TOLERABLE AMOUNT OF GLUTEN FOR PEOPLE WITH COELIAC DISEASE

A systematic review conducted on behalf of the Food Standards Agency (Project T07048)

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1. EXECUTIVE SUMMARY

Although avoidance of gluten is necessary for people with coeliac disease (CD), the exact relationship between the quantity of gluten ingested and the development of symptoms and/or mucosal abnormalities is not clearly defined. The aim of this study was to evaluate articles that had investigated threshold amounts of gluten in the diet which people with CD can tolerate, or threshold concentrations of gluten in food products that can safely be consumed by people with CD. We performed a systematic review of studies published between 1966 and March 2006 that had examined the relationship between the amount of gluten ingested or the concentrations of gluten in food products, and the development of symptoms or mucosal abnormalities. Our data sources included Medline, Embase, Cochrane Central Register of Controlled Trials, Cinahl, and reference lists of retrieved articles.

Twelve studies met the review criteria. A daily consumption of 200 mg or more of gluten clearly induced mucosal abnormalities. In two studies, the ingestion of an average of 34 - 36 mg of gluten daily did not cause histological changes or clinical symptoms. However, in one study, a much smaller dose of gluten (1.5 mg daily) triggered symptoms but it was not clear whether the symptoms were associated with mucosal abnormalities. The effect of the consumption of 'gluten-free' products with different degrees of gluten contamination was also inconsistent between studies. Whilst some people tolerated current Codex standard 'gluten-free' products (less than 200 ppm gluten), others developed histological abnormalities whilst consuming the same products. It is likely that it is the total amount of gluten ingested over time rather than the concentration of gluten in the food products that is important.

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. We, however, found insufficient evidence to allow us to propose a definitive clinical threshold dose of gluten or a threshold concentration of gluten in food products that would be tolerated by all people with CD. It appears that the amount of tolerable gluten varies among people with CD but the reason for this is unclear.

Future studies should be randomised controlled trials and should address the following points:

1. the exact amount of gluten that can be tolerated by people with CD and over what period of time.
2. the exact concentration of gluten in wheat-starch ‘gluten-free’ products and all other foods that can be tolerated by people with CD
3. the potential reasons (e.g. genetic variability) that may explain the variable response to gluten.

2. **INTRODUCTION**

Coeliac disease (CD), also known as gluten-sensitive enteropathy, 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 (Ferenci 1998). The disorder is characterised by intestinal malabsorption, histologic abnormalities of the small bowel mucosa, clinical and histologic improvement on a gluten-free diet, and a relapse on a gluten-containing diet.

CD is a multifactorial disorder that depends on genetic, immunological and environmental factors. 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 to 1/500 in different continents (Schapira et al, 2003). The seroprevalence of CD in the general population of English adults (West et al, 2003) and English children (Bingley et al, 2004) have both been reported to be about 1%.

In infants and young children, CD classically manifests as diarrhoea, steatorrhoea, failure to thrive and abdominal distension that occurs a few weeks to months after the introduction of gluten into the diet, usually between the ages of 6 months and 3 years. Vomiting, irritability, anorexia and constipation are also common. Some patients have more subtle symptoms such as oedema, anaemia, growth retardation and recurrent dental caries (Devlin et al, 2004). In older children and adults, diarrhoea and other gastrointestinal symptoms are less prominent. In older children, short stature, retarded puberty, iron deficiency anaemia and personality problems may predominate. Adult patients often have symptoms of irritable bowel syndrome (Devlin et al, 2004) and may also present with recurrent aphthous stomatitis, iron deficiency without obvious cause, osteoporosis or osteopenia, and short stature. Other non-intestinal manifestations of CD include dermatitis herpetiformis and hepatitis. Certain malignant diseases are also more frequent in patients with CD. These include small bowel adenocarcinoma, oesophageal and oropharyngeal squamous carcinoma, and non-Hodgkins lymphoma (Green and Jabri, 2003).

Lifelong avoidance of gluten ingestion is the cornerstone treatment for CD (Fasano and Catassi, 2001). This involves a diet free of wheat, rye and barley. There is controversy regarding the safety of oats for people with CD. In a recent systematic review, Haboubi et al found that although oats can be symptomatically tolerated by most people with CD, the long term effects of a diet containing oats remain unclear (Haboubi et al, 2006). Treatment of CD is important not only to improve the immediate quality of life of the patient but also to decrease the long-term risks of untreated CD such as growth failure in children, osteopenia and malignancies.

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 (Collin et al, 2004) 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 (Hischenhuber et al, 2006; Collin et al, 2004). 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 1981, the WHO/FAO organization that sets International Standards for foods, Codex Alimentarius, suggested that foods labeled as 'gluten-free' should contain less than 0.05 g nitrogen per 100 g of food products on a dry matter basis (Codex-Alimentarius- Commission, 1981). At the time this standard was set, methods for directly measuring the gluten content of grain were not available; therefore the nitrogen content of food was used as an indirect measure. It has since been approximated that wheat starch-based 'gluten-free' products meeting the 1981 Codex standard may contain up to 40-60 mg of gluten per 100 g or 400-600 ppm {mg per kilogram} (Thompson 2001; Peraaho et al, 2003; Kaukinen et al, 1999). This amount of gluten is equivalent to 200 to 300 ppm gliadin {mg per kilogram} (Thompson, 2001)

In 1998, a draft revised standard for Codex gluten-free foods was proposed (Codex-Alimentarius- Commission, 1998). In the revised standard, it was 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 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. 'Oats'was put in brackets because the committee could not decide whether it was toxic to people with CD. Although the revised Codex standard of not more than 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 (Ciclitira 2005). These different practices reflect the fact that the exact amount of gluten that can be tolerated long term without harmful effects by patients with CD is unclear.

In the current study, we aimed to systematically evaluate the current published 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 will be tolerated by people with CD.

3. RESEARCH QUESTIONS AND AIMS OF THE STUDY

3.1 Research Questions

1. Is there a threshold amount of gluten in the diet that can be tolerated by people with CD?
2. For ‘gluten-free’ labelling purposes, is there a threshold concentration of gluten in food that will be tolerable for people with CD?

3.2 Aims

To systematically evaluate studies in any language, published or unpublished, which had investigated:

a) Threshold amount of gluten in the diet that people with CD can tolerate, and/or

b) Threshold concentration of gluten in food that can safely be consumed by people with CD.

4. STUDY DESIGN AND INCLUSION CRITERIA

The study design was a systematic review of studies that had investigated threshold amounts of gluten in the diet which people with CD can tolerate, or threshold concentrations of gluten in food products that can safely be consumed by people with CD.

4.1 Types of studies

We aimed to include studies that meet the following criteria:

a. Randomised controlled trials (RCT)
b. Cohort studies
c. Case-control retrospective studies
d. Cross-sectional studies
e. Longitudinal surveys
f. Case series

4.1.1 Types of participants

Patients diagnosed as having CD based on histological criteria regardless of age, sex or duration of the disease.

4.1.2 Types of intervention

Studies which had investigated a threshold level of gluten in people with CD.

4.2 Outcome measures

The primary outcome was small intestinal histology. Secondary outcome measures were clinical symptoms such as abdominal pain, diarrhoea, tiredness, and quality of life.

4.3 Search strategy for identification of studies

A. Electronic searching

The following electronic databases were searched for relevant studies:

1. Medline (1966- May 2006)
2. EMBASE (1974 – May 2006)
3. The Cochrane Central Register of Controlled Trials (CENTRAL) – Issue 2, 2006

4. CINAHL (1982 – May 2006)

The search strategy was not limited by language.

MEDLINE on PUBMED was searched using the following search strategy:

1. Coeliac disease OR celiac disease
2. celiac disease (MeSH)
3. Sprue
4. gluten enteropathy
5. gluten sensitive enteropathy
6. gluten intolerance
7. gluten allergy
8. gluten hypersensitivity
9. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8
10. gluten
11. gluten (MeSH)
12. gliadin*
13. gliadin (MeSH)
14. wheat
15. 10 OR 11 OR 12 OR 13 OR 14
16. threshold
17. safe
18. tolerable
19. tolerance
20. 16 OR 17 OR 18 OR 19
21. level*
22. concentration*
23. amount*
24. 21 OR 22 OR 23
25. 9 AND 15 AND 20 AND 24

The above search strategy was adapted and used to search the other databases.

B. Reference searching

The references of all identified studies were inspected for more trials.

C. Personal contacts

Leaders in the field were contacted to try and identify other studies. The final list of included studies was also sent to leaders in the field to ensure that no relevant study was missed.

D. Manufacturers of gluten-free products

Manufacturers of UK gluten-free products were contacted for additional information.

5. METHODS OF THE REVIEW

5.1 Searching

Using the search strategy described above, papers that appeared to be potentially relevant were identified by two reviewers (AKA and AGT). The abstracts of identified studies were reviewed and full manuscripts obtained for those that appeared potentially relevant.

5.2 Assessment of study eligibility

The reviewers, after reading the full texts, independently assessed the eligibility of all trials identified using an eligibility criteria based on the inclusion criteria above. Disagreement among reviewers was discussed and agreement reached by consensus.

5.3 Assessment of methodological quality

The methodological quality of included studies was independently assessed by two reviewers. For randomized controlled trials and cohort studies, we used checklists recommended by the Scottish Intercollegiate Guidelines Network (http://www.sign.ac.uk/guidelines/fulltext/50/annexc.html).

5.4 Data extraction

A data extraction form was developed and used to extract information on relevant features and results of included studies. Two reviewers (AKA and AGT) separately extracted and recorded data on the predefined checklist. Extracted data included the following items:

1. Trial quality characteristics.
2. Participants: number of subjects at baseline, gender, mean age.
3. Interventions: threshold gluten level.
4. Outcome data.
5. Potential confounding factors

5.5 Levels of evidence

The level of evidence of the main findings was determined using the published Scottish Intercollegiate Guidelines Network (SIGN) criteria (Harbour and Miller, 2001).

Levels of evidence

1++ High quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias

1+ Well conducted meta-analyses, systematic reviews of RCTs, or RCTs with a low risk of bias

1− Meta-analyses, systematic reviews or RCTs, or RCTs with a high risk of bias

2++ High quality systematic reviews of case-control or cohort studies *or* High quality case-control or cohort studies with a very low risk of confounding, bias, or chance and a high probability that the relationship is causal

2+ Well conducted case-control or cohort studies with a low risk of confounding, bias, or chance and a moderate probability that the relationship is causal

2− Case-control or cohort studies with a high risk of confounding, bias, or chance and a significant risk that the relationship is not causal

3 Non-analytic studies, e.g. case reports, case series

4 Expert opinion

The definitions of +, ++ or – are as shown below:

++ All or most of the criteria have been fulfilled. Where they have not been fulfilled the conclusions of the study or review are thought <u>very unlikely</u> to alter.

+ Some of the criteria have been fulfilled. Those criteria that have not been fulfilled or not adequately described are thought <u>unlikely</u> to alter the conclusions.

– Few or no criteria fulfilled. The conclusions of the study are thought <u>likely or very likely</u> to alter.

6. DESCRIPTION OF STUDIES

Based on a review of the title and/or abstract of the paper, a total of 34 studies were initially identified as being potentially eligible for inclusion. After reviewing the full manuscripts, 22 of these studies were excluded (see below). Twelve studies met the inclusion criteria and were included in the review. Two of these were randomised controlled trials {RCTs} (Catassi et al, 1993 and Peraaho et al, 2003), one was a cohort study (Chartrand et al, 1997), two were crossover studies (Ciclitira et al, 1984; Ciclitira et al, 1985) and the remaining 7 were cross-sectional studies (Dissanayake et al 1974; Baker et al 1975; Montgomery et al, 1988; Kaukinen et al 1999; Selby et al 1999; Lohiniemi et al 2000; Collin et al 2004).

6.1 Excluded studies

Ten papers (Auricchio and Troncone, 1991; Ciclitira et al, 2005a, Ciclitira et al, 2005b, Ferguson et al, 1996; Ferguson et al, 1998; Hischenhuber et al, 2006; Pena and Crusius, 1998, Stern et al, 2001; Thompson, 2001 and Thompson, 2003) were excluded because they were review articles. One study (Anderson et al, 2005) assessed the T cell response in peripheral blood after a 3 day gluten challenge. Two studies (Ciclitira et al, 1984; Dewar et al, 2006) were excluded because they did not assess the amount of tolerable gluten in the diet but assessed the effect of intra-duodenal infusions of gliadin. One study (Ellis et al, 1998) described the development of an assay to measure gluten in food. Another study (Holm et al, 2006) assessed the effect of oats on small intestinal morphology. One study (Howdle et al, 1984) assessed the in vitro response of jejunal biopsies to 24 hour culture with gliadin or alpha-gliadin. Another study (Johnson et al, 1990) measured antibody responses to wheat and maize grain protein fractions in mice. A further study (van Overbeek et al, 1997) measured gluten intake in relatives of patients with CD. One study (Picarelli et al, 1996) assessed the sensitivity and specificity of anti-gliadin antibodies in adult CD. One study (Restani et al, 2002) measured the antigenicity of gluten-treated wine. Another study (Vader et al, 2002) measured the T cell response to gliadin and glutenin peptides and the final excluded study (Valdes et al, 2003) described the development of an ELISA test to quantify low levels of wheat, barley and rye prolamins in foods for people with CD.

6.2 Brief summary of included studies

A summary of the characteristics of the included studies is shown in Table 1. The included studies fell into two main groups: 1) those which investigated a threshold amount of ingested gluten, and 2) those which investigated a threshold concentration of gluten in food.

6.2.1 Threshold amount of ingested gluten

Dissanayake et al 1974

This cross-sectional study from England assessed jejunal mucosal recovery in CD in relation to the degree of adherence to a 'gluten-free' diet. 38 adult patients with CD were reassessed after a mean of 27.5 (range 6-72) months on a gluten-free diet. Diagnosis of CD was based on symptoms and small intestinal histology. They were classified into 3 groups depending on their gluten intake and jejunal morphometry.

Baker et al 1975

This cross-sectional study, conducted in England, assessed the incidence and effects of continuing gluten ingestion in 51 adults with CD after a mean 63 (range 4-132) months on a gluten-free diet. Diagnosis of CD was based on jejunal biopsy appearances. Outcome was assessed by prospective dietary questionnaire, jejunal morphometry and gluten antibody estimation.

Ciclitira et al 1984

This cross-over study, conducted in England, evaluated a gluten-free product containing wheat gliadin in 7 adults with CD after more than 12 months on a gluten-free diet. They all then followed a gluten-free diet with no commercial gluten-free products for 1 week then followed the same diet plus 6 slices of Juvela gluten-free bread for 1 week. The daily intake of gliadin from the bread was between 1.2 and 2.4 g per day. The diagnosis of CD was confirmed histologically. Outcome was assessed by jejunal morphometry in biopsies taken at the end of each one week period.

Ciclitira et al 1985

The aim of this cross-over study, conducted in England, was to investigate symptoms and enterotoxicity of gliadin-containing gluten-free bread made with wheat starch in 10 adults with CD who had clinically improved on a gluten-free diet for at least a year. They were given 6 slices of gliadin-containing Juvela gluten-free bread for one 6 week period and no gluten-free bread during the other 6 week period. It was not stated in the paper how CD was diagnosed, but on contacting the corresponding author, he confirmed that diagnosis was based on histological criteria. Outcome was assessed by symptom diary, 24 hr ^{51}Cr-EDTA excretion and jejunal morphometry.

Montgomery et al 1988

This English cross-sectional study aimed to determine the effects of a low gluten containing diet on jejunal morphology & gluten antibody levels in adults with CD. The diagnosis of CD was based on a characteristic histological appearance and improvement on a gluten-free diet. Patients studied were either on a low gluten-containing diet (n=13) and consuming 2.5-5 g per day for 3-14 months (median 6 months) or a strict gluten-free diet (n=12) for 6-27 months (median 13 months). The amount of gluten in the 'strict gluten-free diet' was not measured.

Catassi et al 1993

This was a randomised controlled trial from Italy. The authors investigated the effects of chronic ingestion of specified amounts of gliadin on children with CD. Twenty children with CD who had been on a 'gluten-free' diet for a mean of 14 months were randomised to receive either 100mg (Group A, n=10, mean (SD) age 4 (2) years) or 500mg (Group B, mean age 5 (3) years) per day of gliadin for 4 weeks. Diagnosis of CD was based on subtotal villous atrophy on initial biopsy and subsequent improvement on a gluten-free diet. Effects of gliadin were monitored by jejunal morphometry, intestinal permeability (cellobiose/mannitol) & antigliadin antibody.

Chartrand et al 1997

This Canadian cohort study evaluated the tolerance of prolonged consumption of small amounts of gliadin contained in products containing wheat starch. The study was an open

1-year trial of the addition of wheat starch to a gluten free diet in 17 adults with CD who had never consumed wheat starch. Diagnosis of CD was according to ESPGAN criteria (European Society of Paediatric Gastroenterology and Nutrition, 1990). The control group consisted of 14 patients with CD who tolerated wheat starch. Outcome measures included symptoms, antigliadin and endomysial antibodies.

Kaukinen et al 1999

This was a cross-sectional study conducted in Finland. The authors investigated whether wheat starch-based gluten-free products are safe in the treatment of gluten intolerance. Forty one children and adults with CD and 11 adults with dermatitis herpetiformis who had been on a gluten-free diet for a mean of 8 years were studied. 35 newly diagnosed patients with CD at the time of diagnosis and 6-24 months after starting a gluten-free diet, and 27 non-coeliac patients with dyspepsia were investigated for comparison. Diagnosis of CD was according to ESPGAN criteria. Forty out of 52 patients were on strict wheat starch-based gluten-free diet and their mean daily intake of gluten was 34 mg (range, 5-150 mg). 6 patients were on a strict naturally gluten-free diet and another 6 patients were on the wheat starch-based gluten-free diet but also consumed about 1-2 g of gluten every week or at least once per month. Small intestinal mucosal morphometry, mucosal HLA-DR expression, endomysial, reticulin and gliadin antibodies were assessed.

Lohiniemi et al 2000

The aim of this cross-sectional study from Finland was to establish whether a wheat starch-based gluten-free diet has an untoward effect in terms of gastrointestinal symptoms and general well-being in people with CD. The diagnosis of CD was based on typical small bowel biopsy appearances. The Gastrointestinal Symptom Rating Scale was applied to 58 adults on gluten-free diets and 110 non-coeliac controls, 23 of the coeliac patients also had a small bowel biopsy. Fifty patients with CD were on strict wheat starch-based gluten-free diets, 3 had occasional lapses. The mean daily estimated gluten intake from wheat starch was 36mg (range 0-180).

Collin et al 2004

The purpose of this Finnish cross-sectional study was to estimate the safe threshold for gluten contamination in gluten-free products used in the treatment of CD. The gluten content of 59 naturally gluten-free and 24 wheat starch-based gluten-free products were analysed. The daily intake of flours was estimated in 76 adults and 16 children with CD on a strict gluten-free diet for 1-10 years (median 2 years) and the intake compared with mucosal histology and endomysial antibodies. 13 of 59 of the naturally gluten-free products and 11 of 24 of the wheat starch based gluten-free products contained gluten from 20 to 200 ppm. Median daily flour consumption in adults was 80g (range 10-300). It was not stated in the paper how CD was diagnosed, but on contacting the corresponding author, he confirmed that diagnosis was based on histological criteria.

6.2.2 Threshold concentration of gluten in food

Selby et al 1999

The aim of this Australian cross-sectional study was to determine whether persistent villous atrophy in people treated for CD could be due to trace amounts of gluten in "gluten-free" foods. Duodenal biopsy appearances of 89 adults with longstanding CD (duration 0.6 to 29.2 years) were correlated with their form of gluten-free diet. In 78 people, the diagnosis of CD had been confirmed by follow-up biopsy on a gluten-free diet, in the other 11 the study biopsy was used as the second biopsy. Patients were assigned to either Codex-gluten-free diet or non-detectable gluten gluten-free diet.

Peraaho et al 2003

This randomised controlled trial, conducted in Finland, compared the effects of a wheat starch-based and a natural gluten-free diet in adults with newly diagnosed CD. Fifty seven adults with untreated CD were randomised to a wheat starch-based or natural gluten-free diet. The diagnosis of CD was based on small intestinal histological changes. After one year, the effects of the 2 diets were monitored by clinical response, duodenal morphometry, mucosal human leucocyte antigen-DR expression, endomysial, transglutaminase and gliadin antibodies.

6.3 Methodological quality of included studies

Twelve papers were identified that fulfilled the inclusion criteria. These included two randomised controlled trials (RCTs) (Catassi et al, 1993 and Peraaho et al, 2003), one cohort study (Chartrand 1997), two crossover studies (Ciclitira et al, 1984; Ciclitira et al, 1985) and 7 cross-sectional studies (Baker et al, 1975; Collin et al, 2004; Dissanayake et al, 1974; Kaukinen et al, 1999; Lohiniemi et al, 2000; Montgomery et al. 1988; and Selby et al, 1999). All of the included studies addressed an appropriate and clearly focussed question but none of the studies reported a power calculation to determine how many patients would be required to detect significant effects of gluten exposure.

In one RCT (Catassi et al, 1993), the authors stated that the patients were randomly assigned to each group but they gave no further details about the method of randomisation, allocation concealment or blinding. Twelve patients were excluded before randomisation because of poor dietary compliance or did not consent to the protocol. The groups were similar with respect to age but not gender (there were 8 females in group A and 4 in group B). The patients were given a 4-week microchallenge of gliadin (100 or 500 mg) but the rest of their diet does not appear to have been assessed to see whether it contained any additional gluten. There was no difference in the initial morphometric parameters between the 2 groups. All 20 recruited patients completed the study protocol. We judged that there was a low risk of bias in this study.

The method of randomisation was satisfactory in the RCT by Peraaho et al 2003 but it is unclear whether there was adequate allocation concealment. It is stated that all specimens were evaluated by the same investigator who had no previous knowledge of the disease history or laboratory findings. The patients were presumably aware of which diet they were receiving. Eight patients refused to participate in the study. The groups were similar with respect to age, gender, symptoms and initial morphometric parameters. Only 23/29 patients in group I and 26/28 in group II completed the study with a proper diet. We judged that there was a low risk of bias in this study.

In the cohort study by Chartrand et al 1997 it is not stated whether the investigators were blind to the exposure status of the patients. Wheat starch was added to the gluten-free diet of adults with CD. The control group consisted of patients with CD who tolerated wheat starch. The choice of control group appears inappropriate in a study whose aim is to investigate the effects of consumption of small amounts of gliadin contained in wheat starch, and could have introduced bias into the study. The rest of the diet of both groups does not appear to have been assessed to see whether it contained any additional gluten. There was a higher proportion of children in the control group (6 of 14) compared to the experimental group (2 of 17) and a higher proportion of females in the experimental group (12 of 17) compared to the control group (8 of 14). Although the trial was initially intended to continue for 12 months it was stopped after 10 months because only 2 of the 17 patients remained asymptomatic and wished to continue consuming the wheat starch product. We judged that there was a high risk of bias in this study.

In the cross-over study by Ciclitira et al 1984, patients were required to consume known quantities of gliadin containing wheat-starch based gluten-free diet but the rest of their diet does not appear to have been assessed to see whether it contained any additional gluten. The study by Ciclitra et al 1985 was also a crossover study. Patients were given a wheat-starch based gluten-free bread during one 6 week period and no gluten-free bread during the other 6 week period but the rest of their diet does not appear to have been assessed to see whether it contained any additional gluten. It was not stated whether the order of the two 6 week periods was randomised or whether the investigator was blind to exposure status of the patients during each period. It was also not stated in the paper how CD was diagnosed, but on contacting the corresponding author, he confirmed that diagnosis was based on histological criteria.

The remaining 7 studies were all cross sectional. Only in the studies by Dissanayake et al 1974, Ciclitira et al, 1984; Kaukinen et al 1999; and Collin et al 2004 was it stated that the investigators were blind to the exposure status of the patients. The amount of gluten in the gluten-free diet was not measured in the study by Montgomery et al, 1988 and in the study by Dissanayake et al 1974, it is not stated what is meant by “small amounts of gluten in the diet. It was not stated in the article by Collin et al 2004 how CD was

diagnosed, but on contacting the corresponding author, he confirmed that diagnosis was based on histological criteria.

7. RESULTS

We included 12 studies that examined the effect of persistent intake of small amounts of gluten or gliadin in patients with CD. Ten of the 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. The amount of gluten ingested, the length of exposure to gluten, and the assessment of the effect of gluten were not consistent between studies. We, therefore, considered it inappropriate to statistically combine the data of the studies. The results of the individual studies are described below.

7.1 Studies assessing the safe threshold amount of ingested gluten

Dissanayake et al 1974

No histological abnormalities or minor changes were observed in 16 of 18 patients on strict gluten-free diet (gluten content of diet not assessed), 4 of 13 patients receiving small amounts of gluten and none of 7 receiving large amounts of gluten. However the authors did not specify what is meant by ‘small’ and ‘large’ amounts of gluten.

Baker et al 1975

Of those patients found (by a 4-week dietary questionnaire) to be adhering to a strict gluten-free diet (n=18) 3 had positive gluten antibodies and 13 had villous atrophy. Of those consuming <2g/day gluten (n=24) 10 had positive gluten antibodies, and 16 had villous atrophy. Of those consuming >2g/day gluten (n=9) all 9 had positive gluten antibodies and villous atrophy. Gluten content of the ‘gluten-free’ diet was not measured.

Ciclitira et al 1984

At the end of the one week period, people receiving Juvela ‘gluten-free’ bread (containing 1.2 to 2.4 mg gliadin/day) exhibited significant reduction in the mean villous height/crypt depth ratio but other measures of jejunal morphometry were not significantly different.

Ciclitira et al 1985

There was no significant difference in jejunal morphometry in the period of consumption of between 1.2 mg and 2.4 mg of gluten from bread and the period where this product was not ingested. Four patients reported diarrhoea associated with the ingestion of the bread.

Montgomery et al 1988

Some histological abnormalities were found both in patients on a low gluten diet defined as consuming 2.5-5 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) but there was no statistical difference between the two groups. There was also no significant difference in antigluten IgA,G & M. There was a significant increase in intra epithelial lymphocytes in patients on a low gluten diet compared to those on gluten-free diet and control patients.

Catassi et al 1993

After 4 weeks no clinical abnormalities were reported in Group A (100mg gliadin) but 3 patients in Group B (500mg gliadin) had anorexia and loose stools. After the challenge there was a significant reduction in the villous height/crypt depth ratio in both groups and this was more marked in Group B. Intra-epithelial lymphocytes were also significantly increased in both groups. The villous height was significantly reduced and the crypt depth significantly increased in Group B but not Group A.

Chartrand et al 1997

Fifteen of 17 subjects who had not previously received wheat-starch based products developed symptoms within 8 months of consuming 0.75 mg gliadin per day (equivalent to 1.5 mg gluten) from wheat starch-based gluten-free products. The symptoms resolved within weeks of discontinuing the product. The control group who had previously tolerated wheat starch-based products remained well during the study period. No significant difference in the titres of IgA or IgG antigladin antibody were detected after consumption of wheat starch bread. Endomysial antibodies were uniformly negative in both groups. Mucosal histology was not assessed.

Kaukinen et al 1999

Forty of 52 patients adhered to a strict wheat starch-based gluten-free diet (mean daily intake of gluten from this 34 mg; range 5-150 mg) and 6 to a strict naturally gluten-free diet. In these 46 patients, the mucosal morphometry was similar to that of non-coeliacs and better than in short-term treated people with CD. Two of six patients who had dietary lapses (ingesting 1-2 g gluten every week or at least once a month in addition to the wheat starch-based gluten-free diet) developed villous atrophy.

Lohiniemi et al 2000

Fifty adults with CD were on strict wheat starch-based gluten-free diets and 3 had occasional lapses. The mean daily estimated gluten intake from wheat starch was 36 mg (range 0-180). The mean Gastrointestinal Symptom Rating Scale score in these patients did not differ from that in controls. The daily amount of wheat starch had no effect on Gastrointestinal Symptom Rating Scale score. Twenty one of 23 patients had normal villous architecture, 1 had partial and 1 had subtotal villous atrophy. It is unclear whether these were the patients who had the dietary lapses. The daily amount of gluten-free wheat starch consumed did not correlate with symptoms.

Collin et al 2004

Median daily flour consumption in adults was 80 g (range 10-300) and mean daily flour consumption in children was 60 g (range 20-140). One patient on the naturally gluten-free diet and one on a wheat starch-based gluten-free diet were positive for endomysial antibodies after 1 year (titres 1:5 & 1:50). There was no correlation between the amount of flour ingested and intestinal mucosal morphometry. Mucosal recovery was generally good. All the 16 children had normal small bowel morphology.

7.2 Studies assessing the safe threshold limit of gluten concentration in food

Selby et al 1999

Eighteen of 39 of patients taking the Codex gluten-free diet (containing up to 0.03% protein derived from gluten containing grains) had villous atrophy, and 20 out of 50 of patients ingesting non-detectable gluten gluten-free diet (containing less than 0.003%

protein derived from gluten-containing grain) also had villous atrophy. The authors concluded that the persistent abnormalities could not be attributed to the consumption of Codex gluten-free products. The exact amount of gluten ingested by patients was not measured.

Peraaho et al 2003

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, intra-epithelial lymphocytes or coeliac antibodies (gliadin, endomysial, transglutaminase) between the groups. Gluten contamination in both diets was not assessed.

8. DISCUSSION

Whilst it is accepted that the treatment of CD is a gluten-free diet, there is a lot of controversy surrounding the definition of what a 'gluten-free' diet is. 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 will be tolerable for people with CD, and also investigated the evidence base for a tolerable threshold concentration of gluten in foods.

8.1 Threshold amount of ingested gluten

We found that the consumption of about 200 mg or more of gluten per day is clearly associated with the development of intestinal mucosal abnormalities. Catassi et al demonstrated that the ingestion of 100 mg gliadin (200 mg gluten) per day or more induced histological abnormalities after 4 weeks (Catassi, 1993). At least one earlier study had also demonstrated mucosal abnormalities with larger amounts of gluten (Baker et al, 1975).

Kaukinen et al showed that patients who consumed between 5-150 mg gluten daily (mean 34 mg) for about 8 years developed no histological abnormalities, but those who, in addition to this amount of gluten, also ingested between 1-2 g of gluten per week developed villous atrophy (Kaukinen et a, 1999). In another study, patients consuming an average of about 36 mg of gluten per day did not develop histological abnormalities or clinical symptoms (Lohiniemi et al). However, in one study, a much smaller dose of gluten (1.5 mg daily) triggered symptoms in some patients. Chartrand et al showed that some 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 on top of 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. The quality of this study was, however, poor.

It is not clear from the included studies whether very small amounts of gluten per day (say, less than 30 mg per day) induce histological abnormalities. The study by Chartrand et al showed that some patients may develop symptoms on much smaller amounts but unfortunately, histological changes were not assessed in either the patients who remained well or those who exhibited symptoms. Thus whilst 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 manifest symptoms with much smaller amounts. Whether these symptoms are related to mucosal changes is not clear.

The length of exposure to gluten varied considerably between the included studies. For instance, in the study by Kaukinen et al (Kaukinen et al, 1999), patients had been on their diet for a mean of 8 years, whilst in the study by Ciclitira et al (Ciclitira et al, 1984), patients were assessed after a one week gluten challenge. It is likely that the length of exposure would have an effect on outcomes.

8.2 Threshold limit of gluten concentration in food

The evidence in this area 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 100g of food (Codex standard) developed no symptoms or mucosal abnormalities. 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). 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, assessment was made after one year whilst in the study by Selby et al, patients appeared to have been on these products for the duration of their disease (0.6 to 29.2 years).

We can deduce from these primary studies that whilst many people with CD may remain well on consuming products with the Codex concentration of gluten, others may develop symptoms when they consume products with even lower concentrations of gluten. However, in both these studies, the actual amounts of gluten ingested by patients were not assessed. 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.

Thus whilst the current Codex standard gluten-free products may be tolerable for some people with CD, they may not be so for others. There is an argument for any revised standard to be set at a lower concentration. However, we found no evidence in this study to suggest a single definitive threshold concentration of gluten in food products that would be tolerated by all people with CD. Collin and colleagues (Collin et al, 2004) argued that if the daily 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. As earlier discussed, some people with CD may tolerate this amount but others may manifest symptoms even at much lower doses of gluten.

9. SUMMARY OF FINDINGS

1. In people with CD, a daily consumption of 200 mg or more of gluten clearly induced mucosal abnormalities. (Evidence level 1+)
2. In some studies, the consumption of about 34 - 36 mg of gluten per day did not cause mucosal abnormalities (Evidence Level 3) or clinical symptoms (Evidence Level 3)
3. In another study, some patients who consumed much smaller amounts of daily gluten (1.5 mg daily) developed symptoms but it was not clear if these symptoms were associated with mucosal changes (Evidence Level 2–)
4. The effect of the consumption of gluten-free products with different degrees of gluten contamination was inconsistent between studies. Some people who consumed wheat starch-based gluten-free products with a gluten concentration of about 400 mg to 600 mg per kilogram food remained well with normal intestinal mucosa (Evidence Level 1+) but others who consumed similar products or even products with much lower concentrations of gluten developed histological changes (Evidence Level 3)

10. CONCLUSIONS AND RECOMMENDATIONS

1. We found no evidence to allow us to propose a single threshold dose of gluten that would be tolerated by all people with CD.
2. 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.
3. We, however, found no evidence to allow us to propose a single threshold concentration of gluten in food products that would be tolerable for all people with CD.
4. Whilst some people will tolerate the current Codex standard gluten-free products, others may develop histological abnormalities when they consume these products. It is likely that what is most important is the total amount of gluten ingested per unit time, i.e. per day 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.
5. Whilst it is clear that the amount of tolerable gluten varies among people with CD, the reason for this is unclear. Future studies should investigate potential reasons (e.g. genetic variability) that may explain the variable response to gluten.
6. 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.
7. We also recommend that future studies in this area should be well designed randomised controlled trials, and should have adequate statistical power to detect any differences between groups.

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Table 1: Characteristics of included studies

Study ID	Methods	Participants	Diet	Outcomes	Comments
Dissanayake 1974	Cross-sectional study from England.	38 adults with CD.	Patients on strict 'gluten-free' diet, 'small amounts' of gluten, or 'large amounts' of gluten.	Jejunal mucosal histology.	Authors did not define what they meant by 'strict' or 'small' but 'large' was defined as ≥ 0.5 g/day.
Baker 1975	Cross-sectional from England.	51 adults with CD	Patients on 'no gluten', 'small amounts of gluten' (< 2g/day) or 'large amounts of gluten' (≥2g/day)	Jejunal mucosal histology	
Ciclitira 1984	Cross-over study from England.	7 adults with CD.	One week on 'gluten-free' diet, then one week on same diet plus bread containing up to 2.4 mg gliadin/ day.	Jejunal mucosal histology	
Ciclitira 1985	Cross-over study from England.	10 adults with CD.	6 weeks on gluten-free diet and 6 weeks on same diet plus bread containing up to 2.4 mg gliadin/day.	Jejunal mucosal histology and clinical symptoms	
Montgomery 1988	Cross-sectional study from England.	25 adults with CD.	'Low gluten diet' (2.5 – 5 g per day) or 'strict 'gluten-free' diet' (amount of gluten not measured).	Jejunal mucosal histology.	
Catassi 1993	Randomised controlled trial from Italy.	20 children with CD. Age range 1.6 to 9.6 years).	Either 100 mg or 500 mg gliadin per day.	Jejunal mucosal histology.	
Chartrand 1997	Cohort study from Canada.	31 adults with CD.	Wheat starch-based diet.	Symptoms.	17 participants had never been on wheat starch-based products but 14 controls had previously tolerated these products.
Kaukinen 1999	Cross-sectional study from Finland.	41 children and adults with CD and 11 adults with dermatitis herpetiformis.	Either a wheat starch-based gluten-free diet or a naturally gluten-free diet.	Small bowel mucosal histology.	
Selby 1999	Cross-sectional study from Australia.	89 adults with CD.	Codex gluten-free diet or non-detectable gluten gluten-free diet.	Duodenal mucosal histology.	
Lohiniemi 2000	Cross-sectional study from Finland	58 adults with CD, 110 non-coeliac controls.	CD patients were on wheat starch-based 'gluten-free' diet.	Symptoms; small bowel mucosal histology.	
Peraaho 2003	Randomised controlled trial from Finland.	57 adults with CD.	Either a wheat starch-based or a naturally 'gluten-free' diet	Symptoms; duodenal mucosal histology.	
Collin 2004	Cross-sectional study from Finland.	76 adults and 16 children with CD.	Either a wheat starch-based or a naturally 'gluten-free' diet.	Small bowel mucosal histology.	