

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

Participants were recruited from the Manitoba Celiac Disease Inception Cohort study. This prospective longitudinal study enrolled adults (16 years or older) with biopsy-confirmed celiac disease (CD). A central laboratory performs all serum tissue transglutaminase-2 IgA (TTG) testing for Manitoba which facilitated a population-based approach to reduce recruitment bias. Urban as well as rural residents participated; however, none were from the more remote Northern Health Region (53° latitude and above). All participants were referred at diagnosis to a Registered Dietitian with gluten-free diet (GFD) expertise and given information about the Canadian Celiac Association patient support group. Follow-up small intestinal biopsy 24 months after initiation of a GFD was included in the study protocol irrespective of symptoms, dietary adherence or other participant characteristics.

All DOGGIE BAG study participants were instructed to follow their usual dietary patterns, including eating at restaurants and others' homes. At the end of the study, participants completed standardized CD-specific self-report measures, including: Celiac Symptom Index (Leffler et al, *Clin Gastroenterol Hepatol* 2009;7:1328-34), a 16-item measure with possible scores ranging from 16 to 80 on which scores of 30 or less are associated with better quality of life and GFD adherence; Celiac Diet Assessment Tool (Leffler et al, *Clin Gastroenterol Hepatol* 2009;7:530-6), a seven-item self-report measure with questions about symptoms and personal traits with possible scores ranging from 7 to 35 on which scores of 12 or less are associated with adequate adherence; and Gluten-Free Eating Assessment Tool Short (GF-EATs), a single-item self-reported adherence scale with 5 categorical responses.

Gluten-Free Eating Assessment Tool-short

Please describe your current diet

- Frequent gluten (more than once per week)
- Occasional gluten (1-4 times per month)
- Rare intentional gluten ingestion (less than once per month)
- Rare accidental gluten ingestion (less than once per month)
- No gluten

Quantification of gluten in food samples

Gluten content of food (ppm) was determined using an A1-G12 antibody sandwich enzyme-linked immunosorbent assay (ELISA; GlutenTox ELISA Sandwich, Hygiene Diagnostica España, Seville, Spain) according to manufacturer instructions. Gluten concentration in food samples was determined according to the following formula to account for sample dilution:

$$\text{gluten content (ppm)} = \text{ppm gluten in food sample} \times \frac{\text{weight of sample with water added}}{\text{sample weight as collected}}$$

The amount of gluten consumed from a given sample was estimated using the following formula to convert from ppm to mg and account for sampling of ¼ of food as plated (the participant ate the other ¾): $\text{mg gluten consumed} = \text{ppm gluten in sample} \times \text{sample weight (kg)} \times 3$. Following sample dilution, the working limit of quantification of this method is 1.6 ppm gluten.

Quantification of GIP in stool samples

Thawed stool samples were manually homogenized, then stool GIP concentration was determined by sandwich ELISA (iVYLISA GIP Stool kit, iVYDAL In Vitro Diagnostics®, Biomedal S.L., Seville, Spain) according to the manufacturer's protocol. Each sample was run in duplicate and at least four different aliquots of each sample were tested on different days. The accuracy of this method in detecting GFD transgressions has been reported previously (Comino et al, *Am J Gastroenterol* 2016;111:1456-65): analytical sensitivity (limit of quantification 160 ng GIP per gram stool); diagnostic sensitivity and specificity (98.5% and 100%, respectively). GIP are stable in stool stored between -20 and -80°C for more than 12 months, and through multiple freeze-thaw cycles.

Quantification of GIP in urine samples

Urine samples were processed for testing by the iVYCHECK GIP Urine (iVYDAL In Vitro Diagnostics®, Biomedal S.L., Seville, Spain) according to the manufacturer's recommendations. Samples with positive qualitative results were quantified using the iVYCHECK Reader. The validity of this method in detecting GFD transgressions was determined by the analytical sensitivity using α -gliadin 33mer peptide as GIP reference material (limits of detection and quantification 2.2 and 6.3 ng per ml urine, respectively). Each sample was run in duplicate and at least two different aliquots of each sample were tested on different days.

Intestinal histology

Intestinal biopsies obtained from the duodenal bulb (2 specimens) and from the distal duodenum (4 specimens) during sedated endoscopy were processed by the St Boniface Hospital Pathology Laboratory (Winnipeg, Manitoba) and interpreted by a specialist pathologist with expertise in gastrointestinal histology. Findings in the biopsy with the most severe lesion are reported.