Improvement in intestinal permeability precedes morphometric recovery of the small intestine in coeliac disease

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ABSTRACT

It is often difficult to assess small bowel recovery in adults with coeliac disease on a gluten-free diet (GFD). This prospective study compares changes in intestinal permeability with changes in intestinal biopsy at various intervals after commencing a GFD. Intestinal permeability was measured by lactulose/rhamnose absorption from 1 week to 24 months after commencing a GFD. Intestinal morphometry was measured by villus area, crypt length and mitotic count per crypt at diagnosis and after commencing a GFD. Median intestinal permeability values decreased from 0.47 (n = 35) at diagnosis to 0.25 (n = 17) after 1 week and to 0.16 (n = 18) after 2 months of a GFD. Rhamnose absorption improved significantly at an early stage, from 6.6% (untreated) to 15.4% at 3 months of a GFD, whereas the decrease in lactulose permeation took longer: from 3.4% (untreated) to 0.8% after 12 months of a GFD. Mean villus area (n = 29) was reduced to 16% of control values at diagnosis, and improved to a maximum of 48% after 6 months on a GFD, but did not change thereafter. Mean crypt length and mitotic count per crypt were increased by 222% and 356% respectively at diagnosis, and these parameters remained elevated at 172% and 216% above control values after 6 months of a GFD. We conclude that intestinal permeability improves within 2 months after starting a GFD, but that measurable intestinal biopsy improvement requires ingestion of a GFD for at least 3–6 months, and even then remains incomplete.

INTRODUCTION

Diagnosis of coeliac disease is made using intestinal biopsy demonstrating surface villous effacement and atrophy, crypt hyperplasia and increased numbers of intra-epithelial lymphocytes [1]. These changes of intestinal damage should respond to a gluten-free diet (GFD). However, recovery of the intestinal biopsy in adult-onset coeliac disease remains variable [2–5]. Children generally show better improvement of intestinal histology than adults [6]. Earlier studies in small numbers of children and adults at various intervals after starting a GFD indicated that enterocyte height improved within days or weeks (but was subject to wide fluctuation), that dissecting microscopic appearance (flat mosaic to convoluted to leaves) took months to several years to improve, and that sucrase and maltase disaccharidases improved, but lactase had delayed recovery [2–7]. The main problem with these studies has been the arbitrary intervals of tissue sampling and the small number of subjects that were studied. A variable histological response in adults with coeliac disease has been observed even in recent studies [8–12]. This means that it is difficult to assess recovery alone by intestinal biopsy in adults, and the questions arises: how long and to what extent does the small intestine recover in subjects on a GFD?

Key words: coeliac disease, intestinal permeability, small intestine.
Abbreviations: GFD, gluten-free diet; sIL-2R, soluble interleukin-2 receptor.
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Intestinal permeability, as measured by a double sugar permeation technique, is increased in coeliac disease. While it was suggested initially that this represents a primary defect [13], it is more likely to be due to intestinal damage, as abnormal permeability and intestinal damage are associated [14]. We have shown previously that intestinal permeability in eight subjects improved rapidly within 1–2 months after commencing a GFD, but we were unable to detect any morphometric improvement of the intestinal biopsy in six of the eight subjects after 3–6 months of a GFD [10]. Thus the association was not as simple as might be expected, but a limitation of the study was that the follow-up biopsies had been grouped together due to the small number of subjects. The previous study has now been extended prospectively with additional subjects and over a longer duration of a GFD. In particular, intestinal biopsies were taken after 1.5, 3, 6, 12 and 24 months of a GFD, to allow assessment of recovery.

During the course of the present study, food labelling guidelines in Australia became stricter, with the designation that ‘gluten-free food’ should have no detectable gluten on immunoassay (sensitivity < 0.003 g of gluten/100 g of protein) [15]. This was brought into regulation from 15 March 1995. Prior to that date, food guidelines followed the Codex Alimentarius Commission of the World Health Organization and of the Food and Agricultural Organization of the United Nations. The Codex allows gluten-free foods to contain 0.3 g of gluten/100 g of protein. In practice, this meant that the new guidelines excluded malt and wheat starch from a GFD. We therefore used this opportunity to compare biopsies from subjects before and after the introduction of these stricter guidelines.

The aims of the present study were to compare intestinal permeability and morphometry as measures of recovery in coeliac disease, and to discern the relationship between permeability and histological change. Intestinal morphometry was also compared in subjects after 6 months of the old Codex and the new, stricter GFD. As coeliac disease is an immunological intolerance to gluten, the soluble interleukin-2 receptor (sIL-2R) concentration was used to assess total T cell activity, and anti-gliadin and anti-endomysial antibodies were used to assess gluten withdrawal.

**METHODS**

**Subjects**

A total of 36 subjects with coeliac disease (16 males, 20 females; age 18–80 years) were studied following diagnosis by small bowel biopsy that showed villous effacement and atrophy, crypt hyperplasia and increased intra-epithelial lymphocytes. Subjects were recruited between 1987 and 1997. The presentations of coeliac disease included iron- or folate-deficient anaemia, chronic diarrhoea, epilepsy, symptoms suggestive of irritable bowel syndrome, recurrent miscarriage, and metabolic bone disease. One patient had rheumatoid disease, one had Type I diabetes mellitus, and two had isolated IgA deficiency without any complicating symptoms. Three patients had aphthous ulcers in the duodenum at endoscopy. Two patients had infertility that responded to a GFD. A control group of 75 patients underwent small bowel biopsy while being investigated for other medical conditions. They were subsequently found to have normal gastrointestinal function (except for irritable bowel syndrome). Endoscopic duodenal biopsies have been shown to be a satisfactory means of assessing the presence or absence of coeliac disease [16–18].

**Experimental protocol**

The initial permeability test in subjects on a normal diet was performed with the patient unaware of the diagnosis. Nearly all subjects became members of the Coeliac Society of South Australia, and received regular food lists and support. Each subject was interviewed about their dietary compliance and was encouraged to maintain a strict GFD at each interval of testing. We endeavoured to study changes in intestinal permeability in the same patients after commencing a GFD, but this was not always possible. However, we found that the mean value for permeability stabilized with 8–12 patients in each group, but we recruited at least 15 patients in each group to ensure good stability of the mean data. Duodenal biopsies were taken at endoscopy from subjects on a normal diet, and at 1.5, 3, 6, 12 and 24 months after commencement of a GFD. Except for the 1.5 month group, which contained eight subjects, nine subjects had biopsies on a normal diet and at all intervals on the GFD; three further patients had no initial biopsy available for morphometry at diagnosis but completed all biopsy intervals of the GFD. Histological recovery in coeliac subjects was assessed by comparing intestinal morphometry with that of control subjects.

**Intestinal permeability**

Each subject fasted overnight, and emptied their bladder before drinking 100 ml of a hypertonic solution (1500 mOsmol) containing 1.0 g of α-L-rhamnose (R-3875; Sigma, St. Louis, MO, U.S.A.), 5.0 g of lactulose and 22.6 g of glucose. Lactulose was used as a 67% (w/v) syrup (duphalac; Solvay Pharmaceuticals, Sydney, NSW).
Glucose was used as an osmotic filler in preference to lactose or sucrose, because the latter are likely to be malabsorbed in villous atrophy. All urine was collected for the next 5 h. Subjects could drink water throughout the study, and could eat after the first 3 h. Urinary rhamnose and lactulose were measured using HPLC, as previously described and validated [19]. The overall precision of estimation of the probe sugars varies from 4.2 to 6.5% using this method. Intestinal permeability was expressed as the mg ratio of urinary lactulose to rhamnose, with each expressed as the percentage of the ingested dose excreted in urine. Permeability was measured at diagnosis and at 0.25, 0.5, 1, 2, 3, 6, 12 and 24 months after commencement of a GFD. We determined that the mean intestinal permeability of 38 healthy adult subjects was 0.088, with a 95% confidence interval of 0.063–0.112.

### Intestinal morphometry

All duodenal biopsies for morphometry were taken from the distal second or third parts of the duodenum at endoscopy. Duodenal biopsies were stained with Feulgen reagent and microdissected [20]. Briefly, rows of villi and crypts were carefully cut using a cataract knife from stained tissue suspended in water in a Petri dish lid under stereomicroscopy. These fragments were transferred into 45% (v/v) acetic acid before a cover slip was applied. This helps to flatten the tissue slices in the wet film. Measurements were made of villus length, apical villus width and basal villus width using a x10 lens, and of length of crypts using a x20 microscope lens and a calibrated eyepiece graticule. Villus area was calculated using a trapezoid approximation as described previously, with each expressed as the percentage of the ingested dose excreted in urine. Permeability was measured at diagnosis and at 0.25, 0.5, 1, 2, 3, 6, 12 and 24 months after commencement of a GFD. We determined that the mean intestinal permeability of 38 healthy adult subjects was 0.088, with a 95% confidence interval of 0.063–0.112.

### Disaccharidases

Lactase, sucrase and maltase activities were assayed as described previously in detail [22]. The 90% confidence intervals for lactase, sucrase and maltase are 3–14, 6–26 and 13–44 μmol/min·g dry weight respectively. The activity was expressed per wet weight, as this is the simplest denominator. Measurement of activity per mg of protein may be subject to a surface-to-volume error, and assumes that disaccharidase protein is independent of mucosal changes.

### Anti-gliadin and anti-endomysial antibodies

Sera stored at –20 °C were available from 28 coeliac subjects at diagnosis and from 18 subjects after 2 years of a GFD. Serology was assessed at the end of the study using this stored sera. Measurement of anti-gliadin antibodies was performed using an ‘in house’ method adapted to the Boehringer Mannheim ES300 automated ELISA system (Boehringer Mannheim/Roche Diagnostics, Sydney, Australia). Gliadin (G-3375; Sigma-Aldrich, Sydney, Australia) was dissolved in 100% (v/v) ethanol and used to coat plastic tubes. Serum was diluted 1:500 (v/v) and added to the plastic tubes. Bound anti-gliadin antibodies were detected using anti-IgA (AH10104; 1:4000 dilution) and anti-IgG (AH10304; 1:25000 dilution) human antibodies conjugated to peroxidase (BioSource International, Camarillo, CA, U.S.A.). Chromogen (857424; Boeheringer Mannheim/Roche Diagnostics) was added, and the reaction was read after 40 min. A standard curve was constructed using dilutions of a known positive control serum (BP140; The Binding Site, Birmingham, U.K.). ELISA values were calculated in arbitrary units from the standard curve fitted by the Rodbard Evaluation method. The ELISA was originally validated using sera from 20 paediatric coeliac subjects, and yielded a sensitivity of 95% for IgA anti-gliadin antibody. The reference ranges for IgA and IgG anti-gliadin antibodies were 0–58 and 0–78 ELISA units respectively for control subjects. Anti-endomysial IgA antibody was detected by immunofluorescence using monkey oesophagus substrate (FS208.2; The Binding Site). Serum was added ‘near’ and incubated for 30 min. Bound antibody was detected using anti-IgA human antibody conjugated to fluorescein (F0204; 1:20 dilution; Dako Australia, Sydney, Australia).

### sIL-2R levels

sIL-2R levels were measured in sera from coeliac subjects at diagnosis and at the same intervals of the GFD as for the permeability study. sIL-2R levels were measured using a CellFree IL-2R kit (T Cell Sciences, Boston, MA, U.S.A.). The sensitivity of the assay is 50 units/ml.

### Statistics

Data were summarized as mean (S.E.M.), except for intestinal permeability data, for which the median was used to indicate central tendency. Intestinal permeability data were transformed log10(x + 1) to normalize the data and stabilize the variance before significance testing by Peritz’ F test [23]. Anti-gliadin antibody data that were more than the upper or less than the lower level of detection were equated to these respective limits for the purpose of data analysis. Peritz’ F test was also used for significance testing of intestinal morphometry, epithelial cell height, sIL-2R and anti-gliadin antibody data.

### RESULTS

### Intestinal permeability

A total of 36 subjects had intestinal permeability measured within a few days of diagnosis of coeliac disease.
by intestinal biopsy, but only 26 of these subjects were available for sequential prospective permeability testing at intervals after commencement of the GFD. This was because the remaining subjects were diagnosed in rural areas and could not attend for logistical reasons. We investigated early changes in intestinal permeability in 15–19 patients at 1, 2, 4 and 8 weeks after commencing a GFD. Ten of these patients completed all these intervals of testing. Longer-term improvements in permeability were assessed in 15–23 subjects at 3, 6, 12 and 24 months of the GFD, including eight patients who completed all these intervals of permeability testing.

Intestinal permeability lactulose/rhamnose ratios are given in Figure 1, and the individual urinary excretion data are given in Table 1. The sensitivity of the intestinal permeability test for detecting the abnormality of coeliac disease in 36 subjects on a gluten-containing diet was 89%. Intestinal permeability improved rapidly within weeks, with a significant improvement evident from 2 months on a GFD (P = 0.0008). The data for lactulose and rhamnose excretion, expressed as a percentage of total dose, showed that the abnormality in initial permeability was due to a combination of increased lactulose permeation and lowered rhamnose absorption (Table 1). Lactulose permeation remained abnormal despite institution of a GFD, but had improved by 1–2 years of a GFD. Rhamnose excretion was unusual, in that excretion over the period 0.5–3 months after starting the GFD improved to values beyond those normally seen in healthy subjects. Thus rhamnose absorption improved quickly, by 3 months after starting the GFD, whereas

### Table 1 Urinary excretion of lactulose and rhamnose in coeliac subjects at various intervals after commencing a GFD

<table>
<thead>
<tr>
<th>Group</th>
<th>Lactulose</th>
<th>Rhamnose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coeliac/normal diet (n = 35)</td>
<td>3.4 (1.0)</td>
<td>6.6 (1.4)</td>
</tr>
<tr>
<td>Coeliac/GFD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week (n = 17)</td>
<td>3.2 (0.7)</td>
<td>8.2 (2.0)</td>
</tr>
<tr>
<td>2 weeks (n = 15)</td>
<td>4.4 (0.9)</td>
<td>15.5 (5.7)</td>
</tr>
<tr>
<td>1 month (n = 19)</td>
<td>2.3 (0.5)</td>
<td>11.5 (2.9)</td>
</tr>
<tr>
<td>2 months (n = 17)</td>
<td>1.9 (0.4)</td>
<td>14.3 (4.3)</td>
</tr>
<tr>
<td>3 months (n = 23)</td>
<td>2.8 (0.7)</td>
<td>15.4 (3.2)*</td>
</tr>
<tr>
<td>6 months (n = 21)</td>
<td>2.2 (0.7)</td>
<td>9.5 (2.0)</td>
</tr>
<tr>
<td>12 months (n = 17)</td>
<td>0.8 (0.2)**</td>
<td>3.5 (0.7)</td>
</tr>
<tr>
<td>24 months (n = 15)</td>
<td>0.7 (0.1)</td>
<td>7.1 (1.1)</td>
</tr>
<tr>
<td>Control group (n = 38)</td>
<td>0.7 (0.09)</td>
<td>10.9 (1.9)</td>
</tr>
</tbody>
</table>

Intestinal permeability lactulose permeation took 12 months of a GFD to improve (Table 1).

**Intestinal morphometry**

A total of 29 patients had duodenal biopsies collected for morphometry, but 11 of these subjects were not available for subsequent follow-up for logistical reasons, while another 10 subjects had already had their diagnostic biopsy but were available for subsequent biopsies. Changes in intestinal morphometry before and at progressive intervals after starting a GFD for all available subjects are given in Figure 2. Coeliac disease was evident morphometrically at diagnosis, as indicated by a decrease in mean villus area to 16%, by a 222% increase in crypt length, and by a 356% increase in mitotic count compared with control values. Both villus area and crypt hyperplasia had improved significantly by 3 months of a GFD (P < 0.0001). This stabilized after 6 months of a GFD. At this time, mean villus area, crypt length and mitotic count values were 59%, 175% and 224% respectively of control values. There were 14 subjects who had biopsies at diagnosis and at 6 months of a GFD. The mean (S.E.M.) villus area in these subjects was 0.057 (0.025) mm² at diagnosis and 0.165 (0.024) mm² 6 months after 6 months of a GFD. These values were equivalent to those for all subjects who were biopsied at diagnosis and at 6 months of a GFD (Figure 2). There was no further significant improvement in villus area or mitotic count, and only a small decrease in crypt length (P = 0.013), between 6 and 24 months of a GFD. Mean values of villus area for the nine subjects who completed all the biopsy intervals (excluding 1.5 months, which had eight subjects) were similar to those for all subjects biopsied at each of
Coeliac disease and intestinal permeability

Figure 2 Villus area, crypt length and mitotic count in duodenal biopsies from coeliac subjects before and after progressive intervals of a GFD
Data are given as means ± S.E.M.

Table 2 Comparison of intestinal morphometry at 6 months after starting the old Codex GFD or the new GFD after introduction of stricter food labelling regulations in Australia
Data are given as means ± S.E.M.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Villus area (mm²)</th>
<th>Crypt length (μm)</th>
<th>Mitotic count per crypt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codex GFD (n = 18)</td>
<td>0.187 (0.020)</td>
<td>369 (23)</td>
<td>5.5 (0.6)</td>
</tr>
<tr>
<td>New GFD (n = 8)</td>
<td>0.112 (0.012)</td>
<td>324 (17)</td>
<td>5.0 (0.8)</td>
</tr>
</tbody>
</table>

Changes in enterocyte height
Changes in enterocyte height are given in Figure 3. Enterocyte height was still depressed at 80% of control values at 3 months of the GFD, but had improved by 12 months.

Disaccharidases
Lactase activity improved 2.4-fold from initial diagnosis to 2 years of a GFD (P = 0.008), but remained subnormal. Sucrase and maltase activities showed no significant improvement (Table 3).

Anti-gliadin and anti-endomysial antibodies
Anti-gliadin IgA and IgG and anti-endomysial IgA antibodies were analysed from sera collected at diagnosis (n = 28) and after 2 years of a GFD (n = 18). Anti-gliadin IgA antibody was positive in 29% of subjects at diagnosis, and in 0% of subjects after 2 years of a GFD. The mean (S.E.M.) ELISA values were 59 (11) units at diagnosis and 29 (13) units after 2 years (P = 0.06). Anti-gliadin antibody IgG was positive in 54% of subjects at diagnosis, in 22% of subjects after 2 years of a GFD. The mean (S.E.M.) values were 125 (18) units at diagnosis and 67 (19) units after 2 years of a GFD (P = 0.009). Anti-endomysial IgA was positive in 82% of subjects at diagnosis and in 39% of subjects after 2 years of a GFD. Of the five patients negative for anti-endomysial anti-
Table 3  Disaccharidase activities of duodenal biopsies from coeliac subjects on a normal diet, and at various intervals after starting a GFD
Data are means ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>Lactase (µmol·min⁻¹·g⁻¹ wet weight)</th>
<th>Sucrase (µmol·min⁻¹·g⁻¹ wet weight)</th>
<th>Maltase (µmol·min⁻¹·g⁻¹ wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coeliac/normal diet (n = 18)</td>
<td>1.4 (0.2)</td>
<td>5.6 (0.7)</td>
<td>10.3 (1.6)</td>
</tr>
<tr>
<td>Coeliac/GFD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 months (n = 5)</td>
<td>2.2 (0.2)</td>
<td>9.0 (1.5)</td>
<td>17.4 (0.9)</td>
</tr>
<tr>
<td>3 months (n = 18)</td>
<td>1.6 (0.2)</td>
<td>5.5 (0.7)</td>
<td>13.5 (1.7)</td>
</tr>
<tr>
<td>6 months (n = 18)</td>
<td>2.0 (0.4)</td>
<td>6.3 (1.0)</td>
<td>16.5 (2.6)</td>
</tr>
<tr>
<td>12 months (n = 18)</td>
<td>1.7 (0.2)</td>
<td>5.9 (0.6)</td>
<td>14.1 (1.8)</td>
</tr>
<tr>
<td>24 months (n = 16)</td>
<td>3.0 (0.5)</td>
<td>8.0 (1.1)</td>
<td>17.2 (2.5)</td>
</tr>
<tr>
<td>Controls (n = 16)</td>
<td>5.5 (2.9)</td>
<td>9.6 (1.4)</td>
<td>21.8 (2.2)</td>
</tr>
</tbody>
</table>
* This excludes subjects with lactase deficiency (n = 8); the inclusive values were 3.7 ± 0.7.

DISCUSSION

Subjects with coeliac disease often report clinical improvement in symptoms before any improvement is evident on intestinal biopsy [1,2,7]. The present study was undertaken to investigate whether intestinal permeability (a functional test of the small intestine) improved soon after commencing a GFD. Our investigation was prospective, using a large number of subjects with coeliac disease who were investigated at progressive intervals after starting a GFD. Histological improvement was quantified morphometrically, unlike the subjective reporting of the majority of previous studies. Intestinal permeability improved significantly within 2 months of commencing a GFD, with the median value of intestinal permeability decreasing from a ratio of 0.47 to 0.16. This preceded any improvement in villus area, which only began to improve after 3 months on a GFD and showed no further improvement after 6 months of a GFD.

Abnormal intestinal permeability was present in 89% of untreated subjects with coeliac disease, which was greater than the 82% detected by measurement of anti-endomysial IgA antibodies, at least in our population. The major finding in the present study was that intestinal permeability decreased exponentially, and had improved after only 2 months of a GFD (although not completely to normal), compared with the partial improvement of intestinal morphometry by 6 months. The improvement at 2 months was due to a 220% increase in monosaccharide (rhamnose) absorption and a 44% decrease in disaccharide (lactulose) permeation (Table 1). A previous study of intestinal permeability using lactulose/mannitol showed a similar improvement in permeability after 2 months of a GFD [24], and an earlier study also showed improvement in permeability within 5 months of starting a GFD [25]. It has been suggested that coeliac disease involves an intrinsic permeability defect [13], but the rapid improvement after commencement of a GFD indicates that permeability is related to gluten ingestion. Conversely, a single 30 g dose gluten challenge causes a rise in intestinal permeability within 3 days [25].

The simplest possible explanation for the rapid improvement in intestinal permeability is that there was a greater intestinal surface area available within weeks of commencing a GFD [26]. It was interesting that rhamnose permeation increased up to 2.2-fold in the period from 2 weeks to 3 months after starting the GFD. The mucosal surface area of the small intestine in humans is amplified over that of a simple cylinder by submucosal folds, villi and microvilli, with a proximal-to-distal gradient such that 50% of the total intestinal surface area is within the first 25% of the proximal intestine [27]. It is unlikely that the improvement in intestinal permeability was due to an increased mucosal surface area, proximally at least, as intestinal morphometry did not show any detectable change in villous surface area.
and there was no detectable change in disaccharidase activities, indicating that microvilli had not improved at this stage. Therefore this cannot explain the improved rhamnose absorption.

Controversy exists as to the mechanism of monosaccharide permeation. One proposed mechanism is transcellular permeation across the enterocyte, which, in the case of rhamnose, is also mediated passively [26]. This is presumably dependent on differentiated villous rather than immature crypt cells. Interestingly, rhamnose absorption was only reduced to 60% of control values in untreated coeliac disease, even though villous area was reduced to 16% of normal values (Table 1). This suggests the rhamnose permeation is not entirely dependent on villous architecture. Rhamnose absorption increased by 220% between 0.5 and 3 months after starting a GFD, which is difficult to reconcile with continued villous atrophy, although there may have been distal ileal improvement. Another proposed mechanism is paracellular permeation through tight junctions between enterocytes, with a differential effect between the less permeable junctions of villous enterocytes and the more permeable junctions between crypt enterocytes (the Hollander hypothesis) [26]. However, these theories still do not explain our findings of increased rhamnose absorption at 3 months after commencing a GFD. We suspect that other factors, such as improved ileal compensation and changes in motility, may in some measure explain these unusual results.

Changes in lactulose permeation are more easily explained, as being due to an improvement in individual enterocyte function because of a decrease in inflammatory cytokines in the mucosa. Immunological activity decreased rapidly by 45% within 6 weeks after commencing a GFD, as shown by the exponential decrease in sIL-2R levels (Figure 4). Recent studies have indicated that mucosal cytokines disrupt intestinal tight junctions. Fasano et al. [28] described high-level expression of a novel cytokine, zonulin, in the intestinal lamina propria of untreated coeliac subjects, and this was found to disrupt the assembly of tight junctions. Zonulin expression was reduced after treatment, although the period of a GFD was not indicated. Zonulin is a analogue of zonula occulens toxin from Vibrio cholerae [29]. Morphometric studies have shown that crypt epithelial junctions are exposed in untreated coeliac disease, and are relatively structurally abnormal as compared with tight junctions on villus epithelial cells [30,31]. These morphometric abnormalities are correlated with increased paracellular permeability. A recent study has shown that untreated coeliac subjects have a deficiency of ZO-1 protein, which is a constituent of epithelial tight junctions [32]. These studies certainly help to explain why lactulose permeation in our study was increased nearly 5-fold in subjects with untreated coeliac disease, and normalized by 12–24 months of a GFD (Table 1).

The present prospective study also confirmed what has been suspected for a long time in adults with coeliac disease – namely that the proximal small bowel does not entirely return to normal after commencing a GFD. Morphometry demonstrated that villous atrophy persisted even after 2 years of a GFD. We also extended our morphometry to six subjects after 4 years of a GFD, and showed no further evidence of improvement. Our findings agree with those from a study in adult coeliac subjects which showed that villous epithelial thickness improved in subjects on a GFD to 61% of control values [33]. Although deliberate or inadvertent ingestion of gluten would probably explain this effect, it is interesting that no detectable differences in villous atrophy were seen in the first 6 months of a GFD before and after gluten-free labelling (and therefore gluten elimination) became stricter in Australia (Table 2). In addition, no improvement in villous atrophy was seen in subjects who were negative for anti-endomysial antibody, as compared with those who were positive, after 2 years of a GFD. The problem is that gluten exclusion is very difficult, and it is almost impossible to assess accurately minor indiscretion or, more likely, inadvertent ingestion of a minuscule quantity of gluten. A GFD probably represents the ideal end of a continuum rather than a dichotomous reality of dietary exclusion.

In conclusion, this prospective study has shown that intestinal permeability, particularly the component of monosaccharide absorption, improved rapidly after commencing a GFD; nevertheless villous atrophy, as assessed morphometrically, takes at least 3–6 months to improve, and recovery was still incomplete after this duration. This was in spite of the removal of gluten antigen and decreased immunological activity. Thus a degree of villous atrophy may persist indefinitely in coeliac subjects, despite them adhering to an apparent GFD.

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