The Clinical Response to Gluten Challenge: A Review of the Literature

Maaike J. Bruins

DSM Biotechnology Center, Alexander Fleminglaan 1, Delft 2613AX, The Netherlands; E-Mail: maaike.bruins@dsm.com; Tel.: +31-613-207-601; Fax: +31-152-794-110

Received: 4 September 2013; in revised form: 1 November 2013 / Accepted: 6 November 2013 / Published: 19 November 2013

Abstract: The aim of this review was to identify, evaluate and summarize all relevant studies reporting on the clinical response to gluten challenge by adult or pediatric patients with suspected or diagnosed coeliac disease (CD) on a gluten-free diet. We evaluated the effect of gluten challenge on changes in symptoms, intestinal mucosa histology, and serum antibodies. A systematic electronic search was performed for studies published as of 1966 using PubMed and Scopus databases. In the reviewed studies, doses ranged from 0.2 to 30 g/day of wheat gluten or comprised a gluten-containing diet. The onset of symptoms upon gluten intake varied largely from days to months and did not parallel serum antibody or histological changes. Within 3 months of gluten challenge, 70%–100% of pediatric CD patients became positive for AGA-IgA and EMA-IgA antibodies and 50%–70% for AGA-IgG. A limited number of trials suggest that no more than half of adult patients developed positive AGA-IgA, EMA-IgA, tTG-IgA or DGP-IgA/IgG titers. Approximately 50%–100% of pediatric and adult patients experienced mucosal relapse of gluten provocation within 3 months, which was preceded by increased mucosal intra-epithelial lymphocytes within several days of challenge. A 3-month high-dose gluten challenge should be suitable to diagnose the majority of CD patients. In some cases prolonged challenge may be needed to verify diagnosis. Combination testing for antibodies and mucosal histology may fasten the diagnosis.

Keywords: gluten challenge; coeliac disease; diagnosis
1. Introduction

Significant health complications may occur when coeliac patients remain on a normal gluten-containing diet. Diagnosis of coeliac disease (CD) should accurately be established before starting a person on a lifelong gluten-free diet. In children and adults, diagnostic testing includes blood HLA-DQ2 and HLA-DQ8 testing, histological examination of small-intestinal biopsies and serum CD-specific antibodies [1–7]. The diagnosis is confirmed by decline in antibody levels after the exclusion of gluten from the diet. Clinical improvement and histological remission are also supportive clinical endpoint to confirm the disease. Gluten challenge is not necessary, except under unusual circumstances [4] where doubt exists about the initial diagnosis; for example, when the patient is on a gluten-free diet or presents with antibodies or complaints but, nonetheless, normal histology. Moreover, failure to respond to a gluten-free diet may raise doubt regarding the initial diagnosis. Examination of mucosal biopsy, however, involves a potential risk of misdiagnosis since it is subject to large method variability [8,9] and moderate-to-poor inter- and intra-observer reproducibility has been shown [10–12]. Unfortunately, histological findings in CD are characteristic but not specific as several disorders can produce comparable histopathological changes [13]. Over recent years, more reliable, specific and sensitive serological diagnostic tests and markers have become available. Small bowel histology remains the gold standard for diagnosis. Symptomatic relapse is not sufficient for a diagnosis of coeliac disease in isolation. Particularly in children in whom the initial biopsy was performed before two years of age, a gluten challenge may be necessary because of the risk of misdiagnosis due to confusion with other causes of enteropathy at this age [3]. In patients suspected of CD and following a gluten-free diet, diagnosis may be confirmed by reintroduction of gluten into the diet or by an oral gluten challenge followed by clinical relapse [3,4,7,14].

Currently, the monitoring of parameters during a gluten challenge is largely empirical, particularly in those patients who remain asymptomatic, and the optimum duration and dose of a gluten challenge has not been established yet. Some guidelines propose a gluten diet/challenge until relapse, even for up to 2 years or longer if patients remain symptom free. The ESPGHAN guidelines recommend that daily gluten intake during gluten challenge should contain at least the normal amount of gluten intake for children (approximately 15 g/day) [4,15]. There is considerable inter-individual variability of clinical presentation among patients with CD [16,17] but also in clinical response time to gluten intake [17]. The large variability and lack of predictability in the response time and severity to gluten complicate defining recommendations regarding the duration and dose of necessary gluten challenge in the diagnostic setting as well as the clinical trial setting.

A standardized approach regarding the amount and duration of dietary gluten necessary to provoke a clinical response in children and adults could provide guidance to physicians and investigators. Therefore, the aim of this article was to perform a review of the literature reporting on the course of the clinical symptoms, serum CD autoantibodies, and intestinal histological changes in response to a gluten challenge in children and adults with diagnosed or suspected CD.
2. Method

The data sources used for this systematic review of references published between 1966 and July 2013 included PubMed and Scopus. Only publications in English were included. We included studies that evaluated the effect of oral gluten challenge in individuals with CD on clinical parameters, i.e., CD-specific antibodies, histology of small bowel mucosa biopsies, symptoms, and urinary sugar absorption test. We reviewed the studies that described the effect of a gluten challenge on clinical relapse in order to confirm diagnosis of CD in children or adults with diagnosed and/or suspected CD. We also extracted valuable information of patients receiving a gluten challenge in the placebo arm of clinical trials testing CD-related therapies. Gluten challenge studies with the aim to determine the safe threshold of prolonged exposure to trace amounts of gluten were beyond the scope of this paper. Studies reporting on positive anti-gliadin antibodies (AGA), anti-endomysial antibodies (EMA), anti-tissue transglutaminase antibodies (tTG), and anti-deamated gliadin peptide antibodies (DGP) were included. Anti-reticulin antibodies were not reviewed as this test has nowadays been replaced by the more reliable AGA test. While AGA antibodies have been in use for several decades. However, there is a wide variability in their diagnostic accuracy and both AGA-IgA and AGA-IgG have sensitivities and specificities inferior to tTG-IgA and DGP-IgA and are no longer included in the routine testing strategy for CD [5]. Positivity for CD-specific antibodies was defined as concentrations above the assay cutoff value, which varied among assays used in the different studies. The definition of abnormal mucosa histology of small bowel biopsies also varied depending on the biopsy rating scores used (e.g., villous height to crypt depth ratio, Marsh scores). Clinical symptoms in most studies comprised CD-specific symptoms including vomiting, abdominal pain or distension, obstipation, diarrhea, fatty or loose stool, anorexia, weight loss, and growth failure. Only in the summary table we specified whether symptoms constituted mild, moderate or severe symptoms. Studies that were excluded were studies investigating a single-dose gluten challenge, studies investigating oat challenge, rectal gluten challenge, transamidated, hydrolyzed or digested gluten, gluten-specific peptides, and ex-vivo studies. Since gluten intake at baseline is likely to influence the response to a gluten challenge, we excluded studies in which patients were on a normal gluten-containing diet or had positive baseline autoantibodies at start. Trials were categorized into trials enrolling pediatric or adult patients with mean age below 18 years or 18 years and older, respectively. Moreover, less patients suspected of having CD can be expected to respond to gluten than patients with a confirmed diagnosis of CD. Therefore, we classified trials according to “confirmed diagnosis based on a biopsy in the past”, or “diagnosed based on inadequate grounds” referring to as “suspected CD”. If possibly, results were reported separately for subgroups of patients with diagnosed and suspected CD in one study. When the amount of dietary gluten in bread was not reported [18–20], we estimated the gluten content, assuming that a slice of bread weighs 25–30 g and contains 8–11 g/100 g of protein [15,21], which corresponds to approximately 2–3 g of gluten [7,22]. In some studies the gluten dose was expressed per kg of body weight. If body weight was not given, estimates were based on WHO child growth charts [23].
3. Results

Table 1 gives an overview of the included studies. In total, the following studies were identified that investigated the clinical effect of dietary gluten challenge: 16 trials with pediatric patients with biopsy-diagnosed CD, 13 trials with pediatric patients with suspected CD, 11 trials with biopsy-diagnosed adult CD patients and 3 with adolescent or adult patients suspected of having CD. Of the eleven trials with diagnosed adult patients, five reported on the clinical response to a placebo as part of a clinical intervention study [24–29]. In the studies included, a gluten challenge consisted of a gluten-containing diet, wheat-derived food products, wheat flour, or wheat gluten powder. The gluten doses ranged from 0.2 to 30 g/day and duration from 1 day to 8 years.

3.1. CD-Specific Symptoms in Pediatric Patients with Diagnosed or Suspected CD

The response rates and onset of symptoms throughout the course of gluten challenge in the different studies was highly variable (Table 1); when a gluten-containing diet was given to children with diagnosed CD, 4% of them developed symptoms within 1–2 of weeks [30]. At least 10 g/day of gluten caused symptoms in 13% of children within 12 h [31], 33% of children within 4 weeks of gluten challenge [32], and 60% of children within 3-months of challenge [33]. When given a gluten-containing diet or 3–15 g/day of gluten, 32% of children experienced symptoms within 4–5 months [34]. Smaller amounts of about 2 g/day of gluten caused symptoms in 4% children on the fourth day and in 25% of children after 6 months [18].

In children with suspected CD, a gluten-containing diet induced symptoms in about 26%–33% of children within between a few days to 13 months of gluten challenge [35,36], a gluten challenge of at least 10 g/day caused symptoms in approximately 24%–42% of children from 4 weeks to several [32,37–39] months of challenge, only few patients reported severe symptoms during gluten challenge [38]. A gluten-containing diet providing 5 to 15 g/day of gliadin caused symptoms in 59% of children within 45 days of challenge [40]. About 32% of adolescents with diagnosed or suspected CD who received at least 10 g/day of gluten for 2.4 months to 2 years experienced abdominal symptoms at the time of appearance of antireticulin-IgA [41]. In 70% of the cases, the mucosa relapsed before any symptoms had occurred [41]. Lower doses of 0.2–4.3 g/day of gluten, surprisingly triggered symptoms in 79% and 96% of children within 4 and 15 weeks, respectively [42]. No correlation was observed between time of appearance of symptoms and positive antibodies.

Summarizing, in most studies only few children with diagnosed or suspected CD respond by symptoms to a low or high gluten dose during the first 2 weeks. During prolonged low or high dose gluten challenge 24%–42% of children may experience symptoms, although in three studies higher response rates were reported of 60% [33,40], and even 96% [42]. Large variability exists in time of onset of symptoms during gluten challenge: symptoms appear almost immediately in some children while some do not develop symptoms until several months of challenge or develop no symptoms at all. Symptoms are generally mild to moderate. Some studies indicated that clinical symptoms are a very unreliable indicator of antibody response and mucosal relapse [34,41,42].
Table 1. The effect of a gluten challenge in pediatric or adult patients on response rate of clinical symptoms, serology, and histology parameters.

| Author (year)          | Age Group and Age | Diagnosed/ Suspected CD | Time on Gluten-Free Diet | Gluten Type and Dose | Duration of Challenge | CD-Symptoms | CD-Antibodies | Mucosal Immunohistology | Sugar Absorption Test |
|------------------------|-------------------|-------------------------|--------------------------|----------------------|-----------------------|--------------|---------------|-------------------------|----------------------|
| Mayer et al. (1989) [31] | Children 3.5M years (1.8–9.6) | Diagnosed by biopsy (n = 37) | ≥1 year, 17Mdn months | 10 g/day gluten either as biscuit or as powder | 60Mdn (14–205) days | Acute symptoms in 13% (4/32) within 12 h and symptoms in 0% (0/31) within ~7 months | Increased AGA-IgA and IgG in 65% (20/31) within 15 days | Worsening histology score by Whitehead in 68% (21/31) at 2 month, 84% at 3 month, and 97% within 2 years | Decreased blood xylose within 15 days, remained low up to 150 day |
| Packer et al. (1978) [33] | Children 9.9M years (3.0–15.3) | Diagnosed by biopsy (n = 32) | ≥10 g/day as 4 slices white bread | Up to 3 month | Symptoms in 60% (19/32) within 3 months | | Increase in villous atrophy in 78% (25/32) within 3 months | | |
| Hamilton et al. (1972) [18] | Children 7.2M ± 1.5SD years | Diagnosed by biopsy (n = 23) | 3.8M years (0.25–11.0) | 2.25 g/day as wheat gluten followed by 1 slice/day of bread or equivalent flour (~2–3 g of gluten) | 6 days | Symptoms in 4% (1/23) at 4 day, in 8% (1/12) at 1 month, in 25% (3/12) at 6 months | Mucosal lesions in 7% (1/13) within 6 days, 92% (11/12) within 1 year, and 100% within 15 months | | |
| Mavromichalis et al. (1976) [43] | Children 6.5M years | Diagnosed by biopsy (n = 23) | 6.5M year (1.5–10) (n = 11 on gluten-free diet) | 20 g/day as gluten-containing diet | 4–9 weeks | | | Worsening histology score (III or IV on scale I–IV) in 100% (11/11) within 4–9 weeks | Increased IEL in 100% (11/11) within 4–9 weeks |
| Study            | Age Range | Diagnosis Method | Gluten Consumption | Follow-up | Test Results | Change in IEL |
|------------------|-----------|------------------|--------------------|-----------|--------------|---------------|
| Hansson et al.   | 4 years   | Biopsy (ESPGAN)  | 2–3 slices/day of white bread (~4–9 g/day 1 of gluten) | 12 weeks  | Positive     | Increased IEL |
| (1997) [44]     | (1–18)    | (n = 57)         | gluten-containing diet |           | -AGA-IgA in 30% (6/20) within 2 weeks and 75% (21/28) within 12 weeks | 25% (5/20) within 12 weeks |
| Scott et al.     | 5.8 years | Biopsy (n = 10)  | One 20 g slice/day of bread (~2 g/day 1 gluten) followed by gluten-containing diet | 11 month  | Positive     | Increased IEL |
| (1980) [19]     | (2.9–8.8) | (n = 10)         | gluten-containing diet |           | -AGA-IgA in 78% (31/40) within 2 weeks, 89% (41/46) within 12 weeks | 16% (6/38) within 12 weeks |
| Schaad et al.    | 8.1 years | Biopsy (n = 22)  | 1 g raw cooked gluten/kg/day (~25 g gluten/day 1/2) | 30 days    | Positive     | Increased IEL |
| (1981) [45]     | (3.1–13.1) | (n = 22)       | gluten-containing diet |           | -AGA-IgA in 45% (18/40) within 2 weeks, 91% (42/46) within 12 weeks | 100% (22/22) at 30 day |
| Hansson et al.   | 4 years   | Biopsy (ESPGAN)  | 2–3 slices/day of white bread (~4–9 g/day 1 of gluten) | 12 weeks  | Mucosal relapse | Increased IEL |
| (2002) [20]     | (1–16)    | (n = 57)         | gluten-containing diet |           | in 100% (10/10) within 2–11 months (7 months 1/2) | within 2–11 months |

1 gluten/day
1/2 gluten/day

Table 1. Cont.
| Study                  | Children Age Range | Diagnosis Method | Gluten-containing Diet | Follow-up | Positive AGA-IgA | Abnormal Mucosa |
|------------------------|--------------------|------------------|------------------------|-----------|------------------|----------------|
| Bürgin-Wolff et al.    | 4 months–18 years  | Biopsy (n = 135) | Dose not mentioned     | Up to 15 year | 97% (28/29) within 3 months, 85% (73/86) within 1 year, and 49% within ≥3 years | 72% (31/43) within 1 month, 94% (31/33) within 7–10 months, 95% (18/19) within 20 months |
| Ascher et al. (1990)   | 1.4 Mdn (0.5–16.5) years | Biopsy (ESPGAN) (n = 45) | Dose not mentioned | 1 year | 90% (38/42) of not-IgA deficient patients within 10 months | |
| Bodé et al. (1983)     | 2.8 Mdn (0.3–15.5) years | Biopsy (ESPGAN) (n = 14) | ≥10 g/day (type not mentioned) | 3–31 months | 79% (11/14) within 3 months–2 years | |
| Danielsson et al. (1990)| ~2 M (1–5.6) years | Biopsy (n = 67) | 10 g/day as gluten-containing diet | 0.5–4.4 years | 100% (23/23) in patients without symptoms within 4–5 months | Abnormal histology score (II–IV on scale I–IV) in 96% (64/67) within 2 years |
| Berg et al (1997)      | ~1 year            | Biopsy (n = 34) | Gluten-containing diet or 3–15 g/day of gluten | Symptoms in 32% (11/34) within 4–5 months | Abnormal histology in 100% (23/23) in patients without symptoms within 4–5 months | |
| Study                          | Age (years) | Diagnosis | Gluten Intake/Route | Duration | Symptoms | AGA IgA/IgG | EMA IgA | Other Parameters |
|-------------------------------|-------------|-----------|---------------------|----------|----------|-------------|---------|------------------|
| Troncone et al. (1994) [37]   | 4.9–9.8     | Suspected | 10 g/day as biscuits or pasta | 30 days | 42%      | ~AGA-IgA in ~50% at 2 weeks, ~25% at 1 month, ~50% at 2 months, ~70% at 3 month, ~50% at 6 months | ~AGA-IgA 6 months, ~70% at 3 month, 29% within 6 months | Increased urinary cellulose/mannitol ratio in 86% (12/14) within 3 months |
| Korponay-Szabó et al. (1997) [38] | 1.9–15.3   | Suspected | 5–10 g/day as purified gluten | 6 weeks | -Mild symptoms in 34.3% (46/134) -Severe symptoms in 2.9% (4/134) of patients with histological relapse within 6 weeks–2 years | Positive EMA-IgA or -IgG in 66% at 3 month, 90% at 6 month, and 88% (134/153) within 21 months | Abnormal histology score (scale I-III by Fontaine and Navarro) in 88% (134/153) within 2 years. Relapse time (A + B) 5 months (1.8–26.5) and (C) 6 months (1.4–25.3) |
| Rolles et al. (1976) [32]    | 1.5–15     | Suspected | 20 g/day as gluten powder | 4–13 weeks | Mild to severe symptoms in 29% (10/35) within 4–13 weeks | Abnormal histology score (scale 3 or 4 on 0–4) in 51% (18/35) within 4–13 weeks |
| Lancaster et al. (1976) [49] | 5–16.5     | Suspected | 10 g/day as wheat protein | Up to 24 month | Abnormal histology score | Decrease in Vh in 62% (10/16) within 3 months, in 81% (13/16) within 3–24 months | Increased in IEL density in 100% (13/13) within 3 months |
### Table 1. Cont.

| Study            | Age Group                      | Suspected | Time After Diagnosis | Positive |
|------------------|--------------------------------|-----------|----------------------|----------|
| Laurin et al.    | Children 3.8M (2.7–8.8 years)  | (n = 25)  | ≥1 year              |          |
| (2002) [42]      |                                 |           | 1.4M g/day (0.2–4.3) as gluten-containing diet | 13Ms week (5 week–1 year) |
|                  |                                 |           |                      | Symptoms in 79% within 4 weeks, 96% (23/24) within 15 weeks |
|                  |                                 |           |                      | - AGA-IgA in 25% (5/20) within 4 weeks, 75% within 8 weeks |
|                  |                                 |           |                      | - AGA-IgG in 0% (0/19) within 4 weeks, 5% (5/20) within 8 weeks |
|                  |                                 |           |                      | - EMA-IgA in 65% (13/20) within 4 weeks, 75% within 8 weeks |
|                  |                                 |           |                      | - EMA-IgG in 16% (3/19) within 4 weeks, 25% (5/20) within 8 weeks |
|                  |                                 |           |                      | - Abnormal histology score (3 or 4 on scale 0–4 by Marsh) in 91% (21/23) within 1 year |
|                  |                                 |           |                      | - Increased IEL count in 96% (22/23) within 1 year |
| Laurin et al.    | Children 3.8M (2.7–8.8 years)  | (n = 25)  | ≥1 year              |          |
| (2003) [50]      |                                 |           | 1.4M g/day (0.2–4.3) as gluten-containing diet | Up to 3 month |
|                  |                                 |           |                      | - AGA-IgA in 90% (16/18) within 8 weeks |
|                  |                                 |           |                      | - EMA-IgA in 90% within 8 weeks |
| Valletta et al.  | Children 3.8M (2.7–8.8 years)  | (n = 17)  | 0.4–8 years          |          |
| (1990) [40]      |                                 |           |                      | - Symptoms in 59% (10/17) within 20–45 days |
|                  |                                 |           |                      | - AGA-IgA in 94% (16/17) within 15–35 days |
|                  |                                 |           |                      | - EMA-IgA in 90% within 2 months |
|                  |                                 |           |                      | - Worsening histology score in 94% (16/17) within 25–45 days |
|                  |                                 |           |                      | - Increased IEL score in 100% (17/17) within 25–45 days |
| Jansson et al.   | Children 2.7M ± 1SD years       | (n = 54)  | ≥1 year              |          |
| (2001) [51]      |                                 |           | 4–8 weeks            |          |
|                  |                                 |           |                      | - AGA-IgA in 76% (38/50) at 2 week, 88% at 4 week, 94% at 8 week |
|                  |                                 |           |                      | - EMA-IgA in 59% (32/54) at 2 week, 65% at 4 week, 67% at 8 week |
|                  |                                 |           |                      | - Comparing treatment A/B (≥3 increase on scale 4–16) |
|                  |                                 |           |                      | - Comparing treatment A/B in 94% (51/54) at 4 week and 100% at 8 week |
| Study               | Participants          | Suspected (n) | Gluten Powder/Diet | Symptoms | Positive Tests | Abnormal Pathology |
|---------------------|-----------------------|---------------|--------------------|----------|----------------|---------------------|
| Wauters et al. (1991) [39] | Children 5.6M (2–16) years | (n = 17) 46⁺ months (10–168) | Gluten powder: 750 mg/kg bw/day (~14 g/day) with max 20 g/day | Symptoms in 24% (4/17) within 3 months | Positive -AGA-IgA in 90% (9/10) within 6 weeks, and 100% (7/7) within 12 weeks | Villous atrophy in 59% (10/17) within 12 weeks |
| Savilahti et al. (1983) [35] | Children 1.6M years | (n = 19) 0.7–2.3 years | Gluten-containing diet (dose not mentioned) | Symptoms in 26% (5/19) within 0.1–1.1 year | Positive AGA-IgA in 73% (11/15) within 0.1–1.1 year | Abnormal mucosa in 95% (18/19) within 0.1–1.1 year |
| Rolles et al. (1975) [52] | Children 0.5–5.7 years | (n = 16) 0.1–5 years | 20 g/day as gluten powder | Symptoms in 33% (5/15) within 28 days | | |
| Bonamico et al. (2005) [36] | Children and adolescents 9.2M (5.4–19) years | (n = 24) | Three gluten-containing meals/day (n = 24) | Symptoms after in vivo challenge in 33% (8/24) within few days–2 months | Positive EMA-IgA in 63% (15/24) within 2 months | Abnormal histology score (3 on scale 0–3 by Marsh) in 87% (13/15) within 2 months |
| Mäki et al. (1989) [41] | Adolescents 16.6M (14.3–22.1) years | (n = 9 and diagnosed by biopsy (ESPGAN) (n = 20) | Suspected 8⁺ years (3.0–16.0) | Symptoms in 32% (7/22) anti-reticulin positives within 2.4–24 months | Positive -AGA-IgA in 79% (23/29) within 2.4–24 months -AGA-IgG in 62% (18/29) within 2.4–24 months | Lower Vh in 85% (23/27) within 2.4–24 months. 15% (4/27) did not relapse in 2 years |
| Study                          | Age (Gender) | Diagnosis | Follow-up | Gluten Intake | Symptoms/Tests                                                                 |
|-------------------------------|--------------|-----------|-----------|---------------|--------------------------------------------------------------------------------|
| Lancaster-Smith et al. (1975) | Adults (11)  | Biopsy   | 4.3 years | 25 g (n=8) or 20 g (n=4) | A: Increased IEL in 24–48 h (A) B: Increased IEL in 100% within 1 week (B) |
| Lähdeaho et al. (2011)        | Adults: 49M  (21–68) | Biopsy (n=21) | 11.8 (2–34) years | 1–3 g/day (n=8) or 10 or 20 g/day (n=4) | A: Symptoms in 64% (7/11) within 3 months (A) B: Symptoms in 80% (8/10) within 3 months (B) |
| Leffler et al. (2012)         | Adults 43M  ± 14SD years | Biopsy (n=20) | 5 years | 3 or 7.6 g/day (n=20) | Positive tTG-IgA in 25% (5/20) within 2 weeks (increase to 50% 2 weeks post-challenge) DGP-IgA/IgG in 30% (6/20) within 2 weeks |
| Montgomery (1988)             | Adults 46M (17–74) | Biopsy (n=13 on GFD) | 13.8 (6–27) months | 2.5–5 g/day | Positive AGA-IgA in 17% (11/13) within 3–14 months |

Table 1. Cont.
| Study                  | Age Range          | Diagnosed by biopsy | Duration of GFD | Gluten Intake       | Subsequent Effects                                                                 |
|-----------------------|--------------------|---------------------|-----------------|---------------------|-----------------------------------------------------------------------------------|
| Brottveit (2011) [56] | Adults 41 years (16–65 years) | Biopsy (n = 13 on GFD) | 13.9 (0.8–31.6) years | 40 g/day (four slices bread) | 3 days Abnormal histology score (3 or 4 on scale 0–4 by Marsh) in 23% (4/13) within 3 days |
| Daveson (2011) [57]   | Adults 44 years (25–58 years) | Biopsy (n = 10 on GFD in control group) | ≥6 months | 16 g/day (two slices bread) | 5 days Abnormal histology score (3 or 4 on scale 0–4 by Marsh) in 70% (7/10) within 1 week Increased IEL at 1 week |
| Cornell et al. (2005) [25] | Adults 18–70 year | Biopsy (n = 21 on placebo) | Not reported | 3 Cracker biscuits (~1.3 g/day gluten) | 2 weeks >5 Episodes of moderate-to-severe symptoms in 33% (7/21) on placebo within 2 weeks challenge and the following 10 week Positive tTGA >5 U/mL in 19% (4/21) within 2 weeks and 3–15 weeks post-challenge -Increased lymphocyte score in 83% (5/6) at 2 week -Increased epithelial stunting in 50% (3/6) at 2 week |
| Tye-Din et al. (2010) [26] | Adults 41 years (21–67 years) | Biopsy (n = 10 on placebo) | ≥8 weeks | 16 g/day Wheat flour slurry | 3 days Symptoms increased within 1 week, 75% of symptoms were mild No positive tTGA and DGP-IgA/IgG at 6 day |
| Kelly et al. (2013) [27] | Adults 18–65 years | Biopsy (n = 44 on placebo) | ≥6 weeks | 2.7 g/day Gluten powder (3 × daily 0.9 g) | 6 weeks Symptoms increased in 80% within 6 weeks. Plateau at 3 week Positive tTG-IgA > 10 U/mL in 30% (13/44) at 6 week Increase in urinary lactulose:mannitol ratio. Plateau at 4 week |
| Study | Participants | Diagnosis Method | Gluten Intake | Duration | Symptoms | TG-IgA | Increase in urinary lactulose:mannitol ratio |
|-------|--------------|------------------|--------------|----------|----------|--------|-----------------------------------|
| Leffler <i>et al.</i>, 2012b [28] | Adults 18–72 years | Biopsy (<i>n</i> = 14 on placebo) | 2.4 g/day Gluten powder (<i>3 × daily 0.8 g</i>) | ≥6 weeks | Symptoms increased in 50% within 2 weeks | No positive TG-IgA at 2 week | -Increased TG-IgA deposits in 71% (5/7) |
| Tack <i>et al.</i>, 2010 [58] | Adults 55 (20–68) years | Biopsy (<i>n</i> = 7 on placebo) | 7 g/day (<i>5 toasts</i>) | 2 weeks | Symptoms increased in 43% (3/7) within 2 weeks | No positive EMA-IgA within 2 weeks | -Abnormal histology score (3 or 4 on scale 0–4 by Marsh) in 23% (2/7) Within 2 weeks |
| Kumar <i>et al.</i> (1979) | Adolescents: 16.1 (14–21) years<br>Adults: 37.7 (17–59) years | Suspected (<i>n</i> = 28) | ≥4 Slices bread (<i>~10 g/day gluten</i>) | 4–17.5 weeks (<i>23 days</i>)<br>Adults: median 4–25.5 weeks (<i>11.5 days</i>) | Symptoms in 67% (6/9) within 1 h–2 weeks, no symptoms in 33% within 1 year<br>Adults: Symptoms in 84% (16/19) within 4 days–3 months, no symptoms in 16% within 1 year | Decreased Vh in 56% (5/9) within 8 weeks, 100% within 10 months<br>Increased IEL in 100% within 10 months<br>Adults: Decreased Vh in 95% (18/19) within 7 weeks, still 95% within 1 year<br>Increased IEL in 95% within 7 weeks |
| Wahab (2001) [60] | Adults 40 (16–74) years | Suspected (<i>n</i> = 37) | 30 g/day on top of GCD | 2 months | Symptoms in 55% (17/38) within 2 months<br>Positive EMA-IgA in 17% (4/23) within 2 months | Abnormal histology score (2, 3 or 4 on scale 0–4 by Marsh) in 32% (12/38) at 2 month |
| Kaukinen <i>et al.</i> (2005) [61] | Adults 45 (19–70) years | Suspected (<i>n</i> = 21) | ≥15 g/day (<i>5 Slices of bread</i>) | 6 months | -Increased TG-IgA deposits in 24% (5/21) |

Abbreviations: CD: Coeliac Disease, Vh: villous height, Cd: crypt depth, M: mean; Mdn: median; SD: standard deviation. ¹ Assumption made as described in the Methods. ² Assuming an 8-years old child weighs 25 kg. ³ Assuming a 2.7-years old child weighs 13 kg. ⁴ Assuming a 5.6-year old child weighs 19 kg.
3.2. CD-Specific Symptoms in Adults with Diagnosed or Suspected CD

In three studies, effects were reported of gluten challenge on symptoms in diagnosed or suspected adult or adolescent CD patients [54,59,62]. In five clinical trials [25–28,58], the effects of gluten challenge given to diagnosed adult patients in the placebo arm were reported. Reintroduction of a gluten-containing diet induced gastrointestinal symptoms in 77% of patients suspected of CD between 1 and 8 months of challenge and CD was confirmed in 40% of these patients [62]. The diagnosis CD was nevertheless confirmed in 65% of the 33% patients who did not develop symptoms. Symptoms occurred in 67% of patients with confirmed diagnosis of CD. When diagnosed or suspected CD patients received 7 to 10 g/day of gluten, 43% [58] and 67% [59] reported symptoms within two weeks of challenge. Within 3 months 84% had experienced symptoms [59]. Most symptoms occurred after one week of challenge [59]. After a 2-week and 6-week challenge period with about 2.5 g/day of gluten three times daily, about 50% of patients [28] and 80% of patients [27] reported complaints, respectively. The severity of symptoms increased after 2 weeks [27,28] reaching a plateau at 3 weeks [27]. When diagnosed CD patients received a low (1–3 g/day) or a high (3–5 g/day) dose of gluten, 64% and 80% of them, respectively, reported symptoms within 3 months [54]. A 2-week challenge of ~1.3 g/day of gluten triggered symptoms in 66% of patients the following 12 weeks; about 33% had more than five episodes of moderate to severe symptoms [25].

In summary, the number of adult patients reporting symptoms as well as the severity of symptoms may increase throughout gluten challenge. Within 3 months of gluten challenge, about 64%–80% of adult patients can be expected to experience symptoms. A proportion of patients with CD may never develop symptoms during gluten challenge. The onset of symptoms is rather unpredictable. The appearance of symptoms during gluten challenge is no indicator of CD.

3.3. Antibodies in Pediatric Patients with Diagnosed or Suspected CD

3.3.1. AGA-IgA and AGA-IgG Antibodies

Figure 1 illustrates the time course of children with diagnosed or suspected CD responding to a gluten challenge by positive AGA-IgA antibodies.

The proportion of children with diagnosed or suspected CD responding to gluten challenge by AGA-IgA antibodies varied widely. After 2 weeks, about 30% to 78% of children had responded to a challenge providing 3 to 15 g/day of gluten [20,31,44,63,64]. After 2 to 3 months of challenge with 4 to 14 g/day of gluten, about 70%–100% of children showed positive AGA-IgA antibodies in their serum [20,44,46,63,64]. No clear dose-response effect was observed between the different studies. In two studies with a low dose of gluten (0.2–4.3 g/day), the percentage of children responding by AGA-IgA was 90% [50] or 75% after 2 months [42]. Within 10 months to 1 year, 73%–90% of children had developed AGA-IgA antibodies [30,35,46]. Interestingly, the percentage of CD children with AGA-IgA was highest (97%) after a gluten consumption period of about 1 to 3 months and decreases thereafter to 85% at 1 year, and 49% after 3 years or more of gluten intake [46].
Figure 1. Percentage of pediatric patients with diagnosed or suspected coeliac disease (CD) showing an anti-gliadin antibodies (AGA)-IgA response to gluten over time.

Figure 2 shows the proportion of diagnosed or suspected CD children responding with positive AGA-IgG antibodies to a gluten challenge.

Figure 2. Percentage of pediatric patients with diagnosed or suspected CD showing an AGA-IgG response to gluten over time.

Whereas most studies showed less children responding by AGA-IgG than by AGA-IgA throughout the course of gluten challenge [41,42,44,63], two studies showed similar response rates by AGA-IgA and AGA-IgG [31,39]. When children with CD were given a gluten-containing diet or 10 to 14 g/day of gluten, AGA-IgG rose significantly in 15% [44] or 65% [31] of children within 2 weeks and in 71%–100% of children within 3 months of challenge [39,44]. In two studies in which children
received 10 g/day [63] or 0.2–4.3 g/day [42] of gluten, only 25% and 5% of children had responded by AGA-IgA after 2 months of gluten challenge, respectively.

3.3.2. EMA-IgA Antibodies

Figure 3 summarizes the proportion of children with diagnosed or suspected CD developing positive EMA-IgA during gluten challenge.

**Figure 3.** Percentage of pediatric patients with diagnosed or suspected CD showing an anti-endomysial antibodies (EMA)-IgA response to gluten over time.

In a number of trials with children with diagnosed or suspected CD, results on serum EMA-IgA levels were reported during gluten challenge doses from 0.2 to 15 g/day of gluten or a gluten-containing diet. After 2 weeks of challenge, 35% to 59% of children showed positive EMA-IgA antibodies [20,36,40,42,44,46,63–65], 65% to 77% of children after 1 month [42,51,63], between 63% and 100% of children became EMA-IgA positive between 2 and 3 months [20,36,38,40,42,44,46,51], while 84% to 93% of children had become positive from 6 months to 3 years of challenge [38,46,63]. Even small gluten amounts caused relapse by EMA-IgA [42]. There was no clear difference in time to EMA-IgA positivity between the different gluten doses.

3.3.3. tTG-IgA Antibodies

In one study, diagnosed CD children received 4–9 g/day of gluten [20]; positive tTG-IgA levels were detected in 45% and 89% of children within 2 and 12 weeks, respectively.

3.3.4. Antibodies in Pediatric Patients: Summary

In summary, the time it takes for children to relapse by antibodies with a gluten challenge is variable. Moderate-to-high gluten challenge doses given to children with diagnosed or suspected CD increased
AGA-IgA, AGA-IgG and EMA-IgA to positive levels within the first few weeks. Within 3 months of challenge, the majority of children had developed AGA-IgA, AGA-IgG, EMA-IgA or tTG-IgA antibodies. Only few children relapsed by AGA-IgA, AGA-IgG and EMA-IgA after 1 year. No clear difference in relapse rate to gluten was observed between children with diagnosed and suspected CD. Conversion of AGA-IgA positive to negative tests has been reported to occur in some patients.

3.4. Antibodies in Adult Patients with Diagnosed or Suspected CD

3.4.1. AGA-IgA and EMA-IgA Antibodies

In four trials, AGA-IgA antibody titers in gluten-challenged adult patients with suspected or diagnosed CD were reported [26,29,55,60]. The AGA-IgA titers increased in 14% of diagnosed patients in the placebo arm after a 2-week 7 g/day gluten challenge [58] and in 85% of diagnosed patients receiving 2.5–5 g/day of gluten for up to 14 months [55]. Increased AGA-IgA was observed in 22% of borderline patients receiving 30 g/day on top of a normal diet for up to 2 months [60], None of the diagnosed CD patients receiving 16 or 7 g/day of gluten developed positive EMA-IgA antibodies within 2 weeks [29,58]. In borderline patients, 17% became EMA-IgA positive after a 2-month very high-dose gluten challenge [60].

3.4.2. tTG-IgA and DGP-IgA/IgG Antibodies

Figure 4 shows diagnosed adult CD patients responding by tTG-IgA throughout gluten challenge.

**Figure 4.** Percentage of adult patients with diagnosed CD showing a tTG-IgA response to gluten over time.

The effects of gluten challenge on tTG-IgA titers in diagnosed adult CD patients were reported either [24–29] or not [54] as part of a clinical trial. No positive tTG-IgA antibodies were observed at day 6 post-challenge in any of the diagnosed CD patients receiving 16 g of gluten for 3 days [26]. After a 2-week challenge with a dose from 1.3 to 7.6 g/day of gluten, tTG-IgA increased in
0% to 25% of adult diagnosed CD patients [24,25,28,29]. Longer gluten challenge form 6 weeks to 3 months increased the proportion of tTG-IgA-positive patients to 30%–43% [27,54]. A 3-day gluten challenge of 16 g/day did not increase DGP-IgA/IgG titers at day 6 [26], but 3 or 7.5 g/day induced positive DGP-IgA/IgG titers in 30% of diagnosed patients within two weeks and 45% the following two weeks [24].

3.4.3. Antibodies in Adult Patients: Summary

In summary, few diagnosed CD patients responded by AGA-IgA, EMA-IgA, tTG-IgA, or DGP-IgA/IgG antibodies after 2 weeks of gluten challenge. Within 6 weeks to 3 months of gluten challenge, still no more than 50% of patients became positive for these antibodies.

3.5. Mucosal Immunohistology in Pediatric Patients with Diagnosed or Suspected CD

3.5.1. Mucosal IEL

When children with diagnosed or suspected CD received 5 to 25 g/day of gluten, 91% to 100% of them developed increased mucosal IEL within 1 to 2 months [40,42,43,45,50]. Within 3 months of gluten challenge with 10 g/day, all children with suspected CD showed increased mucosal IEL counts [49]. In one study less children, 16% to 25%, responded with increased IEL within 3 months of about 6 g/day of gluten challenge [20,44]. The authors of one study found that the gluten intake dose strongly correlated with the degree of inflammation in the biopsy, as expressed by IEL [42]. A gluten challenge increased IEL in mucosal biopsies before histological changes occurred.

3.5.2. Mucosal Histology

Figure 5 gives an overview of children with diagnosed or suspected CD developing changes in mucosal morphology throughout gluten challenge.

The proportion of children with diagnosed or suspected CD having abnormal mucosal histology gradually increased during the course of gluten challenge. Only 7% of children with diagnosed CD developed mucosal lesions after 1 week when a gluten challenge of 2–3 g/day of gluten was given [18]. However, when doses of 3 to 20 g/day of gluten were given, the proportion of children developing an abnormal small bowel mucosal histology scores within 1 month ranged from 72% to 100% [43,46,64]. Mucosal relapse rates after 2 to 3 months of challenge ranged between 51% and 100% [31–33,36,39,49,64]. After 5 months to 2 years of gluten challenge, the majority of children have relapsed by mucosal abnormalities, with relapse rates of 79% to 100% reported in the different studies [18,19,31,33,34,41,42,46,48,49,52]. After 1 year low-dose gluten challenge (0.2 to 4.3 g/day gluten), the proportion of children showing abnormal small bowel mucosal histology scores did not differ from those receiving a higher dose challenge (5 g/day or higher). For some children it took 2 or even 8 years to relapse on gluten [48]. As expected, the intestinal mucosa relapse rate was higher in diagnosed than in suspected patients: within 3 months of gluten intake, respectively, about 60% and 80% had relapsed.
3.5.3. Mucosal Immunohistology: Summary

In summary, within 1 month of gluten exposure, mucosal IEL counts were increased in almost all children with diagnosed or suspected CD. The percentage of children developing moderate to severe mucosal histological abnormalities within 2 to 3 months of gluten challenge ranged between 51%–100%. When child patients are biopsied after one week of challenge, only a minority show morphological relapse. The majority of children will have relapsed after 2 to 3 months of challenge, and only few children relapse thereafter.

3.6. Mucosal Immunohistology in Adult Patients with Diagnosed or Suspected CD

3.6.1. Mucosal IEL

A single 25-g gluten challenge given to adult patients with proven CD increased IEL in the mucosal biopsy as soon as 24–48 h following challenge [49]. A one-week 10 to 20 g gluten-containing diet increased IEL density in the mucosal biopsy of all patients [49,57]. Lower gluten doses (3–7.6 g/day) also increased mucosal IEL of patients within 2 weeks [24]. Gluten challenges of 10–25 g/day increased mucosal IEL counts in 95% to 100% of adult or adolescent patients with diagnosed or suspected CD within 1 to 2 months [59], and 3 to 14-months [55,59]. Increased IEL were found in 55% and 80% of diagnosed CD patients receiving, respectively, 1–3 g/day and 3–5 g/day of gluten for 3 months [54].
3.6.2. Mucosal Histology

Figure 6 illustrates the proportion of adult diagnosed or suspected CD patients responding by abnormal small bowel mucosal histology throughout a gluten challenge.

**Figure 6.** Percentage of adult patients with diagnosed or suspected CD showing histological response to gluten over time.

Increased biopsy Marsh scores were observed in 23% of diagnosed CD patients receiving 40 g/day of gluten for 3 days [56], and in 70% of diagnosed CD patients receiving 16 g/day of gluten for 5 days [57]. Gluten doses between 1 and 7 g/day induced abnormal histology scores in 23% to 68% of adult diagnosed CD patients within 2 weeks [24,25,29], and 67% of patients within 3 months [54]. In adolescents and adult patients with suspected CD, 10 g/day of gluten triggered mucosal relapse in 56% and 95% of them within 2 months, and 95% and 100% of them within 1 year, respectively [59]. Less patients with borderline CD may respond to gluten; 32% showed abnormal histology scores within 2 months of high-dose gluten challenge [60].

3.6.3. Mucosal tTGA-IgA Deposits

A gluten challenge of at least 15 g/day for 6 months induced positive tTGA-specific mucosal IgA deposits in 24% of suspected CD patients [61].

3.6.4. Mucosal Immunohistology: Summary

In summary, the results of gluten challenge on mucosal histology in adult patients are variable. More than two weeks of high-dose gluten challenge may be required to induce small intestinal mucosal morphology changes in the majority of patients. However, IEL can appear as early as 1 to 2 days after gluten challenge with increased counts in all patients after 4 weeks. Mucosal tTGA-IgA deposits is another marker appearing in the majority of patients within 2 weeks of challenge.
4. Discussion

4.1. Strength and Weaknesses

To our knowledge, this is the first review giving an overview of gluten challenge studies in patients suffering from CD or suspected of having CD and the consequences on symptoms, mucosal damage and CD-specific antibodies. In this review, we excluded studies with patients who were on a regular gluten diet at the time of challenge, as their response to gluten may be lower and not representative for patients on a gluten-free diet. This review has, however, several limitations. The gluten challenges used in all studies were wheat-derived and hence, findings relate to wheat gluten. There is very limited data looking at the effect of barley hordein or rye secalin on CD outcomes in the published literature (e.g., [66,67]), but evidence exists that these prolamins induce effects different to wheat gluten, at least at an immunologic level. Moreover, the quoted gluten amounts in the publications were mostly estimates and probably not accurate. In a few studies, gluten amounts were analyzed by R5 ELISA probably providing better estimates. Although several studies looked at the effect of gluten challenge in pediatric patients, the number of studies with adult patients is limited. The clinical response to gluten is most likely larger in diagnosed CD patients than in patients suspected of having CD in whom part may not have CD. Nevertheless, the results for both groups were combined in the figures. Furthermore, the participants in the different studies convey a heterogeneous group with respect to age, gluten dose, and time on a gluten-free diet, and criteria for diagnosis and are therefore difficult to compare. Also methodologies for measurement of antibodies, biopsies, and histology were different including the cutoff levels used to define antibody or histological positivity. Another limitation is that in most studies in the seventies to nineties, AGA-IgA and AGA-IgG antibodies were most commonly measured. However, particularly AGA-IgA has a poor sensitivity compared to newer antibodies such as EMA-IgA, tTG-IgA, and DGP-IgA/IgG which may have resulted in an underestimation of the patients responding to gluten by positive AGA-IgA titers.

4.2. Occurrence of Symptoms in Response to Gluten

Until recently, no proper guidelines for categorizing symptoms were available, making it difficult to compare the symptoms reported in the different studies. Moreover, symptoms in response to gluten are not CD-specific as approximately half of non-coeliac patients also show exacerbation of symptoms during gluten challenge [62]. Gastrointestinal symptoms are not specific for CD. The predictive value of symptoms after gluten re-introduction or gluten challenge is very low [34,38,39,41,62]. In one study the positive predictive value of symptoms for having CD was 52% [62]. In diagnosed adult patients, the symptom response rate seems to range somewhere between 65% and 85% [26,59] and most symptoms seem to occur within 1 to 2 weeks [26,59]. Less children (24%–42%) than adults (64%–80%) reported symptoms throughout prolonged gluten challenge but this may strongly depend on the methodology used in the different studies.

In summary, symptoms upon gluten challenge are hard to predict and have low positive predictive value. Recently, a validated disease-specific symptom index for coeliac disease was developed, but it remains to be established whether this can be used as an independent outcome measure for the monitoring of coeliac disease [68].
4.3. Occurrence of Antibodies in Response to Gluten

The CD-specific antibody and mucosal response is more predictable than the appearance of symptoms. Nevertheless, considerable variation between patients exists in the time to serological relapse on gluten [17]. On average, about 70%–100% of diagnosed pediatric CD patients given a moderate to high-dose gluten challenge will have responded by AGA-IgA, EMA-IgA, and tTG-IgA antibodies within 3 months of moderate-to-high gluten intake. Less children responded to gluten by AGA-IgG than by AGA-IgA. Compared to diagnosed patients, slightly less patients suspected of CD developed positive antibodies, but the majority had responded by 3 months. Also low dose prolonged gluten challenge caused serological or histological relapse in children with (suspected) CD [25,69]. In these studies, mucosal changes to gluten correlated with the gluten dose given, suggesting a dose-dependent response to gluten. Histological relapse occurred faster in children receiving a larger gluten dose in children with diagnosed CD [51,70], also suggesting a dose-response effect. Therefore, when testing serological antibodies during gluten challenge of approximately 15 g/day on a 3 to 6 monthly basis as recommended by the current ESPGHAN recommendations [4], most cases of CD should be detected. While the majority relapses in three months, for a few patients it may take longer to relapse, and in rare cases it may take years to relapse. Conversion to antibody negativity during prolonged gluten intake has been reported, suggesting that in rare cases gluten tolerance may develop [46,71].

In adult patients, the few available studies suggest that no more than half of the patients develop positive serum antibodies (AGA-IgA, EMA-IgA, tTG-IgA, and DGP-IgA/IgG) in response to a 6-week to 3-month gluten challenge. The few available studies suggest that the AGA-IgA and EMA-IgA response rates of adult CD patients to high-dose gluten challenge was very low. This suggests a lower response in diagnosed adult than pediatric patients. Whether this lower antibody responsiveness to gluten in adults is due to a longer period of gluten withdrawal remains to be established.

4.4. Occurrence of Histological Changes in Response to Gluten

About 50% to 100% of children with diagnosed or suspected CD developed moderate to severe mucosal histological abnormalities within 2 to 3 months of gluten challenge. Comparable response rates were reported for adult patients. As can be expected, the average 3-month relapse rate in patients with diagnosed CD was generally higher than those with suspected CD. Some patients may still show histological relapse on gluten challenge continuing up to 1 or 2 years.

The earliest stages of gluten challenge include increased density of IEL in the mucosa, crypt hyperplasia, and finally, the development of villous atrophy [72], which was confirmed by the reviewed data. The gluten challenge studies showed that mucosal IEL infiltrates respond fast to gluten (days to weeks) whereas the CD-associated antibodies and mucosal morphological deterioration appeared later within weeks to years. In some studies, relapse by abnormal histology of the small bowel biopsy paralleled positive antibodies [38,40,41,51]. In other studies, antibodies and primarily AGA-IgA preceded the worsening of mucosal histology [30,31,39,46] whereas in one study the mucosal changes preceded serum EMA-IgA positivity during gluten challenge [36]. The AGA-IgA antibodies may appear earlier than EMA-IgA during gluten challenge [37,46]. Both in adults and children, symptoms were unpredictable and did not coincide with histological or serological relapse.
Within one month of gluten challenge, serological and histological relapse does not occur in all cases during challenge [31,32]. In contrast, increased mucosal IEL were reported in almost all diagnosed pediatric and adult patients within 1 month of gluten challenge [43,45,49,53]. High IEL counts in the mucosa are therefore a fast and sensitive marker of responsiveness to gluten although not specific for CD [42,73–75]. In addition, mucosal tTG-IgA deposits are considered to appear rapidly in response to gluten and are both a sensitive and specific marker of early stage CD present in biopsy samples with normal mucosal architecture [61,76]. Although not reviewed in this paper, tetramer staining of gluten-specific T-cells may be supportive in the diagnosis of CD due to the fast appearance after start of a gluten challenge [77].

5. Conclusions

To diagnose pediatric patients with suspected CD on a gluten-free diet, a moderate-to-high dose gluten challenge for up to 3 months should be sufficient to induce changes in mucosal histology and antibodies in the majority of patients. In adults on a gluten-free diet, histological and serological relapse rates to gluten may be slower and prolonged challenge may be considered if no relapse is observed. Moreover, testing for combinations of conventional and new early markers with high sensitivity and specificity will significantly shorten the time of gluten challenge to diagnose CD.

Acknowledgments

The author is an employee of DSM Biotechnology Center (Delft, The Netherlands). The author would like to acknowledge Sheryl Roumen (DSM Biotechnology Center) who substantially contributed to searching the literature and compiling the data.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Coeliac Disease: Recognition and Assessment of Coeliac Disease, 2009. Available online: http://www.nice.org.uk/nicemedia/pdf/cg86fullguideline.pdf (accessed on 7 August 2013).

2. Richey, R.; Howdle, P.; Shaw, E.; Stokes, T.; Guideline Development Group. Recognition and assessment of coeliac disease in children and adults: Summary of NICE guidance. BMJ 2009, 338, b1684.

3. Walker-Smith, J.A.; Guandalini, S.; Schmitz, J.; Shmerling, D.H.; Visakopi, J.L. Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. Arch. Dis. Child 1990, 65, 909–911.

4. Husby, S.; Koletzko, S.; Korponay-Szabo, I.R.; Mearin, M.L.; Phillips, A.; Shamir, R.; Troncone, R.; Giersiepen, K.; Branski, D.; Catassi, C.; et al. European society for pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coeliac disease. J. Pediatr. Gastroenterol. Nutr. 2012, 54, 136–160.
5. Rubio-Tapia, A.; Hill, I.D.; Kelly, C.P.; Calderwood, A.H.; Murray, J.A.; American College of Gastroenterology. ACG clinical guidelines: Diagnosis and management of celiac disease. *Am. J. Gastroenterol.* **2013**, *108*, 656–676.

6. Hill, I.D.; Dirks, M.H.; Liptak, G.S.; Colletti, R.B.; Fasano, A.; Guandalini, S.; Hoffenberg, E.J.; Horvath, K.; Murray, J.A.; Pivor, M.; *et al.* Guideline for the diagnosis and treatment of celiac disease in children: Recommendations of the North American society for pediatric gastroenterology, hepatology and nutrition. *J. Pediatr. Gastroenterol. Nutr.* **2005**, *40*, 1–19.

7. Murch, S.; Jenkins, H.; Auth, M.; Bremner, R.; Butt, A.; France, S.; Furman, M.; Gillett, P.; Kiparissi, F.; Lawson, M.; *et al.* Joint BSPGHAN and Coeliac UK guidelines for the diagnosis and management of coeliac disease in children. *Arch. Dis. Child* **2013**, *98*, 806–811.

8. Catassi, C.; Fasano, A. Is this really celiac disease? Pitfalls in diagnosis. *Curr. Gastroenterol. Rep.* **2008**, *10*, 466–472.

9. Ravelli, A.; Bolognini, S.; Gambarotti, M.; Villanacci, V. Variability of histologic lesions in relation to biopsy site in gluten-sensitive enteropathy. *Am. J. Gastroenterol.* **2005**, *100*, 177–185.

10. Corazza, G.R.; Villanacci, V.; Zambelli, C.; Milione, M.; Luinetti, O.; Vindigni, C.; Chioda, C.; Albarello, L.; Bartolini, D.; Donato, F. Comparison of the interobserver reproducibility with different histologic criteria used in celiac disease. *Clin. Gastroenterol. Hepatol.* **2007**, *5*, 838–843.

11. Corazza, G.R.; Villanacci, V. Coeliac disease. *J. Clin. Pathol.* **2005**, *58*, 573–574.

12. Weile, B.; Hansen, B.F.; Hagerstrand, I.; Hansen, J.P.; Krasilnikoff, P.A. Interobserver variation in diagnosing coeliac disease. A joint study by Danish and Swedish pathologists. *APMIS* **2000**, *108*, 380–384.

13. Freeman, H.J. Refractory celiac disease and sprue-like intestinal disease. *World J. Gastroenterol.* **2008**, *14*, 828–830.

14. Guidelines for the Management of Patients with Coeliac Disease. British Society of Gastroenterology. Available online: www.bsg.org.uk (accessed on 8 April 2013).

15. United States Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 26. Available online: http://ndb.nal.usda.gov/ (accessed on 24 October 2013).

16. Setty, M.; Hormaza, L.; Guandalini, S. Celiac disease: Risk assessment, diagnosis, and monitoring. *Mol. Diagn. Ther.* **2008**, *12*, 289–298.

17. Akobeng, A.K.; Thomas, A.G. Systematic review: Tolerable amount of gluten for people with coeliac disease. *Aliment. Pharmacol. Ther.* **2008**, *27*, 1044–1052.

18. Hamilton, J.R.; McNeill, L.K. Childhood celiac disease: Response of treated patients to a small uniform daily dose of wheat gluten. *J. Pediatr.* **1972**, *81*, 885–893.

19. Scott, H.; Ek, J.; Baklien, K.; Brandtzæg, P. Immunoglobulin-producing cells in jejunal mucosa of children with coeliac disease on a gluten-free diet and after gluten challenge. *Scand. J. Gastroenterol.* **1980**, *15*, 81–88.

20. Hansson, T.; Dahlbom, I.; Rogberg, S.; Dannaeus, A.; Hopfl, P.; Gut, H.; Kraaz, W.; Klareskog, L. Recombinant human tissue transglutaminase for diagnosis and follow-up of childhood coeliac disease. *Pediatr. Res.* **2002**, *51*, 700–705.
21. Dutch Food Composition Database Online Version 2013/4.0. Available online: http://www.rivm.nl/en/Topics/Topics/D/Dutch_Food_Composition_Database (accessed on 24 October 2013).

22. Van Overbeek, F.M.; Uil-Dieterman, I.G.; Mol, I.W.; Köhler-Brands, L.; Heymans, H.S.; Mulder, C.J. The daily gluten intake in relatives of patients with coeliac disease compared with that of the general Dutch population. *Eur. J. Gastroenterol. Hepatol.* 1997, 9, 1097–1099.

23. World Health Organisation. The WHO Child Growth Standards. Available online: http://www.who.int/childgrowth/en/ (accessed on 7 August 2013).

24. Leffler, D.; Schuppan, D.; Pallav, K.; Najarian, R.; Goldsmith, J.D.; Hansen, J.; Kabbani, T.; Dennis, M.; Kelly, C.P. Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease. *Gut* 2012, 62, 996–1004.

25. Cornell, H.J.; Macrae, F.A.; Melny, J.; Pizzey, C.J.; Cook, F.; Mason, S.; Bhathal, P.S.; Stelmasiak, T. Enzyme therapy for management of coeliac disease. *Anon. Scand. J. Gastroenterol.* 2005, 40, 1304–1312.

26. Tye-Din, J.A.; Anderson, R.P.; Ffrench, R.A.; Brown, G.J.; Hodsman, P.; Siegel, M.; Botwick, W.; Shreeniwas, R. The effects of ALV003 pre-digestion of gluten on immune response and symptoms in celiac disease *in vivo*. *Clin. Immunol.* 2010, 134, 289–295.

27. Kelly, C.P.; Green, P.H.; Murray, J.A.; Dimarino, A.; Colatrella, A.; Leffler, D.A.; Alexander, T.; Arsenescu, R.; Leon, F.; Jiang, J.G.; et al. Larazotide acetate in patients with coeliac disease undergoing a gluten challenge: A randomised placebo-controlled study. *Aliment. Pharmacol. Ther.* 2013, 37, 252–262.

28. Leffler, D.A.; Kelly, C.P.; Abdallah, H.Z.; Colatrella, A.M.; Harris, L.A.; Leon, F.; Arterburn, L.A.; Paterson, B.M.; Lan, Z.H.; Murray, J.A. A randomized, double-blind study of larazotide acetate to prevent the activation of celiac disease during gluten challenge. *Am. J. Gastroenterol.* 2012, 107, 1554–1562.

29. Tack, G.J.; van de Water, J.M.; Bruins, M.J.; Kooy-Winkelaar, E.M.; van Bergen, J.; Bonnet, P.; Vreugdenhil, A.C.; Korponay-Szabo, I.; Edens, L.; von Blomberg, B.M.; et al. Consumption of gluten with gluten-degrading enzyme by celiac patients: A pilot-study. *World J. Gastroenterol.* 2013, 19, 5837–5847.

30. Ascher, H.; Lanner, A.; Kristiansson, B. A new laboratory kit for anti-gliadin IgA at diagnosis and follow-up of childhood celiac disease. *J. Pediatr. Gastroenterol. Nutr.* 1990, 10, 443–450.

31. Mayer, M.; Greco, L.; Troncone, R.; Grimaldi, M.; Pansa, G. Early prediction of relapse during gluten challenge in childhood celiac disease. *J. Pediatr. Gastroenterol. Nutr.* 1989, 8, 474–479.

32. Rolles, C.J.; McNeish, A.S. Standardised approach to gluten challenge in diagnosing childhood coeliac disease. *Br. Med. J.* 1976, 1, 1309–1311.

33. Packer, S.M.; Charlton, V.; Keeling, J.W. Gluten challenge in treated coeliac disease. *Arch. Dis. Child* 1978, 53, 449–455.

34. Berg, N.O.; Lindberg, T. Incidence of coeliac disease and transient gluten intolerance in children in a Swedish urban community. *Acta Paediatr. Scand.* 1979, 68, 397–400.

35. Savilahti, E.; Viander, M.; Perkkio, M.; Vainio, E.; Kalimo, K.; Reunala, T. IgA antigliadin antibodies: A marker of mucosal damage in childhood coeliac disease. *Lancet* 1983, 1, 320–322.
36. Bonamico, M.; Sabbatella, L.; di Tola, M.; Vetrao, S.; Ferri, M.; Nenna, R.; Mariani, P.; Picarelli, A. Antiendomysial antibody detection in biopsy culture allows avoidance of gluten challenge in celiac children. *J. Pediatr. Gastroenterol. Nutr.* **2005**, *40*, 165–169.

37. Troncone, R.; Auricchio, R.; Granata, V. Issues related to gluten-free diet in coeliac disease. *Curr. Opin. Clin. Nutr. Metab. Care* **2008**, *11*, 329–333.

38. Korponay-Szabo, I.R.; Kovacs, J.B.; Lorincz, M.; Gorác, G.; Szabados, K.; Balogh, M. Prospective significance of antiendomysium antibody positivity in subsequently verified celiac disease. *J. Pediatr. Gastroenterol. Nutr.* **1997**, *25*, 56–63.

39. Wauters, E.A.K.; Jansen, J.; Houwen, R.H.; Veenstra, J.; Ockhuizen, T. Serum IgG and IgA anti-gliadin antibodies as markers of mucosal damage in children with suspected celiac disease upon gluten challenge. *J. Pediatr. Gastroenterol. Nutr.* **1991**, *13*, 192–196.

40. Valletta, E.A.; Trevisiol, D.; Mastella, G. IgA anti-gliadin antibodies in the monitoring of gluten challenge in celiac disease. *J. Pediatr. Gastroenterol. Nutr.* **1990**, *10*, 169–173.

41. Maki, M.; Lahdeaho, M.L.; Hallstrom, O.; Viander, M.; Visakorpi, J.K. Postpubertal gluten challenge in coeliac disease. *Arch. Dis. Child* **1989**, *64*, 1604–1607.

42. Laurin, P.; Wolving, M.; Fälth-Magnusson, K. Even small amounts of gluten cause relapse in children with celiac disease. *J. Pediatr. Gastroenterol. Nutr.* **2002**, *34*, 26–30.

43. Mavromichalis, J.; Brueton, M.J.; McNeish, A.S.; Anderson, C.M. Evaluation of the intraepithelial lymphocyte count in the jejunum in childhood enteropathies. *Gut* **1976**, *17*, 600–603.

44. Hansson, T.; Dannaeus, A.; Kraaz, W.; Sjöberg, O.; Klareskog, L. Production of antibodies to gliadin by peripheral blood lymphocytes in children with celiac disease: The use of an enzyme-linked immunospot technique for screening and follow-up. *Pediatr. Res.* **1997**, *41*, 554–559.

45. Schaad, U.B.; Gaze, H.; Hadorn, B. Intraepithelial lymphocytes before and after gluten challenge in children with celiac disease. *Am. J. Dis. Child* **1981**, *135*, 272–273.

46. Burgin-Wolff, A.; Gaze, H.; Hadziselimovic, F.; Huber, H.; Lentze, M.J.; Nusslé, D.; Reymond-Berthet, C. Antigliadin and antiendomysium antibody determination for coeliac disease. *Arch. Dis. Child* **1991**, *66*, 941–947.

47. Danielsson, L.; Stenhammar, L.; Astrom, E. Is gluten challenge necessary for the diagnosis of coeliac disease in young children? *Scand. J. Gastroenterol.* **1990**, *25*, 957–960.

48. Lancaster Smith, M.; Packer, S.; Kumar, P.J.; Harries, J.T. Cellular infiltrate of the jejunum after re introduction of dietary gluten in children with treated coeliac disease. *J. Clin. Pathol.* **1976**, *29*, 587–591.

49. Rolles, C.J.; Anderson, M.; McNeish, A.S. Confirming persistence of gluten intolerance in children diagnosed as having coeliac disease in infancy. *Arch. Dis. Child* **1975**, *50*, 259–263.
53. Lancaster-Smith, M.; Kumar, P.J.; Dawson, A.M. The cellular infiltrate of the jejunum in adult coeliac disease and dermatitis herpetiformis following the reintroduction of dietary gluten. *Gut* **1975**, *16*, 683–688.

54. Lahdeaho, M.L.; Maki, M.; Laurila, K.; Huhtala, H.; Kaukinen, K. Small-bowel mucosal changes and antibody responses after low- and moderate-dose gluten challenge in celiac disease. *BMC Gastroenterol.* **2011**, *11*, 129.

55. Montgomery, A.M.; Goka, A.K.; Kumar, P.J.; Farthing, M.J.; Clark, M.L. Low gluten diet in the treatment of adult coeliac disease: Effect on jejunal morphology and serum anti-gluten antibodies. *Gut* **1988**, *29*, 1564–1568.

56. Brottveit, M.; Raki, M.; Bergseng, E.; Fallang, L.E.; Simonsen, B.; Løvik, A.; Larsen, S.; Løberg, E.M.; Jhanssen, F.L.; Sollid, L.M.; *et al.* Assessing possible celiac disease by an HLA-DQ2-gliadin Tetramer Test. *Am. J. Gastroenterol.* **2011**, *106*, 1318–1324.

57. Daveson, A.J.; Jones, D.M.; Gaze, S.; McSorley, H.; Clouston, A.; Pascoe, A.; Cooke, S.; Speare, R.; Macdonald, G.A.; Anderson, R.; *et al.* Effect of hookworm infection on wheat challenge in celiac disease—A randomised double-blinded placebo controlled trial. *PLoS One* **2011**, *6*, e17366.

58. Tack, G.J.; van de Water, J.M.; Kooy-Winkelaar, E.M.; van Bergen, J.; Meijer, G.A.; von Blomberg, B.M.; Schreurs, M.W.; Bruins, M.J.; Edens, L.; Mulder, C.J.; *et al.* 379 Can prolyl endopeptidase enzyme treatment mitigate the toxic effect of gluten in coeliac patients? *Gastroenterology* **2010**, *138*, S54.

59. Kumar, P.J.; O’Donoghue, D.P.; Stenson, K.; Dawson, A.M. Reintroduction of gluten in adults and children with treated coeliac disease. *Gut* **1979**, *20*, 743–749.

60. Wahab, P.J.; Crusius, J.B.A.; Meijer, J.W.; Mulder, C.J. Gluten challenge in borderline gluten-sensitive enteropathy. *Am. J. Gastroenterol.* **2001**, *96*, 1464–1469.

61. Kaukinen, K.; Peraaho, M.; Collin, P.; Partanen, J.; Woolley, N.; Kaartinen, T.; Nuutinen, T.; Halttunen, T.; Mäki, M.; Korponay-Szabo, I. Small-bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: A prospective and randomized clinical study. *Scand. J. Gastroenterol.* **2005**, *40*, 564–572.

62. Campanella, J.; Biagi, F.; Bianchi, P.I.; Zanellati, G.; Marchese, A.; Corazza, G.R. Clinical response to gluten withdrawal is not an indicator of coeliac disease. *Scand. J. Gastroenterol.* **2008**, *43*, 1311–1314.

63. Troncone, R.; Caputo, N.; Micillo, M.; Maiuri, L.; Poggi, V. Immunologic and intestinal permeability tests as predictors of relapse during gluten challenge in childhood coeliac disease. *Scand. J. Gastroenterol.* **1994**, *29*, 144–147.

64. Jansson, U.H.; Kristiansson, B.; Magnusson, P.; Larsson, L.; Albertsson-Wikland, K.; Bjarnason, R. The decrease of IGF-I, IGF-binding protein-3 and bone alkaline phosphatase isoforms during gluten challenge correlates with small intestinal inflammation in children with coeliac disease. *Eur. J. Endocrinol.* **2001**, *144*, 417–423.

65. Korponay-Szabo, I.; Kovacs, J.; Lorincz, M.; Körmendy, M.; Sashegyi, J. New cases of celiac disease detected by anti-endomysial antibody test in families of gluten-sensitive patients and among children examined for non-specific gastrointestinal complaints. *Orv. Hetil.* **1993**, *134*, 15–20.
66. Baker, P.G.; Read, A.E. Oats and barley toxicity in coeliac patients. *Postgrad. Med. J.* **1976**, *52*, 264–268.

67. Anand, B.S.; Piris, J.; Truelove, S.C. The role of various cereals in coeliac disease. *Q. J. Med.* **1978**, *47*, 101–110.

68. Leffler, D.A.; Dennis, M.; Edwards George, J.; Jamma, S.; Cook, E.F.; Schuppan, D.; Kelly, C.P. A validated disease-specific symptom index for adults with celiac disease. *Clin. Gastroenterol. Hepatol.* **2009**, *7*, 1328–1334.e3.

69. Anderson, R.P.; van Heel, D.A.; Tye-Din, J.A.; Barnardo, M.; Salio, M.; Jewell, D.P.; Hill, A.V.S. T cells in peripheral blood after gluten challenge in coeliac disease. *Gut* **2005**, *54*, 1217–1223.

70. Catassi, C.; Rossini, M.; Ratsch, I.M.; Bearzi, I.; Santinelli, A.; Castagnani, R.; Pisani, E.; Coppa, G.V.; Giorgi, P.L. Dose dependent effects of protracted ingestion of small amounts of gliadin in coeliac disease children: A clinical and jejunal morphometric study. *Gut* **1993**, *34*, 1515–1519.

71. Hadziselimovic, F.; Bürgin-Wolff, A. P0429: Indication of gluten tolerance development in patients with coeliac disease. *J. Pediatr. Gastroenterol. Nutr.* **2004**, *39*, S218–S219.

72. Murray, J.A. The widening spectrum of celiac disease. *Am. J. Clin. Nutr.* **1999**, *69*, 354–365.

73. Chang, F.; Mahadeva, U.; Deere, H. Pathological and clinical significance of increased intraepithelial lymphocytes (IELs) in small bowel mucosa. *APMIS* **2005**, *113*, 385–399.

74. Collin, P.; Wahab, P.J.; Murray, J.A. Intraepithelial lymphocytes and coeliac disease. *Best Pract. Res. Clin. Gastroenterol.* **2005**, *19*, 341–350.

75. Ferguson, A.; Arranz, E.; O’Mahony, S. Clinical and pathological spectrum of coeliac disease—Active, silent, latent, potential. *Gut* **1993**, *34*, 150–151.

76. Koskinen, O.; Collin, P.; Korponay-Szabo, I.; Salmi, T.; Iltanen, S.; Haimila, K.; Partanen, J.; Mäki, M.; Kaukinen, K. Gluten-dependent small bowel mucosal transglutaminase 2-specific IgA deposits in overt and mild enteropathy coeliac disease. *J. Pediatr. Gastroenterol. Nutr.* **2008**, *47*, 436–442.

77. Raki, M.; Fallang, L.E.; Brottveit, M.; Bergseng, E.; Quarsten, H.; Lundin, K.E.; Sollid, L.M. Tetramer visualization of gut-homing gluten-specific T cells in the peripheral blood of celiac disease patients. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 2831–2836.

© 2013 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).