Gluten intake and gluten-free diet in the Netherlands

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CHAPTER 8

General discussion

Celiac disease can be defined as a permanent intolerance to gluten from wheat and wheat-related cereals and is treated with a life-long, gluten-free diet. It affects 1:100 – 200 individuals and is considered to be the most common food-related enteropathy. Celiac disease can present with a wide range of symptoms or extraintestinal complications. In addition, patients may even be symptom-free. Therefore, the disease is easily missed and the diagnosis is often delayed. Currently, most individuals with celiac disease have not been diagnosed (1,2), although the detection rate is increasing both in children and in adults (3,4). This suggests that in the near future, more individuals will be known celiac patients and that professionals involved in their treatment have to be prepared to take care of an increased number of celiac patients.

To increase the diagnosis of celiac disease, general practitioners should have adequate knowledge about the wide range of symptoms the disease can present with, about which patients are at high risk of having celiac disease, and if they should refer patients to the internist/paediatrician or the (paediatric) gastroenterologist for further diagnosis. Dietitians should be trained to provide adequate support to the patients. It has been recommended that education programmes on celiac disease and its treatment for doctors and dietitians should be developed to speed up diagnosis and reduce the risk of many of the serious conditions associated with untreated celiac disease (5).
A proposed option for better treatment of celiac patients is to explore the possibilities for a combined consultation of the doctor and the dietitian as used in other patients with chronic diseases, like diabetes mellitus. In addition, other approaches for patient care are possible, such as support through telephone or the internet, which has been proven to be (cost-) effective in patient management (6).

Additionally, attempts have been made to unravel the complex mechanisms underlying the origin and development of the disease in order to find preventive interventions.

PREVENTION OF CELIAC DISEASE

Celiac disease is caused by an aberrant T cell mediated immune response to gluten and is known to have a strong association with the HLA haplotypes DQ2 and, to a lesser extent, with DQ8, encoded by the HLA region on the short arm of chromosome 6. About 30% of the general population carries the HLA-DQ2 molecule, but only a minority develops celiac disease. Therefore, other factors must play a role in disease development. Genetic factors outside the HLA region that contribute to the disease have been found, but they only have a small contribution to the genetic risk for the disease (7,8). Furthermore, the existence of a gluten tolerance threshold for development of the disease has been suggested (9), and several environmental factors, like early infant feeding

associated with disease susceptibility or resistance, have been found (10,11). In this context, the term oral tolerance is frequently used. Oral tolerance can be defined as peripheral tolerance in which lymphocytes in the local and peripheral lymphoid tissues are rendered nonfunctional or hypo-responsive by prior oral administration of antigen (12). In celiac disease, the antigens causing the T cell response are gliadins and glutenins (13).

The first oral administration in infants is usually by breastfeeding. Breast milk contains many immunologic factors such as secretory IgA antibodies, cytokines and growth factors that stimulate the infant's immune system (14). The exact mechanism by which breastfeeding prevents or delays celiac disease is not known, but it is known that breastfeeding provides passive and active immunity, playing a role in the decreased development of food allergy and food intolerance (14). It is assumed that breast-fed infants have a better functioning immune system, providing better protection against and a diminished risk of immunologic diseases (14).

Breast milk contains small amounts of food antigens, like gliadin (15,16). These small levels of gliadin may contribute to tolerance induction rather than to sensitization (17). In our study on the detection of gluten peptides in human breast milk (Chapter 2), we indeed were able to detect gliadin and glutenin peptides in the breast milk samples of mothers on a gluten-containing diet. On the other hand, we could also detect gliadin fragments in the milk of mothers on a gluten-free diet, something one would not expect, unless the diet is not kept strictly. However, basal levels of gliadin in breast milk, even before gliadin intake, have been described by others (16). These levels of gliadin peptides may be explained by cross reactivity of the gluten-specific antibodies used for detection or by the existence of human proteins that have some similarity with gluten sequences. Exact characterization of the peptides found in breast milk and improvement of the test used to detect the gluten peptides is necessary for adequate use in future research. Better understanding of the nature and passage mechanism of dietary antigenic proteins into mother's milk could lead to a better understanding of the development of food allergies and food intolerance in infants, such as celiac disease (16), and also to the mechanisms of tolerance for these antigens.

In our study described in chapter 2, we could not find a correlation between the gluten consumption by the mothers and the level of gluten peptides in their breast milk. Other investigators have previously described that there is a great inter- and intra-individual variation in the amount of antigens in breast milk irrespective of dietary regimen (16,17). On the other hand, the method of calculation of the gluten content of food products should be improved. At present, the gluten calculation is based on the Osborne

classification, which indicates a gluten content of 80% of the total wheat protein content; however, this may not exactly reflect the real quantity of the gluten present. In food processing, a diversity of wheat varieties are used because different food products, e.g. gluten-containing bread, in contrast with gluten-containing pasta, require different quality of wheat. It is proposed that wheat varieties differ in amount of protein and possibly differ in gluten content (18). Analyses of the gluten content of specific gluten-containing products can ameliorate the calculation of gluten intake for future research purposes.

Another proposed aspect of early feeding that plays a role in the development or prevention of celiac disease is the introduction of gluten into the infants' diet. Breastfeeding at the time of gluten introduction and ongoing breastfeeding while gluten is already being consumed are factors that have been associated with a reduced risk of development of celiac disease (10,11). An additional factor in this respect is the amount of gluten introduced into the diet (10,19). This is demonstrated by the changes of infant feeding in Sweden leading to an epidemic of celiac disease: before the epidemic of celiac disease, gluten was given from the age of 4 months, an age at which most of the infants were still breast-fed. When in the mid 1980s gluten was introduced at a higher age, it was also implied that more infants had ended breastfeeding and gluten was introduced in larger amounts, leading to the epidemic of celiac disease among children younger than 2 years of age. From these studies, it is suggested that gluten-containing foods should be gradually introduced into the infant's diet before breastfeeding is discontinued (19). It is possible that this gradual introduction will lead to the development of oral tolerance (12,20), which may be one of the possible strategies in the prevention of celiac disease. However, this has never been formally studied.

The timing of the introduction of gluten into the diet of an infant has also been studied among infants with increased risk for type 1 diabetes or celiac disease, and it was concluded that the introduction of gluten into the diet before the age of 3 months and after the age of 7 months was associated with a higher risk of developing celiac disease (21). In that study, however, no attempt was made specifically to calculate the amount of gluten consumed or to correlate the intake of gluten with the presence or absence of breastfeeding.

Recently, a prospective collaborative European study on breastfeeding and gluten intake in newborns from high-risk families has started to find evidence for the hypothesis that gradual introduction of gluten during the period of breastfeeding may play a role in the development of oral tolerance (PreventCD: Influence of the dietary history in the prevention of coeliac disease: possibilities of induction of tolerance for gluten in genetic predisposed children, FP6-2005-FOOD-4-B: 036383, www.preventcd.com).

In the Netherlands, like in most European countries, parents are advised not to introduce gluten into the diet of their child before the age of six months. With respect to the possible preventive role of introduction of gluten during the period of breastfeeding, only a minority of the Dutch infants may profit from this effect, as 76% of the infants receive breastfeeding right after birth, 52% are still being breast-fed at the age of 3 months, and breastfeeding duration longer that 6 months is not very common (31%), though it is increasing compared to previous years (22).

The duration of breastfeeding itself seems to reduce the risk for celiac disease, and a long duration of breastfeeding should thus be promoted (19). However, it is not clear from the above-mentioned studies whether breastfeeding provides a true protection against celiac disease, or just delays its presentation. In a recent prospective observational study including only children with a high risk for autoimmune disease, no protective effect of prolonged breastfeeding was observed with respect to autoimmunity for celiac disease (21).

The exact role of breastfeeding and gluten introduction in the development of oral tolerance still has to be demonstrated. Long-term prospective cohort studies in newborns with high-risk for celiac disease, in which early feeding is taken into account, will be required to further investigate the relationship between breastfeeding, gluten introduction, and celiac disease. For that purpose, we have developed and validated an instrument to assess the use of breastfeeding and to quantify the amount of gluten ingested by infants and young children (Chapter 3). Although some improvements of the questionnaire have to be made for future use among children aged 11 and 12 months, this food questionnaire, the FQ-gluten, gives similar results for children up to the age of 10 months, is less time consuming compared with the food record and easy to use both by parents and researchers in future gluten consumption studies in young infants. The FQ-gluten will allow collaborative prospective studies concerning gluten ingestion by young children in different populations and countries. In these cases, the Dutch FQ-gluten should be validated and adapted to the eating pattern of young children in different countries. This adaptation and validation is already in progress by participants of the ESPGHAN Working Group (European Society of Paediatric Gastroenterology Hepatology and Nutrition) on 'New strategies for prevention and treatment of celiac disease', in which a common research protocol was developed to study the gluten consumption by young children in different European countries. Furthermore, the partners of the above-mentioned collaborative European study, which explores the possibilities of primary prevention of celiac disease are working on adapting and validating the Dutch FQ-gluten (PreventCD, FP6-2005-FOOD-4-B: 03638, www.preventcd.com). In that study children will have follow-up after the age of 12

months and food questionnaires suitable for children in older age categories covering a more extended food package will have to be developed and validated.
The information resulting from such studies may possibly lead to changes in the actual European guidelines for infant feeding, e.g. the introduction of gluten after 6 months of age.

So far, the gluten-free diet is the only available treatment for patients with celiac disease and it must be maintained throughout life. The diet has a great impact on the social life and quality of life of the patients and their families. Many studies have been performed on the burden of the gluten-free diet, including the impact of the diet on social activities, dining out or traveling, and on the variability, availability, taste and cost of the gluten-free products (5, 23-26). We have studied whether the health-related quality of life of adult celiac patients was associated with the degree of compliance with the gluten-free diet (Chapter 6). We found that differences in dietary compliance were not associated with significant differences in health-related quality of life, but compared with the general population, celiac patients had worse general health.

Adhering to the gluten-free diet implicates avoidance of wheat, rye, barley, spelt, kamut, and products derived from these cereals. Examples of gluten-free cereals that can be used as an alternative are rice, maize, buckwheat, millet, quinoa, and amaranth. The industrially-prepared gluten-free substitutes are frequently based on rice or wheat starch, and as a result, the gluten-free diet is low in B complex vitamins and iron (27,28). Food products enriched with vitamins, minerals, or fiber are available on the gluten-free market and may contribute to higher nutrient intakes. It has been suggested that the gluten-free diet has consequences for the adequacy of the nutritional intake and that patients may be at potential risk for comorbid health problems, such as elevated lipids because of the lack of fiber in the gluten-free diet (29) and high plasma homocysteine levels (being a risk factor for cardiovascular disease) because of poor folate, vitamin B6 and vitamin B12 status (30). Studies in celiac patients have confirmed a low intake of pyridoxal 5'-phosphate (vitamin B6) and vitamin B12 compared to controls (30), and the intake of fiber, vitamin B1, B6, iron, calcium, and vitamin D, E, and A were also below the recommendations (31,32). Furthermore, high homocysteine levels, indicating poor vitamin status (folate, vitamin B6 and vitamin B12), have been observed by Hallert et al. (30). Serum ferritin, iron, and heamoglobin levels below the reference values have also been found in up to 20% of a group of celiac patients (31).

In our study of adolescent celiac patients (Chapter 4), we found that their nutrient intake was comparable with that of the general population; however, they had a lower than

recommended intake of iron and fiber and a higher than recommended intake of saturated fat, which represents a potential risk factor for cardiovascular disease.

In this respect, the nutrient intake of the celiac patients can be ameliorated by more attention to the nutritional quality rather than only to the food products allowed or not allowed in the gluten-free diet (33). A way to reach adequate nutritional intake in agreement with the recommendations is through providing proper instructions by a dietitian on balanced food choices and, when indicated, on enriched gluten-free food products or vitamin and mineral supplements. Another possibility for a better nutritional intake is to enlarge the possibilities within the gluten-free diet with (new) gluten-free cereals that have good nutritional value. The use of oats in the gluten-free diet has been extensively studied, and it has been found to be a safe diversification of the diet for most adults and children with celiac disease. However, some patients develop clinical symptoms or mucosal damage after oats consumption (34-36). In the Netherlands uncontaminated oats are not available at present, but efforts are being made to realise their availability in the near future.
Sorghum is another gluten-free cereal that might be added to the gluten-free food package. Sorghum is a naturally gluten-free cereal that, in Western countries, is traditionally used for animal feed, but in Africa and India, it has been used for human food for centuries. A short-term study on the use of sorghum in the gluten-free diet showed that it is safe for celiac patients (37). Future long-term studies are needed to confirm this result.

We have studied the use of a rather new gluten-free cereal, tef, in celiac patients in the Netherlands (Chapter 5). Tef is a naturally gluten-free cereal originating from Ethiopia, and cultivated in the Netherlands for a few years now that can be used for the same purposes as wheat (38). Tef may contribute to a higher nutrient intake by celiac patients in comparison with other frequently-used naturally gluten-free cereals because tef has a high protein, thiamin, iron and fiber content, comparable to that of wheat. We have found that tef is frequently used by Dutch celiac patients and that the patients consuming tef in their gluten-free diet reported fewer symptoms after adding tef to their diet. We conclude that tef may contribute to a better clinical condition of the celiac patients and to a higher nutritional value of the gluten-free diet.

New treatment strategies for celiac disease that may reduce the burden caused by the gluten-free diet are presently being explored. Not all wheat varieties may be equally harmful for the celiac patients. Gluten proteins that lack one or more of the known T-cell-stimulatory sequences have been identified, which enables selecting wheat varieties with a natural low number of T-cell-stimulatory epitopes compared to the wheat

normally used. Such wheat varieties may be suitable for consumption by celiac patients (18). It is expected that these wheat varieties and food products derived from them will have a higher palatability than the gluten-free substitutes available now.

Another alternative in the treatment of celiac disease is to reduce the toxicity of the antigen causing the disease. In this respect, research on enzymatic breakdown of gluten peptides by prolyl endoproteases or prolyl endopeptidases (under conditions similar to those found in the gastrointestinal tract) has shown that intact gluten molecules and T-cell-stimulatory epitopes can efficiently be degraded into harmless fragments before causing damage to the small bowel mucosa. Further studies are required to determine if these enzymes may be used to reduce the toxicity of gluten intake in celiac patients (39,40).

Another novel therapeutic approach to the treatment of celiac disease is to prevent gluten peptides from crossing the mucosal barrier by reducing the intestinal permeability. The safety and tolerability of AT-1001, which is an inhibitor of paracellular permeability, has been tested in celiac patients after a challenge with gluten. It has been found that AT-1001 was well tolerated, and it reduced the intestinal permeability and the production of pro-inflammatory cytokines in celiac patients after gluten exposure (41). These new developments, among others, may contribute to better treatments for the celiac patients. Finally, it is important to find ways to establish a nutrient intake in better agreement with the recommendations and to decrease the burden of the treatment. This may serve to prevent patients from cessation with the diet, and thus to prevent them from the potential risk of complications.

TOLERANCE TO GLUTEN

There is an ongoing debate about the permanency of celiac disease (42-44). In general, celiac disease is considered a permanent disorder. However, although this is exceptional, patients have been described who possibly had become tolerant to gluten consumption, or in whom the intolerance had returned to a latent phase. In a recently published follow-up study, eight adult patients were described who were diagnosed with celiac disease in childhood and consumed a normal gluten-containing diet for more than 14 years, without symptoms or signs of the disease and with a normal small bowel mucosa (44). Apparently these patients had become tolerant to gluten ingestion, and they were considered to have returned to a latency stage of celiac disease.

In our study on the possible development of tolerance to gluten in celiac patients (Chapter 7), we have found two patients who have consumed gluten for 18 and 22 years

without development of immunologic or histologic signs of the disease, and they may be considered to have become tolerant. Further follow-up remains necessary to confirm whether this tolerance will continue or whether these patients will deteriorate or develop complications. We suggest a possible role for genetics in the development of tolerance as one of our tolerant patients did not have the matching HLA-DQ for celiac disease: HLA-DQ2 or -DQ8 is present in 98% of the celiac patients. In our study, however, we found a higher percentage of non-HLA-DQ2 or non-HLA-DQ8 patients, all diagnosed according to the accepted ESPGHAN criteria. The referral to a university hospital, possibly indicating that our patients were not clear CD patients but difficult to be diagnosed, may explain the clustering of haplotypes different from HLA-DQ2 or -DQ8 in our study population.

Furthermore, it is possible that gluten sensitivity may decrease or increase during different periods in life. In this respect, early infancy may be a period of tolerance development (10,11). Puberty seems to be a period in life in which gluten ingestion is clinically well tolerated (43,45,46) and development of resistance to the gluten toxic effect in adults, after a period of gluten-free diet in childhood, was also suggested (47). Refractory celiac disease, a state in which patients are not responding to the treatment with a gluten-free diet, may be present in adulthood celiac disease. Until now, this serious complication of celiac disease that may be associated to the development of enteropathy associated T-cell lymphoma has not been described in childhood celiac disease.

The factors and mechanisms that play a role in the development of tolerance in some exceptional celiac patients are unclear and more studies are needed to unravel this phenomenon. This knowledge may be useful in the development of new treatment strategies.

FINAL CONCLUSIONS

In this thesis, we have described the studies on the intake of gluten and the adherence to the gluten-free diet in celiac patients in different age categories. Starting from birth, the first oral intake by infants is usually via breastfeeding. To assess the first exposure to gluten of young breast-fed infants, we measured the level of gluten peptides in breast milk. For the assessment of the gluten intake when the infant starts weaning we developed and validated an instrument to measure the quantity of gluten consumption up to 12 months of age. The role of both parts of early infant feeding, e.g. breastfeeding and gluten intake, in the development of celiac disease or in oral tolerance to gluten needs further study.

In adolescent celiac patients, we found a high compliance with the gluten-free diet, although compliance with the diet was often experienced as difficult. Their nutrient intake, however, was unbalanced and in need of improvement. Furthermore, we have found that children and adult patients with celiac disease who consume the rather new naturally gluten-free cereal tef in their gluten-free diet report less physical complaints compared to patients without tef consumption. Finally, in adult celiac patients, we found that the compliance with the diet was low; however, differences in the degree of dietary compliance were not associated with differences in their health-related quality of life. We found that celiac patients adhering strictly to the gluten-free diet did not have worse health-related quality of life as compared to patients consuming gluten. This supports the encouragement of strict compliance with the only available treatment for celiac disease in light of prevention of complications while awaiting further development of treatments.

In an attempt to find whether tolerant celiac patients do exist, we have found two exceptional patients with long-term gluten consumption without symptoms or signs of the disease. We suggest that genetic factors may be important in this exceptional development of tolerance, but this needs to be further studied in larger groups of tolerant patients.

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