

Increased intestinal sugar permeability after challenge in children with cow's milk allergy or intolerance

For Prof. Salvatore Auricchio on his 60th birthday

Troncone R, Caputo N, Florio G, Finelli E. Increased intestinal sugar permeability after challenge in children with cow's milk allergy or intolerance. *Allergy* 1994; 49: 142–146. © Munksgaard 1994.

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The diagnosis of cow's milk allergy or intolerance (CMAI) is based on clinical improvement on exclusion diet and relapse after challenge with milk. The aim of this work was to investigate the value of the cellobiose/mannitol (C/M) sugar permeability test, performed before and after cow's milk challenge, as a tool for the diagnosis of CMAI. Thirty-two patients underwent milk challenge at a median age of 13 months (range 3–84 months). A dual sugar (C/M) permeability test with an iso-osmolar solution was performed before and 24 h after challenge. Of the 10 patients who developed symptoms after challenge, nine showed increased postchallenge C/M ratio, whereas such an increase was observed in only one of the 22 nonrelapsed subjects. The postchallenge C/M ratio increase in relapsed subjects is to be attributed to both higher cellobiose and lower mannitol urinary excretion. These results suggest the use of the sugar permeability test, in addition to clinical observation, as an aid in the evaluation of provocation tests in infants with suspected CMAI.

Key words: cow's milk allergy or intolerance;
intestinal permeability.

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Accepted for publication 16 April 1993

Cow's milk allergy or intolerance (CMAI) is one of the commonest causes of morbidity in the first year of life. It is known to cause many symptoms, including malabsorption and failure to thrive, bloody diarrhea, vomiting, infantile colic, anemia, eczema, shock, urticaria, wheezing, and rhinitis (13), none of which are specific to CMAI. The different pathogenic mechanisms probably account for the protean clinical pattern, and, at the same time, for the lack of laboratory tests showing sufficient sensitivity and specificity for the diagnosis. In fact, the diagnosis is based on the clinical improvement on exclusion diet and relapse after challenge with milk. Goldman et al.'s criteria require three repeated challenges (5), but a single challenge is now widely accepted. Sometimes the interpretation of the provocation test may prove to be difficult, particularly when symptoms are delayed. The reliability of the challenge procedure is improved by the addition of simple tests to clinical observation (12).

The aim of the present work was to investigate the value of the cellobiose/mannitol (C/M) sugar per-

meability test, performed before and after cow's milk challenge, as a tool for the diagnosis of CMAI.

Patients and methods

Patients

Thirty-two patients (16 boys and 16 girls) first presented with symptoms at a median age of 2 months (range 0–13 months). The symptoms at presentation were diarrhea ($n = 17$), vomiting ($n = 13$), growth failure ($n = 17$), colitis ($n = 5$), colic ($n = 1$), anaphylactic shock ($n = 1$), asthma ($n = 1$), and atopic dermatitis ($n = 1$), more than one symptom often being present in the same patient. In all cases, a definite disappearance of symptoms was observed after elimination of cow's milk and cow's milk products. The patients underwent milk challenge at a median age of 13 months (range 3–84 months). The challenge was started in the hospital when the patients were free of any symptoms and signs of CMAI or other illness. Cow's milk was given in increasing doses at half-hour intervals (15, 30, and 60 ml). The patients

remained in the ward for 24 h. The challenge was stopped and the patients were examined when any adverse reaction was noted; no milk was given after the development of symptoms. In patients with no adverse reactions to milk, the normal milk intake appropriate for age was begun on day 2. Patients were prick-tested with whole milk and purified milk proteins (Bayropharm Italiana srl, Milan, Italy); the test was considered to be positive in the presence of a wheal diameter 3 mm greater than that of the negative control. Cow's-milk-specific IgE antibodies were measured by the Phadebas RAST kit (Pharmacia Diagnostics, Uppsala, Sweden). Some laboratory tests were performed before and after challenge: they included polymorphonuclear (PMN) count before and 6 h after challenge; a rise at the sixth hour of 4000/ μ l above the starting value was considered to suggest CMAI (8). Occult blood was also sought for in feces before and in at least two samples after challenge (3).

Cellobiose/mannitol permeability test

The C/M sugar permeability test was performed two times, before and 24 h after challenge. After an overnight fast (6–8 h), the test sugar solution was ingested. After 1 h, patients were encouraged to drink water to promote diuresis. All urine passed within 5 h of the sugar drink was collected into 1% thiomersal used as preservative, the volume measured, and an aliquot stored at -20°C . The composition of the sugar test solution was as follows: 2 g mannitol and 5 g cellobiose made up to 100 ml with tap water to give an osmolality of approximately 270 mOsm. Mannitol was measured colorimetrically (2); cellobiose in urine was determined by enzyme hydrolysis to glucose by β -glucosidase, followed by detection of glucose by the hexokinase method with a test kit

(Boehringer) according to Strobel et al. (10). The ratio of their percentage recoveries (C/M ratio) was calculated. Ratio values above the 90th percentile of a large group of normal subjects, namely 0.022, were considered to be abnormal.

Statistical analysis

The statistical comparison of mannitol excretion, cellobiose excretion values, and C/M ratio, before and after challenge, was performed by paired, non-parametric tests (Wilcoxon). A P value <0.05 was considered to be statistically significant.

Results

Clinical reactions

Of the 32 patients undergoing challenge, 10 developed symptoms. All presented gastrointestinal symptoms (mainly vomiting and diarrhea) at different times (from a minimum of 30 min to a maximum of 3 d after the start of the provocation test). Only one in 10 showed both RAST and prick tests positive to milk proteins (Table 1). In the patients who underwent other laboratory tests, PMN count rose and fecal occult blood became positive in two of six and four of 10 cases, respectively. The remaining 22 infants who had improved on a milk-free diet exhibited no clinical modification after milk challenge. There were no age, sex, and clinical differences at first presentation between the infants who had a positive challenge and those with a negative one. Among the nonrelapsed subjects, two were prick-test positive. In those who underwent other laboratory tests, PMN count and fecal occult blood showed modifications in three of 14 and two of 19 of cases, respectively. In no case did both tests have an abnormal result in the same patient.

Table 1. Clinical data of patients who developed symptoms after cow's milk challenge

Case	Sex	Family atopy	Age 1st present (mo)	Symptoms 1st present	Cow's-milk-specific IgE		Cow's milk challenge			
					Prick	RAST (class)	Age (mo)	Symptoms	Time of appearance	Dose (ml)
1	F	+	1	vom, dia	+	2	31	vom, dia	24 h	100
2	F	–	1	vom, dia	–	0	36	colic, dia	30 min	15
3	M	–	2	vom, dia	–	0	7	vom	11 h	60
								dia	90 min	60
4	F	+	2	dia, ecz	–	1	6	dia	3 d	> 100
5	F	–	2	dia	–	0	9	dia	24 h	100
6	M	–	2	vom, dia	–	0	6	dia, colic, shock	90 min	60
7	M	–	2	vom, dia	–	0	6	dia, colic	90 min	60
8	M	+	5	asthma, colic	–	0	12	vom, dia	60 min	30
9	M	–	2	vom, dia	–	0	4	vom	6 h	60
10	F	–	2	vom, dia	–	0	16	vom, dia	90 min	60

vom=vomiting; dia=diarrhea; ecz=eczema.

Cellobiose/mannitol permeability test

Of the 30 subjects for whom prechallenge permeability values were available, 28 presented a normal C/M ratio; of the two with abnormal values, one was affected by cystic fibrosis. Of 32 children who underwent challenge, 10 showed a postchallenge C/M ratio above the cutoff value of 0.022, nine of them having clinically relapsed after provocation (Fig. 1). In fact, only one patient relapsed after challenge showed a postchallenge C/M ratio within normal values; clinically, he presented diarrhea on the third day after the start of challenge. On the other hand, only one subject not relapsed presented postchallenge C/M ratio values immediately above the cutoff; he was carefully followed-up, but no clinical relapse was observed. Altogether, the sugar permeability test performed after challenge shows, for the diagnosis of CMPI, a sensitivity of 90%, a specificity of 97%, a positive predictive value of 90%,

and a negative predictive value of 97%. The postchallenge C/M ratio increase in relapsed subjects is to be attributed to differences in both the cellobiose and the mannitol excretion. In fact, cellobiose excretion values after challenge were higher and mannitol excretion values lower than figures before challenge, although in both cases the differences did not reach statistical significance (Table 2).

Discussion

Over the last few years, tests based on intestinal permeability to sugars have gained great success. This approach is based on the simultaneous administration of two probe molecules (a monosaccharide and a disaccharide), and estimation of the urinary recovery of each molecule. In situations affecting the integrity of the proximal small intestine, the recovery of monosaccharide is reduced, perhaps because of a reduction in absorptive surface area, while that of intact disaccharide is increased, as paracellular pathways are "leakier" and/or more numerous. Expression of the results as a ratio of disaccharide/monosaccharide recovery gives better distinction between normal and abnormal cases. We here report results obtained with a test based on the measurement of 5-h urinary excretion of a standard dose of cellobiose and mannitol. To measure the former, we used a method established by Strobel et al. (10) and based on enzyme assay: cellobiose is hydrolyzed and the resulting glucose is estimated in the urine. This method is more suitable for ordinary diagnostic laboratories, as it dispenses with time-consuming chromatographic analysis, or other more sophisticated procedures.

The results of this study using cellobiose and mannitol as probes show a normal intestinal permeability in CMAI patients on a milk-free diet. These findings indicate that increased intestinal permeability is not a primary abnormality in CMAI. Of the two subjects with abnormal prechallenge values, one had cystic fibrosis, a disorder known to be associated with increased intestinal permeability (7). Our data also show an increased small bowel permeability after challenge with milk in patients with CMAI, these results being consistent with previous observations. With 51 CrEDTA as a permeability marker, increased permeability was shown in a group of 20 children with proven CMAI while on a milk-containing diet (9). A significant correlation between ingestion of an offending food and alteration of intestinal permeability to sugars has also been observed in subjects with allergy to other foods (1). The increase in the C/M ratio in our patients appears to be mainly due to a rise in cellobiose excretion; moreover, this observation is in keeping with other reports (1).

Among the other tests proposed to help interpret

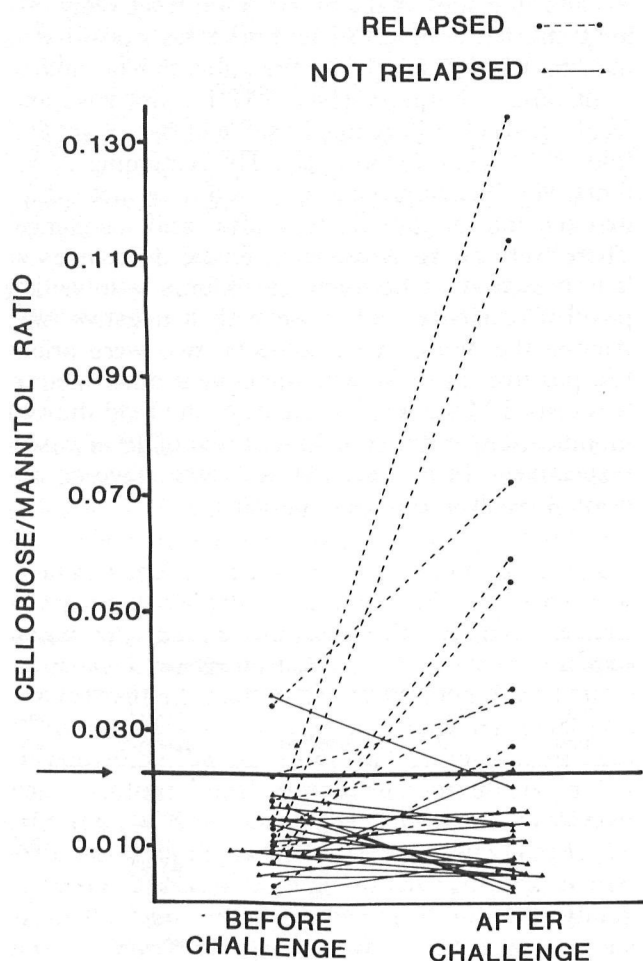


Fig. 1. Cellobiose/mannitol ratio measured in 32 children before and after cow's milk challenge. Relapsed and nonrelapsed patients are identified by dotted and continuous lines, respectively. Arrow indicates cutoff value, set at 90th percentile of control group.

Table 2. Urinary excretion values of cellobiose (C) and mannitol (M), and C/M ratio values, before and after cow's milk challenge

Case	Sex	Cellobiose		Mannitol		C/M ratio	
		before	after	before	after	before	after
1	F	0.059*	0.437*	17.21*	16*	0.003	0.027
2	F	0.850	0.695	39.10	20	0.022	0.035
3	M	0.388	0.464	11.51	6.42	0.034	0.072
4	F	0.023	0.179	2.2	11.24	0.010	0.016
5	F	0.083	0.045	9.24	0.4	0.009	0.113
6	M	0.065	0.064	3.63	2.63	0.018	0.024
7	M	0.054	0.141	4.03	1.5	0.013	0.134
8	M	0.165	0.352	12.10	9.46	0.012	0.037
9	M	0.064	0.273	8.12	4.6	0.007	0.059
10	F	0.037	0.070	4.20	1.27	0.009	0.055
		0.178	0.272	11.13	7.35	0.014	0.057**
11	F	0.066	0.088	7	12.2	0.009	0.007
12	M	0.015	0.094	2	18	0.007	0.005
13	M	N.D.	0.227	N.D.	17	N.D.	0.013
14	F	0.104	0.016	11	2	0.009	0.005
15	F	0.167	0.100	10	7.2	0.017	0.014
16	M	0.202	0.050	14.1	10	0.014	0.005
17	M	N.D.	0.122	N.D.	28	N.D.	0.004
18	M	0.034	0.058	7.1	11	0.005	0.005
19	M	0.014	0.098	6.84	11	0.002	0.008
20	F	0.413	0.098	11.5	5	0.036	0.020
21	M	0.198	0.306	20.32	13.5	0.009	0.023
22	M	0.206	0.095	13.33	5.84	0.015	0.016
23	F	0.098	0.030	6.21	2.75	0.015	0.011
24	F	0.054	0.115	9.47	9.30	0.005	0.012
25	M	0.258	0.100	13.26	7.34	0.019	0.014
26	M	0.175	0.088	19.78	14.70	0.009	0.006
27	M	0.066	0.073	5.94	5.25	0.011	0.014
28	F	0.212	0.001	12.23	0.58	0.017	0.002
29	F	0.003	0.001	0.2	0.32	0.015	0.003
30	F	0.049	0.044	4.12	5.73	0.012	0.007
31	F	0.067	0.133	4.55	8.5	0.015	0.016
32	F	0.210	0.117	23.03	10.42	0.009	0.011
		0.130	0.093	10.1	9.35	0.012	0.010

* Percentage of the dose (5 g cellobiose, 2 g mannitol) excreted in the urine in the 5 h following the oral load.

** $P < 0.002$.

the results of a challenge, xylose oral load, PMN count, and presence of occult blood have been used in the past. Although, taken together, their contribution to the diagnosis may prove to be good (in one large multicenter study (12), at least one of these tests was positive in all patients with symptoms), taken singly, their sensitivity has been reported to range from 23 to 41%. In comparison, the sugar permeability test shows a much higher sensitivity, approaching 90%. In particular, there are several reasons for preferring the C/M sugar test to the xylose oral load. We have already shown that the sugar permeability test is more sensitive than the D-xylose test in the diagnosis of celiac disease (11). As compared with the xylose test, the C/M test, which measures the urinary excretion of two sugars, expressing the results as a ratio, offers the advantage of canceling the interferences by nonintestinal extraneous

factors, such as failure to ingest the entire solution, slow gastric emptying, and incomplete urinary excretion; similarly, the possible drawback resulting from incomplete urinary collection, particularly in infants, is minimized by expressing the results as a ratio between probes.

In this study we have investigated almost exclusively patients with gastrointestinal symptoms. The great majority should be considered to be intolerant rather than allergic, as only a few of them had milk-specific IgE. Nevertheless, we think that the permeability test may help also in the diagnosis of milk-allergic patients. In fact, it has been shown that patients with atopic dermatitis have increased intestinal permeability after challenge, if milk is responsible for the exacerbation of the skin lesions (4). Furthermore, a recent Finnish study (6) has found the same alterations of intestinal permeability after

the milk provocation test, both in relapsed patients with gastrointestinal symptoms (of whom only 20% showed milk-specific RAST) and in patients showing cutaneous symptoms.

In conclusion, on the basis of these results, we recommend the use of the sugar permeability test, in addition to clinical observation, as an aid for the assessment of the results of a provocation test in infants with suspected CMAI.

Acknowledgment

The excellent technical assistance of Maria Cucciardi is acknowledged.

References

1. ANDRÉ C. Diagnosis of food allergy and evaluation of therapeutic effectiveness by measurement of intestinal permeability. *Presse Med* 1986; 15: 105-8.
2. CORCORAN AC, PAGE IH. A method for the determination of mannitol in plasma and urine. *Biol Chem* 1947; 170: 165-71.
3. CUCCHIARA S, DE RITIS G, FOLLO D, et al. Cow's milk protein intolerance: evaluation of clinical, histological, endoscopic and laboratory criteria for post-challenge diagnosis. *Ital J Gastroenterol* 1983; 15: 231-5.
4. DUPONT C, BARAU E, MOLKHOUP, RAYNAUD F, BARBET JP, DEHENNIN L. Food-induced alteration of intestinal permeability in children with cow's milk-sensitive enteropathy and atopic dermatitis. *J Pediatr Gastroenterol Nutr* 1989; 8: 459-65.
5. GOLDMAN AS, ANDERSON DW, SELLERS WA, et al. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963; 32: 425-43.
6. JALONEN T. Identical intestinal permeability changes in children with different clinical manifestations of cow's milk allergy. *J Allergy Clin Immunol* 1991; 88: 737-42.
7. LECLERCQ-FOUCART J, FORGET P, VAN CUTSEN J. Lactulose/rhamnose intestinal permeability in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1987; 6: 66-70.
8. POWELL GK. Milk- and soy-induced enterocolitis of infancy. Clinical features and standardization of challenge. *J Pediatr* 1978; 93: 553-60.
9. SCHRANDER JJP, UNSALAN-HOOYEN RWM, FORGET PP, JANSEN J. 51 CrEDTA intestinal permeability in children with cow's milk intolerance. *J Pediatr Gastroenterol Nutr* 1990; 10: 189-92.
10. STROBEL S, BRYDON WG, FERGUSON A. Cellobiose/mannitol sugar permeability test complements biopsy histopathology in clinical investigation of the jejunum. *Gut* 1984; 25: 1241-6.
11. TRONCONE R, STARITA A, COLETTA S, MAYER M, GRECO L. Antigliadin antibody, d-xylose, and cellobiose/mannitol permeability tests as indicators of mucosal damage in children with coeliac disease. *Scand J Gastroenterol* 1992; 27: 703-6.
12. VENTURA A, GRECO L, BALLI F, et al. Cow's milk allergy in the first year of life. *Acta Paediatr Scand* 1988; 348 (Suppl.): 1-14.
13. WALKER-SMITH JA. Milk intolerance in children. *Clin Allergy* 1986; 16: 183-90.

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