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Bruins Slot, I. D., Bremer, M. G. E. G., Hamer, R. J., & van der Fels, I. (2015). Part of celiac population still at risk despite current gluten thresholds. Trends in Food Science and Technology, 43(2), 219-226. https://doi.org/10.1016/j.tifs.2015.02.011

Accepted Manuscript

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PII: S0924-2244(15)00053-9

DOI: 10.1016/j.tifs.2015.02.011

Reference: TIFS 1634

To appear in: *Trends in Food Science & Technology*

Please cite this article as: Bruins Slot, I.D, Bremer, M.G.E.G., Hamer, R.J., van der Fels-Klerx, H.J., Part of celiac population still at risk despite current gluten thresholds, *Trends in Food Science & Technology* (2015), doi: 10.1016/j.tifs.2015.02.011.

Part of celiac population still at risk despite current gluten thresholds

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Key words: Celiac disease, gluten, thresholds, food safety

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 &

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

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

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

Literature selection

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

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

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

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

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

Current thresholds for gluten-free labelling of food products

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

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

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

Other thresholds concerning the gluten content of food products

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

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

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

Exposure

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

Gluten content of foods

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

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

Consumption

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

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

Tolerable levels

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

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

Average CD population

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

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

Sensitive CD population

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

Recovering CD population

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

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

Thresholds evaluation

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

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

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

Conclusions and recommendations

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

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

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

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

Acknowledgements

This study was financed by the Netherlands Food and Consumer Product Safety Authority (NVWA). The financer had no involvement in the study design; in the collection, analysis and interpretation of data; in writing the report; or in the decision to submit the article for publication.

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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).**

	Amount of gluten-free products consumed (g)					
Gluten content of gluten-free products (ppm)	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.