

# Systematic review: tolerable amount of gluten for people with coeliac disease

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## SUMMARY

### Background

The threshold amount of gluten in 'gluten-free' products that can be tolerated by people with coeliac disease is unclear.

### Aim

To investigate the threshold amount of gluten and the threshold concentration of gluten in food products that can be tolerated by people with coeliac disease.

### Design

Systematic review of studies published between 1966 and May 2007.

### Methods

The data sources used were MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, CINAHL, and reference lists of retrieved articles. We included studies that evaluated the amount of dietary gluten or the concentrations of gluten in food products that can be tolerated by people with coeliac disease whatever their design, method or language of publication.

### Results

Thirteen studies (three randomized controlled, one cohort, two crossover, and seven cross-sectional) met the inclusion criteria. The daily amount of tolerable gluten varied widely between studies. Whilst some patients tolerated an average of 34–36 mg of gluten per day, other patients who consumed about 10 mg of gluten per day developed mucosal abnormalities. The effect of the consumption of 'gluten-free' products with different degrees of gluten contamination was also inconsistent.

### Conclusions

The amount of tolerable gluten varies among people with coeliac disease. Although there is no evidence to suggest a single definitive threshold, a daily gluten intake of <10 mg is unlikely to cause significant histological abnormalities.

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## INTRODUCTION

Coeliac disease (CD) is defined as a permanent intolerance to gluten, a protein found in cereals such as wheat, rye and barley, associated with mucosal disease of the proximal small bowel.<sup>1</sup> The disorder is characterized by intestinal malabsorption, histological abnormalities of the small bowel mucosa, clinical and histological improvement on a 'gluten-free' diet, and relapse on a gluten-containing diet. Lifelong avoidance of gluten ingestion is the cornerstone treatment for CD.<sup>2</sup>

The true prevalence of CD is difficult to ascertain as many affected people are asymptomatic. The prevalence of the disease is estimated to vary between 1/100 and 1/500 in different continents.<sup>3</sup> The seroprevalence of CD in the general population of both English adults<sup>4</sup> and children<sup>5</sup> has been reported to be about 1%.

Whilst it is generally accepted that avoidance of gluten is necessary for people with CD, the relationship between the quantity of gluten ingested and the development of symptoms and histological abnormalities is not clearly defined<sup>6</sup> and the exact amount of gluten that people with CD can tolerate on a daily basis without suffering any deleterious effects has not been established. Total avoidance is also extremely difficult, if not impossible to achieve, as gluten contamination in 'gluten-free' products cannot be avoided completely.<sup>6,7</sup> Thus in CD, it is generally accepted that the term 'gluten-free' refers to a level of gluten that is supposed to be harmless, when ingested indefinitely, rather than to total absence of gluten.

Considerable controversy exists among authorities as to what constitutes a 'gluten-free' diet'. In 1998, the World Health Organisation / Food and Agriculture Organization's Commission that sets international standards for foods, Codex Alimentarius, proposed a revision of their 1981 standard<sup>8</sup> and suggested that naturally 'gluten-free' foods (i.e. food consisting of or made only from ingredients which do not contain any prolamins from wheat or all *Triticum* species such as spelt, kamut or durum wheat, rye, barley, [oats] or their crossbred varieties) should not contain more than 20 parts per million (ppm) of gluten but that foods consisting of ingredients from wheat, rye, barley, oats, spelt or their crossbred varieties, which have been rendered 'gluten-free' should not contain more than 200 ppm gluten.<sup>9</sup> Although the current Codex recommendation of  $\leq 200$  ppm gluten in wheat starch-based

'gluten-free' products has been adopted in a number of countries including the UK and some European countries, it is not universally accepted. In some countries such as the USA, food made from wheat starch is not recommended, and a naturally 'gluten-free' diet is prescribed.<sup>10</sup> These different practices reflect the fact that the exact amount of gluten that can be tolerated without harmful effects by patients with CD remains unclear.

In this study, we aimed to evaluate systematically the current evidence on the potential threshold amount of gluten which people with CD can tolerate, and to explore the evidence base of the Codex threshold level of gluten in 'gluten-free' products that can be tolerated by people with CD.

## METHODS

### Literature search strategy

We searched the following electronic databases: Medline (1966 to May 2007), CINAHL (1982 to May 2007), Embase (1974 to May 2007), and the Cochrane Central Register of Controlled Trials (Issue 2, 2007). The Medline search strategy was of the general structure 'Coeliac disease synonyms' AND 'gluten synonyms' AND 'threshold OR concentration synonyms.' The Medline search strategy was adapted and used to search the other databases. We then searched the reference lists of studies identified by electronic searches to identify additional studies. We also contacted key individuals and organisations to ascertain their knowledge of published and unpublished data or ongoing studies. The search strategy was not limited by language.

### Identification of articles for inclusion

To reflect the difficulties in performing long term randomized controlled trials on this topic, our initial inclusion criteria were deliberately broad. We sought studies on patients with histologically diagnosed CD that reported changes in small intestinal histology or clinical symptoms as a primary or secondary outcome. We aimed to include various study designs including randomized controlled trials (RCTs), cohort studies, case control studies, cross-sectional studies, longitudinal surveys and cross-over studies.

Using the search strategy described above, papers that appeared to be potentially relevant were identified by the two reviewers after reviewing titles and

abstracts of articles. Full manuscripts were obtained for those that appeared potentially relevant. The reviewers, after reading the full texts, independently assessed the eligibility of all studies identified using eligibility criteria based on the inclusion criteria above. Disagreement among reviewers was discussed and agreement reached by consensus.

### Data extraction

The two reviewers independently extracted data from each included study by using a predefined data extraction form. Any disagreement was resolved by consensus. We extracted details of the study design, aim of the study, setting of the study, study quality characteristics, study period, interventions used, outcome data, potential confounding factors and results. The quality of included RCTs and cohort studies was assessed using checklists recommended by the Scottish Intercollegiate Guidelines Network.<sup>11</sup>

## RESULTS

Based on a review of the title and/or abstract of the paper, 35 studies were initially identified as being potentially eligible for inclusion. After reviewing the full manuscripts, 22 of these studies were excluded. A list of the excluded studies with reasons for exclusion is shown in Table 1.

We included 13 studies that examined the effect of persistent intake of various amounts of gluten or gliadin in patients with CD. A summary of the characteristics of the included studies is shown in Table 2. Three of these studies were randomized controlled trials (RCTs),<sup>12-14</sup> one was a cohort study<sup>15</sup>, two were cross-over studies<sup>16, 17</sup> and the remaining seven were cross-sectional studies.<sup>6, 18-23</sup> All but two of the included studies investigated the effect of the ingestion of a specified amount of gluten/gliadin over a specified period of time. The other two studies investigated what happens when people with CD ingested products with a specified concentration of gluten.<sup>13, 22</sup> We originally aimed to perform meta-analyses with this review. However, on closer examination of the results of the primary studies, it became obvious that any form of pooled statistical analyses was not possible. This is because the study designs, amount of gluten ingested, the length of exposure to gluten and the way the effect of gluten was assessed varied greatly between studies.

### Methodological quality of included studies

The three included randomized controlled trials<sup>12-14</sup> were judged to have a low risk of bias. In the cohort study by Chartrand *et al.*, wheat starch was added to the 'gluten-free' diet of patients with CD and the control group consisted of patients with CD who were known to tolerate wheat starch-based products.<sup>15</sup> The choice of control group appears inappropriate. We judged that there was a high risk of bias in this study. The two cross-over studies and the seven cross-sectional studies were judged to have a moderate risk of bias.

### Threshold amount of ingested gluten

Catassi *et al.* found that after 4 weeks, there was a significant reduction in the villous height/crypt depth ratio in patients who received a daily dose of either 100 mg of gliadin (200 mg gluten) or 500 mg gliadin (1 g gluten), but the changes were more marked in the latter group.<sup>12</sup> No clinical symptoms were reported in the 100 mg gliadin group but three patients in the 500 mg gliadin group developed loose stools. In another study by Catassi's group, patients who consumed 50 mg of gluten per day for 3 months had a significant worsening of their villous height:crypt depth ratio compared to patients receiving placebo.<sup>14</sup> Eleven of 13 patients who consumed 50 mg of gluten per day developed worsening of villous height:crypt depth ratio, but seven of 13 patients who ingested 10 mg of gluten per day also had worsening of their villous height:crypt depth ratio. Baker *et al.* found that 16 of 24 patients consuming <2 g gluten per day had villous atrophy and all nine patients consuming >2 g/day gluten had villous atrophy.<sup>19</sup> In this study, 13 of 18 patients adhering to a strict 'gluten-free' diet *al.so* had villous atrophy, but the gluten content of the 'gluten-free' diet was not assessed.

Ciclitira *et al.* showed that after one week, seven adults receiving between 1.2 and 2.4 mg gliadin from bread in addition to their usual 'gluten-free' diet exhibited a significant reduction in the mean villous height/crypt depth ratio.<sup>16</sup> However, the same group of researchers later found no significant difference in jejunal morphometry in a 6-week period during which patients consumed between 1.2 mg and 2.4 mg of gluten from bread compared to another 6-week period where this product was not ingested.<sup>17</sup> Dissanayake *et al.* assessed 38 adults with CD after a mean of 27.5

Table 1. Excluded studies and reasons for exclusion

Study ID	Reason for exclusion
Anderson RP, van Heel DA, Tye-Din JA, <i>et al.</i> T cells in peripheral blood after gluten challenge in celiac disease. <i>Gut</i> 2005; 54: 1217–23.	Unsuitable outcome measure
Auricchio S, Troncone R. Effects of small amounts of gluten in the diet of coeliac patients. <i>Panminerva Med</i> 1991; 33: 83–5.	Review
Ciclitira PJ, Evans PJ, Fagg NLK, <i>et al.</i> Clinical testing of gliadin fractions in coeliac patients. <i>Clin Sci</i> 1984; 66: 357–64.	Gliadin administered intravenously
Ciclitira PJ. Gluten-free diet – what is toxic? <i>Best Pract Res Clin Gastroenterol</i> 2005; 19: 359–71.	Review
Ciclitira PJ, Johnson MW, Dewar DH, Ellis HJ. The pathogenesis of celiac disease. <i>Mol Aspects Med</i> 2005; 26: 421–58.	Review
Dewar DH, Amato M, Ellis HJ, <i>et al.</i> The toxicity of high molecular weight glutenin subunits of wheat to patients with coeliac disease. <i>Eur J Gastroenterol Hepatol</i> 2006; 18: 483–91.	No assessment of gluten intake
Ellis HJ, Rosen-Bronson S, O'Reilly N, Ciclitira PJ. Measurement of gluten using a monoclonal antibody to a celiac toxic peptide of A gliadin. <i>Gut</i> 1998; 43: 190–5.	Did not study tolerable levels of gluten
Ferguson A, Gillett H, O'Mahony S. Active immunity or tolerance to foods in patients with celiac disease or inflammatory bowel disease. <i>Ann NY Acad Sci</i> 1996; 778: 202–16.	Review
Ferguson A, Gillett H, Humphreys K, Kingstone K. Heterogeneity of celiac disease: clinical, pathological, immunological and genetic. <i>Ann NY Acad Sci</i> 1998; 859: 112–20.	Review
Hischenhuber C, Crevel R, Jarry B. Review article: safe amounts of gluten for patients with wheat allergy or coeliac disease. <i>Aliment Pharmacol Ther</i> 2006; 23: 559–75.	Review
Holm K, Maki M, Vuolteenaho N, <i>et al.</i> Oats in the treatment of childhood coeliac disease: a 2-year controlled trial and long-term clinical follow-up study. <i>Aliment Pharmacol Ther</i> 2006; 23: 1463–72.	Describes effect of oats
Howdle PD, Ciclitira PJ, Simpson FG, Losowsky MS. Are all gliadins toxic in coeliac disease? <i>Scand J Gastroenterol</i> 1984; 19: 41–7.	In vitro study
Johnson RB, Labrooy, JT, Skerritt JH. Antibody responses reveal differences in oral tolerance to wheat and maize grain protein fractions. <i>Clin Exp Immunol</i> 1990; 79: 135–40.	Animal study
Van Overbeek FM, Uil-Dieterman IGA, Mol IW, <i>et al.</i> The daily gluten intake in relatives of patients with celiac disease compared with that of the general Dutch population. <i>Eur J Gastroenterol Hepatol</i> 1997; 9: 1097–9.	Describes gluten intake in relatives
Pena AS, Crusius JBA. Food allergy, celiac disease and chronic inflammatory bowel disease in man. <i>Vet Q</i> 1998; 20: S49–52.	Review
Picarelli A, Triglione P, Mariani P, <i>et al.</i> Use of a threshold serum level of anti-gliadin antibodies improves diagnostic efficiency of the test in adult coeliac disease but is unreliable as a screening test. <i>Ital J Gastroenterol</i> 1996; 28: 70–5.	Did not study tolerable levels of gluten
Restanti P, Beretta B, Ballabio C, <i>et al.</i> Evaluation by SDS-page and immunoblotting of residual antigenicity in gluten-treated wine: a preliminary study. <i>Int J Tissue React</i> 2002; XXIV: 45–51.	Unsuitable outcome measure
Stern M, Ciclitira PJ, van Eckert R, <i>et al.</i> Analysis and clinical effects of gluten in celiac disease. <i>Eur J Gastroenterol Hepatol</i> 2001; 13: 741–7.	Review
Thompson T. Wheat starch, gliadin and the gluten-free diet. <i>J Am Diet Assoc</i> 2001; 101: 1456–9.	Review
Thompson T. Oats and the gluten-free diet. <i>J Am Diet Assoc</i> 2003; 103: 376–9.	Review
Vader W, Kooy Y, van Veelen P, <i>et al.</i> The gluten response in children with coeliac disease is directed toward multiple gliadin and glutenin peptides. <i>Gastroenterology</i> 2002; 122: 1729–37.	Did not study tolerable levels of gluten
Valdes I, Garcia E, Llorente M, Mendez E. Innovative approach to low-level gluten determination in foods using a novel sandwich enzyme-linked immunosorbent assay protocol. <i>Eur J Gastroenterol Hepatol</i> 2003; 15: 465–74.	Did not study tolerable levels of gluten

Table 2. Characteristics of included studies

Study ID	Methods	Participants	Diet	Outcomes	Comments
Dissanayake 1974	Cross-sectional study from England.	38 adults with CD reassessed after 6–72 (mean 27.5) months on 'GFD'.	Patients on strict 'GFD', 'small amounts', or 'large amounts' of gluten (assessed by dietary interview, not measured directly).	Jejunal mucosal histology.	Authors did not define what they meant by 'strict' or 'small' but 'large' was defined as $\geq 0.5$ g/day. Symptoms not recorded in detail. Gluten consumption estimated from dietary questionnaire, not measured directly. Symptoms not recorded.
Baker 1975	Cross-sectional from England.	51 adults with CD assessed after 4–132 (mean 63) months on 'GFD'.	Patients on 'no gluten', 'small amounts' (<2 g/day) or 'large amounts of gluten' ( $\geq 2$ g/day).	Jejunal mucosal histology.	Gluten consumption estimated from dietary questionnaire, not measured directly. Symptoms not recorded.
Ciclitira 1984	Cross-over study from England.	7 adults with CD assessed after >12 months on 'GFD' then study diet.	One week on 'GFD', then one week on same diet plus bread containing up to 2.4 mg gliadin/day.	Jejunal mucosal histology after each 1 week period.	Gliadin intake estimated but not measured directly. Symptoms not recorded.
Ciclitira 1985	Cross-over study from England.	10 adults with CD assessed after at least 1 year on 'GFD' then study diet.	6 weeks on 'GFD' & 6 weeks on same diet plus bread containing up to 2.4 mg gliadin/day.	Jejunal mucosal histology & symptoms after each 6 week period.	Gliadin intake estimated but not measured directly.
Montgomery 1988	Cross-sectional study from England.	25 adults with CD.	'Low gluten diet' (2.5–5 g/day for 3–14 (median 6) months ( $n = 13$ ) or strict 'GFD' for 6–27 (median 13) months ( $n = 12$ )).	Jejunal mucosal histology.	Amount of gluten in strict 'GFD' not measured. Symptoms not recorded.
Catassi 1993	Randomized controlled trial from Italy.	20 children with CD aged 1.6 to 9.6 years after mean 14 months on 'GFD'.	Randomized to receive either 100 mg or 500 mg gliadin/day for 4 weeks.	Jejunal mucosal histology.	Amount of gluten in 'GFD' estimated to be about 1 mg/day but not measured directly. Symptoms recorded included anorexia & pale stools.
Chartrand 1997	Cohort study from Canada.	31 adults with CD on 'GFD' for at least 1 year.	Wheat starch added to 'GFD' (gliadin content measured by gluten ELISA).	Symptoms.	17 participants had never been on wheat starch-based products but 14 controls had previously tolerated them. Symptoms recorded included: diarrhoea, abdominal pain, altered stools, flatulence, bone pain, appetite, fatigue, rash).
Kaukinen 1999	Cross-sectional study from Finland.	41 children & adults with CD & 11 adults with dermatitis herpetiformis on GFD for mean 8 years.	Strict wheat starch-based 'GFD', gluten intake 5–150 (mean 34) mg/day ( $n = 40$ ), strict naturally 'GFD' ( $n = 6$ ) or wheat starch based GFD + 1–2 g gluten/week or at least once a month.	Small bowel mucosal histology.	Assumed that wheat starch-based 'GFD' contained maximum amount of gluten allowed by Codex Alimentarius. Amount of gluten not measured directly. Symptoms not recorded.

Table 2. Continued

Study ID	Methods	Participants	Diet	Outcomes	Comments
Selby 1999	Cross-sectional study from Australia.	89 adults with longstanding CD (0.6–29.2 years).	Codex 'GFD' or non-detectable of gluten in diets not measured. Patients on wheat starch-based 'GFD'.	Duodenal mucosal histology.	Symptoms not recorded.
Lohiniemi 2000	Cross-sectional study from Finland	58 adults with CD diagnosed 9–11 years previously, 110 non-coeliac controls.	Gluten intake from wheat starch estimated to be 0–180 (mean 36) mg/day (based on dietary survey).	Symptoms, well-being, small bowel mucosal histology.	Scales used: Gastrointestinal Symptom Rating Scale, Psychological General Well-Being Inquiry.
Peraaho 2003	RCT from Finland.	57 adults with untreated (newly diagnosed) CD.	Either a wheat starch-based or a naturally 'GFD' for 1 year. Amount of gluten in diets estimated by dietitian but not measured directly.	Symptoms; duodenal mucosal histology.	Scales used: Gastrointestinal Symptom Rating Scale, Psychological General Well-Being Inquiry.
Collin 2004	Cross-sectional study from Finland.	76 adults & 16 children with CD on strict 'GFD' for 1–10 (median 2) years.	Either a wheat starch-based or a naturally 'GFD'. Flour consumption in adults was 10–300 (median 80) g/day.	Small bowel mucosal histology.	Gluten content of gluten-free products determined by R5 ELISA. Symptoms not recorded.
Catassi 2007	RCT from Italy.	49 adults with CD on 'GFD' (<5 mg gluten/day) for >2 years.	Randomized to receive 10 mg of gluten, 50 mg of gluten or 50 mg placebo for 90 days.	Small bowel mucosal histology before & after challenge.	Symptoms recorded included: vomiting, diarrhea, abdominal distension. Histological evaluation by 2 histopathologists blinded to subject assignment.

GFD, gluten free diet.

(range 6–72) months where they were supposed to be on a 'gluten-free' diet.<sup>18</sup> They found no histological abnormalities in 16 of 18 patients who were strictly adhering to the 'gluten-free' diet (gluten content of diet not measured), but nine of 13 patients receiving small amounts of gluten and all seven patients receiving large amounts of gluten had some histological abnormalities. The exact gluten content of the 'strict', 'small' and 'large' groups was not measured. Montgomery *et al.* found histological abnormalities in patients who consumed between 2.5 and 5 g gluten per day for 3–14 months (median 6) and those on a strict 'gluten-free' diet for 6–27 months (median 13 months).<sup>20</sup> The content of gluten in the strict 'gluten-free' diet was not assessed.

Kaukinen *et al.* showed that patients who consumed between 5 and 150 mg gluten daily (mean 34 mg) for about 8 years developed no histological abnormalities, but some of those who in addition, also ingested between 1 and 2 g of gluten per week developed villous atrophy.<sup>21</sup> In another study, patients consuming an average of about 36 mg of gluten per day did not develop histological abnormalities or clinical symptoms.<sup>23</sup> However, in one study, a much smaller dose of gluten (1.5 mg daily) triggered symptoms in some patients.<sup>15</sup> In this study, 15 of 17 patients who had never been on wheat starch-based 'gluten-free' products developed significant gastrointestinal symptoms within 8 months of taking a wheat starch-based 'gluten-free' product and consuming the equivalent of 1.5 mg gluten per day in addition to their usual 'gluten-free' diet, although other patients had remained well after taking the same product and consuming the same amount of gluten for about 6 years. Mucosal histology was not assessed in this study. In another study, Collin *et al.* found no correlation between the amount of flour ingested and intestinal mucosal morphology in patients consuming between 10 g and 300 g of wheat-starch based 'gluten-free' flour.<sup>6</sup>

### Threshold limit of gluten concentration in food

Two studies assessed the effect of different concentrations of gluten in food. Selby *et al.* found that 18 of 39 patients consuming a Codex 'gluten-free' diet had villous atrophy, and 20 of 50 patients ingesting non-detectable gluten 'gluten-free' diet (containing less than 0.003% protein derived from gluten-containing grain) also had villous atrophy.<sup>22</sup> The exact amount of gluten ingested by patients was not measured.

Peraaho *et al.* randomized fifty seven adults with untreated CD to a wheat starch-based or natural gluten-free diet.<sup>13</sup> After 12 months, abdominal symptoms were alleviated equally in both the wheat starch-based and natural 'gluten-free' diet groups and there was no significant difference in small intestinal morphology or intra-epithelial lymphocytes between the groups. The exact gluten content in both diets was not measured.

## DISCUSSION

Whilst it is accepted that the treatment of CD is a 'gluten-free' diet, there is a great deal of controversy surrounding what a 'gluten-free' diet should be. This confusion arises because of two main reasons: 1) it is extremely difficult to achieve a diet which is completely devoid of gluten and 2) the exact amount of gluten that people with CD can tolerate without experiencing adverse effects is not clearly established. In this study, we systematically examined the available evidence on the threshold amount of ingested gluten that would be tolerable for people with CD and also investigated the evidence base for a tolerable threshold concentration of gluten in foods.

### Key findings

The available evidence shows that the consumption of about 200 mg gluten per day is clearly associated with the development of intestinal mucosal abnormalities after only 4 weeks in patients with CD.<sup>12</sup> In one study, the ingestion of 10 or 50 mg gluten per day was associated with worsening of the villous height/crypt depth ratio in most patients after 3 months.<sup>14</sup> In two other studies, patients who consumed an average of 34–36 mg of gluten per day did not develop histological abnormalities after an average of at least 8 years<sup>21, 23</sup> but in another study, Chartrand *et al.* found that some patients developed symptoms on much smaller amounts within 8 months, but unfortunately, in this study, histological changes were not assessed in either the patients who remained well or those who exhibited symptoms.<sup>15</sup> Thus, although it appears that some patients may tolerate an average of about 34–36 mg gluten per day, it is likely that some other patients may develop histological changes or manifest symptoms with much smaller amounts.

The length of exposure to gluten varied considerably between the included studies. For instance, in

the study by Kaukinen *et al.*, patients had been on their diet for a mean of 8 years,<sup>21</sup> whereas in the study by Ciclitira *et al.*, patients were assessed after a one week gluten challenge.<sup>16</sup> It is likely that the length of exposure would have an effect on outcomes.

The evidence regarding the threshold limit of gluten concentration in food is also unclear. Peraaho *et al.* found that patients consuming either natural 'gluten-free' diet or wheat starch-based 'gluten-free' diet containing up to 40–60 mg per 100 g of food developed no symptoms or mucosal abnormalities.<sup>13</sup> Selby *et al.*, however, found persistent mucosal abnormalities in patients who were consuming either Codex 'gluten-free' products or 'non-detectable gluten' 'gluten-free' products (containing less than a tenth of the gluten content of the Codex products).<sup>22</sup> It is not clear how the duration of being on Codex 'gluten-free' products contributed to these contradictory findings. In the study by Peraaho *et al.*,<sup>13</sup> assessment was made after one year whereas in the study by Selby *et al.*,<sup>22</sup> patients appeared to have been on these products for the duration of their disease (0.6–29.2 years).

### Implications of the research

There is considerable debate within the food industry regarding the optimum concentration of gluten in 'gluten-free products'. This review has highlighted the limited nature of the evidence base in this area. We can deduce from this study that although some people with CD may tolerate products with the current Codex concentration of gluten, others may develop symptoms and/or histological abnormalities when they consume products with even lower concentrations of gluten. It is likely that what is most important is the total amount of gluten ingested rather than just the concentration of gluten in the food products as the amount of gluten ingested will depend on both the concentration and the volume of food products consumed.

It is clear from this study that the current Codex standard of 200 ppm is not sufficiently protective for *all* people with CD and so there may be a case for lowering the current concentration of gluten permitted in 'gluten-free' food products. However, we found no evidence to suggest a single definitive threshold concentration of gluten in food products that would be tolerated by all people with CD. Collin and colleagues argued that if the daily 'gluten-free' flour

intake of patients with CD is assumed to be that found in their study (300 g or less), a threshold gluten concentration in flour of 100 ppm (100 mg/kg of flour), will mean that patients will not be consuming more than 30 mg gluten per day.<sup>6</sup> However, as Catassi and colleagues have recently shown, some patients who consume an even lower dose of gluten (10 mg daily) will develop histological changes.<sup>14</sup> It can therefore be argued that if the concentration is set at, say, 20 ppm, patients will be consuming around 6 mg per day of gluten which may be less likely to induce mucosal changes.

It is obvious from the results of this study that the amount of tolerable gluten varies among people with CD. The reason for this remains unclear. Future studies should investigate potential reasons (e.g. genetic variability) that may explain the variable response to gluten. Future studies should also assess the exact amount of gluten that can be tolerated by people with CD and over what period of time and the exact concentration of gluten in wheat-starch 'gluten-free' products and all other foods that can be tolerated. Tolerance should be assessed by small bowel histology before and after the intervention and reproducibility of assessments by histopathologists should also be assessed. Assessment of tolerance should also include symptoms and quality of life. Factors influencing latency (e.g. age at diagnosis, initial time on a gluten-free diet and time elapsed from diagnosis) should be standardized and clearly defined. We recommend that future studies in this area should be well designed RCTs and should have adequate statistical power to detect any differences between groups. The necessary sample size of future studies may be influenced by the particular outcome being assessed (i.e. histology, symptoms, quality of life) as well as the magnitude of the intervention (i.e. amount of gluten and length of exposure).

### Strengths and weaknesses of the study

Our review included studies from Europe, North America and Australia. We searched multiple databases and reference lists and also contacted key individuals and organizations so it is unlikely that we missed relevant studies. We minimized subjectivity by carrying out study selection, data extraction, and quality assessment in duplicate. However, the validity of the results of a systematic review depends on the validity of the included studies. Considering the



number of people affected by CD world-wide, it is surprising that so few good quality studies have been conducted to ascertain tolerable amounts of gluten. Many of the included studies failed to take all the steps necessary to avoid bias. Our conclusions were, therefore, limited by the quality of included studies and the information provided. Despite the inclusion of 13 studies, significant variations in study design, amount of gluten ingested, the length of exposure to gluten, and the way the effect of gluten was assessed prevented the use of meta-analysis to summarize results.

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