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Most Patients With Celiac Disease on Gluten-free Diets Consume Measurable Amounts of Gluten

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A gluten-free diet (GFD) is the primary treatment for celiac disease (CD), yet many have persistent villous atrophy despite a "strict" GFD. Several lines of indirect evidence suggest that persistent villous atrophy reflects ongoing gluten exposure; however, only recently have tools become available to test this hypothesis directly.

Self-reported rates of GFD adherence are high[1]; however, unintentional gluten exposures may be more common than realized and not necessarily considered a lapse in an otherwise intentionally strict GFD. Trials in which CD patients were asked to maintain a GFD showed an observed (Hawthorne) effect as villous height-to-crypt depth ratio increased in the placebo group[2]. As well, an ultrastrict "Gluten Contamination Elimination Diet" may resolve villous atrophy even when no gluten sources are identified[3].

Recently, G12 and A1 antibodies have been developed which are specific for gluten immunogenic peptides (GIP) recognized by T cells of patients with CD[4]. We used immunoassays with these antibodies to detect gluten in food ingested and stool and urine

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excreted by community-dwelling adults with CD endeavoring to follow a strictly gluten-free diet. Further, we examined the relationship of gluten exposure to mucosal recovery and non-invasive measures of CD activity.

Methods

Additional details in supplementary methods. Participants in the Manitoba Celiac Disease Inception Cohort Determination of Gluten Grams Ingested and Excreted By Adults eating Gluten-free (DOGGIE BAG) sub-study collected food, urine, and stool during 10 days immediately prior to follow-up biopsy. For 7 days, participants provided a representative ¼ portion of food consumed, including sauces/dressings and flavored beverages. Inherently gluten-free unprocessed foods were not collected (e.g., fruits/vegetables, wine). To account for intestinal transit, stools were collected after day 3. Three urine samples were collected daily. Sterile containers and a 2.1 cubic foot −20°C freezer (Whynter LLC, Brea, CA) were provided for sample storage. Participants also completed standardized CD-specific self-report measures. The University of Manitoba Health Research Ethics Board reviewed the protocol.

Food samples pooled based upon time consumed (04.01–10.00 a.m., 10.01 a.m.–4 p.m., 4:01 p.m.–4.00 a.m.) were homogenized using a 1000W triple blade stainless steel blender (Breville, Saint Laurent, Canada) with deionized water added to facilitate mixing.

All assays used A1/G12 antibodies specific and sensitive for the most GIP[5]. Foods were tested using GlutenTox ELISA Sandwich (Hygiena Diagnostica España, Seville, Spain). iVYLISA GIP Stool kit and iVYCHECK GIP Urine immunochromatographic test were used (Biomedal S.L., Seville, Spain). Duplicates of ≥2 aliquots were tested on different days.

Results

Eighteen participants (12 female; median 41 years, range 21–77) completed the protocol and were generally asymptomatic. No intentional gluten exposures occurred. CSI scores ranged from 19 to 40. Celiac Diet Adherence Test (CDAT) scores were ≤14, yet 77% self-reported “rare accidental gluten exposure” on the Gluten-Free Eating Assessment Tool-short (GF-EATs). Over 24 months, median serum TTG IgA decreased from 9 to 0.6 multiples of upper limit of normal, providing further evidence that this was a relatively adherent cohort. Although all improved from baseline, most (56%) had persistent villous atrophy.

The 25/313(8%) food samples from 9 participants with detectable gluten had a median concentration of 11 ppm (range 4 to >200 ppm); 40% contained >20 ppm and 20% contained >200 ppm gluten. GIP were detected in 30/519(6%) urine samples from 8 participants and 8/75(11%) stool samples from 5 participants. Positive samples were distributed proportionately throughout the day.

Commonly used non-invasive measures of GFD adherence did not correlate closely with gluten exposure (Table 1). Most of those who had a normal TTG had at least one positive sample (7/11; 64%). Two-thirds of those with a positive sample had persistent villous atrophy (Marsh 3a) whereas two-thirds of those whose samples all tested negative had

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normal villous architecture (Marsh 0 or 1). Nevertheless, 4/6 patients with Marsh 0 had detectable gluten in ≥1 sample.

Discussion

In this study, 12/18 CD patients with good or excellent GFD adherence based on standardized self-report measures were exposed to gluten within the 10-day study period. There was some discordance between gluten detection and villous atrophy for which there are several potential explanations. In some cases, extremely low levels of gluten exposure were detected which may have been insufficient to induce mucosal damage. Micro-challenge studies suggest 50 mg gluten daily for three months may induce histologic damage, but 10 mg may not though individual sensitivity varies[6]. Perhaps both amount and duration of gluten exposure are important determinants of histologic damage. Alternatively, abnormalities may reflect slow natural history of mucosal recovery rather than ongoing gluten exposure[7]. Lastly, sample collection was limited to 10 days which may not accurately reflect ongoing gluten ingestion as participants may have intensified their GFD adherence due to their participation.

Notable aspects of this study include use of recently developed tests to directly measure gluten in food collected at the point of consumption, urine and stool over a multi-day period. Participants had diagnostic and follow-up biopsies available and received comprehensive follow-up as part of a longitudinal research study. This may limit generalizability as many CD patients do not receive regular disease-specific follow-up[8] and may be less successful at eliminating gluten. Another potential limitation is that not all foods and beverages were tested.

Even though typical “real world” gluten exposures may have been underestimated, this study confirms that gluten ingestion occurs frequently despite efforts to follow a strictly gluten-free diet. Our results suggest that most CD patients in actuality follow a low gluten diet, and complete elimination of dietary gluten may not be possible to maintain. The Codex Alimentarius definition of “gluten-free” as <20 ppm gluten explicitly allows for gluten in a “gluten-free diet”. Initial determination of the 20 ppm “gluten-free” cut-off was strongly influenced by limitations of available ELISA assays for gluten. While this appears to be tolerated by most CD patients, there is minimal evidence that this level of gluten exposure consistently permits mucosal recovery. Our novel findings support the general concern that a gluten-free diet may be more aspirational than achievable, even by highly committed and knowledgeable individuals. This issue likely underlies persisting symptoms and incomplete mucosal recovery. Additional treatments are needed for this common condition.

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Supplementary Material

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AC is the CEO and shareholder of Biomedal S.L. He is also partner of Glutenostics LLC. Biomedal was the licensor of G12/A1 monoclonal antibodies for detection in food, urine and stool at the time of the study.

DRD has received research funding from Biomedal S.L. and has served as a consultant for Takeda Pharmaceuticals and Shire Canada and is Chair of the Professional Advisory Council of the Canadian Celiac Association

RD is an employee of Biomedal S. L.

CPK has acted as a scientific advisor to Cour Pharma, Glutenostics, ImmunogenX, Innovate and Takeda. He also acts as Principal Investigator on a research grant on Celiac disease supported by Aptalis.

DAL is a Medical Director for Takeda Pharmaceuticals.

FL was an employee of Celimmune at the time of the study, and is currently an employee of Provention Bio. He also serves as a partner in Biomedal and Glutenostics.

JAS serves on an advisory board of Takeda Pharmaceuticals and received research support from Cour Pharma, Glutenostics and the Celiac Disease Foundation.

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Abbreviation

CD	Celiac Disease
EMA	Endomysial antibody
GFD	Gluten-free diet
GIP	Gluten immunogenic peptides
MULN	Multiples of the Upper Limit of Normal

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TTG	Tissue transglutaminase

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Table 1 –

Relationship between Detection Of Gluten Ingestion or Excretion and Measures of Dietary Adherence and Celiac Disease Activity at 24 months from diagnosis

	Gluten detected[d] (N =12) [N (%)]	No gluten detected (N=6) [N (%)]
Celiac Symptom Index (CSI)[a]		
≤30	7(58%)	3 (50%)
31–40	5 (42%)	3 (50%)
Gluten-Free Eating Assessment Tool short (GF-EATs)[b]		
Frequent gluten (>1/week)	--	--
Occasional gluten (1–4 times per month)	1 (8%)	--
Rare intentional gluten ingestion (<1/month)	1 (8%)	--
Rare accidental gluten ingestion (<1/month)	9 (76%)	5 (83%)
No gluten	1 (8%)	1 (17%)
Celiac Diet Adherence Test (CDAT)[c]		
< 13	10 (83%)	4 (66%)
13–14	2 (17%)	2 (33%)
TTG IgA multiples of upper limit of normal		
<1	7 (58%)	4 (66%)
≥1	5 (42%)	2 (33%)
≥2	3 (25%)	--
Marsh Classification		
Marsh 0	4 (33%)	2 (33%)
Marsh 1	--	2 (33%)
Marsh 3a	8 (66%)	2 (33%)
Marsh 3b	--	--
Marsh 3c	--	--

[a] Lower scores more desirable, 16 items with possible range 16 to 80, scores ≤30 suggestive of clinical remission, scores ≥45 suggestive of ongoing active celiac disease. Leffler DA, Dennis M, Edwards-George JB et al. A Validated Disease-Specific Symptom Index for Adults with Celiac Disease. *Clin Gastroenterol Hepatol* 2009;7(12), 1328–34.

[b] See supplementary file for full instrument.

[c] Lower scores more desirable, 7 items with possible range 7–35, scores ≥13 predict inadequate GFD adherence. Leffler DA, Dennis M, Edwards-George JB et al. A simple validated gluten-free diet adherence survey for adults with celiac disease. *Clin Gastroenterol Hepatol* 2009;7(5), 530–6.

[d] Limit of detection: 1.6 ppm gluten in food; 160 ng GIP per gram stool; 2.2 ng GIP per ml urine.