

# Determination of gluten consumption in celiac disease patients on a gluten-free diet

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## ABSTRACT

**Background:** Celiac disease (CD) patients adhering to a gluten-free diet (GFD) are exposed frequently to low levels of gluten that contribute to symptoms and persistent intestinal histologic damage.

**Objective:** We analyzed prior clinical data to determine how much gluten is accidentally consumed while on a GFD. The aim was to understand the range of gluten consumption for a wide distribution of CD patients.

**Design:** A meta-analysis was conducted on data from 2 different clinical programs: 1) measurements of gluten in stool and urine in CD and non-CD populations; and 2) analysis of data from trials for the investigational therapeutic latiglutenase. The stool and urine studies included controlled gluten challenges. A calibration factor was applied that allowed normal ingestion of gluten to be computed from the urine and stool measurements. From the latiglutenase trial data, a determination of gluten consumption was made by estimating how much gluten was eliminated from patients' diets due to a trial effect that led to improved histology even in the placebo group.

**Results:** The average inadvertent exposure to gluten by CD individuals on a GFD was estimated to be ~150–400 (mean) and ~100–150 (median) mg/d using the stool test and ~300–400 (mean) and ~150 (median) mg/d using the urine test. The analyses of the latiglutenase data for CD individuals with moderate to severe symptoms indicate that patients ingested significantly >200 mg/d of gluten.

**Conclusions:** These surrogate biomarkers of gluten ingestion indicate that many individuals following a GFD regularly consume sufficient gluten to trigger symptoms and perpetuate intestinal histologic damage. *Am J Clin Nutr* 2018;107:201–207.

**Keywords:** celiac disease, gluten exposure, gluten-free diet

## INTRODUCTION

Celiac disease (CD) is the most common autoimmune gastrointestinal disease, affecting ~1% of the world population (1–3). There are currently no US Food and Drug Administration (FDA)-approved treatments, other than a gluten-free diet (GFD), which is exceedingly difficult to maintain. The average Western diet contains ~5–15 g gluten/d (4). Gluten ingestion as low as

50 mg/d can be harmful to some celiac patients (5). The elimination of 99% of gluten from a diet may still be insufficient to avoid symptoms and histologic damage. The FDA has established a guideline that foods labeled gluten free must contain <20 ppm gluten (6). However, there are difficulties with currently approved analytical methods for the detection and quantification of gluten in certain foods (e.g., fermented and hydrolyzed foods) (7–9).

There is an unmet need to protect against unintended gluten ingestion, particularly since persistent uncontrolled gluten exposure is known to lead to life-long health issues and comorbidities such as anemia, malnutrition, and lymphoma (10). As such, investigational drugs in clinical development are generally intended to be used as an adjunct to a GFD (11–14).

Despite the obvious need to protect CD patients against exposure to gluten consumption, there is surprisingly very little known about the quantity of gluten that is accidentally consumed episodically and continually for those on a GFD. Much has been written about GFDs and the complexities, difficulties, and challenges associated with maintaining strict adherence across social and demographic groups and behaviors (15–17). However, we are unaware of any studies that attempt to analytically determine the actual quantity of gluten that is consumed while on a GFD.

In this work, we performed a meta-analysis based on data from clinical studies that provided key information needed to determine the amount of gluten that CD patients consume while attempting to follow a GFD.

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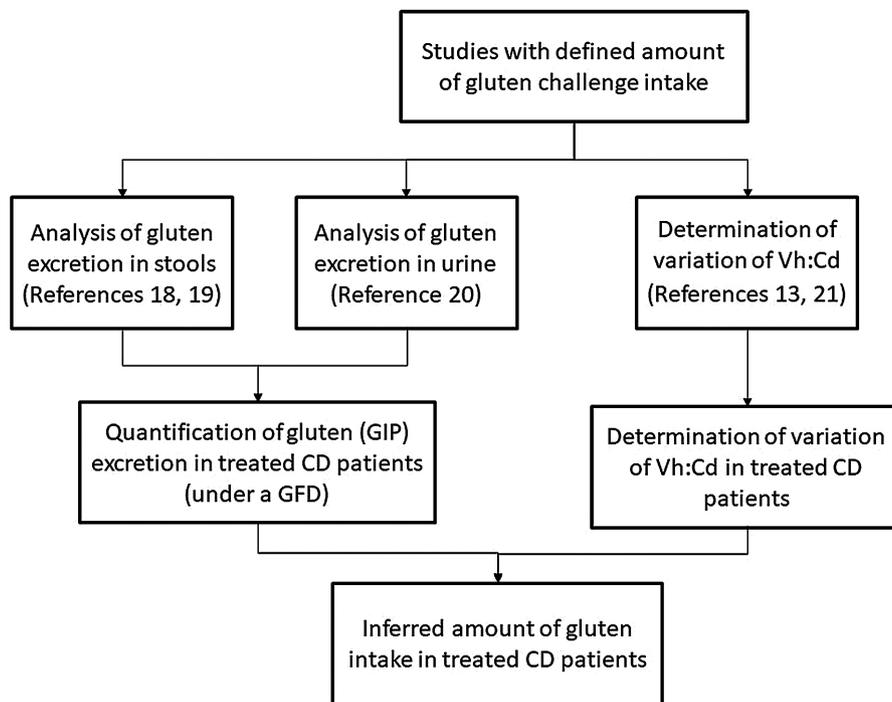
ImmunogenX is a clinical-stage company developing the therapeutic drug latiglutenase for treating celiac disease and also a minimally invasive drug biomarker and blood test for monitoring the villous health of the small intestine.

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Abbreviations: CD, celiac disease; FDA, Food and Drug Administration; GFD, gluten-free diet; GIP, gluten immunogenic peptide; LOD, limit of detection; Vh: Cd, villous height-to-crypt depth ratio.

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**FIGURE 1** Flow chart outlining the basis for selecting the studies used in the present analysis. CD, celiac disease; GFD, gluten-free diet; GIP, gluten immunogenic peptide; Vh:Cd, villous height-to-crypt depth ratio.

## METHODS

The analysis was conducted combining clinical study results from the following sources: 1) measurements of gluten in stool (NCT02711397 and NCT01478867) (18, 19) and urine (NCT02344758) (20) in non-CD and CD populations; and 2) trial effect and symptom results for the study of latiglutenase (NCT01255696) (13) and (NCT01917630) (21). These studies (except for NCT01255696) focused primarily on CD patients experiencing moderate to severe symptoms. The decision process for selecting these studies is delineated by the flow chart in [Figure 1](#).

The measurements of gluten immunogenic peptides (GIPs) in stool were performed using the ELISA Sandwich G12/G21 assay (iVYLISA GIP, Biomedal SL). In urine, the results were determined by a quantitative lateral flow immunoassay (iVYCHECK GIP Urine, Biomedal SL) using a lateral flow reader A1/G12 (iVYCHECK Reader, Biomedal SL). These measurements were accompanied by controlled measurements of gluten exposure utilizing a gluten challenge. A conversion factor (described below) was determined that allowed the ingestion of gluten to be computed. Gluten challenge measurements in stool showed that gluten could be detected in stool for  $\leq 4$  d after a gluten challenge, indicating a sufficiently long residence time (18, 19), such that a measured value of gluten in stool on any given day could be a good indication of the peak gluten level and no additional correction factor was needed. Gluten challenge measurements in urine indicated a residence time of gluten in urine of about half a day (20). Therefore, random measurements of gluten in urine following unintended gluten ingestion would, on average, register a value of about half that for the peak gluten level. The conversion factor from GIP (expressed as ng/mL urine) to gluten ingestion (expressed as mg) was determined by 2 different methods: 1

was based on uncorrelated urine measurements following 42- and 84-mg gluten challenges, and the other was based on correlated urine measurements to 500 mg gluten challenge. The former method used a factor of 2 correction for the gluten residence time. The details of how these conversion factors are computed are given in the Results section.

In the second analysis we estimated the quantity of gluten that was removed from the diets of patients in a Phase 2b latiglutenase study (ALV003-1221) (21). The quantity of gluten eliminated was determined by relating the improvement in villous height to crypt depth ratio (Vh:Cd) for placebo patients to a standard fit that related deterioration of Vh:Cd to a continuous gluten intrusion, as measured in a previous Phase 2a latiglutenase trial (ALV003-1021) (13). Three cohort groups were given 1.5, 3.0, and 6.0 g of gluten daily for 6 wk and experienced changes to their mucosa ( $\Delta$ Vh:Cd) of 2.8–2.2, 2.6–1.5, and 2.8–1.1, respectively. A polynomial fit with a (0,0) intercept led to the following equation:

$$\Delta w_g = 46.5 \Delta \text{Vh:Cd}^2 - 68.2 \Delta \text{Vh:Cd} \quad (1)$$

where  $\Delta w_g$  is the aggregate change in gluten weight to the diets of the patients corresponding to the mucosal change ( $\Delta$ Vh:Cd). The placebo as well as latiglutenase arms improved their mucosal health due to the Hawthorne effect. From this improvement, one can estimate the amount of gluten removed from their diets using Equation 1. We further estimated the amount of gluten that remained in their diets during the treatment phase of the trial, using the trial effect improvement on symptoms (Results section). The total gluten intake for these symptomatic patients before the trial began can thus be estimated.

The statistical methods for the data used in this analysis are published in the referenced manuscripts. The analysis in this

TABLE 1

Gluten consumption as measured in stool<sup>1</sup>

Cohort	n	GIP concentration ( $\mu\text{g/g}$ stool)			Gluten daily consumption (mg)		
		Mean	Median	SD	Mean	Median	SD
Healthy non-CD							
Adults	73	5.23	7.60	2.95	7802	11,699	4757
CD on GFD							
Adults ( $\geq 13$ y)	74	0.22	0.13	0.40	244	141	488
Children (4–12 y)	79	0.32	0.11	0.88	387	118	1216
Children (0–3 y)	35	0.14	0.10	0.19	155	104	214

<sup>1</sup>Conversion factor for  $y = \text{GIP}$  ( $\mu\text{g/g}$  stool) to  $x = \text{gluten daily consumption}$  (mg) is  $y = 0.0649x^2 + 1.0461x$ . CD, celiac disease; GFD, gluten-free diet; GIP, gluten immunogenic peptide.

study used standard polynomial fits and mean, median, and SD calculations. The histogram plots were calculated using the histogram function in Excel and using a bin size to provide sufficient resolution of the gluten distribution.

## RESULTS

### Measurements of gluten in stool and urine

In a series of studies, an estimate of gluten consumption by CD patients following a GFD was determined by measuring GIPs in stool (18, 19) and urine (20). Population groups included healthy non-CD patients and CD patients, each segmented as adults and children. Measurements were also performed on healthy patients under controlled gluten challenge conditions.

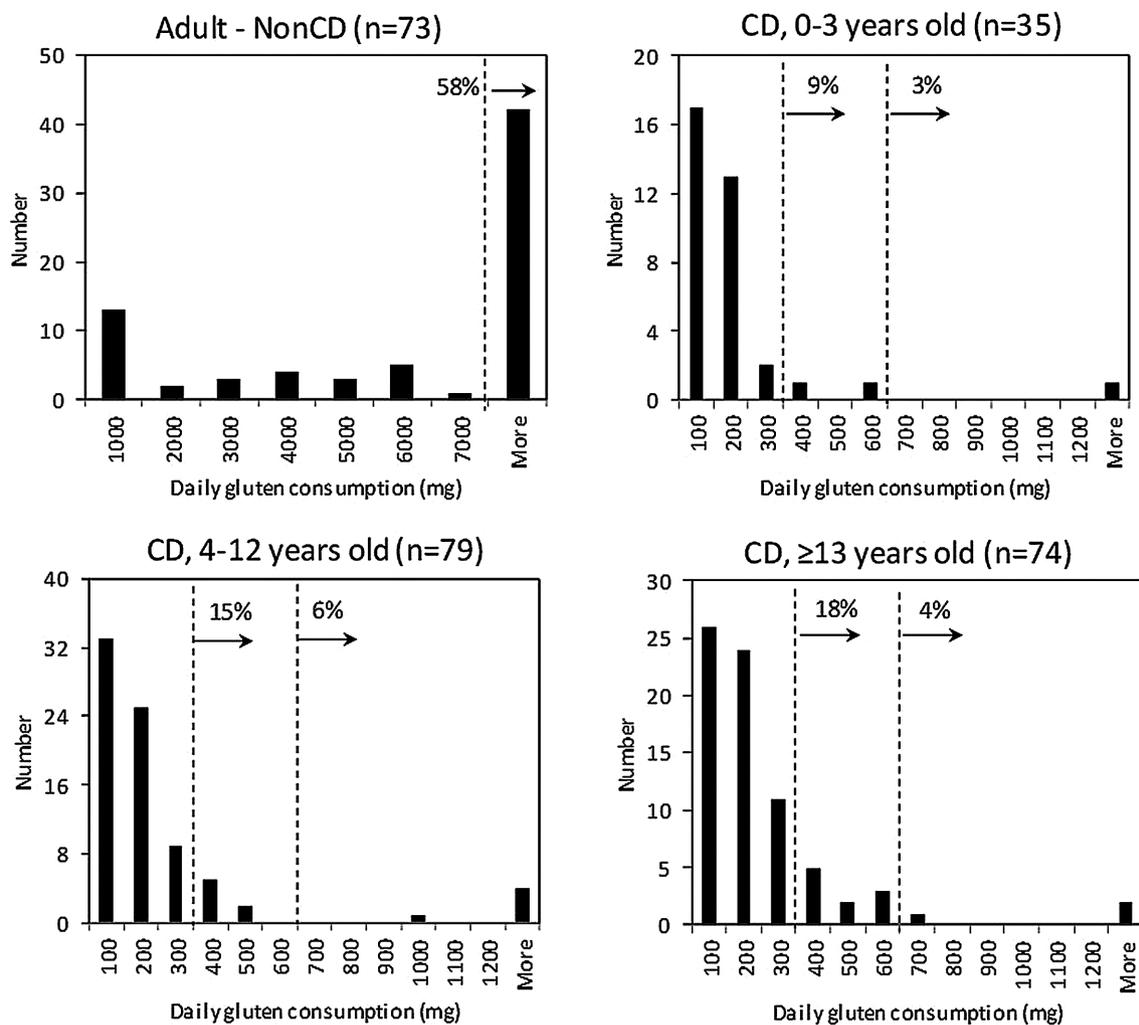
Table 1 shows results for stool samples. In this study a gluten challenge was conducted for several days to equilibrate the gluten content in stool. There was a 1- to 2-d induction period for gluten to be detected in stool and similarly to be eliminated. The factor for converting GIP concentration (in micrograms per gram) ( $x$  variable) to gluten daily consumption (in milligrams) ( $y$  variable) was determined from measured mean values of 6.2 and 14.9  $\mu\text{g/g}$  in stool for daily gluten challenges of 9 and 30 g (18). Fitting to a second-order polynomial going through the origin gave the relation  $y = 0.0649x^2 + 1.0461x + 0.0$ . The computed gluten daily consumption for healthy non-CD adults was found to be 7.8 g (mean) and 11.7 g (median). Non-CD children were not included in this study. This value is consistent with 5–15 g for a typical gluten-containing diet (measured in Denmark) (4). This analysis utilized the complete set of data [compared with the data plotted in Figure 2 of Comino et al. (18)] for values above the quantitation limit.

Both the mean and median values for each population group are reported in Table 1 due to the asymmetry in the distribution of gluten ingestion. The computed daily gluten consumption for CD adults ( $\geq 13$  y old) on a GFD was 244 mg (mean) and 141 mg (median), for older children (4–12 y old) it was 387 mg (mean) and 118 mg (median), and for younger children (0–3 y old) it was 155 mg (mean) and 104 mg (median). These values must be qualified in terms of accuracy as more than half of these individuals recorded GIP (micrograms per gram) values below the limit of quantitation ( $\text{LOQ} = 0.16 \mu\text{g GIP/g}$  stool sample), computing to  $\sim 169$  mg gluten consumed daily ( $\text{LOD} = 0.06 \mu\text{g/g}$  corresponding to 63 mg) (19). As an example, for the  $\geq 13$  y old patients, 45 of 74 measured below the LOQ, which computes to 39%

averaging  $> 169$  mg gluten consumed daily. For children 0–3 and 4–12 y old this figure is 14% and 28%, showing a trend toward increased gluten consumption with age.

Table 2 summarizes the results of urine samples, an independent study from the stool results. Two methods were used to estimate the conversion factor from GIP concentration in urine to daily gluten consumption. The first made use of the original data from Moreno et al. (20) where the LOD was 3.5 ng GIP/mL and gluten challenges of 42 and 84 mg (revised from originally reported values of 25 and 50 mg) were mostly undetected and detected, respectively. We therefore estimate the LOD to correspond to the average of these gluten ingestions, giving 63 mg. The collection of the urine was uncorrelated with the gluten consumption and a factor of 0.5 was introduced, reflecting the  $\sim 0.5$ -d residence time during which gluten remains in urine (essentially the full width, half height of the peak concentration) (20). This led to a conversion factor for gluten daily consumption (in milligrams) per GIP concentration (expressed as ng/mL urine) measured of  $63/(3.5 \times 0.5) = 36$ . A subsequent gluten challenge study gave 18 patients 500 mg of gluten on 2 subsequent days (at dinner time) and then collected urine samples at 3 specific times during the following day (to be published). The LOD was 2.2 ng GIP/mL. These unpublished results (A Cebolla and R Dominguez) indicate a conversion factor of 50 (similar to above), except the 0.5 factor was not necessary because the urine measurement was at a fixed time after ingestion. This value is consistent with the previous factor and is the value that we use in the current analysis as we believe these are more accurate data and remove the need for the 0.5 urine residence time assumption.

We found that the computed daily gluten consumption for healthy non-CD individuals was 5.7 g (mean) and 4.3 g (median) for adults and 4.4 g (mean) and 0.74 g (median) for children. This is lower than the 5–15 g of gluten (cited above) for a typical gluten-containing diet and lower than the measurements in stool (above). This may reflect an upper limit or saturation level for the ELISA assay. The computed daily gluten consumption for CD individuals on a GFD was 363 mg (mean) and 158 mg (median) for adults and 316 mg (mean) and 149 mg (median) for children. Of note, similar to the stool results above, more than half of these individuals recorded GIP (expressed as ng/mL) values below the LOD ( $< 188$  ng gluten). Therefore, we assigned values that linearly decremented from the LOD value to zero. We believe this is a more realistic assumption than assigning values of zero as it is more reflective of the extrapolation of values above the LOD. By comparison, if these values are assigned zero, then



**FIGURE 2** Histogram plots of the distribution of gluten consumption in the different population groups as measured by GIP in stool. Dotted lines represent demarcations for patients (in percentages) with greater-than-indicated gluten consumption. CD, celiac disease; GIP, gluten immunogenic peptide.

for adults the mean decreases from 363 to 319 mg, not a marked difference.

We evaluated the distribution of gluten ingestion for the different CD and non-CD population groups. The stool measurements are presented in Figure 2. For the non-CD adults (healthy controls), there is a large spike in the distributions for gluten consumption of >7 g, which corresponds to the upper signal saturation limit. This corresponds to 58% of the population consum-

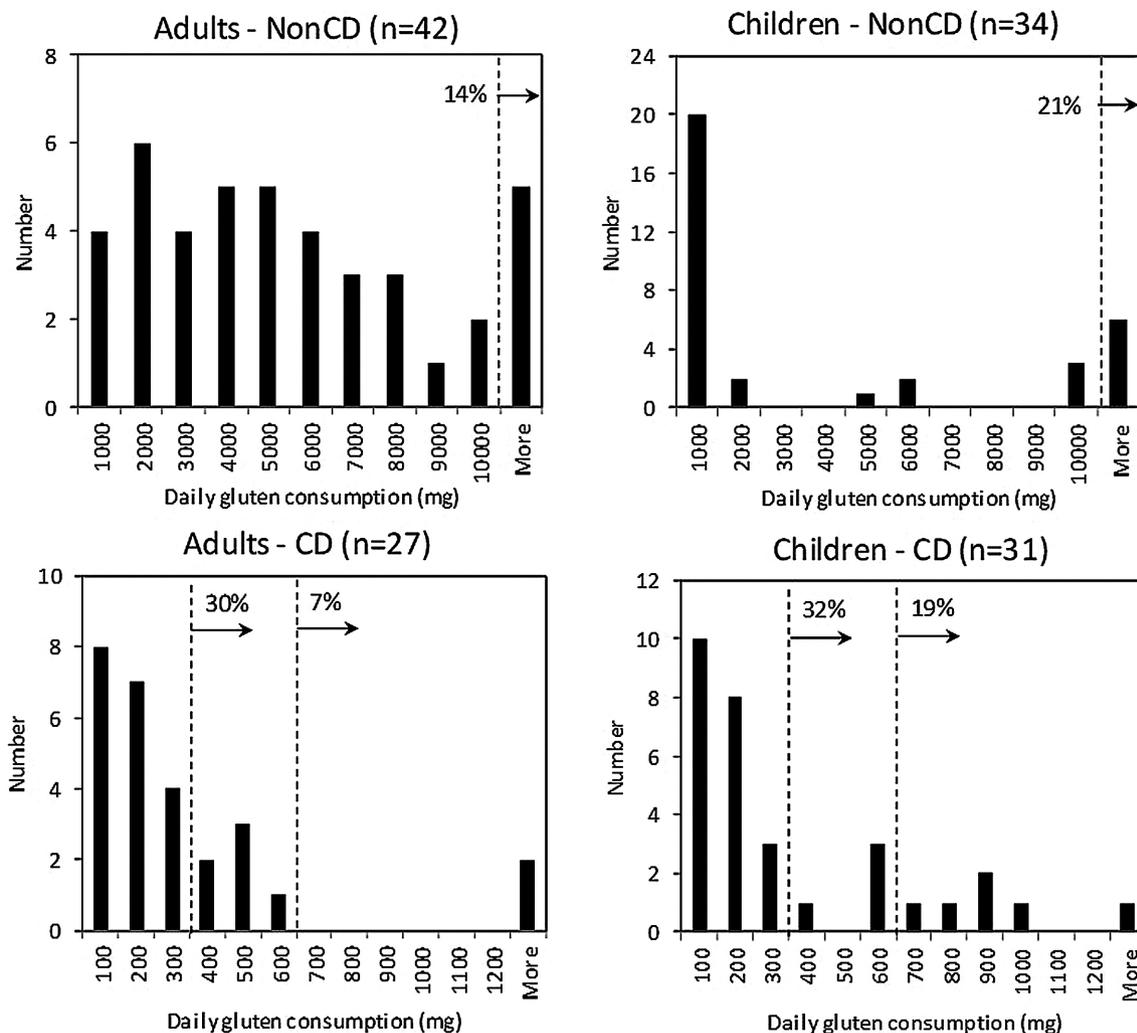
ing >7 g gluten/d. For children (0–3 and 4–12 y old) and adult (≥13 y old) CD populations on a GFD, the gluten consumption is considerably less, but consumption is not insignificant; adult consumption of gluten of >300 mg/d occurs 18% of the time as seen in Figure 2.

Figure 3 shows histogram plots for the distribution of test subjects for the urine measurements. These results show that the large majority of non-CD subjects consume <10 g gluten/d

**TABLE 2**  
Gluten consumption as measured in urine<sup>1</sup>

Cohort	n	GIP concentration (ng/mL urine)			Gluten daily consumption (mg)		
		Mean	Median	SD	Mean	Median	SD
Healthy non-CD							
Adults	42	113.2	85.3	112.1	5658	4264	5605
Children	34	87.9	14.8	117.0	4395	740	5848
CD on GFD							
Adults	27	7.3	3.2	12.7	363	158	634
Children	31	6.3	3.0	7.1	316	149	354

<sup>1</sup>Conversion factor for GIP (ng/mL urine) to gluten daily consumption (mg): 50. CD, celiac disease; GFD, gluten-free diet; GIP, gluten immunogenic peptide.



**FIGURE 3** Histogram plots of the distribution of gluten consumption in the different population groups as measured by GIP in urine. Dotted lines represent demarcations for patients (in percentages) with greater-than-indicated gluten consumption. CD, celiac disease; GIP, gluten immunogenic peptide.

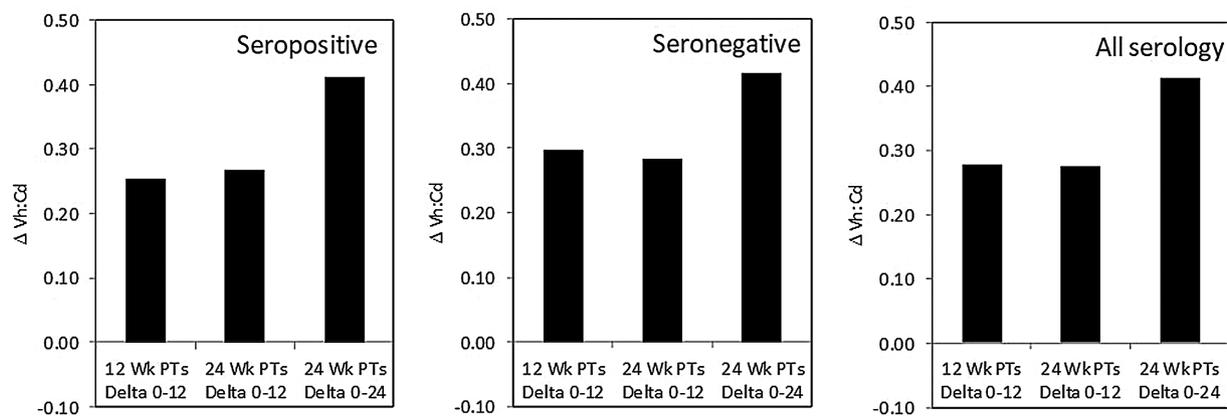
(a low value for a gluten-containing diet). The CD subjects obviously consume considerably less gluten on a daily basis, and these data show 30% of adults and 32% of children consume >300 mg gluten/d which is greater than, but consistent with, the results in Figure 2 for the stool analysis.

**Calculations based on the latiglutenase clinical trials**

The Phase 2b ALV003-1221 trial was a real-world study where patients were instructed to continue their GFD, but not to change their normal dietary behavior. Over the 12-wk trial period, patients, on average, improved their GFD and mucosal health as measured by their Vh:Cd. Figure 4 shows that the improvement was similar for seropositive and seronegative subjects (mean  $\Delta$ Vh:Cd 0.28) and for a small group of patients who continued on for a total of 24 wk the improvement increased further (mean  $\Delta$ Vh:Cd 0.41). If we input 0.28 for  $\Delta$ Vh:Cd in Equation 1, we obtain  $\Delta w_g$  of -15.4 g over the 12-wk treatment period, which computes to a mean of 184 mg/d of gluten removed from individual’s normal GFDs. Of note, Equation 1, which was derived from added gluten to the diet, is not symmetric for positive and negative values of  $\Delta w_g$ . If we make it symmetric, then we

obtain  $\Delta w_g$  of -22.7 g or 271 mg/d. The former calculation assumes that mucosal damage occurs more quickly or more extensively than recovery; the latter calculation assumes they are the same. There is no conclusive understanding of these phenomena, and therefore we treat them as boundary conditions and use the average of 228 mg/d for gluten elimination. This determination is limited by the assumption that cumulative change in Vh:Cd from total exposures over 6 wk of gluten challenge is similar to the same exposure (or removal of gluten) over 12 wk.

The above conditional calculation is based on objective measures that clearly show that trial-bound patients significantly reduced gluten from their diets and sets a lower limit on the amount of gluten typically consumed in their normal GFDs. More difficult is an estimate of the gluten consumption that remained in their diets during the treatment period. There was clearly continued gluten intake as the trial revealed a statistically and clinically significant symptom improvement with the drug from baseline relative to placebo of the order of 30–50% (for seropositive patients) for abdominal pain, bloating, tiredness, and constipation (14). Any further effort to estimate the total gluten consumption in this population of moderately to severely symptomatic patients would invoke unjustifiable assumptions so we instead leave this



**FIGURE 4**  $\Delta$  Vh:Cd for the placebo patients evidencing the trial effect in the Phase 2b trial. Patient numbers are: seropositive:  $n = 54$  (12 wk),  $n = 12$  (24 wk); seronegative:  $n = 68$  (12 wk),  $n = 18$  (24 wk). Seropositive patients are defined as patients registering a positive titer for either of anti-TG2 IgA, DGP IgA, or DGP IgG. Delta, change in villous height-to-crypt depth for patients; DGP, deamidated gliadin peptide; PT, patient; TG2, tissue transglutaminase 2; Vh:Cd, villous height-to-crypt depth;  $\Delta$ , change.

analysis with the knowledge that the amount is  $>228$  mg/d by a factor of  $1/(1-f)$  where  $f$  is the fraction of gluten that was eliminated from the diet due to the trial effect. By way of example, if  $f = 50\%$ , then the implied pretrial gluten consumption would be 456 mg/d.

## DISCUSSION

Gluten is ubiquitous, and continued exposure leads to persistent histologic injury and episodic symptom distress in CD patients following a GFD. The GFD is vulnerable to gluten exposure and it is hoped that these results will inform CD patients of the need to re-evaluate their diets under the guidance of a clinician or dietician and provide a guide for drug development for this autoimmune disease, for which no effective drug therapy exists.

Adult CD patients evidently consume, on average, potentially unsafe levels of gluten while on a GFD (Tables 1 and 2). Mean daily consumptions for adults were determined to be 244 mg (stool analysis), 363 mg (urine analysis) and  $>228$  mg (ALV003-1221 trial analysis), with the latter value likely to be greater than the former values. There is a general consistency in these analyses and the potentially higher value for the latter case may reflect that this trial enrolled a more symptomatic CD population. The fact that the placebo patients improved histologically in a 12-wk trial due to a trial effect substantiates that they were consuming significant amounts of gluten before the trial. The stool and urine analyses were also conducted on a population of children, and although they generally consumed less than adults, the mean gluten consumption may still be regarded as above the recommended level of gluten ingestion for patients with CD (Tables 1 and 2 and Figures 2 and 3).

It should be noted that there is considerable variation in GIP concentration in stool and urine that may impact the accuracy of these analyses. A single ingestion of 0.5 g gluten showed a GIP concentration in the first urine in the morning that varied from undetectable (only 1 out of 18 patients) to 41.9 ng/mL. Differences in GIP concentration in stool of volunteers ingesting the same amount of gluten (9 or 30 g) were found (18). Variation of excreted GIP from identical gluten intake could be due to the interaction with other ingested food, the time from gluten ingestion,

the glutenase activity of the microbiome, the intestinal motility, differential amount of digestive juices and enzymes, etc. In urine, the variation may also be affected by the amount of ingested water and the leakiness of the intestine. The differential modification of the GIP by deamidation by transglutaminase might also contribute to the variability of the results for single gluten ingestion. There is, however, significant correlation between the amount of gluten ingested and the gluten excreted by either stool or urine, and the sample sizes are sufficiently large to statistically average over these variabilities. However, it should be recognized that the stool and urine tests are relatively new and the methods continue to be improved.

Distribution plots for the frequency of occurrence at various gluten ingestion levels showed that although many, if not most, CD patients are able to control their gluten intake reasonably well, others cannot. These analyses were not able to distinguish between individuals who are diligent or not diligent at maintaining their GFD. For example, a low value may reflect a diligent person who consistently consumes a little gluten, or a less diligent person who happened to consume a little gluten on the day of the measurement. Regardless, the frequent measurement of high gluten consumption indicates that a reasonable fraction of the CD population have difficulty controlling their GFD. Data from Figures 2 and 3 show that depending on the subclass of CD patients, anywhere from 3% to 19% of patients consume  $>600$  mg gluten on a daily basis. The ALV003-1221 trial data suggests that individuals on a GFD cannot avoid accidental gluten intrusions and these small amounts are sufficient to trigger severe symptomatic responses and may contribute to histologic damage.

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