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Intestinal absorption of food antigens in coeliac disease

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SUMMARY Serum concentrations of ovalbumin, β-lactoglobulin, and antigen-antibody complexes were measured after jejunal administration of milk and raw egg in 6 children with active coeliac disease and in 4 controls. The results did not support the hypothesis of a generalised increase in absorption of antigens from the intestinal lumen in coeliac disease.

It is well known that antigenically-intact food proteins are absorbed across the healthy gastrointestinal mucosa;[1] the amounts absorbed are insignificant nutritionally but they are sufficient to immunise, and antibodies to cows' milk proteins may be found in most normal children.[2] Increased absorption of food proteins has been demonstrated in infants recovering from diarrhoea[3] and it has been suggested that this is owing to mucosal damage.

In coeliac disease, where profound mucosal damage occurs, it has been proposed that there may be a generalised increase in the absorption of antigens from the intestinal lumen and that an immunological response to these antigens contributes to the lesion of the small intestine.[4] In common with several other gastrointestinal diseases, coeliac disease is associated with increased antibody responses to dietary proteins[5–7] but there is no evidence that these antibodies are directly responsible for the intestinal disease.

To test the hypothesis that absorption of dietary antigens is increased in active coeliac disease we have measured the serum concentrations of β-lactoglobulin, ovalbumin, and antigen-antibody complexes (AAC) after administration of milk and egg to children with active coeliac disease, to children with coeliac disease in remission, and to control children.

Subjects

Ten children were studied while having a jejunal biopsy for investigation and confirmation of the diagnosis of coeliac disease. Six were having a post-gluten challenge biopsy which showed severe partial villous atrophy in 5, and subtotal villous atrophy in the sixth. Two patients were having pre-challenge biopsies while on gluten-free diets; they were free from symptoms and their biopsies were normal. Two further children in whom no organic cause was found were being investigated for suspected malabsorption and their biopsies were normal. The children therefore comprised a group of 6 patients with severe mucosal damage and 4 patients with normal mucosa.

Methods

The study was approved by the hospital's ethical committee and fully informed consent was obtained in each case. All patients were fasted overnight and, before the biopsy specimens were taken, the patients were given a mixture of milk (10 ml/kg; equivalent to 25 mg β-lactoglobulin/kg) and beaten egg (1·5 ml/kg; equivalent to 150 mg ovalbumin/kg) to a maximum volume of 500 ml via a jejunal tube. Indwelling cannulae that had been inserted for drug administration during the biopsy procedure were used for blood sampling and small samples were taken just before administration of the milk/egg mixture and at 30 minutes and 1, 2, 3, 4, and 5 hours thereafter. Serum was separated within 2 hours, placed in aliquots, and stored at −70°C.

Antigen detection assays. Concentrations of ovalbumin and β-lactoglobulin were measured by a two-site solid-phase radioimmunoassay[8] using polyvinyl plates. Results in ng/ml (limits of detection about 1 ng/ml) were read from standard curves and expressed as ng/ml serum/g antigen administered/kg bodyweight.

Antigen-antibody complexes. Circulating AAC were measured by the polyethylene glycol (PEG) precipitation method. This was performed using 12% PEG in EDTA buffer pH 7·6 diluted with sample to give a final PEG concentration of 2% and incubated overnight at 4°C.[9] Precipitated immunoglobulins G, A, and M were measured by radial immunodiffusion[10] and the results expressed as percentages of the total immunoglobulin concentrations of each class.

Results

Levels of ovalbumin and β-lactoglobulin were undetectable before administration of the test meal in each case. After the mixture of milk and egg had been given 4 coeliac patients showed progressive increases in serum ovalbumin concentrations (Fig. 1a) but the remaining 2 showed no evidence of absorption. Two of the 4 children with normal mucosa demonstrated absorption of ovalbumin (Fig. 1b) which was similar in degree to that seen in the 4 patients with mucosal damage.

The 2 children with normal biopsies who showed no detectable ovalbumin absorption were the coeliacs in remission. Although levels of ovalbumin did not correlate with having coeliac disease or with the degree of mucosal damage judged microscopically, the greatest degree of ovalbumin absorption occurred in a child with subtotal villous atrophy (the most severe form of mucosal damage).

There was no good evidence of β-lactoglobulin absorption in 4 of the children with active coeliac disease (Fig. 2a) although the other 2 such children had pronounced increases in β-lactoglobulin concentration after administration of the test meal. The maximum values in those 2 patients were greater than those of the 2 coeliacs in remission while the controls with normal mucosas did not develop measurable concentrations of β-lactoglobulin (Fig. 2b). There was no demonstrable absorption of β-lactoglobulin in the child with subtotal villous atrophy.

None of the subjects had significant changes in IgG, IgA, or IgM complexes after antigen administration. The results for the children with normal mucosas are compared with those of the children with abnormal biopsies in the Table. One child with

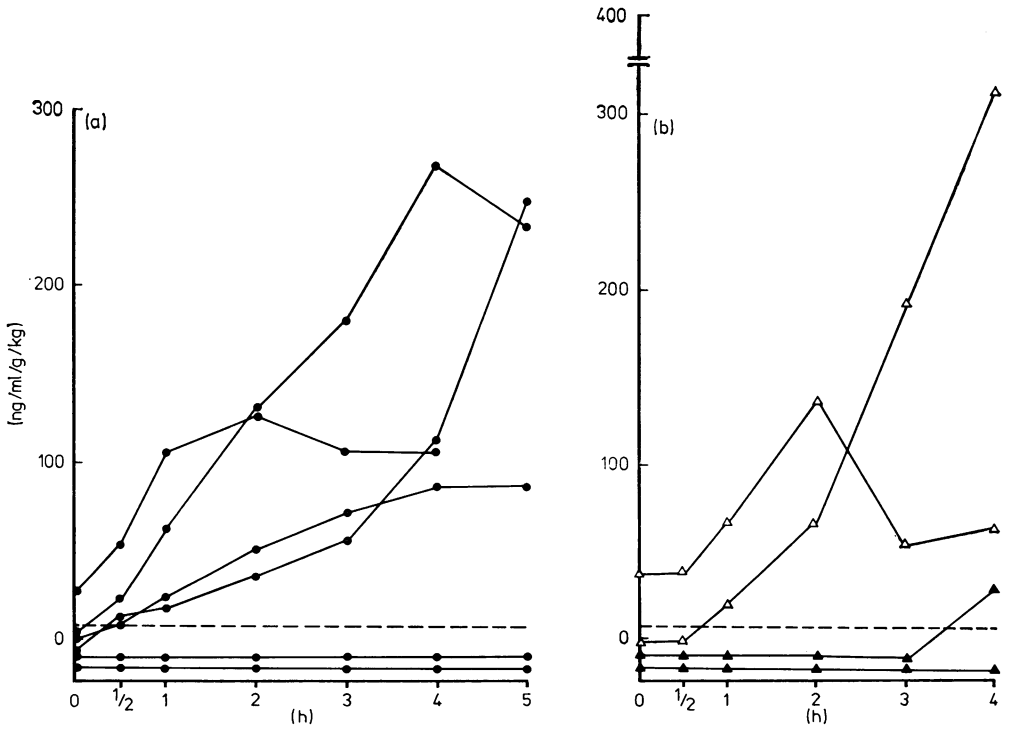

Fig. 1 *Serum concentrations of ovalbumin after food provocation in (a) 6 children with active coeliac disease and (b) 4 children with normal mucosa (open triangles are the normal children and closed triangles are coeliacs in remission). The broken line indicates the limit of sensitivity for the assay.*

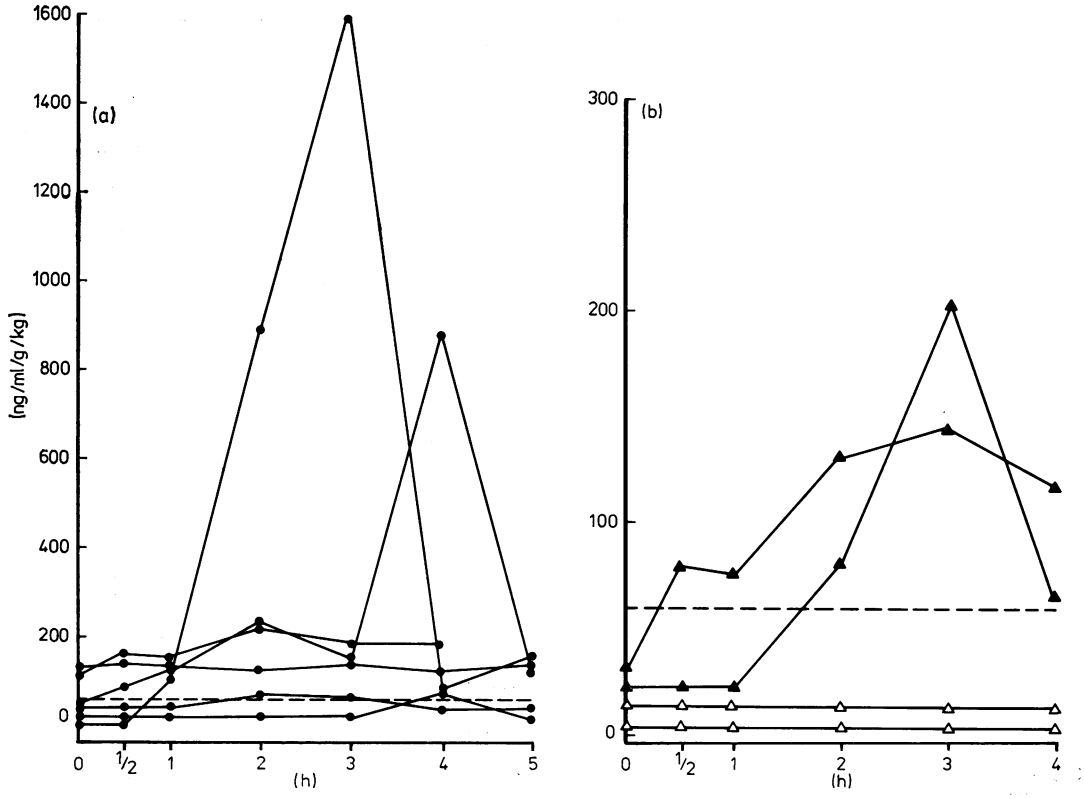

Fig. 2 *Serum concentrations of β-lactoglobulin after food provocation in (a) 6 children with active coeliac disease and (b) 4 children with normal mucosa (open triangles are the normal children and closed triangles are coeliacs in remission). The broken line indicates the limit of sensitivity for the assay.*

Table *Antigen-antibody complex levels (mean and range) after food provocation in 6 children with active coeliac disease compared with 4 controls*

Immunoglobulin	*Before*	*Time after food provocation (hours)*					
		30 min	*1*	*2*	*3*	*4*	*5*
Patients							
IgG	1·88	1·43	1·54	1·94	1·65	1·45	1·60*
	1·36–2·44	0·82–2·44	0·82–2·49	0·62–2·64	0·93–2·46	0·62–2·63	0·94–2·2
IgA	1·01	0·91	0·88	1·20	1·13	0·88	0·78*
	0–1·64	0–1·78	0–1·60	0–1·82	0–1·61	0–1·30	0·34–1·38
IgM	3·89	4·61	4·26	3·71	4·41	4·04	4·21*
	0–6·05	0–9·26	0–8·55	0–6·55	0–9·26	2·08–6·55	0–6·27
Controls							
IgG	1·70	1·18	1·65	1·05	1·17	1·30	—
	0·87–3·03	0·65–2·03	0·70–2·83	0·52–1·96	0·90–1·56	0·85–2·28	—
IgA	1·54	1·33	1·61	1·01	1·31	1·06	—
	0·91–2·72	0·65–2·36	0·78–2·19	0·61–1·65	1·00–2·17	0·82–1·48	—
IgM	5·25	3·98	4·08	3·85	3·74	4·59	—
	32·7–7·81	2·20–5·19	3·13–4·94	3·27–4·73	2·37–5·71	3·07–6·29	—

* Only 5 patients as one did not complete the study.
Results are expressed as the percentage of total immunoglobulin for each class precipitated by 2% polyethylene glycol.

coeliac disease was found to have IgA deficiency; this was the child who also had severe partial villous atrophy on biopsy.

Discussion

These preliminary data do not support the concept of increased permeability of gut mucosa to food antigens in active coeliac disease. The evidence in support of this theory is circumstantial and rests on the demonstration of higher levels of food protein antibodies than normal in patients with coeliac disease[5 6 7] and increased absorption of molecules such as lactulose.[11] However, other studies have shown reduced absorption of small molecules—such as polyethyleneglycol 400 and urea.[12 13] The number of subjects in our study was small because of ethical constraints, but all were given equivalent amounts of antigen according to bodyweight and there were no apparent differences in absorption of β-lactoglobulin and ovalbumin between children with damaged mucosa and those with normal mucosa. The levels were similar to those seen in our earlier study of dietary antigen absorption in term infants.[14] Thus we are unable to confirm the notion that a damaged mucosa is more permeable to food antigens than a normal mucosa. In addition to mucosal permeability other factors—such as gut transit time, pancreatic function, local immunity, and the extent of the damaged mucosa—could theoretically influence circulating concentrations of food antigens. However, we did not feel justified in measuring these other variables in the children. It seems likely that the serum concentrations observed were in the main a function of intestinal permeability to the antigens.

The detection of food protein antibodies in coeliac disease does not necessarily imply markedly increased absorption of food antigens because the amount of antigen required to immunise by the oral route is very small.[15] Such antibodies to food proteins could indeed be associated with decreased systemic absorption of these antigens if AAC form locally and deposition occurs within the gut wall.

Further studies in animals have supported the concept that the mucosa with partial villous atrophy does not allow increased absorption of food antigens. We have studied rats infected with *Nippostrongylus brasiliensis* (which causes partial villous atrophy) and at the time of maximal mucosal disease there was no increase in the absorption of food proteins.[16]

In the earlier studies it was shown that in healthy adults and children the physiological mechanism for clearing food antigens is by the formation of small IgA complexes.[17] In the present study there were no significant changes in the levels of IgG, IgA, and IgM complexes measured by polyethylene glycol precipitation, a method which detects the larger complexes which may be of more pathological significance.[18] A direct assay of the antigen content of the AAC was not performed but increases seem unlikely in view of the low levels of AAC. This suggests that the AAC demonstrated in other studies of coeliac disease in remission and relapse[19 20] may have been non-specific and that they did not necessarily contain food protein antigens.

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