



# Antibody Concentrations Decrease 14-Fold in Children With Celiac Disease on a Gluten-Free Diet but Remain High at 3 Months

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## BACKGROUND & AIMS:

Celiac disease can be identified by a serologic test for IgA against tissue transglutaminase (IgA-TTG) in a large proportion of children. However, the increased concentrations of antibody rarely normalize within the months after children are placed on a gluten-free diet (GFD). Early serologic predictors of sufficient adherence to GFD are required for optimal treatment.

## METHODS:

In a prospective study, we observed the response to a GFD in 345 pediatric patients (67% girls; mean age, 8.4 y) who underwent duodenal biopsy to confirm or refute celiac disease from October 2012 through December 2015. Baseline serum samples were tested centrally for IgA-TTG and IgG against deamidated gliadin. Follow-up serologic analyses of children on a GFD were performed about 3 months later.

## RESULTS:

The geometric mean concentration of IgA-TTG decreased from 72.4-fold to 5.2-fold the upper limit of normal (ULN), or by a factor of 14.0 (95% CI, 12.0–16.4). A substantial response (defined as a larger change than the typical variation in patients not on a GFD) was observed in 80.6% of the children. Only 28.1% of patients had a substantial response in the concentration of IgG against deamidated gliadin. Concentration of IgA-TTG remained above 1-fold the ULN in 83.8% of patients, and above 10-fold the ULN in 26.6% of patients with a substantial response.

## CONCLUSIONS:

Serum concentration of IgA-TTG decreases substantially in most children with celiac disease within 3 months after they are placed on a GFD, but does not normalize in most. This information on changes in antibody concentrations can be used to assess patient response to the diet at short-term follow-up evaluations. Patients with a substantial response to a GFD often still have high antibody levels after 3 months. German Clinical Trials Registry no. DRKS00003854.

**Keywords:** Celiac Disease; Deamidated Gliadin; Gluten-Free Diet; Tissue Transglutaminase.

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**Abbreviations used in this paper:** AbCD, Antibody diagnostics in paediatric Coeliac Disease; BMI, body mass index; CD, celiac disease; GFD, gluten-free diet; IgG-DGL, IgG antibodies against deamidated gliadin; IgA-EMA, IgA antibodies against endomysium; IgA-TTG, IgA antibodies against tissue transglutaminase; SDS, standard deviation scores; ULN, upper limit of normal.



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Celiac disease (CD) is an autoimmune condition in which the production of (auto)antibodies is triggered by dietary gluten from wheat and proteins from related cereals in genetically susceptible individuals. The autoimmune response to tissue transglutaminase (TTG) elicits inflammation of the small intestinal mucosa and results in mucosal injury with villus atrophy accompanied by increased numbers of intraepithelial lymphocytes.<sup>1</sup> Until recently, histologic investigation of mucosal tissue from biopsy specimens was regarded as a cornerstone of the diagnosis of CD. However, assays of IgA antibodies to TTG (IgA-TTG) in serum samples proved to be reliable for diagnosis in a large proportion of patients.<sup>2-4</sup>

This diagnostic paradigm shift from the evaluation of biopsy specimens toward blood tests means that assessing antibody response to a gluten-free diet (GFD) is becoming increasingly important. The guidelines of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition suggest that children with an established diagnosis of CD be followed up regularly for symptomatic improvement and normalization of CD-specific antibody tests.<sup>3</sup> Retrospective analyses have indicated that TTG antibodies normalize after 2 years in 70% to 80% of children on a GFD, that is, the antibody concentration has decreased to less than the manufacturer's upper limit of normal (ULN),<sup>5,6</sup> although there also have been reports of normalization rates as low as 30%.<sup>7</sup> Children and their parents require feedback considerably earlier than after 24 months, and the question that remains is whether antibody tests are useful after 100 days of GFD. Studies have shown that only approximately 10% of values have normalized by then, but that they decrease considerably in that short time.<sup>5,7</sup>

A number of pediatric studies have investigated how antibody levels respond to a GFD.<sup>6-18</sup> Many of those studies used small data sets for children on a GFD ( $n = 20-93$ ),<sup>7-18</sup> used retrospective data,<sup>6-8,12,17,18</sup> did not focus primarily on antibody decrease,<sup>9,11,16,17</sup> or had severe limitations in the methods, such as an upper limit in antibody measurements or assumptions of exponential decrease in antibody concentrations over time.<sup>12,13</sup> Here, we present short-term follow-up data for IgA-TTG and IgA-endomysium antibodies (IgA-EMA) together with IgG-antibodies to deamidated gliadin peptides (IgG-DGL) in children with CD, prospectively recruited in the diagnostic Antibody diagnostics in paediatric Coeliac Disease (AbCD) study.<sup>3</sup> Our aim was to determine if an antibody test could provide valuable information about the success of a GFD after only 3 months and quantify what constitutes a clear response to a GFD.

## Materials and Methods

### *Study Design and Patient Selection*

The AbCD study is a prospective multinational trial to validate the diagnostic accuracy of antibody tests.<sup>3</sup>

Patients between 5 months and 18 years scheduled for duodenal biopsy with the primary aim to confirm or refute celiac disease were eligible if they had not already been diagnosed with celiac disease and were not on a GFD. At baseline, a duodenal biopsy specimen was taken along with a blood sample, which was tested in a central laboratory. Prior to receiving results from the central laboratory, the treating physician then decided if a GFD should be recommended and asked patients to return for a follow-up visit approximately 3 months later. At the follow-up visit, blood samples were foreseen for patients on a GFD and at the discretion of the physician for remaining patients. Adherence to the GFD was assessed as follows: very strict, less strict, no compliance, difficult to estimate, and a diagnosis made, if possible.

The trial was registered in the German Clinical Trials Register (DRKS00003854) and approved by each site's ethics committee.

Patients were considered informative for the current analysis (primary analysis set) if the baseline blood sample was taken fewer than 30 days before recommending a GFD, if at least 1 baseline IgA-TTG or IgG-DGL antibody measurement was higher than normal, if they provided a follow-up blood sample between 2 and 6 months after beginning a GFD, and if they did not present with selective IgA deficiency. A secondary analysis set consisted of a small number of patients without an indication for GFD who optionally provided a follow-up blood sample.

All authors had access to the study data and reviewed and approved the final manuscript.

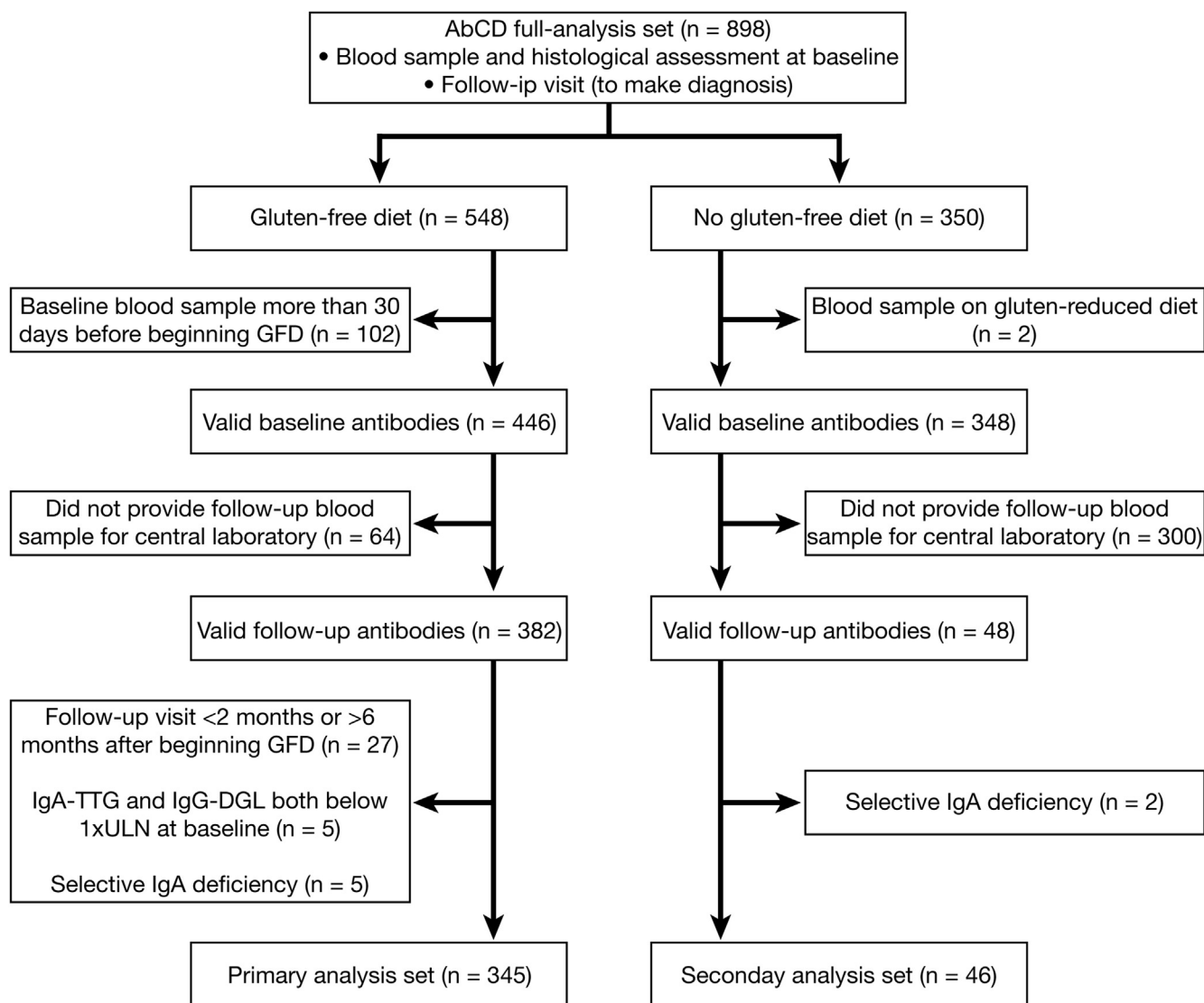
### *Serology*

Sera were stored at  $-20^{\circ}\text{C}$  and shipped to the central laboratory (Dassow, Germany) on dry ice for blinded measurement of IgA-TTG, IgG-DGL, and IgA-EMA with test kits from EUROIMMUN (Lübeck, Germany; cut-off values  $\geq 20$  U/mL,  $\geq 25$  U/mL,  $\geq 1:10$ , respectively). If antibody concentrations were higher than the measurement range, sera were serially diluted and values were corrected by the dilution factor. Further details are available in the article by Wolf et al<sup>3</sup> with the primary analysis. The results of antibody assays performed locally in the trial centers were used for comparison with central assay results.

### *Symptoms and Anthropometric Parameters*

At baseline and follow-up visits, physicians recorded whether the patient had diarrhea, flatulence, constipation, vomiting, nausea, anorexia, fatigue, cramping, abdominal distention/bloating, abdominal pain, aphthous stomatitis, and dermatitis herpetiformis-like rash. The number of symptoms thus was between 0 and 12.

SD scores (SDS), also called z-scores, for body mass index (BMI) and height were determined using the LMS method and standard tables for a representative German population.<sup>19,20</sup>



**Figure 1.** Patient flowchart.

### Statistics and Analysis Methods

All analyses were performed using the software R (The R Foundation for Statistical Computing) version 3.3.3. Antibody concentrations were always treated on a logarithmic scale.

When can a decrease in IgA-TTG concentration be interpreted as a response to a GFD? To distinguish a response from chance fluctuations, we require that the observed decrease after beginning GFD be clearly larger than differences observed when antibodies were measured twice within a short period of time. Such double measurements were analyzed in the Supplementary material to the main AbCD publication.<sup>3</sup> The SD of local and central measurements of IgA-TTG in the same patients before GFD was approximately a factor of 2. The corresponding estimate for IgG-DGL (Supplementary Figure 1) was 1.74. For parsimony, we used the factor 2 for the SD of both IgA-TTG and IgG-DGL. We define a change of 2 SDs (ie, a factor of 4), to be a substantial response.

Antibody data were analyzed with 2 complementary methods. First, linear models containing the baseline value as a covariate were used after having confirmed that trial site as a random term in a mixed model did not affect results meaningfully. Second, repeated-measurement analyses containing interaction terms between the point in time and various covariates were analyzed with generalized estimating equations. We quantified the association between changes in the number of symptoms and antibody concentrations using Kendall's  $\tau$ . As a proxy for thriving and malabsorption, changes in BMI-SDS were examined with a paired  $t$  test and the association to changes in antibody concentration was quantified with the Pearson correlation, using a Fisher transformation for the CI. The latter analysis was repeated for children older than the age of 2 because BMI-SDS is considered unreliable in young children.<sup>21</sup> For single samples of a continuous variable, 95% CIs are found using the  $t$ -distribution and using the Wilson score for count data. Estimates are followed by 95% CIs

**Table 1.** Baseline Demographic and Clinical Characteristics

	All patients (n = 391)	Patients on GFD (n = 345)	Patients not on GFD (n = 46)
Girls, n	253 (65%)	231 (67%)	22 (48%)
Age, y	8.6 (4.7)	8.4 (4.6)	9.8 (4.8)
BMI-SDS	-0.35 (1.20)	-0.38 (1.16)	-0.10 (1.14)
<10th percentile	70 (19%)	64 (19%)	6 (14%)
<3rd percentile	36 (10%)	33 (10%)	3 (7%)
Height-SDS	-0.52 (1.31)	-0.53 (1.26)	-0.40 (1.11)
<10th percentile	90 (24%)	81 (25%)	9 (21%)
<3rd percentile	52 (14%)	46 (14%)	6 (14%)
Diagnosis <sup>a</sup>			
CD	344 (88%)	344 (100%)	0 (0%)
Not CD	36 (9%)	0 (0%)	36 (78%)
No final diagnosis	11 (3%)	1 (0.3%)	10 (22%)
First-degree relative with CD	43 (11%)	42 (12%)	1 (2.2%)
Diabetes (type I)	30 (8%)	25 (7%)	5 (11%)
Down syndrome	4 (1.0%)	4 (1.2%)	0 (0%)
Autoimmune thyroid disease	6 (1.5%)	5 (1.4%)	1 (2.2%)
Turner syndrome	1 (0.3%)	1 (0.3%)	0 (0%)
Autoimmune liver disease	1 (0.3%)	1 (0.3%)	0 (0%)
Number of symptoms <sup>b</sup>			
0	84 (21%)	75 (22%)	9 (20%)
1	109 (28%)	97 (28%)	12 (26%)
2	104 (27%)	90 (26%)	14 (30%)
3	37 (9%)	33 (10%)	4 (9%)
>3	57 (15%)	50 (14%)	7 (15%)

NOTE. Data are means (SD) or numbers (percentages).

BMI, body mass index; CD, celiac disease; GFD, gluten free diet; SDS, standard deviation scores.

<sup>a</sup>See [Supplementary Table 1](#) for details on alternative diagnoses for patients not on a GFD.

<sup>b</sup>See [Supplementary Table 2](#) for a list of symptoms with frequencies.

and statistical tests were defined to be significant for a  $P$  value  $\leq .05$ .

## Results

### Patient Characteristics

Between October 2012 and December 2015 there were 898 children and adolescents who were enrolled in

the AbCD trial.<sup>3</sup> For the 345 CD patients on a GFD and a further 46 patients without CD who were not on a GFD, follow-up data are available ([Figure 1](#)). Basic demographic and clinical characteristics can be found in [Table 1](#) and [Supplementary Tables 1 and 2](#).

### Antibodies at Baseline and Follow-Up Evaluation

The median follow-up time was 98 days (interquartile range, 86–116 d) after the start of a GFD, see [Supplementary Figure 2](#) for more detailed information on the interval between GFD and follow-up evaluation.

Antibody concentrations at baseline and follow-up evaluation can be found in [Table 2](#). IgA-TTG decreased in 97.7% (95.5%–98.8%) of patients on a GFD and decreased substantially (ie, by a factor of 4, cf. Statistics and Analysis Methods) in 80.6% (76.1%–84.4%) of them. In patients with initial concentrations greater than  $10\times$  ULN and with strict diet adherence, 90.1% (85.9%–93.2%) had a substantial decrease and 97.0% (94.1%–98.4%) had a decrease of at least a factor of 2 (1 SD). Of note, even in those with a substantial decrease, it remained greater than  $1\times$  ULN in 83.8% (79.0%–87.7%) and greater than  $10\times$  ULN in 26.6% (21.8%–32.1%) of patients ([Figure 2](#)).

IgG-DGL decreased in 95.7% (93.0%–97.3%) of patients on a GFD and decreased substantially in 28.1% (23.6%–33.1%) of them. Even in those with a substantial decrease, it remained greater than  $1\times$  ULN in 38.1% (29.1%–48.1%), but greater than  $10\times$  ULN in only 5.1% (2.2%–11.5%) ([Figure 2](#)).

The considerably higher proportion of patients on a GFD with a substantial decrease in IgA-TTG compared with IgG-DGL implies that the former is better suited to assess response to a GFD in the short term. A receiver operating characteristic analysis showed that IgA-TTG responses to GFD can be distinguished better from IgA-TTG fluctuation under a normal diet than the IgG-DGL analogue (area under the curve, 0.942 vs 0.880;  $P = .0025$ ) ([Figure 3A](#)). The correlation between IgA-TTG and IgG-DGL response was moderate at 0.42 (0.33–0.50) ([Figure 3B](#)).

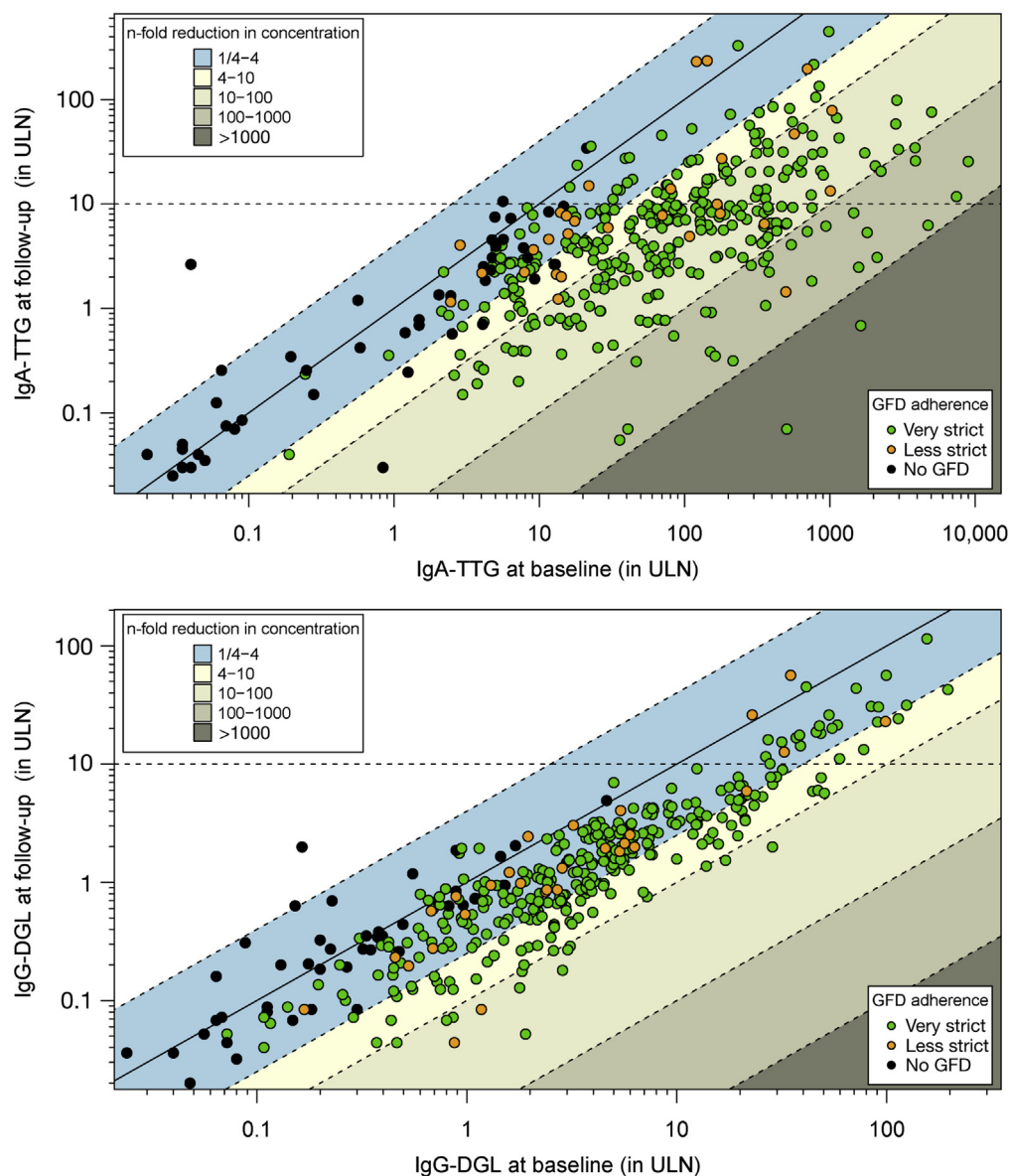
There was very good agreement between changes in IgA-TTG and IgA-EMA in almost all patients

**Table 2.** Antibody Concentrations and Changes

	IgA-TTG			IgG-DGL		
	Concentrations, ULN units		N-fold decrease from baseline to follow-up evaluation (95% CI)	Concentrations, ULN units		N-fold decrease from baseline to follow-up evaluation (95% CI)
	Baseline	Follow-up evaluation		Baseline	Follow-up evaluation	
Patients on a GFD	72.4	5.2	14.0 (12.0–16.4)	3.7	1.3	2.9 (2.7–3.1)
Very strict adherence	75.0	4.9	15.3 (13.0–18.0)	3.8	1.3	3.0 (2.8–3.2)
Less strict adherence	48.9	8.8	5.6 (3.2–9.7)	2.9	1.3	2.2 (1.7–2.9)
Not on a GFD	0.83	0.63	1.3 (1.0–1.8)	0.29	0.29	1.0 (0.8–1.3)

GFD, gluten free diet; IgG-DGL, IgG antibodies against deamidated gliadin; IgA-TTG, IgA antibodies against tissue transglutaminase; ULN, upper limit of normal.





**Figure 2.** Centrally measured antibody values at baseline and follow-up evaluation. Note that IgA-TTG concentrations decreased by more than a factor of 100 for a number of patients, although they remained high at follow-up evaluation.

(Supplementary Table 3). IgA-EMA decreased by at least a factor of 10 in 78.8% (74.2%–82.8%) of patients. In 12 CD patients the baseline IgA-EMA titers already were so low that a substantial decrease by 2 dilution steps was not measurable.

Only 3 patients (0.9%) on a GFD had IgA-TTG and IgG-DGL values that both increased from baseline to follow-up evaluation, 2 of whom were considered to have followed the GFD less strictly. None of these values increased by more than a factor of 2.

Supplementary Figure 3 shows how changes in IgA-TTG concentrations depend on time.

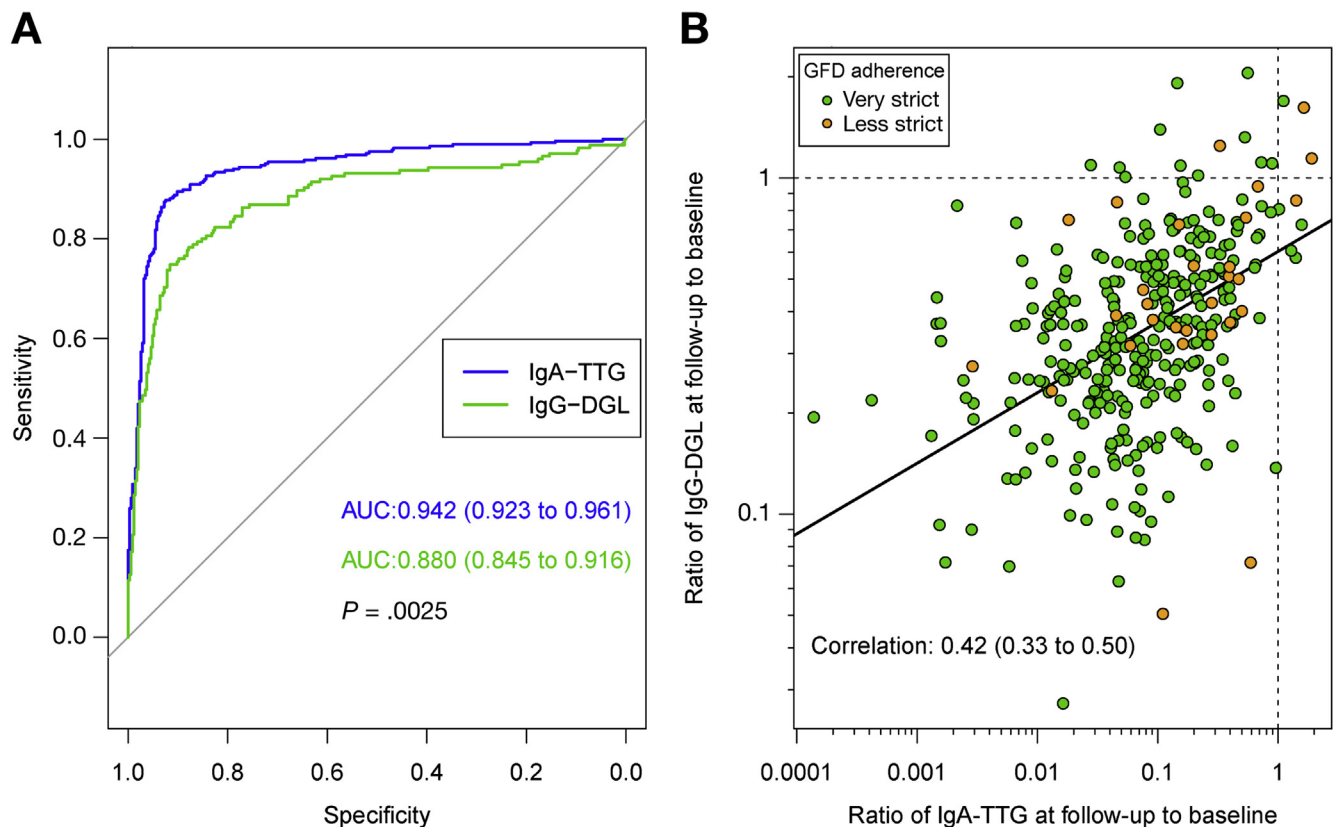
#### Covariates Influencing IgA-Tissue Transglutaminase After a Gluten-Free Diet

IgA-TTG was found to decrease more if concentrations were initially high, in younger children, and in those with strict GFD adherence (Table 3 and

Supplementary Table 4). There is weak evidence for a slight dependence on sex and type I diabetes in Table 2 that cannot be verified in Supplementary Table 4. There was no indication in this model that the effect of age was nonlinear and, in particular, that children younger than the age of 2 responded differently (Supplementary Figure 3). Younger children (eg, age <8 y) tended to have higher baseline antibody concentrations (Supplementary Figure 5). Note that the effects of age and baseline concentration are taken into account as separate covariates in Table 3.

#### Correlation Between Antibodies and Symptoms or Body Mass Index–Standard Deviation Scores

The mean number of symptoms per patient decreased from 1.79 at baseline to 0.54 at follow-up evaluation in patients on a GFD, that is, there was a decrease by 1.25 (1.06 to 1.44;  $P < .001$ ) symptoms. For



**Figure 3.** (A) Areas under the curve (AUC) are shown from a receiver operating characteristic (ROC) analysis. Differences in antibody concentrations (log-scale) on a normal gluten containing diet are treated as characterizing a healthy cohort. Differences between the antibody concentrations at baseline (before beginning a GFD) and follow-up (after about 3 months of a GFD) are treated as characterizing the sick cohort. The  $P$  value arises from a test of the null hypothesis that the 2 areas under the concentration-time curves are equal. The analysis shows that IgA-TTG responses to GFD can be distinguished better from IgA-TTG fluctuation under normal diet than the IgG-DGL analogue. (B) Correlation between IgA-TTG and IgG-DGL responses.

comparison, in patients not on a GFD the number decreased from 2.02 to 0.85, that is, there was a decrease by 1.17 (0.58 to 1.77;  $P < .001$ ) symptoms. Thus, the decrease in the mean number of symptoms in children on a GFD was not larger than in children not on a GFD. No correlation between changes in the number of symptoms and changes in IgA-TTG values could be found for the GFD patients (Kendall's  $\tau$ , 0.06;  $P = .11$ ).

The mean value of BMI-SDS, used here as a proxy for failure to thrive, increased from -0.37 to -0.10 in patients on a GFD (ie, a change of 0.27; 0.19–0.35;  $P < .001$ ). This change was slightly although significantly related to changes in IgA-TTG, with a Pearson correlation coefficient equal to -0.23 (-0.33 to -0.12;  $P < .001$ ). Limiting these analyses to children older than the age of 2 ( $n = 321$ ) had little effect. For comparison, in patients not on GFD it increased marginally from -0.11 to -0.03 (ie, a change of 0.08; -0.05 to 0.21;  $P = .23$ ).

## Discussion

Our data show that adherence to a GFD can be detected reliably after only 3 months of diet. Mean IgA-TTG concentrations in children with CD decrease considerably, but remain well above  $10 \times$  ULN for

approximately a quarter of patients. In addition, changes in IgA-TTG resulting from the GFD are typically larger than the factor 2 seen in random fluctuations on a

**Table 3.** Results of a Linear Model for Covariates Affecting IgA-TTG Response to GFD

	Estimate <sup>a</sup>	95% CI	$P$ value
Baseline concentration, <sup>b</sup> per factor 10 higher	2.76	2.34–3.25	<.001
Age, per year younger	1.068 <sup>c</sup>	1.038–1.098	<.001
GFD adherence, very strict vs less strict	2.15	1.39–3.33	<.001
Sex, male vs female	1.32	1.02–1.71	.036
Type I diabetes, present vs absent	1.57	0.99–2.51	.058

NOTE. See [Supplementary Table 4](#) for more information.

GFD, gluten free diet; IgA-TTG, IgA antibodies against tissue transglutaminase.

<sup>a</sup>Estimates show the relative factor by which antibody concentrations decrease (eg, the IgA-TTG concentrations of boys decrease by a factor 1.32 more than those of girls).

<sup>b</sup>The adjusted  $R^2$  value is 0.43 for the full linear model compared with 0.36 for the linear model containing only baseline concentrations, indicating that the baseline value accounts for roughly 36% of the total variance, whereas the remaining 4 covariates only account for an additional 7%.

<sup>c</sup>This implies that if patient A is 10 years younger than patient B, then patient A's antibody response will be stronger by a factor of  $1.068^{10} = 1.93$  than patient B's on average.

normal diet. Any decrease by a factor of 4 thus is strongly indicative of a response to GFD. This was observed in the majority of patients. Note, however, that this approach required that blood samples be diluted enough to determine the absolute antibody concentration, which is not standard procedure.

IgA-EMA correlated strongly with IgA-TTG. However, IgA-EMA is measured on a discrete scale, relies on a subjective assessment of the immunofluorescence staining pattern, and its measurement is more costly than that of IgA-TTG.

IgG-DGL also decreases in the vast majority of cases, but does not distinguish GFD response from random fluctuations as effectively as IgA-TTG. In standard laboratory procedures, however, IgG-DGL does not reach the upper bound of the measurement range very often, meaning that it could be a pragmatic, albeit inferior, alternative to IgA-TTG.

In a small pediatric study ( $n = 20$ ), Agardh et al<sup>9</sup> observed a decrease in median IgA-TTG levels by a factor of approximately 16 at 3 months, with a small number of nonresponders. A 2-year follow-up study of CD patients ( $n = 93$ ) restricted its attention to patients who had not “committed dietary transgressions” and found that approximately 60% had positive IgA-TTG and IgG-TTG tests after 4 months.<sup>10</sup> These IgG-TTG results agree well with our findings for IgG-DGL, but we had a higher proportion of positive IgA-TTG (85%) patients, possibly because of the sensitive kit we use.

A large retrospective analysis ( $n = 487$ ) showed that the median normalization time for IgA-TTG is approximately 400 days and that type I diabetes and higher baseline values contribute to longer normalization times.<sup>6</sup> One study in children and adults ( $n = 25$ ) found a decrease in IgA-TTG for all patients at 50–1102 days (median, 213 d) after starting a GFD and a subsequent increase upon performing a gluten challenge.<sup>22</sup> A 1-year follow-up study in adults ( $n = 82$ ) found that 80% were positive for IgA-TTG and IgG-DGL after 3 months.<sup>23</sup> At 1 year, the respective rates were approximately 60% and 50%, respectively.

The picture that emerges is that IgA-TTG decreases quickly in most patients who begin a GFD, but may require a long time to normalize and does not decrease in a few patients. Symptoms and failure to thrive appear to be poor indicators of a measurable short-term response to a GFD. Although it is tempting to believe that the GFD led to the marked reduction in the mean number of symptoms per patient seen in our trial, our data show a very similar spectrum of symptoms and reduction in them for the non-GFD group. Regression to the mean may account for a considerable share of the change, but dedicated trials would be required to elucidate this point further. Attempts to identify further factors associated with slow or nonresponders have been only marginally successful: apart from the initial IgA-TTG concentration, only adherence to diet and age seem to play notable roles.

Recently, a meta-analysis concluded that IgA-TTG has “low sensitivity (below 50%) in detection of persistent villous atrophy.”<sup>24</sup> This analysis is based on treating antibody concentrations as a binary variable either above or below  $1 \times \text{ULN}$ . Our new data clearly indicate that, at least in the short term, high antibody concentrations at follow-up evaluation are consistent with good GFD response and, indeed, typical. This implies that classifying antibody response with such a binary variable can lead to vast underestimates in sensitivity. The error one makes by looking only at normalization can lead to inconsistent results as to which antibody test is considered most appropriate to assess GFD response and conclusions drawn are not necessarily based on sound logic.<sup>13,25</sup>

One limitation of this study was the lack of long-term data and follow-up biopsies, which would provide interesting additional information to questions beyond the scope of the current article. As a result, we could not follow up the progress of nonresponders and we could not correlate the antibody response with morphologic changes in the intestine. Moreover, we did not have a CD group without GFD for comparison purposes, did not conduct a gluten challenge, and we did not assess GFD adherence with an established instrument. Although we have data from local antibody tests from different assay manufacturers, these were not performed using serial dilution, meaning that the baseline concentrations could not be used to assess response. As a result, we can only provide data for the Euroimmun test kit.

The strengths of this study were the large number of patients prospectively recruited, the central blinded measurements of antibodies, and the serial dilution of the blood samples so that even very high antibody concentrations could be measured appropriately.

We set out to characterize short-term antibody response to GFD, before substantial histologic changes can be expected. Measurement of IgA-TTG after approximately 3 months can be useful in confirming response to a GFD if initial concentrations are known. A decrease by more than a factor of 4 can be considered a clear indication of such a response and a smaller decrease suggests a rather poor response, perhaps indicating low adherence to the diet and the need for clinical/dietetic advice.

## Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at [www.cghjournal.org](http://www.cghjournal.org), and at <https://doi.org/10.1016/j.cgh.2018.04.008>.

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#### Reprint requests

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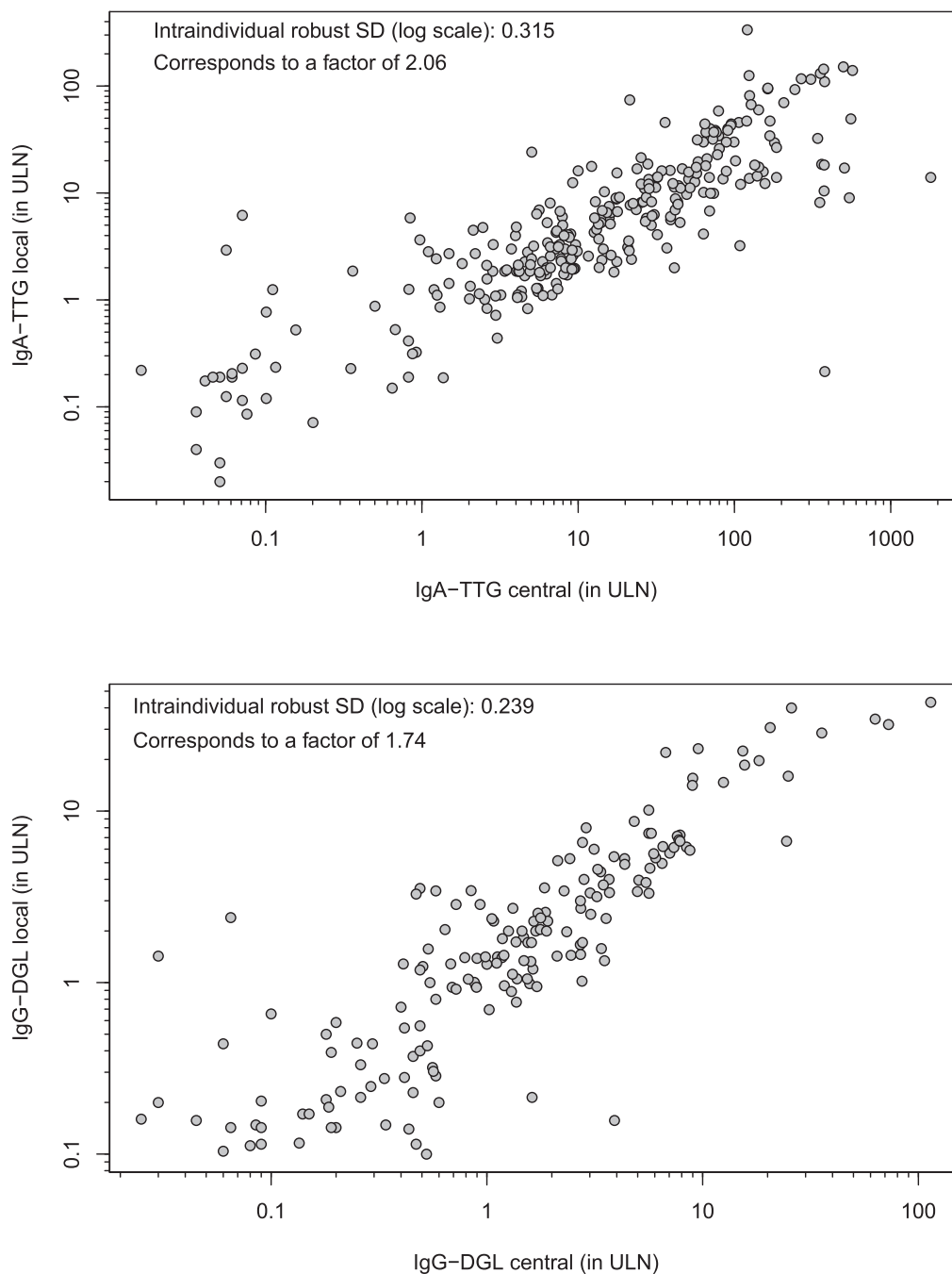
#### Conflicts of interest

These authors disclose the following: Thomas Mothes and Holm H. Uhlig are 2 of the patent holders for "Peptides and Their Use in a Procedure for Diagnostics of Coeliac Disease and Dermatitis Herpetiformis" (German patent DE10005932), with premiums paid by Leipzig University until 2014. Holm H. Uhlig has collaborated on projects with Eli Lilly, UCB Pharma, and Regeneron, and has consulted for Boehringer Ingelheim, AbbVie, and Pfizer; Johannes Wolf has received a grant from the German Coeliac Society and EUROIMMUN (Lübeck, Germany) for a celiac screening project in LIFE Child of the Research Centre of Civilization Diseases (Leipzig, Germany), outside the submitted work; Thomas Richter has received a grant from EUROIMMUN (Lübeck, Germany) for celiac screening in the Children's Hospital St Georg Leipzig, Germany, outside the submitted work; and Marcus K. H. Auth has received nonfinancial support from the British Society for Paediatric Gastroenterology during the conduct of the study and nonfinancial support from Nutricia plus Advance Medical Nutrition outside the submitted work. The remaining authors disclose no conflicts.

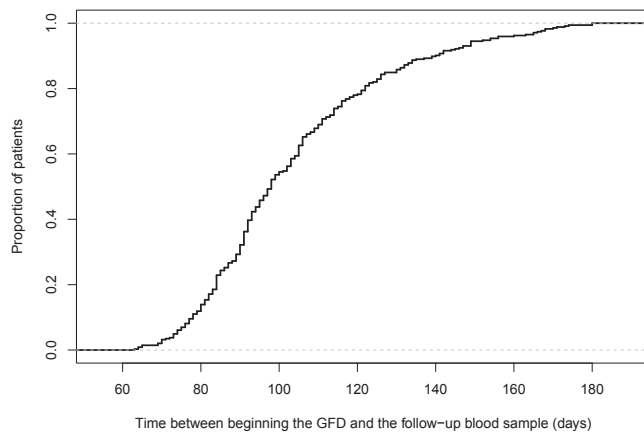
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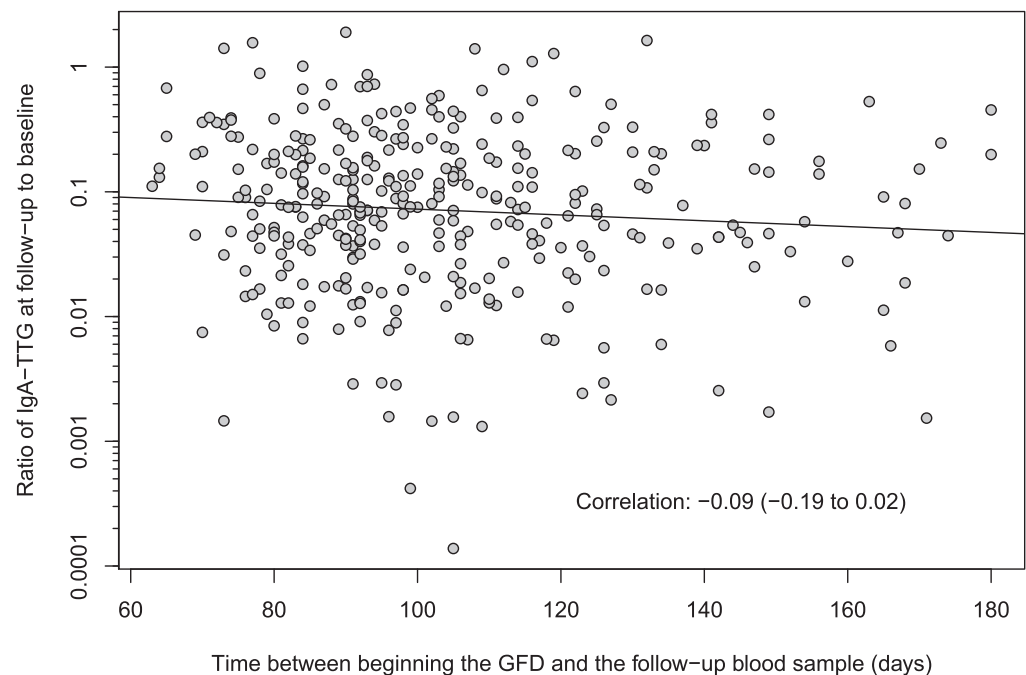
**Supplementary**

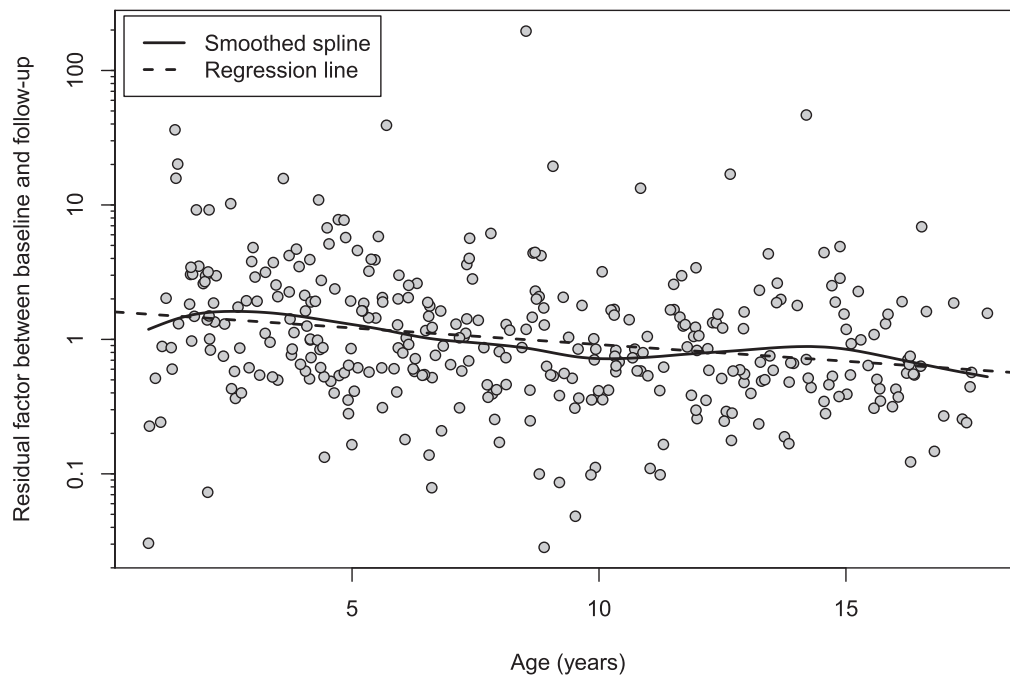
**Figure 1.** Intraindividual variance on a gluten-containing diet. The last available local antibody concentration is plotted against the measured value in the central laboratory in units of the ULN. The robust estimate for SD is based on mean absolute deviations. A slightly different version of the upper panel was presented by Wolf et al as [Supplementary Figure 4](#).<sup>3</sup>



**Supplementary Figure 2.** The median interval between the start of a GFD and the follow-up blood sample was 98 days, with an interquartile range from 86 to 116 days.

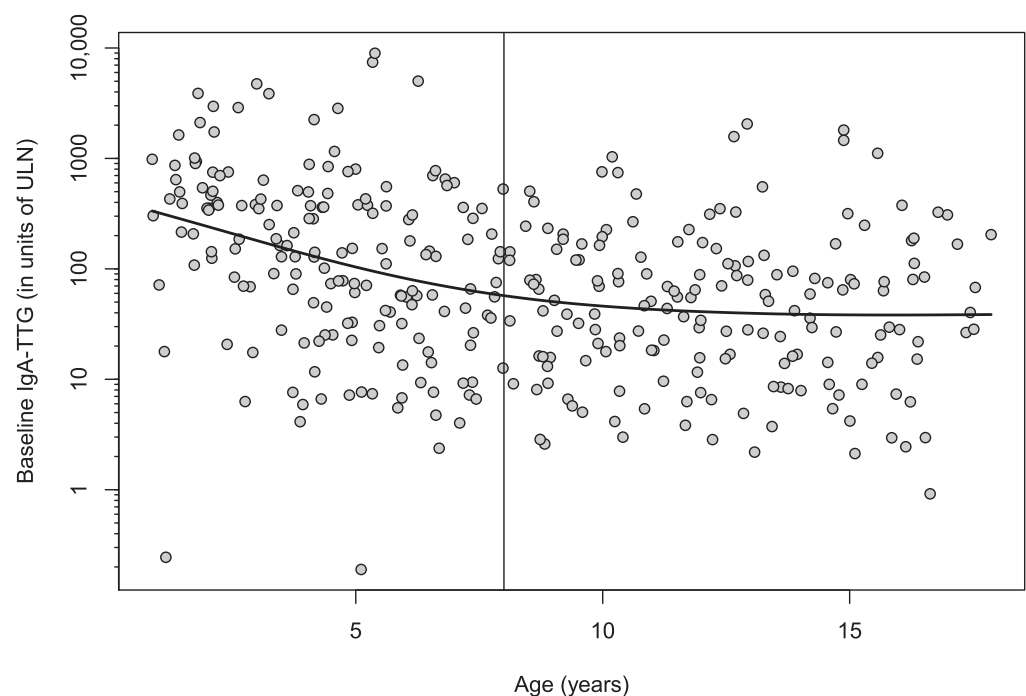
**Supplementary Figure 3.** Little dependence on time is seen for the interval over which data were collected. This was verified by generalized estimating equations indicating a decrease in antibodies by a factor of 0.82 (95% CI, 0.68–0.98) over 30 days within this time period.





#### Supplementary

**Figure 4.** Residuals from a linear model with IgA-TTG response as the dependent variable and baseline IgA-TTG, sex, GFD adherence, and diabetes status as covariates are plotted vs age. There is no indication that the age dependence is nonlinear, or that antibodies of children younger than the age of 2 behave differently from those of older children.



#### Supplementary

**Figure 5.** Baseline concentrations for IgA-TTG are plotted vs age. The smoothed spline suggests that there is a strong dependence on age until an age of approximately 8 years (vertical line).

**Supplementary Table 1.** Alternative Diagnoses for Patients Not on a GFD Listed According to Decreasing Baseline IgA-TTG Concentrations

Sex	Age, y	IgA-TTG at baseline, ULN	IgA-TTG at follow-up evaluation, ULN	Alternative diagnosis
Female	13.8	14.72	9.49	Potential celiac disease
Male	10.8	6.38	7.28	Crohn's disease
Male	16.4	6.25	2.77	Irritable bowel syndrome
Male	3.0	5.03	3.93	Gastrointestinal infection
Female	13.7	4.73	4.53	Iron-deficiency anemia
Female	7.3	4.65	2.33	Gluten sensitivity?
Female	14.6	2.46	1.32	Ulcer ventriculi
Female	14.8	2.04	1.35	Type B gastritis
Female	11.7	1.49	0.69	Functional abdominal pain and mild duodenitis
Female	14.0	1.25	0.24	Type C gastritis
Female	13.5	1.19	0.58	Candidal esophagitis
Female	17.9	0.09	0.08	Fructose and sorbitol intolerance, proctitis
Male	11.5	0.06	0.12	Gastritis, IgA deficiency, irritable bowel
Male	1.7	0.05	0.03	Recurrent diarrhea
Female	15.0	0.04	2.64	Type C gastritis
Female	14.7	0.04	0.04	Functional abdominal pain
Female	15.9	0.03	0.04	Irritable bowel syndrome

NOTE. Of the 46 patients in this group, alternative diagnoses before the end of the study were available for only the 17 listed here. GFD, gluten free diet; IgA-TTG, IgA antibodies against tissue transglutaminase; ULN, upper limit of normal.

**Supplementary Table 2.** Number of Patients at Baseline and Follow-Up Evaluation With the Given Symptoms, Provided as a List on the Case Report Forms

	Patients on GFD (n = 345)		Patients not on GFD (n = 46)	
	Baseline	Follow-up	Baseline	Follow-up
Abdominal pain	191 (55.4%)	58 (16.8%)	27 (58.7%)	12 (26.1%)
Diarrhea	98 (28.4%)	14 (4.1%)	18 (39.1%)	4 (8.7%)
Bloating	79 (22.9%)	37 (10.7%)	10 (21.7%)	6 (13.0%)
Fatigue	54 (15.7%)	30 (8.7%)	3 (6.5%)	1 (2.2%)
Flatulence	46 (13.3%)	34 (9.9%)	8 (17.4%)	8 (17.4%)
Cramping	39 (11.3%)	13 (3.8%)	7 (15.2%)	3 (6.5%)
Constipation	33 (9.6%)	19 (5.5%)	4 (8.7%)	2 (4.3%)
Vomiting	26 (7.5%)	4 (1.2%)	5 (10.9%)	1 (2.2%)
Nausea	26 (7.5%)	9 (2.6%)	7 (15.2%)	0 (0.0%)
Anorexia	12 (3.5%)	9 (2.6%)	1 (2.2%)	4 (8.7%)
Aphthous stomatitis	7 (2.0%)	7 (2.0%)	2 (4.3%)	0 (0.0%)
Dermatitis herpetiformis-like rash	6 (1.7%)	6 (1.7%)	1 (2.2%)	0 (0.0%)

NOTE. Multiple symptoms per patient are possible, hence the sum is not 100%. GFD, gluten free diet.



**Supplementary Table 3.** Changes in IgA-TTG vs Changes in IgA-EMA After Start of GFD

		Change in IgA-EMA				
		Increased ( $\geq 2$ dilution steps)	Increased (1 dilution step)	Unchanged	Decreased (1 dilution step)	Decreased ( $\geq 2$ dilution steps)
Changes in	Increased (0–1 SD)	2	1	2	1	2
IgA-TTG	Decreased (0–1 SD)	0	1	6	7	3
	Decreased (1–2 SD)	0	1	8	15	18
	Decreased ( $>2$ SD)	0	0	5	24	249

NOTE. Dilution steps for IgA-EMA measurements were roughly a factor of 3.

GFD, gluten free diet; IgA-EMA, IgA antibodies against endomysium; IgA-TTG, IgA antibodies against tissue transglutaminase.

**Supplementary Table 4.** The Effect of Different Covariates on IgA-TTG Concentrations Is Estimated Along With a CI Using Generalized Estimating Equations and Robust (Also Called Sandwich) Estimators

	Estimate	95% CI	P value
Relative factor for both baseline and follow-up evaluation			
Age, per year younger	1.141	1.097–1.188	<.001
GFD adherence, very strict vs less strict	1.21	0.64–2.28	.56
Sex, male vs female	0.81	0.55–1.20	.30
Type I diabetes, absent vs present	0.48	0.22–1.08	.076
Relative reduction from baseline to follow-up evaluation			
Age, per year younger	1.132	1.096–1.169	<.001
GFD adherence, very strict vs less strict	2.34	1.40–3.92	.0012
Sex, male vs female	1.20	0.88–1.65	.25
Type I diabetes, absent vs present	1.14	0.58–2.23	.70

NOTE. The upper rows show the shifts in IgA-TTG concentrations for both points in time (ie, the main effects in the model), whereas the lower rows show how covariates affect the reduction in concentrations from baseline to follow-up evaluation (ie, the interaction effects in the model).