

Postheparin plasma diamine oxidase increases in patients with coeliac disease during gluten free diet

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SUMMARY An intravenous injection of heparin releases diamine oxidase (DAO) from villous tip enterocytes. In a previous study, we found that postheparin plasma DAO (PHD) values were significantly lower in patients with malabsorption syndrome and small bowel atrophy at jejunal biopsy than in normal subjects. In this study we performed the PHD test in 14 coeliac patients before and after three and six months of gluten free diet to show whether the enterocytes maturing processes induced by the diet joined with enhanced PHD values and to assess the clinical usefulness of this test. In all subjects jejunal biopsy carried out after six months showed a partial but consistent histological recovery. The clinical status, xylosuria and daily faecal fat excretion improved progressively and there was a significant increase ($p < 0.001$) in mean PHD activity that reached the normal range after three months. After six months a further slight increase of the mean PHD value was recorded. These data indicate that PHD values rise together with the improved intestinal absorptive functions of coeliac patients on gluten free diet and that this test is a useful tool in monitoring recovery of the small bowel mucosa.

Diamine oxidase (DAO) is an enzyme whose low plasma values are enhanced by an intravenous injection of heparin which releases the enzyme from the enterocytes of the villus tip.¹ Diamine oxidase, which is not excreted in active form in urine and bile, is rapidly removed from plasma by liver uptake.² Lower postheparin plasma DAO (PHD) values have been found in subjects with an overt malabsorption syndrome and histologically proven small bowel mucosa atrophy than in normal subjects,³ and a significant inverse correlation between PHD and faecal fat excretion was found.⁴

Coeliac disease, a clinical condition in which small bowel mucosa atrophy is reverted by gluten free diet, is an interesting human model of intestinal adaptation.⁵ The dramatic proliferative changes induced by gluten free diet determine a rapid reconstitution of the mature enterocytic mass;⁶ these mucosal modifications are only indirectly reflected by the currently available tests of intestinal function that assess the absorptive capability of the small bowel. The detection in plasma of enhanced concentrations of DAO which directly mirrors the morphologic and functional integrity of the small bowel mucosa^{3,7} could

be useful in monitoring mucosa recovery in coeliac patients on gluten free diet. To this end we performed the PHD test, xylosuria and faecal fat excretion (the two most widely used tests of malabsorption) in subjects with coeliac disease before and during treatment.

Methods

PATIENTS

Heparin (15 000 IU) was injected into an antecubital arm vein by intravenous bolus in 14 subjects (10 women and four men; mean age 42 ± 17 years) with coeliac disease before and three and six months after starting gluten free diet. At the same time daily faecal fat excretion and xylosuria were carried out.

In all cases diagnosis was made by histological examination of jejunal mucosa samples obtained by Crosby capsule. A control biopsy was carried out after six months of gluten free diet. In each subject morphometric evaluation of small bowel mucosa was done with a LEITZ ocular micrometer.

The patients were referred to hospital because of clinical and laboratory signs of malabsorption syndrome; these progressively reverted after the starting of the gluten free diet. Laboratory signs of altered

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liver function were absent. Informed consent was obtained from all patients. No complications from the heparin bolus or jejunal biopsy occurred. In all subjects partial thromboplastin time was monitored and returned to normal values within five hours after heparin injection.

DAO ASSAY

Blood samples (4 ml) withdrawn by vein catheter were collected in heparinised tubes at 0, 5, 15, 30, 45, 60, 90, 120 minutes after the iv heparin bolus. All samples were centrifuged at 3000 g and the plasma was stored at -20°C and assayed for DAO within a week. Diamine oxidase assay was done by the Okuyama and Kobayashi method⁸ as modified in our previous study.⁹ Diamine oxidase activity was expressed as U/ml (1 U = 1 nM of putrescine dihydrochloride oxidated in one hour at 37°C , pH 7.2). The area under the DAO release curves, expressed as U/ml·min, was calculated by trapezoid method.

FAECAL FAT EXCRETION

Patients received a standard hospital diet containing 100 g fat daily during the three days of faeces sampling. A faecal fat excretion, assayed according to Van der Kamer,¹⁰ of less than 7 g/day was taken as normal.

D-XYLOSE ABSORPTION TEST

D-xylose (25 g) in 250 ml of water was administered to patients the morning after an overnight fast. Urine was collected for five hours. An excretion of 4 g or more was considered normal. The assay was carried out according to a modified method¹¹ of Roe and Rice.¹²

STATISTICAL METHODS

Statistical evaluation includes a two-tailed unpaired Student's *t* test. Results, expressed as mean \pm SD, were considered significant when $p < 0.05$.

Results

Postheparin plasma values in 14 patients with coeliac disease before and after three and six months of gluten free diet are shown in Figure 1. Increased mean PHD values, reaching the normal range, were present after three months; after six months a further slight increase of these values was recorded. Mean \pm SD PHD area values (Fig. 2) were 195 ± 94 on diagnosis and 411 ± 119 and 493 ± 110 ($p < 0.001$; normal values more than 370) after three and six months of treatment respectively.

The test was also undertaken in five patients after only 10 days of diet; a significant increase in PHD values was recorded in three of these. Daily faecal fat

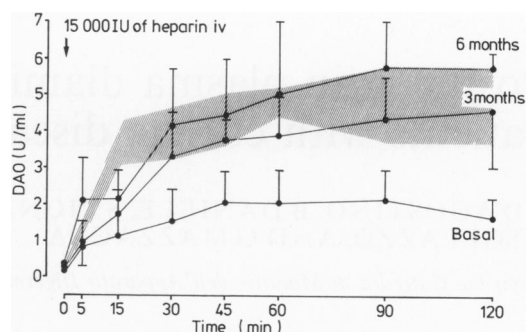


Fig. 1 Postheparin DAO release curves in 14 subjects with coeliac disease before and during gluten free diet. Shaded area represents the normal range.

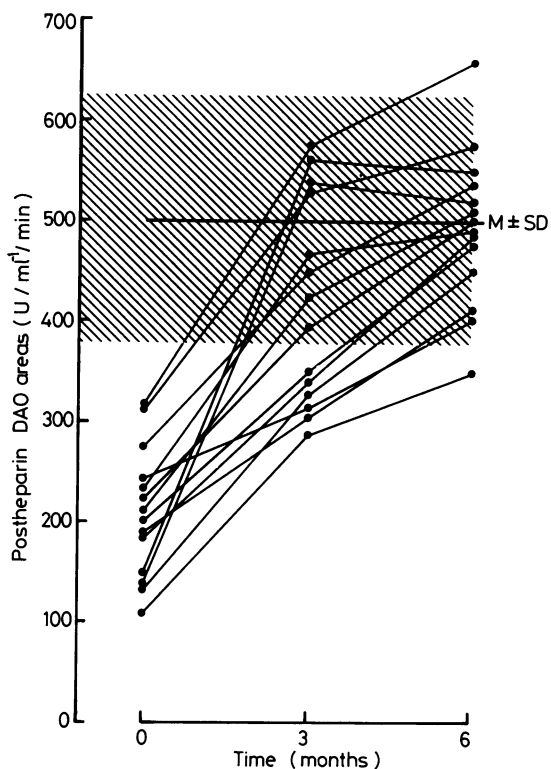


Fig. 2 Postheparin DAO area values in 14 subjects with coeliac disease before and during gluten free diet. Shaded area represents the normal range.

excretion, xylosuria and morphometric data are shown in the Table.

Daily faecal fat excretion progressively decreased and xylosuria increased during treatment. The histological evaluation of jejunal specimens after six

Table Changes in daily faecal fat excretion, xylosuria, villus height and crypt height in 14 subjects before and during gluten free diet.

Months	0	3	6
Faecal fat excretion (g/day)	27 ± 9	13 ± 5	9 ± 2
D-xylose excretion (g)	2.7 ± 1	3.8 ± 2.1	4.2 ± 1.8
Villus height (µm)	35 ± 3		255 ± 105
Crypt height (µm)	328 ± 28		167 ± 63

months of diet showed an incomplete normalisation of villus morphology in all patients.

Discussion

This study shows that in patients with coeliac disease on gluten free diet the functional recovery of small bowel mucosa closely accompanies the rapid normalisation of the PHD test. Diamine oxidase is an enzyme located in villus tip enterocytes of mammals¹ and its activity increases successively from the duodenum to the ileum.^{9,13} This enzyme functions as a plasma marker of small bowel mucosa maturation and integrity¹⁴ and injury and recovery in the rat.¹⁵ Heparin induces high DAO concentrations in plasma by releasing the enzyme from differentiated and non-replicating enterocytes.¹ Patients with small bowel mucosa atrophy and malabsorption syndrome presented PHD values significantly lower than did normal subjects. Postheparin area values in these patients were also inversely correlated with the daily faecal fat excretion, indicating that PHD test explores the remaining mature enterocytic mass.⁴

These data induced us to investigate whether enterocyte maturing processes determined by gluten free diet were connected with increased amounts of releasable intestinal DAO in coeliac patients and to assess the usefulness of this test in monitoring small bowel mucosa recovery.

The results show that three months after starting treatment mean PHD values reached the normal range and that normal values were recorded at the six month evaluation. It is also interesting that the early clinical improvement induced by a few days of gluten free diet was associated with a significant increase in PHD values in three subjects. The absorption tests performed after three and six months showed a progressive improvement, and at six months quite complete normalisation of small intestinal absorptive functions, although the histological picture of jejunal specimens only partially improved. These findings are

consistent with the observation that in coeliac patients on gluten free diet the mucosa of the distal small intestine improves more rapidly than that of the severely involved proximal bowel⁶ and with the prevalently distal location of DAO along the small bowel.^{9,13}

The early increase in release of enterocytic DAO may depend upon the rapid maturing processes induced by gluten free diet. In fact, the diet induces the 'flat' coeliac mucosa, that shows a three-fold increase in the size of the proliferation compartment compared with normal and a net six-fold increase in crypt cell production rate,⁵ to shift to a normal replicative pattern with a progressive increase in the number of the mature enterocytes.

In conclusion, these data suggest that in patients with coeliac disease the functional recovery of the small bowel mucosa, revealed by clinical improvement and near-normalisation of absorptive tests is closely associated with high PHD values. Furthermore, the maturing processes that begin within a few days of gluten free diet, are clearly shown by increased PHD values. For the above reasons this test, carried out in man for the first time by us,³ appears to be a useful tool in the early monitoring of small bowel mucosa recovery.

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