



Skin testing with ultra-heat-treated (UHT) cow's milk in children with cow's milk allergy



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ABSTRACT

Background: A large proportion of cow's milk (CM)-allergic children are able to tolerate extensively heated forms of CM such as baked goods. Little is known about whether ultra-heat-treated (UHT) forms of cow's milk are immunologically similar to extensively heated cow's milk and therefore may be tolerated by these children.

Objective: To determine whether skin test wheal size using UHT CM was significantly different from other forms of CM and CM extracts.

Methods: Children presenting for oral food challenges with either extensively heated or unheated cow's milk underwent skin prick test (SPT) to commercial CM, UHT CM, evaporated CM, and fresh whole CM. The results were compared between groups of children.

Results: At study exit, only 14% of children were avoiding all forms of CM, compared with 70% at study entry. No difference was seen in the mean SPT results for UHT CM between those children that could tolerate heated CM compared with those that could not. The mean SPT result for casein was significantly lower in those that could tolerate heated CM. However, within the group of heated milk-tolerant children, the mean SPT for UHT CM was significantly lower than the SPT for fresh whole CM.

Conclusion: Ultra-heat-treated CM does not behave significantly differently from other forms of CM when evaluated by SPT in heated milk-allergic vs heated milk-tolerant children. This suggests that UHT CM is not sufficiently immunologically different from unheated CM to be tolerated by heated CM-tolerant children.

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Introduction

Cow's milk allergy is very common, affecting between 2% and 3%, and in some reports up to 7%, of young infants and children.¹ It has been found that approximately 75% of cow's milk-allergic children are able to tolerate heated cow's milk, such as in baked goods, while remaining reactive to unheated milk.^{2–4} In addition, regular consumption of heated cow's milk may hasten the development of tolerance to unheated cow's milk in a proportion of cow's milk-allergic children.⁵

Heating cow's milk to high temperatures for a prolonged period is associated with a reduction in allergenicity of cow's milk proteins, mainly because of a disruption of conformational

immunoglobulin E (IgE) epitopes.⁶ In addition, the heating of cow's milk to high temperatures for very short periods is a well-established practice designed to improve its safety and durability. Pasteurization involves heating milk to 72°C for 15 seconds, followed by rapid cooling. Ultra-heat-treated (UHT) cow's milk is produced when raw cow's milk is exposed to a temperature of at least 135°C for 1 to 2 seconds to kill bacterial and fungal spores. Evaporated milk (EM) is fresh milk from which 60% of the water has been removed; after the water has been removed, it is packaged and sterilized at 115 to 118°C for 15 minutes.^{7,8} These processes also allow for storage of the unopened milk container unrefrigerated for prolonged periods until use. Evaporated milk may then be diluted with water and used as a substitute for pasteurized whole milk, whereas UHT milk is usually used undiluted.

Anecdotally we have come across cases in which families have been advised by their health practitioner to introduce UHT cow's milk rather than whole pasteurized cow's milk when weaning babies at approximately 12 months of age. Presumably this advice is given with the belief that UHT cow's milk is hypoallergenic and

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therefore that this practice may reduce their risk of developing cow's milk allergy. Perhaps of more concern, we are aware of some cow's milk–allergic children that have been advised by their treating physicians to ingest UHT milk as a substitute for unheated cow's milk. However, neither are we aware of any cases of cow's milk–allergic children safely tolerating UHT cow's milk nor do there seem to be any such cases reported in the medical literature. We are not aware of any studies examining whether UHT cow's milk may be safely tolerated by extensively heated milk–tolerant children or indeed whether the cow's milk–specific IgE of these children is less likely to bind to protein epitopes in UHT cow's milk in a similar fashion to more extensively heated forms of cow's milk. We therefore hypothesized that if UHT cow's milk, or other forms of extensively heated cow's milk such as evaporated milk, are indeed safe substitutes for pasteurized cow's milk in heated milk–tolerant, unheated cow's milk–allergic children, there would be a significant difference in the skin prick test (SPT) reactivity to these different forms of milk between these groups of children.

This study compares the results of SPT with various forms of cow's milk, including UHT and evaporated cow's milk, between children who reacted to all forms of cow's milk, those that tolerated both heated and unheated cow's milk, and those that tolerated only heated cow's milk. Our aim was to determine whether these forms of processed cow's milk are more immunologically similar to cow's milk heated by more conventional methods, such as baking for a prolonged period, vs unheated cow's milk.

Methods

Between April 2012 and October 2016, we recruited children with cow's milk allergy into our study. Our institution is a tertiary paediatric referral centre, located in Sydney, Australia. Potential participants were identified either at their allergy outpatient clinic appointment or on booking their oral food challenge. Postal and telephone contact was initiated before the oral food challenge (OFC) to inform families of the study. If they chose to participate, the child's parents gave written informed consent for their child's participation in the study, accompanied by a written informed assent for older children. This project was approved by the Sydney Children's Hospitals Network Human Research Ethics Committee (HREC approval number 11/SCHN/220).

At baseline, we recorded patient characteristics, including sex, age at recruitment, age at first cow's milk reaction, history of atopy (asthma, eczema, allergic rhinitis), and history of prescription of an adrenaline autoinjector for cow's milk allergy. We also recorded the nature of any concomitant food allergies and the level of avoidance of cow's milk, such as tolerating heated cow's milk but avoiding unheated cow's milk.

The inclusion criteria were 1) a history of reacting to unheated cow's milk, referred for assessment of current allergy to either unheated or heated cow's milk; and 2) a positive SPT (wheal size >3 mm) to commercial cow's milk extract.

Children who tolerated unheated cow's milk and children with no known prior reactions to cow's milk were excluded from the study. All eligible participants underwent SPT to commercial whole cow's milk extract and commercial casein extract (Stallergenes, France), ultra-heated (UHT) milk (Devondale), evaporated milk (Nestle), and unheated whole cow's milk (Paul's Full Cream Milk), as well as histamine and normal saline positive and negative controls. Skin prick tests were performed on the child's volar forearm with a lancet, using the prick and lift technique. The SPTs were measured 15 minutes later: the size of the longest diameter and its longest perpendicular were recorded and averaged. The SPTs were performed by experienced allergy nurses or the lead investigators.

Challenge protocols and muffin recipes used are available on the website of the Australasian Society for Clinical Immunology and Allergy (ASCIA: www.allergy.org.au). These protocols call for the child to ingest between 1 and 2 muffins or up to 161 mL pasteurized cow's milk. Each muffin contained 21 g cow's milk (approximately 700 mg cow's milk protein), although 161 mL whole cow's milk contains 5.4 g cow's milk protein. Parents baked the muffins themselves at home, and only muffins that were thoroughly cooked through according to the baking instructions were accepted. All children included in this study were referred for a challenge at the discretion of the treating physician after a routine assessment at the outpatient allergy clinic. Therefore, not all children referred for an unheated cow's milk challenge were known to tolerate heated milk before the challenge, because that was not a requirement for referral for an unheated cow's milk challenge. Children who passed a heated cow's milk challenge and re-presented to the clinic for evaluation of unheated cow's milk tolerance during the study period were eligible to be re-recruited into the study as separate cases. Similarly, children who were deemed appropriate for an initial unheated cow's milk challenge and failed were counted as separate cases if they returned for a heated cow's milk challenge. At oral food challenge, we recorded the amount of whole unheated cow's milk or muffin tolerated, as well as the severity of any reactions and the treatment required. Children were placed in 3 different groups based on their oral food challenge outcome (Fig 1):

- Group 1 included children who were unable to tolerate cow's milk in any form (ie, those who failed the heated cow's milk challenge).
- Group 2 included children who tolerated heated but not unheated cow's milk.
- Group 3 included children who tolerated unheated cow's milk (ie, those that passed an unheated whole milk challenge).

Statistics

One-way analysis of variance and χ^2 tests were used to compare the demographics between groups. The mean of the SPTs for the various forms of cow's milk were compared between groups, using a Student's *t* test. Next, the mean of the SPT for the various forms of cow's milk was compared with the mean of the SPT for UHT milk and separately with the mean of the SPT to whole fresh cow's milk within group 1 and within group 2, using a single-sample Student's *t* test. SPSS Version 24 was used for the analysis.

Results

Patient Demographics

One hundred two children were enrolled in the study. The mean age at study entry was 4.98 years (± 3.32), and 61.8% were male. At study entry, 30% of subjects overall were avoiding unheated forms of milk only, whereas the rest were avoiding all milk. Significantly more children who ended up in group 3 were avoiding unheated milk only at study entry compared with the other groups, whereas significantly more that ended up in group 2 avoided all heated and unheated cow's milk at study entry ($P < .001$). Seven percent of the cohort overall avoided products with precautionary labeling statements. High rates of atopy were seen at baseline, with asthma diagnosed in 54% of our cohort, eczema in 74%, and allergic rhinitis in 31%. An adrenaline autoinjector had been prescribed in 82% of patients, although not always as a result of their cow's milk allergy. Most (88.1%) had at least 1 other food allergy, with the highest proportions being documented as egg 70%, tree nuts 59%, peanut

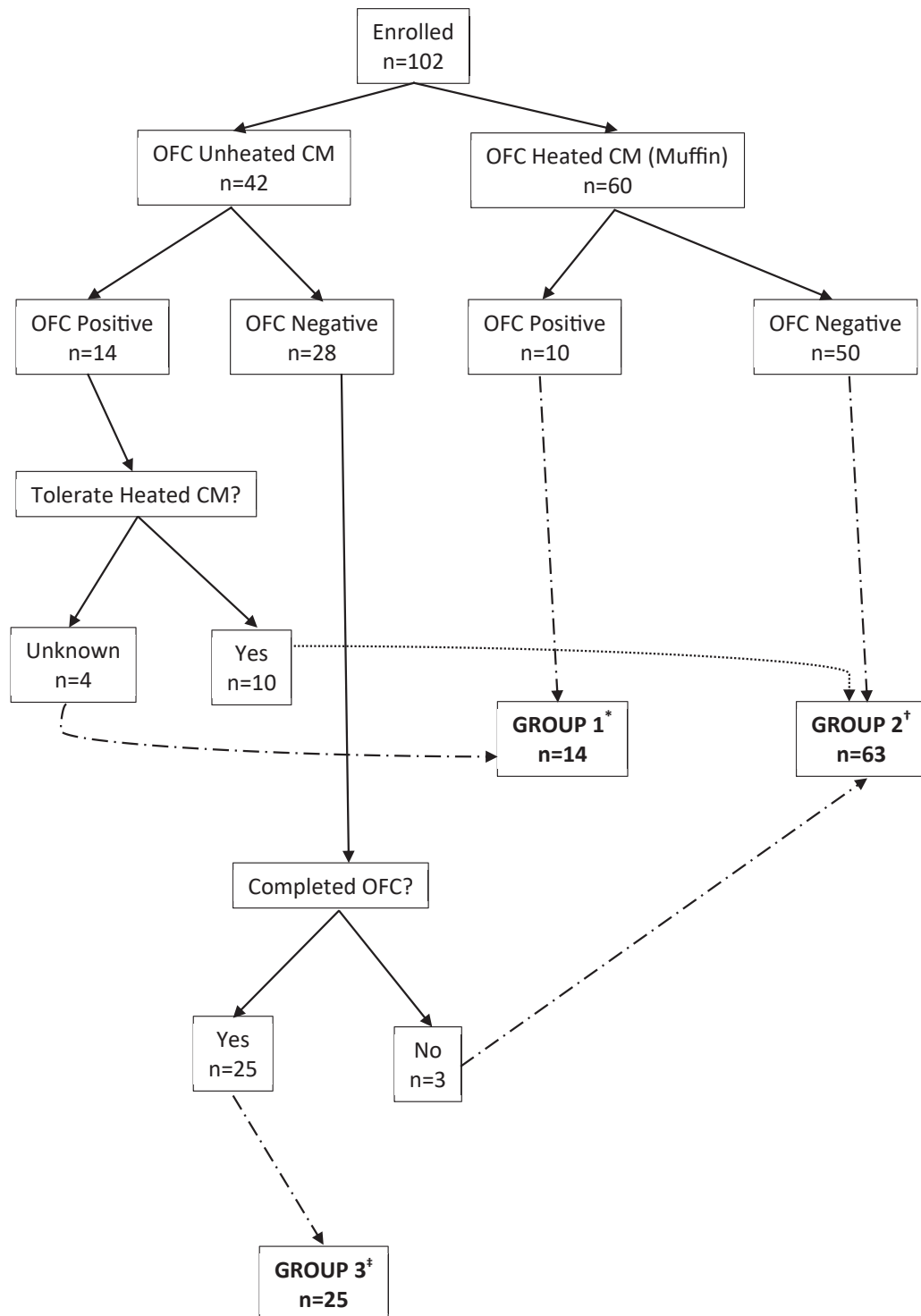


Figure 1. Enrollment flowchart. *Heated and unheated CM reactive. †Heated CM tolerant but unheated CM reactive. ‡Heated and unheated CM tolerant.

45%, seafood (including fish) 25%, and sesame 14%. The demographics of included subjects are shown in Table 1.

Results of Oral Challenges

Forty-two (41.6%) patients underwent an OFC with unheated cow's milk (fresh whole milk) and 60 (58.4%) with heated cow's milk (muffin) (Fig 1). The mean doses consumed were 118.4 mL (± 62.5 mL) unheated whole milk (approximately 3.97 $\pm$ 2.19 g

cow's milk protein) and 1 $\frac{1}{3}$ ($\pm \frac{1}{2}$) whole muffins (approximately 910 $\pm$ 350 mg cow's milk protein). Ten (17%) of the 60 patients that underwent a heated cow's milk OFC reacted and were thus placed in group 1, whereas 50 (83%) were placed in group 2 because they tolerated the muffin but were known to react to unheated cow's milk before the OFC. Fourteen (33%) of the 42 patients that underwent an unheated cow's milk OFC reacted. Of these 14 patients, 4 were not known to tolerate heated cow's milk and so were considered to be reactive to heated cow's milk and placed in group

Table 1
Demographics

	Group 1 ^a (n = 14)	Group 2 ^b (n = 63)	Group 3 ^c (n = 25)	P
Age at entry (years ±SD)	5.07 (3.10)	5.22 (3.50)	4.31 (2.96)	.512
Avoiding unheated milk only (%)	21	15	72	<.0001
Avoiding all milk (%)	64	78	24	<.0001
Asthma (%)	54	42	44	.492
Eczema (%)	85	77	60	.158
Allergic rhinitis (%)	38	31	28	.800
Adrenaline autoinjector prescribed (%)	77	86	76	.488

Bold values indicate statistical significance ($P < .05$).

^aHeated and unheated CM reactive.

^bHeated CM tolerant but unheated CM reactive.

^cHeated and unheated CM tolerant.

1. The other 10 patients were placed in group 2, because they were known to tolerate heated cow's milk before the OFC. Of the remaining 28 patients, 3 were placed in group 2 because they consumed a very small amount of milk during the unheated cow's milk OFC, and the outcome was considered inconclusive, whereas the other 25 patients were considered tolerant of unheated cow's milk. Thus, 14 patients were placed in group 1, 63 in group 2, and 25 in group 3 (Fig 1).

Before study entry, 70% of patients were avoiding milk completely, whereas on study completion only 14% continued to do so. Eighty-eight patients (86%) were tolerating some form of milk: 63 (72%) tolerated heated milk only, and 25 (28%) tolerated heated and unheated milk.

SPT Results—Comparison Between Groups

The mean of the SPTs for each group are shown in Table 2. In terms of whether any SPT could differentiate between the groups, the mean of the SPT results for commercial casein extract was significantly larger in the heated milk–reactive group (7.27 mm; group 1) vs the heated milk–tolerant group (5.34 mm; group 2; $P = .03$). No difference was seen in the mean of the SPT results for UHT cow's milk between the heated cow's milk–reactive group (8.58 mm; group 1) and the heated cow's milk–tolerant group (7.88 mm; group 2; $P = .42$). However, the means of the SPT results with all the various forms of milk or extracts were significantly smaller ($P < .0001$) for the milk-tolerant group (group 3) compared with a combined group 1 and 2, that is, those who could not tolerate unheated cow's milk, regardless of whether they tolerated heated cow's milk.

SPT Results—Comparison Within Groups

First we compared the mean of the UHT SPT with the means of the SPTs with the other forms of milk within the heated

milk–reactive group (group 1). In this group, no differences were found in the means of the SPTs with the various forms of milk compared with the mean of the SPT for UHT (Table 3). Next we compared the mean SPT results within the heated milk–tolerant group (group 2). In this group, the mean of the commercial extract SPT (7.68 mm) did not differ significantly from the mean of the SPT to UHT (7.88 mm), whereas the means of the SPTs for casein (5.34 mm; $P < .0001$) and evaporated milk (6.92 mm; $P < .01$) were significantly smaller than the mean SPT for UHT (7.88 mm). The mean SPT for unheated fresh whole milk (9.41 mm; $P = .001$) was significantly bigger (Table 3).

Discussion

This study describes the outcome of oral food challenges with either heated cow's milk (muffin) or fresh whole unheated cow's milk in 102 children with a history of cow's milk allergy based on clinical history and demonstration of sensitization. Similar to what has been reported in previous studies,^{4,9} 72% of the included children who remained allergic to whole unheated cow's milk were found to have developed tolerance to heated cow's milk, and 28% had outgrown their milk allergy completely. Many cow's milk–allergic children are able to tolerate heated cow's milk that has been exposed to high temperatures for a prolonged period (usually around 30 minutes) during a process such as baking and that regular ingestion of heated cow's milk may accelerate the rate of tolerance development in these children.⁵ Ultra-heat treated (UHT) milk is subject to temperatures far exceeding that of normal pasteurized cow's milk but for a very short duration, and data regarding the effect of that processing on the allergenicity of cow's milk proteins are lacking.

It was not an aim of the current study to describe cutoffs of the SPT to various forms of cow's milk that may predict tolerance to unheated milk; however, similar to several previous studies,^{9–11} we found that the mean SPT wheal sizes for all forms of cow's milk were significantly smaller in children who tolerated unheated cow's milk compared with those that did not, regardless of whether they tolerated heated cow's milk (Combined groups 1 and 2 vs group 3; Table 2).

Only the mean casein SPT was found to be significantly smaller in those children who tolerated heated (but not unheated) cow's milk compared with those that did not (group 1 vs group 2; Table 2); however, the absolute difference in wheal size between the 2 groups was only 2 mm, making a cutoff difficult to use clinically. In a retrospective study, Bartnikas et al¹² reported that a casein SPT cutoff of 9 mm had a sensitivity of 86% and a negative predictive value of 92% for predicting tolerance to baked cow's milk (muffin). A cutoff of 15 mm was 100% specific for failing a baked milk OFC.¹² In addition to SPT with a casein extract, other studies have described significantly lower levels of casein specific IgE in children who tolerate heated cow's milk compared with those who

Table 2
Skin Prick Test Means: Comparison Between Groups

	CommWhole mm (SD)	CommCasein mm (SD)	UHT mm (SD)	Evaporated mm (SD)	Whole mm (SD)
Group 1 ^a	8.29 (3.22)	7.27 (2.45)	8.58 (2.61)	8.63 (2.91)	8.33 (1.50)
Group 2 ^b	7.68 (3.07)	5.34 (2.50)	7.88 (2.88)	6.92 (2.83)	9.41 (3.46)
Group 3 ^c	4.17 (2.39)	2.91 (2.14)	4.16 (1.85)	3.36 (2.67)	5.15 (2.85)
Group 1 + 2 ^d	7.79 (3.09)	5.69 (2.58)	8.01 (2.83)	7.20 (2.89)	9.23 (3.23)
Grp 1 c/w 2 P	.53	.03	.42	.08	.09
Grp 1 + 2 ^d c/w 3 P	<.0001	<.0001	<.0001	<.0001	<.0001

Abbreviations: CM, cow's milk; c/w, compared with; UHT, ultra-heat-treated.

Bold values indicate statistical significance ($P < .05$).

^aHeated and unheated CM reactive.

^bHeated CM tolerant but unheated CM reactive.

^cHeated and unheated CM tolerant.

^dCombined means for group 1 and group 2.

Table 3
Skin Prick Test Means—Comparison Within Groups

	CommWhole mm (SD)	CommCasein mm (SD)	UHT mm (SD)	Evaporated mm (SD)	Whole mm (SD)
Group1 ^a	8.29 (3.22)	7.27 (2.45)	8.58 (2.61)	8.63 (2.91)	8.33 (1.50)
Group2 ^b	7.68 (3.07)	5.34 (2.50)	7.88 (2.88)	6.92 (2.83)	9.41 (3.46)
Within group 1 c/ w UHT P	.74	.11	—	.96	.58
Within group 2 c/ w UHT P	.61	<.0001	—	.01	.001
Within group 2c/ w whole fresh P	<.001	<.001	<.001	<.001	—

Abbreviations: CM, cow's milk; c/w, compared with; UHT, ultra-heat-treated.

Bold values indicate statistical significance ($P < .05$).

^aHeated and unheated CM reactive.

^bHeated CM tolerant but unheated CM reactive.

are reactive.^{4,9,12,13} Caubet et al¹³ suggested that only children with a casein specific IgE level less than 20 kU/L should be offered a baked milk OFC to determine tolerance.

Caseins are far more heat stable than other allergenic cow's milk proteins such as β -lactoglobulin and α -lactalbumin, and specific IgE in the sera of children who are reactive to heated cow's milk bind to caseins even after prolonged heating.¹⁴ This may be explained by preferential binding of conformational B cell epitopes on, for example, β -lactoglobulin, by specific IgE from heated milk-tolerant children that are lost when the tertiary structure of the protein is disrupted by heating.¹⁵ It therefore makes sense that heated milk-tolerant children will have lower specific IgE levels to heat-stable cow's milk proteins such as caseins. In the current study, the mean SPT results for UHT cow's milk and evaporated cow's milk were not significantly different in the heated cow's milk-tolerant group compared with the heated cow's milk-reactive group (group 1 vs group 2; Table 2). Based on this finding, we hypothesize that the content of heat-labile proteins still present in UHT cow's milk and evaporated cow's milk is similar to that of unheated cow's milk, presumably because the UHT or evaporative process is not sufficiently prolonged to affect the tertiary structure of heat-labile cow's milk proteins.¹⁴ This suggests therefore that extensively heated milk-tolerant children (baked cow's milk) are still at high risk of reacting to UHT and evaporated cow's milk.

A second interesting finding in this study was that within the heated milk-tolerant group (group 2) the mean SPT result for UHT cow's milk was significantly smaller than that of whole unheated cow's milk (Table 3). This may be taken to mean that the short duration of heating of UHT cow's milk may indeed be sufficient to disrupt the tertiary structure of particular heat-labile epitopes in UHT cow's milk. Therefore, heated milk-tolerant children who predominantly recognize these particular epitopes may demonstrate lower levels of reactivity to UHT or evaporated cow's milk compared with unheated cow's milk. However, the finding that within the same group the UHT cow's milk SPT mean wheal size was also significantly larger than the mean casein SPT (Table 3) suggests that, despite processing, UHT cow's milk still possesses noncasein proteins or conformational epitopes recognized by many heated milk-tolerant children. The mean UHT SPT result in group 2 was also significantly bigger than that of evaporated cow's milk, suggesting that perhaps the heating and evaporative process does result in fewer heat-labile cow's milk proteins in evaporated cow's milk compared with UHT cow's milk. However, taken together, these findings suggest that both UHT and evaporated cow's milk likely contain sufficient heat-labile cow's milk proteins to cause reactions in most, but perhaps not all, heated cow's milk-tolerant children.

A weakness of this study is that we have drawn conclusions regarding the allergenicity of proteins in UHT, evaporated, and fresh whole cow's milk using the SPT results of milk-allergic children as a surrogate marker of IgE-binding to these proteins. We did not

perform laboratory-based analysis to confirm these findings, but this could be done in a subsequent study.

In conclusion, this study suggests that processing of cow's milk using high-temperature, short-duration heating methods such as UHT and evaporation are unlikely to result in significant conformational changes in the epitopes of heat-labile milk proteins to the point where these types of cow's milk could routinely be considered safe for consumption by heated milk-tolerant children. However, there may be some selected heated cow's milk-tolerant children that would tolerate these types of cow's milk as a step between unheated cow's milk and more extensively heated cow's milk, but further studies are needed to confirm this. As such, UHT or evaporated cow's milk should not be recommended as an unheated milk substitute in cow's milk-allergic children even if they are known to tolerate extensively heated cow's milk.

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