

# Asthmatic Response to Milk Ingestion Challenge in Adults: A Comparison of the Open and Double-Blind Challenges

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## Key Words

Bronchial asthma · Asthmatic responses to milk · Ingestion challenge with milk

## Abstract

**Background:** Cow's milk allergy can participate in pathophysiological mechanisms underlying bronchial asthma in some adult patients. This role should ultimately be confirmed by means of a milk ingestion challenge. In this study, the diagnostic value of the open food ingestion challenge (OFICH) and the double-blind placebo-controlled food challenge (DBPCFC) techniques with milk were compared in adult patients suffering from bronchial asthma with suspected milk allergy. **Methods:** In 87 asthmatics with a suspected history and positive skin tests for milk, the 87 OFICHs and DBPCFCs were performed in combination with spirometry and followed up to 72 h after the challenge. **Results:** Of 87 patients, 74 (85%) developed a positive asthmatic response (AR) (20 immediate, 33 late, 6 dual late and 15 delayed;  $p < 0.01$ ) and 13 (15%) developed a negative AR ( $p > 0.1$ ) to OFICH. Seventy-five (86%) developed a positive AR (17 immediate, 35 late, 8 dual late and 15 delayed;  $p < 0.01$ ) and 12 (14%) developed a negative AR ( $p > 0.05$ ) to DBPCFC. The correlation between the OFICH and DBPCFC was statistically significant

( $p < 0.01$ ). All placebo control challenges were negative ( $p > 0.2$ ). **Conclusions:** In some adults with bronchial asthma, involvement of an allergy to cow's milk results in the appearance of various AR types (immediate, late, dual late or delayed). The milk allergy can be confirmed by open or double-blind techniques, combined with spirometry. No significant differences were found between the OFICH and DBPCFC results. OFICH with natural milk combined with spirometry seems, therefore, to be an adequate technique for the detection of milk allergy in asthmatics. The DBPCFC can be performed as an additional check, if necessary.

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## Introduction

Food allergy is a multifaceted clinical manifestation of an immunological process in which foods or their components act as antigens, stimulating the production of specific antibodies or sensitizing the particular T lymphocyte subsets and subsequently interacting with them [1–30]. Food allergy was previously classically attributed to the involvement of the immediate (IgE-mediated) hypersensitivity mechanism [1–11, 17, 22, 23, 25–34]. However, evidence has been found for the possible involve-

ment of other, so-called non-IgE-mediated, hypersensitivity types, such as late (immune-complex-mediated) and delayed (cell-mediated) types of hypersensitivity in food allergy [1, 3, 5–30]. Food allergy can also be a factor in disorders of the respiratory tract, such as bronchial asthma [1–4, 8–11, 21, 23–31, 34–36]. This involvement can occur either in a primary form, where foods act as the sole causal factor of this disorder, or in a more frequently observed secondary form, where the foods play a role in the already existing bronchial asthma, in addition to the inhalant allergens [5–8, 11, 26–31].

Cow's milk allergy is one of the frequently occurring forms of food allergy, owing to the widespread consumption of milk by almost all age groups [1–3, 5–8, 11, 26, 28, 30, 33, 37]. The possible role of cow's milk allergy in bronchial asthma has been frequently studied in children [4, 9, 11, 14–16, 30, 33, 38–40], but comparable data in adults is relatively uncommon [2, 25–27, 31, 41, 42]. The role of food allergy among patients with bronchial asthma can lead to the development of various types of asthmatic response (AR). The immediate/early (IAR), late (LAR), dual late (DAR; a combination of IAR and LAR) and delayed (DYAR) responses to the food ingestion challenge, described in our previous papers [28, 29, 32, 41] and reported also by other investigators [1–4, 8, 25, 30, 32–34, 36–38, 40–46], seem to be analogous to the AR types induced by inhalant allergens [29, 47–49].

Food-induced IAR, LAR, DAR and DYAR differ from each other with respect to their clinical features, their association with other diagnostic parameters and to the possible underlying immunological mechanisms [1–4, 9, 12–19, 23, 25–27, 30, 32, 34, 40, 42, 43]. The provocation tests with foods should be considered to be the definite confirmation of the contribution of a certain food to the patient's complaints, e.g. bronchial asthma [1–8, 10, 25–30, 32–37, 40–46, 49–51]. These tests can be performed by (1) an open food ingestion challenge (OFICH), (2) a double-blind placebo controlled food challenge (DBPCFC) or (3) a single-blind food ingestion challenge, all of the above having their own advantages and disadvantages [1–10, 25–30, 32–34, 36, 37, 40–46, 50, 51].

The purpose of this study was: (1) to investigate the clinical features of the particular AR types induced by cow's milk allergy in some patients with bronchial asthma and (2) to compare the ARs produced by OFICH and DBPCFC and thus assess the suitability and diagnostic value of these techniques in combination with the monitoring of objective spirometric parameters in order to reach diagnostic confirmation of the possible participation of cow's milk allergy in adult asthmatics.

## Materials and Methods

### *Patients*

Eighty-seven patients with bronchial asthma in which cow's milk allergy was suspected to play a role, who had been referred to our Department of Allergology and Immunology (Institute of Medical Sciences 'De Klokkenberg', Breda, The Netherlands) for more extensive diagnostic analysis, volunteered to participate in this study.

These patients, 17–41 years of age, included 71 subjects, with existing bronchial asthma to an inhalant allergen, in whom it was suspected that milk allergy participated in their bronchial complaints (group I), and 16 subjects in whom milk was suspected to be the sole cause of their bronchial complaints (group II) (table 1).

They showed suspect/positive history (97%), positive skin tests (59%) and in some cases (13%), positive specific IgE antibodies for milk (tables 1, 2). They had no history of anaphylactic reaction, did not suffer from any airway infections or any systemic/serious disorder, did not use oral corticosteroids or immunotherapy and the possibility of pregnancy was excluded. They all demonstrated negative lactose intolerance tests. The patients were examined by routine diagnostic procedure, serving also as an exclusion-inclusion check [26–28], i.e. confirmation of a patient's eligibility for the study and the exclusion of possible contraindications [1–3, 26–28, 32, 37, 39, 42, 43, 50–53]. This procedure consisted of (1) the general part: disease history, physical examination, basic laboratory tests, X-ray of chest and sinuses, lung function, determination of blood gases and bacteriological examination of the sputum, (2) the allergologic part: skin tests with inhalant and food allergens, bronchial histamine thresholds [52, 53] and serum immunoglobulin determination, (3) 121 bronchial challenges with inhalant allergens (BPT) [47–49, 52] and (4) 87 OFICH and 87 DBPCFC with milk, according to the randomized sequence schedule.

A 7-day interval was always inserted between the consecutive tests, to prevent carry-over effects. All challenges were performed during a period without manifest symptoms and during a short hospitalization. Patients avoided all dairy products for 4 weeks before the challenges.

Inhaled glucocorticosteroids ( $n = 36$ ) and long-acting  $\beta_2$ -sympathomimetics ( $n = 33$ ) were withdrawn 4 weeks, disodium cromoglycate ( $n = 8$ ) and nedocromil sodium ( $n = 10$ ) 2 weeks before and other treatments 48 h before each of the challenges. If the FEV<sub>1</sub> values decreased after the allergen challenge by 50% or more, with respect to the predicted values, the patients ( $n = 6$ ) were treated with a single dose of 400  $\mu$ g salbutamol aerosol. The local ethical committee (IRB-MCK) approved this study and written informed consent was obtained from all study participants. The study was conducted according to the WMA Declaration of Helsinki concerning the principles for medical research involving human subjects.

### *Allergens*

Dialyzed and lyophilized allergen extracts (Allergopharma, Reinbek, Germany) diluted in PBS were used in concentrations of 50–500 BU/ml for skin tests and in concentrations of 1,000–3,000 BU/ml for BPTs. The concentrations recommended by the manufacturer were 100–500 BU/ml for skin prick tests (SPTs) and 5,000 BU/ml for intradermal tests and BPTs.

**Table 1.** Characteristics of the patients and control subjects

|                                                           | Patients          |                 |                 |                |                  |                 | Controls<br>(n = 15) |
|-----------------------------------------------------------|-------------------|-----------------|-----------------|----------------|------------------|-----------------|----------------------|
|                                                           | Total<br>(n = 87) | IAR<br>(n = 20) | LAR<br>(n = 33) | DAR<br>(n = 6) | DYAR<br>(n = 15) | NAR<br>(n = 13) |                      |
| Age, years                                                | 32 ± 5            | 29 ± 8          | 31 ± 6          | 34 ± 5         | 30 ± 3           | 33 ± 4          | 29 ± 5               |
| Gender, M/F                                               | 38/49             | 9/11            | 14/19           | 2/4            | 6/9              | 7/6             | 5/10                 |
| Disease history, years                                    | 4.9 ± 0.8         | 5.5 ± 0.4       | 4.7 ± 1.1       | 5.3 ± 0.8      | 4.6 ± 1.3        | 4.3 ± 0.5       | 4.2 ± 1.5            |
| FEV <sub>1</sub> , % predicted                            | 95 ± 4            | 95 ± 5          | 97 ± 3          | 94 ± 6         | 96 ± 3           | 92 ± 7          | 94 ± 4               |
| FVC, % predicted                                          | 98 ± 1            | 97 ± 2          | 100 ± 1         | 95 ± 4         | 101 ± 2          | 99 ± 1          | 102 ± 3              |
| Blood leukocyte count (×10 <sup>9</sup> /l) <sup>1</sup>  | 7.8 ± 0.9         | 7.3 ± 1.0       | 7.9 ± 0.6       | 6.8 ± 1.5      | 9.3 ± 0.8        | 8.2 ± 0.4       | 7.9 ± 1.5            |
| Blood eosinophil count (×10 <sup>6</sup> /l) <sup>2</sup> | 346 ± 41          | 381 ± 36        | 319 ± 29        | 374 ± 43       | 330 ± 11         | 325 ± 32        | 347 ± 23             |
| Blood neutrophil count (×10 <sup>9</sup> /l) <sup>3</sup> | 5.7 ± 0.5         | 4.3 ± 0.7       | 5.9 ± 0.5       | 5.1 ± 1.0      | 7.1 ± 0.3        | 6.4 ± 0.7       | 4.5 ± 0.9            |
| Bronchial histamine threshold <sup>4</sup>                |                   |                 |                 |                |                  |                 |                      |
| ≤2.0 mg/ml                                                |                   | 3               | 2               | 1              | 0                | 2               | 3                    |
| 4.0 mg/ml                                                 |                   | 5               | 8               | 2              | 2                | 3               | 4                    |
| 8.0 mg/ml                                                 |                   | 7               | 5               | 2              | 2                | 5               | 5                    |
| 16.0 mg/ml                                                |                   | 2               | 12              | 0              | 7                | 3               | 0                    |
| 32.0 mg/ml                                                |                   | 2               | 3               | 0              | 2                | 0               | 2                    |
| >32.0 mg/ml                                               |                   | 1               | 3               | 1              | 2                | 0               | 1                    |

Values = Mean ± SD. NAR = negative asthmatic response.

<sup>1</sup> Normal value: 4.0–10.0 × 10<sup>9</sup>/l. <sup>2</sup> Normal value: <300 × 10<sup>6</sup>/l. <sup>3</sup> Normal value: 2.0–7.2 × 10<sup>9</sup>/l. <sup>4</sup> Normal value: >32.0 mg/ml [52, 53].

#### Skin Tests

The SPTs in concentrations of 500 BU/ml were evaluated after 20 min and after 24 h. The intradermal (intracutaneous, i.c.) tests were performed in concentrations of 100 BU/ml and evaluated 20 min after and 6, 12, 24, 36, 48, 72 and 96 h after the injection. Histamine diphosphate was used as a positive control and PBS as a negative control. The SPTs and i.c. tests with milk extracts were performed in all 87 patients studied. A skin wheal (>7.0 mm in diameter) appearing 20 min after the injection was considered to be an immediate skin response, the skin infiltration observed between 6–12 h to be a late skin response and the skin induration recorded later than 48 h was designated a delayed skin response [26–29, 47–49].

#### Spirometry

The ARs were monitored by spirometry (Spirograph D-75; Lode NV, Groningen, The Netherlands), recording the FVC and FEV<sub>1</sub>, and were evaluated by the following criteria: (1) the decrease in FEV<sub>1</sub> of <10% with respect to the prechallenge values as a negative response, from 10 to 20% as doubtful and of ≥20% as a positive response, (2) a decrease in FEV<sub>1</sub> values being recorded at at least 3 consecutive time intervals was considered to be a positive response and (3) the response appearing within 2 h after the challenge was considered to be an IAR, that occurring between 4 and 24 h to be an LAR and that appearing later than 24 h after the challenge to be a DYAR [26–29, 47–49].

#### Food Used for the Ingestion Challenge

The quantities of milk used for both the OFICH and DBPCFC were similar to those usually consumed by the patients, in order

to obtain the highest degree of reproducibility [28–30, 41]. The OFICH was performed using 100 ml of natural milk (3.5 g of protein and 3.5 g of fat per 100 ml). The DBPCFC was carried out using 20 g of powdered whole milk (containing 2.9 g of protein and 2.85 g of fat) dissolved in 80 ml of water. The 5% glucose solution was used as a control for OFICH, while 20 g of tablet inactive ingredients (excipients), including dibasic calcium, sucrose, corn starch and microcrystalline cellulose, dissolved in 80 ml water, was used as a control (placebo) for DBPCFC. Both the powdered milk and the tablet excipients were enriched with 4 g of glucose to mask their taste. The control challenges were performed according to the same schedule as those with the experimental foods [28–30, 41]. The DBPCFC was carried out according to a triple-blinded schedule, which means the blind applied to the laboratory technician preparing the test materials, the nurse performing the challenge and the patient.

#### Schedule of the Food Challenge

The OFICH and DBPCFC were performed according to European and international standard procedures [2, 4, 32, 36, 37, 43, 51] and modified them [26–29] according to the following schedule. (1) The initial (baseline) values were recorded at 0, 5 and 10 min. (2) The food was consumed within 10 min, followed by a 1-hour waiting interval to allow the food to be ingested; during this interval the parameters were measured 4 times to exclude a reaction considered as 'too early'. (3) Post-challenge values were recorded at 0, 5, 10, 20, 30, 45, 60, 90 and 120 min, every hour up to the 12th hour and every second hour during the 22nd- and 38th-hour interval and the 46th- and 58th-hour interval [26–29, 47–49].

**Table 2.** Survey of other diagnostic parameters

|                                            | Patients          |                 |                 |                |                  |                 | Control subjects <sup>10</sup><br>(n = 15) |
|--------------------------------------------|-------------------|-----------------|-----------------|----------------|------------------|-----------------|--------------------------------------------|
|                                            | total<br>(n = 87) | IAR<br>(n = 20) | LAR<br>(n = 33) | DAR<br>(n = 6) | DYAR<br>(n = 15) | NAR<br>(n = 13) |                                            |
| Positive SPT response <sup>1</sup>         |                   |                 |                 |                |                  |                 |                                            |
| Immediate                                  | 25                | <b>11</b>       | 9               | 2              | 0                | 4               | 1 <sup>2</sup>                             |
| Negative SPT response <sup>2</sup>         | 62                | 9               | 24              | 4              | 15               | 9               | 14                                         |
| Positive i.c.test response <sup>3</sup>    | 51                | 14              | 25              | 2              | 5                | 5               | 0                                          |
| Immediate                                  | -24               | <b>-10</b>      | -9              | <b>-1</b>      | -0               | -4              |                                            |
| Late                                       | -23               | -4              | <b>-16</b>      | <b>-1</b>      | -1               | -1              |                                            |
| Delayed                                    | -4                | -0              | -0              | -0             | <b>-4</b>        | -0              |                                            |
| Negative i.c. test response <sup>3</sup>   | 36                | 6               | 8               | 4              | 10               | <b>8</b>        | 15                                         |
| Increased total IgE (serum) <sup>4</sup>   | 2                 | 1               | 1               | 0              | 0                | 0               | 0                                          |
| Positive specific IgE (serum) <sup>5</sup> | 11                | 4               | 2               | 3              | 0                | 2               | 1                                          |
| Increased total IgG (serum) <sup>6</sup>   | 3                 | 0               | 2               | 1              | 0                | 0               | 0                                          |
| Increased subclasses (serum) <sup>7</sup>  |                   |                 |                 |                |                  |                 |                                            |
| IgG <sub>1</sub>                           | 1                 | 0               | 1               | 0              | 0                | 0               | 0                                          |
| IgG <sub>2</sub>                           | 0                 | 0               | 0               | 0              | 0                | 0               | 0                                          |
| IgG <sub>3</sub>                           | 1                 | 0               | 1               | 0              | 0                | 0               | 0                                          |
| IgG <sub>4</sub>                           | 1                 | 0               | 0               | 1              | 0                | 0               | 0                                          |
| Increased total IgM (serum) <sup>8</sup>   | 0                 | 0               | 0               | 0              | 0                | 0               | 0                                          |
| Increased total IgA (serum) <sup>9</sup>   | 4                 | 1               | 1               | 2              | 0                | 0               | 0                                          |
| Concomitant disorders                      |                   |                 |                 |                |                  |                 |                                            |
| Allergic rhinitis                          | 13                | 4               | 6               | 2              | 1                | 0               | 1                                          |
| Atopic eczema                              | 15                | 1               | 10              | 3              | 1                | 0               | 0                                          |
| Urticaria                                  | 2                 | 0               | 1               | 1              | 0                | 0               | 0                                          |
| Angioneurotic edema                        | 1                 | 0               | 1               | 0              | 0                | 0               | 1                                          |
| Gastrointestinal complaints                | 11                | 1               | 4               | 2              | 3                | 1               | 0                                          |

NAR = Negative asthmatic response.

<sup>1</sup> SPT with milk extract. <sup>2</sup> Negative skin response to milk extract. <sup>3</sup> Intracutaneous skin test with milk extract. <sup>4</sup> Total IgE in the serum (PRIST), normal value: <500 IU/ml. <sup>5</sup> Positive allergen-specific IgE in the serum for milk (ImmunoCAP): >0.70 U/ml

(more than class 1). <sup>6</sup> Total IgG in the serum (single radial immunodiffusion and ELISA), normal value: <15.0 g/l. <sup>7</sup> Normal values: IgG<sub>1</sub> <5.0 g/l, IgG<sub>2</sub> <2.6 g/l, IgG<sub>3</sub> <0.4 g/l, IgG<sub>4</sub> <0.5 g/l. <sup>8</sup> IgM: <3.8 g/l. <sup>9</sup> IgA: <4.0 g/l. <sup>10</sup> Control subjects: 7 OFICH + 8 DBPCFC food ingestion challenges with milk.

#### Control Group

Fifteen patients with perennial bronchial asthma developing 5 IARs, 9 LARs and 1 DYARs to BPTs with *Dermatophagoides pteronyssinus* and demonstrating a negative history, skin test and specific IgE for milk (ImmunoCAP), volunteered to participate as controls. The OFICH was performed in 7 patients and the DBPCFC was performed in 8 patients with milk according to the same schedule as the experimental patient group.

#### Statistical Analysis

ARs were analyzed by the generalized multivariate analysis of the variance (MANOVA) model [54]. The polynomials were fitted to the mean curves over time (8 time points within 120 min and 23 time points up to 72 h after the challenge), and the appropriate hypotheses were tested by the MANOVA computerized system [54]. In every patient, the post-challenge FEV<sub>1</sub> values measured at each time interval were compared with the pre-challenge FEV<sub>1</sub> values and evaluated by means of the Wilcoxon matched-pair signed-rank test. The mean post-challenge FEV<sub>1</sub> values were

compared with the corresponding post-challenge control FEV<sub>1</sub> values at each of the time points and analyzed by means of the Mann-Whitney U test. The correlation between the OFICH and DBPCFC was evaluated by means of the Wilcoxon matched-pair signed rank test. A p value of <0.05 was considered to be statistically significant.

#### Results

In 87 patients, 87 OFICHs with milk resulted in 74 (85%) positive and 13 (15%) negative ARs (tables 3–5). The 74 positive ARs included 20 (23%) IARs (p < 0.01), 33 (38%) LARs (p < 0.01), 6 (7%) DARs (p < 0.05) and 15 (17%) DYARs (p < 0.05) (tables 3, 5; fig. 1a–d; online suppl. tables 1–4, for all online suppl. material, see [www.karger.com/doi/10.1159/000345130](http://www.karger.com/doi/10.1159/000345130)). Both the positive

**Table 3.** Survey of bronchial complaints during the particular asthmatic response types to milk ingestion by means of OFICH

| Bronchial complaints | Patients     |              |             |               |              | Control subjects (n = 15) |
|----------------------|--------------|--------------|-------------|---------------|--------------|---------------------------|
|                      | IAR (n = 20) | LAR (n = 33) | DAR (n = 6) | DYAR (n = 15) | NAR (n = 13) |                           |
| Dyspnea              |              |              |             |               |              |                           |
| –                    | 0            | 0            | 0           | 0             | <b>92</b>    | <b>100</b>                |
| +                    | 15           | 9            | 17          | 13            | 8            | 0                         |
| ++                   | <b>80</b>    | <b>67</b>    | <b>83</b>   | 20            | 0            | 0                         |
| +++                  | 5            | 24           | 0           | <b>67</b>     | 0            | 0                         |
| Wheezing             |              |              |             |               |              |                           |
| –                    | 0            | 0            | 0           | 40            | <b>92</b>    | <b>94</b>                 |
| +                    | 20           | 6            | 17          | <b>48</b>     | 8            | 6                         |
| ++                   | <b>75</b>    | <b>85</b>    | <b>66</b>   | 6             | 0            | 0                         |
| +++                  | 5            | 9            | 17          | 6             | 0            | 0                         |
| Cough                |              |              |             |               |              |                           |
| –                    | 35           | 45           | 50          | <b>94</b>     | <b>92</b>    | <b>100</b>                |
| +                    | <b>50</b>    | <b>52</b>    | <b>50</b>   | 6             | 8            | 0                         |
| ++                   | 15           | 3            | 0           | 0             | 0            | 0                         |
| +++                  | 0            | 0            | 0           | 0             | 0            | 0                         |
| Expectoration        |              |              |             |               |              |                           |
| –                    | <b>90</b>    | <b>94</b>    | <b>83</b>   | <b>100</b>    | <b>100</b>   | <b>100</b>                |
| +                    | 10           | 6            | 17          | 0             | 0            | 0                         |

Numbers are percentages. Bronchial complaints according to author's modified score system: absent (–), slight (+), moderate/intermittent (++), pronounced/regularly (+++). NAR = negative asthmatic response.

and negative ARs corresponded to the disease history, skin tests and other diagnostic parameters to various degrees (tables 2–4).

In 71 asthmatics of the group I, in whom food allergy was only an additional cause, 71 OFICHs with milk resulted in 59 positive ARs (83%;  $p < 0.01$ ) and 12 negative ARs (17%;  $p > 0.1$ ) (table 5). The 59 positive ARs included 15 IARs, 26 LARs, 5 DARs and 13 DYARs.

In 16 patients of the group II, in whom BPTs with inhalant allergens were negative and the milk allergy was suspected to be the sole cause of their bronchial complaints, 16 OFICHs with milk resulted in 15 positive ARs (94%;  $p < 0.01$ ) and 1 negative AR (6%;  $p > 0.1$ ) (table 5). The 15 positive ARs consisted of 5 IARs, 7 LARs, 1 DAR and 2 DYARs. All OFICH control challenges were negative ( $p > 0.1$ ) (fig. 1a–d).

The OFICH positive ARs ( $n = 74$ ) were associated with oral itching in 16%, gastrointestinal symptoms (slight nausea, abdominal pain or diarrhea) in 19% and skin itching/activation of atopic eczema in 10%. No accompanying symptoms were recorded during the negative ARs ( $n = 13$ ).

**Table 4.** Agreement between OFICH, disease history and skin tests

|                       | History + skin + (n = 48) | History ± skin + (n = 3) | History + skin – (n = 36) | Total (n = 87) |
|-----------------------|---------------------------|--------------------------|---------------------------|----------------|
| OFICH (n = 87)        |                           |                          |                           |                |
| 74 positive responses | 43 (49)                   | 3 (4)                    | 28 (32)                   | 74 (85)        |
| 13 negative responses | 5 (6)                     | 0 (0)                    | 8 (9)                     | 13 (15)        |
| Total                 | 48 (55)                   | 3 (4)                    | 36 (41)                   | 87 (100)       |

Numbers in parentheses are percentages. + = Positive; ± = unknown; – = negative.

**Table 5.** Agreement between particular types of AR generated by OFICH and DBPCFC

| OFICH             | DBPCFC         |                |               |                |                   |
|-------------------|----------------|----------------|---------------|----------------|-------------------|
|                   | IAR (n = 17)   | LAR (n = 35)   | DAR (n = 8)   | DYAR (n = 15)  | Negative (n = 12) |
| IAR (n = 20)      | <b>17</b> (85) | 2              | 1             | 0              | 0                 |
| I = 15            | 13             | 2              | 0             | 0              | 0                 |
| II = 5            | 4              | 0              | 1             | 0              | 0                 |
| LAR (n = 33)      | 0              | <b>31</b> (94) | 1             | 0              | 1                 |
| I = 26            | 0              | 25             | 1             | 0              | 0                 |
| II = 7            | 0              | 6              | 0             | 0              | 1                 |
| DAR (n = 6)       | 0              | 0              | <b>5</b> (83) | 0              | 1                 |
| I = 5             | 0              | 0              | 4             | 0              | 1                 |
| II = 1            | 0              | 0              | 1             | 0              | 0                 |
| DYAR (n = 15)     | 0              | 1              | 0             | <b>14</b> (93) | 0                 |
| I = 13            | 0              | 1              | 0             | 12             | 0                 |
| II = 2            | 0              | 0              | 0             | 2              | 0                 |
| Negative (n = 13) | 0              | 1              | 1             | 1              | <b>10</b> (77)    |
| I = 12            | 0              | 1              | 1             | 1              | 9                 |
| II = 1            | 0              | 0              | 0             | 0              | 1                 |

Numbers in parentheses are percentages. I = group I ( $n = 71$ ); II = group II ( $n = 16$ ).

The 87 DBPCFC with milk demonstrated 75 positive ARs (86%) and 12 negative ARs (14%) (table 5). The 75 positive ARs included 17 IARs (20%;  $p < 0.01$ ), 35 LARs (40%;  $p < 0.001$ ), 8 DLARs (9%;  $p < 0.05$ ) and 15 DYARs (17%;  $p < 0.05$ ) (table 5; fig. 1a–d; online suppl. tables 1–4). The 71 asthmatics of the group I, suffering from inhalant allergen-related bronchial asthma, developed 61 (86%) positive ARs (13 IARs, 29 LARs, 6 DARs and 13 DYARs) and 10 (14%) negative ARs. The 16 patients of the group II, in whom the milk allergy was suspected to be the sole

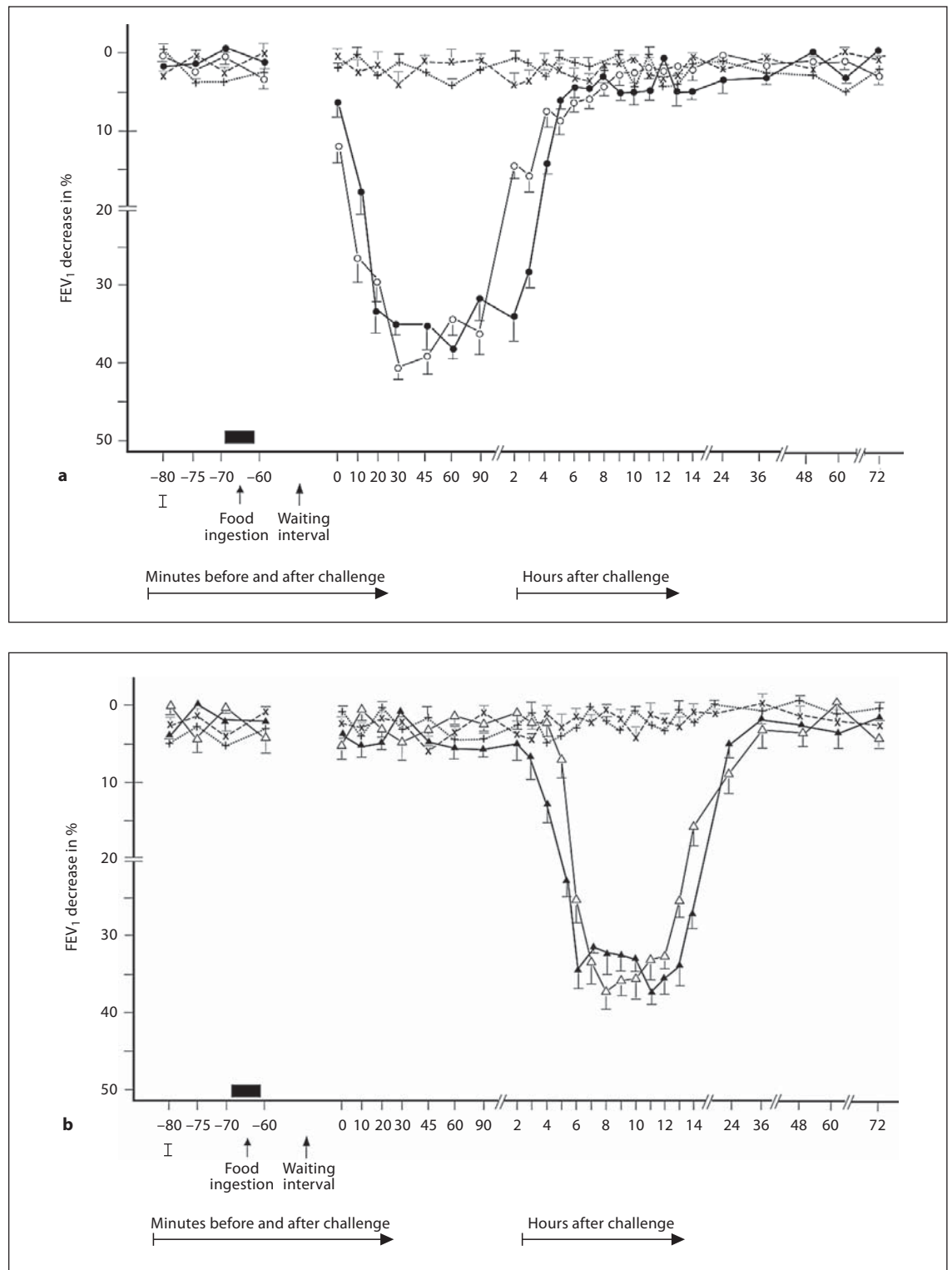


**Fig. 1. a** IAR to the milk ingestion challenge. The mean percentage changes in the FEV<sub>1</sub> values calculated from all patients with positive IAR. OFICH: IAR (○; n = 20), placebo (x; n = 20). DBPCFC: IAR (●; n = 17), placebo (+; n = 17). **b** LAR to the milk ingestion challenge. The mean percentage changes in the FEV<sub>1</sub> val-

ues calculated from all patients with positive LAR. OFICH: LAR (△; n = 33), placebo (x; n = 33). DBPCFC: LAR (▲; n = 35), placebo (+; n = 35). **a-d** I = Initial (baseline) values; food ingestion = OFICH or DBPCFC with milk; waiting interval = 1 h. Bars: means ± SEM.

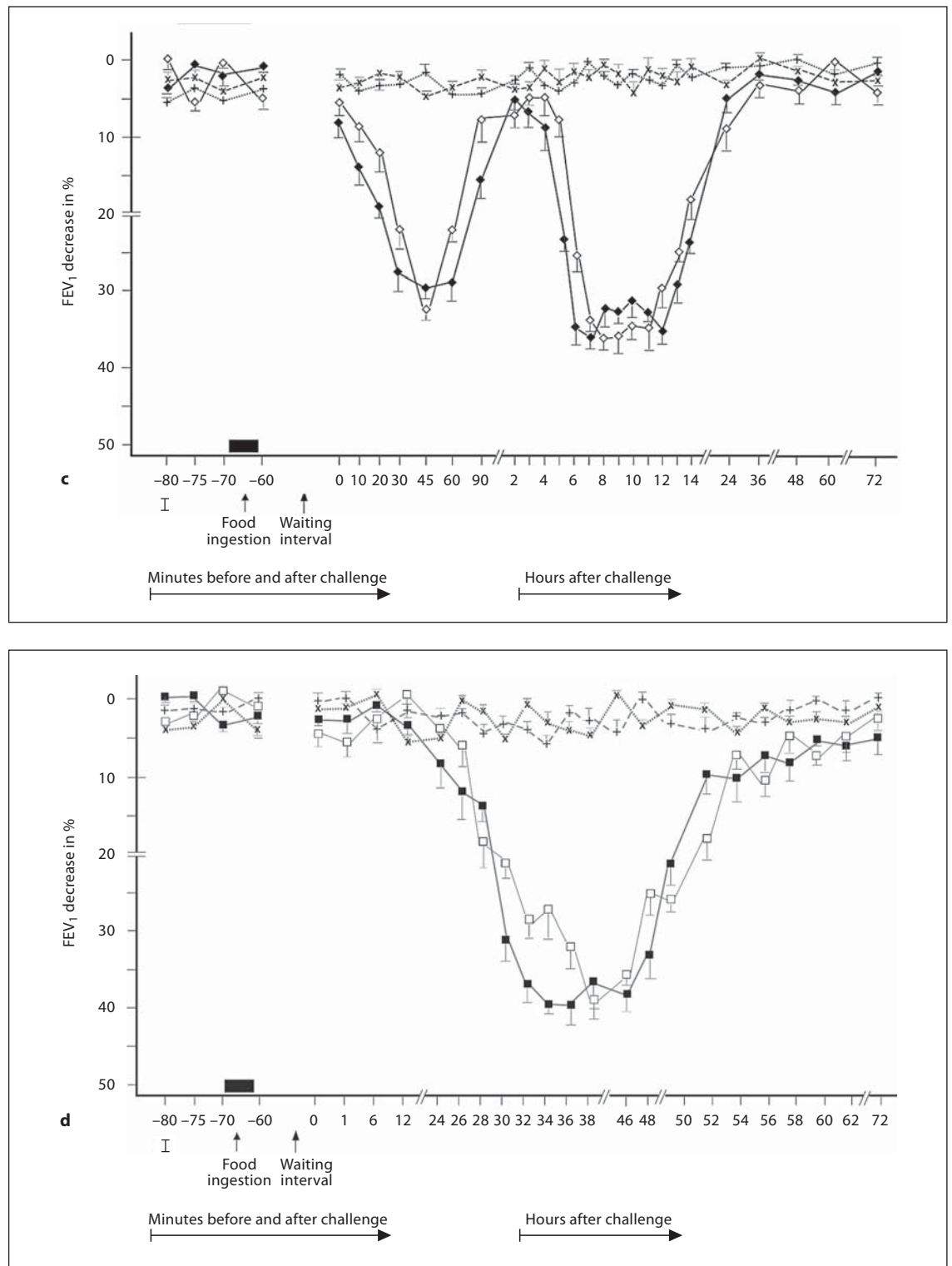


**Fig. 1.c** DAR to the milk ingestion challenge. The mean percentage changes in the FEV<sub>1</sub> values calculated from all patients with positive DAR. OFICH: DAR (◇; n = 6), placebo (x; n = 6). DBPCFC: DAR (◆; n = 8), placebo (+; n = 8). **d** DYAR to the milk ingestion challenge. The mean percentage changes in the FEV<sub>1</sub> val-

ues calculated from all patients with positive DYAR. OFICH: DYAR (□; n = 15), placebo (x; n = 15). DBPCFC: DYAR (■; n = 15), placebo (+; n = 15). **a-d** I = Initial (baseline) values; food ingestion = OFICH or DBPCFC with milk; waiting interval = 1 h. Bars: means ± SEM.

cause of their bronchial asthma, developed 14 (88%) positive ARs (4 IARs, 6 LARs, 2 DARs and 2 DYARs) and 2 (12%) negative ARs.

All DBPCFC placebo-control challenges were negative ( $p > 0.1$ ). DBPCFC positive ARs were accompanied by gastrointestinal complaints in 23% and skin itching/activation of atopic eczema in 12%.

The total agreement between the OFICH and DBPCFC results was 94%. The overall correlation of the OFICH and DBPCFC ARs was highly significant for both the positive ( $p < 0.01$ ) and the negative responses ( $p < 0.05$ ) (table 6). The particular AR types (table 5; fig. 1a–d; online suppl. tables 1–4) caused by OFICH and DBPCFC demonstrated the following statistical correlation: IAR (85%;  $p < 0.02$ ), LAR (94%;  $p < 0.01$ ), DAR (83%;  $p < 0.05$ ), DYAR (93%;  $p < 0.01$ ) and negative AR (77%;  $p < 0.01$ ).

#### Control Group

In the 15 control patients, all 7 OFICHs and 8 DBPCFCs with milk were negative ( $p > 0.1$ ).

## Discussion

The suspected involvement of food allergy, especially of cow's milk allergy, in adult patients with bronchial asthma remains underestimated. The diagnostic confirmation of its role of food allergy in the clinical manifestations is usually performed by means of disease history, skin tests and/or the determination of the food-specific IgE antibody in the serum. However, these tests demonstrate various degrees of correlation with the clinical manifestation of bronchial asthma and their prediction value does not appear to be completely satisfactory [1–8, 10, 25–29, 36–38, 42, 43, 51].

The involvement of food allergy can be confirmed conclusively only by means of the food ingestion challenge, demonstrating particular types of asthmatic response induced by certain food(s), which can be recorded quantitatively by means of objective functional parameters, such as lung function, in their dynamic course [2–4, 25–29, 32–34, 36, 37, 40, 41, 43–46, 50, 51].

The IAR due to the IgE-mediated food allergy has been most extensively investigated [1–11, 17, 23, 25, 26, 30, 32, 34, 36, 37, 40, 43]. The LAR to foods has also been reported, but its immunological mechanism has not yet been sufficiently clarified [2, 9, 10, 14, 17, 18, 23, 26, 27, 38]. In the DYAR to food challenge that we described previously [26, 27], which is analogical to the DYAR induced by inhalant allergens [49], an involvement of the cell-me-

**Table 6.** Overall correlation between OFICH and DBPCFC

| OFICH (n = 87)                                           | DBPCFC (n = 87)      |                      |       |
|----------------------------------------------------------|----------------------|----------------------|-------|
|                                                          | positive<br>(n = 75) | negative<br>(n = 12) | total |
| 74 positive OFICH                                        | <b>72**</b>          | 2                    | 74    |
| 13 negative OFICH                                        | 3                    | <b>10*</b>           | 13    |
| Total                                                    | 75                   | 12                   | 87    |
| Statistical significance: * $p < 0.05$ ; ** $p < 0.01$ . |                      |                      |       |

diated hypersensitivity mechanism could be presumed [13, 15, 16, 18–24, 26, 27, 49]. However, there is still a dearth of structural information concerning the immunological mechanisms underlying the particular food-induced AR types investigated in large groups of well-defined patients [1–3, 6–8, 17, 23, 25–27, 30, 37–39, 41, 42].

The results of this study, consistent with our previous observations [26, 27], demonstrate unequivocally the existence of 4 AR phenotypes, namely IAR, LAR, DAR and DYAR, due to food allergy, simulated by the food ingestion challenge. All these AR types were similarly detected both by the OFICH and by the DBPCFC techniques, without any significant differences. However, our results are not always in agreement with data in the literature, showing an unsatisfactory correlation between the OFICH and DBPCFC results. The DYAR to food ingestion challenge has also not been reported previously in the literature. The discrepancy may probably be explained by a number of factors, such as differences in patient populations, standard diagnostic procedures and measured diagnostic parameters, differences in the food challenge techniques, the materials (foods) tested and the practical experience of the laboratory staff, and finally by the length of the post-challenge period during which the relevant diagnostic parameters, in this case the lung function, had to be measured. In many studies, the post-challenge parameters were followed for a relatively short period of time, i.e. for one or a few hours, but according to our schedule the lung function parameters were routinely registered up to 72 h after the food challenge, applying both techniques, during the hospitalization of the patients.

Our results, together with findings by other investigators, suggest that the food ingestion challenge is a more reliable diagnostic test for the detection of possible AR to the food ingested than data obtained from single skin

tests or the determination of specific IgE antibody in the serum by means of ImmunoCAP [27, 34–37, 39–43, 51]. Moreover, our results (table 2, 4) also indicate that particular AR types to ingested milk cannot be predicted to a significant degree from the skin tests or estimation of the allergen-specific IgE in the serum.

However, the food ingestion challenges, besides their advantages, also have some limitations and contraindications, and require some special precautions [6, 8, 10, 26, 28, 32, 34, 36, 37, 39–43, 50, 52]. The absolute contraindications are suspected anaphylactic reactions to particular food(s), pregnancy, lung functions under 50% of the predicted values and all life-threatening disorders. The relative contraindications are conditions which can lead to any undesirable complications or which can distinctly influence the food challenge results, such as treatment with certain drugs. Food challenges where the vital organ functions should be monitored or serious clinical manifestations may be expected should be performed during the patient's hospitalization. The foods tested should be avoided for at least 2 weeks prior the challenge. The food challenges require a number of precautions, such as the careful evaluation of indications and contraindications, reliable materials and a provocation system, the monitoring of representative, if possible objective, parameters, resuscitation facilities and experienced staff under supervision by a physician. The food challenge can be performed by using the following techniques: (1) a DBPCFC, (2) an OFICH, alternatively combined with a placebo food challenge or (3) a single-blind food ingestion challenge [1–8, 10, 25–29, 32–34, 36, 37, 39–46, 50, 51]. The OFICH is a simple, less time-consuming method using natural, not specially processed, foods and the results are directly available. Moreover, during the natural food ingestion, all parts of the digestive system can participate in the processing of the food and in the food allergy mechanism(s) resulting finally in a clinical, organ-associated response. The OFICH is appropriate in cases where the objective parameters, such as lung function, can be monitored quantitatively, but it is not suitable in cases where only subjective parameters can be recorded or where the results can be influenced by the patient or investigator [2, 6–8, 10, 26, 28, 33, 36, 37, 39, 41–46].

The DBPCFC is required in cases where the particular organ-associated response can be measured and evaluated only by means of subjective parameters, such as itching, headache, tiredness, general malaise, gastrointestinal symptoms and in pharmacological studies [1–8, 10, 27, 28, 32, 36, 37, 39, 40, 42, 43, 50]. Nevertheless, the DBPCFC also has some disadvantages, i.e. (1) the pro-

cessing of the foods, required for concealment of their identity, such as color, taste and odor, can substantially change their physical and chemical properties, (2) production of a suitable placebo matching the tested foods may sometimes be a technical problem, (3) the number of capsules to be swallowed is relatively high, (4) the administration of food in capsules excludes the mouth and oesophagus, often being the first site of the reaction to foods and (5) the placebo itself can sometimes induce a false-positive reaction [1–8, 10, 27, 28, 32, 36, 37, 39, 40, 42, 43, 50].

The results of this study emphasize the importance of the food challenge for the diagnosis of food allergy in bronchial asthma patients. The definite confirmation of this role should be provided by a food ingestion challenge combined with the monitoring of lung function for a sufficiently long period of time (e.g. 36–48 h), demonstrating the particular AR types in their dynamic course. Regarding the above-listed results, our previous papers [28–31, 34, 41] and findings by other investigators [2, 6, 8, 33, 34, 36, 37, 41–46, 50, 51], the diagnostic value of the food ingestion challenge seems to be superior to other diagnostic parameters.

The significant overall correlation of the OFICH and DBPCFC results, found in this study, would suggest that in bronchial asthma, where the ARs can be measured by objective lung function, the DBPCFC is not superior to the OFICH.

In most of the patients studied, the inhaled glucocorticosteroids had been withdrawn before the study without any problems, which indicates that they suffered from mild to moderate asthma. The lack of patients with severe (burdensome) asthma who would not be able to interrupt the corticosteroid treatment can be regarded as a certain limitation of this study. It may also be notified that the majority of the positive ARs due to the OFICH as well as DBPCFC with cow's milk, recorded in the study (74%), were of the so-called 'non-immediate type' and different from the classical IgE-mediated ARs. It can be concluded that cow's milk allergy involved in some adult patients with bronchial asthma results in the appearance of various AR types, such as IAR, LAR, DAR and DYAR. The OFICH with natural cow's milk, combined with spirometry, may definitely confirm this involvement. The DBPCFC should be performed in cases in which the OFICH results are inconclusive.

Further studies are nevertheless necessary to clarify the exact immunological mechanisms underlying the individual AR types to food and milk allergy.

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**ON-LINE REPOSITORY (SUPPLEMENT)**

to “Asthmatic response to milk ingestion challenge in adults- A comparison of open and double-blind challenge” [Ms No.201206021]

**Table S 1. Immediate asthmatic response (IAR) to milk ingestion performed by OFICH and DBPCFC**FEV<sub>1</sub> values in liters per second (mean ± SEM) and their decrease with respect to the baseline, in %;

|        |                   | Baseline   | 1-hour<br>waiting<br>interval | After milk ingestion<br>minutes |           |           |           |           |           |           |
|--------|-------------------|------------|-------------------------------|---------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|
|        |                   |            |                               | 0                               | 10        | 20        | 30        | 45        | 60        | 90        |
| OFICH  | -FEV <sub>1</sub> | 3.27±0.11  |                               | 2.88±0.09                       | 2.42±0.15 | 2.32±0.14 | 1.92±0.06 | 2.02±0.11 | 2.13±0.10 | 2.09±0.12 |
|        | - %               |            |                               | 12                              | 26        | 29        | 41        | 38        | 35        | 36        |
| DBPCFC | -FEV <sub>1</sub> | 3.15± 0.14 |                               | 2.96±0.10                       | 2.58±0.14 | 2.11±0.12 | 2.05±0.07 | 2.05±0.14 | 1.98±0.07 | 2.14±0.09 |
|        | - %               |            |                               | 6                               | 18        | 33        | 35        | 35        | 37        | 32        |

|        |                   | After milk ingestion<br>hours |           |           |           |           |           |           |           |           |
|--------|-------------------|-------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|        |                   | 2                             | 3         | 4         | 5         | 6         | 7         | 8         | 9         | 10        |
| OFICH  | -FEV <sub>1</sub> | 2.78±0.07                     | 2.75±0.10 | 3.00±0.11 | 2.97±0.09 | 3.04±0.05 | 3.07±0.06 | 3.10±0.04 | 3.14±0.05 | 3.14±0.04 |
|        | - %               | 15                            | 16        | 8         | 9         | 7         | 6         | 5         | 4         | 4         |
| DBPCFC | -FEV <sub>1</sub> | 2.08±0.13                     | 2.27±0.09 | 2.70±0.05 | 2.96±0.06 | 2.99±0.07 | 2.99±0.04 | 3.05±0.05 | 2.99±0.08 | 2.99±0.10 |
|        | - %               | 34                            | 28        | 14        | 6         | 5         | 5         | 3         | 5         | 5         |

|        |                   | After milk ingestion<br>hours |           |           |           |            |           |           |           |           |
|--------|-------------------|-------------------------------|-----------|-----------|-----------|------------|-----------|-----------|-----------|-----------|
|        |                   | 11                            | 12        | 13        | 14        | 24         | 36        | 48        | 60        | 72        |
| OFICH  | -FEV <sub>1</sub> | 3.17±0.05                     | 3.17±0.03 | 3.17±0.04 | 3.17±0.07 | 3.24±0.0.3 | 3.17±0.04 | 3.17±0.03 | 3.17±0.05 | 3.14±0.05 |
|        | - %               | 3                             | 3         | 3         | 3         | 1          | 3         | 2         | 3         | 4         |
| DBPCFC | -FEV <sub>1</sub> | 3.02±0.05                     | 3.12±0.06 | 2.99±0.10 | 2.99±0.06 | 3.02±0.10  | 3.02±0.05 | 3.12±0.05 | 3.02±0.03 | 3.12±0.04 |
|        | - %               | 4                             | 1         | 5         | 5         | 4          | 4         | 1         | 4         | 1         |

**Table S 2. Late asthmatic response (LAR) to milk ingestion performed by OFICH and DBPCFC**FEV<sub>1</sub> values in liters per second (mean  $\pm$  SEM) and their decrease with respect to the baseline, in %

|        |                   | Baseline        | 1-hour<br>waiting<br>interval | After milk ingestion<br>minutes |                 |                 |                 |                 |                 |                 |
|--------|-------------------|-----------------|-------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|        |                   |                 |                               | 0                               | 10              | 20              | 30              | 45              | 60              | 90              |
| OFICH  | -FEV <sub>1</sub> | 3.31 $\pm$ 0.20 |                               | 3.14 $\pm$ 0.08                 | 3.27 $\pm$ 0.06 | 3.17 $\pm$ 0.05 | 3.14 $\pm$ 0.11 | 3.17 $\pm$ 0.06 | 3.23 $\pm$ 0.05 | 3.20 $\pm$ 0.05 |
|        | - %               |                 |                               | 5                               | 1               | 4               | 5               | 4               | 2               | 3               |
| DBPCFC | -FEV <sub>1</sub> | 3.08 $\pm$ 0.12 |                               | 2.95 $\pm$ 0.04                 | 2.92 $\pm$ 0.07 | 2.92 $\pm$ 0.06 | 3.02 $\pm$ 0.04 | 2.92 $\pm$ 0.03 | 2.89 $\pm$ 0.07 | 2.89 $\pm$ 0.04 |
|        | - %               |                 |                               | 4                               | 5               | 5               | 2               | 5               | 6               | 6               |

|        |                   | After milk ingestion<br>hours |                 |                 |                 |                 |                 |                 |                 |                 |
|--------|-------------------|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|        |                   | 2                             | 3               | 4               | 5               | 6               | 7               | 8               | 9               | 10              |
| OFICH  | -FEV <sub>1</sub> | 3.23 $\pm$ 0.06               | 3.20 $\pm$ 0.04 | 3.20 $\pm$ 0.08 | 3.07 $\pm$ 0.10 | 2.48 $\pm$ 0.14 | 2.18 $\pm$ 0.13 | 2.05 $\pm$ 0.10 | 2.11 $\pm$ 0.08 | 2.11 $\pm$ 0.13 |
|        | - %               | 2                             | 3               | 3               | 7               | 25              | 34              | 38              | 36              | 36              |
| DBPCFC | -FEV <sub>1</sub> | 2.92 $\pm$ 0.09               | 2.86 $\pm$ 0.15 | 2.67 $\pm$ 0.11 | 2.37 $\pm$ 0.13 | 2.03 $\pm$ 0.09 | 2.09 $\pm$ 0.05 | 2.06 $\pm$ 0.14 | 2.06 $\pm$ 0.10 | 2.03 $\pm$ 0.07 |
|        | - %               | 5                             | 7               | 13              | 23              | 34              | 32              | 33              | 33              | 34              |

|        |                   | After milk ingestion<br>hours |                 |                 |                 |                 |                 |                 |                 |                 |
|--------|-------------------|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|        |                   | 11                            | 12              | 13              | 14              | 24              | 36              | 48              | 60              | 72              |
| OFICH  | -FEV <sub>1</sub> | 2.21 $\pm$ 0.12               | 2.24 $\pm$ 0.07 | 2.48 $\pm$ 0.08 | 2.77 $\pm$ 0.11 | 3.04 $\pm$ 0.10 | 3.20 $\pm$ 0.09 | 3.20 $\pm$ 0.06 | 3.30 $\pm$ 0.05 | 3.17 $\pm$ 0.04 |
|        | - %               | 33                            | 32              | 25              | 16              | 8               | 3               | 3               | 0               | 4               |
| DBPCFC | -FEV <sub>1</sub> | 1.91 $\pm$ 0.09               | 1.97 $\pm$ 0.05 | 2.00 $\pm$ 0.09 | 2.24 $\pm$ 0.10 | 2.92 $\pm$ 0.07 | 3.02 $\pm$ 0.04 | 2.97 $\pm$ 0.06 | 2.95 $\pm$ 0.09 | 3.02 $\pm$ 0.04 |
|        | - %               | 38                            | 36              | 35              | 27              | 5               | 2               | 3               | 4               | 2               |

**Table S 3. Dual late asthmatic response (DLAR) to milk ingestion performed by OFICH and DBPCFC**  
 FEV<sub>1</sub> values in liters per second (mean ± SEM) and their decrease with respect to the baseline, in %

|                               |                   |           | 1-hour<br>waiting<br>interval | After milk ingestion<br>minutes |           |           |           |           |           |           |    |
|-------------------------------|-------------------|-----------|-------------------------------|---------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|----|
| Baseline                      |                   |           |                               | 0                               | 10        | 20        | 30        | 45        | 60        | 90        |    |
| OFICH                         | -FEV <sub>1</sub> | 3.38±0.26 |                               | 3.21±0.07                       | 3.10±0.09 | 2.74±0.11 | 2.64±0.10 | 2.26±0.08 | 2.67±0.09 | 3.14±0.13 |    |
|                               | -%                |           |                               | 5                               | 8         | 12        | 22        | 33        | 21        | 7         |    |
| DBPCFC                        | -FEV <sub>1</sub> | 3.35±0.17 |                               | 3.12±0.08                       | 2.88±0.10 | 2.71±0.05 | 2.44±0.08 | 2.38±0.04 | 2.41±0.11 | 2.84±0.10 |    |
|                               | -%                |           |                               | 7                               | 14        | 19        | 27        | 29        | 28        | 15        |    |
|                               |                   |           |                               |                                 |           |           |           |           |           |           |    |
| After milk ingestion<br>hours |                   |           |                               |                                 |           |           |           |           |           |           |    |
|                               |                   |           | 2                             | 3                               | 4         | 5         | 6         | 7         | 8         | 9         | 10 |
| OFICH                         | -FEV <sub>1</sub> | 3.17±0.08 | 3.24±0.05                     | 3.24±0.04                       | 3.14±0.10 | 2.54±0.09 | 2.23±0.08 | 2.16±0.06 | 2.19±0.10 | 2.23±0.07 |    |
|                               | - %               | 6         | 4                             | 4                               | 7         | 25        | 34        | 36        | 35        | 34        |    |
| DBPCFC                        | -FEV <sub>1</sub> | 3.19±0.07 | 3.15±0.09                     | 3.08±0.11                       | 2.57±0.05 | 2.21±0.08 | 2.14±0.05 | 2.27±0.09 | 2.24±0.06 | 2.31±0.07 |    |
|                               | - %               | 5         | 6                             | 8                               | 23        | 34        | 36        | 32        | 33        | 31        |    |
|                               |                   |           |                               |                                 |           |           |           |           |           |           |    |
| After milk ingestion<br>hours |                   |           |                               |                                 |           |           |           |           |           |           |    |
|                               |                   |           | 11                            | 12                              | 13        | 14        | 24        | 36        | 48        | 60        | 72 |
| OFICH                         | -FEV <sub>1</sub> | 2.19±0.14 | 2.26±0.10                     | 2.54±0.06                       | 2.46±0.10 | 3.10±0.12 | 3.28±0.05 | 3.24±0.06 | 3.37±0.03 | 3.24±0.05 |    |
|                               | - %               | 35        | 33                            | 25                              | 17        | 8         | 3         | 4         | 0         | 4         |    |
| DBPCFC                        | -FEV <sub>1</sub> | 2.24±0.04 | 2.17±0.05                     | 2.34±0.08                       | 2.55±0.05 | 3.18±0.07 | 3.26±0.03 | 3.22±0.02 | 3.20±0.04 | 3.26±0.02 |    |
|                               | - %               | 33        | 35                            | 30                              | 24        | 5         | 2         | 3         | 4         | 2         |    |

**Table S 4. Delayed asthmatic response (DYAR) to milk ingestion performed by OFICH and DBPCFC**FEV<sub>1</sub> values in liters per second (mean ± SEM) and their decrease with respect to the baseline, in %;

|                               |                   |           | 1-hour<br>waiting<br>interval | After milk ingestion<br>hours |           |           |           |           |           |           |    |
|-------------------------------|-------------------|-----------|-------------------------------|-------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|----|
| Baseline                      |                   |           |                               | 0                             | 1         | 2         | 4         | 6         | 8         | 12        |    |
| OFICH                         | -FEV <sub>1</sub> | 3.16±0.19 |                               | 3.03±0.07                     | 3.00±0.08 | 3.06±0.06 | 2.87±0.10 | 3.06±0.11 | 2.97±0.07 | 3.16±0.09 |    |
|                               | -%                |           |                               | 4                             | 5         | 3         | 9         | 3         | 6         | 0         |    |
| DBPCFC                        | -FEV <sub>1</sub> | 3.24±0.22 |                               | 3.14±0.02                     | 3.14±0.06 | 3.05±0.05 | 2.98±0.07 | 3.20±0.03 | 3.01±0.10 | 3.11±0.05 |    |
|                               | -%                |           |                               | 3                             | 3         | 6         | 8         | 1         | 7         | 4         |    |
|                               |                   |           |                               |                               |           |           |           |           |           |           |    |
| After milk ingestion<br>hours |                   |           |                               |                               |           |           |           |           |           |           |    |
|                               |                   |           | 24                            | 26                            | 28        | 30        | 32        | 34        | 36        | 38        | 46 |
| OFICH                         | -FEV <sub>1</sub> | 3.03±0.03 | 3.00±0.11                     | 2.59±0.12                     | 2.49±0.05 | 2.28±0.06 | 2.31±0.13 | 2.15±0.11 | 1.96±0.07 | 2.05±0.04 |    |
|                               | - %               | 4         | 5                             | 18                            | 21        | 28        | 27        | 32        | 38        | 35        |    |
| DBPCFC                        | -FEV <sub>1</sub> | 2.98±0.14 | 2.85±0.14                     | 2.78±0.07                     | 2.20±0.10 | 2.04±0.08 | 1.98±0.04 | 1.98±0.07 | 2.07±0.12 | 2.04±0.06 |    |
|                               | - %               | 8         | 12                            | 14                            | 32        | 37        | 39        | 39        | 36        | 37        |    |
|                               |                   |           |                               |                               |           |           |           |           |           |           |    |
| After milk ingestion<br>hours |                   |           |                               |                               |           |           |           |           |           |           |    |
|                               |                   |           | 48                            | 50                            | 52        | 54        | 56        | 58        | 60        | 62        | 72 |
| OFICH                         | -FEV <sub>1</sub> | 2.37±0.09 | 2.33±0.05                     | 2.62±0.09                     | 2.94±0.05 | 2.81±0.07 | 3.00±0.08 | 2.94±0.03 | 3.00±0.06 | 3.06±0.03 |    |
|                               | - %               | 25        | 26                            | 17                            | 7         | 11        | 5         | 7         | 5         | 3         |    |
| DBPCFC                        | -FEV <sub>1</sub> | 2.14±0.10 | 2.56±0.08                     | 2.92±0.05                     | 2.88±0.10 | 3.01±0.05 | 2.98±0.07 | 3.08±0.02 | 3.05±0.05 | 3.08±0.05 |    |
|                               | - %               | 34        | 21                            | 10                            | 11        | 7         | 8         | 5         | 6         | 5         |    |