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Intestinal permeability after single dose gluten challenge in coeliac disease

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Abstract

The changes of intestinal permeability before and after a gluten load were studied. The study group comprised 27 patients with coeliac disease (mean age 12·3 years) and 19 healthy controls matched by sex and age. Intestinal permeability was studied by measuring the urinary excretion of two sugars, lactulose and L-rhamnose, before and six hours after the ingestion of five palatable biscuits made with 50 g of gluten powder. The patients with coeliac disease had been on a gluten free diet during the previous two years. After the gluten load lactulose and L-rhamnose urinary excretion changed significantly in patients, and a significant increase in the lactulose:L-rhamnose ratio was also observed. No significant changes were observed in the controls. In view of the modification of the three biopsies diagnostic protocol made by the European Society for Paediatric Gastroenterology and Nutrition, permeability tests associated with single gluten challenges may be an added contribution to the accuracy of the diagnosis in childhood.

Abnormalities of intestinal permeability have been shown by several authors in a series of gastroenterological diseases.[1–7] The sensitivity of the permeability test is considerable, as has been shown in transient gastrointestinal mucosa alterations such as those found in gastroenteritis.[8] Typical mucosal alterations of patients with coeliac disease are indeed responsible for considerable abnormalities in sugar permeability.[1 9 10]

Passive intestinal permeability can be studied in man by various probe markers: polyethyleneglycol 400, ^{51}Cr-EDTA, mannitol, cellobiose, lactulose, or L-rhamnose.[7–14] All are excreted nearly unmodified in urine. Lactulose (with a molecular weight of 340) permeates the intestinal mucosa through large 'pores' of low incidence, which are probably associated with the paracellular 'tight junction' complexes. Less than 1% of ingested lactulose permeates the intestinal mucosa and appears in the urine. L-Rhamnose is a monosaccharide (with a molecular weight of 164) which permeates mainly via a transcellular route through smaller water filled pores in the enterocyte cell walls that larger molecules, such as lactulose, cannot penetrate. Intestine mucosal damage decreases the area for absorption. This is a factor which negatively influences the high transcellular permeation of small molecules like L-rhamnose. It increases the incidence of large pores (paracellular and extrusion cell zones) which enhances the passive permeation of large molecules like lactulose.[14] The ratio of lactulose:L-rhamnose in urine is appreciably raised during severe derangement of intestinal integrity and allows an excellent diagnostic discrimination of patients with severe gut disease.

Sugar permeability has been extensively used in the adult gastroenterological patient, but fewer reports are available about its application in the paediatric patient.[15] Hamilton *et al* studied children with coeliac disease and cows' milk intolerance and showed the low specificity of the test.[16] Adult coeliac patients have been studied by the same group in different phases of the disease. Sugar permeability improves with the start of the gluten free diet and deteriorates with the reintroduction of gluten in the diet. Six adult patients were challenged with a single dose of gluten and their permeability was assessed before and 24 hours after the challenge. All showed a significant gluten induced modification. The authors concluded that a single dose gluten challenge with an intestinal permeability evaluation could be an adequate substitute for the recommended long term challenge.[16] Gluten challenge has been proved to be associated with a consistent reduction of growth velocity in children, with clinically relevant symptoms, and with a considerable degree of anxiety in children and families.[17]

The objective of this study was to evaluate the intestinal permeability by an oral load of lactulose and L-rhamnose in patients with coeliac disease after a single dose of gluten. The aim was to minimise the time and damage caused by prolonged gluten challenge in these subjects.

Subjects and methods

Twenty seven patients with coeliac disease participated in the study. They were included in the study if (1) they were aged 7 years or above and were aware of what a gluten challenge would involve. (2) The diagnosis of coeliac disease had been confirmed by a full diagnostic protocol with flat mucosal biopsy in the florid phase and histological relapse on gluten challenge, according to the indications of the European Society for Paediatric Gastroenterology and Nutrition (1982). (3) They had been on a gluten free diet for at least two years. (4) Antigluten antibodies were within normal limits. Nineteen healthy subjects were recruited as controls and they were age and sex matched with the cases.

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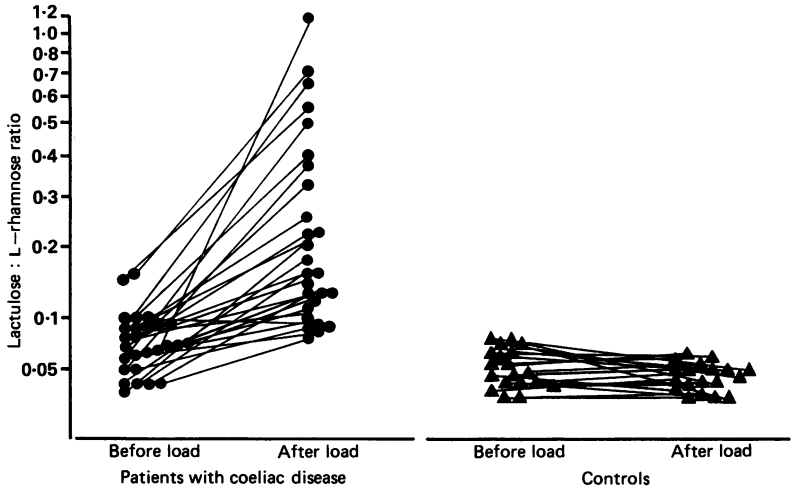
Excretion vaues before and after gluten challenge. Results are mean % oral dose (95% confidence intervals)

	Lactulose	*L-rhamnose*	*Lactulose:L-rhamnose ratio*
Controls:			
Before gluten	0·29 (0·32 to 0·25)	5·63 (6·14 to 5·11)	0·052 (0·06 to 0·04)
After gluten	0·27 (0·30 to 0·24)*	5·93 (6·44 to 5·42)*	0·045 (0·05 to 0·04)*
Mean change†			−9·3 (−11·8 to −6·8)
Patients with coeliac disease:			
Before gluten	0·43 (0·48 to 0·37)	4·94 (5·62 to 4·26)	0·106 (0·15 to 0·07)
After gluten	0·65 (0·79 to 0·52)**	2·87 (3·43 to 2·03)***	0·316 (0·42 to 0·21)***
Mean change†			+240 (260 to 220)

Student's *t* test for paired samples before and after gluten (log transformed values).
*p>0·2, **p<0·005, ***p<0·00001.
†Mean change has been calculated as the antilog of the log transformed value of the percent ratio change before and after the gluten load.



Lactulose:L-rhamnose ratio before and after gluten load in patients with coeliac disease and in controls.

PROCEDURE

Intestinal permeability was assessed twice: once after an overnight fast before the gluten challenge and again six hours after the ingestion of five palatable biscuits made with 50 g of gluten powder that was wheat germ free (Salsa), cooked with sugar and flavours but no yeast. The bladder was emptied, and 5 g of lactulose and 1 g of L-rhamnose dissolved in 100 ml water were administered. The solution was nearly isotonic (220 mosm/l). The osmolarity of the test solution was kept below the level known to induce an alteration of intestinal permeability or motility.[5] A five hour urine collection was performed, the volume was recorded, and a sample, preserved with thiomersal (Merthiolate, 1 mg/10 ml urine), was stored at −20°C before analysis using a method previously described.[5] The conditions used were a starting temperature of the oven at 110°C, with an increase of 3°C/minute up to 255°C, injector temperature 275°C, detector temperature 305°C, gas carrier (nitrogen) flow 35 ml/min, attenuation input 1:100 and output 1:2, and back off 1:100.

The lactulose retention time relative to that of turanose was 0·92, and the retention time of L-rhamnose relative to that of 3-O-methyl-D-glucose was 0·79.

STATISTICAL ANALYSIS

All study variables were assessed for normality. The distribution of lactulose, L-rhamnose, and the ratio before the challenge did not show any major skewness. The values after the challenge, in cases but not in controls, showed a significant skewness when compared with a normal distribution. A log transformation was therefore adopted in order to reduce the skewness for the data of all the groups when differences between the means were assessed.

Results

No sex difference was noted in the two groups. Mean age was 13·8 (range 5–24) in controls and 12·3 (range 7–21) in cases. The frequency distribution of age in cases was not significantly different than the controls (χ^2 test). None of the controls showed any discomfort after gluten ingestion. Ten cases did show significant disturbances, mostly transient vomiting and abdominal pain two hours after gluten ingestion.

Before gluten challenge, permeability was normal in all controls and in 25/27 cases. The two cases with abnormal permeability before the challenge (lactulose:L-rhamnose ratio above 0·1) claimed to be on a strict gluten free diet. The L-rhamnose excretion before the challenge was not statistically different in cases compared with controls (unpaired Student's *t* test), while the lactulose excretion showed a statistical difference between the two groups (unpaired *t* test=−3·9, p<0·001). After the gluten challenge, the controls showed on average no alteration of their permeability (no statistical difference for lactulose, L-rhamnose, and the ratio). In the patients with coeliac disease the lactulose excretion increased about 50%, the L-rhamnose halved, and the ratio almost trebled (table).

If the limit of the mean+1·96 SD of the lactulose:L-rhamnose ratio of the controls after the challenge (0·0646) is assumed as the level of normal absorption, none of the cases show a normal absorption after gluten challenge. All the controls are within normal limits after having ingested the same amount of gluten biscuits. The figure shows the distribution of the lactulose:L-rhamnose ratio in cases and controls before and after the gluten challenge.

Discussion

A hypertonic test solution was often adopted to increase sensitivity in adults,[18 19] while experience in most investigations of children is

actually confined to the use of an isotonic test solution to avoid the modifications that the hyperosmolarity would induce in vivo.[10] Recently Hodges *et al* demonstrated that a hypertonic sugar absorption test is well tolerated even by young children.[20]

Intestinal permeability was outside the normal range in all treated subjects with coeliac disease after the challenge. Two of them showed significantly higher values than controls before gluten challenge. They claimed to be on a gluten free diet. Minor permeability alterations have often been described in treated coeliac patients.[9 16]

The single dose load of a consistent amount of gluten powder that was free of wheat germ does not produce any permeability change in healthy controls. It produces a consistent alteration of the intestinal permeability in most of the cases, including the ones with slightly abnormal permeability before the challenge.

The lactulose:L-rhamnose ratio allows a good discrimination between cases and controls after the gluten challenge. Cases who developed temporary symptoms on gluten ingestion did not show a higher ratio after the challenge than the whole coeliac group. In five of these cases, the ratio after challenge ranged from 0·09 to 0·20, in three there was a very clear rise in the ratio due to the challenge (from 0·095 to 0·14, from 0·07 to 0·095, and from 0·09 to 0·21) and in two cases the ratio moved from 0·09 to 0·11. A biological variation of the response to gluten ingestion is not uncommon in studies of coeliac disease.

Results from 27 cases are not enough to state anything about specificity and sensitivity, but the observed differences are sufficiently consistent to make the test a useful tool to evaluate gluten challenge in coeliac children. Those subjects responding sufficiently strongly to the test can be regarded as successful and the few others, who respond less sharply, should go on to a formal long term gluten challenge.

Sensitivity to gluten in patients with coeliac disease is often age dependent and children appear to be more sensitive than adults to occasional gluten introduction. For this reason a single dose of gluten is likely to produce an intestinal response in coeliac children. Histological abnormalities may not have sufficient time to develop after a single dose of gluten, but physiological mechanisms may well be affected by the offending agent.

The response to gluten, in most of our cases, was not moderate or borderline, but sharp and consistent.

The final confirmation of the diagnosis of coeliac disease in children is much too important for the child to be based on the results of a single test, with no mucosal evaluation, as suggested by Hamilton *et al*.[16] We found, from a cohort of 426 cases, that after a mucosal relapse further challenges were required by teenage coeliacs and their families in order to maintain the diet. Many of these tests may be carried out in the future with no invasive techniques by a single dose gluten challenge and permeability evaluation. We have previously used the combination of antigluten antibodies with oral xylose load to follow up the gluten challenge,[21] but most cases required at least two months to show a clear relapse. Although no symptoms appeared in such a time interval, growth arrest occurred in a few patients. A six hour gluten challenge does reduce the effect on the patient and make the challenge a routine ambulatory test with an answer within a few days. More work will help to identify the best gluten dose and osmolality of the sugar solution to improve the sensitivity of the test, but no absolutely perfect method will remove the biological variability in the expression of the intestinal permeability.

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