

## Intestinal trehalase activity in a UK population: establishing a normal range and the effect of disease

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Trehalose is a disaccharide, the main dietary source being mushrooms. It has been approved as an additive in the preparation of dried food. Isolated intestinal trehalase deficiency is found in 8% of Greenlanders, but is rare elsewhere. The normal range of trehalase activity and the incidence of isolated trehalase deficiency in the UK have not been reported. Patients ( $n$  400) were investigated for suspected malabsorption. Endoscopic distal duodenal biopsies were taken for histological assessment and maltase, sucrase, lactase and trehalase estimation. Disaccharidase activities were determined by Dahlqvist's technique (Dahlqvist, 1968). Most patients ( $n$  369) had normal duodenal histology. In these, square root transformation of trehalase activity produced a normal distribution. The normal range (mean  $\pm$  2 SD) was 4.79–37.12 U/g protein. One patient had an isolated borderline trehalase deficiency. The thirty-one patients with villous atrophy had significantly reduced disaccharidase activities. With ingestion of a gluten-free diet, maltase, sucrase and trehalase activities recovered to normal in most patients, whereas lactase activity did not. The normal range and very low incidence of isolated enzyme deficiency is comparable with that described in populations from the USA and mainland Europe. Activity is significantly reduced in untreated coeliac disease and recovers with treatment with a gluten-free diet. There is no place for routine determination of trehalase activity in the UK population and there should be no concern over the introduction of trehalose-containing dried foods.

### Disaccharidase: Coeliac disease

Trehalose is a disaccharide found in mushrooms, algae and insect haemolymph. The high concentration of trehalose in cryptobiotic plants is responsible for their remarkable ability to go through cycles of desiccation and rehydration without injury. This has led to interest by the food industry, as the addition of trehalose to foodstuffs improves the quality of dried food (Roser, 1991*a, b*; Roser & Colaço, 1993).

Intestinal trehalase is a  $\beta$ -galactosidase which catalyses the hydrolysis of trehalose to two glucose molecules for absorption. It is present throughout the small intestine with highest levels in the proximal jejunum. Isolated trehalase deficiency occurs in at least 8% of the Greenland population (Gudmand-Høyer *et al.* 1988) but only three cases have been reported elsewhere (Bergoz, 1971; Madzarovová-Nohejlova, 1973), two of whom were first degree relatives. There are no reported studies of the incidence of intestinal trehalase deficiency in a British population.

The activities of other disaccharidases (maltase, sucrase and lactase) are low in patients with untreated coeliac disease. After treatment with a gluten-free diet, there is recovery of maltase, sucrase and sometimes lactase activities, although the latter only after several months.

The aim of this present study was to assay trehalase

activity in duodenal biopsies from patients presenting with suspected malabsorption and to determine a reference range in those with normal histology. We also determined the effect of coeliac disease and its treatment on duodenal trehalase activity.

### Methods

#### Patients and biopsies

Patients ( $n$  400) with suspected malabsorption (251 women and 149 men; mean age 46.9 years, range 16–88 years), and thirty-four patients with known coeliac disease (twenty-one women and thirteen men; mean age 43.9 years, range 26–72 years), taking a gluten-free diet for at least 6 months, attended for endoscopic duodenal biopsies following overnight fast. Of the 400 patients taking a normal diet, fourteen were of South Asian origin, nine Afro-Caribbean, one Oriental, six Caucasian originally from Southern or Eastern Europe and the remainder were Caucasians of British descent. They were prepared with xylocaine throat spray and intravenous midazolam. At least three 3.7 mm diameter biopsies were taken from the second part of the duodenum. One was immediately flash frozen and stored in liquid

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N<sub>2</sub> until disaccharidase estimation was performed within 2 weeks of biopsy.

#### *Disaccharidase estimation*

The specimen was placed in distilled water and homogenized (Ultra-Turrax, Ika-Kunke, Staufen, Germany; maximum speed for 15 s). Disaccharidase activities (maltase, sucrase, lactase and trehalase) in the homogenate were determined by the method of Dahlqvist (1968), all assays being run in duplicate and corrected for glucose already present in the homogenate and in the substrate. Protein content was estimated by Lowry's technique (Lowry *et al.* 1951) using lyophilized bovine serum albumin (Sigma, Poole, Dorset, UK) as standard. Activity is expressed as U/g protein, equivalent to  $\mu\text{M}$  disaccharide hydrolysed/g protein per min. Intra-assay variation using this method is < 6% while inter-biopsy variation in samples taken from the same level in a single patient is up to 32.4% (McIntyre *et al.* 1994).

#### *Statistical methods*

The frequency distributions of disaccharidase activities were examined and transformed into a normal distribution by square root transformation (McIntyre *et al.* 1994). Transformed values were plotted on a probit (percentage cumulative frequency) plot where the gradient of the line is a function of the variance. Values lying on the straight line were used to determine reference ranges (mean  $\pm$  2 SD) for each disaccharidase. Patients were considered to have an isolated disaccharidase deficiency if a single activity of only one enzyme lay below the normal range determined by this method. Lactase deficiency was diagnosed if the sucrase:lactase value was > 5 (Bergoz *et al.* 1981; Smith *et al.* 1989; McIntyre *et al.* 1994). As lactase and trehalase values are similar in those without an isolated trehalase deficiency (Gudmand-Høyer *et al.* 1988), we considered that a sucrase:trehalase value > 5 indicated an isolated trehalase deficiency.

The difference in disaccharidase activities between groups with histology was examined by ANOVA with Scheffé's F test used for *post hoc* comparisons. Differences in activities before and after commencing a gluten-free diet in coeliac patients were analysed by Student's paired *t* test (two-sided). A value of  $P < 0.05$  was considered statistically significant.

## **Results**

#### *Patients on normal diet*

Of the 400 patients with suspected malabsorption, 369 had normal histology of the second part of the duodenum; eighteen had partial villous atrophy and thirteen had total villous atrophy. All patients with villous atrophy had coeliac disease. No patient had giardiasis.

For the 369 patients with normal histology, maltase, sucrase, lactase and trehalase activities were positively skewed (coefficients of skewness were 3.71, 1.67, 1.49 and 1.73 respectively). Square root transformation of the disaccharidase activities gave a near normal distribution in

each case (coefficients of skewness 1.26, 0.52, 0.05 and 0.33 respectively).

*Maltase, sucrase and trehalase activities.* When transformed values were plotted on a probit (percentage cumulative frequency) plot, values for  $\sqrt{\text{maltase}}$ ,  $\sqrt{\text{sucrase}}$  and  $\sqrt{\text{trehalase}}$  were found to lie on straight lines, i.e. were from a single population with a normal distribution (Fig. 1). There was some discrepancy at the upper ranges. The reference ranges for maltase, sucrase and trehalase are 49.01–382.79, 15.12–97.16 and 4.79–37.12 U/g protein respectively. Two patients had trehalase activities below this normal range and sucrase:trehalase values > 5. The first also had low lactase and maltase activities. Repeat biopsies showed completely normal disaccharidase activities. The second had a trehalase activity of 4.48 U/g protein (sucrase:trehalase value 9.0).

Disaccharidase activity in biopsies from the second part of the duodenum are 40% lower than those from biopsies at the ligament of Treitz or more distal (Bergoz *et al.* 1981; Smith *et al.* 1989). Hence it is possible to compare normal ranges with other studies where jejunal biopsies were performed (Table 1).

#### *Lactase activity*

$\sqrt{\text{Lactase}}$  values were from three subpopulations with different gradients seen on the probit plot (Fig. 1). The first group with  $\sqrt{\text{lactase}}$  activity > 2.7 ( $n$  313) gave a reference range of 5.71–54.89 U/g protein. There was a second group of patients with very low lactase activity of < 2.25 U/g protein ( $n$  16) and a third group with intermediate lactase activity ( $n$  40). Sucrase:lactase values > 5 were seen in the entire group with low lactase activity and in twenty-six of forty patients with intermediate activities. Two of six Southern or Eastern Europeans (33%), eleven of fourteen (78.6%) South Asians, eight of nine Afro-Caribbeans (88.9%), the only Oriental subject and twenty of 339 (5.9%) British Caucasian subjects had isolated lactase deficiency.

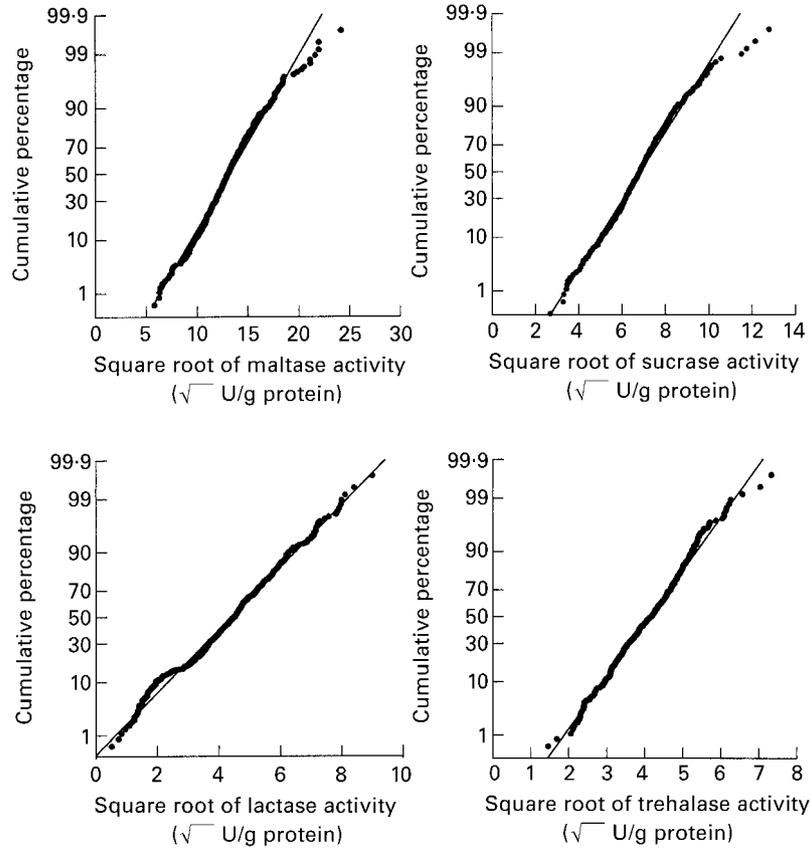
#### *Disaccharidase activity seen in untreated coeliac disease*

All disaccharidase activities were significantly reduced in those with partial and total villous atrophy compared with patients with normal histology (Table 2). Although activities for all disaccharidases were lower in total villous atrophy than in partial villous atrophy, this did not achieve statistical significance.

#### *Patients on a gluten-free diet*

Of the thirty-four patients on a gluten-free diet, twelve had normal villous architecture of the second part of the duodenum, twenty had partial villous atrophy and two had total villous atrophy. Disaccharidase activities correlated well with histological appearance with statistically significant lower values in partial and total villous atrophy compared with those on normal diet (Table 2).

Patients ( $n$  12) had duodenal disaccharidase estimation performed before treatment and after taking a gluten-free diet for at least 3 months. The trehalase activity increased in



**Fig. 1.** Cumulative frequency plots for disaccharidase activities measured in the second part of the duodenum from 369 patients with normal histology.

**Table 1.** Studies of intestinal trehalase activity

| First author/date   | Country of origin | Sample site | Range of trehalase activity (U/g protein)* | Patients studied (n) | Primary trehalase deficient (%) |
|---------------------|-------------------|-------------|--|----------------------|---------------------------------|
| Asp, 1971           | Finland           | Jejunal     | 6.2–27.8                                   | 35                   | 0                               |
| Malis, 1972†        | Czechoslovakia    | Jejunal     | 15.6–24.0                                  | 15                   | 0                               |
| Berg, 1973          | Sweden            | Duodenal    | 14 (median)                                | 178                  | 0                               |
| Asp, 1975           | Denmark/Sweden    | Jejunal     | 4.2–18                                     | 19                   | 0                               |
| Welsh, 1978         | USA               | Jejunal     | 8.4–37.2                                   | 187                  | 0                               |
| Bergoz, 1981        | Switzerland       | Duodenal    | 6–58                                       | 34                   | 0                               |
| Jönsson, 1986       | Sweden            | Duodenal    | 1–21                                       | 10                   | 0                               |
| Gudmand-Høyer, 1988 | Denmark/Greenland | Jejunal     | 8–67                                       | 97                   | 0                               |
|                     |                   |             | 0–67.1                                     |                      | 8                               |
| Present study       | UK                | Duodenal    | 4.8–37.1                                   | 369                  | 0.3                             |

\* If jejunal, corrected to duodenal activity by multiplying by 0.6.

† Disaccharidase activity in supernatant fraction of homogenate measured.

**Table 2.** Disaccharidase activity (U/g protein) in second part of duodenum

| Histology | n   | Diet   | Maltase    |        | Sucrase    |        | Lactase* |        | Trehalase |        |
|-----------|-----|--------|------------|--------|------------|--------|----------|--------|-----------|--------|
|           |     |        | Range      | Median | Range      | Median | Range    | Median | Range     | Median |
| Normal    | 369 | Normal | 49.0–382.8 | 170.6  | 15.1–97.2  | 46.7   | 5.7–54.9 | 20.5   | 4.8–37.1  | 17.6   |
| PVA       | 18  | Normal | 2.9–147.0  | 47.5   | 0–29.5     | 14.1   | 0–15.5   | 2.9    | 0–11.3    | 2.5    |
| TVA       | 13  | Normal | 1.6–111.0  | 33.8   | 0.1–26.2   | 8.2    | 0–7.8    | 1.2    | 0–5.0     | 1.6    |
| Normal    | 12  | GFD    | 68.2–251.5 | 143.6  | 11.7–101.1 | 42.9   | 0.4–50.6 | 11.6   | 2.5–27.6  | 10.5   |
| PVA       | 20  | GFD    | 11.7–191.2 | 82.1   | 0.3–59.8   | 19.9   | 0–12.9   | 3.1    | 0–16.5    | 3.9    |
| TVA       | 2   | GFD    | 19.4–36.5  | 27.3   | 2.2–12.0   | 6.1    | 1.0–3.8  | 2.2    | 0.8–2.11  | 1.4    |

PVA, partial villous atrophy; TVA, total villous atrophy; GFD, gluten-free diet.

\* Lactase value for normal histology excludes lactase deficient (see p. 242).

ten patients and recovered to normal levels in eight, compared with recovery to normal levels in nine patients for maltase and sucrase and only in four for lactase. The change in activity of every disaccharidase with a gluten-free diet was statistically significant.

### Discussion

Mushrooms and products containing baker's yeast are the only sources of trehalose in the human diet. However, there is interest in the food industry in trehalose, as its addition to foodstuffs before air-drying produces dried food with a long shelf-life. Rehydration produces food with the same taste and even aroma of the fresh product.

Ingestion of a disaccharide in an individual who cannot digest it results in osmotic diarrhoea, abdominal pain and increased rectal flatulence. It is therefore important to establish the incidence of trehalase deficiency in a population before introducing trehalose-dried foods.

In the present study we have examined 400 patients with suspected malabsorption and found one with possible primary trehalase deficiency. The incidence of primary trehalase deficiency in the UK must be extremely low. In Greenland, there is an incidence of at least 8% (Gudmand-Høyer *et al.* 1988) but the incidence in the USA (Welsh *et al.* 1978), Finland (Asp *et al.* 1975), Czechoslovakia (Malis *et al.* 1972), Sweden (Berg *et al.* 1973; Jönsson *et al.* 1986), Denmark (Gudmand-Høyer & Skovbjerg, 1996) and Switzerland (Bergoz *et al.* 1981) has been zero. Although these studies are not directly comparable, the normal ranges vary little throughout the USA and northern Europe (Table 1). The discrepancy from the normal range at higher disaccharidase activities has previously been noted for maltase and sucrase (McIntyre *et al.* 1994).

In untreated coeliac disease, the activity of all intestinal disaccharidases is greatly reduced. The median activity in untreated coeliac disease (total villous atrophy compared with those with normal histology) was reduced by 80.2%, 82.5%, 94.0% and 91.1% for maltase, sucrase, lactase and trehalase activity respectively. This is similar to that seen in previous studies (Berg *et al.* 1973; Langman & Rowland, 1990). Of our thirty-one patients with coeliac disease taking a normal diet, six patients had no detectable trehalase activity, seven no detectable lactase activity and four no detectable sucrase activity. All had generalized decreases in disaccharidase activities.

In those patients studied before and after starting a gluten-free diet, maltase, sucrase and trehalase activities recovered in almost all when measurement of activities was repeated after 3–35 months (median 7 months) on the diet. Histological improvement may be seen within 4 weeks of commencing the diet (Dissanayake *et al.* 1974). Delayed recovery of lactase activity measured in proximal jejunal biopsies is well recognized, but may not be clinically important. Bodé & Gudmand-Høyer (1988) found low lactase activity in 56% of jejunal biopsies but found an abnormal lactose tolerance test in only 12% and a specific deficiency in only half of these. As the incidence of primary lactase deficiency in a Danish population is 6%, it was concluded that the distal small bowel compensated for low levels proximally and that in well-treated coeliac disease, low lactase activity measured

on biopsy was of no clinical relevance. Colonic bacteria in lactose intolerant subjects adapt to lactose ingestion and reduce the symptomatic response to lactose (Hertzler & Savaiano, 1996). The incidence of primary lactase deficiency in British white subjects is 3.2–6% (Ferguson *et al.* 1984; Duncan *et al.* 1994; Gudmand-Høyer & Skovbjerg, 1996) so it is unlikely that 67% with low lactase activity represents primary lactase deficiency in treated coeliac disease. Using sucrase:lactase and sucrase:trehalase values in patients with coeliac disease is less reliable than in patients with normal villous architecture because of the variable effect the disease has on enzyme activity. We did not investigate the relevance of low lactase levels in our coeliac patients who were taking a gluten-free diet, but none of them suffered from diarrhoea or weight loss on the diet.

Two separate populations of lactase-deficient patients, one with 'intermediate low' levels of lactase and one with very low levels have been reported in previous studies (Asp *et al.* 1971; McIntyre *et al.* 1994). Examining the sucrase:lactase values shows that 65% of the latter group have an isolated lactase deficiency.

In conclusion, we have examined 369 UK patients with normal duodenal histology and defined a normal range for trehalase activity in biopsies from the second part of the duodenum of 4.79–37.12 U/g protein. One patient may have a primary intestinal trehalase deficiency, although activity was only just below the normal range. Trehalase activity is decreased in untreated coeliac disease and recovers to normal in most patients within 6 months of starting a gluten-free diet. We cannot recommend routine intestinal trehalase estimation in the UK for the investigation of unexplained diarrhoea.

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