Might gluten traces in wheat substitutes pose a risk in patients with celiac disease? A population-based probabilistic approach to risk estimation1–3

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ABSTRACT
Background: In patients with treated celiac disease (CD), the ingestion of gluten traces contained in gluten-free (GF) wheat substitutes (eg, GF bread, flour, and pasta) could cause persistent intestinal mucosal damage.

Objective: The objective was to evaluate the proportion of CD patients at risk of mucosal damage due to the consumption of GF products in 4 European countries (Italy, Spain, Germany, and Norway).

Design: A probabilistic modeling approach was used to assess the risk of gluten intake at the population level. The input variables were 1) consumption of GF products, 2) concentration of gluten traces in GF products determined by the sandwich R5 ELISA method, and 3) the gluten threshold for mucosal damage of 10 to 50 mg/d. Different population and product availability scenarios were examined for risk assessment.

Results: The gluten content of 205 commercially available GF products ranged between <5 and 27.8 mg/kg. Overall, 99.5% of the analyzed samples had a gluten concentration <20 mg/kg. Most (94%) had a gluten concentration below the limit of quantification (5 mg/kg). The mean percentage of the CD European population at risk of mucosal damage resulting from consumption of GF products ranged between 0.01 (Germany) and 0.15 (Italy) and remained very low, even in the worst-case scenario (<1%).

Conclusions: The adoption of a single gluten threshold (20 mg/kg) for gluten contamination is suggested. GF products in Europe constitute a very safe option for patients with CD. The dietary follow-up of CD patients should focus on other potential sources of gluten contamination. Am J Clin Nutr 2013;97:109–16.

INTRODUCTION
Celiac disease (CD)4 is an immune-mediated enteropathy in susceptible individuals that is triggered by the ingestion of gluten—the major protein complex (including gliadins and glutenins) in wheat, rye, and barley. Because alternative therapies are not yet available, CD patients have to rely on the exclusion of gluten-containing products from their diet as the only treatment. An increasing number of gluten-free (GF) wheat substitutes are available on the market to facilitate a varied gluten-free diet (GFD). Compliance with the GFD has been positively correlated with the availability of GF products, which ultimately affects the quality of life of CD patients and their families (1–4).

It is well known that full mucosal recovery does not occur in most treated patients (5–7). The persistent inflammation of the small intestinal mucosa caused by exposure to gluten (5, 8, 9) has been related to the increased risk of complications, such as low bone mineral density (10) and cancer (11). Because a minimal degree of gluten contamination is difficult to avoid in the daily diet, the concept of a “daily tolerable intake” of gluten has received special attention (5, 12–20). According to recent systematic reviews (21, 22), a daily intake of <10–50 mg gluten is unlikely to cause significant histologic abnormalities. First, this threshold should not be exceeded by the consumption of GF wheat substitutes specifically marketed for CD patients (hereinafter referred to as GF products).

The regulation on the composition and labeling of foodstuffs suitable for CD patients has recently changed. In 2008, the FAO/WHO Codex Alimentarius Committee on Nutrition and Foods for Special Dietary Uses adopted a revised standard (23) with a dual threshold: 1) 20 mg/kg for products to be labeled “gluten-free” and 2) 100 mg/kg for products labeled as “very low gluten” (processed to reduce the gluten content to <100 mg/kg). These rules were adopted by the European Union in 2009 (24).

The provision of manufacturers with a legal framework for the use of the claims “gluten-free” and “very low gluten,” via this new regulation, represents a tangible improvement in labeling policies. However, it is not known to what extent the variable gluten content of products for treatment of CD (ranging from 0 to 100 mg/kg) may influence the risk of exceeding the tolerable

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2 Supported by the Celiac Associations of Italy (Associazione Italiana Celiachia), Germany (DZG Deutsche Zöliakie Gesellschaft e.V), and Spain (Asociación de Celiacos de Catalunya). R-Biopharm (Darmstadt, Germany) donated the diagnostic kits.
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4 Abbreviations used: CD, celiac disease; GF, gluten-free; GFD, gluten-free diet; LoQ, limit of quantification; P95, 95th percentile.

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daily intake of 10 to 50 mg gluten, given the variable consumption of GF products in different countries (16).

Probabilistic modeling is now considered the best approach for assessing the risk of allergen intake at the population level (25). This method was previously used to estimate the risk of unintended exposure to food allergens (26–28). In this multicenter European work, we applied the probabilistic model to evaluate the risk of mucosal damage in the CD population related to the possible gluten contamination of commercially available GF products.

SUBJECTS AND METHODS

Study design

The probabilistic approach was contextualized in 3 different scenarios to calculate 1) the percentage of the CD population at risk of mucosal damage resulting from the consumption of GF products in 4 different European countries (Italy, Spain, Germany, and Norway), 2) the percentage of the CD population at risk of mucosal damage in one of these countries should GF products from one country become available in other countries, and 3) the percentage of the CD population at risk of mucosal damage due to the consumption of GF products in Europe.

The input variables required for the implementation of the probabilistic approach described below were as follows: 1) the range of consumption of GF products by patients on treatment of CD, 2) the range of gluten concentration in these products, and 3) the distribution of gluten thresholds among patients with CD.

GF food-consumption data

The GF food-consumption data used in the modeling were based on the research performed by Gibert et al (16). They collected data regarding the food consumption of GF products among CD patients in Italy (n = 1359), Spain (n = 273), Germany (n = 56), and Norway (n = 226) by means of a food questionnaire. Data were collected on the consumption of 7 food groups: bread, pasta, pastry, biscuits, pizza, breakfast cereals, and bread crumb–coated food. For the risk assessment, the cumulative distribution of the consumption data was used.

Concentration of gluten in GF products

Representative GF products among 6 food groups (bread, pasta, pastry, biscuits, pizza, and breakfast cereals) were sampled from the market of the same countries investigated in the consumption study (16), ie, Italy, Spain, Germany, and Norway with the collaboration of the celiac societies of Italy (AIC Associazione Italiana Celiachia), Spain (Asociación de Celiacos de Cataluña), Germany (DZG Deutsche Zöliakie Gesellschaft e.V.), and Norway (NCF Norsk Celiakiforening).

Each celiac society suggested the choice of products to be purchased among the most commonly consumed products in each country. The samples were purchased in local stores from January 2010 to July 2010. For each food item, the samples were chosen from different manufacturers and, when possible, 2 different lots were bought for each product. The samples were preserved at 4–7°C until shipped for analysis.

The samples were extracted by using the cocktail solution and were measured with a sandwich R5 ELISA method (29, 30) (RIDASCREEN Gliadin ELISA art. no. R7001; R-Biopharm AG). This method is validated (31) and is the recommended method for determining the gluten content of foods by the Codex Alimentarius Commission (23). The results were expressed as mg/kg gluten. The limit of quantification (LoQ) was 5 mg gluten/kg, and the limit of detection was 3 mg gluten/kg.

For the risk assessment, the number of samples with a value above the LoQ was determined. Because the number of samples above the LoQ was at most 6 samples per food group, which was judged insufficient to fit a distribution, the maximum concentration above the LoQ was used in the risk assessment.

Determination of gluten threshold

Mucosal deterioration is the most sensitive measurement of a reaction to gluten in CD patients, because neither the clinical nor the serologic findings are always indicative (6, 7, 20). For the gluten threshold estimate, we used the data of the only prospective, double-blind, and placebo-controlled gluten challenge study that is available in the literature (5). In that trial, 49 adults with biopsy-proven CD who had adhered to a GFD for > 2 y were challenged for 3 mo with a placebo or with either 10 or 50 mg gluten/d. Most of the subjects exposed to 50 mg gluten/d showed a worsening of morphometric variables based on small intestinal biopsy testing. The value of 50 mg/d was therefore taken as the lowest observed adverse effect level in our study. Because no statistical differences in morphometric variables were observed in the group exposed to 10 mg/d, we considered 10 mg/d to be the no observable adverse effect level. Because no information about the distribution of the individual gluten threshold is available, we tested the following hypotheses: 1) normal distribution of the gluten threshold with a mean (±SD) of 30 ± 10 mg/d (the normal distribution was adjusted to be truncated at zero, so no negative threshold values could occur) and 2) point estimates of the gluten threshold at 10, 20, 30, 40, or 50 mg/d.

Probabilistic risk assessment

The probabilistic approach uses distributions to represent the input variables, which made it possible to account for both variability and uncertainty to quantify the risk. Consumption of the food (ie, the chance that a person with CD consumes a particular product and what amount of this product is consumed) and the concentration of gluten in the food (ie, whether this food contains gluten and at what concentration) determine the exposure to gluten. The third variable is the sensitivity of the individual for gluten, also referred to as the gluten threshold. By comparing the distribution of the thresholds to the distribution of the exposure to gluten, the probability of an adverse reaction—in this case the mucosal damage—is determined. A schema of the probabilistic approach, as applied to CD risk assessment and adapted from Spanjersberg et al, is shown in Figure 1 (26).

The outcome of the model is determined by running the model for a number of iterations. In each iteration, a drawing is made from every input variable to determine the risk in this iteration. By combining all the iterations, it is possible to estimate the distribution of the outcome, ie, the chance of the adverse reaction (mucosal damage in this case).

The probabilistic risk assessment was performed in the context of 3 different scenarios to address the 3 different situations mentioned in the study design.

Scenario 1: For each country (Italy, Spain, Germany, and Norway), the data on food consumption and gluten concentration were...
combined with the threshold estimates (distribution mean $\pm$ SD = 30 $\pm$ 10 mg/kg or point estimate at 10, 20, 30, 40, and 50 mg/d).

Scenario 2: For each country (Italy, Spain, Germany, and Norway), the data on food consumption were combined with the gluten concentration data based on the 4 countries together and the threshold estimates (distribution mean $\pm$ SD = 30 $\pm$ 10 mg/kg or point estimate at 10, 20, 30, 40, and 50 mg/d). This scenario represents a risk estimate for each country in the case that GF products from other European countries were also available.

Scenario 3: The data on food consumption for the 4 different countries were grouped together to form a single pattern of consumption. This was combined with the overall concentration data (4 countries together) and various threshold estimates. This scenario represents the risk of the CD population in Europe. An overview of the scenarios for the probabilistic risk assessment is shown in Table 1.

Statistical analysis

All models were run by using Microsoft Excel 2003 and @RISK version 4.5 with the use of 10,000 iterations and 25 simulations (26). In each simulation the model was run 10,000 times (iterations), each time giving an estimate of an adverse reaction. On the basis of 10,000 iterations, a mean number of adverse reactions was estimated. A distribution of the mean number of adverse reactions and the 95th percentile (P95) were estimated by repeating this procedure 25 times.

RESULTS

A summary of the results obtained from the analysis of 205 samples of commercially available GF products in the different countries in the study, according to the different scenarios, is shown in Table 2. The concentration of gluten ranged from <5 to 27.8 mg/kg. All the analyzed samples had a gluten concentration <100 mg/kg, and 99.5% were <20 mg/kg. All samples from Italy, Spain, and Norway and 98% of the samples from Germany had a gluten concentration <20 mg/kg. Most (94%) had a gluten concentration below the LoQ (5 mg/kg). The percentage of samples with a gluten content below the LoQ was 93% in Italy, 95% in Spain, 90% in Germany, and 100% in Norway. The 100% result of the Norwegian products may have been influenced by the smaller sampling performed in this country. In this sample of commercially available GF foods, wheat starch–based derivatives were not used to formulate GF products in Spain and occasionally in Italy (one product), whereas there were 6 products in Germany and 4 in Norway; however, most of

<table>
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<th>Scenario</th>
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<th>Concentration data (maximum concentration above LoQ)</th>
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<sup>1</sup> Combined data from Italy, Spain, Germany, and Norway. LoQ, limit of quantification.
them had a gluten content below the LoQ (5 mg/kg). Only one lot of the wheat starch–based products from Germany was minimally above the 20-mg/kg limit (27.8 mg/kg). Overall no remarkable difference was observed between the concentrations of gluten in the GF products from the different countries. However, Germany had more products above the LoQ, whereas no food in Norway contained gluten above the LoQ.

The results of the probabilistic risk assessment in the 3 different scenarios designed to answer the 3 corresponding questions are shown in Table 3.

**Question 1.** What is the percentage of the CD population at risk of mucosal damage due to the consumption of GF products in a specific country (ie, Italy, Spain, Germany, and Norway)?

Overall, the percentage of the CD population at risk (mean and P95) was <0.1, with the exception of Italy, where the mean percentage at risk was 0.15 (P95 = 0.20). According to the mean values, of 10,000 patients, 15 in Italy, 5 in Spain, 1 in Germany, and 2 in Norway were at risk of mucosal damage.

Assuming a point sensitivity estimate of 10 mg gluten/d, the mean CD population at risk was 0.1%, with the exception of Italy, where the mean percentage at risk was 0.16 (P95 = 0.20). Of 10,000 patients, 16 patients in Italy and 20 in Spain would be at risk of mucosal damage. With the exception of Italy, the higher thresholds (ie, 20, 30, 40, and 50 mg/d) all resulted in a negligible risk in each country.

**Question 2.** What would be the percentage of the CD population at risk of mucosal damage in a specific country if GF products from one country were also available in other countries?

Overall, the mean percentage of the CD population at risk was <0.1%, with the exception of Italy, where the mean percentage at risk was 0.16 (P95 = 0.20). Of 10,000 patients, 16 patients in Italy,
4 in Spain, 2 in Germany, and 5 in Norway would be at risk of mucosal damage. In all countries except Spain, the risk increased slightly if foods from other countries were made available.

Assuming a point threshold estimate of 10 mg gluten/d, the mean risk was <0.1% for Spain and Norway, 0.59% for Italy, and negligible for Germany. Of 10,000 patients, 59 in Italy, 7 in Spain, and 9 in Norway would be at risk of mucosal damage. With the exception of Italy, the higher thresholds (ie, 20, 30, 40, and 50 mg/d) did not result in a quantifiable risk in each of the countries. In this scenario, Spain and Germany were the countries at lower risk and Italy was the country at higher risk.

**Question 3.** What would be the percentage of the CD population at risk of mucosal damage due to the consumption of GF products in Europe?

Overall, the mean percentage of the CD population at risk of an adverse reaction was 0.18% in Europe. Assuming a point threshold estimate of 10 mg gluten/d, the risk increased to 0.47% and decreased along with the point estimate to 0.03% at 50 mg/d.

An overview of the 3 different scenarios for Italy, the country with the highest risk in scenarios 1 and 2 as compared with scenario 3 (all countries), is shown in Figure 2. Overall, the estimated risk in Italy is slightly increased should GF products from other countries enter the Italian market (from 0.15% in scenario 1 to 0.16% in scenario 3). As expected, the risk decreased when the threshold point estimate increased from 10 to 50 mg/d.

**DISCUSSION**

The analytic data collected in this study on gluten quantification in commercial GF products clearly demonstrate a successful effort by the specialized food industry to obtain products with very low gluten contamination. Even wheat starch-containing products mostly showed gluten values below or close to the LoQ (5 mg/kg).
In past years, the difficulty of producing wheat-substitute products with a gluten content <200 or 100 mg/kg was a concern for manufacturers. It was postulated that zero gluten was unrealistic or impossible to achieve (17, 19, 32) because of the existence of residual gluten in specially processed GF products. It was also suggested that too strict limits might lead to poor availability of GF products (17, 19) and consequently to lower compliance with dietary treatment. However, the finding that minimal amounts of gluten (10–50 mg/d) could still cause intestinal damage led to a more prudent view in the long run. The compromise of having food for persons with CD with a gluten content of either 10 or 100 mg/kg was accepted by many experts. The Codex standard for “foods for special dietary use for persons intolerant to gluten” and the recent regulation in Europe are based on dual thresholds of gluten contamination at 20 mg/kg (“gluten-free”) and 100 mg/kg (“very low gluten”).

Use of a representative sample of products from 4 different European countries in this study clearly showed that 1) no product contained gluten at a content close to 100 mg/kg, as the maximum content established was 27.8 mg/kg; 2) most products (99.5%) were <20 mg/kg; and 3) 94% of these products had a gluten concentration below the LoQ (5 mg/kg). These results show that the production of GF products at a threshold of 20 mg/kg (“gluten-free”) and 100 mg/kg (“very low gluten”).

Our results show an evolution toward high-quality GF products with minimal gluten contamination, which is of great importance for the avoidance of diet-related CD complications. The finding that 94% of the samples had a gluten content <5 mg/kg questions the need for having dual thresholds (20 and 100 mg/kg), instead of a universal GF denomination. It could even be argued that the threshold should be reduced to 5 mg/kg, because the vast majority of products are in that range. However, lowering to this threshold would increase the margin of error of the test because of loss of linearity of the ELISA determination at such lower levels, which increases the chance of frivolous law suits with major harm to the industry and consumers alike. A single gluten threshold of 20 mg/kg would be in line with the proposal endorsed by the Food and Drug Administration (33–35). This threshold could be applicable worldwide and guarantee the free movement of food across countries.

In general, the proportion of the CD patient population at risk of harmful gluten traces ingestion by commercial GF products was found to be very low in Europe. Assuming that the sensitivity to gluten of CD patients has a normal distribution (mean ± SD = 30 ± 10 mg/d), a statement that is consistent with current views (21, 22), the population at risk of mucosal damage under scenarios 1 and 2, respectively, was well below 0.1% in Spain (0.04–0.05%), Germany (0.01–0.02%), and Norway (0.02–0.05%) and only slightly higher in Italy (0.15–0.16%). The availability of GF foods produced in different countries (scenario 2) would not significantly increase the risk in any European country. Even in the worst-case hypothesis (threshold for intestinal damage at 10 mg/d), the percentage of the CD patient population at risk of mucosal damage was tiny in most countries and very small in Italy. Because the gluten concentration data were not worse in Italy than in other countries, this higher risk in Italy was primarily related to the higher consumption of GF wheat substitutes (mostly pasta) in this country, as previously shown by food-consumption data (16). The between-countries “risk dilution” observed under scenario 3 was basically an artifact. Indeed, this estimate was influenced by the unbalanced origin of the food-consumption data, 71% of which were collected in Italy.

There was a huge discrepancy between the percentage of the CD patient population at risk of excessive gluten intake from commercial GF products shown by our study (well below 1%) and the proportion of treated patients presenting persistent damage of the small intestinal mucosa (range: 50–80%) (5–7). Other factors should be considered to explain these histologic findings, such as analytic bias. The R5 method used in this study...
to quantify gluten is superior to older methods in terms of sensitivity and accuracy of gluten analysis; however, it mainly quantifies gliadins and to a lesser extent glutenins (36). Because the role of glutenin traces in the activation and persistence of intestinal damage is still unclear (37, 38), the effect of this possible bias is, at most, only minor in our opinion. Other sources of gluten contamination should also be considered to explain the histologic findings. In our experience, more attention should be paid to voluntary transgressions, particularly in vulnerable subjects such as adolescents, and to the gluten contamination of meals consumed outside the patient’s household (eg, in restaurants and pizzerias). The histologic findings may also be explained by the lack of a cause-effect relation. It cannot be excluded that, in some patients, the mucosa is so deranged by years of consuming a gluten-containing diet before diagnosis that it can never fully recover even if a strict GFD is consumed.

In summary, this study showed that the percentage of the CD patient population at risk of significant contamination of the GFD because of the consumption of commercial GF substitutes is very low in Europe and is not influenced by the producer’s country within the European market. Our analytic results highlight that a single gluten threshold of 20 mg/kg could not only protect the consumer but also simplify the trading of these “niche” products. At the current gluten contamination of commercial GF products, the risk of ingesting clinically significant traces of gluten with these products in Europe is more related to the overall amount of ingested products than to their gluten content. Because persisting intestinal damage caused by ongoing gluten ingestion is a common problem in the follow-up of CD patients consuming a GFD, the dietary interview should focus on other potential sources of diet contamination.

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The authors’ responsibilities were as follows—CC, AG, and AGK: were primarily responsible for the conception and design of the study; SN and AG: collected and analyzed the data; MAC: provided the consumption data; and CC has served as a consultant for Menarini Diagnostics (a healthcare company) and Schür (gluten-free food brand). AF is a shareholder of Alba Therapeutics (Biopharmaceutical Company). AG is a Board Member of the Catalan Celiac Society (Asociacio´ de Celiacs de Catalunya). SN is an employee of the Italian Celiac Society (Associazione Italiana Celiachia). None of the other authors had a conflict of interest.

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