

Clinical Tolerance to Lactose in Children With Cow's Milk Allergy

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ABSTRACT. *Objective.* Adverse reactions following the ingestion of lactose have been reported in children with cow's milk (CM) allergy. Whether this is attributable to the contamination of lactose with CM proteins is unknown. In this paper, we assessed clinical tolerance of lactose derived from CM whey in children hypersensitive to CM from 2 university hospital pediatric departments.

Design. Twenty-four children (5 girls and 19 boys, median 25 months old; range: 2–107 months) with immediate CM allergy confirmed at history or during double-blind, placebo-controlled food challenge (DBPCFC) were enrolled. DBPCFC with CM could be conducted in 11 of 24 patients. Children with a history of immediate/delayed reactions to soy formula (SF) were excluded. Clinical tolerance to CM, SF, and SF + lactose was assessed by: 1) skin prick test with casein, lactalbumin, soy commercial allergen preparations, fresh CM, SF, SF and lactose, lactose (Official Pharmacopoeia) in 4 concentrations (0.01%, 0.1%, 1%, 10%); 2) specific serum immunoglobulin E determination by CAP system technology; 3) DBPCFC in 8 incremental doses of SF + lactose and using SF as a placebo to make up a total of 240 mL of reconstituted formula.

Results. With a positive cutoff point of ≥ 3 mm wheal diameter at SPT, all patients were sensitized to fresh CM, lactalbumin, and/or casein. Twenty-three of 24 patients (95.8%) were SPT-positive to CM formula, 16 of 24 to lactalbumin (66.6%), 14 of 24 to casein (58.3%), and none to SF, SF + lactose, or lactose alone at all dilutions. Complexed immunoglobulin E determinations were positive for CM in 23 of 24 cases and negative in all cases for soy. Challenge with SF + lactose was negative in all cases.

Conclusions. Even children hypersensitive to CM are clinically tolerant to lactose and can safely consume foods and drugs with lactose from bovine sources as an ingredient. Lactose exclusion is unwarranted from soy preparations on grounds of potential allergic reactions to CM protein residue. *Pediatrics* 2003;112:359–362; *children, lactose, cow's milk allergy, soy.*

ABBREVIATIONS. AEDS, atopic eczema/dermatitis syndrome; CM, cow's milk; CMA, cow's milk allergy; DBPCFC, double-blind, placebo-controlled food challenge; SF, soy formula; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IgE, immunoglobulin E; SPT, skin prick test; cSPT, soy commercial allergen extract.

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There are 2 reasons why infant formulae used in the treatment of cow's milk allergy (CMA) do not contain lactose. Lactose produced following the refinement and separation of dairy products may retain an allergizing protein fraction, and there is a known crossover between symptoms of lactose intolerance and CMA allergy, such as diarrhea, which most infant formulae are intended to treat. Thus, many pediatric allergists believe that no lactose is better than any lactose for children with CMA. Textbooks,¹ reviews,² and position papers^{3,4} allude to possible adverse reactions in children with CMA and attribute this to milk protein contamination of lactose. This concern could translate into a blanket ban for these children of not only lactose-containing foods but also of many pharmaceutical preparations, and even toothpastes, which may contain this sugar as an excipient, bulking agent, or nutritional supplement. However, a computer search of the literature using the algorithm [lactose AND CMA OR adverse reactions children 1975–2002] did not retrieve a single report of an adverse reaction to lactose among children with CMA. Lactose, however, assists in calcium assimilation⁵ and is a slow energy release sugar. Although it is unclear whether a lactose-free diet plays a role in the clinical neurologic abnormalities of children with galactosaemia,⁶ it is well known that galactose is a functional component of myelin galactolipids. These rare autosomal recessive disorders may be fatal to neonates lacking intestinal lactase and should be considered in the risk/benefit assessment when planning diets for infants and children with CMA.⁷ The aim of this study was to prospectively assess clinical tolerance to lactose from a bovine source in a consecutive series of 24 children with CMA.

PATIENTS

Patients diagnosed with CMA at both our institutions presenting for follow-up visits between June 1, 2001 and December 31, 2001 were recruited. All were tolerant of soy formula (SF), which they consumed openly. Informed parental consent was obtained for participation and blood tests after institutional review of the ethics of the study aim and protocol. Five girls and 19 boys (median 25 months old; range: 2–107 months) were recruited. Presenting allergic symptoms were anaphylaxis ($n = 1$); asthma ($n = 1$); atopic eczema/dermatitis syndrome (AEDS; $n = 3$); urticaria ($n = 2$); AEDS and anaphylaxis ($n = 3$); AEDS and asthma ($n = 1$); AEDS and urticaria ($n = 3$); anaphylaxis, asthma, and urticaria ($n = 2$); ana-

phylaxis, AEDS, and asthma ($n = 2$); AEDS, asthma, and urticaria ($n = 1$); lip edema, asthma, and anaphylaxis ($n = 1$); AEDS, anaphylaxis, lip edema, and urticaria ($n = 1$); anaphylaxis, lip edema, and urticaria ($n = 1$); lip edema and anaphylaxis ($n = 1$); and AEDS, anaphylaxis, asthma, and urticaria ($n = 1$). A double-blind, placebo-controlled food challenge (DBPCFC) was performed to confirm the diagnosis of CMA in all cases except in children with a history of anaphylaxis. Fresh, pasteurized cow's milk (CM) was administered in 4 doubling doses of 16, 32, 64, and 128 mL at 2-hour intervals for 6 hours. Neocate (Numico, Liverpool, United Kingdom) was used as placebo. Challenge was discontinued at the first onset of symptoms or with a negative response after the fourth dose. All patients were asymptomatic at the time of lactose challenge.

METHODS

Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis (PAGE) and Immunoblotting

Samples were separated according to a protocol published elsewhere,⁸ on a polyacrylamide gradient gel (9%–19% of acrylamide) with SDS as a denaturing agent. After an electrophoresis run (90 V at room temperature for ~6 hours), the gels were dyed with Coomassie brilliant blue G-250.⁹ After SDS-PAGE, immunoblotting was performed as described.¹⁰ The membranes were incubated overnight in 10 mL of 0.25% gelatin solution containing 0.3 mL of serum from children allergic to milk proteins. The antigen-immunoglobulin E (IgE) complex was detected using goat anti-human IgE antibodies (Sigma Aldrich, St. Louis, MO) labeled with alkaline phosphatase. The secondary antibody commercial stock was diluted 1:1000 (v:v) in 0.25% gelatin solution. After incubation in bromochloroindolyl phosphate/nitro blue tetrazolium solution, an intense black-purple precipitate developed at the site of enzyme binding. The developing solution contained 15% bromochloroindolyl phosphate and 30% nitro blue tetrazolium in alkaline phosphatase buffer (100 mM Tris, 100 mM NaCl, 5 mM MgCl₂, pH 9.5). To concentrate the protein fraction, lactose was suspended in 10% trichloroacetic acid (10 mg/mL). After 30 minutes at 4°C, samples were centrifuged at 10 000 rpm for 30 minutes at 4°C. Pellets were suspended in 0.5 N NaOH and diluted with sample buffer (1:1 v:v). Sample buffer contained 0.25 M Tris-HCl buffer pH 6.8, 7.5% glycerol, 2% SDS, and 5% β -mercaptoethanol. Gels and membranes were elaborated using a gel scanner (Sharp JX-330; Pharmacia Biotech, Uppsala, Sweden) and the Image Master Total lab version 1.11 software (Amersham Pharmacia Biotech, Cologno Monzese, Italy).

Skin Prick Test (SPT) and Determination of Specific IgE Antibodies (CAP)

Clinical sensitization assessed by SPT with casein, lactalbumin and soy commercial allergen extracts (cSPT; from ALK, Hørsholm, Denmark), fresh CM, SF (Humana Sinelac 1 from Humana Italia, Milan, Italy), SF + lactose (Humana Italia), and α -D-lactose (Official Italian Pharmacopoeia) at 4 different concentrations (0.01%, 0.1%, 1%, 10%). The lactose used in all tests was extracted from CM whey and comes from a single batch supplied by Humana Italia. Wheal diameters ≥ 3 mm on a millimetre caliper disk up to 30 minutes after pricking were considered positive. Ten mg/mL histamine phosphate in 50% glycosaline and glycosaline alone were used as positive and negative controls, respectively. Food-specific IgE determinations were performed on patients' sera for the following foods: CM, casein, α -lactalbumin, β -lactoglobulin, and soy (CAP System; Pharmacia Biotech).

DBPCFC

DBPCFC in 4 incremental doses of CM (equivalent to 240 mL of reconstituted formula) was performed at 2-hour intervals on 2 alternate days, following protocols current at this institution. Neocate was used as a placebo, but was not added to challenge doses administered to children under 3 years. The formula used in

DBPCFC with lactose consisted of 4 incremental doses of α -D-lactose up to a maximum dose of 11.6 mg in the SF used as placebo.¹¹

RESULTS

History of CMA was confirmed by DBPCFC in 11 children only, as patients with anaphylactic reactions were not challenged for fear of inducing shock (Table 1). With the cutoff point for positivity at SPT set by a wheal diameter larger than 3 mm, all patients were sensitized by fresh CM, lactalbumin, and/or casein. Twenty-three of 24 patients (95.8%) were positive to cSPT with CM, 16 of 24 to cSPT with α -lactalbumin (66.6%), 14 of 24 to cSPT with casein (58.3%). Although tolerant of soy, 4 patients positive at cSPT showed soy-specific IgE. However, no patient was SPT-positive to SF, lactose, or SF + lactose. CM-specific IgE were identified by CAP in 23 of 24 children. Using a cutoff point of 3.5 kU/L, RAST showed negative in all cases with soy. The outcomes of DBPCFC with SFL were negative in all cases. Both SDS-PAGE (Fig 1) and immunoblotting (Fig 2) showed the lactose sample not to contain proteins and the absence of trace proteins was confirmed by the protein-enriched sample (lactose precipitate). No patient showed a positive reaction to lactose samples at immunoblotting, even when samples were enriched in protein fraction by precipitation by trichloroacetic acid.

DISCUSSION

The concept of the allergenicity of lactose is so ingrained that lactose-induced reactions are proposed in the differential diagnosis of adverse reactions to foods when the cause is unclear. An allergic reaction to lactose has even been contemplated by a case study reporting an immediate reaction to a preparation of royal jelly.¹² Manufacturers prefer to use milk-extracted, rather than synthesized, lactose by reason of its cost but rarely label products accordingly. Thus, allergists recommend that children with CMA should avoid lactose-containing foods for fear of exposure to CM protein residue. This is the first study of whey-derived lactose allergenicity by serology and DBPCFC investigation of children with confirmed CMA. In this limited sample, allergy symptoms were not induced on formal oral challenge, nor were CM protein traces found by immunochemistry to be associated with lactose. A second finding was that lactose did not elicit a positive SPT response among children sensitive to CM. As patients presented with IgE-mediated symptoms to CM, and 9 of 24 had a history of anaphylaxis, our data support the idea that even children acutely sensitive to CM will be tolerant of whey-derived lactose. In consequence, either removing lactose from the diet or lactose residues from foods intended for children with CMA may not be necessary. Some nutritional imbalance or deficiency may result from restricting dairy products in general¹³ or lactose specifically, as future research may find. A limitation of this study is that lactose from a single batch from 1 manufacturer was used to forestall source variation. Further studies are needed to investigate whether introducing this variable is

TABLE 1. Clinical Characteristics, SPT, Specific IgE Determinations (CAP), and DBPCFC With Lactose in 24 Children With CMA

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sex (M,F)	M	M	M	F	F	M	M	M	M	M	F	M	M	M	M	M	M	M	M	M	M	F	F	M
Age (mo)	47	31	59	8	23	10	30	15	77	11	58	28	5	42	107	56	9	10	4	27	3	20	2	63
Diagnosis	AEDS, A, AS	AEDS, U	A, AS	AS, AED, S	AEDS, U	AS	A	U	U, A, AS	AEDS, U	AEDS, AEDS, A	AEDS, AEDS, A	AEDS, AEDS, A	AEDS, AEDS, A	AEDS, AEDS, A	AEDS, AEDS, U	AEDS, AEDS, S, AS, A	AEDS, AEDS, A	AEDS, AEDS, A	AEDS, AEDS, U, LE, AS	AEDS, AEDS, U, A	AEDS, AEDS, S, AS	AEDS, AEDS, LE, AS	AEDS, A, AS
DBPCFC with CM		+			+		+	+		+	+	+	+	+	+	+					+			
Formula	NC	SF	SF	BM, SF	SF	SF	SF	HRF	NC	SF	SF	SF	SF	SF	HRF	SF	NC	HRF	NC	NC	SF	SF	BM	SF
SPT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fresh CM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactalbumin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Casein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Soy	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Soy formula	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SFL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CAP																								
Cow's milk	43,8	3,5	2,2	28,4	2,7	53,9	10,5	1,2	48,3	4,7	1,0	1,4	<0,35	5,2	6,8	1,8	50,1	29,5	45,2	38,3	1,9	>10	15,5	5,6
Casein	38,3	2,1	2,4	39,8	2,5	47,6	8,6	1,0	46,3	4,7	0,8	1,4	<0,35	7,1	7,4	3,5	41,4	5,6	31,0	41,3	2,6	>10	17,6	5,7

BM indicates breast milk; NC, neocate; SFL, soy formula + lactose; HRF, hydrolysed rice formula; A, asthma; U, urticaria; LE, lip oedema; AS, anaphylactic shock.

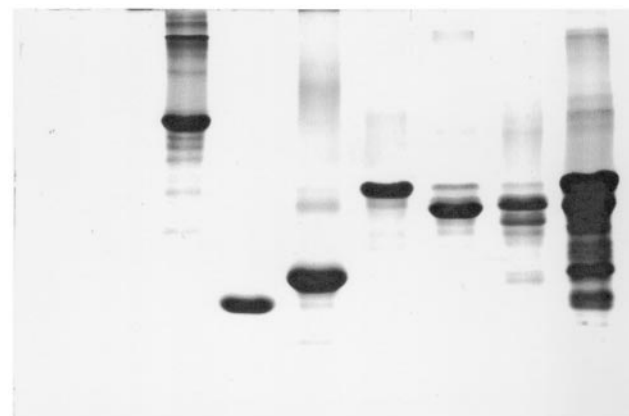


Fig 1. SDS-PAGE of commercially available lactose and CM proteins.

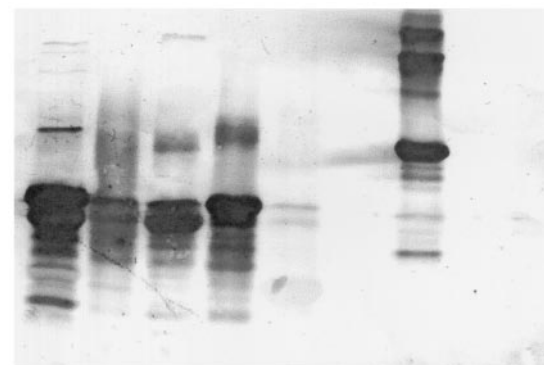


Fig 2. Immunoblotting of commercially available lactose and CM proteins: serum of a child with CMA.

clinically relevant. The European Society for Paediatric Allergology and Clinical Immunology/European Society for Paediatric Gastroenterology, Hepatology, and Nutrition guidelines on hypoallergenic formula use⁴ recommend “products without lactose, or with lactose processed to remove any residual allergenic protein.” However, we were unable to find clinical evidence to substantiate this precautionary stance. Milk-sourced lactose may thus be acceptable as a sugar for processed foods, nutritional supplements, and weaning formulas or as an additive in excipients for children with CMA, thereby containing costs. In the absence of positive DBPCFC to lactose extracted from whey, our data suggest that this bioactive carbohydrate need not be removed from CM hydrolysates and may be added to soy-based formula. We conclude that incurring this nutritional risk—however small—through lactose restriction may be in fact more “routine practice” than is justified clinically in children with CMA.

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HYPERTENSION REPORT DISPUTES EARLIER STUDY

“A backlash is brewing among some doctors against a recent government-sponsored clinical trial that found diuretics to be the best, first option for treating high blood pressure. Critics say the results from the 10-year study, called Allhat and published in December (2002) to wide publicity, overstated the case for diuretics and contradicted much of their own clinical experience with the pills, which are an older, generally cheaper hypertension treatment. The critics are getting some substantial backing in a new report in the *New England Journal of Medicine*. The study suggests that newer drugs called ACE inhibitors are a better first choice for elderly patients, leading to fewer deaths, heart attacks, strokes, and other cardiovascular problems. The results are certain to further roil the debate over how to best treat the more than 50 million Americans who have high blood pressure. The seemingly conflicting reports also underscore the difficulty of using evidence from major studies to standardize medical practice—a cornerstone of the drive to improve the quality of health care. It's common in medicine to come up with different answers to similar questions, making it sometimes daunting to know which evidence to believe and when there is enough of it to change practice. Even more challenging is trying to apply results of large studies to individual patients. . . Ochsner Clinic's Dr Edward D. Frohlich cautions against seeing the 2 classes of medicines as in a contest. Both work and the main thing, he says, is to get more patients well-treated. Current estimates are that about 70% of Americans with hypertension aren't being effectively treated.”

Hensley S, Winslow R. *Wall Street Journal*. February 13, 2003

Noted by JFL, MD