

# Allergy to *Theobroma cacao*

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## Introduction

Cocoa bean is obtained from the *Theobroma cacao* tree fruit and is the source of the edible cocoa (fermented and roasted) used to prepare chocolate and other manufactured products as cocoa powder, shakes, etc.<sup>1</sup> Food allergy to cocoa is a rare condition and very few cases have been reported with an allergy study.<sup>2</sup> The risk of suffering an allergic reaction after cocoa consumption in patients with food allergy, especially to tree nuts, is not recognized.

## Case History

We present a case of a 24-years-old woman with atopic dermatitis, eosinophilic allergic asthma and cat allergy with previously history of severe anaphylaxis to walnut and almond (sensitization to storage proteins). The patient also has a history of bronchospasm with (non-occupational) airborne exposure to trace amounts of almond.

The patient was evaluated for anaphylaxis to a piece of dark chocolate. She developed ocular, nasal, oral and pharyngeal pruritus, lip angioedema, sneezing, rhinorrhea and intense abdominal pain within 5 minutes of ingestion. Her symptoms resolved after treatment with IM epinephrine in an emergency department. She had previously tolerated cocoa.

## Methods and results

Subsequent allergy evaluation included skin prick test (SPT) to commercial extracts of common foods with positive results for almond, hazelnut and walnut. A pure cocoa prick-prick test (PPT) was also positive (4 mm) (Figure 1). SPT to common aeroallergens included positive results for *Olea europaea* pollen and cat dander.

Results of in vitro analysis included a total serum IgE 310 (kU/L), specific IgE (ImmunoCAP; kU<sub>A</sub>/L) to hazelnut: 20.30, walnut 0.14, and cocoa 0.12; and ImmunoCAP ISAC (ISU-E) being positive with following species-specific food components: nCor a 9 (11S globulin): 1.8, rJug r 1 (2S albumin): 28, nJug r 2 (7S globulin): 3.6, rAra h 1 (7S globulin): 0.5, nGly m 6 (Glycinin): 0.6, rOle e 1: 0.1 and Fel d 1: 1.2.

Protein extracts from edible cocoa powder (fermented and roasted cocoa) were prepared by homogenization in phosphate-buffered saline (10% V/V) (50 mM phosphate buffer, 100 mM NaCl, pH 7.5), dialyzation against distilled water and liophilization. In order to avoid contamination by tree nut proteins that sometimes appears in some edible manufactured cocoa products, we prepared a protein extract from a raw cocoa bean following the same method above described.

SDS-PAGE immunoblotting method according to Laemmli <sup>3</sup>was used to estimate the molecular mass of IgE-reactive proteins of the following samples: cocoa bean powder, cocoa powder extract, raw cocoa bean, raw cocoa bean extract as well as walnut and almond extracts. IgE binding was detected in a band of

approximately 17.5 kDa in three cocoa samples: cocoa powder, raw cocoa bean, raw cocoa bean extract, and a broad band between approximately 21-17.5 kDa in cocoa powder extract. IgE-reactive bands between approximately 45 – 35 kDa and 20-9 kDa were observed in walnut extract, and bands between approximately 58-13 kDa in almond extract (Figure 2.I).

With the aim to assess the presence of IgE cross-reactivity to proteins in cocoa, walnut, and almond, an SDS-PAGE Immunoblotting-inhibition assay was carried out using cocoa extract in solid phase and walnut and almond extracts as inhibitors. Both walnut and almond extracts produced a partial IgE-binding inhibition, although walnut extract showed a higher inhibition capacity, however, neither produced a total IgE-binding inhibition to cocoa extract (Figure 2.II).

## Discussion

Allergy to cocoa is rare, but cocoa has been reported previously as both an occupational and food allergen. A survey of Salvadorian schoolchildren demonstrated an incidence of cocoa allergy between 0.5-0.7%, they report symptoms including sneezing, rhinorrhea, wheezing, hives; however most of these cases have not been objectively demonstrated.<sup>4</sup>

In another study, 40 occupational cocoa workers had a high prevalence of positive skin tests to cocoa powder extract (60.2%) compared to the unexposed population, and 2 out of 3 sensitized subjects with bronchoconstriction-related underwent a bronchoprovocation test to cocoa powder with objective changes in FEV1. These suggest that workers employed in cocoa processing are at risk of developing allergic sensitization and respiratory impairment.<sup>5</sup>

As allergy to chocolate and cocoa is commonly attributed to the presence of other allergenic sources that often accompany cocoa in manufactured foods, such as tree nuts or milk, *Lopes et al.* confirmed specific cocoa allergy in 3 patients via supervised oral challenges and suggested raised awareness for cocoa as a potential true food allergy.<sup>2</sup>

To date, no major cocoa allergen has been defined, although there are studies of cocoa bean proteins<sup>6</sup> as well as a publication identifying a potential allergen belonging to the PR-10 group.<sup>7</sup>

Pereira Menezes *et al.*, to demonstrate the allergenic nature of TcPR-10 protein in a mouse model, modified by genetic engineering the allergenic motif of this protein trying to reduce its allergenic character.<sup>8</sup>

Cocoa bean 2S Albumin storage polypeptides have been described that have high sequence similarity (>52%) to several 2S albumins from known allergenic sources such as English walnut, rape seed and Brazil nut.<sup>9</sup>

Assuming that primary sensitization was produced by tree nuts proteins, we would expect a total inhibition of IgE binding (which could not be observed in our study), however, our inhibition studies demonstrated cross-reactivity between cocoa, walnuts and almond proteins.

We hypothesize that primary tree nut allergy predisposed our patient to develop an allergy cocoa.

## Conclusion

In conclusion, we demonstrate that while rare, true cocoa allergy is possible and may be associated with certain tree nut allergies. Major cocoa allergens still need to be confirmed, and further study is needed to better characterize cocoa allergy.

## Author Contributions:

Ana Rosa Cristina Nin Valencia: Conceptualization, Validation, Writing – original draft, Writing – review & editing.

Lorena Soto-Retes: Supervision, Conceptualization, Validation, Writing – review & editing.

Gustavo Perdomo Gutierrez: Validation.

Teresa Bigorra: Methodology, Validation.

Borja Bartolome: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing.

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