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Celiac Disease and Non-Celiac Gluten Sensitivity

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Meet the editor

Dr. Luis Rodrigo, PhD, is currently Emeritus Professor at the University of Oviedo, Spain. He studied in the School of Medicine of Madrid University and obtained his PhD in Medicine in 1975. He has been the Head of Gastroenterology at the University Hospital Central of Asturias since 1996, Titular Professor at the Oviedo University since 1983, and Full-Time Professor since 2010. He has published a total of 255 scientific papers in English and 282 in Spanish. His total Impact Factor is 1581.96, total citation numbers are 5696, and the h-index is 39. He has written a total of 58 chapters and eight edited books about "Treatment in gastroenterological diseases" and the other digestive and liver diseases. He has participated in 50 clinical trials, in 15 as main investigator.

Contents

Preface

Celiac disease (CD) is described as a chronic, genetically based gluten-sensitive immune-mediated enteropathic disease, primarily affecting the small intestinal mucosa. CD did not occur before the Neolithic period (beginning about 9500 BC) because the grains have been cultivated by humans only since this time, in the Fertile Crescent in Western Asia.

In 1930, during World War II, a Dutch pediatrician William Dicke observed that a lack of access to wheat improved the status of children with celiac disease, and in 1952, he was acknowledged for linking the ingestion of wheat proteins as the cause of celiac disease. The first biopsy technique of CD was developed by Margot Shiner, a pediatric gastroenterologist in 1950; she observed the small intestine and the pathologic changes in the celiac disease.

In 1966, dermatitis herpetiformis was linked to gluten sensitivity. In the 1980s, celiac disease was associated with other autoimmune diseases such as thyroid diseases, type 1 diabetes mellitus, and Down syndrome. In the 1990s, genetic markers HLA-DQ2 and HLA-DQ8 and the antitransglutaminase antibodies were identified.

The prevalence of CD is approximately 1% within the US and European populations and may be higher in Northern European countries, approximately 1.5%. CD is a common disorder in North Africa, the Middle East, and India. The diagnostic rate is low in these countries due to low availability of diagnostic facilities and poor disease awareness. The highest CD prevalence in the world (5.6%) has been described in an African population originally living in Western Sahara, the Saharawi, of the Arab-Berber origin.

Initially, it was thought that exogenous gluten products were directly toxic to the mucosa in celiac disease. In contrast with earlier suggestions, intraepithelial lymphocytes (IELs) are now thought to actively contribute to mucosal damage. Antigen exposure in celiac disease causes rapid in situ activation of α/β T-cell IELs. These cells may then damage enterocytes through contributions from several possible mechanisms, including the NKG2D-major histocompatibility complex class I chain-related gene A pathway.

CD diagnosis includes three major steps: (1) blood tests (including serology) positive, (2) small bowel biopsy and histological confirmation, and (3) implementation and response to a gluten-free diet (GFD). At present, the only effective treatment available for CD individuals is a strict lifelong gluten-free diet (GFD). There is a need for an alternative, because GFD is costly and not universally available and compliance is difficult.

Non-celiac gluten sensitivity (NCGS): It is a new syndrome of gluten intolerance, a condition where intestinal and extraintestinal symptoms are triggered by gluten ingestion, in the absence of CD and wheat allergy, as defined by discussions held at three different international consensus conferences. The clinical picture of NCGS is a combination of IBS-like

symptoms, behavior disturbances, and systemic manifestations. In the medical literature, some other names have been suggested for this disorder, such as gluten sensitivity (GS), gluten hypersensitivity, or nonceliac gluten intolerance.

This new entity was described around 30 years ago, but it was necessary to wait until 2011, when it was proposed by members of the First Expert Meeting on gluten sensitivity. The new definition (the Oslo definition) of CD suggested that the disorder should be named NCGS, which made it distinguishable from CD. The Second Expert Meeting on GS that was held in Munich in 2012 decided to change the name of this disorder to NCGS in order to avoid confusion with CD. The first case reports of NCGS in children were described in 2012. NCGS can be diagnosed in those patients with gluten intolerance who do not develop antibodies that are typical neither of CD nor of wheat allergy (WA) and who do not suffer from lesions in the duodenal mucosa, which are characteristic of CD. The gluten-free diet leads to complete regression of symptoms in the same way and efficacy that is achieved in celiac patients.

The prevalence of NCGS is at least six times higher than that observed in CD. Half of the NCGS patients have the genes encoding DQ2 or DQ8 molecules in their HLA system. The genes encoding DQ2 or DQ8 molecules are present in 95% of the CD patients. Negative results for both HLA-DQ2 and HLA-DQ8 excluded the diagnosis of CD in at least 95%. These genes are present in healthy people as well (30%), but less frequently than in the case of the NCGS patients (50%).

The adaptative immune response may play a role in the NCGS pathogenesis. Contrary to CD, where the secondary immune response is upregulation, the NCGS patients demonstrate mainly upregulation of the primary response, and there is no increased expression of the genes of the secondary immune response including IL-6, IL-21, and IFN-gamma, which is characteristic of CD. The NCGS patients' gastrointestinal tracts and their intestinal permeability are normal, and the lesions in the histological picture of their duodenal mucosa are minor (Marsh 0 classification, in the majority of cases).

NCGS treatment is identical to CD and consists in the prescription of a gluten-free diet, which must be followed in a strict form during long life, avoiding all the crossed contaminations and also the possible unannounced interruptions of the diet.

Finally, I want to thank all the authors for their wonderful contributions, as well as the speed and efficiency in the delivery of their chapters. A special gratitude should be mentioned to all the excellent team from the Editorial Board of InTech, especially to Ms. Marijana Francetic, for their continued support and final condition of this book.

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Celiac Disease

Chapter 1

Differential Hallmarks of Celiac Versus Non-Celiac Gluten Sensitivity

Mahesh Mohan and Karol Sestak

Additional information is available at the end of the chapter

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Abstract

Non-celiac gluten sensitivity (NCGS) is an intestinal tissue transglutaminase (TG2)- and IgE-independent form of GS. NCGS is approximately 6× more prevalent than the classical celiac disease (CD), and its incidence is on the rise. Because of its high relative prevalence and striking resemblance to other forms of GS, there is a greater need to develop new and accurate diagnostic assays to facilitate its definitive diagnosis. As the presence of serum anti-gliadin antibodies (AGA) in the absence of TG2 antibodies is suggestive of NCGS, several reports have recommended AGA immunoassays for differential diagnosis. Although AGA immunoassays are in general suitable for diagnostic purpose, to corroborate NCGS and to distinguish it from CD, a simultaneous use of CD-specific diagnostics, i.e., TG2 antibody-based assay, is also required. Due to lower accuracy of AGA assays than those of TG2-based ones, there will always be a chance (estimated to 5–10%) of misdiagnosing NCGS. Moreover, AGA-based diagnostics would not take into consideration the fact that NCGS is potentially triggered by not only gluten but also other molecules such as fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs). Therefore, a second generation of assays needs to be developed to differentiate NCGS from CD with high accuracy.

Keywords: celiac, gluten, NCGS, tissue transglutaminase, differential diagnosis, gut microbiome, gluten-free, diet, IBS, chronic inflammation, small intestine, GI tract

1. Introduction: NCGS, CD, and irritable bowel syndrome

Similarities between non-celiac gluten sensitivity (NCGS) and irritable bowel syndrome (IBS) were first noted in 1978 when it was reported that an adult female patient with IBS but not

celiac disease (CD) showed dramatic relief of chronic diarrhea and abdominal pain symptoms after administration of gluten-free diet (GFD) [1–6]. More recent studies corroborated that some but not all IBS patients show significant onset of clinical diarrhea upon mucosal challenge with gluten [7, 8]. There is an emerging consensus that tissue transglutaminase (TG2) antibody-negative and anti-gliadin antibodies (AGA)-positive (TG2−AGA+) IBS patients with DQ2/8-negative haplotype qualify as NCGS candidates [3]. Such an assumption can be confirmed by placing suspect NCGS patients on GFD with subsequent relief of clinical/immunological symptoms. Conversely, if AGA test is used alone, without other corroborative/exclusionary assays, its predictive value for NCGS is poor [4]. Taken together, it appears that NCGS and IBS patients share several clinical and histopathological symptoms. NCGS should therefore be differentiated from IBS based on complete CD/NCGS serology, and diagnosis can be confirmed by performing a mucosal gluten challenge. To simplify and to expedite diagnostic steps, new molecular assays need to be developed to differentiate NCGS from IBS and CD.

2. Composition of host gut microbiome and NCGS/CD

Given the unprecedented rise of food allergies and autoimmune disorders in urban populations during recent decades, several studies have indicated that a potential causative association exists between some of these disorders and composition of the host's gut microbiome [9, 10]. Since both CD and NCGS are inflammatory disorders of not only gastrointestinal (GI) tract but also other organs, including dysfunction of the gut-brain axis [11, 12], studies aimed at identification of specific hallmarks of gut dysbiosis of these disorders are the focus of current investigations.

It has been reported that bacteria involved in gluten metabolism predominantly belong to phylum *Firmicutes*, in particular, those from the genus *Lactobacillus*, followed by *Streptococcus*, *Staphylococcus,* and *Clostridia* [13, 14]. Recently, it was shown that GFD treatment significantly altered proportions of these bacterial groups and that restoration of normal bacterial flora took many months and possibly years [14, 15]. It was also shown that increased presence of some of the bacterial species involved in gluten metabolism leads to enteritis [13]. Our group recently demonstrated that *Streptococcaceae* and *Lactobacillaceae* families were enriched in GS rhesus macaque model of CD, while *Coriobacteriaceae* predominated in healthy animals [14]. In the future, studies to elucidate specific dysbiotic pathways that distinguish NCGS from CD need to be done.

3. Host luminal shedding of fecal microRNAs

Recently, a novel concept concerning the capability of intestinal epithelial cells to release luminal regulatory microRNAs (miRNAs) was described [16]. It was demonstrated that

these fecal miRNAs could potentially enter bacterial cells and regulate their replication and growth. In this context, it is possible that inflammation-induced miRNAs could enter commensal bacteria and posttranscriptionally suppress or promote their growth by binding to specific sequences on bacterial genes [16]. This in turn, depending on the outcome, may give pathogenic bacteria an opportunity to expand leading to dysbiosis. [16]. These findings have therapeutic implications as oral supplementation of stable miRNA mimics capable of targeting specific dysbiotic or probiotic members of the gut microflora relevant to disease relapse and/or remission may be implemented. In our recently published studies, we hypothesized that GS disorders including CD and NCGS have their own unique signatures of dysbiosis. In addition, it is also likely that regulatory miRNAs secreted by host epithelial cells in response to dysbiotic events are also disease specific. Recently, we identified and reported several miRNAs (miR-203, miR-204, miR-23b, and miR-29b) with perfect complementarity between miRNA seed nucleotides (5′ prime nt position 2–7) and 16S rRNA sequence of dysbiotic bacterial species in the rhesus macaque model of CD (**Figure 1**) [14].

Dysbiotic bacterial species that could be potentially regulated in this fashion by inflammatory miRNAs included members of the *Streptococcaceae* and *Lactobacillaceae* families that are known to play roles in metabolism of gluten [13]. As biological and regulatory functions of miRNAs include host cell effects such as expression of epithelial tight junction proteins, more work remains to be performed to characterize regulatory relationships and pathways pertinent to miRNA molecules that influence dysbiotic gut microbiota in NCGS and CD individuals.

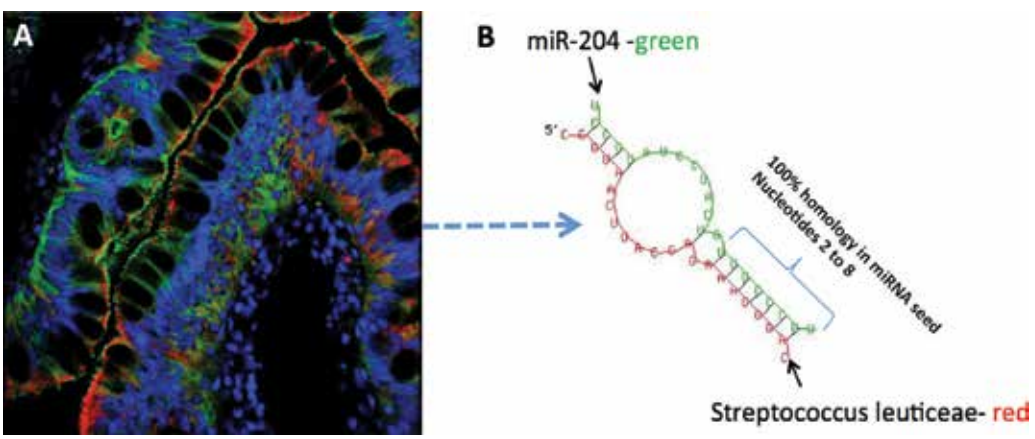

Figure 1. Small intestinal epithelial cells of gluten-sensitive rhesus macaque (A) were visualized by immunofluorescent triple labeling of cytokeratin-1 (red), tight junction protein claudin-1 (green), and nuclear DNA (blue) antigens. Epithelial cells of gluten-sensitive but not healthy, normal primates produced regulatory fecal microRNAs (miRNA) species complementary with dysbiotic bacterial species such as *Streptococcus leuticeae* (B) and others. It was proposed that intensity of such interactions can shape the gut microbiome dysbiosis either toward remission or relapse [14, 16].

4. Dietary gluten and neurodevelopmental disease markers

The first report suggesting an association between increased occurrence of neurodevelopmental disorders and consumption of gluten-containing cereal grains dates back to 1966 [17]. In the same year, it was reported that some but not all GS patients develop neurological dysfunctions referred to as gluten ataxia, gluten neuropathy, or gluten encephalopathy [18, 19]. Since then, several studies have suggested that symptoms of the autism spectrum disorders (ASD) could be improved upon changes in diet. One of these diets is GFD [20]. Despite its widespread use, the efficacy of GFD for the treatment and prevention of ASD has not been conclusively proven. More recently, a case report involving NCGS patients with gluten psychosis was reported [21]. The molecular mechanisms underlying ASD/psychosis vs. dietary gluten relationship are highly complex and understudied [22, 23]. Therefore, a transition from the "clinical phenomena" to "basic research" type of studies is needed. We propose that perturbation levels (measured by the extent of mRNA expression) of ASD predisposition genes need to be elucidated in preclinical, humanlike models first in the context of experimental introduction/withdrawal of dietary gluten.

For this and other purposes, we developed the rhesus macaque (*Macaca mulatta*) model of GS [14, 24–30]. The presence of AGAs, gluten-sensitive enteropathy (GSE), increased intestinal permeability, and genetic predisposition were all documented. Consistent with human disease, GSE in macaques is characterized by a wide range of severity, ranging from the subclinical to severe form that includes decreased absorption of nutrients, decreased xenobiotic metabolism, cancer predisposition, diarrhea, dermatitis, decreased diversity of gut microbiome, as well as the perturbations in expression of several neurodevelopmental disorder-associated genes including those of ASD and down syndrome. One of these genes that showed significant upregulation in GS rhesus macaques was the Ca^{2+}-dependent activator protein for secretion 2 (*CADPS2*). In humans, the *CADPS2* gene is located within the autism susceptibility locus 1 on chromosome 7q. It was shown that *Cadps*2-knockout mice exhibit cellular and behavioral traits consistent with ASD [31]. The CADPS2 protein regulates exocytosis of synaptic vesicles in neurons and neuroendocrine cells. In accordance with these findings, analysis of the ASD-associated genetic predisposition factors by a group at Harvard School of Medicine revealed that ASD is not restricted to not only humans but also apes, monkeys, and dolphins [32]. Remission and relapse stages of GSE can be accomplished in GS macaques by feeding gluten-free and gluten-containing diets, respectively. Similar to human gluten-sensitive patients, AGA and GSE are reversibly dependent in GS macaques by exposure to dietary gluten [24, 33, 34]. Thus, an extensive use of GS rhesus macaque model in experimental and translational studies involving neurodevelopmental disorder-associated genes and their corresponding pathways is desired—as a new preclinical tool for not only ASD research but also for the development of NCGS vs. CD differential diagnostics.

5. NCGS vs. CD microbial signatures

Based on the assumption that CD is caused by an autoimmune reaction to TG2, while NCGS is caused by chronic bacterial intestinal infections, a recent study by Columbia University

researchers focused on the identification of differential, bacterial byproduct-specific diagnostic markers to distinguish the two conditions [35]. Their findings suggested that enteropathy could occur in individuals who report GS in the absence of CD, while it is associated with increased serum antibodies recognizing bacterial lipopolysaccharide (LPS) and/or its CD14 ligand [35]. Although several antibodies were evaluated for their potential to be used as differential diagnostic tools including anti-LPS, anti-flagellin, and anti-soluble CD14 (sCD14), the best predictive values were attributed to antibodies targeting LPS and sCD14. These results corroborated that NCGS and CD have common and differential features that can be further exploited for the development of more sensitive and accurate differential diagnostic assays.

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Chapter 2

Celiac Disease and HBV Vaccination

Caterina Anania, Francesca Olivero,
Eugenia Olivero and Lucia Pacifico

Additional information is available at the end of the chapter

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Abstract

Celiac disease (CD) is an immune-mediated systemic disorder elicited by gluten and related prolamins in genetically susceptible individuals, characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 and HLA-DQ8 haplotypes, and enteropathy. Hepatitis B virus (HBV) infection is an important global public health problem that can cause chronic liver disease, and it is associated to a high risk of death from cirrhosis and hepatocellular carcinoma. Since 1982, a safe and effective HBV vaccine has been available, and recommendation for HBV vaccination has been extended to all infants to achieve protection against HBV infection. HBV vaccination is highly effective in eliciting a sustained immune response in immune-competent individuals. However, research papers have suggested that celiac patients may have low rate of protective antibodies after HBV vaccination. The failure of CD subjects to respond to HBV vaccination has great importance for public health policies as the nonresponders could be regarded as a reservoir for HBV. The aim of our work is to revise and to discuss the scarce literature on this field in order to provide clinical practice guidelines to establish the best surveillance program of response to HBV vaccine in CD pediatric patient.

Keywords: celiac disease, children, hepatitis B vaccine, HLA, gluten-free diet

1. Introduction

Celiac disease (CD) is an immune-mediated systemic disorder elicited by gluten and related prolamins in genetically susceptible individuals, characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 and HLA-DQ8 haplotypes, and enteropathy. Genetic, immunological, and environmental factors therefore appear to be responsible for the disease. HLA-DQ2 is present in 90%–95% of patients with CD, whereas 5% carry the HLA-DQ8 haplotype and the remaining 5% at least one of the two DQ2 alleles [1, 2]. The prevalence of CD is high in the European and

North American population (1%), reaching 10%–15% in patients who have first-degree relatives with this disease [1, 2].

HLA system has a fundamental role in identifying the antigens inoculated with the vaccines and in the production of specific antibodies [3, 4], and some HLA phenotypes seem to be predictive of a less effective immunological response [5].

In particular, the immunogenic peptides in the hepatitis B (HBV) vaccine determine the protective immune response to the virus through the HLA-DR and DQ molecules [6, 7], with the DR3-DQ2 and DR7-DQ2 haplotypes generally having a lower response rate [7–10].

HBV infection is one of the major causes of chronic liver disease, associated with a high risk of death from cirrhosis and hepatocellular carcinoma, and therefore represents an important global public health problem [11, 12]. To prevent it, since 1982, a safe and effective hepatitis B vaccine has been available. The one currently in use is a recombinant vaccine that contains HBV surface antigen (HBsAg) and causes the production of specific antibodies (anti-HBs) that protect against the infection [13]. Many epidemiologic studies have been conducted to determine the efficacy of the vaccine. A positive immune response to the vaccine is defined as the development of HBV anti-HBs at a titer of >10 mIU/mL, after a complete and appropriate immunization schedule, measured preferably 1–3 months after the last vaccine administration [14, 15]. The optimum response, conferring seroprotection against HBV infection, is defined as an anti-HBs titer ≥100 IU/l [14, 15]. Subjects that develop an anti-HBs titer between 10 and 100 IU/ml are referred to as "poor responders." Vaccinated subjects with an anti-HBs titer <10 mIU/ml after completion of primary vaccine series are called "nonresponders" [16]. HBV vaccination is very effective, showing a sustained immune response in immune-competent individuals: the antibody response has been found to occur in more than 90% of the healthy subjects vaccinated with the standard dose regimen of 20 μg HBV vaccine given at 0, 1, and 6 months of intervals [17, 18]. However, among healthy immunocompetent subjects, approximately 4–10% do not produce protective levels of anti-HBs after immunization [19] depending on age, male gender, obesity, inappropriate vaccine storage conditions, route of administration, smoking, drug abuse, state of immunosuppression, and presence of specific HLA haplotypes.

2. Responses to vaccinations in celiac children

Data concerning antibody response of patients with CD to vaccine are scanty. Most studies in this field are addressed to HBV vaccination response, while fewer works are available about the immunological response to other vaccinations.

Several research papers have suggested that celiac patients may have low rate of protective antibodies after vaccinations such as HBV. The failure of CD subjects to respond to HBV vaccination has great importance for public health policies as the nonresponders could be regarded as a reservoir for HBV [20]. The studies that have addressed the relation between CD and HBV vaccination in children are summarized in **Table 1** [21–29]. In the earliest report involving 26 celiac patients aged 9.2 ± 4.6 years and 18 age-matched controls, receiving the full complement of childhood vaccination (HBV, tetanus, rubella, *Haemophilus influenzae* type b), Park et al. [21] demonstrated that a significantly higher proportion of subjects in the CD group failed to

respond to HBV vaccine compared with controls (53.9% versus 11.1%; $p < 0.05$). However, all subjects in both groups tested positive for other vaccinations. These results led the authors to support the role of HLA haplotypes in response to HBV vaccine. Nemes et al. [22] evaluated HBV vaccine response in CD patients in relation to disease activity and examined the possible role of dietary gluten in the failure to achieve protective antibody titers. The authors studied 128 biopsy-proven CD children and adolescents and 113 age-matched control subjects; 22 patients with CD were prospectively vaccinated with a recombinant HBV vaccine after the diagnosis of CD during dietary treatment, while 106 CD patients received a recombinant HBV vaccine unrelated to CD diagnosis and dietary compliance. They found that a seroconversion rate for anti-HBs was 95.5% (95% CI: 78.25–99.2%) after vaccination in the patients prospectively immunized, while the response rate was 50.9% and correlates with gluten intake (untreated patients 25.9%, non-strict diet 44.4%, strict diet 61.4%) when HBV immunization was performed unrelated to diagnosis and diet status suggesting that disease activity may play a primary role in vaccination failure rather than specific HLA alleles [22]. Subsequently, Ertem et al., to assess the response to HBV vaccine prospectively in a group of CD children and to explore the potential link between CD and HBV vaccine nonresponse, evaluated serologically for anti-HBs status 63 previously biopsy-proven CD patients on a strict gluten-free diet (GFD) and 54 healthy children. CD children who were anti-HBs negative at baseline were fully vaccinated prospectively and reevaluated for the response to HBV vaccine. The authors found that the response rate to HBV vaccine in CD patients prospectively vaccinated was 96.9%, which was as high as the response rate obtained in healthy population, and they concluded that treatment with GFD and compliance to the treatment rather than the specific HLA alleles may improve the immune response to HBV vaccine in CD patients [23]. Balamtekin et al. conducted a study to compare the response rates to HBV vaccination in the first year of life, using two different immunization protocols. The total study group included 64 CD children (group 1 who received HBV vaccination at birth, 2 and 9–12 months of life, and group 2 at birth, 1 and 6 months of life) and 49 healthy controls. The authors found that the response rate to HBV vaccine and anti-HBs titers in CD patients who completed the HBV vaccination before 1 year of age were significantly lower compared to healthy controls, whereas no statistically significant difference was observed with the two different HBV vaccination schedules [24]. Ertekin et al. compared the response to HBV vaccine between children with CD and healthy children and investigated the relationship between the patients' responses to HBV vaccine, the clinical presentation of CD, and the dietary compliance in the patients. They evaluated the production of specific anti-HB surface antigen (HBsAg) in 52 CD patients and 20 age- and sex-matched healthy children who received HBV vaccination according to the standard immunization schedule. The authors found that anti-HBs titers of CD patients were positive in 32 (61%) and negative in 20 (38.5%) patients, while 18 (90%) of control subjects had positive anti-HBs titers. They found also statistically significant differences between negative anti-HBs titers, clinical presentation of CD, and dietary compliance in patients with CD ($P < 0.05$). Therefore, they concluded that, in children with CD, the immune response to HBV vaccination may be improved by compliance to the GFD [25]. Leonardi et al. [26] in a retrospective report confirmed that CD patients have a lower percentage of response to HBV vaccination than healthy subjects. In fact, they found that 30 (50%) of 60 CD patients were nonresponders to HBV vaccination, compared to 7 (11.6%) of 60 controls. The same authors also found that a significantly higher number of nonresponders in adolescent patients older than 14 years and concluded that a very early

diagnosis of CD seems to increase significantly the percentage of responders suggesting that a short time of gluten introduction seems to play a favorable effect on the antibody memory [26]. Leonardi et al. [27] in a subsequent retrospective study, including 66 CD patients and 50 healthy children, analyzed and compared the immunologic response against obligatory vaccination (HBV, diphtheria and tetanus component, and *Bordetella pertussis*) and against recommended vaccination (*Measles virus*, *Paramyxoviridae*, and *Rubella virus*) in the two groups. The authors found similar response to obligatory and recommended vaccines into the two groups, except for HBV vaccine. Moreover, they compared patients whose diagnosis was made before or after 18 months of age and found that an early or a delayed diagnosis does not significantly modify the immunological response, except for that one involved in the HBV vaccination. Thus, the immunologic response did not seem to be influenced by the natural history of CD [27]. Urganci and Kalyoncu determined the rate of response to hepatitis A (HBA) and HBV vaccine, the duration of protection against HAV and HBV, and the incidence of acute HAV or HBV infections during follow-up in 30 pediatric patients with CD and compared them with 50 healthy age-, sex-, and body mass index-matched controls [28]. They found that 14 (46%) of 30 CD patients and 15 (30%) of the controls had natural immunity for HAV, whereas all patients and controls did not show evidence of earlier exposure to HBV. Sixteen patients and 35 controls received HAV vaccine, and HBV vaccine was administered to all CD patients and controls; protective anti-HAV antibodies were developed in 12 (75%) of the patients and all the controls (75% versus 100%, respectively). Thirty patients and 50 controls received HBV vaccine, and 70% of the patients versus 90% of the controls achieved seroprotection. The authors concluded that the rate of seroconversion to the HBV and HAV vaccine is lower in CD patients than in healthy controls. Finally, in a very recent paper, Leonardi et al. comparing a group of patient affected by diabetes mellitus type 1 (DMT1) and CD and a group affected by DMT1 without CD (both groups had similar HLA haplotype) found a higher nonsignificant percentage of nonresponders in DMT1/CD group than in DMT1 (53.3% versus 38.2%); comparing the DMT1/CD group with CD group, the authors found a similar percentage (53.3% versus 50%) of nonresponders, and this result indirectly confirmed that gluten can favor a further decrease of efficacy to HBV vaccine, beyond the HLA system [29].

Author/ references	Year	Country	Study design	Patient population and sample size	Vaccine	(%) of nonresponders	HLA
Park et al. [21]	2007	Japan	Prospective	26 (mean age 9.2 ± 4.6 years) untreated CD vs 18 (mean age 10.4 ± 3.8) controls	HBV	53.9% vs 11.1%; $P < 0.05$	NA
Nemes et al. [22]	2008	Finland	Prospective	22 (mean age 8.8 years) treated CD prospectively immunized; 27 (mean age 16.7 years) untreated CD; 79 (mean age 16.7 years) treated CD vs 113 (mean age 16.1 years) controls	HBV	0.5% 74.0% 38.6% vs 24.8%; $P < 0.001$, $P < 0.001$, $P = 0.102$	Group 1 (22 treated CD): HLA DQ2 Group 2 (53/106 treated and untreated CD): 51: HLA DQ2 2: HLA DQ8

Author/ references	Year	Country	Study design	Patient population and sample size	Vaccine	(%) of nonresponders	HLA
Leonardi et al. [26]	2009	Italy	Retrospective	60 (mean age 9.32 years) treated CD vs 60 (mean age 10.1 years) controls	HBV	50% vs 11.6%; $P < 0.0001$	15/60: 13 HLA-DQ2 2 HLA-DQ8
Ertem et al. [23]	2010	Turkey	Retrospective Prospective	40 vaccinated (mean age 12.4 ± 5.4 years) treated CD vs 54 (mean age 9.8 ± 3.6 years) controls 28 prospectively vaccinated treated CD	HBV	32.5% vs 14.8%; $P < 0.05$ 3.6%	37.5% CD 23.8% controls: HLA DRB1*03 21% CD 2.4% controls: HLA DRB1*07 55% CD 14.6% controls: HLA DQB1*02 30% CD 47.6% controls: HLA DQB1*03
Ertekin et al. [25]	2011	Turkey	Retrospective	52 (mean age 10.7 ± 4 years) CD vs 20 (mean age 10.7 ± 4 years) controls	HBV	38.5% vs 10%; $P < 0.05$	NA
Balamtekin et al.[24]	2011	Turkey	Retrospective	64 (mean age 4.69 ± 2.31 years) treated and untreated CD vs 49 (mean age 5.45 ± 2.92 years) controls	HBV	21.9% vs 4.1%; $P = 0.001$	NA
Urganci and Kalyoncu [28]	2013	Turkey	Prospective	30 (mean age 6.15 ± 4.1 years) treated and untreated CD vs 50 (8.13 ± 1.7 years) controls	HBV	30% vs 10%; $P = 0.03$	NA
Leonardi et al. [27]	2011	Italy	Retrospective	66 (mean age 8.34 ± 3.47 years) CD vs 50 (mean age 7.58 ± 3.51 years) controls	HBV	53% vs 16%; $P < 0.0001$	NA
Leonardi et al. [29]	2015	Italy	Prospective	30 (mean age 6 years) CD/DMT1 vs 100 (mean age 13.6 years) DMT1 vs 60 (mean age 8.6 years) CD	HBV	53.3% vs 38.2% vs 50%; $P > 0.02$	NA

HBV hepatitis B virus; *CD* celiac disease; *HLA* human leukocyte antigen; *NA* nonavailable; *DMT1* diabetes mellitus type 1.

Table 1. Response to HBV vaccination in CD children and adolescents compared to healthy subjects.

3. Pathogenetic role of HLA system in vaccination unresponsiveness in celiac disease

The mechanism for hepatitis B vaccination failure in patients with CD is not clear. A few hypotheses have been proposed. Multiple candidate genes influence the ability to respond

to the recombinant HBV vaccine [9, 30–32]. HLA is believed to contribute significantly to the genetic susceptibility immune response variations to the vaccine [33]. Poor or nonresponsiveness to HBV vaccine has been associated with HLA-DQ2, DR3, and DR7 alleles, which are also associated with CD [9, 10, 34]. In particular, HLA genotype DQ2, found in 90–95% of celiac patients, may have a fundamental role in the predisposition to a weaker immunization to recombinant hepatitis B vaccine in these patients. The HLA is coded by the major histocompatibility complex (MHC) group of genes located on chromosome 6 in the human genome, and they are essential for determining the specificity of an individual's immune response [35]. There are three classes of HLA: HLA class I, HLA class II, and HLA class III. Among them, HLA class II molecules have the task of presenting antigens to the T lymphocytes from outside the cell. Antibody-producing B cells are then stimulated to produce specific antibodies by these antigens [36]. HLA-DQ2 haplotype would be responsible for the failure of induction of the Th2 response needed to promote the differentiation of B cells and the formation of memory B cells necessary for immunization.

Defective or insufficient HBsAg-specific T-helper cells, inadequate T-helper 1, and T-helper 2 cytokine production [37–39], or diminished expression of cell contact signal between activated T and B cells, like CD40L [40] may also be responsible for the lack of response to HBsAg [41, 42]. On this regard, interleukin genotypes (IL10, IL12, IL18) were associated with the anti-HBs antibody development in response to HBsAg in hemodialysis patients [43, 44]. Chen et al. in 2011 found that serum anti-HBsAg response to HBV vaccine in healthy population was closely related to four specific single-nucleotide polymorphism (SNPs) in the IL4, IL4RA, IL13, and Toll-like receptor (TLR2) genes and suggested that variation in these structures may influence the duration and intensity of HBV vaccine-induced immune response [45].

Other studies suggested that compliance with a GFD is responsible for the response to the hepatitis B vaccine in patients with CD. Several studies have hypothesized gluten intake as a cause of failed immunity upon vaccination. Gluten may be implicated because both HBsAg protein fragments and gliadin peptides bind to HLA-DQ2 molecules and induce proliferation of T lymphocytes. Defective antibody production may result from competition between the proteins [22, 23].

4. New approaches in hepatitis B vaccination in celiac children

Inadequate response to HBV immunization in CD patients represent a public health concern because the group of nonresponder patients could act as an HBV infection reservoir. For this reason, response to HBV vaccine should be investigated in children with CD. To protect this population and to achieve the goal of universal protection, new immunization strategies were proposed for CD: the first one is the use of booster and/or higher doses of HBV vaccine by intramuscular (IM) route, and the second one addresses on the use of intradermal route (ID). The studies that have addressed new immunization strategies in CD are summarized in **Table 2** [22, 23, 46, 47].

Author/references	Year	Country	Study design	Patient population and sample size	VAC Ag	Type of vaccine	Route	Number of booster doses	% Seroconversion
Nemes et al. [22]	2008	Finland	Prospective	37 (mean age 16.7 years) nonresponders CD on GFD	HBV	Recombinant	IM	1	97.3%
Ertem et al. [23]	2010	Turkey	Prospective	28 (12.4 ± 5.4 years) nonresponders CD	HBV	Recombinant	IM	Three doses of HBV vaccine	96.4%
Leonardi et al. [46]	2010	Italy	Prospective	20 nonresponders CD to IM vaccination	HBV	Recombinant	ID	4	90%
Leonardi et al. [47]	2012	Italy	Prospective Randomized	58 (mean age 9.8 ± 6.2 years) nonresponders CD	HBV	Recombinant	30 ID vs 28 IM	3	After first dose: ID:76.7% vs IM: 78.6% After third dose: ID: 90% vs IM: 96.4% High responders (anti-HBs >1000 IU/l): ID: 40% IM: 7%; $P < 0.01$

CD celiac disease; *IM* intramuscular; *ID* intradermal; *VAC* vaccine; *Ag* antigen; *GFD* gluten-free diet; *HBV* hepatitis B virus; *anti-HBs* anti-hepatitis B surface

Table 2. Seroconversion rate in CD children and adolescents after IM or ID HBV vaccination.

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Nemes et al. administered intramuscularly to 37 nonresponder CD children on GFD, the booster dose of 20 μg of recombinant HBV vaccine, and found that 36 out 37 (97.3%) showed seroconversion 4 weeks after vaccination. However, success with the booster vaccination after controlled GFD suggests that disease activity may play a primary role in vaccination failure [22]. Few studies that exist about HBV vaccine administered by ID route in CD patients unresponsive to IM recombinant vaccine. Leonardi et al. revaccinated 20 CD children and adolescents with a 2 μg dose of recombinant intradermal HBV vaccine. After 4 weeks they found that 15 out 20 patients (75%) showed a protective titer of anti-HBs [22, 23].

Subsequently, Leonardi et al. conducted a prospective, randomized study on 58 CD patients, vaccinated in the first year of life, without protective HBV antibodies as demonstrated by blood analysis. They performed in all patients randomly an HBV vaccination booster dose by ID or IM route. In 30 CD children, a 2 μg dose of recombinant HBV vaccine was administered by the ID route, while 28 CD patients received by IM route 10 μg dose of the same vaccine. Four weeks after every booster dose, 90% of ID patients and 96.4% of IM subjects showed a protective anti-HBs titer after a third booster dose. The authors concluded that both routes are effective in revaccinating CD patients; however, the ID route seems to produce a significantly higher percentage of higher responders [47].

Data suggest that the ID route offers greater immunogenicity due to direct delivery of antigen to the skin immune system, using even lower doses of antigen than IM route [47]. Moreover, the presence of a skin reaction on the site of the intradermal injection could represent a less expensive strategy to test serum anti-HBs response after the booster dose [48]. Economic studies suggest that the substantial cost-saving benefits could be achieved using a fraction of the IM dose via an ID route [48, 49].

5. Conclusions

The available literature shows that HBV vaccine response is lower in celiac subjects compared with healthy ones. Some authors hypothesize that the failure to respond to HBV vaccination is related to specific HLA association, whereas others argue that exposure to gluten at the time of vaccination may play an important role in unresponsiveness to the HBV vaccine. Therefore, nonresponsiveness to the HBV vaccination in CD patients represents a serious public health problem because of the large diffusion of CD that affects about 1% of the European population. Consequently, new vaccination strategies have been proposed to achieve full protection in this context, including the administration of booster doses of HBV vaccine by the intramuscular or the intradermal route. An evaluation of the response to HBV vaccine should be considered as a routine assessment in children newly diagnosed with CD who were previously vaccinated for HBV. Whenever unresponsiveness occurs, certain measures must be taken into account, such as revaccination utilizing ID route, which offers a potentially greater immunogenicity than the IM one, even using lower doses, due to the direct delivery of antigen to the skin immune system. Moreover, the revaccination should be done after the decrease of specific antibodies, which usually occurs after about 1 year of GFD, seen as some studies support GFD

as crucial to vaccine responsiveness. More randomized controlled studies with a prospective design are needed for CD patients in order to clarify this topic.

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Section 2

Gluten Determination Techniques

Chapter 3

Measurement of Gluten in Food Products: Proficiency-Testing Rounds as a Measure of Precision and Applicability

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Additional information is available at the end of the chapter

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Abstract

In 2008, Codex Alimentarius endorsed the R5 Enzyme-Linked Immunosorbent Assay (ELISA) method as Method Type 1 for gluten measurement in gluten-free foods. The most recognized R5 ELISA test kit is the RIDASCREEEN® Gliadin (R7001; manufacturer R-Biopharm). Beside collaborative tests that led to several international approved methods of this test kit, proficiency-testing (PT) rounds are regularly performed in Europe by different PT providers. Results from these rounds were analyzed regarding the number of participating labs with acceptable results for the RIDASCREEN® Gliadin. All PT rounds document the excellent consistency and comparability of results. The data show that the RIDASCREEN® Gliadin R5 ELISA is also applicable to cake mix, oat-based foodstuff, infant soya formula, cookies, canned boiled sausage, gravy thickener, pasta, and potato dumpling. These rounds also included the analysis of blank matrices. It was found that more than 95% of all participating laboratories correctly detected these samples as negative. Other gluten test kit manufacturers were analyzed as well, but due to the low number of participants using these test kits results were often only analyzed in a qualitative manner questioning the comparability of these kits to the RIDASCREEN® Gliadin R5 ELISA.

Keywords: gluten, gliadin, R5, RIDASCREEN, ELISA, proficiency test, precision, applicability, method comparability, Codex Alimentarius

1. Introduction

In the context of coeliac disease (CD), gluten is the protein fraction from wheat, rye, barley, oats, or their crossbred varieties and derivatives thereof, which induces intestinal symptoms in patients and that is insoluble in water and 0.5 mol/l NaCl [1]. Gluten proteins can be divided into the alcohol-soluble prolamin fraction and the alcohol-insoluble glutelin fraction, which is only soluble after addition of reducing and disaggregating agents. The prolamin content of gluten is generally taken as 50% [1]. The Codex threshold of 20 mg/kg gluten (including a security factor) was endorsed in parallel and derived from challenge studies in coeliac patients using the commercially marketed Working Group on Prolamin Analysis and Toxicity (WGPAT) or Prolamin Working Group (PWG) gliadin [2]. This threshold was adopted by many national legislations, including the USA and the EU, so that food not exceeding 20 mg/kg gluten can be labeled as gluten free in these countries. Although oats is part of the Codex definition of gluten, this crop is considered safe for the vast majority of persons intolerant to gluten, if it is not contaminated with other gluten-containing cereals [3]. The Codex explicitly mentions that oat may be allowed at the national level. At the moment, the most precise definition and explanation on oats is given in the US regulation [4].

So far, the only treatment for celiac disease is the strict adherence to a gluten-free diet. Specific and sensitive immunochemical methods are therefore needed to ensure quality control and compliance testing for gluten measurement in gluten-free food. The sandwich enzyme-linked immunosorbent assay (ELISA) RIDASCREEEN® Gliadin (R7001) is based on the R5 monoclonal antibody [5] for the detection of intact gluten and was laid down as a Codex Alimentarius type 1 method for the analysis of gluten [1]. It is calibrated to the PWG gliadin and therefore results are traceable to the threshold value of 20 mg/kg gluten determined in challenge studies as mentioned above. Furthermore, it has been adopted as official or approved method by AOAC International [6], ICC [7], and the AACC International [8]. Raised against rye ω-secalins, the R5 antibody primarily recognizes the epitope QQPFP, which is present in wheat gliadins, rye secalins, and barley hordeins, and part of many CD-toxic or -immunogenic peptides [9–11].

Beside collaborative tests [8, 12] that led to AOAC-, ICC-, and AACC-approved methods of this test kit for corn- and rice-based matrices, proficiency-testing (PT) rounds are regularly performed in Europe by three different PT providers. Mostly accredited laboratories participate in these PT rounds to prove their analytical competence. This publication will analyze all PT rounds between 2011 and 2016 with regard to precision and applicability of the official R5 gluten test kit RIDASCREEN® Gliadin. Other test kits that claim to be comparable with the R5 reference method were analyzed as well but the number of participants using these kits were often not enough for robust quantitative statistics. Therefore, these kits were often only analyzed in a qualitative manner.

2. Materials and methods

Results from 33 different PT rounds with different food matrices were analyzed regarding the number of participating laboratories with acceptable results for the RIDASCREEN®

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Gliadin. These rounds also included the analysis of blank matrices with gluten concentrations below the limit of quantification of the test kit. The following PT providers were analyzed: Food Analysis Performance Assessment Scheme (FAPAS; www.fapas.com), Dienstleistung Lebensmittel Analytik GbR (DLA; www.dla-lvu.de), and Durchführung von Laborvergleichsuntersuchungen GbR (LVU; www.LVUs.de).

2.1. RIDASCREEN® Gliadin (R7001)

RIDASCREEN® Gliadin is a sandwich enzyme-linked immunosorbent assay (ELISA) for the quantification of gliadin/gluten derived from wheat and related prolamins derived from rye and barley and other gluten containing varieties in various foodstuffs. The test is based on a microtiter plate coated with the specific monoclonal anti-gliadin R5-antibody. Bound gliadin is finally detected with a peroxidase-labeled specific antibody (R5). The factor of two is used to convert quantitative gliadin results into gluten results.

A pre-ground sample is extracted by the use of a special solvent (Cocktail, patented; Mendez extraction) and can then be analyzed in less than 100 minutes. The standard calibration curve of the ELISA covers a range from 5 to 80 mg/kg gluten (including the dilution factor from sample preparation) and is standardized against the WGPAT gliadin reference standard. The assay is applicable to the detection of gluten with a limit of quantitation (LoQ) of 5 mg/kg gluten and a limit of detection (LoD) of 1 mg/kg gluten. This method was developed to detect traces of gluten in gluten-free food, not for quantifying the gluten content in wheat, rye, or barley flour. It is not suitable for analysis of fragmented gluten, for example, in beer.

2.2. *Z*-scores

To evaluate results provided by each participating laboratory, a *z*-score is calculated for each participant. The basis for calculation differs slightly when comparing proficiency test providers which are explained in Chapters 2.2.1 and 2.2.2.

2.2.1. Using assigned values (for one method) and target standard deviations

$$z = \frac{(x - x_a)}{\sigma_p} \tag{1}$$

where x denotes the result delivered by a participant and x_a, the assigned value, derived from the consensus of the results submitted by the participants according to the test kit they used. The standard deviation for proficiency, σ_p, was set at a value that reflects best practice for the analyses in question. In case of gluten, σ_p was set to a relative standard deviation of 25% using fitness-for-purpose criteria based on expert advice. This approach is used by FAPAS and DLA. Further explanations are given in each PT report from FAPAS or DLA.

2.2.2. Using median and robust standard deviation (data from all participants)

$$z = \frac{(x - x_M)}{\sigma_{\text{robust}}} \tag{2}$$

where x denotes the result delivered by a participant and x_M, the median, derived from valid results submitted by all participants. The robust standard deviation, σ_{robust}, calculated from all participants was used as a target standard deviation. Reported values that were obviously erroneous were not included in the calculation. This approach is used by LVU and based on the procedure described in ISO 5725-5. Further explanations are given in each PT report from LVU.

2.2.3. Interpretation of z-scores

The z-score characterizes the difference between an individual result and the median or assigned value compared to a target standard deviation in a normalized way. Normally 95% of all results can be found within the range $-2 \leq z \leq 2$. Occasionally scores in the range $2 \leq |z| \leq 3$ are to be expected at a rate of 1 in 20. Whether or not such single scores are of importance can only be decided by considering them in the context of the other scores obtained by that laboratory. Scores were $|z| > 3$ are to be expected at a rate of about 1 in 300. Given this rarity, such z-scores strongly indicate that the result is not fit-for-purpose and almost certainly requires investigation. The consideration of a set or sequence of z-scores over time provides more useful information than a single z-score.

2.3. FAPAS

Twenty rounds were provided by FAPAS which consisted of spiked and blank cake mix, infant soya formula, and oat-based foodstuff in the time between 2011 and 2016. The spiking material was gluten powder in all cases. The spiking concentration was not provided by FAPAS and assigned values were calculated from the results of participants using the test kit RIDASCREEN® Gliadin (**Table 1**). The number of participants ranged from 30 to 114.

2.4. DLA

Six rounds were provided by DLA and consisted of spiked and blank cake mix, infant formula, cookie, and cake mix in the time between 2012 and 2014. The spiking material was wheat flour in all cases. The spiking concentration is provided by DLA as a target value (**Table 2**) on the basis of assumed gluten contents in wheat flour taken from the literature. The spiking concentrations were between 19 and 34 mg/kg gluten. Mean values were calculated from the results of participants using the test kit RIDASCREEN® Gliadin (**Table 2**). The number of participants ranged from 11 to 21. Uncontaminated materials were also provided to the participants.

2.5. LVU

Nine extensive rounds were provided by LVU and consisted of spiked, naturally contaminated, and blank matrices. As can be seen in **Table 3**, a wide variety of matrices was evaluated: flour substitute, mashed potato powder, canned boiled sausage, potato dumplings, cake mix,

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gravy thickener, pasta, bread mix, bread crumbs, and cornflakes. In case of spiked matrices, flours from wheat, rye, and barley were used beside gluten and wheat proteins. It should be noted that in a few cases oat meal was also used for spiking. The spiked target concentrations ranged from 15 mg/kg gluten up to 120 mg/kg gluten. The number of participants using the RIDASCREEN® Gliadin ranged from 14 to 33.

Report no.	Year	Matrix	Assigned value	Labs total	Labs $\|z\| \leq 2$	Blank, labs correct
			mg/kg	n	%	%
27179	2016	Cake mix	44.5	114	97	94
27173	2016	Cake mix	16.1	30	90	-
27173	2016	Cake mix	28.1	30	93	-
27168	2016	Oat-based foodstuff	17.9	41	88	98
27164	2016	Cake mix	21.5	102	89	-
27160	2015	Cake mix	35.3	88	95	100
27156	2015	Infant soya formula	35	85	96	100
27150	2015	Oat-based Foodstuff	44	60	93	95
27146	2015	Cake mix	26.1	73	96	-
27142	2014	Cake mix	27.3	81	94	97
27138	2014	Infant soya formula	15.1	59	92	100
27133	2014	Oat-based Foodstuff	26.7	50	92	100
27129	2014	Cake mix	15.8	68	93	-
27125	2013	Cake mix	20.5	69	94	99
27121	2013	Infant soya formula	21.0	90	96	96
27113	2013	Cake mix	51.4	61	90	-
27109	2012	Cake mix	76.2	81	95	95
27106	2012	Infant soya formula	53.0	76	99	97
2799	2012	Cake mix	58.3	39	90	-
2795	2011	Cake mix	58.5	67	97	97

Table 1. Results from 20 different FAPAS proficiency testing rounds between 2011 and 2016 using the R5-based ELISA RIDASCREEN® Gliadin in cake mix, oat-based foodstuff, and infant soya formula spiked with gluten.

Report no.	Year	Matrix	Target value	Mean	Labs total	Labs $\|z\| \leq 2$	Blank, labs correct
			mg/kg	mg/kg	*n*	%	%
03/2012	2013	Infant formula	34	33.4	21	95	100
02/2012	2012	Biscuit	32	33.2	11	100	Contaminated
03/2013	2013	Infant formula	19	14	14	100	
02/2013	2013	Cookie	22	29.8	14	79	100
02/2014	2014	Cake mix	20.3	29.4	19	100	92
03/2014	2014	Infant formula	22	32.8	11	91	82

Table 2. Results from six different DLA proficiency-testing rounds between 2012 and 2014 using the R5-based ELISA RIDASCREEN® Gliadin in cake mix, biscuits, and infant formula spiked with gluten.

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Report no.	Year	Matrix	Gluten added as	Target mg/kg
264-23-Gluten 2011	2012	Flour substitute	-	gf
264-23-Gluten 2011	2012	Mashed potato powder	-	gf
264-23-Gluten 2011	2012	Flour substitute	Naturally contaminated	35
264-23-Gluten 2011	2012	Flour substitute	Naturally contaminated	15
264-23-Gluten 2011	2012	Flour substitute	Naturally contaminated	30
264-32-Allergene 2011	2011	Canned boiled sausage	Wheat flour	16
264-32-Allergene 2011	2011	Canned boiled sausage	-	gf
264-32-Allergene 2012	2012	Canned boiled sausage	Wheat flour	74
264-32-Allergene 2012	2012	Canned boiled sausage	-	gf
264-23-Gluten 2012	2013	Potato dumpling	-	gf
264-23-Gluten 2012	2013	Cake mix	Naturally contaminated	30
264-23-Gluten 2012	2013	Canned boiled sausage	Wheat flour	30
264-23-Gluten 2012	2013	Gravy thickener	Wheat flour	120
264-23-Gluten 2012	2013	Pasta	Gluten	50
264-23-Gluten 2012	2013	Bread mix	Gluten	80
264-23-Gluten 2013	2014	Canned boiled sausage	Wheat proteins	-
264-23-Gluten 2013	2014	Mashed potato powder	-	gf
264-23-Gluten 2013	2014	Gravy thickener	Naturally incurred	40
264-23-Gluten 2013	2014	Cake mix	Naturally contaminated	15
264-23-Gluten 2013	2014	Bread mix	Naturally incurred	30
264-23-Gluten 2013	2014	Potato dumpling	Naturally incurred	25
264-32-Allergene 2013	2013	Cookies	Rye, oat	-
264-32-Allergene 2013	2013	Canned boiled sausage	-	gf
264-32-Allergene 2014	2014	Cookies	Wheat, rye, barley	-
264-32-Allergene 2014	2014	Canned boiled sausage	Wheat flour	-
264-23-Gluten 2015	2015	Cake mix	Wheat flour	**

Report no.	Year	Matrix	Gluten added as	Target mg/kg
264-23-Gluten 2015	2015	Bread crumbs	Wheat flour	**
264-23-Gluten 2015	2015	Flour substitute	Wheat flour	**
264-23-Gluten 2015	2015	Pasta	Wheat flour	**
264-23-Gluten 2015	2015	Bread mix	Wheat flour	**
264-23-Gluten 2015	2015	Cornflakes	-	gf
264-32-Allergene 2015	2015	Cookies	Wheat, barley, oat	-
264-32-Allergene 2015	2015	Canned boiled sausage	-	gf

** Threefold higher values than expected; preparation error.

Table 3. Description nine different LVU proficiency-testing rounds between 2011 and 2015 using the R5-based ELISA RIDASCREEN® Gliadin in flour substitute, mashed potato powder, canned boiled sausage, potato dumplings, cake mix, gravy thickener, pasta, bread mix, bread crumbs, and cornflakes.

3. Results and discussion

FAPAS provided three different gluten-containing matrices with gluten concentrations that bracket the threshold of 20 mg/kg gluten (**Table 1**). Except for two of 20 rounds, the percentage of participants with a *z*-score equal to or smaller than 2 was 90% or more. The relative target standard deviation of 25% is realistic since relative reproducibility standard deviations calculated from an AACC collaborative test were between 18 and 25% [8]. Therefore, due to statistical reasons, 5% of all participants will not reach a *z*-score range of ±2.

Three rounds were based on oat-based foodstuff and it is clear that the RIDASCREEN® Gliadin is suitable not only for gluten-containing oat samples but also for oat samples itself, showing no cross-reaction. This is an important requirement since oats are a crucial component for gluten-free food. Other test kits as, for example, the ELISA based on the G12 monoclonal antibody show a significant cross-reactivity to certain oat varieties which make this system not suitable for oat-based materials [13]. Another conclusion that can be drawn from **Table 1** is the fact that blank soya materials do not exert positive results after Cocktail (patented) extraction. This possible cross-reactivity was alleged repeatedly over the last years but was never underpinned with reliable scientific data. The most probable explanation for this (unproven) observation is a contamination of soya with wheat, rye, or barley, due to agricultural commingling. If a gluten contamination of a material is assumed, this should be verified by PCR (e.g., SureFood® ALLERGEN ID Gluten; S3106; R-Biopharm). In consideration of the fact that FAPAS is the most important PT provider in Europe, we recommend delivering homogeneity data reports to participants on request and to include spiked gluten values for each material in the PT report.

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DLA provided six rounds between 2012 and 2014 (**Table 2**) with gluten concentrations slightly higher than 20 mg/kg gluten. The most interesting information from these PT schemes is the fact that target concentrations are provided. The mean recovery ranged from 74% for round 03/2013 up to 149% for round 03/2014. Since the wheat flour used for spiking the matrices is not characterized for its gluten content, the PT providers used data from the literature to estimate the gluten content within the total protein fraction. Therefore, differences between the theoretical and practical value may occur. For five out of six rounds, the percentages of participants that fulfill the *z*-score requirement of equal to or smaller than 2 is 91% up to 100%. The fact that for round 02/2013 the results for the spiked cookie material showed more variability between participants may be explained by the fact that a homogeneity test was only performed for soya which was the second analyte in this PT round and not for gluten. As for the FAPAS rounds, we strongly recommend publishing the homogeneity data and following international guidelines for homogeneity testing [14]. Additionally, the benefit of having a target concentration would significantly improve if the gluten content of the flour used for spiking would be measured and provided.

The PT rounds provided by LVU show an impressive range of different matrices (**Table 4**) with up to six different matrices in one round. The target gluten concentrations not only bracket the threshold of 20 mg/kg but also include higher values of more than 50 mg/kg. The target values given in **Table 4** were calculated using conversion factors from the literature.

For the last gluten round in 2015, an error seemed to happen during preparation of the PT samples since values three times higher than expected were measured during homogeneity testing. Therefore, no target values are given for this round in **Table 4**. Regarding the percentage of participants that fulfill the *z*-score criterion of ±2, 90% or more participants tested 26 of 33 matrices within this criterion. For the other seven matrices, it can be speculated that perhaps the sample homogeneity was lower than for the other materials. Since even highly problematic matrices, for example, canned boiled sausage, were often analyzed with very good results, the performance of the participants is not (primarily) responsible for the seven matrices that exert a higher variation. Another indication of a lower homogeneity is the fact that each round consist of up to six samples and "outlying" matrices are analyzed in a row with matrices that came out very well. Wherever a target value is given, a recovery can be calculated using the median derived out of all results. The range is from 67% for a bread mix to 117% for a flour substitute with a total mean of 94% (not shown). Again, the relative target standard deviation used for FAPAS and DLA calculations is confirmed by calculations for the relative robust standard deviations for each matrix that contain gluten. The range of relative deviations is 14–31%. These calculations also included values derived from other test kits than the RIDASCREEN® Gliadin R5 ELISA. The influence of other test kits is low because the number of participants that do not use the RIDASCREEN® system is low. Blank samples were analyzed in a qualitative way. Nevertheless, 95% or more of the participants found these samples negative.

Matrix	Target mg/kg	All assays	All assays	R7001	R7001
		Median mg/kg	Robust SD, rel. %	Labs total *n*	Labs, correct %*
Flour substitute	gf			18	100
Mashed potato powder	gf			18	100
Flour substitute	35	32.5	23	17	100
Flour substitute	15	17.6	26	17	82
Flour substitute	30	31.2	23	18	94
Canned boiled sausage	16	10.2	31	20	90
Canned boiled sausage	gf			20	100
Canned boiled sausage	74	57.1	26	18	94
Canned boiled sausage	gf			18	100
Potato dumpling	gf			17	100
Cake mix	30	28	18	19	100
Canned boiled sausage	30	25.6	20	17	100
Gravy thickener	120	129	26	14	100
Pasta	50	45	31	18	100
Bread mix	80	53.2	26	17	100
Canned boiled sausage	-	52.2	31	18	78
Mashed potato powder	gf			20	100
Gravy thickener	40	43.7	23	20	95
Cake mix	15	13.9	14	20	85
Bread mix	30	34.4	24	20	100
Potato dumpling	25	24.1	26	20	95
Cookies	-	19.2	-	32	72
Canned boiled sausage	gf			33	100
Cookies	-	46.8	-	26	96
Canned boiled sausage	-	15.2	-	30	100
Cake mix	**	77.6	24	22	77
Bread crumbs	**	27.4	16	23	91

Matrix	Target mg/kg	All assays	All assays	R7001	R7001
		Median mg/kg	Robust SD, rel. %	Labs total *n*	Labs, correct %*
Flour substitute	**	16.5	27	22	82
Pasta	**	119	27	23	96
Bread mix	**	49	21	23	96
Cornflakes	gf			22	95
Cookies	-	54	-	27	89
Canned boiled sausage	gf			28	100

* |z-score| ≤ 2 or blank correct.

** Threefold higher values than expected; preparation error.

Table 4. Results from nine different LVU proficiency-testing rounds between 2011 and 2015 using the R5-based ELISA RIDASCREEN® Gliadin in flour substitute, mashed potato powder, canned boiled sausage, potato dumplings, cake mix, gravy thickener, pasta, bread mix, bread crumbs, and cornflakes.

4. Other test kit manufacturers

Due to the restricted number of participants, we will only describe and analyze the FAPAS PT rounds for other test kits in the time from 2014 to 2016. Rounds from DLA or LVU show a negligible number of participants for other test kits than the RIDASCREEN® Gliadin R5 ELISA.

Table 5 shows the results of 13 different FAPAS rounds with spiked and blank cake mix, oat-based foodstuff, and infant soya formula. Results (assigned value) for the R5 reference method are also presented for comparison. The alternative test kit from the Neogen company uses the same monoclonal antibody as the reference. In case of only two or three participating laboratories, FAPAS provides no assigned value; therefore, we decided to estimate proficiency by calculating the mean concentrations and standard deviations. A correlation analysis between both methods is not possible due to the small number of pairs of results. Instead, a difference plot is presented where the absolute difference between both methods is plotted over the R5 reference value (**Figure 1**).

This graphical presentation clearly indicates that there is a difference between both methods at least for concentrations at the threshold level of 20 mg/kg gluten. More parallel determinations using both methods are necessary to characterize the comparability between both methods. It should be kept in mind that the threshold level of 20 mg/kg gluten is a decision level. In practice, it is therefore possible that a food product was labeled gluten-free (due to the measurement with the alternative R5 method) but an official control laboratory will use the R5 reference method which maybe results in a value higher than 20 mg/kg gluten. The producer of this food may be confronted with a recall situation. All participants that used the alternative R5 method for the analysis of blank matrices got correct results (**Table 5**).

		R5 reference	R5 alternative				
Year	Matrix	mg/kg	Assigned value mg/kg	Mean (SD) mg/kg	Labs n	Labs $\|z\| \leq 2$ %	Blank labs correct %
2016	Cake mix	44.5	50		13	77	100
2016	Cake mix	16.1	-	12.7 (5.0)	3	-	-
2016	Cake mix	28.1	-	32.7 (7.6)	3	-	-
2016	Oat-based foodstuff	17.9	-	21.5 (2.1)	2	-	100
2016	Cake mix	21.5	23.5	-	12	100	-
2015	Cake mix	35.3	28	-	8	100	100
2015	Infant soya formula	35	27.4	-	6	83	100
2015	Oat-based foodstuff	44	-	28.2 (6.0)	3	-	100
2015	Cake mix	26.1	22.4	-	13	100	-
2014	Cake mix	27.3	20.6	-	9	78	100
2014	Infant soya formula	15.1	15	-	5	80	100
2014	Oat-based foodstuff	26.7	27.6	-	7	100	100
2014	Cake mix	15.8	12.5	-	9	89	-

Table 5. Comparison of results from 13 different FAPAS proficiency-testing rounds between 2014 and 2016 between the reference R5-based ELISA method (RIDASCREEN® Gliadin) and an alternative R5 ELISA test kit in cake mix, oat-based foodstuff, and infant soya formula.

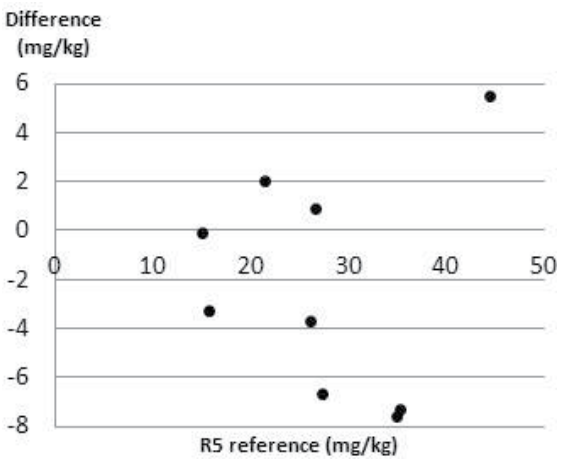

Figure 1. Plot of differences between the R5 reference method and R5 alternative method for samples that bracket the 20 mg/kg gluten threshold level.

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Table 6 summarizes results that participants obtained when using the G12 monoclonal test format. Since only one to four participants used the G12 kit, FAPAS did not calculate any assigned value for the G12 method because minimal numbers of participants are not sufficient for any realistic calculation. Instead, mean concentrations were calculated (where possible) and standard deviations. Although the amount of data is very limited, the data in **Table 6** clearly show that the methods are not comparable. The most "reliable" results can be found in two rounds with four participants. In both cases, the G12 overestimated the gluten content by a factor of two or more compared to the R5 reference method. Even more troublesome is the analysis of blank samples where the G12 often failed, perhaps due to oat in the sample. This is not a problem for coeliac patients but for the gluten-free-producing food industry. All G12 results in **Table 6** were submitted by participants using the G12 ELISA by Romer Labs. Since the G12 is promoted at an international level by the manufacturer, running a proper method comparison is strongly recommended to protect celiac patients from any relapse of symptoms. This study should include spiked samples from different matrices, naturally contaminated samples, problematic matrices like spices, and oats since the G12 is reported to cross-react with varieties of this important gluten-free grain source. Following a guideline from clinical laboratory analysis, a minimum of 100 samples should be run in parallel [15].

		R5 reference	G12		
		Assigned value	Mean (SD)	Labs total	Blank labs correct
Year	Matrix	mg/kg	mg/kg	n	%
2016	Cake mix	44.5	96 (100)	4	50
2016	Cake mix	16.1	12.4	1	-
2016	Cake mix	28.1	29	1	-
2016	Oat-based foodstuff	17.9	n.a.		
2016	Cake mix	21.5	24.2	1	-
2015	Cake mix	35.3	113 (61.7)	4	0
2015	Infant soya formula	35	13.9	1	100
2015	Oat-based foodstuff	44	n.a	1	0
2015	Cake mix	26.1	33.6 (22.1)	2	-
2014	Cake mix	27.3	32.9 (43.7)	2	0
2014	Infant soya formula	15.1	9.3	1	n.a.
2014	Oat-based foodstuff	26.7	19.2	1	100
2014	Cake mix	15.8	18.1	1	-

Table 6. Comparison of results from 13 different FAPAS proficiency-testing rounds between 2014 and 2016 between the reference R5-based ELISA method (RIDASCREEN® Gliadin) and the G12 containing ELISA test kit in cake mix, oat-based foodstuff, and infant soya formula.

5. Recommendations for PT participants

Regular participation in proficiency test is a prerequisite in Europe for laboratories that are accredited according to ISO 17025. Therefore, it is of great importance to handle PT results that are not within the expected *z*-score range. There are the following possible explanations and corrective measures for results outside this range:

(1) Check if the result for a control sample is within its specifications for this run; use samples from older PT rounds if available and compare.

(2) Is the zero calibrator as low as expected? If not or if the results are equivocal, check for contamination of buffers and surfaces using the dip-stick RIDA® QUICK Gliadin (R7003; R-Biopharm); install a proper cleaning procedure and control system.

(3) Check for complete extraction.

(4) Check if the correct extraction procedure in case of an unknown sample was used. It is strongly recommended to always use cocktail extraction as described in the test kit insert.

(5) Compare the actual result with older PT results for any regularities, for example, permanent overestimation of gluten.

(6) Ask the PT provider for a homogeneity data report if not included in the PT report.

(7) Establish in-house control material: a blank and a gluten-containing sample should be tested at minimum.

(8) Check the course of calibration graph for any irregularities, for example, bumps.

(9) Check calculation of results, for example, missing factor of two for conversion from gliadin to gluten.

(10) Did a skilled technician perform the extraction and analysis?

(11) Verification of validation data using PWG-spiked samples.

6. Conclusion

The data show that the RIDASCREEN® Gliadin R5 ELISA is also applicable to cake mix, oat-based foodstuff, infant soya formula, cookies, canned boiled sausage, gravy thickener, pasta, and potato dumpling. These independent data show once again that the R5 ELISA has no cross-reactivity to soy-based food. All PT rounds document the excellent consistency and comparability of results when using the RIDASCREEN® Gliadin R5 ELISA. Based on a comparatively small amount of data for other test kits, slightly different results were observed for test kits from other manufacturers using the R5 monoclonal antibody and considerably different results were observed for kits using the G12 monoclonal antibody.

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Chapter 4

Determination of Gluten Peptides Associated with Celiac Disease by Mass Spectrometry

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Additional information is available at the end of the chapter

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Abstract

Gluten is a big protein network composed of monomeric fraction (prolamins) and polymeric fraction (glutelins), occurring in many cereal-based products, especially in those containing wheat. Gluten peptides can trigger food allergies and intolerances, including inflammatory reactions as the celiac disease, an autoimmune disorder of the small intestine characterized by mucosal degeneration and villous atrophy. The treatment is the permanent exclusion of gluten from diet. However, gluten analysis is a very difficult task, due to the high complexity of polypeptides and the lack of consensus on the most appropriate analytical method. Proteomics approaches, combining liquid chromatography and mass spectrometry *in tandem* (LC-MS/MS), have been pointed as the most promising non-immunological techniques for gluten detection. LC-MS analyses associated with bioinformatics and specific-prolamin database can solve methodological limitations since it is based on the accurate molecular mass of peptide biomarkers. One of the major contributions of proteomics has been the identification of epitopes of gluten peptides responsible for wheat-related diseases. Recent works have defined grain-specific gluten peptides and also the lowest concentration at which peptides could be confidently detected. Proteomic application for gluten quantification should support not only regulatory limits in processed foods, but also the safety of consumers about food labeled as gluten-free.

Keywords: gluten peptides, LC-MS/MS, prolamins, proteomics, wheat

1. Introduction

Gluten is defined as a complex protein network present in the cereal endosperm, responsible to confer viscoelasticity to pasta. It is composed by the cereal storage proteins, divided into two protein fractions: monomers, formed by alcohol-soluble prolamins, and polymers, formed by alcohol-insoluble glutelins [1]. This insoluble complex occurs when the gluten proteins are

hydrated and submitted to mechanical force. Dry gluten is composed about of 75–85% proteins and 5–10% lipids, the rest being residual starch and non-amylaceous carbohydrates [1].

The wheat gluten network presents exclusive rheological properties as viscosity, extensibility, and elasticity conferred by the storage proteins: gliadins and glutenins [2]. An appropriate proportion of both protein fractions in dough is essential to guarantee the viscoelastic properties and end-product quality [1]. Due to these properties, wheat is recognized as the most suitable raw material for bread and pasta-making. Vital wheat gluten is a raw material widely added in gluten-based food products to improve quality and sensory properties and can be obtained from washing the viscoelastic dough, removing the water-soluble components [3, 4].

Besides the technological aspect, the gluten proteins can trigger food allergies and intolerances, including inflammatory reactions in patients with celiac disease (CD). CD is a gluten-sensitive enteropathy defined as an immune-mediated disorder triggered by gluten in genetically predisposed individuals.

The family of storage proteins of gluten occurs in wheat grains (*Triticum* spp.; gliadins and glutenins), barley (*Hordeum vulgare*; hordeins), rye (*Secale cereale*; secalins), and oats (*Avena sativa*; avenins). In the context of gluten intolerance, one of the most common definitions of gluten is provided by the European Commission Regulations: "protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives thereof, to which some persons are intolerant and which is insoluble in water and 0.5 M sodium chloride solution" [5].

The gluten proteins are present in various types of cereal-based food products, mainly in wheat-based products. However, due to the incorporation of gluten as an ingredient in foods that traditionally does not contain wheat proteins, there is also a growing concern about gluten allergenicity in hidden sources of gluten, incorrect labeling or cross contamination in manufacturing, transportation, and storage [3]. Hence, because of its nutritional and economic importance, there is a big effort to characterize these proteins. Since the treatment for gluten sensitivity is the exclusion of gluten from diet, the detection and quantification of these proteins are extremely important, not only due to its direct effect on the food quality but also for food safety reasons.

Nevertheless, the gluten analysis in food products is a very difficult task, due to the need to properly extract the proteins before analysis and to the high complexity and homology of polypeptides. Hence, the first point to be addressed is the appropriate protein extraction, whose steps involve sequential buffers to perform prolamin extraction and the reduction of disulfide bonds of glutenins, formerly insoluble, releasing their polypeptides [6].

The second point is about the lack of consensus on the most appropriate analytical method to identify and quantify gluten in food. The most commonly used methods are based on enzyme-linked immunosorbent assay (ELISA), PCR, and also electrophoresis, but these methods differ in terms of sensitivity and present several drawbacks. The main faced problem is related to the lack of certified reference material [7]. In fact, the immunological methods are based on the use of developed antibodies for the detection of gliadins and, therefore, are not suitable for all classes of gluten proteins. In addition, current methods are unable to distinguish the source of cereals.

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The protein composition of the grain varies among different species and varieties, and it leads to methodological difficulties in the allergenic food analysis. In this context, modern proteomic approaches based on sensitive and reliable techniques combining liquid chromatography (LC) coupled with mass spectrometry *in tandem* (MS/MS) have been pointed as the most promising non-immunological techniques for identification and quantification of gluten proteins, even in trace level [7–10].

2. Cereals

Cereal grains are essentially composed by endosperm (~83%, on weight basis), germ (or embryo, ~3%), and bran (or external layers, ~14%) [11, 12]. The endosperm contains about 80–90% of starch and can contain 8–20% protein (on dry basis) that correspond mainly to gluten proteins [12]. These proteins are important due to its impact on technological processing of cereals.

The most representative species of this class are rice, wheat, rye, barley, and corn. Wheat is one of the most important and most consumed cereals in the world and is considered the most suitable raw material for baking and pasta-making. Its production and consumption have remained constant over the years, being the second most produced and consumed cereal (the first one is corn and rice is the third one) [13].

Rye, barley, and oats also have significant production and consumption, and they are mainly used for baking, especially in the case of rye; barley malt is an important ingredient for beer production but can also be found in the form of meal, flakes, or flour, whereas bran and other oat-based products are largely available for immediate consumption [14].

The cereal proteins are classically divided according to Osborne [15], in four groups consistent with its solubility, being albumins soluble in water; globulins in diluted saline solutions; prolamins in alcoholic solutions; and glutelins in diluted acids or bases. Albumins and globulins are metabolic proteins, which represent 20% of total protein content and participate in important functions in plant development and responses to environment [16], while prolamins and glutelins, cumulatively referred to as gluten, represent the major class of storage proteins (i.e., 80% of total protein), which function is to store nutrients, providing nitrogen during seed germination [12].

3. Gluten proteins

Gluten proteins are represented by the storage proteins that are divided into two groups: prolamins (e.g., gliadin, hordein, secalin, avenin), which are monomerics, and glutelins (e.g., glutenin, D-hordein, secalinin, or simply HMW secalin), which are polymerics. The last ones, as a result of the numerous covalent associations between polypeptides, may remain insoluble even in strongly denaturant buffers such as sodium dodecyl sulfate (SDS) [2, 17].

The gluten proteins present common structural characteristics. The primary structure of these proteins is subdivided into distinct domains that may have repeated sequences of some specific amino acids [2]. These proteins are unique in terms of amino acid composition, characterized by high levels of proline (P) and glutamine (Q)[1] and low levels of amino acids with charged side groups. Glutamine generally predominates (15–31%), followed by proline in the case of wheat, rye, and barley (12–14%) [18]. Cysteines represent only 2% of the amino acids of the gluten proteins but are extremely important to the structure and functionality of gluten [1]. The nutritionally essential amino acids tryptophan (0.2–1.0%), methionine (1.3–2.9%), histidine (1.8–2.2%), and lysine (1.4–3.3%) are also present only at very low levels [18].

Breeding and genetic engineering have been successfully applied to improve the content of essential amino acids, such as the case of high-lysine barley and corn. However, these approaches may be used to develop celiac-safe wheat; this remains a formidable challenge due to the complex multigenic control of gluten protein composition, besides the requirement of acceptable technological properties for bread and pasta-making [19, 20].

The cereals present variable levels of Osborne's fractions (albumins, globulins, prolamins, and glutelins). The amino acid composition of prolamins can be correlated to the botanical genealogy of cereals, where wheat, rye, and barley belong to the subtribe *Triticeae* and oat to *Aveneaea* [21]. The amino acid composition is similar in wheat, rye, and barley, whereas in oats, the prolamin composition is intermediate between the *Triticeae* and other cereals. The amount of glutamine in oat prolamins is similar of the *Triticeae*, while the amounts of proline and leucine in oat prolamins are smaller and larger, respectively, to those found in *Triticeae* [21].

Gliadins are the group of monomeric proteins present in wheat gluten, whose molecular weight (MW) ranges from 30 to 75 kDa. Gliadins are regrouped based on its electrophoretic mobility and structural similarity: α/β-gliadins, γ-gliadins, and ω-gliadins. As the other cereal prolamins, they are all soluble in alcohol, a characteristic of this group [22]. The α/β- and γ-gliadins are smaller (30–60 kDa) than the ω-gliadins ($<$75 kDa) [2]. The first ones have very similar primary sequences and present N-terminal domain with repetitive sequences with 7–11 amino acids (P/Q) and C-terminal homologue domains, with 6–8 cysteines able to form intrachain disulfide bonds [17]. The ω-gliadins show the highest levels of proline and glutamine, with repetitive sequences of 8–10 of these amino acids.

The wheat glutenins are formed by a heterogeneous mixture of polypeptides with high molecular weight, which can reach until 1 million Da. They are considered one of the biggest proteins found in nature [23]. Depending on the polymerization degree, these polymers remain insoluble even in denaturating buffers such as SDS, leading to a difficult solubilization. Glutenin polymers are formed by monomeric glutenin subunits (GS), subdivided according to the MW and stabilized by interchain disulfide bonds. The high-molecular-weight glutenin subunits (HMW-GSs) present MW ranging between 65 and 90 kDa and can be subdivided into x-type and y-type, while the low-molecular-weight glutenin subunits (LMW-GSs) present 30–60 kDa and are subdivided into B, C, and D groups according to electrophoretic mobility [22, 24].

[1]Typical of all cereal flours is the fact that glutamic acid almost entirely occurs in its amidated form as glutamine.

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In other cereals, HMW group contains HMW secalins and D-hordeins, respectively, in rye and barley. They comprise polymers (glutelins) possessing around 600–800 amino acid residues, MW of 70 and 90 kDa, and a high content of glutamine, glycine, and proline, which represent around 60% of residues [18]. HMW and MMW proteins are missing in oats. The MMW group consists of monomeric ω-secalins and C-hordeins, including 300 and 400 amino acid residues and MW around 40 kDa. They are characterized by high contents of glutamine, proline, and phenylalanine, which together account for 80% of residues.

The LMW group not only includes monomers such as γ-40 k-secalins, γ-hordeins, and avenins of oats, but also polymers including γ-75 k-secalins and B-hordeins. They have between 200 and 430 amino acid residues, with MW ranging from 23 to 50 kDa, and its amino acid composition is dominated by glutamine and proline and by relatively high levels of hydrophobic amino acids, leucine and valine [25].

Wheat gluten is of great importance in the food industry because it promotes the dough ability to retain carbon dioxide produced during fermentation, resulting in the rising of dough that presents good gas-holding properties. Barley and rye flours are also able to form gluten because of its chemical composition, whose proteins are similar to gliadins and glutenins. However, the gluten network formed by them is more fragile since these proteins are present in a smaller amount than in wheat flour [21]. Due to the unique viscoelastic characteristics conferred by the wheat gluten proteins, wheat flour becomes an essential ingredient for the food production [3].

4. Celiac disease (CD)

CD is an autoimmune disorder of the small intestine characterized by mucosal degeneration and villus loss, mainly affecting the capacity of nutrient absorption. Its origin is related with the presence of genes human leukocyte antigen (HLA)-DQ2 or HLA-DQ8, and both genotypes cause the predisposition for the disease [26], but 95% of CD patients exhibit the DQ2 serotype class [25]. In predisposed individuals, it can manifest in any stage of life, since that the contact with the protein fraction of wheat, barley, or rye was established [27].

Diagnosed patients cannot consume foods containing gluten or its traces, because even a minimal amount of this protein can trigger the reaction, causing the most varied symptoms, ranging from abdominal pain, bloating, and diarrhea to osteoporosis and infertility in long term. The severity of the reaction can be due to the degree of intolerance of each individual [28, 29].

Current knowledge about the pathogeneses of CD has been associated with the long chain and amino acid composition of the peptides generated during gastrointestinal digestion of the gluten proteins [20]. Due to the lack of lysine and arginine residues in gluten proteins, the action of the proteases, such as trypsin, but also chymotrypsin and pepsin, is very difficult, making the proteolysis practically ineffective. Because of its hard cleavage, those proline- and glutamine-rich polypeptides act as mediators of immune reactions in the intestinal epithelium cells of the predisposed subjects [25].

The most celiac-active T-cell epitopes are present on the α-gliadins, but T-cell epitopes derived from either γ- or ω-gliadins as well as from HMW and LMW-GS have been reported in Refs. [19, 30]. However, T-cell epitopes from hordeins and secalins have been also described; it can be explained by their high homology to those found in wheat [30]. While the consumption of wheat, rye, and barley has been proved to cause harm to CD patients, there is still a discussion about the safety consumption of oats by CD patients.

In this context, there are controversies about the reactivity of oat gluten, since only a few numbers of celiac patients have demonstrated to be affected by oat consumption [28, 31]. Recent reports suggest a tolerated oat consumption for a great part of celiac patients, showing a safe long-term feeding [32, 33]. Although some authors consider oats a gluten-free cereal, the main problem is the risk of cross contamination by gluten-based cereals during harvest, milling, or industry processing [5, 34, 35]. For this reason, this cereal cannot be completely discarded as CD trigger, and its consumption by celiacs is still considered unsafe [36, 37].

The *Codex Alimentarius* proposed in 2008 a standard international labeling, where products labeled as "gluten-free" must not exceed the limit of 20 ppm of wheat, barley, or rye gluten, which corresponds to approximately 1 mg of gluten in 50 g of food [38]. The maximum amount of gluten tolerated by celiac patients is not completely known, because of the variable reactivity of gluten among different species and also the unpredictable sensitivity among individuals. However, several studies have indicated that 10 mg of gluten daily are well tolerated, while intestinal mucosa damage has been observed with doses around 50 mg (as reviewed by Ref. [39]).

The difference in the amino acid composition of prolamins and glutelins from each cereal has been pointed as responsible by the different reactivity associated with the CD [11, 21]. Compared to other cereals, grains belonging to subtribe *Triticeae* (wheat, barley, and rye) contain significantly higher levels of glutamine and proline than others, being these amino acids the principal responsible for triggering the immune response in celiac disease [25]. A direct correlation between the immunogenicity of the different oat varieties and the presence of specific peptides with differential reactivities has been proposed as the origin of the wide range of variation of potential immunotoxicity of oat cultivars [40].

Triticum species exhibits an important genetic variability, resulting in different toxicities, what can be a promising alternative for obtaining suitable varieties for consumption by celiac patients [19, 41, 42]. Higher levels of immunogenic peptides related to CD were attributed to a modern Canadian wheat when compared to old varieties of common wheat and tetraploid wheat [43]. Despite the importance of genotypic variation within species and cultivars, specific knowledge about CD, especially regarding the structure of the allergens and the immunoreactive epitopes is not fully known and requires new information.

5. Gluten detection techniques

Several methods have been developed to guarantee the safety of foods labeled or expected to be gluten-free for celiac patients. However, there is no consensus about the analytical method

considered more appropriate to identify and quantify gluten in foods [37]. The main used methods are based on different techniques for the detection of DNA sequences, related proteins, such as the enzyme-linked immunosorbent assay (ELISA) and the polyacrylamide gel electrophoresis (PAGE) methods or more recently the detection of digested peptides by means of liquid chromatography and mass spectrometry (LC-MS).

These methods differ widely from each other, especially in terms of sensitivity, specificity, and cost. Other reasons for this divergence can be related with food processing (heat or hydrolysis steps); matrix type; polymorphic variants of wheat, rye, and barley; type of extraction; and possible cross-reaction with other prolamins.

5.1. Enzyme-linked immunosorbent assay (ELISA)

Currently, the ELISA method is the most common and recognized approach for detection of gluten, because it presents low cost; it is easy to perform and promotes results quickly. It is the technique recommended by the Codex Alimentarius for the detection of gluten in industrialized foods [44]. This technique is based on the immunological reaction between known toxic peptides from gluten proteins and mono- or polyclonal antibodies.

There are two variations of the method, the R5 ELISA sandwich and competitive R5 ELISA. In ELISA sandwich, samples containing the antigens are incubated to form an antibody-antigen complex, and then a labeled antibody is incubated and conjugated to another antigen epitope, forming two layers of antibodies. This method requires at least two binding sites (epitopes) for the antibody and is only suitable for large peptides or intact protein quantitation, being unfeasible to detect partially hydrolyzed gluten (e.g., fermented foods).

The competitive ELISA only requires one epitope and is indicated for detecting minor antigens, present in partially degraded gluten. In this method, a competitive binding process performed by original antigen (sample antigen) and the added antigen, leading to the competition of the antigens by the limited number of epitopes, occurs. When available, quantification can be done through calibration curves with reference proteins [45].

Some ELISA-based studies were successfully applied in the detection of wheat, barley, and rye contamination, with confirmation of the results by MS and PCR [34, 35]. However, measurements by commercial ELISA kits are inconsistent and require standardization of results due to the lack of certified reference material and the diversity of kits using different test conditions [7, 46].

Current methods are based on the use of antibodies that are not accurate and may have false-negative results. These antibodies were especially developed for the detection of gliadins and therefore are not suitable for all classes of gluten, especially in matrices that are difficult to analyze [7, 47–49]. The accuracy of ELISA method is also compromised since the result is converted into gluten by multiplication by two, assuming that the gliadin/glutenin ratio is constant. Moreover, the current methods are not able to distinguish the cereal source (wheat, barley, rye) or cultivar [50, 51].

The development of standardized gluten material represents significant progress toward the accurate analysis of gluten in low levels. However, this is a challenging task due to

polymorphism of gluten proteins, which vary from sample to sample [7, 46, 48]. When comparing the use of modern techniques such as LC-MS and ELISA, previous studies show no correlation between ELISA results and the relative content of peptides determined by MS [48]. The authors concluded that ELISA methods are no longer sufficient for gluten quantification and should eventually be replaced by MS-based methods.

In this context, methods based on MS have been alternatively proposed for gluten quantification, since it can detect specific and comprehensive peptides with good sensitivity and precision, due to the high-throughput data analysis capacity [10, 46]. A progressive number of approaches using MS have been developed, offering great potential in this area [9, 37, 46, 52].

5.2. Proteomic tools for gluten detection

Proteomics is the large-scale analysis of the set of proteins encoded by the genome responsible for controlling almost all biological processes in a particular biological system at a certain time. Proteomics includes not only the structural and functional knowledge of proteins but also the study of their modifications, interactions, localization, and quantification. The proteome of an organism is dynamic; it will reflect the momentaneous response of those cells to determinate stimulus. It means that a single genome can give origin to infinite different proteomes [53].

The most practical application of proteomics refers to the analysis of target proteins as opposed to the entire proteomes [53]. The use of proteomics in food analysis has become a key technological tool for characterization and quantification of proteins and peptides, especially when it comes to the evaluation of biological markers [54].

The protein composition of cereals is variable between different species and varieties, leading to methodological difficulties for food allergen analysis and also for selection of genotypes. The high similarity of amino acid sequences of the different prolamins, together with limitations on the available methodologies, makes the exact identification of the allergens and immunoreactive epitopes related to CD, as well as its genotypic frequency, variability, and stability, difficult [55].

In this context, proteomic approaches based on reliable and sensitive techniques such as high-resolution LC-MS reveal themselves as important tools for the identification, quantification, and also discrimination of gluten proteins, since it is based on accurate molecular mass of peptide biomarkers.

In the last years, MS techniques have overcome some limitations associated to antibody-based methods, such as cross-reactivity and discriminating capacity of gluten protein sources in a single run [46]. Recently, label-free MS experiments have been improved in order to quantify specifically CD epitopes [43].

This type of research is very important, since accurate quantification and identification of the cereal source and protein type of contamination is critical to the health and well-being of celiac patients [8]. Furthermore, labeled “gluten-free” food products have shown contamination with gluten-containing protein fractions above the acceptable (20 ppm) [56].

One of the major contributions of proteomics related with gluten sensitivity diseases, especially CD, has been the identification of epitope sequences of gluten peptides of known

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immunogenic action. A number of gluten T-cell epitopes restricted by CD associated HLA-DQ molecules have been characterized over the last few years, and a compiled list of epitopes from gluten peptides able to activate the immune system was proposed (**Table 1**) (as reviewed by Ref. [30]). It is interesting to note that the identified sequences were not only from prolamins but also from glutelins. A website dedicated to these epitopes was created to update the list, but until now presented no recent inputs [30].

Epitope	Sequence of peptides recognized
DQ2.5-glia-α1a	PFPQPELPY
DQ2.5-glia-α1b	PYPQPELPY
DQ2.5-glia-α2	PQPELPYPQ
DQ2.5-glia-α3	FRPEQPYPQ
DQ2.5-glia-γ1	PQQSFPEQQ
DQ2.5-glia-γ2	IQPEQPAQL
DQ2.5-glia-γ3	QQPEQPYPQ
DQ2.5-glia-γ4a	SQPEQEFPQ
DQ2.5-glia-γ4b	PQPEQEFPQ
DQ2.5-glia-γ4c	QQPEQPFPQ
DQ2.5-glia-γ4d	PQPEQPFCQ
DQ2.5-glia-γ5	QQPFPEQPQ
DQ2.5-glia-ω1	PFPQPEQPF
DQ2.5-glia-ω2	PQPEQPFPW
DQ2.5-glut-L1	PFSEQEQPV
DQ2.5-glut-L2	FSQQQESPF
DQ2.5-hor-1	PFPQPEQPF
DQ2.5-hor-2	PQPEQPFPQ
DQ2.5-hor-3	PIPEQPQPY
DQ2.5-sec-1	PFPQPEQPF
DQ2.5-sec-2	PQPEQPFPQ
DQ2.5-ave-1a	PYPEQEEPF
DQ2.5-ave-1b	PYPEQEQPF
DQ2.2-glut-L1	PFSEQEQPV
DQ8-glia-α1	EGSFQPSQE
DQ8-glia-γ1a	EQPQQPFPQ
DQ8-glia-γ1b	EQPQQPYPE
DQ8-glut-H1	QGYYPTSPQ
DQ8.5-glia-α1	EGSFQPSQE
DQ8.5-glia-γ1	PQQSFPEQE
DQ8.5-glut-H1	QGYYPTSPQ

Table 1. List of gluten peptide epitopes recognized by immune system (Adapted from Sollid et al. [30]).

More recently, a database (ProPepper™) built from in silico results was proposed to assist the identification of epitopes, peptides, and prolamins associated with DC and other types of wheat and cereal disorders [55]. This database contains sequences of specific peptides, in silico digested, from prolamins available in public databases (UniProtKB, NCBI GenBank), and currently presents 37,914 peptides and 833 epitopes.

5.2.1. Liquid chromatography coupled with mass spectrometry (LC-MS)

LC-MS is an analytical technique that consists in the separation process based on differential interaction of sample components of a mixture, combining a powerful technology of the generation of molecular ions (ionization), which are separated and detected based on their mass/charge ratio (*m*/*z*) [57].

In nowadays, *tandem* designs (also referred to as MS/MS) make up most of the instruments in research laboratories. In this configuration, high energy is applied to produce fragments from precursor ions; hence, the selected peptides are then submitted to fragmentation in order to elucidate the amino acid sequence, allowing the confirmation and identification of sequences differing from one single amino acid [53, 58]. LC-MS/MS is considered a gold standard for the analysis of biomolecules in complex samples, due to high levels of sensitivity and specificity, and has been used in food analysis and forensic science [59–61].

The main current strategies to identify gluten markers use both discovery (known as shotgun analysis) and targeted-based proteomic approaches. Basically, combined strategies can be applied based on primary fractionation of gluten proteins using RP-HPLC or SE-HPLC followed by a multi-enzymatic-based digestion of the protein resulting fractions and high-resolution MS or MS/MS measurements [7–9]. The investigated gluten marker peptides can be identified by comparison via theoretical (in silico) and experimental results (e.g., de novo peptide sequencing), using current protein databank (NCBI, UniProtKB) or specific cereal prolamin epitopes involved in CD pathogenesis [55].

For the selection of gluten markers, the main used MS technique is the selected or multiple reaction monitoring (SRM or MRM) that allows targeted analysis, especially for quantification even in trace levels. The MRM method uses a mass spectrometer of triple quadrupole type (QqQ), where the precursor ions will be selected and focused on the first quadrupole (Q1). The second quadrupole (q2) is actually a collision cell, where the injection of a collision gas (usually argon) leading to ion fragmentation occurs. The third quadrupole (Q3) is the mass analyzer, responsible for defining which the fragments in the collision cell according to their *m*/*z* are generated [62].

In recent studies, some authors evaluated the presence of gluten peptide markers in beers by using MRM techniques [48]. These authors revealed the superiority of LC-MS in relation to the ELISA method when comparing analytical methods to quantify low levels of gluten peptides, since MS quantification is undertaken using peptides that are specific and unique, enabling the quantification of individual hordein isoforms.

Looking for more reliable results for celiac patients, other studies have sought to define gluten-specific peptides in an attempt to validate the MS as high-sensitivity analytical method for gluten detection. Fiedler et al. [9] applied MS to identify grain-specific peptide marker for

wheat, barley, rye, and oats, to assess gluten contamination in various types of commercial flours. Martinez-Esteso et al. [7] identified a set of unique wheat gluten peptides and proposed its use as markers for the presence of gluten related to CD manifestation. The same authors reinforce the idea that this strategy can be applied to other food allergens and may be considered the first step for developing certified reference materials and defining a new methodology, more sensitive than ELISA, to detect gluten in foods.

For complex samples, such as gluten proteins, multiplex methods of acquisition, called data-independent acquisition (DIA) or MS^E, allow to recover sample of all the ions and minimize data loss (e.g., non-fragmented precursors) [10, 63]. In MS^E methods, all the ions generated at ionization source are transmitted to the collision cell, which alternates between high and low energy (c.a. from 15 to 55 eV), sending to the TOF analyzer, simultaneously, the precursors, and fragments of the peptides [64].

Modern technologies can be applied to surmount cross-reactivity problems associated to antibody recognition that are particularly challenging in gluten analysis due to high level of homology between different prolamins. For a consistent analysis of primary structures, showing a high degree of homology, it is also possible to separate peptides applying the ion-mobility system (IMS) that consists of an orthogonal separation technique, where for each value of m/z, a spectrum of *drift time* (dt) is added. The dt corresponds to the time taken by the ion to cross the ion-mobility cell, full of an inert gas, allowing the determination of cross shock sections [65].

The integration of IMS into MS^E workflows provides an additional dimension of separation, improving system peak capacity while concomitantly reducing chimeric and composite interferences; ions can be distinguished by size, shape, and charge, besides to the m/z [66]. MS^E is also able to provide absolute quantitative analysis by examining the signal response of a known internal standard spiked into the sample [10]. Developing MS^E methods to quantitatively measure gluten peptides could support advancement in understanding the natural variability in protein expression of clinically relevant wheat grain allergens. Proteomic application for gluten quantification should support not only regulatory limits in processed foods but also the safety of consumers about the food labeled as gluten-free.

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Chapter 5

Are Gluten-Free Foods Just for Patients with a Gluten-Related Disease?

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Additional information is available at the end of the chapter

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Abstract

Gluten, the set of wheat proteins that gives properties for food processing, is the cause of celiac disease (CD), and patients require a gluten-free diet lifelong. There are other bad-called gluten-related diseases as non-celiac gluten sensitivity and irritable bowel syndrome, for which triggering compounds are unknown, while wheat allergies and carbohydrate intolerances are associated with other wheat proteins and fructans, respectively. The boundaries of each disease are not clear, inducing confusion for diagnosis and dilemma about the right diet. Nowadays, the people who are currently in a gluten-free diet exceed several times the expected number of those requiring dietary gluten exclusion. It is because people consider themselves as affected and dangerously decide to self-diagnose as gluten intolerant and adopt a gluten-free diet. The alternative compounds used in gluten-free foods to obtain the technological properties given by gluten could induce problems in some disease conditions or lead to undernutrition especially in children and adolescents. It is because some gluten-free foodstuffs are limited in vitamins and minerals and contain more fat and sodium than their conventional wheat analogues. Therefore, gluten-free is not a good option for persons without diagnosis; it should be understood as a therapy, prescribed and followed by specialists.

Keywords: gluten-free, celiac disease, gluten-/wheat-related diseases, industrialized food products

1. Introduction

Gluten, the set of proteins that gives technological properties for bread making and other multiple processes, has been recognized since more than 60 years as the cause of celiac disease (CD).

Gluten/wheat is also the cause of other disorders such as wheat allergies and more recently non-celiac gluten/wheat sensitivity [1]. Treatment of these conditions requires gluten/wheat dietary restriction. Gluten-/wheat-related disorders are more frequent now than a couple of decades ago; nowadays, their global prevalence is estimated by 5% [2]. However, due to their highly variable clinical expression, an important proportion of patients could be undetected. Considering this prevalence, the proportion of people who are currently on a gluten-free diet exceeds in more than five times the number of those who require gluten exclusion as a treatment for a diagnosed medical reason [3].

There are reports that about 30% of American people would like to eliminate gluten from their diet and the reasons are wide, perhaps influenced by misunderstood propaganda. Consumers stand out the idea that gluten-free products are healthier or that gluten-free diet would help them to lose weight and to improve their mental health or because symptoms they attribute to ingestion of wheat-containing foods [4, 5]. However, does the intake of gluten-free products really offer those benefits? And especially, is the gluten-free diet safe if auto-administered, or is it an adequate food regimen to be adopted by healthy people in the long term?

In this chapter, we discuss the main indications for a gluten-free diet and refer the current trends in food industry related to gluten-free foods market. Finally, we try to clarify the main benefits, disadvantages and metabolic risks that a gluten-free diet represents for patients with different bowel inflammation diseases or for other way healthy people without a medical diagnosis.

2. The gluten-free diet and the gluten-/wheat-related diseases

2.1. The intricate spectrum of gluten-/wheat-related diseases

Celiac disease is an immune-mediated disease that affects the small intestine, precipitated by dietary gluten in genetically predisposed persons [6]. Patients suffering from this condition develop flattening and atrophy of intestinal villi, decreasing the absorption surface, and therefore there are reduction of digestive enzymes bound to membrane and poor absorption of vitamins and minerals. As a result, in addition to gastrointestinal manifestations, extra-intestinal alterations such as iron deficiency anaemia, loss of bone density and hormonal and skin disorders can be common components of celiac disease [7].

In spite of the recognition since so many years, until recently celiac disease was considered rare in the South American population and even inexistent in the Asian population. It is because of its association with the HLA-DQ2 genotype that is present in more than 95% of white European celiac patients, in which the expressed molecule is needed, but not sufficient for developing the enteropathy. Currently, it is well known that the Amerindian people expressing HLA-DQ8 haplotype alone or in combination with an HLA-DQ2 allele could suffer celiac disease [8, 9]. More recently, Wang et al. [10] found that Asian individuals with HLA-DQ9.3 are also prone to celiac disease.

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Celiac disease prevalence has increased worldwide between 1 and 2% in general population during last decades, possibly due to that is better known and diagnosed and/or to changes in lifestyles, including more acceptation of the high wheat content of the occidental diet, in the last years [7].

In addition to celiac disease, there are wheat allergies with pathogenesis mechanisms well studied, and they have been characterized since long time ago [1]. According to the route of wheat exposure, they are classified as occupational asthma, rhinitis, hives, wheat-dependent exercise-induced anaphylaxis as well as food allergy that can manifest at the skin, gastrointestinal or respiratory level [1]. The common feature of wheat allergies is that they are IgE-mediated conditions and that their symptoms appear in minutes or up to 2 h after ingestion of wheat. It usually occurs in young children with a history of atopy, in whom gastrointestinal symptoms dominate, while in older people, the expression is dermatitis, respiratory disorders and, in extreme cases, anaphylaxis [11].

Since 2012, a new clinical entity related to the ingestion of gluten-containing food emerged as non-celiac gluten sensitivity, characterized by intestinal and extra-intestinal symptoms [12]. Because of the unknown pathogenesis of such gluten sensitivity, there are no biomarkers for diagnosis but exclusion of either celiac disease or wheat allergy [13]. The terminology non-celiac gluten sensitivity is a matter of debate because patients describe symptoms after ingestion of wheat products but the causative compound is unknown; therefore, it should been called non-celiac wheat sensitivity or just wheat sensitivity or wheat intolerance.

To identify specific sensitivity to wheat compounds, a strict exclusion of dietary wheat (including gluten) should be done and then two different challenges using purified wheat without gluten or gluten alone, to evaluate symptoms [13, 14]. However, an additional trouble for result interpretation could be that some non-gluten wheat proteins such as serpins, purinins, α-amylase/protease inhibitors, globulins and farinins are also antigens involved in the celiac disease humoral immune response [15].

On the other hand, irritable bowel disease, considered the prototype of all functional intestinal disorders, has a high prevalence among adults. Its diagnosis relies upon symptoms evaluation according to the Rome III criteria, because there are no specific biomarkers identified yet [14]. Its symptoms are exacerbated by food ingestion especially wheat, recognized as one of the relevant triggers. However, the responsible component among gluten, non-gluten proteins or fructans is unknown because all of them coexist in wheat and may induce symptoms associated with intestinal inflammation in human beings. Fructans belonging to fermentable oligo-, di- and monosaccharides and polyols (FODMAP) are compounds that elicit the clinical picture of irritable bowel syndrome, found in addition to wheat, in several dietary items, as some vegetables and fruits [14].

Therefore, as related to gluten- and/or wheat-related diseases, there are a wide spectrum including celiac disease, non-celiac gluten (or wheat) sensitivity, wheat allergies, FODMAP intolerance and other functional bowel diseases. The boundaries of each wheat-related disease are not always clear, inducing confusion regarding diagnosis [6] and dilemma on which diet and treatment should be applied [14]. In that way, it is not surprising that many people

in developed countries decide by themselves to follow a gluten-free diet; only 15–16% of such diet followers have a medical diagnosis and prescribed treatment of gluten or wheat exclusion [16].

2.2. Gluten-free market and regulation of gluten-free foods

According to the *Codex Alimentarius* [17], the International Food Standards, foods for special dietary uses are "those foods which are specially processed or formulated to satisfy particular dietary requirements which exist because of a particular physical or physiological condition and/or specific diseases and disorders and which are presented as such" (Codex standard 146-1985; last modified: 2009). Additionally, there is a special standard for gluten-free foods defined as "foods for special dietary uses that have been formulated, processed or prepared to meet the special dietary needs of people intolerant to gluten" (Codex standard 118-1979; last modified 2015).

The former definitions mean that unlike foods for special medical purposes, which should be prescribed and supervised by medical doctors, gluten-free foods for special dietary uses do not need any prescription to buy them. They are commercially available, not only for gluten-intolerant individuals but also for any consumer which voluntary follows a gluten-free diet or eventually buy some of these popular products [18]. Nowadays, due to marketing strategies and trends related to healthy foods, as well as self-diagnosis of gluten-/wheat-related disorders, an important part of the population in developed countries is following a gluten-free diet. This diet is currently one of the three most popular food regimen in the world along with the low-carbohydrate and fat-free diets [5].

The trends have boosted the gluten-free and reduced-gluten foodstuffs market over the world. However, the boost of gluten-free foods is peaking because sales of gluten-free products grew more than 30% annually between 2010 and 2014. In the United States (USA), sales increased 47–86% (according to the source) between 2012 and 2013 [19, 20], while the growth was just 6% from 2015 to 2016 [19]. More than two years ago, the reason for eating gluten-free foods were 51% for improved health, 38% to feel better and 27% to lose weight; only 6% followed a medical-prescribed gluten-free diet [20]. In 2016 just 10% think gluten-free foods help to manage their weight, and few people consider that gluten-free foods are higher in quality than those with gluten, probably because people are better informed [19]. However, the market cost of gluten-free foods is still very important, reaching $1328 million of American dollars in 2016 [19].

Additionally, the *Codex Alimentarius* [17] indicates that foods made from naturally gluten-free ingredients or ingredients specially processed to remove gluten should contain no more than 20 mg/kg of gluten and foods specially processed to reduce gluten should content between 20 and 100 mg/kg, which is a decision determined at the national level. Thus, the actual gluten intake in a strict gluten-free diet, consuming 0.5–1.0 kg of labelled foods, is about 10–20 mg/day, while eating a reduced-gluten diet can accomplish up to 100 mg/day. It is not expected to eat more than 1 kg/day of labelled gluten-free foods because dietary intake includes other non-labelled as gluten-free as fresh fruits and vegetables.

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The complete exclusion of dietary gluten is almost impossible due to the ubiquitous nature of gluten in industrialized foods, cross-contamination and inadequate food labelling. Therefore, the actual problem of celiac disease patients sometimes diagnosed as refractory to treatment, because symptoms do not abate, is the consumption of unknown gluten content of some called gluten-free foods. For instance, a study was carried out in the USA analysing 275 gluten-free–labelled foods and 186 non-labelled as gluten-free without wheat, rye or barley. Three of the gluten-free labelled (1.1%) were mislabelled, meaning almost 99% of compliance, while 36 out of 186 of non-wheat containing had more than 20 ppm of gluten, and 19 of them were higher than 100 mg/kg of gluten [21].

According to the Food and Drug Administration (FDA) of the USA [22], the labelled gluten-free foods that include "wheat" in the ingredient list as wheat starch, either before or after hydrolysis (glucose syrup or maltodextrin), should specify in the label that wheat has been processed to remove gluten according to the FDA regulation. This is because while these products are safe for celiac disease patients, these could be risky for people with any other wheat-related disease.

2.3. Health effects and safety considerations of gluten-free products

Currently, to prepare gluten-free bakery products, ingredients such as rice and corn flour, mixed with hydrocolloids and enriched with milk, egg or soybean proteins, are used [23]. In addition, there are alternative grains used as buckwheat, amaranth, quinoa and teff, as well as different starch sources as these of potato and cassava. To improve the overall quality of the products, enzymes as microbial transglutaminase and proteases are used [24]. The European Food Information Council (EUFIC) and the FDA of the USA recognize all of the cited food additives as safe; also, different regulations accept them as gluten-free. However, some of them could affect the health of patients with gluten-/wheat-related and/or other gastrointestinal diseases.

Some of the food additives as emulsifiers and microbial transglutaminase could alter the integrity of tight junctions between the epithelial cells of the small intestine, increasing the paracellular intestinal permeability. Tight junction dysfunction or "leaky gut barrier" is a common feature in several autoimmune disease pathogenesis such as celiac disease and type 1 diabetes [6, 25]. It is because the opened tight junctions allow the entry of dietary antigens and trigger an immune cascade that can lead to autoimmunity in susceptible people [26]. Furthermore, leaky gut barrier function and immune activation are also important factors associated with irritable bowel disease. In this syndrome, the ingested food components can induce infiltration and activation of mast cells after passing the gut barrier, leading to the development of symptoms [14].

The problem is that a considerable proportion of the general population consider themselves as affected due to symptoms and dangerously decide to self-diagnose with gluten or wheat intolerance or sensitivity and adopt self-prescribed diets [27]. Even more, the non-celiac gluten or wheat sensitivity described in adults has little evidence in children, with no data supporting the health benefits of a gluten-free diet. If the children follow a gluten-free diet

without guidance of an experienced nutritionist or physician, it can lead to unbalanced nutrition and health complications [28].

Another additive used in gluten-free foodstuffs is inulin-type fructans because of their prebiotic properties which provide structure and gas retention during baking. Its addition to the mix dough improves the quality of gluten-free bread, enhances sensorial acceptance and increases the fibre content, reducing glycaemic response and inducing a better nutritional quality [29]. Furthermore, to help to improve the decreased calcium absorption of patients with celiac disease, the gluten-free bread can be fortified and added with inulin-type fructans increasing calcium bioavailability [30]. However, for patients with irritable bowel disease and/or FODMAP-intolerant people consuming such gluten-free bread added with fructans, its intake could be a problem.

The transglutaminase family is a set of enzymes capable to bind a protein chain with other one, through covalent bonds, inducing a net formation. If there is no amino acid residue with an amino lateral in each two neighbour chains, transglutaminases release the amino radical, producing glutamate instead glutamine, for instance, in a called deamidation process. The microbial transglutaminase (mTG) is widely used in the food industry, especially for industrially processed products that naturally do not contain gluten as well as in gluten-free bakery products for enhancing quality. Its addition simplifies the elaboration processes and reduces the production cost while improves the texture, elasticity and appearance and even reduces the caloric content. However, the products of the enzymatic activity and homology of mTG with the human tissue transglutaminase (tTG), a key component in celiac disease pathogenesis because of its deamidation capacity, could elicit the exacerbated immune response of celiac patients.

The enzyme mTG can deamidate/transamidate gluten in the same way as tTG does and can change the protein antigenicity leading to a higher antigenic load [31]. Regarding this, Dekking et al. [32] found that gluten-specific T cells recognize gluten peptides deamidated by mTG, so they recommend that patients with celiac disease should avoid the consumption of products containing it, in order to control the disease. In another study, the reactivity of IgA of celiac patients against prolamins of wheat and gluten-free breads (maize and rice flours), mTG-treated or not, was evaluated. Sera pool from celiac patients presented IgA higher titres against prolamins of mTG-treated wheat or gluten-free breads than against mTG-untreated ones. The electrophoretic pattern of gluten-free bread prolamins was modified by the mTG treatment, and a new 31 kDa band originated in maize was recognized by IgA of some patients with CD [33].

Therefore, some additives of the gluten-free foods can induce negative effects for patients with different wheat-related diseases and even for those with celiac disease depending of the added compounds. The gluten-free food formulations should be carefully designed to prevent complications as described above.

2.4. Nutrition quality of the gluten-free diet

A recognized problem associated with gluten-free diets is that it could induce nutritional imbalance, especially in children. Gluten-free cookies and some sweet products at

the Italian market have more than 20% of fat, and 7.5% of them are saturated fat [34]. In addition, gluten-free products contain less vitamins and minerals and frequently have lower protein content than the wheat-containing food products. It is because wheat flours are fortified with vitamins and minerals in several countries; hence, alternative flours for gluten-free foods should be also fortified. Despite this, most of the gluten-free products commercially available are not fortified, and those already fortified premium products are not widely available and have higher costs that are not accessible to the majority of consumers [35].

In addition to other limiting issues, gluten-free breads had a higher glycaemic index than the conventional gluten-containing breads according to the results of a comparison of 20 commercial breads of the major European brands [24]. Formerly, Mazzeo et al. [34] published a food composition database including 60 gluten-free food representatives of different categories sold on the Italian market (more than 50% of them are distributed all over Europe). Almost all the gluten-free products were high in available carbohydrates, with approximately 50% of sugar content in cookies, breakfast and sweet products, which results in a high glycaemic load when consumed.

However, due to their hydrocolloid content, half of the Italian gluten-free foods formerly described presented a dietary fibre content at least of 3% [34]. In contrast, Vici et al. [36] in a wide review between 1990 and 2015 found gluten-free diet to be poor in fibre due to avoidance of grains and because products are usually made with starches and/or refined flours. Coinciding with them, Estevez et al. [35] found, when analysing the basic basket of gluten-free products available in Chile, that regular wheat-based foods like biscuits and noodles contain 50% more fibre than their gluten-free equivalents, which also contain 24% less protein, on average. In addition, dairy products, like gluten-free cheese and yogurts, can have up to 52.4% more sodium than the regular ones.

All these compositional characteristics of gluten-free products have a direct impact for evaluation of diet as a whole. Thus, in another study in Spain [37], the average diets of 58 adults with celiac disease were analysed, finding that women in this regimen tend to decrease the consumption of protein and fibre, increasing fat, while men increase their intake of animal protein, compensating the excluded protein from cereals. In both genres, the authors detected an increase in the total caloric intake when comparing to a similar diet composed of regular foods.

As stated by Pellegrini and Agostoni [18], the gluten-free foods should improve their nutritional quality to decrease the risk of later chronic degenerative disorders.

To follow a strict gluten-free diet is a broad dietary change, which can be associated with several risks. Nutritional inadequacy of this diet can compromise the intake of minerals, especially in children and adolescents. The gluten-free diet can be associated with reduction of fibre intake because patients elude wheat and other cereal fibre sources. The lack of fortification can affect health and quality of life, increasing the risk of anaemia, osteoporosis and constipation, mainly in patients whose disease, which includes deficient intestinal absorption, already predisposes them to these complications.

All of the previous comments together do not mean that the gluten-free diet was a bad choice. As shown in **Table 1**, for people with diagnosed celiac disease, the dietary gluten exclusions are the only treatment option to avoid and/or reduce symptoms and prevent complications in the medium and long term. Even for individuals with wheat allergy, non-celiac gluten/wheat sensitivity and intestinal bowel syndrome, except for those with FODMAP intolerance who need additional dietary changes, the gluten-free diet could be able to reduce gastrointestinal symptoms because the gluten-free foods do not contain wheat with its triggering compounds.

Figure 1 shows a hypothetic design to discriminate among the wheat components possibly related to each wheat-related disease, for dietary recommendation. Therefore, if were possible to know the type of compounds responsible or related to non-celiac gluten/wheat sensitivity and irritable bowel syndrome, the gluten-free diet could be not the best treatment for patients suffering from such diseases. In many cases, a low FODMAP diet may offer a higher chance of symptomatic response; however, the gluten-free diet involves attacking a specific pathogenic factor for celiac disease based in its pathogenesis mechanism. If the injurious nature of other wheat proteins is part of the genesis of visceral hypersensitivity or other gut-related physiological changes, it is very important to look for the key compound to design the right diet [14].

Due to the technological difficulty for obtaining high-quality gluten-free products and to their lower market demand than their conventional food counterparts, they can be up to 300% more expensive [35]. Therefore, an option for people with gluten-related disorders is the home preparation of foods from unprocessed sources to devise a balanced gluten-free diet. Anyway, it is highly recommended to follow the nutritionists or medical specialist instructions as well as to do the periodic analysis of the nutritional status, especially for children, adolescents and risk persons, to maintain the best balance.

After the previous discussion, it is clear that the gluten-free diet is not a good option for persons without diagnosis of gluten hypersensitivity, especially when it relies in the abuse of industrialized products. If healthy people decide to follow a gluten-free diet because they consider it is healthier or fashionable, they should be very careful to accomplish the nutrition balance.

Advantages	Disadvantages	Risks
• Control of celiac disease, correcting the intestinal absorption problems • Prevents chronic celiac disease complications • Reduce gastrointestinal symptoms triggered by wheat in wheat allergy, non-celiac sensitivity and irritable bowel syndrome	• Unbalance of some nutrients if not well supervised • High costs • Difficult to socialize, social stigma • Palatability • Poor variety and nutritious quality	• Malnutrition • Chronic diseases related to lack of vitamins, minerals and fibre and excess of fat and sodium • Development of eating disorders • Difficult for celiac disease diagnosis and other diseases if self-administrated

Table 1. Characteristics of the gluten-free diet.

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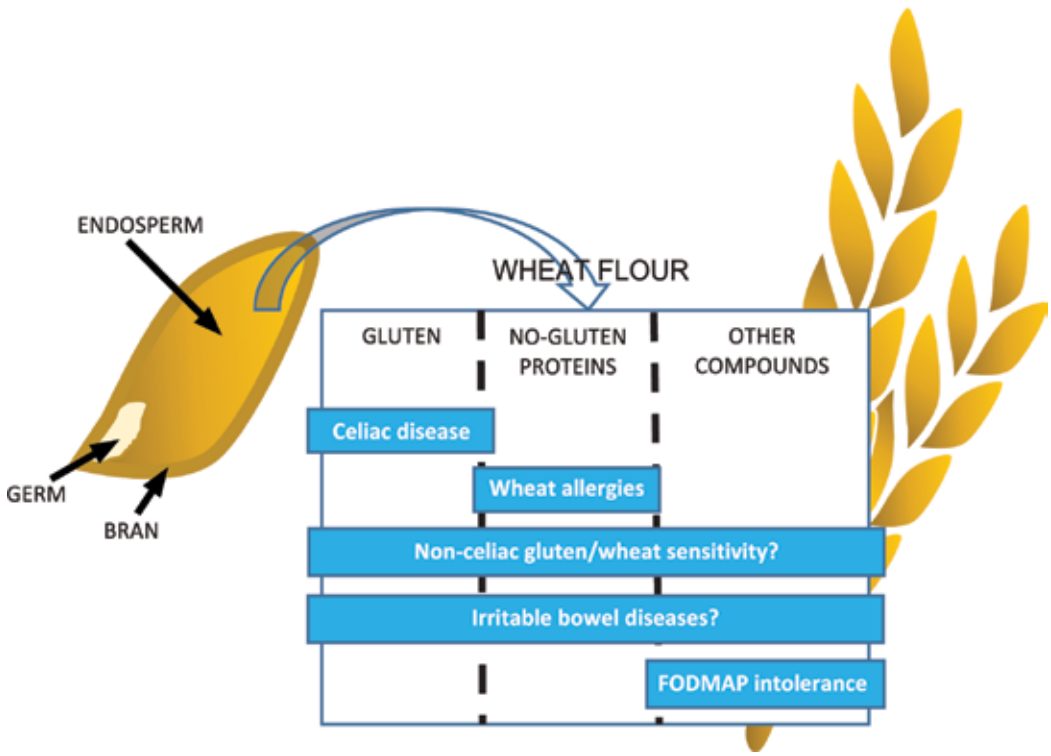

Figure 1. Hypothetic design to discriminate among the wheat components possibly related to each wheat-related disease, for dietary recommendation.

2.5. Gluten-free diet as a therapy and as a self-prescribed food regimen

The differential diagnosis of the actual disease among gluten-/wheat-related disorders has important prognostic and therapeutic implications for the patient. An undiagnosed celiac disease in children may result in growth restriction, emaciation, osteoporosis, dental problems and in acute cases electrolyte imbalance and hypocalcaemia that can be life-threatening [38]. In contrast, following a gluten-free diet in the absence of symptoms or diagnosis might mask underlying diseases.

In the last decade, due to easier access to information, self-diagnosis of gluten-/wheat-related disorders has increased, and because there is more wide availability of gluten-free foods, many people follow a gluten-free diet. This implies that one person recognizes his own symptoms and without medical advice associates them with any of the previously described diseases. Subsequently, one adopts the gluten-free diet looking for improvement. This can generate benefits in the short term in those cases where the symptoms decrease. Golley et al. [16] published a study of 1184 Australians surveyed from the general population, where 10.6% were already on a gluten-free diet, while just 1.2% of them had formal diagnosis of celiac disease. However, 80% of the followers do to relieve symptoms such as bloating, abdominal discomfort, asthenia and adynamia. Perhaps the placebo effect is part of these results while a strict following was not registered.

Self-prescription of gluten-free diet may cause unsuccessful medical diagnosis because gluten consumption is necessary to obtain an accurate and reliable blood test and biopsy. When the patient is already on a gluten-free diet, a challenge with gluten is done for a long time before performing the diagnostic test [39].

Having a medical diagnosis influences the type of diet to follow, for example, gluten-free, wheat-free or restricted in FODMAP (**Figure 1**). In addition, depending on the nature of the disease, the duration of treatment may differ between being temporary or required lifelong as for celiac disease. Favourably, a diagnosis can bring relief to a person, eliminates uncertainty, generates social acceptance, facilitates adherence to the diet and can help convince the family about the importance of the diet and the negative consequences of lack of attachment [39].

Some followers of the gluten-free diet do not have gastrointestinal symptoms and decide to enrol in a gluten-restricted regimen for other causes. In the Golley study [16], this subgroup represents up to 20% of the people in a gluten-free diet, referring reasons such as having a family member with celiac disease, personal taste or preference. In the USA, the main reason to follow a gluten-restricted diet is that people believed that gluten-free industrialized products were healthier than regular ones [40]. Although it is changing nowadays, the market cost of gluten-free foods is still extremely high, which means that there are so many undiagnosed people following a gluten-free diet.

The fact that such a large proportion of the population is in such gluten-free–restrictive regimen forces health personnel to be alert and well informed about the nutritional adequacy of the gluten-free diet in general, in order to guide and counsel this special group. Healthy people on a gluten-free diet may unnecessarily limit the variety and quality of their diet [41]. Believing that these products are healthier or considering them suitable for weight loss can cause an overconsumption of gluten-free energy-rich and nutrient-poor products and could result in the opposite way, promoting weight gain [42].

A new aspect in gluten-related disease and gluten-free diet is the intestinal microbiota. The gut microbiota and its products play an important role in the pathophysiology of celiac disease, and dietary composition can modulate the structure of microbiota. Bonder et al. [5] found that in healthy people, the gluten-free diet did not induce major inflammatory or metabolic changes in gut function after 1-month intervention, in contrast to people with celiac disease. However, they observed a decrease in the proportion of *Veillonellaceae*, considered a pro-inflammatory family frequently reported in patients with inflammatory bowel syndrome. Possibly, it could be another reason explaining why the gluten-free diet benefits this group of patients.

A current very common practice in athletes is to follow the gluten-free diet, perhaps influenced by news about a famous tennis player with celiac disease diagnosed five years ago. According to Lis et al. [43], more than 40% of endurance athletes follow it at least half the time. They consider the idea that these foods, in addition to being healthier and useful for controlling their weight, relieve systemic inflammation and improve athletic performance. However, the double-blind, placebo-controlled, crossover study by Lis et al. [43], in 13 competitive cyclists,

found neither positive nor negative effects on performance, gastrointestinal symptoms or systemic inflammation measured as cytokine responses: IL1A, IL-6, IL-8, IL-10, IL-15 and tumour necrosis factor alpha. For all these reasons, it is recommended that athletes seek nutritional advice to ensure that their diet meets the special requirements that their sport implies, before deciding to start a gluten-free plan.

3. Conclusion

Due to the global epidemic of malnutrition, where extreme problems such as obesity and emaciation prevail together, public health policies should implement a promotion to increase the consumption of real healthy foods, such as whole grains, fresh fruits and vegetables, and reduce the consumption of discretionary foods [41].

Thus, the awareness of gluten effects on healthy and diagnosed individuals is summarized in that a gluten-free diet should be understood as a therapy and therefore only to be prescribed and supervised by specialists. Finally, to eat occasionally a gluten-free foodstuff does not need any prescription but money to buy it.

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Complementary Information

Chapter 6

Novel Endoscopic Techniques in Celiac Disease

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Jinga Mariana

Additional information is available at the end of the chapter

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Abstract

Celiac disease (CD) is a systemic, immune-mediated illness that primarily affects the small bowel. A few decades ago, in the era of Watson and Crosby capsules, we used to sample the small bowel without even looking at it. Nowadays, with the continuous developing field of digestive endoscopy, we can even see the duodenal villi up closely, allowing for an optical, real-time diagnosis of villous atrophy. Advanced endoscopic techniques such as magnification, chromoendoscopy (dye-based and digital), water immersion, confocal endomicroscopy, endocytoscopy, and optical coherence tomography (OCT) have been evaluated in CD with good results: good agreement with histology, allowing for targeted biopsies and a reduction in the number of biopsies needed for diagnosis. Moreover, with the growing use of open-access endoscopy in many parts of the world, endoscopy is now contributing to increasing the diagnostic rate of CD, by recognition of endoscopic markers in patients without clinical suspicion of this disease. This is however an observer-dependent method; to overcome the endoscopists subjectiveness in assessing villous atrophy, in the last years, many papers have looked at means of computerized analysis of endoscopic images. Currently available data show that these automated, quantitative methods hold very promising for the future.

Keywords: celiac disease, advanced endoscopy, capsule endoscopy, computer-aided, diagnosis, endoscopic marker

1. Introduction

Celiac disease (CD) is a systemic autoimmune disease triggered by ingestion of gluten in genetically susceptible individuals. Although so much is known about this disease (its trigger, autoantigen, genetic predisposition, target organ damage, and diet treatment), it remains heavily underdiagnosed. In this setting, new diagnostic strategies are being searched for, and

great attention has been pointed toward the role of endoscopy in increasing the diagnostic rate of celiac disease. Some have even proposed systematic biopsies for patients with abdominal pain or reflux symptoms undergoing upper digestive endoscopy, but this has proven to have a low yield for diagnosing celiac disease, at a high cost [1–4]. However, with the growing use of open-access endoscopy in many parts of the world, endoscopy can be a great opportunity to identify new celiacs, by recognizing suggestive endoscopic markers in previously unsuspected patients. The premises for this window of opportunity are a thorough examination of the duodenum and appropriate training for the endoscopists to recognize endoscopic markers of villous atrophy.

Moreover, endoscopy with tissue sampling is mandatory to establish a correct diagnosis, at least in adults [5]. In children, the 2012 ESPGHAN guideline proposed a triple diagnostic criteria to avoid biopsy (tissue transglutaminase antibodies over 10 times the upper limit of normal, confirmed with positive anti-endomysial antibodies in a separate blood sample; characteristic symptoms of celiac disease; positive HLA-DQ2/DQ8) [6]. Some studies have validated this rule, while others have questioned it [7–10].

But, endoscopy is more than just a mean to get the duodenal mucosal samples. If we think back a few decades, in the era of Watson and Crosby capsules, we used to sample the small bowel mucosa without even looking at it. Nowadays, with the continuous development of technology, endoscopy has turned into a very powerful tool as we can even see the duodenal villi up closely, allowing for an optical, real-time diagnosis of villous atrophy. Advanced endoscopic techniques such as magnification, chromoendoscopy (dye-based and digital), water immersion, confocal endomicroscopy, and optical coherence tomography (OCT) have been evaluated in CD with promising results: good agreement with histology, allowing for targeted biopsies and a reduction in the number of biopsies needed for diagnosis.

Even more, computer processing of images captured during endoscopy or capsule examination have been studied in diagnosing villous atrophy in celiac disease patients. These novel computerized methods are based on texture analysis or other image features and offer a quantitative assessment of mucosal atrophy, so that someday maybe they will replace the biopsy.

2. Advanced endoscopic techniques in celiac disease

Diseased small bowel mucosa is often difficult to recognize in standard, white light endoscopy. In order to enhance the subtle mucosal abnormalities of celiac disease patients, a special focus has been given to advanced endoscopic techniques: from water immersion and dye-based chromoendoscopy to digital (dyeless) chromoendoscopy, magnification, confocal endomicroscopy, endocytoscopy and optical coherence tomography [11, 12]—these have all improved the way we macroscopically evaluate the duodenal villous pattern and increased the diagnostic accuracy of endoscopy for celiac disease.

Besides better delineation of the subtle mucosal changes compared to standard white light endoscopy, these techniques help in accurately characterizing these changes and driving

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targeted biopsies. By targeting the most diseased area of the mucosa, use of advanced endoscopic techniques has the potential to reduce the number of biopsies needed for diagnosis, or even making a real-time, in vivo diagnosis of atrophy (the so-called concept of "virtual" biopsy). In vivo histology could be very useful, especially in patients who refuse biopsy and keeping in mind the frequent low quality of duodenal biopsy samples. However, most of the advanced endoscopy techniques available in daily practice can only assess villous atrophy and not the other features of celiac-type enteropathy (intraepithelial lymphocytosis and crypt hyperplasia) and this is considered an issue as villous atrophy can have a wide differential diagnosis. Not least, mucosal changes in celiac disease can be patchy and the use of advanced endoscopy could be of great help to identify the patchiness and orient biopsy sampling in these areas.

The **water immersion technique** is a simple, quick, and safe method, which can be used routinely to enhance the duodenal villous pattern during upper digestive endoscopy (**Figures 1** and **2**). Developed by the Italians [13], it consists of two steps: first, suction of the air from the duodenal lumen and second, rapid instilling of up to 150 ml water through the channel of the scope (either manually by connecting a syringe to the biopsy channel port or by using an external water-pump) [14]. This adds only about 30 sec to a standard examination and has very good diagnostic accuracy for villous atrophy (100% sensitivity, 99.7% specificity, 85.7% positive predictive value, and 100% negative predictive value for total villous atrophy, and 75, 99.5, 60, and 99.7%, respectively for partial villous atrophy) [14]. It has a favorable profile regarding the tolerability and examiner's learning curve [15]. In a scenario of a biopsy-avoiding approach,

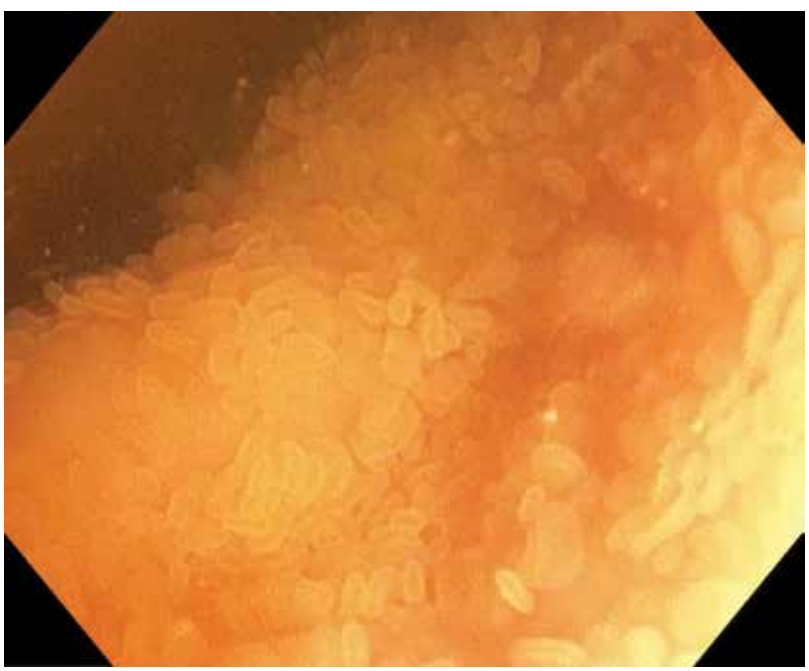

Figure 1. Water-immersion examination of the duodenum showing normal villous pattern.

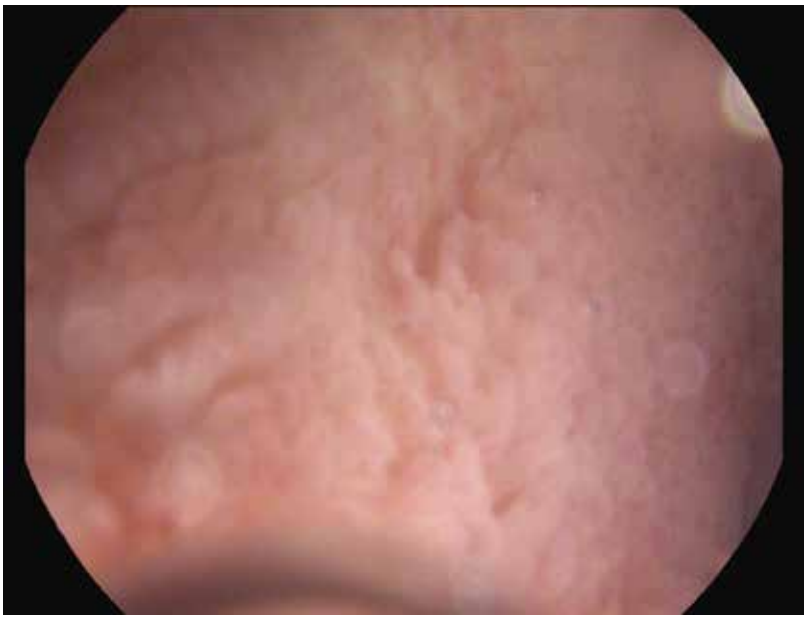

Figure 2. Water-immersion examination in a patient with partial villous atrophy.

using water immersion to diagnose celiac disease has proven to be cost effective [16]. It has also been evaluated with good results in cases of villous atrophy limited to the duodenal bulb only [17] and in the follow-up of celiac disease patients to assess histological recovery after gluten-free diet [18]. As we will see in the following paragraphs, water immersion can also be used in combination with other techniques (digital chromoendoscopy and magnification) in evaluating the duodenal villous pattern.

Dye-based chromoendoscopy is, as the water-immersion technique, a simple, inexpensive method, which can be used to better delineate changes in the mucosal surface of the gastrointestinal tract. It consists in topically administering a colorant (methylene blue, indigo carmine) over the digestive mucosa, by using a spray catheter. The principle of chromoendoscopy is based on the fact that the human eye can better discriminate the contrast given by methylene blue or indigo carmine (which colors the depressed areas of the mucosa and highlights the surface pattern) than the red-pink hue of standard white light endoscopy. Its use in examining the mucosa of celiac disease patients dates back in 1976, as reported by Stevens [19]. Others have followed with small number of patients [20–25], some using combination of chromoendoscopy with magnification, but the most recent study on topic comes from the British; 300 patients with no previous history of CD were evaluated, with 89/300 (30%) being newly diagnosed celiac disease patients; the authors reported an increase of 12% in the identification of endoscopic markers of celiac disease with chromoendoscopy vs. white light endoscopy (48/89 meaning 54% vs. 37/89 meaning 42%, $p = 0.001$), but the overall diagnostic accuracy was poor compared to serology (**Table 1**) [26].

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Standard endoscopy	42	98	90	80
Chromoendoscopy	54	97	89	83

Table 1. Diagnostic accuracy of chromoendoscopy in celiac disease [26].

Virtual chromoendoscopy is even simpler than dye-based chromoendoscopy as it only requires the press of a button on the scope during the examination to get the desired enhancement of the digestive mucosa. Therefore, it saves the additional costs needed for dye spraying and avoids the prolonged procedure time that comes with conventional chromoendoscopy. To get the maximum from a chromoendoscopy examination, the recommendation is to use premedication with an antispasmodic and antifoaming agent and to record images during the examination (for later analysis). Some of the currently available technologies (see **Table 2**) are based on using electronically activated filters to select certain wavelengths, others use post-processing of images.

Most studies have used narrow band imaging (NBI) (**Figure 3**) and i-Scan to better visualize the duodenal mucosa. In the study of Singh et al. [27], NBI performed very good in identifying villous atrophy—93.3% sensitivity and 97.8% specificity (with k values for interobserver and intraobserver agreement of 0.82 and 0.86, respectively) and also in discriminating partial from total villous atrophy (83.3% sensitivity and 100% specificity, k at 0.73 and 0.68, respectively). Even better results have been reported with the combination of NBI and magnification in the study of De Luca [28], with 100% sensitivity, 92.6% specificity, 95% accuracy, and kappa 0.9 when compared to histology (detecting partial villous atrophy in 12 patients which was missed by standard endoscopy). In the paper of Valitutti et al., when combined with water immersion, NBI showed a diagnostic sensitivity of 87.5% with high interobserver agreement (k 0.884) [29]. The Indian experience of Dutta and Goswami has also shown good diagnostic performance for NBI with sensitivity of 87.5 and 95% and specificity of 95.2 and 90.2%, respectively [30, 31]. Goswami even proposed a NBI classification of villous pattern—NBI type I for normal finger-like villi, type II for short and stubby villi (cerebriform pattern), type III for patchy villous atrophy, and type IV for flat mucosa, without villi.

Scope company	Chromoendoscopy technology
Olympus (Tokyo, Japan)	Narrow band imaging (NBI)
	Autofluorescence imaging (AFI)
Pentax (Tokyo, Japan)	i-Scan (surface enhancement/SE, contrast enhancement/CE, tone enhancement/TE)
Fujifilm (Tokyo, Japan)	Flexible spectral imaging color enhancement (FICE)
Fujifilm (Kanagwa, Japan)	Blue laser imaging (BLI)
Karl Storz (Tuttlingen, Germany)	Storz professional image enhancement system (SPIES)

Table 2. Currently available digital chromoendoscopy technologies.

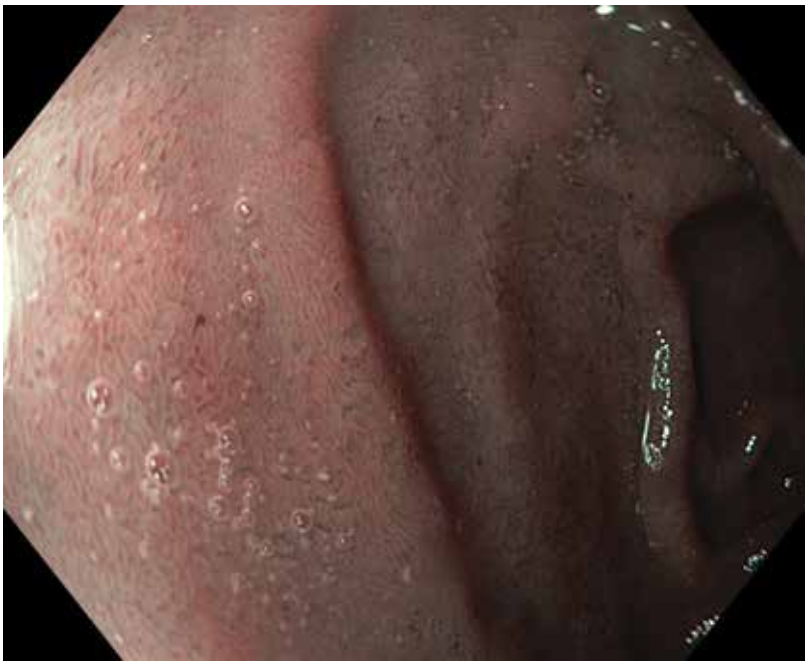

Figure 3. Normal villous pattern, endoscopy with narrow band imaging (Olympus, Tokyo, Japan).

For the i-Scan technology, Cammarota reported accuracy of 100% for detection of marked villous atrophy and 90% for partial villous atrophy and normal villous pattern [32]. Strong correlation with histology (r = 0.732) and high sensitivity (96%) was obtained by Iacucci by combining i-Scan with water immersion [33]. In a comparative study with or without i-Scan, Penny et al. concluded that it is the high definition endoscopy that increases the detection of celiac disease during routine endoscopy, irrespective of the use or not of i-Scan [34].

Good results have also been reported with flexible spectral imaging color enhancement (FICE), on small numbers of patients—100% accuracy in evaluation of villous patterns (marked villous atrophy, partial villous atrophy, and normal villi) [35].

Not least, digital chromoendoscopy techniques such as NBI can be used to detect patchy villous atrophy (**Figure 4**) [36].

Magnification or zoom endoscopy allows the endoscopist to get high-resolution, magnified images (up to 135×) in real time (**Figure 5**), which undoubtedly outperforms the standard endoscopy in assessing the villous pattern [15, 37]. It has been studied in combination with other techniques—water immersion, chromoendoscopy, and acetic acid instillation ("acetowhitening"' or enhanced magnification endoscopy), with very good results (see **Table 3**) [37–42]. It is also been shown to be useful in detecting patchy celiac disease [43]. However, contrasting these supporting results, the study by Kiesslich et al. [21], on assessing duodenal abnormalities (not necessarily focusing on villous atrophy) by dye staining and magnification, the latter did not further increase the diagnostic yield of chromoendoscopy.

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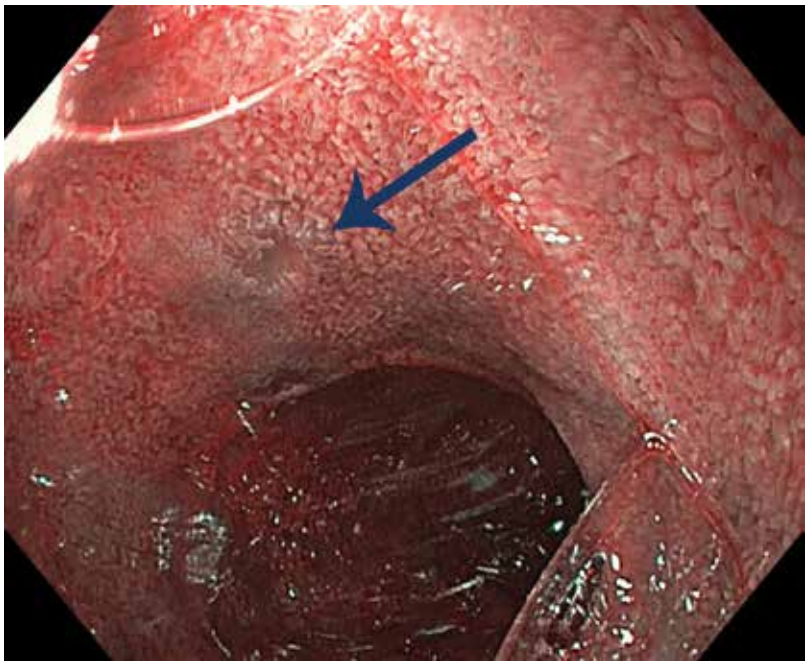

Figure 4. Patchy loss of villi pattern, endoscopy with narrow band imaging (Olympus, Tokyo, Japan).

By using a multimodal approach (standard esogastroduodenoscopy combined with zoom and chromoendoscopy), some authors have even proposed an endoscopic classification of celiac disease (types 0, I, II, and III), with good correlation between the endoscopic changes and the histologic findings (reported as Marsh grade) [44].

Confocal laser endomicroscopy (CLE) was first introduced in practice in 2004 by the team of Ralf Kiesslich (Mainz, Germany). CLE is based on integrating a confocal microscope in the distal tip of a conventional scope, which illuminates the mucosa with a 488 nm wave, allowing for cellular-level imaging (1000× magnification) up to 250 μm in depth. By offering mucosal architectural and cellular details, CLE is considered a method of in vivo histology, thus offering so-called virtual or optical biopsies. Currently, CLE can be done either with a dedicated scope, which has the confocal scanner integrated into the tip of the scope (integrated or endoscope-based CLE, eCLE, or iCLE—available from Pentax, Tokyo, Japan) or by using miniprobes which fit into the working channel of the scope (probe-based CLE, pCLE—available from Cellvizio Endomicroscopy System; Mauna Kea Technologies, Paris, France) [45]. Irrespective of the method used, CLE requires contrast agents, the most commonly used being intravenous fluorescein and topical acriflavine. Because image acquisition during CLE examination is greatly artefacted by peristalsis, respiratory, and circulatory movements (especially in the upper GI tract), the procedure usually consists in capturing the images and analyzing them after.

In addition to the advanced endoscopic techniques previously discussed, CLE also allows for assessment of crypt hyperplasia and intraepithelial lymphocytosis, which brings it closer

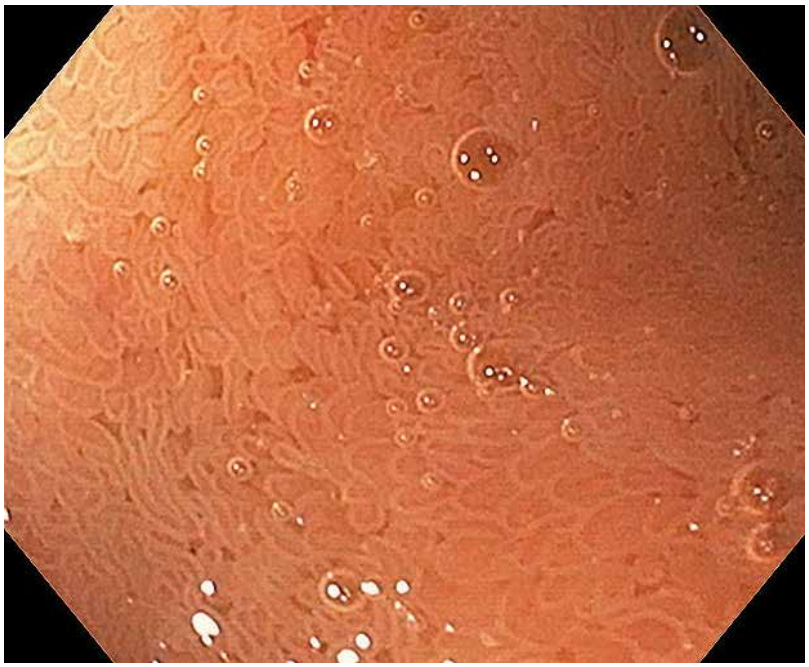

Figure 5. Magnification image of normal, finger-shaped duodenal villi.

to histology when considering all the features of celiac-type enteropathy and not only villous atrophy. Therefore, it can provide a real-time, full diagnosis of celiac disease (avoiding time and cost of processing and difficulty in interpreting biopsy samples), as shown in a case report by Trovato [46]. Furthermore, CLE overcomes the disadvantage of nonrepresentative biopsies of conventional endoscopy by allowing targeted biopsies to relevant mucosal areas [47].

As shown by Zambelli et al., the images acquired by CLE are similar to those obtained by histology, in both normal and celiac disease patients, with best visibility and quality for epithelial architecture and less for inflammatory infiltrate and crypt [48].

Experience of CLE in celiac disease is not very large; three studies have shown good diagnostic performance compared to histopathology—sensitivity of 100, 94, and 73%, specificity of 80, 92 and, 100%, respectively [49–51]. In the study by Leong et al., CLE achieved an excellent AUROC (receiver operator characteristics area under the curve) of 0.946. It is worth mentioning that the CLE has a limited ability to evaluate the crypt depth, as Gunther reported modest agreement with histology for crypt hyperplasia (sensitivity 52%, compared to 74% for villous atrophy and 81% for intraepithelial lymphocytosis). In the same study by Gunther, high interobserver agreement was seen for all three histologic features of celiac disease.

Despite being a very valuable tool, the use of CLE is limited in clinical practice because it is very time consuming, and it is burdened by a high cost of the equipment and need for training.

Author, year	Technique used	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value
Banerjee, 2007	Magnification endoscopy	100	91	83	100
Siegel, 1997	Magnification endoscopy + indigo carmine-chromoendoscopy	94	88		
Badreldin, 2005	Zoom endoscopy (115×)	90.7	62.9	83	77.2
Cammarota, 2004	Magnification endoscopy	95	99	95	99
	Magnification endoscopy + water-immersion technique	95	98	92	99
Lo, 2007	Magnification endoscopy + acetic acid instillation (enhanced magnification endoscopy)	100	–	–	–

Table 3. Summary of studies evaluating magnification endoscopy in the detection of villous atrophy [38–42].

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Optical coherence tomography (OCT) combines the ultrasound and infrared technologies, and is mostly known for its use in ophthalmology. With OCT, we get 1.5 mm in-depth examination of the digestive mucosa, and the images generated resemble closely the histological architecture. Studies done by Masci et al. have shown 100% concordance with histology in both diseased and normal individuals, also with good discrimination between the various degrees of villous atrophy [52–54].

Endocytoscopy is another novel endoscopic technique that allows in vivo, real-time visualization of mucosa under 450× magnification, by using a high power objective lens. Similar to CLE, it is also available as probe-based and endoscope-based equipment and it requires placing the scope/probe in contact with the mucosa to generate images [55]. The study of Matysiak-Budnik et al. on 16 celiac disease patients and seven non-celiac controls have found good concordance between endocytoscopy imaging and conventional histology [56]. The method is not used in daily practice.

Capsule endoscopy is a non-invasive, but expensive method to examine the small bowel. With 8× magnification lens and the ability to capture images at a rate up to 6 frames/second, capsule endoscopy is an excellent method to evaluate the villous pattern. It is usually reserved for special situations, mainly where there is suspicion of refractory or complicated celiac disease (malignancy and ulcerative jejunitis). However, it can also be used as a diagnostic tool for patients unwilling or unable to undergo upper GI endoscopy or to assess the extent of small bowel involvement [57]. Theoretically, it could be also used to search for villous atrophy in seropositive patients with normal histology on duodenal biopsy, although the study by Lidums et al. does not support this [58]; however, in a small case series, celiac disease was diagnosed on the basis of changes visualized by capsule endoscopy, when upper digestive endoscopy and biopsy were unable to provide a diagnosis [59].

As shown in several studies (**Table 4**), capsule endoscopy has high accuracy in recognizing endoscopic markers of villous atrophy, but its major drawback is the lack of possibility for tissue sampling, which is currently the cornerstone for adult celiac disease diagnosis. Also, another limitation is the need to get training in order to get proficient in this technique. Not least, although it has the highest specificity for detecting total villous atrophy in celiac disease patients (**Table 4**), it performs less well in partial villous atrophy [57].

Author, year	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Petroniene, 2005	70	100	100	77
Hopper, 2007	85	100	100	88.9
Rondonot, 2007	87.5	90.9	96.5	71.4
Biagi, 2007	93.6	63.6	100	77
Maiden, 2009	67	100	100	60
Lidums, 2011	93	100	100	89

Table 4. Summary of studies evaluating the diagnostic accuracy of capsule endoscopy in the detection of villous atrophy [60–64].

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Enteroscopy has changed the way we think of the small bowel—if a few decades ago, we thought of it as unreachable beyond the limited examination possible during upper and lower GI endoscopy, with the latest technology, we are now confident that we can do an extensive evaluation of the small bowel. The advantage over capsule endoscopy is that enteroscopy allows for tissue sampling and therapy.

Main indications for enteroscopy are patients with positive serology but normal or equivocal findings in duodenal biopsies [65] and patients with suspected refractory or complicated celiac disease [66, 67].

As capsule endoscopy, enteroscopy should be considered as a complementary method in the diagnosis and management of celiac disease.

All in all, there is strong evidence for the use of advanced endoscopic techniques in the evaluation of the duodenal mucosal pattern (**Table 5**), as it brings several benefits: improving detection of mucosal changes (especially in the setting of partial villous atrophy, where endoscopic markers are not that evident), delineating their extent, identification of patchy disease, and targeting biopsies. This latter aspect allows for a reduction in number of biopsies needed for diagnosis by focusing on relevant mucosal areas and it could be of great significance to optimize the endoscopic evaluation, as several studies have shown low compliance with the currently recommended number of biopsies [68, 69].

As some of these techniques are readily available, being just a press of a button away, endoscopists should be trained to use them routinely. Besides equipment costs and training, another major limitation of these techniques is that while they are very accurate in detecting villous atrophy, most of them cannot establish the full extent of histologic injury, as they cannot asses for intraepithelial lymphocytes and crypt hyperplasia. For techniques that offer in vivo histology, solid expertise and histological knowledge is mandatory.

Author, year	Endoscopic tool	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Oxentenko, 2002	Standard endoscopy	59	92	–	–
Cammarota, 2004	Water immersion	90.9	99.5	83.3	99.7
Johnson, 2014	Chromoendoscopy	54	97	89	83
Singh, 2010	NBI	93.3	97.8	93.6	96.7
Iaccuci, 2016	i-Scan + immersion	96	63	–	–
Banerjee, 2007	Magnification endoscopy	100	91	83	100
Lo, 2007	Enhanced magnification endoscopy	96	–	–	–

Table 5. Summary of studies evaluating the diagnostic performance of various endoscopic techniques in the detection of villous atrophy [14, 26, 27, 33, 38, 42, 78].

3. Endoscopic markers in celiac disease

Over the time, several endoscopic features suggestive of villous atrophy have been described, and many studies have investigated their diagnostic accuracy for celiac disease. The endoscopic markers described in celiac disease are [70–73]:

- mucosal atrophy, with visible submucosal vascular pattern (**Figure 6**),
- mosaic appearance (**Figure 7**),
- nodular pattern of the mucosa (**Figure 8**),
- presence of mucosal fissures (grooves), leading to a "cracked-mud" appearance (**Figures 9** and **10**),
- reduction or complete loss of folds in the distal duodenum (**Figure 11**), and
- scalloping or a dented aspect of the Kerckring folds (**Figure 12**).

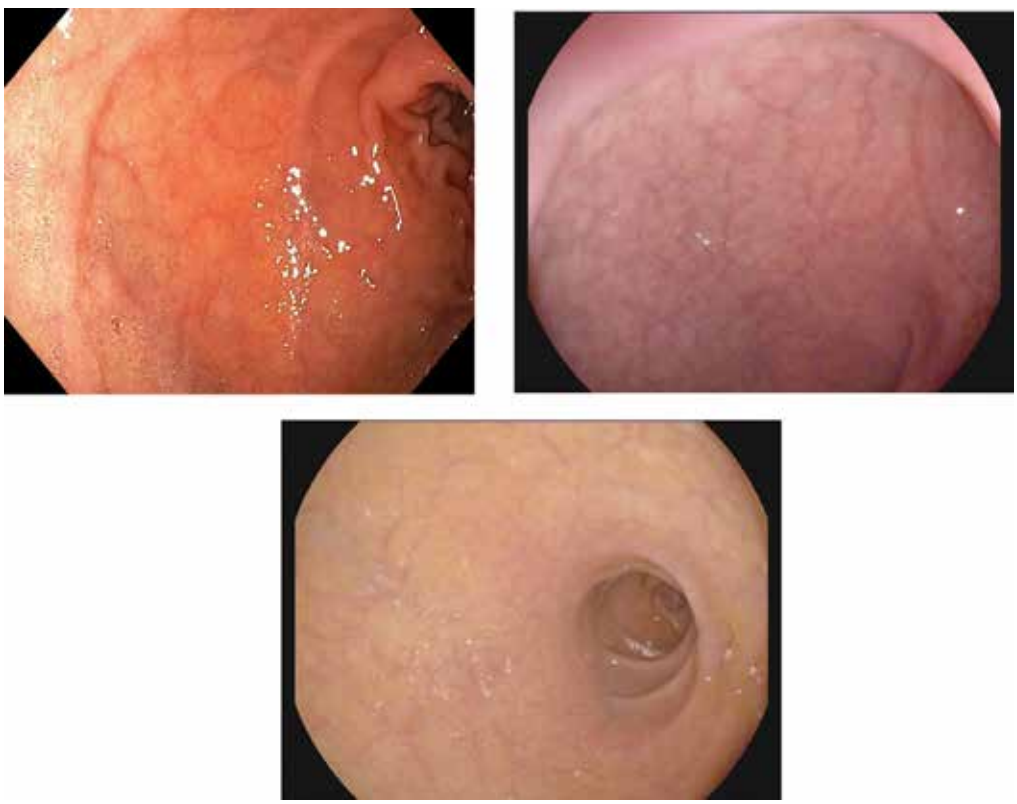

Figure 6. Standard endoscopy showing atrophic mucosa of the duodenal bulb.

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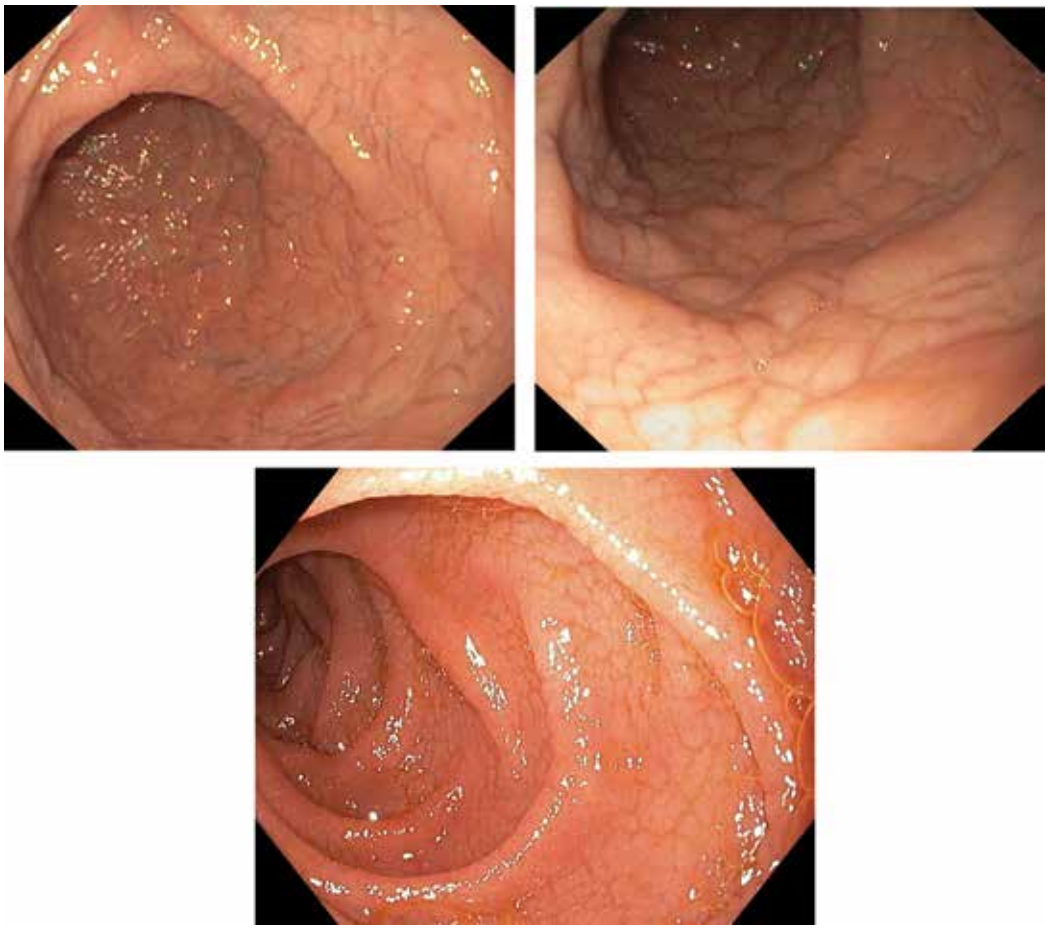

Figure 7. Mosaic pattern of the duodenal mucosa.

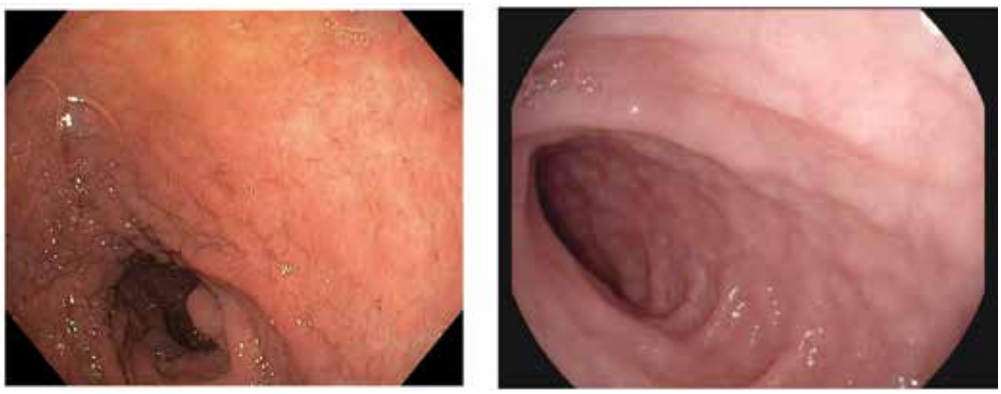

Figure 8. Fine nodular pattern of the duodenal mucosa.

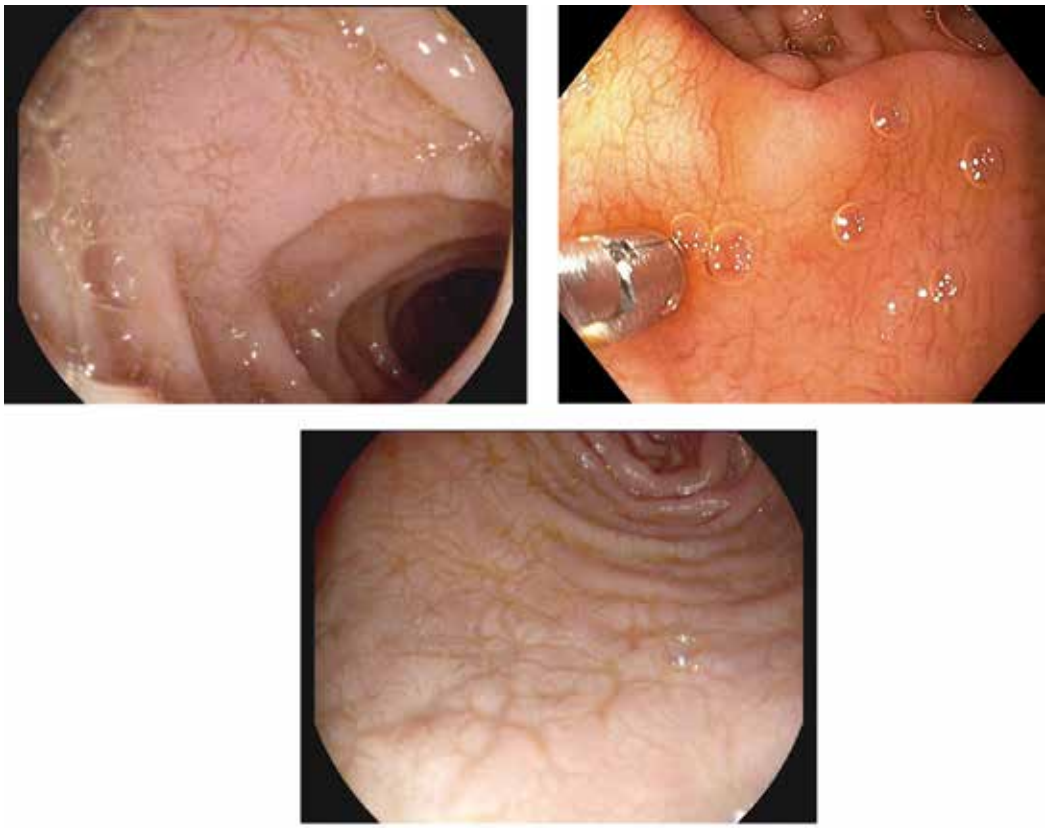

Figure 9. Standard endoscopy showing fissuring of the mucosa.

Erosions in the duodenum have also been described in celiac disease, but they are more frequently related to peptic injury or non-steroidal anti-inflammatory drug use [74].

Studies assessing the diagnostic performance of aforementioned markers have shown highly variable results, with sensitivity ranging from 6 to 96.7% and specificity from 83 to 100% [75]. This could be explained by the heterogeneity of the studies regarding inclusion criteria, the subjectiveness of the examiners in evaluating the endoscopic markers, and by the different pre-test probability of having celiac disease (as reported in comparative studies with low- and high-risk groups) [76, 77].

Because of the conflicting results of studies investigating the diagnostic accuracy of these endoscopic markers, one cannot rely on their presence or absence to decide whether to do or not to do biopsies in case of suspected celiac disease. Current recommendation is to perform biopsies when there is clinical suspicion of celiac disease, regardless of the presence of endoscopic markers [73], although some proposed that owing to their high negative predictive value, biopsy avoidance could be accepted with a normally appearing duodenum on careful examination in a low-prevalence population [77].

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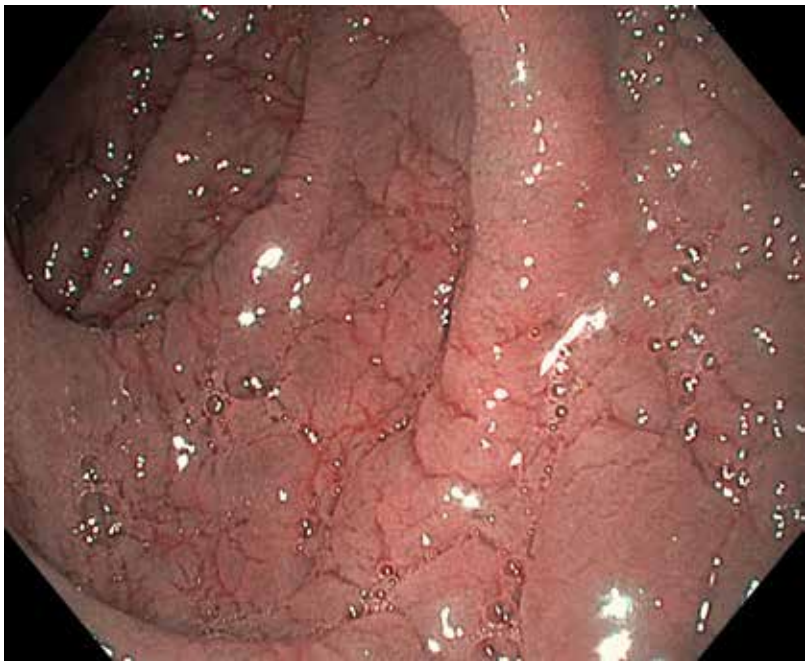

Figure 10. Mucosal fissuring seen with NBI (Olympus, Tokyo, Japan).

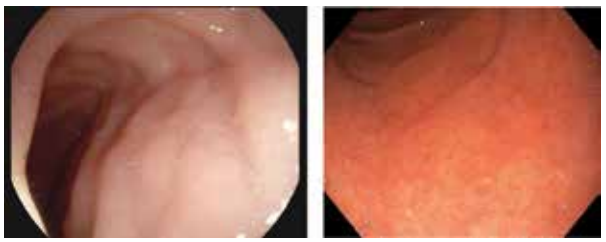

Figure 11. Loss of folds in the distal duodenum.

Another point is that such endoscopic markers are usually described and searched for in the distal duodenum, while bulb changes are frequently neglected [78]—this could be a major pitfall in the practice of endoscopists, especially in light of recent evidence about ultrashort celiac disease (meaning celiac disease with histopathologic changes limited to the duodenal bulb only) [79]. In our paper on this issue [75], we evaluated both the duodenal bulb and the distal duodenum with respect to the presence of endoscopic markers. We have shown high specificity for scalloping, mosaic pattern and fissures, concordant to results of others who even stated that a normal duodenum, with the absence of scallops or grooves, excludes subtotal villous atrophy [80]. Scalloping was reported to be a reliable endoscopic marker for celiac disease from the study of Kasirer also [81].

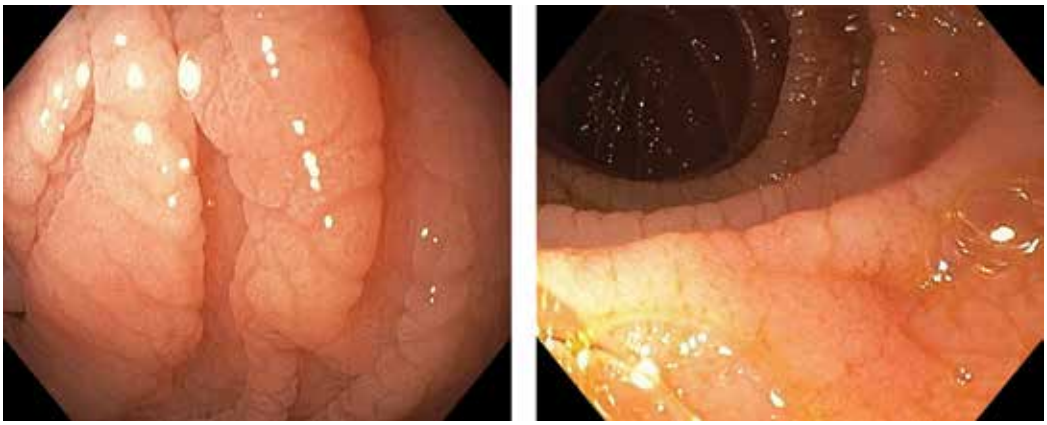

Figure 12. Scalloping of the duodenal mucosa.

At the opposite, reduction in number or loss of folds had a low diagnostic yield, as Niveloni et al. also reported and explained it by the subjectiveness of the endoscopists in the evaluation of folds (interobserver agreement 0.41 compared to 0.76 for mosaic pattern and 0.83 for scalloping) [82]. This finding is supported by the paper of Reyes et al., who stated that reduction or loss of folds are not reliable unless other endoscopic features are also present [77]. On the other hand, Maurino et al. had previously found the opposite—the changes in folds were both sensitive and specific for celiac disease [83]. Regarding the number of markers detected during endoscopy, we found that the presence of two or more markers performed well in predicting celiac disease, with an AUROC (under the curve receiver operating characteristics) of 0.885 [75].

Another issue of these endoscopic markers is that they are present in case of marked villous atrophy, but are usually absent in milder degrees of atrophy (such as Marsh 3a), nondestructive enteropathy (Marsh 1 or 2, meaning infiltrative and hyperplastic enteropathy) or in patchy disease. It has been shown that prevalence of endoscopic markers is lower in partial villous atrophy than subtotal or total villous atrophy (58 vs. 82%) [84]. This is an additional argument, why a no-biopsy strategy, with an apparently normal duodenum, is not feasible.

Also, endoscopic markers are not always that obvious on a gross examination of the duodenum, so that use of novel endoscopic techniques such as chromoendoscopy may be useful to detect these markers by enhancing the subtle changes in the duodenal mucosal pattern. As shown in the study by Niveloni et al., use of chromoendoscopy better delineated the endoscopic markers but did not provide any additional diagnostic yield; however, dye staining improved the interobserver agreement for some of the endoscopic markers (folds changes—k at 0.41 in standard endoscopy, 0.59 with chromoendoscopy) [82]. Other authors have even proposed a key role for these advanced endoscopic techniques in the decision to perform tissue sampling; according to them, biopsy should be done only in patients with villous atrophy detected by image-enhancing endoscopic techniques; however, they also acknowledge that this would miss Marsh 1 patients [85].

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In summary, recognition of endoscopic markers during routine endoscopy could represent a great opportunity to increase the diagnostic rate of celiac disease. In the era of open-access endoscopy, this incidental action to detect unsuspected celiac disease patients could have a significant diagnostic impact [86]. Careful examination of the duodenum is needed to detect endoscopic markers of villous atrophy, which should trigger the endoscopist to do biopsies. As shown by Castro et al., detection of endoscopic markers is associated with a high probability of diagnosing celiac disease (15.6 positive likelihood ratio) [87], so they should be attentively searched for, especially in high-risk patients.

However, absence of endoscopic markers does not rule out celiac disease. Not doing biopsies in a normal-appearing duodenum is associated with a significant miss rate [88]. On the other hand, excessive biopsies without any clinical, laboratory workup or endoscopy-guided selection of patients could represent an unnecessary burden to both endoscopists and pathologists. The best approach to maximize the diagnostic rate with limiting unnecessary biopsies is to use a prediction model that combines pre-endoscopic with endoscopic findings [88].

Not least, one should keep in mind that detection of villous atrophy on endoscopy does not necessarily imply celiac disease, as the differential is very wide (peptic injury, infectious enteropathy, common variable immune deficiency, collagenous sprue, autoimmune enteropathy, drug-induced enteropathy, and eosinophilic enteropathy) [89].

4. Computer-aided diagnosis in celiac disease

During routine examinations, analysis of endoscopy images to detect villous atrophy can be quite difficult because of peristalsis, and presence of luminal foam and bubbles; also, mucosal changes are frequently subtle and are not so easy to spot in the above-mentioned conditions. In the last years, great attention has been paid to processing and analyzing of images captured during endoscopy (especially capsule endoscopy), with regard to several image-related characteristics, in evaluating celiac disease patients. The strong point of using such techniques is that it provides a quantitative, automated evaluation compared to the subjectiveness of assessing the presence of endoscopic markers of villous atrophy—it thus eliminates the interobserver bias reported for other techniques [90].

First studies on this matter looked at the texture, brightness, and motility of the small bowel in videoclips from videocapsule examinations of celiac disease patients and controls [91–94].

Later, Ciaccio et al. converted the original images from capsule endoscopy in grayscale and performed an automated histogram analysis, with good results in discriminating celiac disease patients from controls [95]. An interesting feature was that of using shape-from-shading modeling to assess the architecture of the mucosa, which was validated by the same group of Ciaccio et al. [96]—the number of villous protrusions/image was statistically significant lower in celiacs versus controls ($p < 0.0001$). Other methods tested for the quantitative, computerized assessment of villous atrophy in celiac disease are the degree of fissuring [97] and spectral analysis [98]. They even proposed that such quantitative, automated analysis of the

structural features of the mucosa could be done in real time and displayed as a score during endoscopy [99].

Other research groups have also studied some advanced image processing techniques (wavelets, feature vectors, and distortion correction) in optimizing the computer-aided diagnosis of celiac disease [100–102]. However, these methods are not yet ready for current practice.

But, although histology is the current gold standard in diagnosing celiac disease, computer-aided diagnosis holds very promising for the future. Such computerized methods have been studied on imaging from non-treated celiacs at endoscopy, capsule, and even confocal laser endomicroscopy [103]. Compared to an endoscopy + histology approach, which is invasive, costly, time-consuming and subject to interobserver variability, a computer-based decision strategy is less invasive, time-sparing, and observer independent. Even in the current biopsy-based diagnostic approach, computer-assisted image analysis could be useful by helping endoscopists to target the areas with significant mucosal alterations, which would be otherwise difficult to detect. Not least, the result of using such computational means is numeric, which makes it more accurate in differentiating pathology from normal and in monitoring patients on a gluten-free diet. They need however to be validated in larger cohorts and in gluten-free–treated celiac disease patients. Also, strong collaboration with image engineering techs should be developed in order to optimize descriptors for image processing in celiac disease [104, 105].

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Chapter 7

Examining Non-Celiac Consumers of Gluten-Free Products: An Empirical Evidence in Spain

Tiziana de-Magistris, Hind Belarbi and Wajdi Hellali

Additional information is available at the end of the chapter

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Abstract

This chapter investigates the personal factors that influence intention to purchase gluten-free products (GFPs) in Spain by non-celiac consumers. To achieve this objective, a survey was conducted with 222 consumers in a medium-sized Spanish town, Zaragoza, during March–April 2014 and, ordered bivariate probit model was estimated. The results suggest that intention to purchase is affected not only by self-reported GFP knowledge but also by attitudes toward GFPs, gender, and education level.

Keywords: gluten-free, non-celiac consumers, intention to purchase, bivariate probit

1. Introduction

Celiac disease (CD) is an autoimmune pathology associated with a permanent intolerance to a protein called gluten to which the immune system responds abnormally, generating damage in the small intestine. Although CD cannot be cured, the main treatment for this pathology is to follow a diet without all cereal grains and their derivatives in order to prevent damage to the intestine [1, 2]. In the past decade, the gluten-free (GF) demand trend has dramatically increased even if people with CD represent only 1–2% worldwide [3]. One of the major reasons for the increase in the popularity of gluten-free products (GFPs) is obesity epidemic that has encouraged also people who do not suffer from CD to adopt different eating habits and to show some interest in GFPs. Several beliefs and facts related to food intolerance have emerged, for example, that gluten may increase the risk of attention deficit hyperactivity disorder (ADHD), irritable bowel syndrome (IBS), and autism [4]. Even though there is no scientific consensus about the existence of relation between gluten and these diseases, many non-celiac consumers are choosing a GF diet to preserve their health. This fact is also confirmed by a study carried out by packaged facts [5], which revealed that the main reasons why consumers

intentionally purchased gluten-free products are because they considered GFP healthier, helpful for weight loss, and higher quality. Hence, trends in the GF market has been increasing around 28% since 2008 suggesting that the supply of GFPs could satisfy the demand not only of celiac individuals but also of people without CD who decide to preserve their health status by excluding gluten from their diets [6]. Hence, understanding of the predictors of purchase behavior of non-celiacs people is critical in light of potential consequences associated with elimination foods containing gluten from their diet when there is no medical necessity. Indeed, several people believe that a GF diet may result in a diet that is high in fat and low in carbohydrates and fiber, as well deficiencies in proteins, minerals, and vitamin B-12 [7, 8].

Empirical evidence on non-celiac behavior toward GFP is still scares. To our knowledge, there are just three other investigations on GF consumers examined non-celiac consumers' preferences for some GF attributes. To illustrate, Laureati et al. [9] compared the sensory and hedonic perceptions between celiac and non-celiac people. The authors found that there was no difference between the two groups in the description and perception of GF bread, and that the choice of bread was based upon the softness and porosity of GF bread. Likewise, de-Magistris et al. [10] explored the effects of organoleptic attributes on preferences expressed in terms of willingness to pay (WTP) for GF snack assessed by non-celiac consumers in Spain. The results indicated that the texture of the GF snack was the only significant and positive attribute on consumers WTP values. Finally, de-Magistris et al. [11] reported that taste and GF label use did not influence the non-celiac consumers' WTP values.

Nevertheless, since there remain significant gaps concerning the analysis of determinants affecting the intention to purchase of GFPs by non-celiac consumers, our study aims to fill this gap in the literature. Therefore, the aim of this study is to analyze the intention to purchase GFPs in Spain by non-celiac consumers. To assess the determinants of intention to purchase, an ordered bivariate probit model is specified and estimated by using data for a survey conducted in Spain in 2014. To the best of our knowledge, this is the first study to investigate the intention to buy GFPs by non-celiac people in Spain. This chapter is structured as the following. Section 2 describes the legislation on gluten-free products while Sections 3 and 4 explain the Spanish Federation of Celiac Association (FACE association) and gluten-free label, respectively. Then, Section 5 describes the methods to conduct the investigation while Sections 6 and 7 discuss the results and conclusions.

2. Legislation on "gluten-free" food

The levels of gluten in the gluten-free products can vary greatly, misleading the consumer and potentially impacting on their health. Defined labeling terms will act, as protection measures, which will ensure that all food labeled, are suitable for people intolerant to gluten. In addition, consistent labeling will help consumers to better understand how much gluten there might be in the foods they buy and help them manage their risk of exposure to gluten [6].

Stemming from a joint Food Agriculture Organization of United Nations (FAO) and World Health Organization (WHO) Food Standards Program, the Codex Alimentarius Commission

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procedure manual is giving guidance to government's member for food legislation and industry, especially when participating in global trade. In the revised Codex Alimentarius publication about standard for foods for special dietary use for persons intolerant to gluten [12], gluten-free food is a dietary food naturally containing no wheat prolamins and/or consisting from wheat which have been specially processed to remove gluten; however, the gluten level should not exceed 20 mg/kg in total. Codex standards also recognizes another category of food namely "Foods specially processed to reduce gluten content to a level between 20 and 100 mg/kg" that is consisting of one or more ingredients from wheat, which have been specially processed to reduce the gluten content to a level above 20 up to 100 mg/kg in total.

Likewise, in the European Union, the rules concerning the composition and labeling of food intended for people suffering from an intolerance to gluten are common, the terms gluten-free and very low gluten are covered by the Commission Regulation (EC) No. 41/2009 for the labeling of gluten-free foods [13], that set levels of gluten for all categories of foods, non-pre-packed, pre-packed, or sold loose, in health food stores or in catering establishments, claiming to be either "gluten-free" or "very low gluten", which came into force in January 2012. These levels are:

- "Gluten-free": at 20 parts per million of gluten or less.
- "Very low gluten": at 100 parts per million of gluten or less; however, only foods with cereal ingredients that have been specially processed to remove the gluten may make a "very low gluten" claim.

Further, the Regulation (EC) No. 1169/2011 established the mandatory labeling for all foods of ingredients such as gluten containing ingredients [14], with clarity and more consistency, and that is by:

- a minimum font size of information to make labeling clearer,
- indicating allergens in the ingredients list, and
- emphasizing allergen information for non-pre-packed food, including in restaurants and cafes.

For this reason, later the Regulation (EC) No. 609/2013 amend the Regulation (EC) No. 1169/2011 on the provision of food information to consumers as regards information on the absence or reduced presence of gluten in food [15].

Ultimately, the new Regulation (EC) No. 828/2014 clarifies how operators can inform consumers of the difference between foods that are naturally free of gluten and products that are specially formulated [16].

3. Spanish Federation of Celiac Associations (FACE)

As the Association of European Celiac Societies (AOECS) cover 35 members from 29 European countries to increase the awareness of celiac disease, to facilitate the accessibility of information

and the availability of gluten-free products. In Spain, the Spanish Federation of Celiac Associations (FACE) was legally established on June 27, 1994 as a non-profit organization, its main aim is to ensure the well-being and quality of life of those suffering from celiac disease. This federation groups together with 16 Celiac Associations from the autonomous regions of Andalusia, Aragón, Asturias, the Balearic Islands, the Basque Country, the Canary Islands, Cantabria, Castile-La Mancha, Castile-León, Community of Valencia, Extremadura, Galicia, La Rioja, Melilla, Murcia, and Navarre. In each region of Spain, there is an official association for celiac people. All of them, except the Celiac Association of Madrid (ACM) and the Celiac Association of Cataluña (SMAP), are part of the FACE.

Furthermore, it coordinates and supports the efforts undertaken by the member associations/federations in defense of their rights, with an emphasis on unity of action leading to great success in achieving joint aims. It also takes into account safety regulations, manufacturing processes, and an evaluation of the ingredients listing for products sold in Spain to publish listing of gluten-free products that are "Safe for Celiac" by manufacturer and a FACEMOVIL application that offers assistance to celiac.

Its affiliate in Aragon, the Celiac Aragonese Association (ACA), is a non-profit organization that provides information about the celiac illness and the gluten-free diet. It also provides information about restaurants, hotels, and other establishments that collaborate with them.

4. The quality label

In addition to the general labeling provisions reclaim in the General Standard for the Labeling of Prepackaged Foods [17] and the General Standard for the Labeling of and Claims for Prepackaged Foods for Special Dietary Uses [18], and any specific labeling provisions set out in a Codex standard applying to the particular food concerned, the Association of European Celiac Societies (AOECS) has created a licensing system (**Figure 1**) for the use of the crossed grain symbol, which is the international emblem for the gluten-free products. Only the companies and organizations meeting their criteria can use it [19].

The AOECS has also established a:

- Registration no.
- Gluten content.
- Oats content. A product containing oats as an ingredient or pure oats, shall be labeled "gluten-free" and may use the symbol as long as the word "OATS" is displayed under it.
- And gluten-free Standard based on a Hazard Analysis and Critical Control Point System (HACCP) for producers and food safety inspectors to avoid contamination with gluten at any stage during the manufacturing, packaging, and storing processes.

Even more, the Spanish Federation of Celiac Associations has settled a quality label "Controlado por FACE" to assure to the celiac consumers that any products carrying it is complying with the requirements proposed by FACE concerning maximum content in gluten, making them safe for their consumption (**Figure 2**).

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Figure 1. The crossed grain symbol (by AOECS).

Figure 2. The quality label "Controlado por FACE" (by FACE).

Any enterprise which produces gluten-free products may use the quality label. However, this label can be used also by those companies that produce foodstuffs that can be consumed by celiac when the absence of gluten in the food product is guaranteed.

Furthermore, the quality label also requires control over suppliers of raw materials to avoid the risk of gluten contamination, by means of which a more efficient control is exercised over food products aimed at celiac.

Even though, it may exist in the market some legends and symbols of "gluten" or "gluten free" that are usually used by private brands and do not have official character.

5. Materials and methods

5.1. Data gathering and questionnaire

As mentioned previously, the aim of the study is to investigate the intention to purchase GFPs by non-celiac consumers in Spain. Therefore, a survey was conducted in Spain from March to April 2014. The sample size of the research consisted 222 subjects randomly chosen across the city. The population was considered infinite since Zaragoza has more than 70,000 citizens. Zaragoza was chosen because it is a town widely used by food marketers and consulting companies since the socio-demographic profile of people living in this town is representative of the entire Spanish population.

The error was calculated to the following equation (1) taking into account the proportional data and the population of Zaragoza:

$$N = 4 * p * q/\varepsilon^2 = \text{ 222 Surveys} \quad (1)$$

where N is the total sample size, $P = 0.5$ for a maximum sample size, $Q = 1 - p$, ε is the error term which was set at 6.71% for an inferential error 0.995.

The technique chosen for framing the sample was probabilistic proportional sampling.

5.2. The questionnaire and variables definitions

Consumers were asked to complete a questionnaire concerning questions on consumer purchase behavior for GFPs (**Table 1**). The questionnaire was divided in several parts. The first section analyzed knowledge toward GF. An opening question evaluated the self-reported knowledge of the participants. As showed in **Table 1**, the level of GFPs knowledge (KNOW) was measured by asking respondents their self-reported level of knowledge from 1 to 3, where 3 indicates the highest level of knowledge.

The second part of the questionnaire focused on health status and purchase habits. The first question was to ask the respondents if they suffered from any disease or intolerance related with gluten (SUFFER). This variable was measured on a 5-point Likert scale with 5 meaning strongly disagree. The second question was if non-celiac individuals used to taste new food and beverages (NEW) and it was measured on a 5-point Likert scale with 5 meaning strongly disagree. Then, another question was to determine if consumers ate sweet snacks when they were sad (SWEET), measured by a 5-point Likert scale with 5 meaning strongly disagree.

The last question in the questionnaire was the importance of the gluten-free label by asking the participant whether they seek or not for this type of labeling on the products they purchase (LABEL). The question was coded as dummy variables meaning 1 if individuals seeked for GF labeling when shopping, 0 otherwise.

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Name (Type)	Variable definition	Sample
Endogenous variables		
INTENTION	Intention to purchase GFP	
	Yes (5)	12.6%
	Probably yes (4)	20.7%
	Indifferent (3)	32.4%
	Probably no (2)	19.8%
	No (1)	14.4%
KNOWLEDGE	Consumer's GFP knowledge	
	High (3)	5%
	Medium (2)	34%
	Low (1)	61%
Exogenous variables		
FEMALE (dummy)	Gender	
	Male	49%
	Female	51%
AGE	Age of respondent (average)	47.8
UNIVERSITY (dummy)	Education of respondent	
	Elementary School	27%
	High School	43%
	University	30%
INCOME	Average household monthly net income	
	Between 900 and 1500 Euros	46.8%
	Between 1501 and 3500 Euros	39.2%
	More than 3500 Euros	14.0%
	Attitudes toward healthfulness of GFPs and its taste	
HEALTH (Likert scale)	I believe that GFP are healthier than conventional ones	2.82
EFFECTS (Likert scale)	I believe that GFP have secondary effects	3.28
CHEAP (Likert scale)	I believe that GFP are expensive	3.54
	Health status and lifestyles (dummy or average)	
LABEL (dummy)	I usually pay attention to GF label before buying some products	21%
DESEASE (Likert scale)	I have some disease linked to intolerance	4.7
NEW (Likert scale)	I usually like to taste new food and beverages	3.8
SWEET (Likert scale)	When I am sad I usually eat sweet snack	3.1

Table 1. Sample characteristics (%, unless stated) and definition of the variables [21].

In the third part of the questionnaire, the attitudes toward GFP were evaluated. In particular, individuals were asked if they believed that GFP were healthier than conventional ones (HEALTH), that GFPs had secondary effects (EFFECTS), and they were expensive (CHEAP).

The fourth section of questionnaire consisted of the intention to purchase GFPs measured by asking respondents whether they intended to buy these products (GFP) if they were available at the place they usually do their purchases. This variable was measured on a scale from 1 (definitely no) to 5 (definitely yes). The last part of the questionnaire provided information on demographic characteristics of the respondents. They were asked to indicate their year of birth, gender, number of household members, monthly incomes, level of studies (Primary, Secondary, and University), and neighborhood.

5.3. Model specification

In the model of intention to purchase gluten-free products, we consider two discrete variables: knowledge (KNOW) and intention to buy (INTENTION), as showed in **Table 1**. Since it is likely that the intention to purchase GFP and the knowledge toward them are correlated, a bivariate ordered probit model is specified to take into account for the possible correlation of error terms between the equations.

Eq. (2) in our model is the level of knowledge on GFPs (K) specified as:

$$K_i^* = \omega y_i + \xi_i \tag{2}$$

where y_i represents all the exogenous variables such as personal and socio-demographic characteristics attitudes toward healthfulness of GFPs and its taste and, the importance attached to GF labels for each "i" respondent and ξ_i is the normally distributed error term N $(0, \sigma_\zeta^2)$. K_i^* is the unobserved knowledge about GFPs but the knowledge (K) stated by the respondents (K) is observed and has been measured by three levels (**Table 1**) as follows:

$$K_i = 1 \; if \; K_i^* \leq \psi_1 \tag{3}$$

$$K_i = 2 \; if \; \psi_1 \leq K_i^* \leq \psi_2 \tag{4}$$

$$K_i = 3 \; if \; \psi_2 \leq K_i^* \tag{5}$$

The second question in the model is consumers' intention to purchase gluten-free products (IP), specified as follows:

$$IP_i^* = \lambda K_i^* + \beta x_i + u_i \tag{6}$$

where K_i^* is the consumer's GF knowledge defined above; x_i contains all exogenous variables such as socio-demographic characteristics, attitudes toward healthfulness of GFPs, and its taste and lifestyles and eating habits, and, u_i is the error term normally distributed N$(0, \sigma_e^2)$. IP_i^* is an unobserved variable but the stated intention to purchase (IP) was measured by five levels, as follows:

$$IP_i = 1 \; if \; IP_i^* \leq \tau_1 \tag{7}$$

$$IP_i = 2 \; if \; \tau_1 \leq IP_i^* \leq \tau_2 \tag{8}$$

$$IP_i = 3 \; if \; \tau_2 \leq IP_i^* \leq \tau_3 \tag{9}$$

$$IP_i = 4 \; if \; \tau_3 \leq IP_i^* \leq \tau_4 \tag{10}$$

$$IP_i = 5 \; if \; \tau_4 \leq IP_i^* \tag{11}$$

As mentioned before, to estimate the two Eqs. (2) and (6), we assumed that the error terms (u_i and ξ_i) may be correlated and follow a normal distribution N$(0,\sum)$ and the bivariate ordered probit has been estimated using the STATA 11 statistical software package (see Sajaia [20], for an explanation of the estimation procedure).

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6. Results

Summary statistics showing the characteristics of the sample and the population are presented in **Table 1**. About 49.1% of the samples were male while 50.9% were female. The group age "more than 60" represented the majority of the sample with the 28.4% and the group age "18–30" represented the minority of the sample with the 21.6%. In addition, the table indicates that the percentage of subjects living alone or in pairs was 43.7% and the percentage of subjects living in small or medium families, three to four members, was 41.9%. With regard the household monthly incomes, the sample was considered to have low and average household incomes, 46.8% of the subjects stated incomes up to 1500€, 49.2% between 1500 and 3500€, and only 14% above 3500€. Finally, around 27% of the participants had primary education level, 39.2% secondary education level, and 33.8% university level.

The estimated parameters for the model defined by Eqs. (2) and (8), using the variables defined in **Table 1**, are presented in **Table 2**. First, we estimated the model with all explanatory variables reported in **Table 1**. Those variables individually and/or jointly insignificant were dropped one by one in the subsequent estimations until we got the final model presented in **Table 2**.

Coefficients	Knowledge			Intention to purchase		
	Estimates	*t*-ratio		Estimates	*z*-ratio	
Female	-	-		0.220	1.65	*
University	-	-		−0.351	−2.27	
Desease	0.351	1.78	*			
Label	1.066	5.31	***			
New	0.141	1.80	*			
Health	-	-		0.169	2.07	**
Effects	-	-		0.130	1.92	**
Cheap	-	-		−0.151	−2.43	***
Sweet	-	-		1.37	2.18	***
Know	-	-		0.53	4.11	***
N						
Wald test χ^2 (3)	34.30					
Prob > χ^2 = 0	0.000					
Log Likelihood=	−430.922					
ρ = (*z*-ratio = **)	−0.601	−2.87	**			

*,** denotes statistical significance at the 5 and 10% significance levels.

Table 2. Estimates of the bivariate ordered probit model.

In the estimations, we considered only those exogenous variables statistically different from zero at the 5% significant level. First, the *p* value was statistically significant at 5% suggesting that errors for the two equations are indeed correlated. Therefore, we can conclude that the simultaneous estimation of both equations is the appropriate approach to obtain consistent parameter estimates since equations are not independent of each other.

Only three variables have been found statistically significant at 5% level in the GFP knowledge equation: DESEASE, LABEL, and INNOVATION. All variables had positive and significant effect on GFP knowledge. These results indicated that consumers who declared to have some member of their family with disease, usually paid attention to GFP label when shopping and they like to taste new food products were more likely to have a high knowledge toward GFPs. Self-reported consumer's knowledge (KNOW) variable was statistically significant on the intention to purchase equation. The positive estimated coefficient associated with the KNOW variable indicated that consumers more knowledgeable on GDPs were more likely to be willing to buy them. As Azjen stated, there was a significant relation between the intention to purchase GFPs (INTENTION) and the attitudes toward GFPs [22]. For example, as expected, people who stated that GFP were healthier than conventional ones (HEALTH), did not have secondary effects (EFFECTS) and they were not expensive (CHEEPS), they were more likely to buy GFPs (SWEET).

Finally, regarding socio-demographic variables, as we expected, the estimated coefficient for the variable UNIVER, was negative meaning that people who had lower educational degree were more likely to buy GFPs. Finally, FEMALE variable had positive and significant effects meaning on GFP knowledge meaning that women were more likely to have higher knowledge of GFPs.

The marginal effects were calculated to assess if the exogenous variables affected on the KNOW and INTENTION variables which were ordinal. In the case the exogenous variables were continuous, the marginal effects were calculated by means of the partial derivatives of the probabilities with respect to a given exogenous variable. Nevertheless, if exogenous variables were dummy variables, the marginal effects were calculated taking the difference between the predicted probabilities in the respective variables of interest, changing from 0 to 1 and holding the rest constant.

In **Table 3**, the marginal effects for the continuous variables and for the dummy variables are reported.

With respect to self-reported knowledge on GFPs, the marginal effects indicated that non-celiac consumers who declared to have some member of their family with disease, they used to pay attention to GFP label were more likely to state a medium or higher level of knowledge on GFPs.

Regarding the intention to purchase GFPs, results indicate that female consumers with lower level of education and self-reported GFP knowledge were more likely to buy GFPs. As consumers presented more positive attitudes toward GFPs, they were more likely to buy. Finally, results reported that those consumers who believed that GFPs had secondary effects was not available in the shops, they were less likely to buy them

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	Know = 1	Know = 2	Know = 3	Inten = 1	Inten = 2	Intent = 3	Intent = 4	Intent = 5
				−0.05	0.05	0.00	0.05	0.04
Female				0.07	0.05	−0.08	−0.06	−0.05
University								
Disease	0.34	−0.13	0.11					
Label	1.03	0.39	0.29					
New	0.14	−0.05	0.04					
Health				−0.04	0.03	0.00	0.04	0.04
Effect				0.03	0.02	0.00	0.03	0.03
Cheap				−0.03	0.02	0.00	0.02	0.02
Sweet				−0.02	−0.02	0.00	0.02	0.02
Know				−0.10	−0.08	0.01	0.09	0.09

Table 3. Marginal effects of knowledge and purchase intention.

7. Conclusions and final remarks

The GFP demand has been increasing in popularity among non-celiac consumers since the past decade. In this study, we investigated factors affecting the intention to buy GFP by non-celiac consumers in Spain. To achieve this objective, we conducted a survey in Spain with 222 non-celiac consumers. Generally, results confirmed that knowledge, positive attitudes toward GFPs, tasting new products, gender, and education level influence the intention to buy GFPs.

The marketing implications of these findings are several. Increasing knowledge on GFPs is paramount important to increase intention to purchase and therefore consumption of GF in Spain. Because more knowledgeable consumers are more prone to buying gluten-free products, information campaigns on gluten-free products should be implemented to increase demand for these products. These campaigns should target mainly consumers with lower levels of knowledge, particularly men with no university degree because they were found to be less knowledgeable. On the other hand, paying attention for GF label when shopping, willingness to try new food and beverages, and to have some intolerance to gluten were two distinctive characteristics for knowledgeable consumers. Hence, our findings support that media advertising campaigns providing clear information about GFPs could be a good strategy for GF companies to ensure that their products become known in the Spanish market, targeting women and people with lower level of education.

Further, our findings also showed that consumers who believed that GFPs are healthy, cheap, and did not have secondary effects were more likely to buy GFPs. Hence, in order to encourage the purchase of GFPs, an excellent communication strategy for enterprise is to focus on healthiness of GFP because they do not present secondary effects and they are not expensive with respect to conventional products. In this way, non-celiac consumers would be more prone to buy them.

Finally, GF companies in order to penetrate the Spanish market and to increase their sales afterward could promote tasting promotions at the supermarkets, especially targeting those wine consumers who are more prone to trying new food and beverages. Actually, trying the product for the first time represents the precursor to liking and re-buying.

The main limitation of this study is the hypothetical bias due to the use of self-reported intention to buy GFPs in the questionnaire. Hence, future studies might analyze the final behavior rather intention to buy using non-hypothetical valuation methods, such as Real Choice Experiment and auctions in order to estimate the truthful preferences toward GFPs.

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Chapter 8

I Can't Eat That! Sticking to a Gluten-Free Diet

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Additional information is available at the end of the chapter

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Abstract

Despite the benefits of a gluten-free diet (GFD), rates for strict adherence range from 42% to 91%. Studies have established the maximum tolerable daily dose at 50 mg/day and led the European Union to restrict labelling 'gluten-free' products to those with less than 20 mg/kg. Qualitative studies have determined that patients experience social problems in five areas: eating in the workplace, shopping, travelling, eating out and eating at home with others. These situations may lead to negative emotions and affect relationships. Therefore, further research into investigating the underlying factors behind effective adherence is essential, as is the need for a theoretical framework to design programmes to improve adherence and quality of life in coeliac patients. Albert Bandura´s Social Cognitive Theory can provide a better understanding of adherence and, moreover, a theoretical framework to design self-management programmes. Within this framework, the Health Action Process Approach (HAPA) model could provide a theoretical mechanism to better understand GFD adherence. The main aim of this paper is to review the factors related to GFD adherence and to present the HAPA model as a useful framework for the design of interventions to improve perceived self-efficacy, adherence to the diet and, thus, enhance quality of life in coeliac patients.

Keywords: coeliac disease, gluten-free diet, self efficacy expectation, adherence, quality of life

1. Introduction: the GFD challenge

The only treatment to date for coeliac disease (CD) is a strict lifelong gluten-free diet (GFD). However, let´s analyse this sentence carefully and think about what we are conveying to coeliac patients with this recommendation.

When we refer to '*only treatment*', we are saying that there is no other option, take it or leave it, but there is currently no alternative treatment for CD.

What do we mean by '*strict*'? How much gluten can a coeliac patient consume? In fact, we only have a few studies that focus on this issue. Carlo Catassi, in a now classic study [1], shows that a 50 mg/day intake of gluten over a period of 90 days may cause intestinal damage in coeliac patients. In other words, we are telling these patients that they cannot consume above 10 mg of gluten in each of their five daily meals. How can we ensure this? Gluten-free-labelled products have to contain less than 20 ppm (20 mg/kg). At home, it seems difficult but attainable but how can you ensure you do not surpass these levels when eating out, at work or when travelling? Logically, this strict diet is far more important in the case of CD or wheat allergy than in a non-coeliac gluten sensitivity (NCGS).

'Lifelong', with this word we convey the message to our patients that they must learn to deal with a chronic disease, that, the patient can no longer consume those appetizing products he or she sees on TV and enjoyed as a kid, not so long ago, or that tempting aroma of freshly baked bread or cookies.

These two paragraphs above refer to two well-differentiated issues: the first one to whether the patient will be able to follow the GFD, while the second refers to whether the patient considers giving up all those things he or she once loved and that are now banned for life, worthwhile. This distinction between confidence and motivation is what we are going to deal with in this chapter. Among people suffering from CD or wheat allergy, this confidence plays a more important role than in those suffering from NCGS, as the latter can regulate their GFD according to their tolerance to the adverse symptomatology without having to face other medical complications.

On the other hand, human beings like to celebrate events with food and drink. Frequently, coeliac patients feel obliged to choose between their physical health and their social integration——"Which do you prefer: to follow your GFD or participate in your community?"—"Both". Wrong! Too often this is not possible and they have to make a choice.

Despite the benefits of a strict GFD, we know that only 42–91% of coeliac patients show a correct adherence, depending on what we consider *strict* and how we measure it [2]. But why is it some coeliac patients really do stick to a GFD and others do not? These underlying principles have received scant attention so far, and we propose here an explanatory model.

2. Consequences of adherence and non-adherence

It seems obvious that physical and social consequences of adherence and non-adherence may be the most powerful motivators to initiate a GFD in coeliac patients. Non-adherence has well-known physical consequences as we know that small intake of gluten can lead to a varied gastrointestinal symptomatology such as abdominal pain, diarrhoea, bloating, constipation or more serious consequences such as osteoporosis, sterility in men and women or some types of tumours.

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Researchers have paid less attention to the consequences of adherence to a GFD, in other words, the social costs that the correct adherence to a strict GFD has for coeliac patients. These costs are more social than nutritional. In an interesting qualitative study, Sverker [3] interviewed 43 coeliac patients and found five areas where they had problems: shopping, eating out, meals at home with others, when travelling and at work. At an emotional level, these problems led to feelings of isolation, shame, fear of being contaminated with gluten or bothering others. Because of this, coeliac patients often restrict their participation in social activities, especially in those with food, as they think that their participation may condition others' choices and they, therefore, prefer not to be a bother. Adhering to a GFD may also affect relationships as coeliac patients have unwanted visibility at social events, fear of being rejected or forgotten and, when they do participate, they must always identify themselves as coeliac patients and give detailed explanations, or if not, they must take important risks that could jeopardize their strict GFD.

In their daily lives, they perceive restricted product choice when shopping or eating out, double work and that they have to be constantly on alert to keep up with their GFD. Often, they have to go to several shops and supermarkets to buy the goods they need for their GFD or cook different meals for each family member. In addition, they must be constantly on call while cooking to avoid cross-contamination.

Moreover, GFD adherence is expensive. Some studies estimate that the increase in the cost of shopping per affected family member reaches 1.200€/year [4, 5]. If we take into consideration that CD is genetically mediated, these differences could easily be twice or three times this amount. Therefore, some families could probably not afford a GFD.

3. Social Cognitive Theory, self-efficacy and gluten-free diet

The concept of self-efficacy has been widely studied in Psychology [6]. Albert Bandura proposed the self-efficacy expectation in 1977 in the article 'Self efficacy: towards a unifying theory of behavioural change' [7] where he defines self-efficacy *'as the conviction that one can successfully execute the behaviour required to produce the outcomes'* (page 193). From this first moment, Bandura distinguishes between outcomes and self-efficacy expectations stating: '*outcomes and self-efficacy expectations are distinguished because individuals can believe that a particular course of action will produce certain outcomes, but if they entertain serious doubts about they can perform the necessary activities such information does not influence their behaviour'*. Later, in 1985, he defined self-efficacy *as 'one self-evaluation of one´s capabilities to organize and execute the required courses of action to achieve certain outcomes. Then, it is not about the skills one has but rather about the assessment one makes on his or her own abilities'* [8].

To sum up, therefore, according to Bandura, '*self-efficacy refers to one´s believes in own capabilities to organize and execute the necessary courses of action to produce certain outcomes'* [6] while outcome expectation refers to the belief regarding the most likely results of the action (**Figure 1**). Concerning a GFD adherence, one thing is the belief in being able to take the necessary steps to follow a strict GFD and something very different are the expected outcomes of strictly adhering, or not, to the diet. The first belief is what we know as self-efficacy expectation, whereas the latter is what we call outcome expectation.

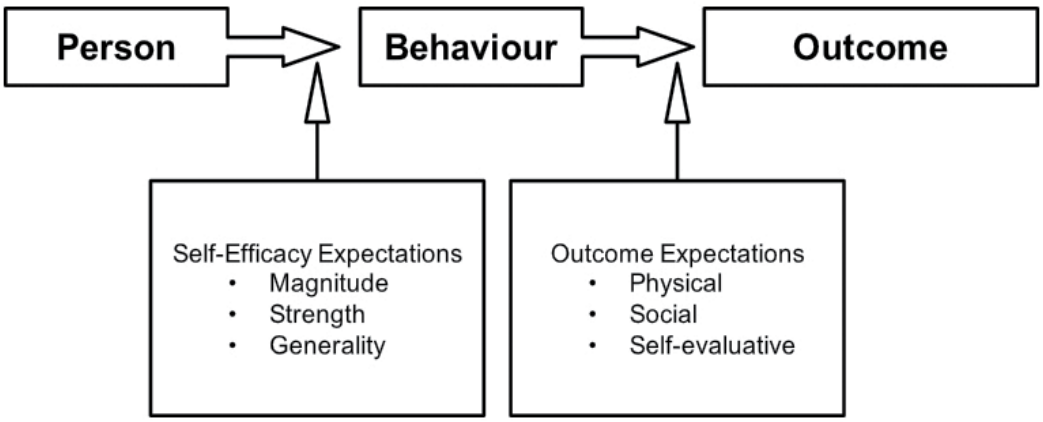

Figure 1. Self-efficacy and outcome expectations [7].

The Social Cognitive Theory suggests three types of outcome expectations: physical, social and self-evaluative and they can all be either positive or negative. While positive consequences will increase willingness towards the GFD adherence, negative ones will decrease it. Physical consequences refer to physiological sensations such as nerves, anxiety or well-being associated with the correct adherence, while social consequences are others' understanding or rejection as well as the cost arising from the diet. The third kind of outcome expectations is self-evaluative expectations, positive and negative, derived from suffering CD and being bound to follow a strict lifelong GFD. These may come together with feelings of pride, belonging or self-assertion or, on the contrary, negative feelings of self-devaluation or depression.

On the other hand, as **Figure 1** shows, self-efficacy expectation has three dimensions: magnitude, strength and generality. The strength refers to the level of the expectation, in other words, the higher the expectation the higher the confidence in one´s own ability to stick to a GFD and the associated tasks such as rejecting a dish or talking to a cook to ensure a gluten-free meal. Self-efficacy strength refers to one's resistance to failure. Finally, generality refers to the range of similar behaviours to which one can apply that given expectation.

Perceived self-efficacy has been applied to many different domains such as self-regulated behaviour, and patients with arthritis [9], physical activity [10], multiple sclerosis [11] or addictive behaviours [12] but it has received scant attention in relation to CD.

Although Bandura [6] proposes a specific self-efficacy expectation narrowly linked to each situation, some authors [13, 14] work with the hypothesis of a more general self-efficacy belief that accounts for behaviour in different domains in life.

Higher levels of general self-efficacy correlate with positive feelings, higher achievements, better quality of life and the perception of potentially stressful situations as challenges rather than as potential threats [6]. Self-efficacy, therefore, is linked to a wide number of psychological constructs and affects not only coping behavior, but human functioning in general.

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According to Bandura, levels of general self-efficacy are related to the perception of well-being and healthy behaviours, while he finds negative correlations with negative feelings. According to this author, a high sense of general self-efficacy also correlates with lower levels of depression in patients with heart problems, less pain and low levels of anxiety in individuals with gastrointestinal problems. There is also evidence of greater adherence to physical exercise and healthy eating in those with high general self-efficacy. In the same way, Luszczynska finds that gastrointestinal patients use less passive coping techniques and more active techniques of pain management [13]. This author, together with Scholz, has carried out several studies to search for evidence to consider self-efficacy a universal construct [14]. Because of all this, we think self-efficacy beliefs may play a major role in the adherence to a GFD and this relation has only just begun to be studied in recent years.

4. How do we increase self-efficacy to improve adherence to a GFD?

According to Bandura [6], there are four sources of self-efficacy: performance accomplishments, vicarious experience through model observation, verbal persuasion on own capabilities and, lastly, the evaluation of emotional arousal during performance. Any change in the level of self-efficacy expectation is going to take place through one of these sources or a combination of any of them.

4.1. Performance accomplishments

According to Bandura´s Social Cognitive Theory, performance accomplishments are the strongest source of self-efficacy as is the real evidence that a person can perform a task successfully. Generally speaking, success events help to build a high level of self-efficacy while failures tend to lower it. Although this is the general rule, this does not always work this way as success and failure need to be cognitively processed. After this analysis, and depending on, for instance, attribution mechanisms, a higher or lower belief of self-efficacy will be instilled. Other factors such as skills assessment, perceived task difficulty, the effort made, the situation or former successes or failures will also condition the sense of self-efficacy. Failure is especially negative in early stages before a strong belief of personal efficacy has been developed. On the other hand, if success comes too soon, the self-efficacy belief instilled could be high, but weak and vulnerable to failure. It is success after overcoming difficulties and setbacks that builds high and strong self-efficacy beliefs, in other words, resilient to future adversities. This source of self-efficacy also builds up an expectation easier to apply to new situations than those obtained through the other three sources. In adhering to a GFD, the successful management of the diet at home, when travelling or eating out may lead to a high and strong sense of self-efficacy while conflicts in those areas, the failure in lowering serological markers or symptomatology may reduce self-efficacy beliefs.

4.2. Vicarious experience

Vicarious learning has been widely studied during the 1960s in the last century and the underlying mechanisms have been well established. People do not learn only by direct experience but also by

imitation or vicarious observation. So, self-efficacy expectations are also affected by individual´s exposition to models that execute, successfully or not, a certain task. The higher the similarity to the model, the higher the effect in the observer´s self-efficacy beliefs. If the model is too different from the observer, the expectation may not be altered significantly as the observer may consider himself or herself to be incomparable. There are a number of circumstances in which this source is especially effective: the greater similarity in sex, age and race between the model and the observer, the greater the influence conveyed. On the other hand, models facing self-doubts and difficulties but controlling masterfully them seem to be more effective than those who perform perfectly. This source is especially useful with people who have not executed the task before and have not faced failure or success yet. But a competent model not only conveys a sense of self-efficacy but also the knowledge and skills of how a task should be executed. The model not only transmits that the goal is achievable but also shows how the task needs to be performed. Those who appear to be confident and persevere in the task help to develop stronger beliefs of self-efficacy in the observer. Vicarious experience emphasizes predictability and controllability. Through observation, the observer anticipates what is going to happen at the same time that he or she learns to control and manage difficulties, reducing stress and increasing self-efficacy beliefs.

In adhering to a GFD, this source of self-efficacy is especially useful, developing efficacy beliefs among siblings, friends or class or workmates who have been diagnosed at the same time. Support groups promoted by patients' associations illustrate clearly how this source can be useful in real settings. This source of self-efficacy must be taken into account, therefore, when designing self-managed health programmes where new members can observe the required behaviour and strategies put into practice by veterans or by recently diagnosed patients.

4.3. Verbal persuasion

Verbal persuasion is the third most effective source of self-efficacy when trying to install a healthy habit. It is easier to develop a sense of self-efficacy when others believe in your capabilities. Its effects may be limited when trying to generate high and long-lasting levels of self-efficacy but it is effective if kept within a realistic contest. On the other hand, people seem more motivated when avoiding the negative costs of a certain habit than for the gains that the adoption of a new habit may bring. Meyerowitz and Chaiken [15] reported that emphasis in potential losses of not adhering to a healthy habit is more effective and builds a stronger sense of self-efficacy than the emphasis on the advantages of adhering. It seems that the efficacy of a message based on gains and losses depends on the pre-existing efficacy beliefs. So, emphasis on losses is more effective for those high in self-efficacy while those with a lower pre-existing sense of self-efficacy have their effort undermined. This leads us to think about the need to adapt the message depending on the pre-existing levels of self-efficacy in the coeliac patient. If he or she is confident in being able to follow a GFD strictly we must emphasise the costs of non-adherence while we must moderate the message for those with lower self-efficacy beliefs.

4.4. Self-evaluation of emotions and feelings

According to Bandura, self-appraisal of affective and physiological states is the fourth source of self-efficacy beliefs. When patients evaluate their capabilities, they often integrate information

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from their physiological response. People differ in the amount of attention they pay to their emotions and feelings: the less immersed they are in their activities the more likely they are to concentrate on inner sensations and physiological reactions to difficulties. Diseases and physical deficiencies may focus their attention on their own limitations.

A coeliac patient excessively focussed on the internal sensations and anxiety may develop a lower and weaker self-efficacy expectation due to the anxiety generated by the activities required when following a GFD menu, such as talking to waiters, cooks, rejecting food, and so on. This also happens if he or she pays much attention to associated symptomatology.

5. Self-efficacy expectation and health management

Since Bandura published the theory of self-efficacy in the 1970s, it has been applied to many areas such as adherence to medical treatments, rehabilitation, sexual risk behaviour, physical exercise, nutrition and weight control, breast and prostate examinations or drug addiction [6].

The World Health Organization (WHO) defines health not only as a lack of illness but as a complete feeling of biological, psychological and social well-being. It is not only about being healthy but also about perceiving a good health status and a good quality of life.

Since the end of the twentieth century, western countries adopted this biopsychosocial model in which health and disease are consequences of the interaction of biological and psychological factors. Healthy habits have a beneficial effect on the organism while the absence of them may have an accumulative impact that leads to the development of chronic diseases; this is why it is necessary to develop self-managed health programmes as the most effective medicine nowadays. Fuchs [16] reported that medical expenditure has only a moderate influence on life expectancy and that, apart from genetics, it is their lifestyle and environmental conditions that are the most important factors in determining patient´s health. People suffer from physical problems and die prematurely because of pernicious habits and from preventable causes.

These are the main reasons why we think that self-efficacy expectation and the Social Cognitive Theory offer a suitable framework for intervention in CD. The self-efficacy expectation seems to play a major role at two different levels and both have been widely investigated in the last decades. The former refers to the effects of perceived self-efficacy in neurophysiological systems in coping situations and an extensive summary can be found in Bandura's 'Self-efficacy: The exercise of control'. This first level is of great importance if we link it to the recent research about the role of self-immune mechanisms and intestinal microbiota in the etiopathogenesis of coeliac disease [17–20]. A second level, and more relevant for the adherence to a GFD, is the role of self-efficacy expectation in the instillation of healthy habits and the elimination of risky behaviours. The Social Cognitive Theory offers, therefore, the necessary knowledge to develop effective health promotion programmes. In this case, how to improve GFD adherence in coeliac patients in order to enhance quality of life is shown.

The Social Cognitive Theory studies three basic change processes: the adoption of new habits, their maintenance through time, and their generalization to new situations. In other words,

how self-efficacy affects the establishment of a strict GFD, its persistence in time, recovery after transgressions and the generalization of those strategies to correctly maintain the diet in different areas such as at home, when travelling, at work or eating out.

5.1. Initiating a gluten-free diet

People´s beliefs about their own ability to motivate themselves and organize their behaviour play a central role when giving up unhealthy habits and adopting medical treatments as the GFD in coeliac patients. If they hold discouraging beliefs, they will not be able to do what is needed to go on a GFD, they will simply not begin. According to the Social Cognitive Theory, those with high pre-existing self-efficacy expectations will succeed better in definitively adhering to a GFD than those with self-doubts and frequent voluntary or involuntary transgressions. Even those who realize that their current habit is not healthy will not go on a GFD while they lack the self-efficacy required to resist temptations and cope with mood alterations. Di Clemente studied the changes in self-efficacy expectations along different stages of habit change and concluded that patients with weak self-efficacy beliefs give up preventive behaviour faster than those with stronger beliefs [12, 21].

According to Bandura, patients need to have sufficient knowledge about the disease and risk behaviours without being frightened by the message. What patients need are clues about how to behave and the strong conviction of being able to change their concerns about their health into preventive behaviour. That is, as we explain below, the intention-action gap is bridged with planning. So, those patients lacking enough self-efficacy to adhere to a GFD must enrol in self-managed programmes that provide them with gradual experiences that will increase their competence and self-efficacy levels while those fostering high beliefs can start a GFD with the medical recommendation alone. The problem is that today these programmes neither exist nor are scientifically based.

The messages, therefore, must be tailored to suit the chronic patient. Some authors have designed programmes of this type to individualize messages for each patient in tobacco addiction, healthy eating or preventive behaviour in cancer but we have not found any for CD and we think that programmes like these may be useful in clinical settings [22].

5.2. Sticking to the gluten-free diet

In order to stick to a GFD, intention alone will not suffice to develop the intention, patients will need self-regulatory skills. They must learn to design the menu, to set short- and long-term goals to focus the effort, such as travelling, eating out or in different places and to be able to anticipate positive and negative consequences of adherence. Once empowered with these skills and with strong self-efficacy beliefs, patients are ready to adopt the necessary behaviours and habits for following a strict GFD.

Over the past decades, the authors have found strong evidence that adherence to healthy habits are mediated by strong expectations of self-efficacy [6]. The higher this expectation is, the more likely the patient is to adhere to treatment and the more intense will be their efforts

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made to keep up with the new habit. This relationship has been found in different health topics such as obstructive lung disease, heart function recovery, pain reduction in patients with arthritis, chronic pain, stress reduction, weight loss, control of bulimic behaviour, cholesterol reduction through diet, adherence to physical exercise and many others. Bandura makes a systematic review of this extensive research [6] but this link with CD has scarcely been studied.

GFD has few positive consequences unless it is strict and maintained for a long time. Patients not only have to be able to start the diet but also be able to cope with potentially conflictive situations such as temptations or voluntary and involuntary transgressions. The development of these self-regulatory skills requires a resilient sense of self-efficacy to resist temptations and return to the GFD after transgressions.

5.3. The generalization of GFD to different settings

The easiest setting to install a GFD is, logically, at home and with naturally gluten exempt food as fish, meat or vegetables but we are social animals and we need to generalize those self-efficacy beliefs developed at home to other settings like restaurants, when we are at work or travelling. This generalization process is not easy, and it is important not only to control the disease but, more specifically, to achieve an adequate quality of life. Coeliac patients must force themselves to conquer new settings and gain confidence without putting themselves at risk. They have to overcome their feelings of 'being forgotten', 'being a bother' or their fear of 'be contaminated by gluten' and to fully participate in the activities of their communities. We are, therefore, speaking about the third of Bandura´s dimensions: magnitude, strength and *generality*. This is about applying the specific self-efficacy from one setting to others until reaching full social integration.

6. An explanatory mechanism for adherence: the HAPA model

It might be easy to go on a GFD, but sticking to it is a very different thing. Traditional explanatory models of change fail to explain the gap between intention and action. The HAPA model [23] tries to address this question and we think it fits very well with the GFD. This model was suggested by Schwarzer in 1988 and deeply reviewed recently by the author as an attempt to integrate the Heckhausen and Gollwitzer's [24] action phases model with Bandura´s Social Cognitive Theory [8]. Five principles help to define the model:

6.1. Principle 1: motivation and volition

The model distinguishes between preintentional motivational process and postintentional volitive processes that lead to healthy habits. Therefore, HAPA is a two-phase model: It is in the initial motivational phase, when the individual still has to develop the intention to acquire a healthy habit, which in this case is adherence to a GFD. In this phase, risks are assessed as

threatening but unlikely, especially by asymptomatic patients, and not important enough to build an intention but they motivate the patient towards a contemplation stage and an evaluation of the capabilities needed to take up a GFD (social skills, facing temptations, etc.) and the consequences (giving up to certain foods, identifying oneself as coeliac, changing habits or extra work associated with the diet). Analogously, positive consequences are important at this motivational phase (e.g. a healthier diet or symptomatology improvement). In addition, in this time, high self-efficacy beliefs, together with positive outcome expectations, play a major role and both are necessary to develop an intention.

But the development of an intention is not the end of the road. Once developed, this has to be turned into action and, ultimately, into a strict adherence for which self-regulation skills and strategies are required. In this postintentional moment, volitional phase, planning and the self-efficacy beliefs to face transgression (recovery self-efficacy) play a central role.

This distinction is important because, while action self-efficacy predicts intention, maintenance and recovery self-efficacy beliefs are better predictors of adherence. So, individuals that go back to a GFD after a transgression need different self-efficacy beliefs than those that keep their adherence. As the saying goes, it is better to fall and rise again than never have fallen at all.

6.2. Principle 2: two volitive phases

Once the intention has been developed and the patient enters the volitive phase, we can distinguish between those with the intention to go on a GFD (intenders) and those who have already adhered to the new diet (actors) (**Figure 2**).

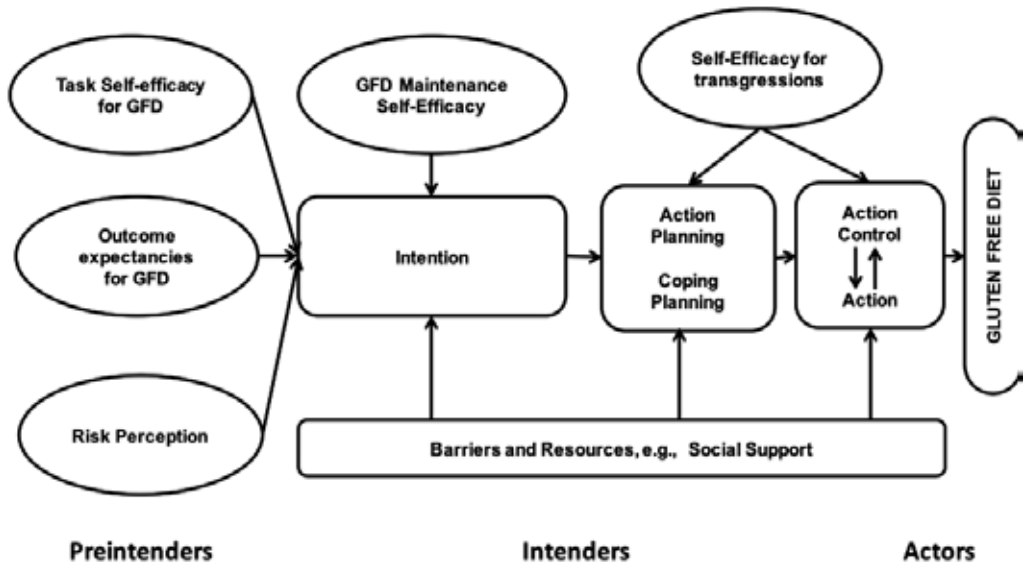

Figure 2. The HAPA model [23] (adapted).

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6.3. Principle 3: postintentional planning

To adhere to a habit, intentions need to be transformed into actions through detailed planning, for which people need to imagine themselves in different settings and the different strategies that they can deploy to get a GFD.

6.4. Principle 4: two types of planning

Schwarzer distinguishes between action planning and maintenance planning. *Action planning* goes beyond intention because it obliges patients to specify when, where and especially how to stick to a GFD. Leventhal [25] suggests that aversive communications in health promotion are only effective if they come with the correct action plan with instructions about when, how and where to execute the proper tasks that lead to establishing a high *maintenance self-efficacy*. Patients are less likely to forget their intentions when these have been expressed in terms of when, where and how they are going to maintain their diet. *Maintenance planning* is about foreseen barriers, difficulties and alternative behaviours to overcome them. This second type of planning is more important as it implies action planning, designing contingency plans and coping strategies before difficulties may arise.

6.5. Principle 5: specific self-efficacy for each phase

Self-efficacy expectation is necessary along all this adherence processes to a GFD but this expectation is slightly different depending on each phase. Marlatt et al. distinguish between initial, maintenance and recovery self-efficacy.

Initial self-efficacy (or action self-efficacy) refers to the motivational moment in which the coeliac patient does not go into action yet but has the confidence to begin a GFD. At this moment, individuals with high self-efficacy foresee the success, and outcomes and are more likely to start the diet. Those with a low self-efficacy expectation imagine themselves failing, are vulnerable to self-doubts and prone to procrastination. The other two types of self-efficacy take place during the volitive phase. *Maintenance self-efficacy* refers to the belief that one is going to be able to cope with the difficulties of guaranteeing a gluten-free meal; recovery self-efficacy deals with the belief that a person holds that he or she is going to be able to go back to a GFD after a transgression. In this context, Marlatt defines the abstinence violation effect (AVE) when an individual makes a stable, internal and global attribution of his or her relapse or abandons the healthy habit. Patients with high *recovery self-efficacy* beliefs avoid this effect as they attribute their relapse to external or controllable causes that allow them to rekindle their hopes of following with the diet. Therefore, people with high self-efficacy trust their capabilities to reinstall their abandoned diet after a transgression and to reduce its negative consequences.

The HAPA model points out the necessary constructs to work on each phase in a self-management health programme. Patients and professionals need to work on the following variables for the motivational phase: action self-efficacy, risk perception, outcome expectations and goal setting while the constructs to work on the volitive phase are action planning, coping planning, social support, maintenance self-efficacy, recovery self-efficacy and action control.

In addition, McLean [26], following a systematic review about adherence to treatments, concludes that this is higher when (1) this follows a cognitive, motivational and behavioural approach, (2) it helps patients to overcome barriers and face relapses and (3) it takes into account the conditions that come from health organizations.

To conclude, we must say that we think that the HAPA model can provide a valid framework for the design and implementation of programmes to improve adherence to a GFD in primary-care settings.

7. Psycho-CD: a programme to improve adherence to GFD

Due to advances in medicine and the subsequent increase in life expectancy in western countries, chronic disease has become a prevalent type of illness and disability in the last decades. Most people with chronic illnesses receive a treatment more based on medication than on education or the development of healthy lifestyles that allow them to manage their illness in a more effective way. This medical treatment is not possible in coeliac disease as there is no other cure besides sticking to a strict GFD for life. According to the Social Cognitive Theory, problems with adherence are more related to a poor belief in the benefits of the treatment or the perceived lack of capacity to stick to it than to the difficulties directly derived from the disease.

Holman and Lorig [27–31] have designed a prototypic programme for the self-management of different chronic diseases. These programmes include the development of technical skills such as pain control, relaxation, short-term goal setting, self-reinforcement, problem solving, heath changes interpretation, community resource finding, medication management and they can be promoted in primary care settings.

Different chronic diseases present very similar problems concerning how to manage symptomatology and how to overcome difficulties when adhering to the treatment or the control of emotions associated to the loss of quality of life. Programmes of this kind are, therefore, generic models that can be adapted to different chronic diseases (e.g. coeliac disease). This research team has not found any scientifically based programme for improving adherence to a GFD and because of this, we would at least like to present the outline of a proposal in this chapter.

Cunningham and Lookwood [32] found that the more the coping self-efficacy for chronic disease is improved through a programme, the higher the improvement is in terms of quality of life. These studies show the need to combine medical treatments with psychosocial interventions based on self-management programmes and we think that coeliac disease treatment would benefit from this approach.

Psycho-CD has the following objectives:

7.1. General objectives

The general objectives include the following:

- To improve adherence to GFD in coeliac patients.
- To develop a level of quality of life in coeliac patients to match non-sufferers.

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- To develop high, strong and generalized self-efficacy expectations in different areas to reduce stress and increase the sense of competency in adhering to GFD.

7.2. Specific objectives

The specific objectives include the following:

- To improve knowledge about coeliac disease and adherence to a GFD.
- Develop self-efficacy in specific settings such as eating at home, at work, eating out, shopping and when travelling.
- To increase social support and referents in the self-management of the disease.
- To learn to manage emotions associated with the disease.

7.3. Theoretical framework

We propose to adapt Schwarzer's HAPA model (**Figure 2**) within the wider framework of Bandura´s Social Cognitive Theory with three phases:

7.3.1. Preintentional phase

In this phase, patients will work on self-efficacy expectations to start a GFD, outcome expectations and risk perception in order to develop the intention to stick to a GFD.

7.3.2. Intentional phase

During this intentional phase, patients will mainly work on the maintenance of self-efficacy as well as barriers to and resources for adherence to a GFD. The objective of this phase is to work on the intention-action gap with patients through the detailed planning of the diet and how to overcome difficulties.

7.3.3. Action phase

During the action phase, together with barriers and resources, patients will work on planning to follow the diet correctly in the five areas identified by Sverker, as well as the social skills and coping strategies together with the development of recovery self-efficacy after transgressions.

7.4. Principles

7.4.1. Principle of motivation and volition

According to HAPA model principles, along the programme, two different stages will be distinguished depending on the patient´s expectations:

- Motivational moment (sessions 1 and 2) when the patient still needs to develop his or her intention (preintender) to follow a GFD.

- Volitive moment (sessions 3–10) when some patients have already developed their intention (intender) but have not gone into action and those who already have (actors).

7.4.2. Principle of empowerment

Responsibility is transferred to the patient. Coeliac disease is a chronic disorder and the only treatment to date is a lifelong strict GFD and, therefore, once the treatment has been set up through adequate training, it is the patient who must take accountability for the adherence.

7.4.3. Principle of self-efficacy

Self-efficacy plays a central role in the programme. Professionals must evaluate specific self-efficacy to initiate, maintain and manage transgressions during the GFD.

7.4.4. Principle of postintentional planning

According to the HAPA model, the programme is based around a detailed plan to ensure adherence, in other words, professionals will help the patients to plan how to prevent relapses and avoid transgressions.

7.4.5. Principle of evaluation

Professionals will carry out several evaluations throughout the programme:

1. Initial evaluation

   a. Evaluation of the diet.

   b. Evaluation of specific self-efficacy.

   c. Evaluation of quality of life.

2. Final evaluation

   a. Evaluation of diet after intervention.

   b. Evaluation of levels of specific self-efficacy after the programme.

   c. Evaluation of quality of life after the programme.

3. Evaluation of the programme as a whole

7.5. Setting

This programme is designed to be implemented in primary care or by Patients´ Associations.

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7.6. Variables of intervention

7.6.1. Motivation

Professionals will adapt motivational intervention depending on whether the patient is in a preintentional, intentional or behavioural phase. Messages will be designed according to previous levels of self-efficacy, thus grading the level of threat and the discrepancy between current behaviour and the new demands of adherence.

7.6.2. Knowledge and risk behaviour

The programme will be based on solid scientific evidence regarding coeliac disease from which professionals will define risk behaviour and make their corresponding recommendations.

7.6.3. Self-efficacy

Self-efficacy expectation is a central factor in the programme. Self-efficacy expectations will be developed using Bandura´s sources: previous achievements in programmed behavioural trials in which the required social skills can be put into, use of models through mates and mentors´ support, verbal persuasion with the messages designed by professionals and emotional appraisal through the control of symptomatology and the anxiety associated with social interaction that can threaten adherence to a GFD.

7.7. Agents

7.7.1. Professionals

This programme will be managed by dieticians with specific training and experience in coeliac disease.

7.7.2. Patients

Patients are responsible for the correct management of their disease, achieving access to a more normalized life through careful planning.

7.7.3. Doctor

Doctors are in charge of initial diagnosis and motivation as well as the derivation to this programme of adherence improvement.

7.7.4. Mentor

Patients will be assigned a mentor among more experienced coeliac patients and, preferably, who have undertaken the programme before. Mentors, according to the Social Cognitive Theory, will be similar to the patients to better help the development of empathy and self-efficacy.

The mentor will be a veteran in managing coeliac disease and will guide the patient through the programme, serving as a reference during and after, as a way of increasing his or her social support.

7.8. Timing

The programme is designed for 10 sessions, preferably in groups of five to eight patients, with a weekly frequency and an estimated duration of 2 h. It would be possible to offer five 4-h sessions.

7.8.1. Session 1: presentation, relationship creation, mentor assignment and contingency measures

In the first session, dieticians give an introduction to CD and GFD, introduce the mentor and offer a tailored GF menu for the next 15 days along with the basic recommendations for starting the diet. Mentors do not need to attend the rest of the sessions but they should be available according to the patient´s needs.

7.8.2. Session 2: coeliac disease and gluten-free diet: developing the intention

During session 2, dieticians will give a detailed explanation of CD and GFD and will motivate patients towards adherence customizing messages based on patient's moment of change (preintention, intention or action). Dieticians will work on action self-efficacy, positive and negative outcome expectations (physical, social and self-evaluative) and risk perception.

7.8.3. Session 3: emotion management in coeliac disease

In this session, dieticians will review emotions associated with coeliac disease such as stress, anxiety, sadness, frustration and others as a strategy for preventing relapses and improving quality of life.

7.8.4. Session 4: planning for shopping

In this session, dieticians will explain concepts related to packaging and labelling as well as the acquisition of unpacked goods. Dieticians will review risk behaviours and associated recommendations.

7.8.5. Session 5: planning eating at home with others

Dieticians will review possible problems associated with eating at home with family and friends. Patients will act out role plays about how to correct inadequate behaviour in guests that may be a risk to their diet as well as how to reject or accept invitations.

7.8.6. Session 6: eating out planning

Dieticians will review risks when eating out. Patients will act out role plays associated with the social skills needed when ordering gluten-free food, rejecting an unsafe dish and other similar situations.

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7.8.7. Session 7: at work and at school planning

Dieticians will review problems that arise at work, school or university, associated legislation, if there is any, and patients will plan how to get gluten-free food in those settings.

7.8.8. Session 8: planning for travelling

Dieticians will help to plan trips and patients will learn to find patients' associations in other cities and countries, as well as other valuable information for following the GFD when travelling.

7.8.9. Session 9: first follow-up session

Dieticians will carry out a follow-up interview at 6 months to assess adherence.

7.8.10. Session 10: final session

In this last session, dieticians will evaluate again self-efficacy expectations, adherence and quality of life as well as the programme as a whole.

7.9. Session structure

Sessions 1–3 will combine technical expositions with presentations of patients and mentors' experiences.

Sessions 4–8 will have the following structure:

- Review of former achievements.
- Technical presentation.
- Objectives for next session: Design of behavioural trial.
- What could go wrong? Contingency plans.
- Closing summary and commitment.

Sessions 9 and 10 will combine quantitative and qualitative evaluation of adherence and quality of life together with the sharing of the benefits of the programme.

8. Conclusion

This chapter presents a theoretical framework that can be useful to improve adherence to a GFD for patients affected by gluten-related disorders, in particular for coeliac patients. The difficulty for a correct adherence lies mainly on how strict the diet needs to be as we understand that it needs to be very strict in the case of CD and wheat allergy, and it could be more relaxed in the case of NCGS.

Self-efficacy expectations play a key role in adherence and quality of life of these patients and the HAPA model offers not only an explanatory mechanism but also the contents that need to be present in any programme to improve adherence.

Psycho-CD is a self-management programme designed to improve adherence and quality of life when adhering to a GFD that can be implemented in primary-care settings or from patients' associations.

As there is currently no alternative treatment for CD, programmes of this type may result not only in an improvement of the quality of life of the patient but also in a reduction of the costs associated with expensive diagnostic procedures and severe complications arising from inadequate adherence.

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This book contains recent advances about CD and NCGS written in eight chapters and is divided in three sections. In the first section, the main hallmarks of both diseases are described, together with the current diagnostic criteria of CD and its influence on the response to the vaccination against hepatitis B virus infection. The second section is dedicated to the description of several techniques for gluten determination in foods and if its consumption is good for nonceliac people. Finally, the third section contains complementary information related to the description and application of novel endoscopic techniques for confirming the diagnosis of CD. Another topic describes the growing consumption of gluten-free products and the adherence to this type of diet.

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