

Allergic reactions to raw, pasteurized, and homogenized/pasteurized cow milk: a comparison

A double-blind placebo-controlled study in milk allergic children

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Five children aged 12-40 months with IgE-mediated adverse reactions to cow milk (immediate onset clinical pattern of cow milk allergy) were orally challenged double-blind in random order with three different milk preparations processed from the same batch of milk 1) raw untreated cow milk, 2) pasteurized cow milk, 3) homogenized and pasteurized cow milk, and 4) Nutramigen® (a commercial hypoallergenic infant formula based on hydrolysed casein) as placebo. Skin prick tests with the same preparations were also performed. On oral challenge the three different processed milk types provoked significant and similar allergic reactions in each child, and no adverse reactions followed the challenge with placebo (Nutramigen). Skin prick test with the same milk products were positive in all children and comparable to the results with an extract of purified raw cow milk protein (Soluprick®), whereas Nutramigen did not elicit any skin reactions. A tendency towards a lower threshold of reaction and larger skin reactions induced by the processed milk preparations might indicate an increased ability of pasteurized and homogenized/pasteurized milk to evoke allergic reactions in patients allergic to milk.

Key words: milk allergy; milk challenge; cow milk; homogenization; pasteurization; infant milk formula.

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CLINICAL ASPECTS

In children with immediate onset clinical pattern of cow milk allergy neither raw untreated cow milk nor unhomogenized pasteurized milk are tolerated. Self-appointed provocations with these products in milk allergic patients may lead to serious reactions and, therefore, should be warned against.

In recent years an increasing number of parents to milk allergic children claim that their children tolerate raw untreated cow milk and unhomogenized low pasteurized cow milk, but show allergic reactions towards homogenized and pasteurized commercial milk and milk products. Only a few reports deal with the possible increased allergenicity of homogenized and pasteurized milk. In a murine anaphylactic shock model pasteurization did not seem to change the ability of milk to induce anaphylactic reactions (9), but increasing fat contents in combination with

homogenization increased the ability of milk to induce anaphylactic reactions in intravenously challenged mice. However, in a clinical study in 30 milk allergic children open challenges with unhomogenized pasteurized and raw untreated cow milk resulted in definite allergic reactions in 92 % and 84 %, respectively (5). As commercial milk products and raw milk from different farms had been used in the latter open study, the possible influence of different feeding, spraying of fields, etc., could not be ruled out.

To eliminate the above mentioned possible

bias and uncertainties we carried out this double-blind placebo-controlled study in milk allergic children with three different types of bovine milk processed from the same batch of raw cow milk.

The aim of the study was to investigate and compare the ability of raw cow milk, pasteurized cow milk and homogenized/pasteurized cow milk to induce allergic reactions in proved milk allergic children with immediate IgE-mediated reactions to cow milk.

Further, the skin test reactivity of the same milk samples was tested double-blind compared to placebo, a purified extract of raw cow milk, a positive reference (histamine dihydrochloride) and a negative reference (saline).

MATERIAL AND METHODS

Five milk allergic children aged 12–40 months (median 14 months) were studied. Clinical data are shown in Table 1. The diagnosis of cow milk hypersensitivity had been established by generally accepted criteria (1, 10) including definite subsidence of symptoms after each of two eliminations and recurrence of symptoms after one challenge. At the time of diagnosis lactose intolerance and possible coincidental infections had been excluded.

Milk preparations

Three different preparations of milk were processed from the same batch of fresh raw untreat-

ed cow milk ("Enighedden" Dairy, Copenhagen, Denmark) 1) raw untreated milk, 2) pasteurized milk (low pasteurization 75°C for 15 s), 3) homogenized and pasteurized milk. Homogenization was performed at 60°C and a pressure of 175 kg/cm². The same procedures of low pasteurization and homogenization are used in the production of commercial consumption milk products. 3000 ml of each type of milk were distributed in bottles of 100 ml, and the 90 bottles were stored at -20°C. Thus, all the milk samples should be chemically identical.

A commercial casein hydrolysate formula (Nutramigen®, Mead-Johnson) was used as placebo. Previously, all patients had tolerated Nutramigen without adverse reaction.

Milk challenges

The children had been on a milk-free diet for at least 4 weeks before the first challenge, and the milk-free diet was continued during the whole challenge period. The challenges with the three different milk preparations and placebo were performed in random order. Before each challenge a clinical investigation was performed and baseline symptoms recorded. The next challenge was not performed until a symptom-free interval of at least 1 week had elapsed. All provocation challenges were done within a period of 3 months.

The challenges, which were carried out in hospital, were performed double-blind by the

Table 1
Clinical data on five children allergic to cow milk¹

Patient No.	Age (months)	Sex ²	Atopic heredity	Milk allergy symptoms ³	Cow milk introduced age (weeks)	Onset of milk allergy age (weeks)	AI-RAST to milk (class)	Total IgE PRIST (kU/l)
1	36	F	mother	A,AD,R,U	2	12	3	484
2	40	F	mother	R,U,VO	8	10	4	254
3	12	F	-	A,AD,R	2	24	2	23
4	14	F	mother/ father	AD,R,VO	4	4	2	15
5	14	M	mother	A,AD,R,U	32	32	4	150

¹Verified by milk-free diet/challenge with cow milk (homogenized and pasteurized)

²F: female, M: male

³A: asthma; AD: atopic dermatitis; R: allergic rhinitis; U: urticaria; VO: vomitus

Results of AI-RAST and total IgE date from the latest milk challenge (1–3 months before this study)

same nurse and the same doctor and the children were kept under close observation. Each sample had been labelled with a figure by the dairy and the code was not broken until all challenges were finished.

The same scheme was followed. Initially, 5 ml of the sample in question was given and every 2 h the amount was increased (10, 20, 40, 80 ml) up to 160 ml, or until a definite clinical reaction was observed. The taste and colour of each milk type was disguised with an appropriate amount of Nutramigen (10, 20, 40 ml, etc.). If no symptoms had occurred after 8 h (a total of 75 ml) the child was sent home with instructions to continue the increasing intakes (80–160 ml). If late reactions occurred at home the patient was reinvestigated in hospital as soon as possible, and with a suspected positive reaction the last dose was repeated once.

A challenge was considered positive if:

- the child displayed definite allergic reactions in keeping with the child's history of cow milk allergy, i.e. skin rash including urticaria, angioedema, protracted conjunctivitis/rhinitis, protracted vomiting, wheezing.
- the provoked symptoms disappeared after withdrawal of the milk preparation in question, and
- coincidental infection could be excluded.

A challenge was considered negative, if the child remained symptom-free after ingestion of more than 315 ml of the sample concerned.

Skin test procedure

All skin prick tests (SPT) were performed on the volar side of the forearm by the same investigator and the procedure was double-blind concerning milk samples. Single droplets of the investigated samples were placed 2 cm apart on the skin and a prick-lancet (Bayer-Kemi) with a 1 mm point (13) was jabbed through the drop at an angle of 90° to the skin. The wheal reactions were read after exactly 10 min (histamine dihydrochloride)

Table 2

Double-blind placebo-controlled provocation challenges with three different milk preparations and a casein hydrolysate (Nutramigen®) in five children allergic to cow milk

Patient No.	Raw untreated cow milk				Pasteurized cow milk				Homogenized and pasteurized cow milk				Placebo (Nutramigen®) ¹	
	Total dose ² (ml)	Symptom ³	Reaction time ⁴	Duration of symptom	Total dose ² (ml)	Symptom ³	Reaction time ⁴	Duration of symptom	Total dose ² (ml)	Symptom ³	Reaction time ⁴	Duration of symptom	Total dose (ml)	Symptom ³
1	5	A,R	2 min	1 h	5	CO,A,R,AO	30 sec	3 h	5	CO,R,A,V,AO	30 s	2 h	> 315	none
2	75	R	6 h	4 h	15	R	3 h	6 h	15	R	4 h	6 h	> 315	none
3	35	R,E	4 h	4 h	15	R,E,U	2 h	24 h	35	R,E,A	4 h	4 h	> 315	none
4	75	V,R,E	8 h	24 h	75	R,E	8 h	48 h	75	R,E	8 h	72 h	> 315	none
5	15	U,R,E	2 h	32 h	5	E,U,A	30 min	24 h	5	U,E	1 min	24 h	> 315	none

¹Nutramigen® is a casein hydrolysate containing 22 g protein/l

²Total dose before challenge was stopped

³Symptoms in order of appearance. A: asthma; AO: angioedema; CO: colic; E: erythema; R: rhinitis; U: urticaria; V: vomitus

⁴Reaction time: from beginning of challenge to onset of symptoms

and 15 min (all other test extracts). The wheals were outlined by a soft ballpoint pen. The markings were transformed to mm² paper by means of tape, and the largest diameter (D_1) and the smallest diameter (d_2) of each wheal were measured. The results were given as $(D_1 + d_2)/2$.

Extracts

The following test extracts were used; whole raw cow milk, whole pasteurized cow milk, whole homogenized/pasteurized cow milk, 1/20 w/v raw cow milk in 50 % glycerol diluent (Soluprick, Allergologisk Laboratorium, Denmark), and casein hydrolysate (Nutramigen). Positive reference was histamine dihydrochloride 3 mg/ml in 50 % glycerol diluent (Soluprick), and negative reference 0.5 % NaCl + 0.25 % NaHCO₃ in 50 % glycerol diluent (Soluprick).

RAST

Al-RAST was performed according to (11, 12) and total IgE by Phadebas® IgE PRIST.

Statistics

Analysis of significance was performed with McNemar's test for paired alternatives, in this

case successive provocations of the same individuals.

RESULTS

The results of the challenge with three different milk types and placebo (casein hydrolysate, Nutramigen) are shown in Table 2.

All provocations with milk samples were positive irrespective of the type of milk, and all challenges with placebo (Nutramigen) were negative. This difference is significant ($P < 0.025$). The reaction time (from beginning of challenge to onset of symptoms) varied both intra- and interindividually with a tendency to a shorter reaction time after challenge with pasteurized and especially homogenized/pasteurized milk. The duration of symptoms appeared prolonged after the same milk types.

The results of skin prick tests are summarized in Table 3. All SPT with raw cow milk and processed milks provoked wheals of at least the same size or larger than the wheals induced by the histamine reference. Controls with saline were all negative as well as SPT with the casein hydrolysate (Nutramigen). The differences between the results with each of the milk samples and both Nutramigen and control solution were significant ($P < 0.025$). No significant difference between each of the milk samples was shown.

Table 3

Skin prick tests with three different whole milk preparations, a raw cow milk extract (Soluprick®)¹ and a casein hydrolysate (Nutramigen®)² in five children allergic to cow milk

Patient No.	Raw untreated (mm)	Pasteurized (mm)	Homogenized/ pasteurized (mm)	Raw extract ² (mm)	Casein hydrolysate (Nutramigen®) (mm)	Positive ref. ³ (mm)	Neg. ref. ⁴
1	9	11	10	5	0	4	0
2	4	4	6	3	0	2	0
3	2	2	2	2	0	2	0
4	3	6	8	3	0	3	0
5	3	5	6	3	0	3	0

Values are given as $(D_1 + d_2)/2$; D_1 = largest wheal diameter, d_2 = smallest wheal diameter

¹1/20 w/v raw cow milk extract in 50 % glycerol diluent (Soluprick), ALK, Denmark)

²Nutramigen is a casein hydrolysate containing 22 g protein/l

³Histamine dihydrochloride 3 mg/ml in 50 % glycerol diluent (Soluprick) was used as positive reference

⁴0.5 % NaCl + 0.25 % NaHCO₃ in 50 % glycerol diluent (Soluprick®) was used as negative reference

DISCUSSION

Many earlier reports on the antigenicity of processed cow milk products have mainly dealt with the effect of heat treatment on milk proteins (4, 6, 7, 8). Low pasteurization (75°C for 15 s) effectively ensures the bacteriological quality of milk and does not seem to cause any significant reduction of the antigenicity. However, severe heating destroyed the anaphylactic sensitizing capacity of whey proteins (beta-lactoglobulin and alpha-lactoglobulin) and reduced that of casein in experiments on guinea pigs (7). Strong heat treatment (121°C for 20 min) also causes a loss of vitamins, and marked browning and Maillard reactions may even exacerbate milk allergic reactions (7). Thus severe heat treatment can have undesirable and unpredictable consequences for nutrition and allergenicity. In general, heat treatment reduces, but does not eliminate the allergenicity of milk proteins (4).

Only a few reports (5, 9) have investigated the postulated decreased allergenicity of unhomogenized milk. Since the 1960s homogenization has been used worldwide both in the preparation of normal commercial milk products and in most infant formulae (produced by spray drying homogenized cow milk). During homogenization, raw milk at 60°C is pressed through narrow valves under pressure (150–200 kg/cm²) and the large fat globules (1–15 µm diameter) are split into many smaller (< 0.8 µm diameter). This procedure prevents creaming and increases keeping quality. Homogenization causes an increase in the total surface area of the fat globules. About 25% of the casein micelles, and to some extent the whey proteins, are adsorbed to these new fat globules. Thus, the amount of protein "increases" in homogenized milk from 3.4% to 6.9% if the fat-content is 3.5%, and physiologically, the homogenized milk acts as a milk with 6.9% protein. This might explain the results from an experiment in cow milk sensitized mice, which showed increased anaphylactic reactions to intravenously injected homogenized milk preparations with increasing fat content (9). However, one should be cautious about applying such results from experimental animal study-models to the human infant, and in a clinical trial

with open challenges (5) most milk allergic children reacted against both raw untreated cow milk and commercial unhomogenized low pasteurized milk.

The present data from our double-blind placebo-controlled trial in proven milk allergic children confirm that neither raw untreated cow milk, pasteurized milk nor homogenized/pasteurized milk are tolerated by children allergic to cow milk protein. Because of the study design including the usage of three different cow milk preparations processed from the same batch of raw milk, we were only able to study a very small number of patients. These patients all belong to the group of milk allergics with an (IgE-mediated) immediate type of reaction (Table 1), and all had positive SPT with the three milk preparations and the commercial SPT extract (Soluprick). These results are in agreement with earlier reports (3). Therefore, in children with immediate type cow milk allergy, uncontrolled and self-appointed introduction or provocation trials at home with either unhomogenized, pasteurized or raw untreated cow milk is not advocated. The present data do not allow any conclusions concerning cow milk allergic children with a delayed onset clinical pattern (mainly gastrointestinal symptoms). However, in a previous open study (5) children with a delayed type reaction to cow milk did not tolerate unhomogenized or raw milk. A double-blind study of this group might be useful. Although no qualitative differences between the different processed milk products were shown in this study, the tendency towards a lower threshold of reaction and large skin reactions induced by pasteurized milk and homogenized/pasteurized milk may point to quantitative differences (Tables 2, 3).

The casein hydrolysate (Nutramigen) also proved hypoallergenic in this study (2) and provoked no symptoms on challenge or wheal reactions to SPT.

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REFERENCES

1. Bahna, S. L. & Gandhi, M. D.: Milk hypersensitivity. II. Practical aspects of diagnosis, treatment and prevention. *Ann. Allergy* 50, 295-301, 1983.
2. Estham, E. J., Lichauco, T., Grady, M. J. & Walker, A.: Antigenicity of infant formulas: Role of immature intestine on protein permeability. *J. Pediatr.* 93, 561-564, 1978.
3. Ford, R. P. K., Hill, D. J. & Hosking, C. S.: Cow's milk hypersensitivity: Immediate and delayed clinical patterns. *Arch. Dis. Child.* 58, 856-862, 1983.
4. Gjesing, B., Østerballe, O., Schwartz, B., Wahn, U. & Løwenstein, H.: Allergen-specific IgE antibodies against antigenic components in cow milk and milk substitutes. *Allergy* 41, 51-56, 1986.
5. Hansen, L. G., Høst, A. & Østerballe, O.: Allergic reactions to unhomogenized milk and raw milk. (In Danish). *Ugeskr. Læger* 149, 909-911, 1987.
6. Heppel, L. M. J., Cant, A. J. & Kilshaw, P. J.: Reduction in the antigenicity of whey proteins by heat treatment: a possible strategy for producing a hypo-allergenic infant milk formula. *Br. J. Nutrition* 51, 29-36, 1984.
7. Kilshaw, P. J., Heppel, L. M. J. & Ford, J. E.: Effects of heat treatment of cow's milk and whey on the nutritional quality and antigenic properties. *Arch Dis Child* 57, 842-847, 1982.
8. McLaughlan, P., Anderson, K. J., Widdowson, E. M. & Coombs, R. R. A.: Effect of heat on the anaphylactic-sensitising capacity of cow's milk, goat's milk, and various infant formulae fed to guinea-pigs. *Arch. Dis. Child* 56, 165-171, 1981.
9. Poulsen, O. M., Hau, J. & Kollerup, J.: The effect of homogenization and pasteurization on the allergenicity of bovine milk analysed by a murine anaphylactic shock model. *Clin. Allergy* (in press) 1987.
10. Savilahti, E.: Cow's milk allergy. *Allergy* 36, 73-88, 1981.
11. Vanto, T., Viander, M., Koivikko, A., Schwartz, B. & Løvenstein, H.: RAST in the diagnosis of dog dander allergy. A comparison between three allergen preparations using two variants of RAST. *Allergy* 37, 75-85, 1982.
12. Weeke, B.: Aluminium hydroxide absorbed allergens used in a modified RAST (Al-RAST). (Abstract). *Allergol. Immunopathol.* 4, 333, 1977.
13. Østerballe, O. & Weeke, B.: A new lancet for skin prick testing. *Allergy* 34, 209-212, 1979.

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