

Changes in N-terminal glucagon-like immunoreactivity and insulin during short-term gluten challenge in childhood coeliac disease

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SUMMARY Sixteen patients (aged 3.5-14.3 years) with normal jejunal mucosa, originally diagnosed as having coeliac disease at least 18 months before, were started on gluten challenge. The 'end point' of challenge was significant deterioration in jejunal mucosa morphologically and morphometrically. Studies carried out both before and after challenge included intestinal absorption of D-xylose and glucose, and release of insulin and N-terminal glucagon-like immunoreactivity (N-GLI). After gluten challenge, there were significant increases in plasma N-GLI at both 45 ($P<0.05$) and 120 minutes ($P<0.03$) after oral glucose. Significant reduction occurred in glucose absorption at 45 minutes ($P<0.04$), in one-hour D-xylose absorption ($P<0.01$) and fasting serum cholesterol ($P<0.01$). Plasma N-GLI showed significant negative correlations with D-xylose absorption ($P<0.003$) and serum cholesterol ($P<0.004$).

The development of specific radioimmunoassay methods for measurement of gastrointestinal hormones in blood has provided new methods of studying familiar alimentary disorders. In adults with active coeliac disease for example, Besterman and colleagues¹ have shown enhanced release of 'enteroglucagon' after a 530 calorie test breakfast, which was not the case in either healthy individuals or those with treated coeliac disease. Also, others have shown in coeliac disease, impaired release of secretin after intraduodenal infusion of dilute hydrochloric acid.² To date, all studies have investigated gastrointestinal hormone profiles in patients with established clinical disease and most have been concerned with adult patients. The purpose of this study was to investigate gastrointestinal hormones in children with coeliac disease and to relate these to other biochemical measurements during gluten challenge.

Methods

PATIENTS

Sixteen children, aged 7.3 ± 3.3 ($M\pm SD$) (range 3.5-14.3) years who had been on a gluten free diet for

4.6 ± 2.9 (1.6-12.6) years, were challenged orally with gluten powder. This was done primarily to confirm a clinical diagnosis of coeliac disease in accordance with criteria enunciated by the (then) European Society of Paediatric Gastroenterology.³ Fifteen of the patients had had original jejunal biopsies which were 'flat' or almost so. Gluten withdrawal had begun at the age of 2.6 ± 2.4 (0.6-8.7) years and in each patient had produced clinical improvement in appetite, bowel habit, nutrition, and growth.

Gluten challenge was discussed with the parents and with the older children themselves and all participated enthusiastically. Patients fasted overnight and the following morning a fine, polyethylene cannula was introduced into a peripheral vein. Blood was withdrawn for fasting blood glucose, plasma insulin, N-terminal glucagon-like immunoreactivity (N-GLI), haemoglobin, serum electrolytes, albumin, cholesterol, and iron; specimens for gastrointestinal hormone assays were taken into iced, heparinised glass tubes. Oral glucose (1.75 g/kg body weight) was given in 50 ml of distilled water and 45 and 120 minutes later blood withdrawn for glucose, insulin, and N-GLI. At this point, D-xylose (5 g in 30 ml of water) was given by mouth and 60 minutes later blood withdrawn for the one hour (absorption) value.⁴

Jejunal biopsy was performed⁵ and assessed qualitatively by two independent observers who examined

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coded biopsies. Provided this was histologically normal, or showed only minimal inflammatory cell infiltrate or changes in villus morphology, the patient was started on gluten powder†—15 g daily for those less than 6 years of age and 25 g for older children. The patients were carefully assessed at frequent clinic visits and the consumption of gluten calculated by reweighing consignments of powder. Gluten challenge was terminated only when jejunal mucosae demonstrated shortened, blunted villi, crypt hyperplasia, and heavy inflammatory cell infiltration. Before and after gluten challenge, surface to volume ratio (SVR) was measured according to Dunhill and Whitehead⁶ and the intraepithelial lymphocyte count as described by Ferguson and Murray.⁷

Serum electrolytes, albumin, cholesterol, and iron were determined on a SMAC autoanalyser, blood glucose by the glucose oxidase method, and D-xylose according to Roe and Rice.⁸

Insulin radioimmunoassay was carried out using antibody GP 25,⁹ raised against porcine insulin and cross-reacting identically with both human and pork insulin; 50% cross-reactivity was present with porcine pro-insulin. This assay detects 0.5 mU/l insulin with 95% confidence limits. N-GLI was measured using antibody YY57 with N-terminal specificity for glucagon.¹⁰ This antibody shows marked cross-reactivity with GLI species of gut origin¹¹ and can detect 8 ng/l glucagon with 95% confidence limits.¹² Data were analysed using the Statistical Package for the Social Sciences (SPSS) and paired Student's *t* test.

Results

The mean length of gluten challenge was 0.34 (range 0.14–0.83) years. The mean gluten consumption was 87% of that suggested; five of the 16 patients developed symptoms and abnormal jejunal histology and challenge was immediately stopped. After three months' gluten challenge, normal jejunal mucosa persisted in four patients, and a further period of challenge was needed before significant histological deterioration occurred. Qualitative abnormality was accompanied by a reduction in mean SVR ($P < 0.0005$)

†Powder is 70% pure gluten. Energen Ltd., Ashford, Kent.

and increase in mean intraepithelial lymphocyte counts ($P < 0.0005$); there were no significant differences in these measurements between symptomatic and asymptomatic patients.

After gluten challenge, there were significant increases in mean plasma N-GLI at both 45 ($P < 0.05$) and 120 minutes ($P < 0.02$) after oral glucose (Figure). Four patients whose jejunal mucosa remained normal at three months failed to show this increase. Significant reductions were also observed in fasting serum cholesterol ($P < 0.01$), blood glucose at 45 minutes ($P < 0.04$), and D-xylose absorption ($P < 0.01$). The fasting and 120 minute blood glucose levels were not significantly changed, nor were the insulin concentrations (Table). Plasma N-GLI levels, fasting and at 45 and 120 minutes each showed significant negative correlations with the D-xylose absorption ($r = -0.67$, $P < 0.003$)[‡] and fasting cholesterol ($r = -0.66$, $P < 0.004$).[‡] N-GLI concentrations in the five symptomatic patients were similar to those in the asymptomatic group. The relationship between the differences in SVR and 45 minutes N-GLI before and after gluten challenge just failed to reach significance ($P = 0.05$).

Discussion

The presence of persisting gluten sensitivity in this group of patients was established by means of a carefully controlled short-term gluten challenge. Morphological and morphometric abnormalities were accompanied by changes in small gut function as shown by reduction in D-xylose absorption⁴ and possibly the fall in fasting cholesterol. Changes in plasma glucose after gluten challenge were minor compared with studies principally carried out in adults with untreated coeliac disease. In these, the releasing stimulus was usually a test meal.¹ In our study, the expected rise in insulin was maintained after gluten challenge by contrast with the poor insulin response previously described in children with active coeliac disease.¹³ These findings suggest that any changes in either glucose absorption or insulin secretion are features of long-standing coeliac disease.

‡*r* and *P* values relate to the 120 minute N-GLI levels.

Table Blood glucose and plasma hormone values ($M \pm SEM$) before and after gluten challenge both fasting and after oral glucose

	Fasting		45 minute		120 minute	
	Pre-GC	Post-GC	Pre-GC	Post-GC	Pre-GC	Post-GC
Blood glucose (mmol/l)	5.08 ± 0.18	4.92 ± 0.16	8.20 ± 0.74	7.02 ± 0.36*	6.35 ± 0.41	6.18 ± 0.39
Plasma insulin (mU/l)	4.37 ± 0.84	5.47 ± 1.11	23.87 ± 4.60	20.91 ± 4.14	15.87 ± 3.05	19.72 ± 5.99
Plasma N-GLI (ng/l)	111.1 ± 40.7	184.1 ± 48.4	96.4 ± 29.7	210.7 ± 53.8*	79.6 ± 20.9	172.9 ± 39.1*

*A significant difference ($P < 0.05$) compared with the corresponding pre-gluten challenge value.

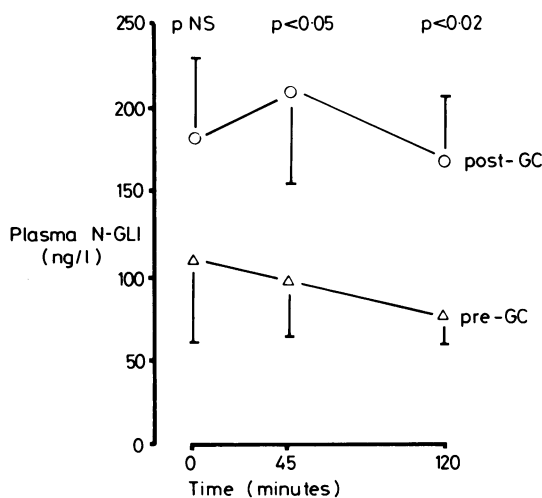


Fig. Plasma N-GLI levels ($M \pm SEM$) in relation to time after oral glucose, both before (pre-GC) and after (post-GC) gluten challenge.

After gluten challenge, the most striking alterations after oral glucose were found in N-GLI release beyond 45 minutes (Figure). In an attempt to keep the volume of blood samples to a minimum, C-terminal GLI, which is a more specific estimate of pancreatic glucagon, was measured in only three patients both before and after gluten challenge. A rise in C-GLI was not observed after oral glucose as reported previously;¹⁴ values before and after challenge were similar. Previous abnormalities with respect to glucagon have been demonstrated using N terminally directed antisera, which measures all known species of GLI.¹⁴

A significant increase in N-GLI was not apparent until appreciable deterioration in jejunal histology had occurred. The degree of morphological change as measured by the fall in SVR appeared to correlate with the rise in N-GLI at 45 minutes, but the relationship in this series just failed to reach statistical significance at the 5% level.

GLI peptides of gut origin are thought to influence mucosal growth and to slow bowel transit.^{15,16} It may be, therefore, that the increased enterocyte turnover and reduced transit time often seen in untreated coeliac disease are consequences of these higher peptide levels. Nevertheless, the increases found are not as great as previously reported by Besterman and colleagues.¹ This may be related to the younger age of our patients, coupled with the fact that they were studied after short-term gluten challenge, rather than at the initial diagnosis of active coeliac disease. The nature of the oral stimulus also may have contributed to these differences. Bloom's group have used a test

breakfast consisting of various nutrients,¹ as compared with a glucose load much of which may have been absorbed before transit to the ileum, the principal site of N-GLI secreting cells.¹⁷

That the increase in N-GLI release is related to abnormalities which develop in the mucosa seems likely, as our patients were studied during carefully controlled gluten challenge, the 'end point' of which was a degree of deterioration in jejunal histology consistent with a diagnosis of coeliac disease. Moreover, plasma N-GLI release remained normal so long as a normal jejunal mucosa was maintained. Also, N-GLI concentrations after gluten challenge were significantly related to D-xylose absorption, the latter finding having been previously used during gluten challenge to indicate the earliest point whenever re-biopsy was likely to show deterioration diagnostic of coeliac disease.¹⁸ This suggests that N-GLI values at 45 or 120 minutes after glucose may also help indicate the timing of such a biopsy during gluten challenge.

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