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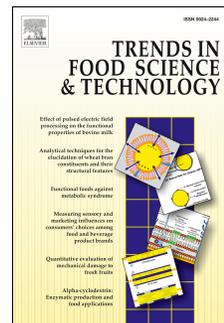
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Part of celiac population still at risk despite current gluten thresholds

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Abstract

In order to assist celiac disease (CD) patients in making safe food choices, gluten-free food products are labelled as such. The exact meaning of the gluten-free label differs throughout the world. This paper discusses the different thresholds that are currently used to label products gluten-free and compares tolerable gluten levels to the gluten levels CD patients can be exposed to with these thresholds in place. Currently, the most applied gluten threshold to label products gluten-free does not protect the most vulnerable patients. Therefore, we propose to lower the threshold for products with a gluten-free label to 3 ppm gluten.

Introduction

Approximately 1% of the world population is afflicted with celiac disease (Lionetti & Catassi, 2011; Reilly & Green, 2012). These persons have an intolerance to gluten, a group of storage proteins found in wheat, rye and barley. When a CD patient ingests gluten, an inflammatory response is triggered in the intestinal tract. This inflammation can lead to atrophy of the mucosal villi and, as a consequence, to malabsorption and malnutrition. The symptoms of CD vary between persons. Symptoms in a typical manifestation are mainly gastrointestinal, whereas atypical manifestations have mostly extra-intestinal symptoms. Furthermore, CD can manifest asymptomatic. In this case, the patient does not show symptoms other than villous atrophy or serological changes. Especially in the asymptomatic cases, CD can remain undetected for a long period of time (Lionetti &

32 Catassi, 2011). A wrong interpretation of biopsy results can also lead to a delay in CD diagnosis (Marsh, 2013).
33 When left untreated, CD can lead to serious complications. In the worst case scenario, these can include
34 lymphomas and intestinal adenocarcinoma (Green & Cellier, 2007). Although multiple new therapies are
35 investigated, at this moment the only treatment is to adhere to a strict lifelong gluten-free diet.

36 In order to make safe food choices, CD patients rely heavily on the correct labelling of food products.
37 This is not an easy task for the patient. Gluten are often added to foodstuffs which are naturally gluten-free, in
38 order to improve product quality and stability (Day, Augustin, Batey, & Wrigley, 2006). Ingredients on the label
39 are sometimes difficult to interpret for gluten presence, since gluten can be hidden in names as, for instance,
40 'flavourings' or 'hydrolysed vegetable protein'. A gluten-free label on a product makes finding the right
41 products for a gluten-free diet much easier. However, labels can be confusing too. Gluten-free labelling
42 legislations differ throughout the world and, as a result, the acceptable gluten content of a product labelled
43 gluten-free can differ per country.

44 According to the Dutch Celiac Disease Association (NCV), CD-related complaints are still often
45 reported by CD patients who have been following a gluten-free diet. Sometimes, the supposedly gluten-free
46 product is found to be contaminated with gluten above the legal threshold, but often the reason for these
47 complaints remains unknown as the products seem to comply with the current European legislation for gluten-
48 free foods. The aim of this literature study is to investigate whether the currently applied gluten thresholds are
49 suitable to protect CD patients, or adjustments should be considered.

50

51 **Literature selection**

52 Systematic literature searches were performed in order to investigate the gluten content of foods and the amounts
53 of gluten tolerated by CD patients. The following databases were included: Medline, Cochrane Library and
54 Scopus. Studies had to be written in the English language to be included

55 Search terms for the gluten contents of food were "gluten traces" OR "gluten content" AND "gluten-
56 free" AND "food". Subsequently, the reference lists of the studies identified by the electronic databases were
57 searched to identify additional studies. Results were filtered to include only original research articles. Full
58 manuscripts were obtained for all potentially relevant articles. Studies had to be performed in the last 10 years to
59 be included. Studies that estimated instead of quantifying the gluten content of foods were excluded, as were
60 studies that did not specify if the tested products were intended for CD patients to use. Furthermore, studies that
61 only assessed the gluten content of raw materials such as flour were excluded, as for this study the gluten content

62 of final products is most relevant to determine exposure. Finally, studies assessing the gluten content of beer
63 were excluded. Beer contains mostly hydrolysed gluten, which are known to be overlooked by the most
64 commonly applied method to detect gluten in food; the sandwich format enzyme-linked immunosorbent assay
65 (ELISA).

66 Search terms for the tolerated amounts of gluten were “coeliac disease OR celiac disease” AND
67 “gluten” AND “threshold OR gluten challenge” NOT “in vitro”. Again, the reference lists of the studies
68 identified by the electronic databases were searched to identify additional studies. Results were filtered to only
69 include original research articles and case reports describing effects on humans. Full manuscripts were obtained
70 for all potentially relevant articles. Since only a limited amount of gluten threshold studies has been performed in
71 total, the time frame for including these studies was increased compared to the studies evaluating the gluten
72 content of food products. Studies had to be performed in the last 20 years to be included. Dietary recall studies
73 concerning wheat starch intake were included if they made at least an estimation of the gluten content of the
74 wheat starches. These dietary recall studies do not give an exact gluten content that CD patients are exposed to,
75 due to their retrospective set-up. However, they do give relevant information on a different approach to gluten
76 exposure; the effect of smaller doses of gluten spread over several meals per day, as compared to the effect of a
77 single, larger dose. Studies concerning gluten challenges given in combination with pharmacological treatment
78 were excluded.

79 The current applied legislations concerning gluten-free labelling of food products were retrieved for the
80 European Union, the United States of America, Canada and Australia and New Zealand. For this, the websites of
81 government authorities responsible for food standards and regulations were consulted.

82

83 **Current thresholds for gluten-free labelling of food products**

84 For the European Union, the United States of America and Canada, products with a gluten-free label cannot
85 contain more than 20 mg/kg (ppm) gluten. However, there are some differences in legislation between these
86 countries. In Europe, the definition of gluten-free products and the recommended limits of the Codex
87 Alimentarius standard 118-1979 were implemented in Commission Regulation 41/2009 in 2012 (The
88 Commission of the European Communities, 2009) and later the new Commission Regulation 1169/2011 in
89 December 2014 (The European Parliament and the Council of the European Union, 2011). Gluten is defined as
90 “the protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives thereof, to which
91 some persons are intolerant and which is insoluble in water and 0.5 M sodium chloride solution”. According to

92 this legislation, in order to label a product gluten-free, the ingredients derived from gluten-containing cereals
93 must have been processed to reduce the gluten content or these ingredients must have been replaced by gluten-
94 free cereals. There is a specific addition for the use of oats. Oats must have been specially produced and
95 processed in a way that avoids contamination by gluten-containing cereals and the maximum of 20 ppm gluten is
96 still valid. The US adopted a legislation on gluten and gluten-free products in 2013. According to this legislation
97 and contrary to the European legislation, the gluten-free label may also be applied to food that does not contain a
98 gluten-containing grain, including naturally gluten-free foods, as long as the gluten content of the final product
99 does not exceed 20 ppm (U.S. Food and Drug Administration, 2013). The Canadian legislation differs from both
100 the European and American legislation by stating that gluten-free products can not contain wheat, including spelt
101 and kamut, or oats, barley, rye, triticale, or any part thereof (Canadian Food and Drug Regulations, 2013). In this
102 case, the 20 ppm threshold is used to set a maximum level of allowed cross-contamination with gluten.

103 The gluten legislation of Australia and New Zealand is very different from the abovementioned
104 legislations and is considered to be most strict worldwide. Their definition of gluten is the main protein in wheat,
105 rye, barley, oats, triticale and spelt, relevant to the medical conditions CD and dermatitis herpetiformis (Australia
106 New Zealand Food Standards Code, 2011). A product can be labelled gluten-free if it contains no detectable
107 gluten. This means that the tolerable amount of gluten in these products is decreasing over time as the detection
108 methods become more sensitive. At this moment, the type I method R5 as recommended by Codex Alimentarius
109 has a limit of detection (LoD) of 3 ppm.

110

111 *Other thresholds concerning the gluten content of food products*

112 Apart from the thresholds that are used to define gluten-free, the European Union, Australia and New Zealand
113 have a second category for products that are low in gluten, yet exceed the threshold to be labelled gluten-free. In
114 the European Union, a product may be labelled 'very low in gluten' if the gluten-containing cereals have been
115 processed to reduce the gluten content, and the product does not contain more than 100 ppm gluten (The
116 European Parliament and the Council of the European Union, 2011). In Australia, products with a gluten content
117 that does not exceed 200 ppm may be labelled 'low in gluten' (Australia New Zealand Food Standards Code,
118 2011).

119

120 The differences between worldwide legislations imply that the same product can have different labels, depending
121 on the country it is brought on the market. A product with wheat as one of its ingredients that contains 10 ppm

122 gluten after processing could be labelled gluten-free in the US and in Europe, but not in Canada and Australia. A
123 naturally gluten-free food such as milk can be labelled gluten-free in the US, but not in Europe. In addition to
124 gluten-free labels, 'low in gluten', 'very low in gluten' and 'may contain' labels are used as well. For CD
125 patients, these different labels can be confusing as all that really matters to them is whether or not a product is
126 safe for them to eat. The gluten thresholds have been and still are under much debate. The bottom line is that
127 these labels should allow CD patients to make safe food choices. When looking at the legislations above, four
128 different thresholds can be distinguished: (a) No detectable gluten (which currently translates into < 3 ppm
129 gluten), (b) ≤ 20 ppm gluten, (c) ≤ 100 ppm gluten and (d) ≤ 200 ppm gluten. The remaining sections discuss
130 whether these thresholds are suitable to protect the CD patients, or adjustments should be considered.

131

132 **Exposure**

133 The gluten-free diet consists of a combination of naturally gluten-free foods such as fruits, vegetables, meat, fish,
134 eggs and dairy products with gluten-free substitutes for cereal-based foods such as bread and pasta. For most
135 naturally, non-processed gluten-free foods such as fruit and eggs, the chance of cross-contamination with gluten
136 is small. Cross-contamination of gluten-free cereals and, as a result, products made from these cereals is much
137 more common. Also naturally gluten-free foods that are processed, such as lunch meats, are often prone to cross-
138 contamination if gluten-containing products are processed on the same locations. The total amount of gluten that
139 CD patients are exposed to depends on both the gluten contents of the products that they consume and the
140 amount of product consumed.

141

142 *Gluten content of foods*

143 Thompson and Grace evaluated the gluten content of 112 food products labelled gluten-free, using a R5 ELISA
144 (Thompson & Grace, 2013). Four products (i.e. bread, hot cereal, tortilla, cookie) contained 20 ppm gluten or
145 more, although the exact gluten contents above 20 ppm were not reported. Gibert *et al* used a R5 ELISA to
146 determine the gluten content of 205 commercially available products labelled gluten-free (Gibert, et al., 2013).
147 One pastry product contained more than 20 ppm gluten; namely 27.8 ppm. In 191 of the 205 products, no gluten
148 could be detected above the limit of quantification (LOQ) of 5 ppm. Agakidis *et al* determined the gluten content
149 of 41 processed food products that are on the safe lists of either the Greek National Food Intolerance Database,
150 the local Celiac Association, or both, chosen according to the preference of the patients (Agakidis, et al., 2011).
151 These products included flours, dairy products, cereals, pasta, sweets, processed meat, sausage, cakes and tomato

152 sauce. The analysis was performed with an ELISA targeted against ω -gliadin. Of these 41 products, 13 did not
153 contain any detectable gluten at all. Two dairy products and two flour products contained gluten ranging from 21
154 to 39 ppm. The gluten content of the remaining products was below 20 ppm. Catassi *et al* performed a
155 prospective trial to establish a safe gluten threshold and did a background analysis on the gluten-free products
156 consumed by the patients in their study (Catassi, et al., 2007). A total of 42 commercially available gluten-free
157 products, randomly chosen from the dietary assessment of the patients, was analysed with a R5 ELISA. The
158 gluten content of these products was found to range from < 3-50 ppm, with an average of 5.2 ppm.
159 Unfortunately, the exact number of products with a gluten content above 20 ppm is not given. Collin *et al*
160 compared the gluten content of 46 naturally gluten-free flours and 13 naturally gluten-free products with 17
161 wheat starch-based flours and 7 wheat starch-based products (Collin, Thorell, Kaukinen, & Mäki, 2004).
162 Analysis was performed with a R5 ELISA. In the naturally gluten-free group, 35 flours (76%) and 11 products
163 (85%) contained less than 20 ppm gluten. The remaining flours and products contained gluten in the 20-200 ppm
164 range. For the wheat starch-based group, 11 flours (65%) and 3 products (43%) contained less or equal than 20
165 ppm gluten. The remaining flours and products contained 30-150 ppm gluten. The results from these studies
166 show that in most cases, food products that are labelled gluten-free do not contain more than 20 ppm gluten and
167 many of them contain less than 3 ppm gluten. Wheat-starch based flours and foods exceed the threshold of 20
168 ppm gluten relatively more often.

169

170 *Consumption*

171 Gluten exposure for CD patients is not only dependent on the amount of gluten present in their foods, but also on
172 the amount of products consumed by these patients. Catassi *et al* kept records of the daily consumption of
173 commercially available gluten-free products consumed by the patients in their study (Catassi, et al., 2007). The
174 type of products were not specified, but the average daily consumption of the CD patients was 332 g (range 177-
175 574). Collin *et al* estimated the use of gluten-free flours from 4-day food records of 76 adults and 16 children
176 with CD and found a daily median of 80 g (range 10-300) flour in adults and 60 g (range 20-140) in children
177 (Collin, et al., 2004). Gibert *et al* compared the gluten-free food consumption in Italy, Spain, Germany and
178 Norway (Gibert, et al., 2006). Gluten-free bread was the most consumed gluten-free product in all four countries
179 and together with pasta this made up to 64%, 56%, 71% and 71% of the total daily intake, respectively. Other
180 gluten-free substitute products that were consumed often included pastry, biscuits and breakfast cereals. The
181 total daily intake of gluten-free products at the 90th percentile of the studied population was 400-411 g/day in

182 Spain, Germany and Norway, and 531 g/day in Italy, the latter mainly due to a higher pasta consumption than in
183 the other included countries.

184

185 **Tolerable levels**

186 In order to set a proper threshold for gluten, the amount of gluten that is tolerated by CD patients needs to be
187 known. These exact amounts can differ per person, but in general three groups of CD patients with different
188 needs can be distinguished: the average CD population; the sensitive CD population; and the recovering CD
189 population. **Table 1** gives an overview of the studies included in this paper. Specific information on the tolerable
190 levels of gluten intake derived from these studies is given in **Table 2**.

191 Depending on the study, the gluten contents were assessed differently. This influences the accuracy of
192 the reported amounts of gluten to which the patients were exposed. The studies performed by Chartrand *et al*,
193 Collin *et al*, Biagi *et al* and Greco *et al* have determined gluten content by ELISA, which is currently the most
194 applied detection method in food. Gluten ELISAs are sensitive enough to detect gluten in the mg/kg range. Both
195 studies performed by Catassi *et al* made use of a purified gluten standard. The studies performed by Kaukinen *et*
196 *al* and Lohiniemi *et al* calculated gluten content based on the amount of wheat starch consumed, assuming that
197 the gluten content of this wheat starch was the maximum amount allowed by European legislation. This method
198 is less accurate than detection with ELISA or using a gluten standard. Overestimation is likely, since not all
199 wheat starches will contain the maximum allowed amount of gluten. However, it is also possible that the wheat
200 flour in these studies contained more than the maximum allowed amount of gluten, which would lead to an
201 underestimation of the total gluten content. Troncone *et al* and Laurin *et al* calculated gluten content based on
202 food records. With this method, underestimation of the total amount of gluten is likely, as underreporting is a
203 known problem with collecting food records. Finally, the study performed by Srinivasan does not specifically
204 mention how the gluten content of their challenge was assessed. This means the reported amount of gluten could
205 be an estimation and could either be over- or underestimated.

206

207 *Average CD population*

208 Greco *et al* found that the 8 ppm gluten that remains in wheat flour after full hydrolysis, does not cause
209 mucosal atrophy or lead to clinical complaints in CD patients if they consume 200 g flour per day (Greco, et al.,
210 2011). This is in agreement with the study performed by Catassi *et al*, who found that a consumption of 10 mg
211 gluten/day can be tolerated by most CD patients (Catassi, et al., 2007). In the same study, a dose of 50 mg

212 gluten/day was found to cause mucosal atrophy. Troncone *et al* saw a histological relapse in some patients who
213 were exposed to 60 mg gluten/day (Troncone, Mayer, Spagnuolo, Maiuri, & Greco, 1995). Studies examining
214 the effects of 200 mg gluten/day or more, all found that these amounts are harmful to CD patients (Catassi, et al.,
215 1993; Greco, et al., 2011; Laurin, Wolving, & Fälth-Magnusson, 2002; Srinivasan, et al., 1996). Several groups
216 determined the gluten content of wheat starch, which is already used in the gluten-free diet of many patients.
217 Some wheat starch products contain up to 200 ppm gluten. An estimation of the gluten exposure for CD patients
218 using these products can be made by looking at the average and maximum intake. In three separate studies, the
219 average intake of gluten via these contaminated wheat starch products was below 50 mg/day (Collin, et al., 2004;
220 Kaukinen, et al., 1999; Lohiniemi, Mäki, Kaukinen, Laippala, & Collin, 2000). Although all three studies
221 reported some CD patients with histological changes, these changes could not be correlated to the amount of
222 wheat starch used. These results would suggest that the tolerable level of gluten for most CD patients lies
223 between 10-50 mg/day.

224

225 *Sensitive CD population*

226 For part of the CD population however, a gluten intake of 10 mg/day seems to be too much. In the study by
227 Catassi *et al*, one participant out of a group of fifteen receiving 10 mg gluten/day quit the study due to relapse
228 symptoms (Catassi, et al., 2007). In the study of Chartrand *et al*, 17 CD patients were exposed to 0.75-3.38 mg
229 gluten/day (Chartrand, Russo, Duhaime, & Seidman, 1997). Within 8 months, 11 (65%) patients experienced
230 clinical symptoms, including those who consumed 0.75 mg gluten/day. Apparently, some CD patients are very
231 sensitive to gluten, but it is currently unknown what part of the celiac population they represent. Gluten
232 challenge studies trying to establish a gluten threshold might be biased, as sensitive CD patients are probably
233 less likely to accept exposure to gluten. Furthermore, they might drop out early as a result of relapse symptoms
234 or their values might be seen as outliers and are therefore not considered. This makes it difficult to establish a
235 threshold for this group, as available data is limited. According to the results of Chartrand, the tolerable level of
236 this group lies below 0.75 mg/day.

237

238 *Recovering CD population*

239 Recovering from previous gluten intake is a very different challenge as compared to remaining gluten-free. In
240 the study by Catassi *et al*, half of the 13 subjects being exposed to 10 mg gluten/day did not worsen their villous
241 height/crypt depth ratio, but also did not improve (Catassi, et al., 2007). Also, half of the subjects showed an

242 increase in intraepithelial lymphocytes (IELs), although this increase was not significant. Biagi *et al* presented a
243 case report of a woman who had persisting villous atrophy and increased IELs, but no clinical symptoms, due to
244 the consumption of 1 mg gluten/day in her communion wafer, after she had removed all other gluten-containing
245 foods from her diet (Biagi, et al., 2004). The study of Kaukinen *et al* showed that the mucosal recovery of newly
246 diagnosed patients was not complete after 10 months of gluten-free diet (Kaukinen, et al., 1999). Hollon *et al*
247 studied a group of diet-adherent non-responsive CD patients (Hollon, Cureton, Martin, Leonard Puppa, &
248 Fasano, 2013). After these patients had followed a diet without all gluten-free food products with a high risk of
249 being contaminated by gluten for at least 3 months, 13 out of 16 patients (81%) became asymptomatic. Of this
250 group, 79% remained symptom-free after returning to a traditional gluten-free diet. This indicates that at least
251 part of the recovering CD population has lower tolerance levels for gluten than they will have after they have
252 been fully recovered. For these persons, an exposure of 10 mg gluten/day as mentioned above may be too much
253 to be exposed to as long as they are recovering from previous gluten intake.

254

255 **Thresholds evaluation**

256 To evaluate the current thresholds for gluten, it is important to compare the amount of gluten that CD patients
257 would be exposed to, to the amount of gluten that can be tolerated. The amount of gluten exposure is dependent
258 on the amount of intake of gluten-free products and the maximum gluten content of these products, as shown in
259 Table 3 (adapted from Collin, et al. (2004)). As discussed above, the total intake of gluten-free products per day
260 would on average be between 300 and 400 g for most CD patients, with some individuals consuming up to 600
261 g. With the Australian threshold of < 3 ppm, patients would on average be exposed to 0.9-1.2 mg gluten/day, up
262 to 1.8 mg gluten/day. In other countries in which the threshold is currently 20 ppm, patients would on average be
263 exposed to 6-8 mg gluten/day, up to 12 mg gluten/day, given an average amount of gluten-free product
264 consumption up to 600 g. As shown above, an intake of 10 mg gluten/day was safe for most CD patients. The
265 studies that assessed the gluten content of wheat starch found that on average, a CD patient using 70-80 g wheat
266 starch per day is exposed to 16-36 mg gluten/day. This shows that at least a part of the average CD population
267 could tolerate more than 10 mg gluten/day, assuming that they are not in the process of recovering anymore.
268 However, there is also a group of sensitive CD patients that do show signs of inflammation after consuming 10
269 mg gluten/day or less, starting at 0.75 mg/day. This group is not protected by the threshold of 20 ppm. For them,
270 a gluten threshold at the limit of detection, 3 ppm, would allow them to safely eat up to 250 g gluten-free
271 product. The group of CD patients that is still recovering, would also be helped by a lower gluten threshold than

272 20 ppm. Therefore, for this group a gluten threshold of 3 ppm would also be more suitable. Once full recovery
273 has been achieved, most of these patients will be able to consume the same kind and amount of products as the
274 average CD population.

275 'Very low in gluten' products can contain up to 100 ppm gluten, which implies that CD patients with a
276 total product consumption of 300-400 g/day would be exposed to 30-40 mg gluten/day. Patients with a high
277 product intake would be exposed to 60 mg/day. No data is available for the 30-50 mg/day range, but intake of 50
278 mg gluten/day caused villous atrophy in the majority of CD patients (Catassi, et al., 2007). Therefore, patients
279 with a high consumption of 'very low in gluten' products would be exposed to unsafe amounts of gluten.
280 Patients consuming products 'low in gluten' would be exposed to even higher amounts of gluten, as the
281 thresholds for these products is 200 ppm gluten. In that case, patients with an average product intake of 300-400
282 g/day would be exposed to 60-80 mg gluten/day, up to 120 mg/day for patients daily consuming up to 600 g
283 products. This is more than twice the amount known to cause villous atrophy. These results show that the current
284 thresholds of both the 'very low in gluten' and 'low in gluten' products are too high for CD patients to safely
285 consume these products. The 'low in gluten' label is irrelevant and harmful for CD patients when misinterpreted
286 and should, therefore, be withdrawn. To make the 'very low in gluten' label meaningful again, it should be based
287 on gluten content that is safe for CD patients to consume after the mucosa has been recovered from previous
288 gluten intake. Unfortunately, very little literature on tolerable doses of gluten is available, especially in the range
289 10-50 mg gluten/day. When looking at the average gluten concentrations in wheat starch products that are
290 tolerated by CD patients, exposure up to 36 mg gluten/day might still be well tolerated. By halving the threshold
291 for 'very low in gluten' products to 50 ppm, CD patients with an average product intake would be exposed to 15-
292 20 mg gluten/day, well below the average gluten exposure from wheat starch. Even CD patients consuming up to
293 600 g 'very low in gluten' products per day would not exceed 30 mg gluten/day. More randomized, placebo-
294 controlled trials, such as performed by Catassi, *et al.* (2007), are needed to come up with a safe threshold for
295 'very low in gluten' products.

296

297 **Conclusions and recommendations**

298 With the current legislations in place, a product can be labelled gluten-free in the European Union, the United
299 States of America and Canada if the gluten content does not exceed 20 ppm gluten. In Australia and New
300 Zealand, this label is only given if gluten cannot be detected in the product, which – with our current detection
301 methods – implies a threshold of 3 ppm gluten. When looking at the average gluten-free product intake of CD

302 patients, these thresholds are safe for a large part of the celiac population. However, the 20 ppm threshold does
303 not protect the sensitive and recovering patients. These patients are exposed to amounts of gluten that can
304 prevent mucosal recovery, cause relapse of symptoms and progress the disease. Thus, patients that are most
305 reliant on gluten-free labelling are still at risk when consuming products that are labelled gluten-free. Especially
306 for this group, the gluten-free label for products containing up to 20 ppm gluten is misleading. If 3 ppm were to
307 be set as the threshold for foods to carry the gluten-free label, like Australia and New Zealand do, this would
308 allow the vulnerable and recovering group to consume up to 250 g/day gluten-free products in a safe manner.
309 Furthermore, the label would no longer be deceptive, as gluten-free would then really implicate 'free of gluten',
310 at least as far as can be detected.

311 Currently, in Europe, products with a gluten content of 20-100 ppm can be labelled 'very low in gluten'
312 and Australia allows products that contain less than 200 ppm gluten to be labelled 'low in gluten'. It is
313 questionable what purpose the 'very low in gluten' and 'low in gluten' labels serve, as they hold little to no value
314 for CD patients. The majority of CD patients can, after mucosal recovery, tolerate a small daily amount of
315 gluten. Therefore, an extra threshold apart from the 3 ppm for gluten-free products would be very useful and this
316 could give the 'very low in gluten' label meaning again. More research on disease-eliciting doses of gluten is
317 needed, especially in the 10-50 mg gluten/day range, in order to come up with a safe threshold for 'very low in
318 gluten' products.

319 By setting the gluten-free threshold to 3 ppm and the 'very low in gluten' threshold to a value relevant
320 for CD patients worldwide, these labels will be informative and safe for all CD patients again.

321

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386

387 **Table 1: Characteristics of included studies.**

Authors	Study	Participants	Duration	Exposure	Results
Greco, et al. (2011)	Randomized trial, Italy	Adolescents, 13	60 days	-Flour (16025 mg gluten/day) -Extensively hydrolysed flour (496 mg gluten/day) -Fully hydrolysed flour (1.6 mg gluten/day)	-Mucosal atrophy in 100%, 100%, 0%, respectively -Clinical complaints in 33%, 0%, 0%, respectively
Catassi, et al. (2007)	Randomized controlled trial, Italy	Adults, 49	90 days	-50 mg gluten/day -10 mg gluten/day -50 mg placebo/day	-50 mg/day decreases Vh/Cd significantly -10 mg/day safe for most patients
Biagi, et al. (2004)	Case report, Italy	Adult, 1	18 months	-1 mg gluten/day	-No clinical complaints -Severe villous atrophy and increased number of intraepithelial lymphocytes
Collin, et al. (2004)	Cross-sectional study, Finland	Adults, 76 Children, 16	1 year	-Wheat starch-based diet -Naturally gluten-free diet	-Gluten-free flours contain trace amounts of gluten (<10 – 200 ppm) -No correlation between flour used in both diets and mucosal histology
Laurin, et al. (2002)	Cross-sectional study, Sweden	Children, 24	5-51 weeks	-0.2-4.3 g gluten/day	-Symptoms in 82% within 5 weeks -Elevated antibodies in 72% within 5 weeks
Lohiniemi, et al. (2000)	Cross-sectional study, Finland	Adults, 53	9-11 years	-Wheat starch-based diet (0-180 mg gluten/day)	-Villous atrophy in 2 patients -No correlation between symptoms and amount of wheat starch consumed
Kaukinen, et al. (1999)	Cross-sectional study, Finland	Adults, 25 Children, 16	8 years on average	-Wheat starch-based diet (5-150 mg gluten/day) -Wheat starch-based diet (1-2 g gluten/week) -Naturally gluten-free diet	-Mucosal integrity was not dependent on the daily intake of wheat starch
Chartrand, et al. (1997)	Cohort study, Canada	Adults, 23 Children, 8	0.5-10 months	-Wheat starch added to gluten-free diet, (0.75-3.38 mg gluten/day)	-Symptoms in 64% within 8 months
Srinivasan, et al. (1996)	Cross-sectional study, Ireland	Adults, 2	6 weeks	-500 mg gluten/day	-Both patients developed histological evidence of relapse
Troncone, et al. (1995)	Cross-sectional study, Italy	Adolescents, 23	>10 years	-Strict gluten-free diet -<0.5 g gluten/day -0.5-2 g gluten/day ->2 g gluten/day	-Changes in mucosal architecture in 0%, 50%, 83% and 100%, respectively
Catassi, et al. (1993)	Randomized controlled trial, Italy	Children, 20	4 weeks	-100 mg gliadin/day -500 mg gliadin/day	-Minimal morphometric changes in jejunal histology for 100 mg/day -Profound morphometric changes in jejunal histology for 500 mg/day

388 **Table 2: Tolerable levels of gluten intake.**

Study	Outcome
Greco, et al. (2011)	-496 mg gluten/day results in mucosal atrophy -1.6 mg gluten/day is safe
Catassi, et al. (2007)	-50 mg gluten/day results in mucosal atrophy -10 mg gluten/day is safe for most CD patients
Biagi, et al. (2004)	-1 mg gluten/day leads to persisting villous atrophy
Collin, et al. (2004)	-in the worst case scenario, CD patients are already exposed up to 60 mg gluten/day -on average, CD patients are already exposed up to 16 mg gluten/day
Laurin, et al. (2002)	-200 mg gluten/day results in CD symptoms
Lohiniemi, et al. (2000)	-in the worst case scenario, CD patients are already exposed to 180 mg gluten/day -on average, CD patients are already exposed to 36 mg gluten/day
Kaukinen, et al. (1999)	-in the worst case scenario, CD patients are already exposed to 150 mg gluten/day -on average, CD patients are already exposed to 34 mg gluten/day
Chartrand, et al. (1997)	-0.75 mg gluten/day results in CD symptoms
Srinivasan, et al. (1996)	-500 mg gluten/day results in histological relapse
Troncone, et al. (1995)	-60 mg gluten/day results in histological relapse in some
Catassi, et al. (1993)	-200 mg gluten/day results in histological relapse

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390 **Table 3: Estimated amount of daily gluten exposure (mg).**

Gluten content of gluten-free products (ppm)	Amount of gluten-free products consumed (g)					
	100	200	300	400	500	600
200	20	40	60	80	100	120
100	10	20	30	40	50	60
50	5	10	15	20	25	30
40	4	8	12	16	20	24
30	3	6	9	12	15	18
20	2	4	6	8	10	12
10	1	2	3	4	5	6
5	0.5	1	1.5	2	2.5	3
3 ^a	0.3	0.6	0.9	1.2	1.5	1.8

391 ^a Considered the lowest limit of detection for gluten at this moment.

Highlights

- The threshold of 20 ppm does not protect sensitive and recovering celiac patients.
- A threshold of 3 ppm would be more suitable to protect these vulnerable groups.
- Labels “low in gluten” and “very low in gluten” hold no value for celiac patients.

ACCEPTED MANUSCRIPT