 |       |
|-----------------|-------|
| Total serum IgE | 694.0 |
| Egg white       | 78.5  |
| Egg yolk        | 16.8  |
| Ovalbumin       | 67.9  |
| Ovomucoid       | 66.2  |
| Lysozyme        | 15.0  |
| Ovotransferrin  | 34.0  |

**Table 2.** ImmunoCAP inhibition of fresh egg white (solid phase) with various types of egg whites as inhibitors (protein concentration at 1 mg/ml)

| Solid phase     | Liquid phase          | Percent inhibition of ImmunoCAP |
|-----------------|-----------------------|---------------------------------|
| Fresh egg white | Fresh egg white       | 97.05%                          |
| Fresh egg white | Pasteurized egg white | 97.20%                          |

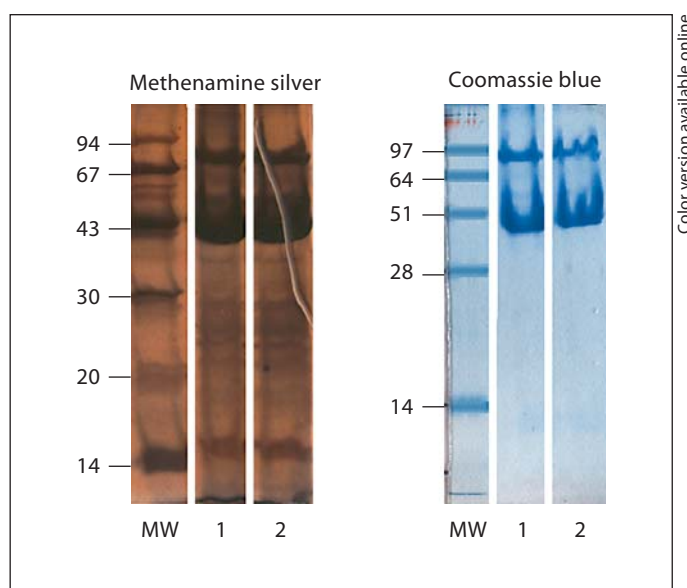
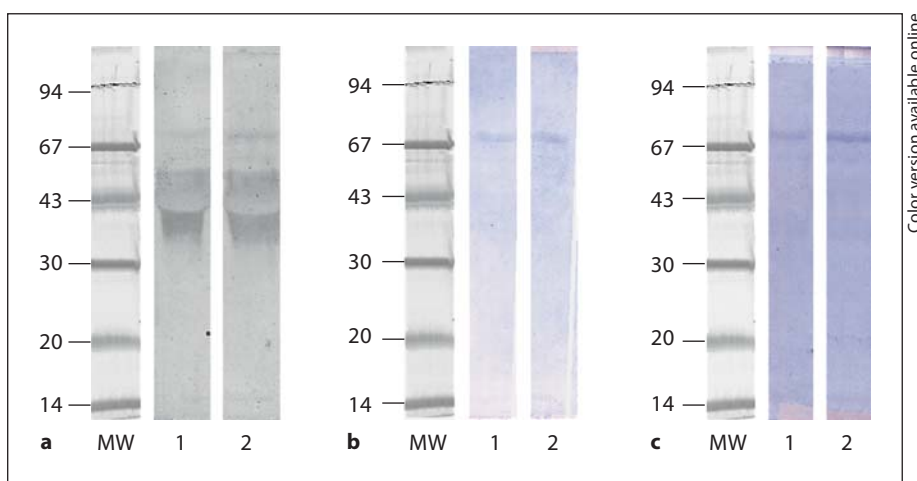


**Fig. 1.** SDS-PAGE with fresh (lane 1) and pasteurized (lane 2) egg white using 2 different stains. MW = Molecular weight.

**Fig. 2.** IgE immunoblotting (a) and IgE immunoblotting inhibition with fresh (b) and pasteurized egg white (c). Lane 1 = Fresh raw egg white; lane 2 = pasteurized egg white. MW = Molecular weight.



en children (63.64%) had symptoms with a dose of 1/8 (4 ml), 3 children (27.27%) with a dose of 1/4 (8 ml) and 1 child (9.09%) with a dose of 1/2 (16 ml). All children with a positive challenge who suffered cutaneous symptoms such as erythema or hives/itching were treated with dexchlorpheniramine (Polaramine®), except 1 child who also had abdominal pain and was treated with adrenaline. Twenty-one children (65.62%) tolerated pasteurized as well as fresh raw egg white. These results support that pasteurized raw egg white is at least as allergenically po-

tent as fresh raw egg white, since no child who had tolerated pasteurized raw egg white experienced symptoms with fresh raw egg white.

## Discussion

Liquid egg white, which is widely used in the food industry, needs to be pasteurized to reduce *Salmonella enteritidis* which causes food poisoning in humans [15].

Thus, the use of pasteurized egg white in food challenges may be of great interest in the diagnosis of egg allergy.

In this study, we have observed that pasteurization of egg white does not change its allergenic activity or IgE-binding profile, as determined by IgE immunoblotting, but still there might be more subtle changes in terms of modifications of epitopes or on the three-dimensional protein shape. However, these possible changes do not appear to have clinical relevance, as we have confirmed by means of oral allergen challenge tests.

In this study, all children who tolerated pasteurized egg white also tolerated fresh raw egg white, that is, the negative predictive value was 100%. The children that did not tolerate pasteurized egg white were not challenged again for ethical reasons since the positive provocation with pasteurized egg white confirmed the diagnosis of persistent egg allergy.

Ovalbumin and ovomucoid are partially thermostable, maintaining their immunogenicity after 20 min of boiling, and they are described as the most important egg allergens. For some authors, sensitization to ovomucoid, which is more heat resistant than ovalbumin, is linked to symptom persistence and can be used to predict tolerance to cooked egg [16]. Ovotransferrin (also called conalbumin) and lysozyme are less stable to heat and are weaker allergens [17], but there is interest in them, since lysozyme is found as a hidden allergen in other foodstuffs (cheese) and some medications [18].

The majority of egg white proteins are globular with an electrical charge that can be modified by adding energy such as thermal energy or heat as well as mechanical energy such as beating. In fact, beaten egg whites show a changed color and consistency. Whipped egg whites also suffer structural changes and their allergenicity has not yet been investigated. When cooking foods, various protein-denaturing agents are used (such as heat, mixing and beating), whose objective is to achieve a more pleasant texture, taking advantage of organoleptic properties [19].

Egg white and egg yolk behave differently upon denaturing. Egg white beaten until white (as happens when making meringue) introduces air, pulls apart protein macromolecules and, if it is vigorous, can even break less stable protein links [19]. Egg white coagulates at around 60°C and yolk coagulates at around 70°C. Coagulation is a thermodynamical adverse event, because while it happens, the temperature stabilizes [19].

When pasteurization is performed at a temperature of less than 60°C, it can be assumed that the proteins of the egg remain intact, because egg proteins coagulate above 65°C [8]. It has been shown that heating liquid egg white

(pH 7) at 60°C for 5 min caused only the aggregation of ovotransferrin, one of the major proteins in egg white [20–22]. Wanatabe et al. [23] and Matsudomi et al. [24] have previously reported that heat-treated ovalbumin inhibited the aggregation of ovotransferrin, and the authors considered that the effect was derived from ionic interaction. In conclusion, the effects of anions on the thermostability of ovotransferrin, especially sulfate, repressed the heat aggregation of liquid egg white [25].

Recently, Roth-Walter et al. [26] analyzed the impact of pasteurization on the antigenicity of the individual milk proteins. Pasteurization of cow's milk proteins caused aggregation of  $\beta$ -lactoglobulin [27] and  $\alpha$ -lactalbumin [28] (but not of casein that already exists in a micellar form), inhibiting uptake by intestinal epithelial cells in vitro and in vivo [26]. These findings support the hypothesis that food processing may contribute to the increasing prevalence of sensitization to food proteins in westernized countries [26].

We conclude that there are no allergenic differences in vitro or in vivo between fresh and pasteurized raw egg whites. The use of pasteurized egg white in the oral controlled challenge avoids the risk of food poisoning (*Salmonella*, avian flu) and complies with current legislation. Therefore, the present study supports the use of pasteurized egg whites for the diagnosis of allergy to raw egg proteins. Besides, raw pasteurized egg whites are easier to handle than fresh raw egg whites.

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