

# Most children who are allergic to cow's milk tolerate yogurt

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## Abstract

**Objective:** Cow's milk allergy is the most common food allergy in childhood. Changes occur in the protein structure of milk during yogurt fermentation. This study aimed to determine whether children who are diagnosed with a cow's milk allergy can tolerate yogurt.

**Methods:** We performed a yogurt challenge test on 34 children who were diagnosed with a cow's milk allergy in our Pediatric Allergy Outpatient Clinic. The mean age of 24 male and 10 female children was  $24 \pm 13$  months.

**Results:** A reaction was observed in 17 (50%) patients, whereas no reaction was observed in the other 17 (50%) during an oral yogurt challenge test that was performed in all of the 34 patients with a cow's milk allergy. Cow's milk-specific immunoglobulin E levels were significantly lower in the group of children who could tolerate yogurt than in the group of children who could not tolerate yogurt.

**Conclusion:** Yogurt is tolerated by half of children with a cow's milk allergy when subjected to a challenge test performed with yogurt, which is consumed as much as milk in Turkey.

## Keywords

Cow's milk allergy, specific immunoglobulin E (IgE), skin prick test, yogurt tolerance, children, challenge test

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## Introduction

Cow's milk allergy is the most common food allergy in childhood, with a prevalence of 2% to 3%.<sup>1,2</sup> Cow's milk allergy is the most commonly encountered food allergy during the first year of life.<sup>3</sup> Among children with a cow's milk allergy, 80% tolerate

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cow's milk at approximately 3 years old.<sup>4</sup> Studies on cow's milk allergy in Turkey have reported that the prevalence of this allergy varies between 0.16% and 1.55%.<sup>5-7</sup> The oral food challenge test is the main method for showing the presence or absence of cow's milk allergy.<sup>8,9</sup> Children with a cow's milk allergy are allergic to more than one type of milk protein. Cow's milk proteins consist of casein and whey proteins.<sup>10</sup> Milk proteins comprise 80% casein and 20% whey. Both protein fractions have five major components each. Alpha 1 casein and beta-casein comprise 70% of the casein fraction. Beta-lactoglobulin constitutes 50% of whey proteins and it is not present in human milk. In recent studies, immunoglobulin E (IgE)-binding sections were shown for the epitopes of allergic areas in food.<sup>10</sup> There are two epitopes in food; conformational epitopes and linear epitopes. Allergic reactions to all food types occur in patients with allergies associated with specific IgE for linear epitopes. Conformational epitopes change the tertiary structure of the protein as a result of heat/hydrolysis and tolerance to food can develop by processes, such as cooking and heating.<sup>10</sup> Boiling milk for 10 minutes reduces the skin prick test response in patients who develop reactions to bovine serum albumin and beta-lactoglobulin.<sup>10</sup> Furthermore, boiling milk at a high temperature (at 121°C for 20 minutes) can change the allergic quality. Therefore, some children who are diagnosed with a cow's milk allergy can tolerate products, such as cake and cookies, that are made with cooked milk.<sup>11,12</sup> Studies have also shown that children who are fed with cooked dairy products can tolerate these foods at a high level.<sup>11,13</sup>

In Turkey, yogurt is consumed as much as milk. The word "yoğurt" is Turkish in origin. This study aimed to identify, by using a food challenge test, whether children who are allergic to cow's milk protein can tolerate yogurt, which is made of cow's

milk, albeit with changes to the protein structure during fermentation.

## Methods

### Subjects

Thirty-four children who were found to have a cow's milk allergy by skin prick test, milk-specific IgE levels, a cow's milk challenge test, and a cow's milk allergy test were included in the study. The study period was between March and September 2015 in the Department of Pediatric Allergy and Immunology, Gaziantep University. A hemogram, IgE measurement, skin prick test, cow's milk-specific IgE measurement, skin prick test with natural milk and yogurt, and a cow's milk food challenge test were repeated for all children. Children who showed allergic symptoms, such as urticaria, eczema, itching, diarrhea, vomiting, wheezing, and conjunctivitis, as positive findings in the oral food challenge test performed with cow's milk were included in the study. Patients with a history of anaphylaxis due to cow's milk were not included in the study. The food challenge test was performed with yogurt in patients with a final diagnosis of cow's milk allergy 15 days after the cow's milk challenge test. Approval for this study was obtained from Gaziantep University Clinical Research Ethics Committee (reference number: 2015/86). Relevant written consent was received from the parents of the children.

### *IgE and specific IgE measurements*

Using the chemiluminescence method with the Immulite 2000 XPI commercial kit (Siemens, Erlangen, Germany), total IgE, cow's milk-specific IgE, and Food Panel 1 (cow's milk, egg white, soya, wheat, peanut, morina fish) were tested in serum samples of patients. Results were obtained in kU/L

units, and values that were  $\geq 0.35$  kU/L were considered to be positive.

### **Skin prick test**

A skin prick test was performed in all of the patients to identify their atopies, and their sensitivity to milk, natural milk, and natural yogurt was measured. Antihistamine use was prohibited for 10 days before the skin test. This test was applied on the volar forearm, face, and back area of small children, using the Quintest Multiple Test skin test device (Hollister Stier Company, Spokane, WA, USA). Allergens produced by ALK Company (Hørsholm, Denmark) were used as the skin prick test solutions. For skin prick testing, natural milk and yogurt products of SEK Company (Süt Endüstrisi Kurumu, Istanbul, Turkey) were used. A volume of 10 mg/mL histamine and physiological saline solution were administered as a positive control and negative control, respectively. Reactions in the application area were evaluated 20 minutes later. A mean induration diameter of  $\geq 3$  mm was interpreted to be positive.

Children with a mean skin prick test diameter  $>3$  mm or a cow's milk-specific IgE level  $>0.35$  kU/L were considered as having IgE-mediated cow's milk allergy, and others were considered as having non-IgE cow's milk allergy.

### **Challenge tests**

We performed yogurt challenge tests in children who were positive for the cows' milk challenge test. The oral milk challenge test was repeated in patients who were on milk elimination and follow-up for cow's milk allergy, and persistence of cow's milk allergy was confirmed. The children were put on a milk and dairy product elimination diet 15 days before the test. Families were informed about which foods contained milk and dairy products. Children who

were fed with milk were fed with amino-acid based formulas during the elimination diet. Antihistamine use was prohibited for 10 days before the test. All of the patients underwent a detailed medical examination before the test. Patients with findings that suggested infection and rashes were treated before the test and the test was performed after the complaints completely disappeared. The test was started with 0.1 cc (3 mg), and then 0.3 cc (10 mg), 0.9 cc (30 mg), 3 cc (100 mg), 9.1 cc (300 mg), 30 cc (1000 mg), and 90 cc (3000 mg) SEK milk was provided at intervals of 15 minutes. The test was then concluded.<sup>14</sup> Patients with findings, such as dermatitis, eczematous rash, urticaria, wheezing, vomiting, and conjunctivitis, were regarded as positive. The duration of the reactions of these patients with positive findings was recorded, and the test was considered positive and ended.

Fifteen days later, the yogurt challenge test was performed in patients who had a positive milk challenge test. Similar to before the milk challenge test, patients underwent a detailed medical examination, and those with positive findings in the examination were treated first in accordance with their symptoms. Patients were questioned about whether they complied with the milk elimination diet. Similar to the milk challenge test, SEK yogurt was provided in logarithmically incremental doses. Patients with findings, such as dermatitis, eczematous rash, urticaria, wheezing, vomiting, and conjunctivitis, were interpreted as positive. The amount of yogurt (in cc) that resulted in a reaction was recorded. The test was started with 0.1 cc (3 mg), and then 0.3 cc (10 mg), 0.9 cc (30 mg), 3 cc (100 mg), 9.1 cc (300 mg), 30 cc (1000 mg), and 90 cc (3000 mg) SEK yogurt was provided at intervals of 15 minutes. The test was then concluded.<sup>14</sup> The duration of the reactions of these patients with positive findings was recorded,

and the test was considered positive and ended.

### Statistical analysis

The SPSS 22.0 package program (IBM Corp., Armonk, NY, USA) was used in statistical analysis of data. Descriptive statistics methods were used. Additionally, the chi-square test was used for comparison of categorical variables. Differences between numerical variables were tested with the Student's *t* test, the Mann–Whitney *U* test, and Pearson correlation test. A *p* value < 0.05 was considered to indicate a statistically significant difference.

### Results

In this study, we evaluated 34 children with cow's milk allergy. When we questioned the children about symptoms of cow's milk allergy, 34 had symptoms of wheezing, 10 had wheezing, 22 had atopic dermatitis, and four had gastrointestinal findings (vomiting, diarrhea). Two of these children had atopic dermatitis accompanied by wheezing.

Cow's milk-specific IgE levels < 0.35 kU/L and a mean diameter < 3 mm in the cow's milk skin test were found in five (14.7%) of the 34 patients. Therefore, these children were considered to have non-IgE cow's milk allergy. During the cow's milk challenge test, 10 (29.4%) patients had wheezing, 22 (58.9%) had skin findings (itchy rash, urticaria), and four (11.7%) had gastrointestinal symptoms (abdominal pain, diarrhea, vomiting). When the yogurt challenge test was performed in all patients who had a positive cow's milk challenge test, half of the children (17/34) tolerated yogurt. During the yogurt challenge test, we observed no reaction in 17 patients, while nine (26.4%) had urticaria, seven (20.5%) had wheezing or a cough, and one (2.9%) had vomiting and diarrhea. The children were divided into two groups of those who could and those who could not

tolerate yogurt. There were no significant differences in sex, presence of IgE-mediated cow's milk allergy, history of breastfeeding by the mother, yogurt consumption, atopic dermatitis history, and the prevalence of urticaria caused by cow's milk, wheezing caused by cow's milk, nausea and vomiting caused by cow's milk, and conjunctivitis caused by cow's milk between these two groups. There were also no significant differences in allergic diseases and use of preventive medication for asthma in the family between the groups (Table 1).

There were no significant differences in age, age of diagnosis, duration of breastfeeding, total IgE, eosinophil count, mean diameter in the skin test with commercial milk, and Food 1 panel-specific IgE between children who tolerated and those who did not tolerate yogurt (Table 2).

In evaluation of the cow's milk skin test that was performed with natural milk, the mean skin test diameter in children who tolerated yogurt was smaller than that in those who could not tolerate yogurt (*p* = 0.03). In evaluation of the cow's milk skin test performed with natural yogurt, the mean skin test diameter in children who tolerated yogurt was smaller than that in those who could not tolerate yogurt (*p* = 0.033). Cow's milk-specific IgE levels were lower in children who tolerated yogurt than in those who could not tolerate yogurt (*p* = 0.023) (Table 2).

### Discussion

The number of people with cow's milk allergy is continuously increasing, and thus it remains a problem. Total avoidance of milk consumption, the current prevailing treatment, involves some difficulties. Recent studies have shown that such a restriction may not be necessary.<sup>15,16</sup> Allergenic characteristics of proteins in food can undergo changes during food processing. Allergenic characteristics of

**Table 1.** Clinical characteristics of the children included in the study.

	Could not tolerate yogurt (n = 17)	Could tolerate yogurt (n = 17)	p*
Male sex	11 (64.7)	13 (76.5)	0.45
IgE-mediated cow's milk allergy	15 (88.2)	14 (82.4)	0.62
Breastfeeding	5 (35.3)	8 (47.1)	0.48
Presence of yogurt consumption	7 (41.2)	10 (58.8)	0.30
History of atopic dermatitis	10 (58.8)	12 (70.6)	0.47
Urticaria caused by cow's milk	9 (52.9)	10 (58)	0.73
Wheezing caused by cow's milk	5 (29.4)	4 (23.5)	0.69
Nausea and vomiting caused by cow's milk	7 (41.2)	4 (23.5)	0.27
Conjunctivitis caused by cow's milk	1 (5.9)	3 (17.5)	0.28
Allergic disease in family	10 (58.8)	12 (70.6)	0.47
Use of preventive medication for asthma	7 (41.2)	3 (17.6)	0.13

\*Analyzed by the chi-square test. Values are n (%). IgE: immunoglobulin E.

**Table 2.** Comparison of children who could and those who could not tolerate yogurt.

	All cases (n = 34)	Could not tolerate yogurt (n = 17)	Could tolerate yogurt (n = 17)	p
Mean age (months)	24 ± 13	22 ± 14	26 ± 12	0.398*
Age at diagnosis age (months)	12 ± 8	12.2 ± 9.6	12 ± 6	0.951*
Duration of breastfeeding (months)	10 ± 4	9 ± 3	11 ± 5	0.213*
Total IgE (Ku/L)	219 ± 199	187 ± 162	252 ± 233	0.358*
Eosinophil count	417 ± 339	425 ± 303	409 ± 384	0.895*
Commercial milk skin test (mean diameter in mm)	4.1 ± 3.3	5.0 ± 3.6	3.2 ± 2.9	0.113*
Natural milk skin test (mean diameter in mm)	4.6 ± 3.8	6.0 ± 4.2	3.2 ± 2.8	<b>0.030*</b>
Natural yogurt skin test (mean diameter in mm)	3.3 ± 3.0	4.5 ± 3.3	2.2 ± 2.4	<b>0.033*</b>
Food I (Ku/L)	8.7 ± 19.8	13.0 ± 24.7	4.2 ± 6.1	0.191*
Cow's milk-specific IgE (Ku/L)	1.7 (0.4–15.9)	12.9 (9.2–24)	1.7 (0.4–15.5)	<b>0.023**</b>

Values are mean ± standard deviation or median (25%–75%).\*t test (mean ± standard deviation); \*\*Mann–Whitney U test (median [25%–75%]). Bold values indicate p < 0.05. IgE: immunoglobulin E.

many proteins diminish with heating to high temperatures. This could be caused by changes in protein structures as a result of the loss of conformational epitopes at high temperatures.<sup>17</sup> An example of this phenomenon is the birch tree pollen allergen Bet v 1, which leads to cross-reactivity

with apple protein allergen (Mal d 1) and carrot (Dau c) allergens, causing oral allergy syndrome. However, these allergenic characteristics of apples and carrots disappear when they are cooked.<sup>18</sup> Previous studies have shown that allergenic characteristics of cow's milk are reduced, but do

not completely disappear, when heated.<sup>19</sup> Casein and alpha-lactoglobulin are more resistant against a high level of heat compared with other cow's milk proteins such as whey proteins, beta-lactoglobulin, and serum albumin.<sup>20</sup> A previous study showed that allergenic characteristics of beta-lactoglobulin, a cow's milk protein, are eliminated by high temperatures. This is because conformational epitopes of this protein are not resistant against heat, while the allergenic characteristics of casein, which has heat-resistant linear epitopes<sup>21</sup>, do not disappear at high temperatures.

Our study showed that half of the children who had been diagnosed with a cow's milk allergy tolerated yogurt. This was shown by a food challenge test that we performed with yogurt, which is derived from fermented milk and is commonly consumed in Turkey. In this study, we found that yogurt tolerance was lower in children with high cow's milk-specific IgE levels. We also found that children with a larger allergy test diameter during the skin allergy test performed with natural milk and yogurt showed tolerance for yogurt.

A previous study that was performed in the field of dairy technology showed that the allergenicity of whey protein was significantly decreased in yogurt that was produced from milk fermentation with the bacterium *Lactobacillus*.<sup>22</sup> Another study showed that in yogurt produced by milk fermentation with *Lactobacillus* bacterium, allergenicity of beta-lactoglobulin was remarkably reduced.<sup>23</sup> In a recent study, allergenic characteristics of whey protein, as well as casein protein, were also decreased as a result of milk fermented by *Lactobacillus casei*.<sup>24</sup> In a study performed in Turkey, a baked milk and yogurt challenge test was performed in children with cow's milk allergy who were aged younger than 2 years, and two thirds of the children tolerated yogurt.<sup>25</sup> More than half of the children tolerated baked milk. In another

previous study performed on children with cow's milk allergy, 75% developed a tolerance for heated milk after a food challenge test that was performed with overheated cow's milk. Our study also showed that development of tolerance was significantly lower in children with a high level of cow's milk-specific IgE. Development of tolerance was also reported to be significantly lower in children with a larger allergy skin test diameter.<sup>26</sup>

This study has several limitations. One limitation is the selection of patients and safety. Although a stepwise approach is considered safer for patients, we did not use this approach in this study.<sup>27</sup> A cross-sectional study was performed in a small number of cases because only 34 patients were followed in our clinic in the study period. Another limitation is that we were unable to include patients with anaphylaxis. Not including patients with a history of anaphylaxis may have slightly lowered the proportion of children who tolerated yogurt.

Our study shows that children with a larger diameter of swelling following the allergy skin test with natural milk and yogurt tolerate yogurt less than children with a small diameter of swelling following this skin test. Our study suggests that yogurt, which is consumed as much as milk in Turkey, is well tolerated by children with a cow's milk allergy when subjected to a challenge test with yogurt.

### **Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

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