"Tolerance to Baked Foods in Children Under 6 Years Old Allergic to Cow's Milk and Egg Proteins"

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TOLERANCE TO BAKED FOODS IN CHILDREN UNDER 6 YEARS OLD ALLERGIC TO COW'S MILK AND EGG PROTEINS

1) CURRENT STATUS OF THE TOPIC

Food allergy is a condition with increasing prevalence in Western countries. It is estimated that 5–8% of children in our environment are allergic to at least one food, compared to 1–2% in adults. This condition significantly impacts quality of life and social activities, causing emotional and familial stress. The most common IgE-mediated food allergens are cow's milk, egg, nuts, fish, and shellfish. To date, the accepted treatment is the strict avoidance of the allergen, which carries the ongoing risk of accidental exposure and potential nutritional deficiencies.

Oral immunotherapy (OIT) for specific food allergens has emerged as a treatment option for patients who do not outgrow their allergy in early childhood. Current studies suggest a higher likelihood of developing tolerance in patients undergoing OIT compared to those following only an elimination diet; however, local and systemic adverse effects, sometimes severe, can occur during treatment. Therefore, including a patient in OIT requires a careful risk-benefit analysis of possible severe reactions during treatment versus the long-term benefit of immune tolerance.

Recently, studies have focused on the potential for tolerance to baked foods. Heating food changes the protein's structure, reducing allergenicity by destroying conformational IgE-binding epitopes. This appears to be key in acquiring tolerance to milk and egg proteins. Additionally, baking within a cereal matrix further reduces allergenicity by altering digestibility or making epitopes less accessible. This effect varies depending on the food; for example, heating peanut proteins may actually increase allergenicity due to glycosylation.

The immunological changes observed in a diet including baked milk or egg are similar to those seen with OIT, but the baked food approach is generally safer and provides a more natural immunomodulatory strategy. OIT can be challenging due to the risk of serious adverse effects. Introducing baked foods into the diet not only improves quality of life by increasing dietary variety but also reduces stress in social settings. According to recent studies, this strategy may help shorten the time needed to achieve full tolerance.

Since 2006, the Hospital Sant Joan de Déu in Esplugues has successfully implemented OIT protocols for milk and egg in children over 5 years of age, accepting the associated risks. However, complete permanent desensitization has not been achieved in all patients. Many still experience occasional adverse reactions during maintenance. The success rate is estimated at around 70%, with failure mainly affecting children with anaphylaxis—those who would most benefit from protection.

2) OBJECTIVES

Main objective: To evaluate tolerance to baked foods in children under 6 years old who are allergic to cow's milk or egg proteins.

Secondary objectives:
- To evaluate tolerance to raw foods after one year of controlled intake of baked foods containing milk or egg in increasing amounts.
- To assess the immune response by identifying biomarkers that may predict the acquisition of tolerance.

- To compare quality-of-life questionnaires between patients tolerant to baked foods and their families versus those in the control group.
- To determine the individual reaction threshold dose that would allow patients to consume trace amounts of the allergen, thereby improving their quality of life and nutritional status.

3) METHODOLOGY

This is a prospective interventional study.

Inclusion criteria for cow's milk (CM):
- Children aged 12 months to 6 years with a clinical history suggestive of cow's milk protein allergy, and positive specific IgE and/or skin prick tests.

Inclusion criteria for egg:
- Children aged 12 months to 6 years with a clinical history suggestive of egg allergy, and positive specific IgE and/or skin prick tests.
- Children aged 12 months to 6 years sensitized to egg proteins (positive specific IgE and/or skin prick tests) without prior ingestion.

Exclusion criteria:
- Patients whose guardians do not sign the informed consent form (Annex 1).
- Patients with heart disease or conditions contraindicating the use of epinephrine.
- Patients who are already consuming baked products with milk or egg.

Complementary evaluations:
Skin prick tests, specific IgE, IgA, and IgG4 levels, regulatory T cells, IL-4, IL-5, IL-10, and TGF-β will be assessed at the beginning of treatment (T0) and after 12 months (T1). Basophil activation tests with cow's milk or egg proteins will also be performed before and after treatment.

Eligible patients will undergo a baked food oral food challenge (OFC) at baseline. After 12 months of controlled intake, they will undergo another OFC with cooked and raw forms of the allergen.

Validated quality-of-life questionnaires for food allergy will be administered to families and children (depending on age) at baseline, during hospitalization for OFC, and at 12 months.

All OFCs will be performed at the hospital in a setting equipped to manage potential adverse reactions. Skin and blood tests will be performed 15 to 30 days before starting treatment.

BAKED FOOD CHALLENGE PROTOCOLS

Initial baked milk challenge:
Conducted using commercially available 6-cereal cookies (Almirón®), each containing 0.275 g of milk protein.

- Dose 1: 0.0375 g protein (1/8 cookie)
- Dose 2: 0.075 g protein (1/4 cookie)
- Dose 3: 0.15 g protein (1/2 cookie)
- Dose 4: 0.275 g protein (1 full cookie)
- Dose 5: 0.55 g protein (2 cookies)

Doses administered at 30-minute intervals. Vital signs are monitored before and after each dose. Observation continues for 3 hours post-final dose.

Initial baked egg challenge:
Conducted using Damira® cookies (free of cow's milk):

- Dose 1: ½ cookie (0.11 g protein)
- Dose 2: 1 cookie (0.22 g protein)
- Dose 3: 2 cookies (0.44 g protein)
- Dose 4: 5 cookies (1.1 g protein)

Doses administered at 30-minute intervals for the first two, then 60-minute intervals. Vital signs are monitored before and after. Observation continues for 3 hours post-final dose.

Cow's milk challenge:
Using pasteurized milk (3g/100mL) per PRACTALL protocol, followed by 125–200 mL final dose:

CM-OFC (ml)	CM-OFC (g)	Interval (minutes)
0.1	0.003	30
0.3	0.01	30
1	0.03	30
3	0.1	30
9	0.3	30
30	1	30
90	3	120
125/200	3.75/6	180 observation

Cooked egg challenge:
6-step protocol: 0.3, 1, 3, 9.5, and 30 g of hard-boiled egg white + 1 well-cooked omelet (7.5 g protein). Administered at 30-minute intervals (first 5 doses) and 120 minutes for final dose.

6-month dose escalation:

- Baked milk: 4 Almirón® cookies = 1.1 g milk protein.
- Baked egg: 1 homemade muffin (recipe in Annex 1) = 2 g egg protein. 3-hour observation post-dose.

4) STATISTICAL ANALYSIS

All data will be analyzed using SPSS Version 26 (IBM Corp., USA) and/or GraphPad Prism Version 9 (GraphPad Software, Inc., La Jolla, Calif). Descriptive statistics will be performed for the total sample, as well as for the control and intervention groups. Results with p-values < 0.05 will be considered statistically significant.

Participants will be categorized as tolerant or non-tolerant based on their ability to tolerate 1 g of cow's milk protein or 2 g of egg protein. Student's t-test will be used to compare means of predictive variables. Differences in the distribution of clinical variables will be assessed using the Chi-square test or Fisher's exact test for categorical variables. For non-parametric data, the Mann–Whitney U test or Kruskal–Wallis test will be applied.

Receiver Operating Characteristic (ROC) curve analysis will be conducted to evaluate the diagnostic performance of each individual biomarker in predicting tolerance to baked

products. The accuracy of the test will be assessed using the area under the ROC curve (AUC), with an AUC > 0.7 considered acceptable. The performance of optimal cut-off points will be described in terms of sensitivity and specificity.

5) WORK PLAN AND TIMELINE

The principal investigator will be Dr. Montserrat Álvaro Lozano. Each study group will be led by a physician: Dr. A. Machinena for egg-allergic patients and Dr. O. Dominguez for cow's milk protein-allergic patients. Data collection and processing will be handled by Dr. A. Machinena and Dr. O. Dominguez. Laboratory determinations will be carried out by Dr. M. Pascal (Hospital Clínic) and Dr. M. Batllori (Hospital Sant Joan de Déu). All physicians and nurses in the department will be direct collaborators.

Patient recruitment will last 2 years. Approximately 80 patients will be included: 30 allergic to egg, 30 allergic to cow's milk protein, and 20 controls. Each patient will be followed for 12 months after randomization.

6) BUDGET

The principal investigator, responsible physicians, and collaborators will conduct the project within regular working hours without additional personnel costs. The main expenses are for laboratory studies, detailed below:

Study	Price per sample (€)
PBMC isolation	15
Antigen-specific proliferation assay	30
TH1/TH2/FOXP3 cytometry panel	60
BAT	99
Specific IgA determination	12
IgG4 determination	19

Estimated costs for 60 patients (T0)	**Price 60 patients (euros)**
PBMC isolation	900
Antigen-specific proliferation	1800
BAT	5940
Specific IgA	720
TOTAL	**9360**

This does not include cytokine determination via Luminex, cytometry panel TH1/TH2/FoxP3 (€3600), and IgG4 determination (€1140), which will require other grants. T1 determinations are also pending funding.

Luminex cytokine analysis is the most expensive: one plate (~€2200-2500) analyzes 8 patients. For 60 patients, the cost is approximately €20,000, which exceeds this grant's budget. Therefore, supernatants will be frozen and analyzed once funding is available.

ANNEX 1 – BAKED EGG MUFFIN RECIPE

(Homemade muffin without milk or nuts)

For 6 muffins:
- 2 eggs
- 130 g wheat flour
- 80 g sugar
- 60 g vegetable margarine or 1 tbsp olive oil
- 2 tsp baking powder

Beat egg whites until stiff, then add sugar, margarine, yolks, baking powder, and flour. Mix until smooth. Rest dough for 15 minutes.

Optional: add lemon/orange juice or zest, fruit pieces (apple, banana, etc.), chocolate chips, or jam, depending on allergies.

Bake in a preheated oven at 180ºC for 20 minutes.