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RESEARCH ARTICLE

Dietary exposure to fumonisins and evaluation of nutrient intake in a group of adult celiac patients on a gluten-free diet

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Scope: The main objectives of this study were to estimate dietary fumonisin exposure and nutrient intake in a group of patients diagnosed with celiac disease compared to non-celiac subjects.

Methods and results: The fumonisin level in 118 frequently consumed corn-based products was determined and dietary habits were recorded using a 7-day weighed food record. Data were then compared to those obtained for a control group. The fumonisin intake in the celiac patients was significantly higher than in controls, with mean values (± SE) of 0.395 ± 0.049 and 0.029 ± 0.006 μg/kg body weight per day, respectively. With regard to nutritional habits, celiac patients showed a preference for a high fat diet, coupled with a high intake of sweets and soft drinks and a low intake of vegetables, iron, calcium and folate.

Conclusion: These findings may have serious health implications for the celiac population due to the widespread occurrence of fumonisins in most of the widely consumed gluten-free products, leading to continuous exposure to this particular mycotoxin. Moreover, the recorded nutritional quality of the celiac patient's diet raises concerns regarding its long-term adequacy and its potential impact on chronic conditions such as type 2 diabetes and cardiovascular diseases.

Keywords:
Celiac disease / Dietary habits / Fumonisins / Gluten-free diet / Risk assessment

1 Introduction

Fumonisins are a group of mycotoxins that are mainly produced as secondary metabolites by *Fusarium* spp. Fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3) are the most abundant of the naturally occurring analogs and predominantly contaminate maize and maize-based products [1]. Fumonisin toxicity is due to their structural similarity with sphingosine, which results in the inhibition of ceramide synthase and thus disruption of sphingolipid biosynthesis [2]. Fumonisins induce hepatic carcinogenicity in rodents and epidemiological data indicate an association with esophageal cancer in populations consuming fumonisin-contaminated maize, in whom the latter represents a major component of the diet [3, 4]. The consumption of fumonisin-contaminated maize has also been associated with the occurrence of neural tube defects [2]. For these reasons FB_1 has been classified as a 2B carcinogen by the International Agency of Research on Cancer [5]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has determined a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight per day for FB_1, FB_2 and FB_3 alone or in combination [6]. Legal limits for fumonisins in raw commodities and foods for human consumption were established by the European Union in 2007: 4000 μg/kg for unprocessed maize, 1000 μg/kg for maize used for direct human consumption, 800 μg/kg for maize-based breakfast cereals and snacks and 200 μg/kg for baby foods (EC No. 1126/2007).

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Abbreviations: 7D-WR, 7-day weighed food record; **BW**, body weight; **CD**, celiac disease; **EMAN**, European Mycotoxin Awareness Network; **FB_1**, fumonisin B_1; **FB_2**, fumonisin B_2; **FB_3**, fumonisin B_3; **FBs**, total fumonisins (the sum of FB_1, FB_2 and FB_3); **GFD**, gluten-free diet; **JECFA**, Joint FAO/WHO Expert Committee on Food Additives; **PMTDI**, provisional maximum tolerable daily intake; **Sa/So**, sphinganine-to-sphingosine ratio

Populations at risk of fumonisin toxicity are generally those who consume maize and/or maize-based products as a major component of the diet and do not have access to non-contaminated maize, as is usually the case in Third World Countries. However, other populations at risk of fumonisin toxicity may include individuals diagnosed with celiac disease (CD), an autoimmune inflammatory disorder triggered by the ingestion of gluten in susceptible individuals [7]. The permanent adoption of a gluten-free diet (GFD) is essential for the normalization of the structure and function of the intestinal mucosa [8]. Cereals such as wheat, barley and rye must be excluded and substituted with gluten-free (GF) cereals such as corn and rice. Despite a prevalence of CD of 0.5–1% worldwide [8], little is known about the nutritional adequacy of GFD [9] and the associated exposure of fumonisin due to diets consisting of high levels of corn and/or corn-based products [10, 11].

The primary objective of this study was to estimate the dietary intake of total fumonisins (FBs, as the sum of FB_1, FB_2 and FB_3) in a group of individuals diagnosed with CD. Our secondary objective was to establish the main dietary sources of FBs in GDF and to assess nutrient intake in this population. For this purpose, the FBs concentration in a number of frequently consumed corn-based products (n = 118) was determined and dietary habits were recorded using a 7-day weighed food record (7D-WR). Data were then compared to those obtained from a control group. Finally, the presence of urinary biomarkers of fumonisin intake was also investigated by collecting a 24-h urine sample at the end of the 7D-WR period.

2 Materials and methods

The flowchart of the overall study is illustrated in Fig. 1.

2.1 Participants and study design

A total of 80 subjects (40 celiac patients with histologically confirmed CD and 40 healthy non-CD control subjects) participated in the study. Exclusion criteria for celiac patients were: (1) diagnosis of CD of less than 6 months, (2) age under 18 or over 70, (3) metabolic or chronic diseases (diabetes mellitus, etc.), (4) pregnancy or lactation, (5) being vegetarian or vegan. Exclusion criteria for the control subjects were the same with the exception of the diagnosis of CD. During their first visit, patients were instructed on how to fill in the dietary questionnaire and received a flask for the 24-h urine collection.

All patients were recruited between March and August 2008 in Emilia Romagna (Italy) by means of an advertisement posted in pharmacies in the city of Reggio-Emilia and at the University of Parma. The protocol was approved by the local Ethical Committee for Human Research of the City of Parma and all patients gave their written informed consent.

2.2 Dietary records

Total food and beverage consumption was assessed by means of a questionnaire filled in everyday for a total of 7 days. All participants were trained by a dietitian to record all food consumed. Participants were asked to weigh all food and drink consumed and to provide a detailed description of each food, including methods of preparation and recipes whenever possible. In the case of GF foods, subjects were asked to precisely note the name of the manufacturer or to provide the food label. All subjects returned the 7D-WR at the second visit during which a dietitian reviewed the 7D-WR with participants to check for errors or omissions and to estimate the amount of food eaten outside the home using a book of photographs and standard household measures. Nutrient and FBs intake was calculated using a Microsoft Access application linked to the food database of the European Institute of Oncology (EIO), covering the nutrient composition of more than 700 Italian foods [12] plus FBs values of the foods analyzed (see below). The nutrient composition of commercial GF foods was evaluated on the basis of information given by suppliers or written on the package.

The computer output consisted of the mean daily intake of macro- and micronutrients and of FBs for each subject. Food items consumed were also retrieved and collapsed into food categories: pasta, breads (including crackers and salty snacks), potatoes, flours, other cereals (including corn, oat and rice), fruit, vegetables, pulses, meat, preserved meat, milk (including yogurt and cream), cheese, eggs, fish, oils and fats, sweets (including biscuits, sweet snacks, breakfast cereals, ice cream, candies and chocolates), soft drinks, juices, coffees plus teas, and alcoholic beverages. For each subject, the mean daily intake of each food category was then calculated.

2.3 Analytical methods: Chemicals

Standard fumonisin solutions (a mixture of FB_1 and FB_2 and FB_3, each 50 μg/mL, in acetonitrile/water, 1:1 v/v) were purchased from Biopure (Tulln, Austria). Sphingosine, sphinganine and phytosphingosine hydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). Methanol (HPLC grade) was obtained from Carlo Erba (Milan, Italy), acetonitrile (HPLC grade) was from J.T. Baker (Mallinckrodt Backer, Phillipsburg, NJ); bidistilled water was produced in our laboratory utilizing an Alpha-Q system (Millipore, Marlborough, MA).

2.4 Analytical methods: GF sample collection for fumonisin analysis

In order to obtain a comprehensive estimate of the occurrence of FBs in GF products purchased in the Italian market, a survey was carried out over a 12-month period. Samples analyzed included 118 of the most frequently purchased GF

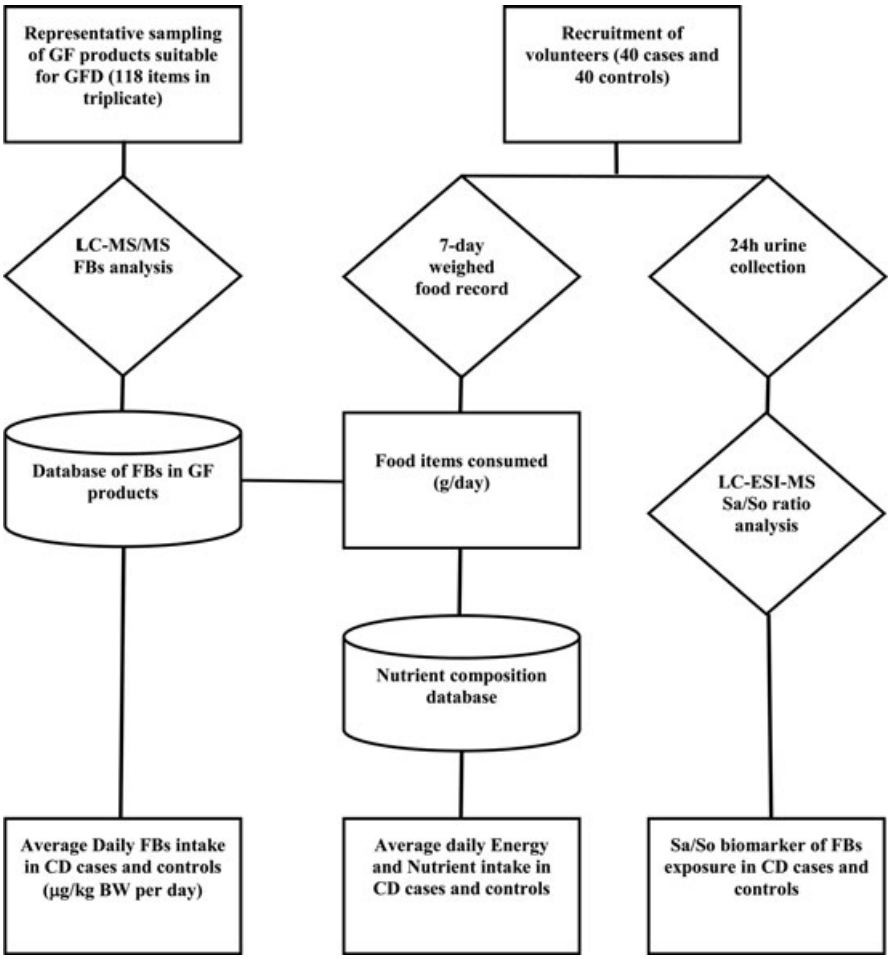

Figure 1. Conceptual flowchart for the determination of FBs exposure in the diet.

products according to the data collected by 37 pharmacies located in Reggio-Emilia. All products were purchased from retail shops specializing in dietetic foods and were labeled with the "gluten-free approved" symbol of the Italian Celiac Association. For each product, a representative sample (100 g) was obtained by thoroughly grinding and mixing the content of three different packages purchased from different retail shops over the territory. The sample was mixed again, and a subsample (10 g) was taken for analysis.

2.5 Analytical methods: Fumonisin in GF products

FBs extraction and analysis were performed according to Dall'Asta et al. [13] on 10 g of finely ground sample. Triplicate from each sample were analyzed. After filtration, 4 mL of extract was evaporated to dryness under a stream of nitrogen and the residue was redissolved in 1 mL of water/methanol (30:70, v/v) prior to LC-MS/MS analysis [13].

2.6 Analytical methods: Urine collection

A 24-h sample of urine was collected on the last day of the dietary record. All participants were instructed to record the total volume of urine using the scale on a graduated flask. A 40 mL sample was collected and divided into four 10 mL tubes and stored at –20°C until delivery to the laboratory. Urine samples were then stored at –80°C until analysis.

2.7 Analytical methods: Urinary biomarkers

The extraction procedure used in this study for urinary sphinganine and sphingosine analysis was a modification of that described by Castegnaro et al. [14]. Briefly, 5 mL of urine were centrifuged at 1000 *g* for 15 min. The supernatant was diluted with 5 mL of distilled water, alkalinized with 125 μL of 1 mol/L KOH (final pH value: 9.5) and extracted with 5 mL of ethyl acetate for 20 min on a shaker at 18 rpm. The organic phase was collected after centrifugation at 1000 *g* for 20 min. The aqueous layer was extracted again and the two organic fractions were pooled. Recovery was 80.5% and 91.7% after the first and second extraction, respectively. After evaporation to dryness with N_2, the residue was reconstituted in 90 μL methanol:water (9:1, v/v), combined with 10 μL of phytosphingosine internal standard (1 μg/mL) and analyzed by LC-ESI-MS, according to Seefelder et al. [15].

2.8 Statistical analyses

Results from the fumonisin studies were subjected to statistical analyses (ANOVA test, $\alpha = 0.05$). The incidences of samples containing FB_1, FB_2 and FB_3 (% positive) were expressed as the percentage of samples containing levels above LOQ (25 μg/kg). The mean was calculated using half of the LOQ for results lower than the LOQ. This procedure is commonly used in risk assessment in order to consider also the contribution to the overall intake of those samples which present a FB concentration lower than the LOQ but higher than the LOD (5 μg/kg). The nutritional data collected from the celiac patients and the control group were checked for normality using the Kolmogorov–Smirnov test. Normally distributed data or log-transformed data were then compared using Student's *t*-test ($\alpha = 0.05$). Statistical analysis was performed using SPSS® version 16.0 for Windows (SPSS®, Chicago, IL).

3 Results

3.1 Fumonisin contamination in GF products

The analyses of GF products showed widespread contamination. In particular, 105 out of 118 samples (89%) were found to be contaminated above the LOD (4 μg/kg for FB_1, 8 μg/kg for FB_2 and FB_3).

Although 8 out of 118 sample were contaminated above the EU legal limits (800 μg/kg for corn-based products and 1000 μg/kg for corn flour), the overall median value (309 μg/kg) was far below the EU limit (Table 1). However, FBs reached high levels in several samples (maximum concentration: 3308 μg/kg). Gluten-free pasta and breads (n = 51) showed very low levels of contamination, with a maximum concentration of 514 μg/kg. Extruded products (n = 19) also showed low levels of contamination (median value: 262 μg/kg), with the exception of one case (2253 μg/kg). On the contrary, cornmeal flour (n = 10) was found to be highly contaminated with a median value of 1190 μg/kg, which is above the legal limit, and a maximum value of 3308 μg/kg. With regard to GF snacks (n = 17), the data showed widespread contamination (14 out of 17 products) but at low levels (median value: 443 μg/kg) with only one exception (maximum concentration: 2625 μg/kg).

Table 1. Occurrence of fumonisins in gluten-free products: median values and contamination ranges

	Positive/ Total samples	Median value (μg/kg)	Contamination range (μg/kg)
Extruded products (n = 19)	16/19	262	39–2253
Pasta (n = 29)	27/29	124	27–335
Bread and surrogates (n = 22)	20/22	138	54–514
Cakes and biscuits (n = 17)	16/17	301	LOQ[a)]–555
Cornmeal (n = 10)	10/10	1190	101–3308
Flours (n = 4)	2/4	67	122–210
Snacks (n = 17)	14/17	443	LOQ[a)]–2625
Total (n = 118)	105/118	309	LOQ[a)]–3308

a) LOQ: 25 μg/kg.

3.2 Dietary data

All participants completed the study. The gender and the associated means for age, height, weight and BMI of both groups are given in Table 2. The high percentage of females in both groups reflects the gender ratio of CD prevalence. Based on self-reported heights and weights, 31 (78%) celiac patients had a BMI in the healthy range (18.5 to 24.9), 3 (8%) were in the overweight range (25.0 to 29.9) and 6 (15%) were in the underweight range (less than 18.5). Thirty out of 40 (75%) healthy subjects had a BMI in the healthy range, 6 (15%) were in the overweight range, 3 (8%) were in the underweight range and 1 (3%) was in the obese range (30.0 or more).

Regarding the dietary intake of nutrients (Table 3), celiac patients and controls reported a similar intake of energy, proteins, carbohydrates, including starch and sugars, fiber and cholesterol. Conversely, the mean daily intake of total and saturated fats, and the percentage of energy from protein and total and saturated fats, were significantly higher for celiac patients than for controls (Table 3).

Subjects on a GFD consumed significantly less vitamin E ($p < 0.01$), β carotene ($p = 0.036$), folate ($p = 0.006$) and iron ($p < 0.01$) (Table 4). The mean daily intake of other micronutrients was comparable between the two groups.

In terms of food categories, celiac patients consumed significantly fewer vegetables ($p = 0.013$) and drank significantly more soft drinks ($p = 0.033$) compared to controls (Table 5).

Table 2. Characteristics of celiac and control subjects[a)]

	Celiac patients n = 40	Control subjects n = 40
Females, n (%)	34 (85)	32 (80)
Age (year)	40.2 ± 2.2 (19–70)	39.4 ± 1.9 (24–69)
Height (cm)	165.3 ± 1.1 (152–178)	167.7 ± 1.3 (150–190)
Weight (kg)	57.8 ± 1.5 (42–89)	64.3 ± 1.7 (46–90)
BMI (kg/m^2)	20.8 ± 0.4 (16–29)	22.3 ± 0.5 (17–33)

a) Mean ± SE, range value (min and max).

The mean daily intake of the other food categories did not differ between the two groups.

3.3 Evaluation of fumonisin intake

In order to compare FBs intake in celiac patients and the control group, the average FBs contamination of each maize-based food category was matched with the 7-day dietary data, obtaining the mean daily intake of FBs. For each subject, this value was converted in the average daily intake for kg of body weight (BW). This procedure was described in Fig. 1. FBs intake in the celiac patients was significantly higher than in controls, with mean (± SE) values of 0.395 ± 0.049 and 0.029 ± 0.006 μg/kg BW per day, respectively ($p < 0.001$).

Fumonisin exposure was also evaluated by monitoring urinary biomarkers, particularly the sphinganine-to-sphingosine ratio (Sa/So) [10,11], measured in the 24-h urine sample collected on the last day of the dietary record. The results were normalized for creatinine content and statistically analyzed. Interestingly, the Sa/So ratio in celiac patients (0.66 ± 0.12 mg/L) did not differ significantly from the control (0.47 ± 0.04 mg/L) ($p = 0.163$).

4 Discussion

In Italy CD has an estimated prevalence of one in 184, very similar to that estimated for the EU as a whole [8]. In a GFD, maize flour is used as a substitute for wheat and other gluten-containing cereals, mainly as an ingredient in preparations such as bread substitutes, pasta, cakes and biscuits or salty snacks. Due to the high toxicity of FBs and their predominant contamination of corn-based products, evaluation of the fumonisin content in ready-to-eat GF products, rather than just flours, is central to conducting an exposure assessment of fumonisins in celiac populations. The literature concerning the occurrence of fumonisin in GF products is currently limited. Ostrý et al. [16] analyzed a total of 127 GF food samples from the Czech market: 88% of the corn-based foods were found to be positive for total fumonisins. The highest fumonisin contamination levels were recorded in extruded corn products (up to 1808 μg/kg) and in cornmeal (up to 1243 μg/kg). Lower contamination levels were found in other commodities, such as corn flour (up to 487 μg/kg), instant corn porridge (up to 788 μg/kg), and corn pastes (up to 511 μg/kg). A more recent study concerning the occurrence of free and bound fumonisins in GF products from the Italian

Table 3. Dietary intake of celiac and control subjects[a)]

	Celiac patients (n = 40)		Control subjects (n = 40)		
	Mean ± SE	Range	Mean ± SE	Range	*p*-Value
Energy (MJ)	8.5 ± 0.3	5.4—14.0	8.0 ± 1.8	5.1–12.9	0.196
Energy from protein (%)	13.3 ± 0.4	10.0–18.9	14.8 ± 0.4	10.5–20.1	0.005
Energy from total fat (%)	36.6 ± 0.7	25.6–49.3	33.9 ± 0.8	22.6–47.0	0.011
Energy from carbohydrate (%)	49.5 ± 0.9	33.6–60.0	51.3 ± 1.2	32.5–71.0	0.225
Energy from saturated fat (%)	19.6 ± 0.8	5.2–34.8	12.4 ± 0.3	2.1–17.2	<0.001
Energy from sugar (%)	18.4 ± 0.7	9.1–31.4	19.2 ± 0.8	9.3–40.5	0.493
Total fat (g)	82.6 ± 3.0	57.0–133.6	72.2 ± 3.3	44.9–133.5	0.024
Saturated fat (g)	44.3 ± 2.5	15.9–85.0	26.4 ± 1.4	8.6–48.2	<0.001
Protein (g)	66.2 ± 2.5	40.0–100.1	68.8 ± 2.5	37.2–103.7	0.464
Carbohydrate (g)	252.1 ± 11.2	138.4–438.4	242.0 ± 9.3	149.6–367.4	0.493
Starch (g)	140.6 ± 7.7	22.9–297.0	144.1 ± 6.1	90.7–244.4	0.723
Sugar (g)	100.6 ± 6.0	47.7–206.8	96.6 ± 5.3	40.4–201.5	0.619
Fiber (g)	18.8 ± 1.4	6.9–40.5	21.6 ± 1.2	9.1–42.9	0.137

a) Comparisons were performed using Student's *t*-test.

Table 4. Micronutrient intake of celiac and control subjects[a)]

	Celiac patients (n = 40)		Control subjects (n = 40)		
	Mean ± SE	Range	Mean ± SE	Range	*p*-Value
Vitamin C (mg)	122.8 ± 12.9	14.7–403.9	123.0 ± 8.9	24.8–277.6	0.989
Vitamin E (mg)	8.0 ± 0.4	3.6–14.9	11.4 ± 0.5	6.1–21.3	<0.010
β-carotene (μg)	3166.2 ± 310.9	470.9–8495.8	4275.1 ± 414.8	1017.2–11 833.7	0.036
Folate (μg)	218.0 ± 18.3	32.8–573.4	281.0 ± 12.3	130.8–518.8	0.006
Sodium (mg)	2456.2 ± 139.4	1148.0–5325.9	2458.6 ± 135.5	933.0–4754.7	0.990
Iron (mg)	8.8 ± 0.7	2.5–19.1	13.0 ± 0.6	5.3–23.6	<0.010
Calcium (mg)	773.0 ± 45.9	258.9–1544.1	801.9 ± 45.4	407.1–1581.2	0.655

a) Comparisons were performed using Student's *t*-test.

Table 5. Daily intake of food groups by celiac and control subjects[a)]

	Celiac patients (n = 40)		Control subjects (n = 40)		
	Mean ± SE	Range	Mean ± SE	Range	*p*-Value
Pasta (g)	39.3 ± 4.5	0.0–102.9	40.0 ± 5.2	0.0–147.1	0.917
Breads (g)	111.3 ± 9.9	0.0–319.6	125.1 ± 8.0	14.3–254.3	0.282
Potatoes (g)	29.5 ± 4.8	0.0–128.6	24.2 ± 4.9	0.0–160.0	0.437
Flours (g)	5.9 ± 2.1	0.0–73.3	7.9 ± 1.6	0.0–34.9	0.444
Other cereals (g)	38.0 ± 8.4	0.0–302.9	19.9 ± 4.4	0.0–131.4	0.060
Fruit (g)	211.1 ± 23.1	0.0–535.0	275.7 ± 30.1	0.0–752.0	0.093
Vegetables (g)	174.2 ± 16.1	22.14–527.86	239.7 ± 20.1	64.3–570.1	0.013
Pulses (g)	10.4 ± 3.0	0.0–102.9	15.5 ± 4.3	0.0–143.4	0.342
Meat (g)	67.9 ± 6.6	4.3–251.4	57.3 ± 5.9	0.0–150.0	0.240
Preserved meat (g)	29.3 ± 3.8	0.0–128.6	24.7 ± 4.1	0.0–151.7	0.411
Milk (g)	157.4 ± 18.5	0.0–501.43	137.4 ± 15.8	0.0–353.6	0.413
Cheese (g)	46.5 ± 4.0	7.1–92.9	37.1 ± 3.3	1.4–97.9	0.074
Eggs (g)	9.1 ± 1.2	0.0–25.7	7.7 ± 1.4	0.0–37.1	0.465
Fish (g)	29.1 ± 5.1	0.0–128.6	38.3 ± 5.4	0.0–185.4	0.222
Oils and fats (g)	23.3 ± 1.5	4.3–52.1	26.9 ± 2.1	4.6–69.7	0.170
Sweets (g)	131.3 ± 9.3	17.1–267.4	107.0 ± 10.7	23.7–353.1	0.091
Soft drinks (g)	64.6 ± 13.1	0.0–362.1	33.0 ± 6.3	0.0–172.9	0.033
Juices (g)	59.5 ± 23.3	0.0–771.4	43.0 ± 9.8	0.0–242.9	0.518
Coffees and teas (g)	209.8 ± 39.6	0.0–1496.4	192.0 ± 22.9	57.1–842.9	0.697
Alcoholic beverages (g)	63.0 ± 17.6	0.0–508.6	121.3 ± 23.6	0.0–731.4	0.051

a) Comparisons were performed using Student's *t*-test.

market also showed widespread contamination [13]: fumonisins were found in 82% of the samples (n = 40) and the overall median value was close to the EU legal limit for foods for human consumption. Finally, Lo Magro et al. [17] recently reported that 54% of GF products (n = 13) on the Italian market were contaminated with FBs (up to 618 μg/kg).

Our results confirm the occurrence of fumonisins in GF corn-based products, showing widespread contamination not only in maize flour and cornmeal but also in treated products such as corn-flakes or mixed breakfast cereals, bread and pasta substitutes and snacks. Although the contamination levels found in this study exceeded the EU legal limits established for food only in two cases out of 118 (a cornmeal sample and a corn-flakes sample), this widespread contamination cannot be considered negligible, particularly for population groups whose diet is mainly based on these products, such as CD patients. Thus, with the aim of measuring the fumonisin exposure in the CD population, the dietary habits of a group of celiac patients following a GFD were recorded and compared to those of a control group of healthy non-CD subjects. The overall dietary results demonstrated that the celiac diet differed from that of the control group in terms of the intake of macro and micronutrients and food selection. In fact, even though the intake of energy, proteins and carbohydrates – including starch and sugars – was similar in celiac and control subjects, celiac patients consumed more total and saturated fats than their healthy peers. Such findings confirm previous results obtained in children [18] and adults with CD [19]. In the present study the differences observed for total and saturated fats were probably due to the higher consumption of cheese and sweets by celiac patients than control subjects, although the difference was not statistically significant. As also found by other authors [18–20], celiac patients tended to choose more unhealthy foods, such as cheese and snacks (included in the sweet food group in the present study) with a high content of total and saturated lipids. Conversely, although the intake of fiber was lower in both groups than that recommended by the Italian Society of Nutrition (30 g/day) [21], the fiber intake in patients did not differ significantly from the controls. This contradicts earlier studies, which have shown that a GFD results in reduced fiber intake [22–25]. However, Kinsey et al. [26] suggested that, in recent years, manufacturers of GF products have brought out high fiber ranges of breads and flour mixes.

Despite the comparable intake of fiber, celiac patients consumed significantly fewer vegetables than controls, resulting in a significantly lower intake of β-carotene and folate. These findings are consistent with previous studies [23, 27]. Moreover, although the mean daily intake of folate by patients with GFD was slightly above the recommended amount of 200 micrograms [21], only 19 out of 40 celiac patients met the current recommendation.

Similarly to fiber intake, there was no significant difference between the two groups in terms of calcium intake. However, less than half (33% of celiac patients and 38% of control subjects) of the participants consumed the amount of calcium recommended by the Italian Society of Nutrition (1200 g/day for young people and 800 mg/day for adults) [21], which was consistent with previous findings [24, 26]. This result is particularly worrying for celiac patients because

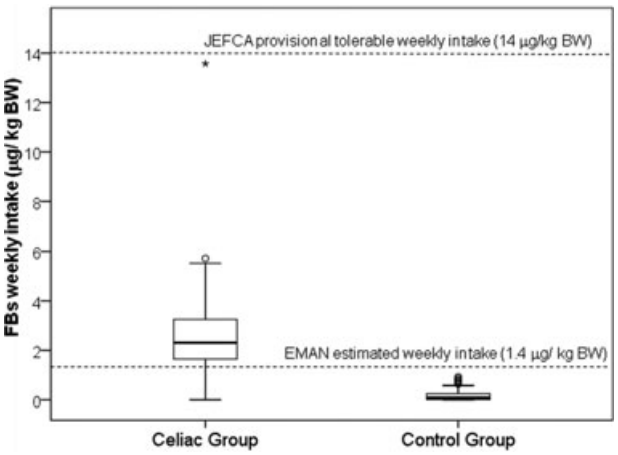

Figure 2. Box chart comparison of the estimated weekly fumonisin intake (μg/kg BW per week) for celiac patients and the control group. The EMAN estimated weekly intake and the JEFCA provisional tolerable weekly intake are also reported (dotted lines).

bone disease, including osteoporosis, may be a common feature of CD [24, 28]. Finally, the dietary analysis showed that the mean daily intake of iron was lower in the celiac group than in controls, possibly due to the consumption of lower amounts of whole grains, pulses and iron-containing vegetables. It is notable that the consumption of iron by both groups was below the recommended level for the Italian population (10 mg for male and postmenopausal women and 18 mg for premenopausal women) [21], as found previously [24, 26].

Regarding FBs exposure in our subjects, the data showed a significantly higher intake in celiac patients (0.39 μg/kg BW per day) compared to the control group (0.03 μg/kg BW per day), with a risk factor, calculated as the ratio between PMTDI and FBs intake, of 5.06 and 68.96, respectively. Figure 2 shows that at least one celiac subject was exposed to very high levels of FBs due to the high amount of corn-based products consumed during the recording week. This case, although treated as an outlier in the statistical analysis, cannot be considered negligible since the calculated daily intake (1.94 μg/kg BW per day) was very close to the PMTDI. Moreover, the weekly estimated intake of FBs in our celiac patients (2.77 μg/kg BW per week) was lower than the provisional tolerable weekly intake given by the JECFA (14 μg/kg BW per week), but higher than the weekly intake for people in the EU (1.4 μg/kg BW per week) reported by the European Mycotoxin Awareness Network (EMAN), as shown in Fig. 2 [29]. This can be attributed to the fact that, in our study, exposure was calculated on the basis of the overall total fumonisin contamination as the sum of FB_1, FB_2 and FB_3, whereas in most previous studies only FB_1 levels were considered. Moreover, it should be noted that the EU risk assessment data were established by taking into consideration the average maize consumption in Europe and the average FB_1 maize contamination estimated by several surveys. However, maize consumption differs among countries (for example, for maize flour, from 2.0 g/capita/day in Norway, Finland and Sweden up to 15.4 g/capita/day in Italy, Spain and Greece), as reported in the latest FAO database [30].

To our knowledge, limited data are available concerning the fumonisin intake derived from a GFD. Only one paper assessed the exposure of people suffering from CD to fumonisins by evaluating the average fumonisin intake deriving from a GFD [16]. In that study, the highest estimate of exposure to total fumonisins was recorded from corn-extruded bread as 3.2 μg/person per day. Very recently, the same authors presented an update of the survey, showing that the Czech population and people suffering from CD were exposed to 3 ng/kg BW per day and 13 ng/kg BW per day, respectively (Ostrý et al., personal communication). Although these data clearly show increased exposure in people suffering from CD, the estimated fumonisin intake was lower for both celiac and control groups than in our study. This could be because our consumption data arose from a 7D-WR, a gold standard tool for monitoring dietary habits, whereas the dietary data collected by Ostrý et al. were derived from a national survey, not specifically intended for the purpose. Moreover, the fumonisin levels in Italy are known to be higher than those reported for many North-Eastern EU Countries such as the Czech Republic.

In addition to estimating FBs exposure based on dietary analysis, the use of biomarkers has been proposed as a suitable method for assessing individual exposure, since it reflects variability due to food contamination levels, cooking, individual consumption, variations in toxicokinetics and toxicodynamics [11]. Various animal studies have successfully investigated the Sa/So as a biomarker of fumonisin exposure [11]. The same biomarker was also investigated in blood and urine in several human studies [10, 11, 31, 32]. Other recent studies have reported the measurement of urinary fumonisin as an alternative biomarker for human exposure. However the latter approach, although very effective and reliable, is only suitable for highly exposed people, given the very low urinary excretion of fumonisins [33, 34]. Given this limitation, and considering the lower exposure calculated for the celiac group compared to that reported in the literature for high risk groups, the direct determination of fumonisin in urine was not appropriate in our study. Thus, to evaluate whether the increased fumonisin intake in the celiac group gave rise to a significant alteration in the sphingoid base metabolism, the Sa/So in 24-h urine in the celiac and control groups was compared, with the finding that there was no significant difference between the two groups. This apparently contradictory result could be attributed to the fact that, in humans, the basal levels of sphingoid base are subject to high individual variability due to factors such as gender, age, health conditions, physical activity, stress, and exposure to other environmental or food contaminants [35, 36]. This high individual variability is more evident when a small number of subjects is considered, as in our study.

The study described herein represents the first attempt to evaluate the exposure of a celiac population to a mycotoxin

using a 7-day food diary. This approach also allows the association of toxicological data with nutritional adequacy data. The main strength of the present study is the comparison of dietary habits of celiac patients with a matched group of healthy subjects with comparable characteristics, such as age, lifestyle, social status and place of origin. Moreover, we collected dietary data over 7 days, whereas previous studies used less accurate food records [19, 20, 24, 25, 27].

The main weakness of this study was the relatively small sample size, which could have had an impact on the risk of bias for both the assessment of fumonisin intake and the evaluation of the nutritional data. However, the sample size was comparable to that of almost all previous studies on the nutritional adequacy of the diet of celiac patients [23, 24, 27]. Finally, even though the dietary habits were recorded using a relatively accurate tool, they only describe dietary intake during one week and do not take into account the seasonal variation in food consumption.

In conclusion, these findings may have serious implications for the health of the celiac population, due to the widespread occurrence of fumonisins in most of the widely consumed GF products, which leads to continuous exposure to this mycotoxin. Moreover, the preference of celiac patients for a high fat diet, coupled with a high intake of sweets and soft drinks and a low intake of vegetables, iron, calcium and folate raises concerns regarding the adequacy of a long-term GFD and its potential impact on chronic conditions such as type 2 diabetes and cardiovascular diseases. An additional concern regards the pregnant CD women, who could be at more risk to neural tube birth effects due to the fumonisin exposure [2] and the low folate intake.

Although these data should be confirmed by further studies in a larger population, performed at least at a national level, our findings raise the alarm concerning the overall diet followed by people suffering from CD and, in particular, their exposure to fumonisins.

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5 References

[1] Sweeney, M. J., Dobson, A. D., Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. *Int. J. Food Microbiol.* 1998, *43*, 141–158.

[2] Marasas, W. F., Riley, R. T., Hendricks, K. A., Stevens, V. L., *et al.*, Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. *J. Nutr.* 2004, *134*, 711–716.

[3] Gelderblom, W. C., Galendo, D., Abel, S., Swanevelder, S., *et al.*, Cancer initiation by fumonisin B(1) in rat liver—role of cell proliferation. *Cancer Lett.* 2001, *169*, 127–137.

[4] Rheeder, J. P., Marasas, W. F. O., Thiel, P. G., Sydenham, E. W., *et al.*, Fusarium moniliforme and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* 1992, *82*, 353–357.

[5] International Agency for Research on Cancer, IARC Monographs, 2002, *82*, 301–366 http://monographs.iarc.fr/ENG/Monographs/vol82/mono82-7B.pdf

[6] Bolger, M., Coker, R. D., DiNovi, M., Gaylor, D., *et al.*, Fumonisins. In: *Prepared by the Fifty-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Safety evaluation of certain mycotoxins in food*. WHO Food Additives Series No. 47, FAO Food and Nutrition Paper No. 74, WHO, Geneva, Switzerland, 2001, pp. 103–279.

[7] de Nijs, M., van Egmond, H. P., Nauta, M., Rombouts, F. M., Notermans, S. H., Assessment of human exposure to fumonisin B1. *J. Food Prot.* 1998, *61*, 879–884.

[8] Fasano, A., Catassi, C., Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology* 2001, *120*, 636–651.

[9] Robins, G., Akobeng, A., McGough, N., Merrikin, E., Kirk, E., A systematic literature review on the nutritional adequacy of a typical gluten-free diet with particular reference to iron, calcium, folate and B vitamins. *Food Standards Agency Report*. http://www.foodbase.org.uk/results.php?_report_id=301.

[10] Shephard, G. S., Van der Westhuizen, L., Sewram, V., Biomarkers of exposure to fumonisin mycotoxins: a review. *Food Addit. Contam.* 2007, *24*, 1196–1201.

[11] Turner, P. C., Nikiema, P., Wild, C. P., Fumonisin contamination of food: progress in development of biomarkers to better assess human health risks. *Mutat. Res.* 1999, *443*, 81–93.

[12] Salvini, S., A food composition database for epidemiological studies in Italy. *Cancer Lett.* 1997, *114*, 299–300.

[13] Dall'Asta, C., Galaverna, G., Mangia, M., Sforza, S., et al., Free and bound fumonisins in gluten-free food products. *Mol. Nutr. Food Res.* 2009, *53*, 492–499.

[14] Castegnaro, M., Garren, L., Galendo, D., Gelderblom, W. C., et al., Analytical method for the determination of sphinganine and sphingosine in serum as a potential biomarker for fumonisin exposure. *J. Chromatogr. B Biomed. Sci. Appl.* 1998, *720*, 15–24.

[15] Seefelder, W., Schwerdt, G., Freudinger, R., Gekle, M., Humpf, H. U., Liquid chromatography/electrospray ionisation-mass spectrometry method for the quantification of sphingosine and sphinganine in cell cultures exposed to fumonisins. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2002, *780*, 137–144.

[16] Ostrý, V., Ruprich, J., Determination of the mycotoxin fumonisins in gluten-free diet (corn-based commodities) in the Czech Republic. *Cent. Eur. J. Public Health* 1998, *6*, 57–60.

[17] Lo Magro, S., Campaniello, M., Nardiello, D., Muscarella, M., Assessment of fumonisins B1 and B2 levels in commercial maize-based food products by liquid chromatography with

fluorimetric detection and postcolumn chemical derivatization. *J. Food Sci.* 2011, *76*, T1–4.

[18] Ferrara, P., Cicala, M., Tiberi, E., Spadaccio, C., et al., High fat consumption in children with celiac disease. *Acta Gastroenterol. Belg.* 2009, *72*, 296–300.

[19] Bardella, M. T., Fredella, C., Prampolini, L., Molteni, N., et al., Body composition and dietary intakes in adult celiac disease patients consuming a strict gluten-free diet. *Am. J. Clin. Nutr.* 2000, *72*, 937–939.

[20] Mariani, P., Viti, M.G., Montuori, M., La Vecchia, A., et al., The gluten-free diet: a nutritional risk factor for adolescents with celiac disease? *J. Pediatr. Gastroenterol. Nutr.* 1998, *27*, 519–523.

[21] Livelli di assunzione raccomandati di energia e nutrienti per la popolazione italiana. 1996. Società Italiana di Nutrizione Umana. http://www.sinu.it/pubblicazioni.asp.

[22] Kemppainen, T., Uusitupa, M., Janatuinen, E., Järvinen, R., et al., Intakes of nutrients and nutritional status in coeliac patients. *Scand. J. Gastroenterol.* 1995, *30*, 575–579.

[23] Grehn, S., Fridell, K., Lilliecreutz, M., Hallert, C., Dietary habits of Swedish adult coeliac patients treated by a gluten-free diet for 10 years. *Scand. J. Nutr.* 2001, *45*, 178–182.

[24] Thompson, T., Dennis, M., Higgins, L. A., Lee, A. R., Sharrett, M. K., Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods? *J. Hum. Nutr. Diet* 2005, *18*, 163–169.

[25] Wild, D., Robins, G. G., Burley, V. J., Howdle, P. D., Evidence of high sugar intake, and low fibre and mineral intake, in the gluten-free diet. *Aliment. Pharmacol. Ther.* 2010, *32*, 573–581.

[26] Kinsey, L., Burden, S. T., Bannerman, E., A dietary survey to determine if patients with coeliac disease are meeting current healthy eating guidelines and how their diet compares to that of the British general population. *Eur. J. Clin. Nutr.* 2008, *62*, 1333–1342.

[27] Hallert, C., Grant, C., Grehn, S., Grännö, C., et al., Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years. *Aliment. Pharmacol. Ther.* 2002, *16*,1333–1339.

[28] Farrell, R.J., Kelley, C.P., Celiac Sprue. *N. Engl. J. Med.* 2002, *346*, 180–188.

[29] European Mycotoxin Awareness Network (EMAN), a thematic network of the 5th Framework Programme R&D call funded by the European Union. http://www.mycotoxins.org/

[30] Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food). http://www.who.int/foodsafety/chem/gems/en/index1.html

[31] van der Westhuizen, L., Shephard, G. S., Rheeder, J. P., Burger, H. M., Individual fumonisin exposure and sphingoid base levels in rural populations consuming maize in South Africa. *Food Chem. Toxicol.* 2010, *48*, 1698–1703.

[32] Xu, L., Cai, Q., Tang, L., Wang, S., et al., Evaluation of fumonisin biomarkers in a cross-sectional study with two high-risk populations in China. *Food Addit. Contam. Part A Chem. Anal. Control Expo Risk Assess.* 2010, *27*, 1161–1169.

[33] Gong, Y. Y., Torres-Sanchez, L., Lopez-Carrillo, L., Peng, J. H., et al., Association between tortilla consumption and human urinary fumonisin B1 levels in a Mexican population. *Cancer Epidemiol. Biomarkers Prev.* 2008, *17*, 688–694.

[34] van der Westhuizen, L., Shephard, G. S., Burger, H. M., Rheeder, J. P., et al., Fumonisin B1 as a urinary biomarker of exposure in a maize intervention study among South African subsistence farmers. *Cancer Epidemiol. Biomarkers Prev.* 2011, *20*, 483–489.

[35] van der Westhuizen, L., Brown, N. L., Marasas, W. F., Swanevelder, S., Shephard, G. S., Sphinganine/sphingosine ratio in plasma and urine as a possible biomarker for fumonisin exposure in humans in rural areas of Africa. *Food Chem. Toxicol.* 1999, *37*, 1153–1158.

[36] van der Westhuizen, L., Shephard, G. S., Rheeder, J. P., Somdyala, N. I., Marasas, W. F., Sphingoid base levels in humans consuming fumonisin-contaminated maize in rural areas of the former Transkei, South Africa: a cross-sectional study. *Food Addit. Contam. Part A Chem. Anal. Control Expo Risk Assess.* 2008, *25*, 1385–1391.

