Gluten intake and gluten-free diet in the Netherlands

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Cover illustration gluten structure obtained by washing out dough.
Cover design Anja Bakker
Printed by PrintPartners Ipskamp B.V., Enschede, The Netherlands

ISBN 978-90-9023381-9

Financial support for the publication of this thesis was kindly provided by Willem-Alexander Kinder- en Jeugdcentrum (Leiden University Medical Center), Celiac Disease Consortium, Dienst Diëtetiek (Leiden University Medical Center), Divisiebestuur Divisie 2 (Leiden University Medical Center), Nederlandse Coeliakie Vereniging, Friso Kindervoeding, Mead Johnson Nutritionals, Nutricia Advanced Medical Nutrition, Sorgente B.V., Nutri-akt b.v. - arbeidsmarktintermediair binnen Food, Technology, Nutrition & Health.

Gluten intake and gluten-free diet in the Netherlands

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. P.F. van der Heijden,
volgens besluit van het College van Promoties
te verdedigen op donderdag 25 september 2008
klokke 15.00 uur

door

Geertruida Dorothea Hopman

geboren te Den Burg, Texel
in 1965

Promotiecommisie:

Promotor:	Prof. dr. J.M. Wit
Copromotor:	Dr. M.L. Mearin-Manrique
Referent:	Prof. dr. C.J.J. Mulder (VU Amsterdam)
Overige leden:	Dr. L.J.W.J. Gilissen (Wageningen Universiteit, Wageningen) Prof. dr. D.W. Hommes Prof. dr. J.A. Romijn

In herinnering aan mijn vader

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CHAPTER 1

General introduction, aim and outline of this thesis

In the early 1930's the Dutch paediatrician W.K. Dicke discovered that the elimination of wheat from the diet was beneficial for celiac patients (1). This benefit was confirmed later during the period of food shortage in the Second World War (1944-1945), during which bread was unavailable, and Dicke observed that the clinical condition of the hospitalized children with celiac disease improved. Further studies showed that gluten and specifically its alcohol-soluble component, gliadin, was harmful for celiac patients (2). Gluten is the storage protein of wheat and wheat-related grains and can be subdivided in the gliadin and glutenin protein families, both of which are involved in celiac disease. Since then, a gluten-free diet has been the basis of the treatment of celiac patients.

In celiac patients, gluten causes histological alterations of the small bowel that may lead to disturbances in nutrient absorption and symptoms such as diarrhea, failure to thrive, abdominal pain, and extraintestinal complications such as osteoporosis, infertility and cancer (3). The treatment of celiac disease consists of a life-long gluten-free diet to heal the duodenal mucosa, improve symptoms, and protect against development of complications (4,5).
The diagnosis of celiac disease is based on characteristic histological alterations of the small bowel mucosa during gluten consumption and clear clinical remission with a gluten-free diet. In asymptomatic patients, however, a control biopsy is needed to prove mucosal recovery after treatment (6). In earlier days, a third biopsy after gluten challenge was needed to confirm the diagnosis in children (7).
Celiac disease is considered to be a life-long disorder, but there are studies describing patients diagnosed with celiac disease in childhood who seem to tolerate gluten later in life for an extended period of time (8).

Until now, the only effective treatment for the disease has consisted of a gluten-free diet in which wheat, rye, barley, spelt, kamut, and products derived from these cereals are avoided. To what extent oats belong to the list of banned cereals is still debated. Both long-term follow-up studies (9-11) and laboratory studies on oats being less toxic than wheat (12), support that oats are permitted in the gluten-free diet for adults and children. However, some patients do show mucosal damage after oat consumption, and individual differences in oat tolerance have been found (11,13). In the Netherlands, as in many other countries, fear of wheat contamination in commercially available oat products has led to a reluctance to recommend oats to celiac patients (14,15).

Wheat cereal is a staple food in many countries in Europe and is widely used in the food industry. Therefore, wheat is difficult for celiac patients to avoid; hence, the prescription to follow a gluten-free diet has a big impact on the patients' daily and social life (16), and even on the lives of family members. The availability of the gluten-free products is

limited, and consequently, celiac patients have difficulty finding gluten-free foods. Furthermore, taste as well as higher expenses can be limiting factors for compliance. For example, in the Netherlands, only a few health insurance companies contribute to the added costs of the gluten-free products. All these factors may affect the health-related quality of life of celiac patients (17,18). Furthermore, the nutritional value of gluten-free food products is lower compared to the gluten-containing equivalents, which may lead to inadequate nutrient intake (19-22).

Genetics play a role in the development of the disease: as much as 98% of the celiac patients are HLA-DQ2 (95%) or –DQ8 (3%) positive. However, the majority of people with these genetic factors do not develop celiac disease. This suggests that additional genetic and/or environmental factors play a role in disease development.
Many genetic and immunological studies have been performed in an attempt to unravel the complexity of this multi-factorial disease (23-25). In addition, the possible role of environmental factors, such as early feeding, in the development or prevention of celiac disease has been studied (26-28). The Swedish 'experiment of nature' causing the rise and fall of 'an epidemic' of gluten intolerance after changes in infant feeding suggests that early feeding may be an important factor (26). Breastfeeding at the time of gluten introduction, ongoing breastfeeding while gluten is already being consumed, as well as timing and amount of gluten introduced into the diet, may play a preventive role in the development of celiac disease (27-29).

Breastfeeding and weaning influence the development of the gastro-intestinal tract, and it is possible that gradual introduction of antigens will lead to the development of oral tolerance (30,31). It is also likely that the response of the immune system to gluten is modified by breastfeeding (32,33). The presence of gluten peptides in breast milk leading to an early exposure to gluten, even before gluten is introduced into the infants' diet, has been studied as a possible factor in the development of oral tolerance (34,35).

AIMS OF THIS THESIS

Gluten is essential for the development of celiac disease: in the absence of gluten, celiac disease will not be expressed. The aims of this thesis were to explore the relationship of celiac patients with gluten and the gluten-free diet at different ages, their ability to develop tolerance to gluten, and the impact of the gluten-free diet on health-related quality of life. Furthermore, this thesis also aims to measure some of the environmental factors such as breastfeeding and gluten intake in early life considered to play a role in the prevention of celiac disease and in the possible development of oral tolerance.

Chapter 1 consists of a general introduction and description of the aims and outline of the thesis. In chapter 2, the implication of the presence of gluten proteins in breast milk for the development of celiac disease is discussed. This relates to one of the aims of the study, i.e. to measure some of the environmental factors possibly involved in celiac disease, such as early feeding. Breastfeeding has been shown to prevent, or at least delay, the development of celiac disease (28). Breast milk contains many immunological factors that stimulate the infant's immune system, but its exact role in the prevention of celiac disease is not known. Furthermore, breast milk contains small amounts of food antigens, like gluten peptides, that may contribute to tolerance induction. As the first contact with gluten may be important in this respect and the level of gluten peptides in breast milk may vary with intake of gluten by the mother, we studied the level of gluten peptides in breast milk of mothers on a gluten-containing diet and of mothers on a gluten-free diet. Expecting to find gluten peptides in the breast milk of mothers on a normal diet, but not in the breast milk of mothers on a gluten-free diet.

In chapter 3 we describe the development and testing of a food questionnaire to assess gluten intake, since another possible factor in the development of oral tolerance is the timing of gluten introduction and the quantity of gluten consumption in early life. However, this hypothesis is only based on observational studies. The role of gluten introduction should be confirmed by intervention studies before cause-effect conclusions can be drawn and changes in advice considering early infant feeding can be proposed. For such studies, it would be necessary to have an instrument available to assess the amount of gluten consumed. Such an instrument should be accurate, easy to use, and should be easily accessible by both researchers and parents. Since such an instrument was lacking, we developed and validated one for this purpose.

In exploring the attitude of celiac patients towards the gluten-free diet, we studied the management of the gluten-free diet by adolescent celiac patients. The results of that study are described in chapter 4. Until now, the gluten-free diet was the only effective treatment for the disease. From the perspective of the celiac patient, this treatment is quite a burden. Gluten intake is difficult to avoid since wheat is the cereal most used in staple food and widely used in the food industry. Furthermore, adherence to a gluten-free diet may have negative nutritional consequences. Dietary compliance in adolescents with celiac disease has been studied frequently and was shown to vary between 52% and 81% in European countries. In the Netherlands, however, we did not have information on this topic. Therefore, we studied the situation of dietary compliance in our country and the consequences for the nutrient intake in young celiac patients.

In an attempt to enlarge the gluten-free food choices, we studied whether the (new) naturally gluten-free cereal, tef (*Eragrostis tef*) can be safely used by celiac patients (Chapter 5). Adherence to the diet is often reported as being difficult. Next to the aforementioned aspects, the limited availability, the variety and the taste of gluten-free food products may have negative effects on the compliance with the gluten-free diet. A greater variety of tasteful products may contribute to a better compliance with the diet.
We studied the health-related quality of life (Chapter 6) in an adult population of celiac patients. Having a chronic disorder as well as having to adhere to a dietary regimen may affect quality of life. The health-related quality of life of celiac patients adhering to the gluten-free diet has been frequently studied in children as well as in adults. The adult population we studied was recruited for the study described in chapter 7 and consisted of celiac patients with strict adherence to the gluten-free diet and of celiac patients with gluten transgression or consuming a normal gluten-containing diet. This gave us the opportunity to compare the results of the health-related quality of life survey between compliers and non-compliers.

In chapter 7 we describe the results of a study on possible development of tolerance to gluten. As celiac disease is considered to be a permanent disorder, the diet has to be followed for life. However, patients consuming gluten without developing symptoms or signs of the disease have been described. Therefore, it is important to investigate which factors (genetic, immunologic or environmental) determine which patients with celiac disease remain intolerant to gluten for life and which few may regain tolerance. We studied the possible existence of adult celiac patients developing tolerance to gluten in the Netherlands and whether we could identify immunological or genetic factors that might contribute to this.
Finally, the overall results of the studies described in this thesis are discussed in chapter 8.

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CHAPTER 2

Presence of gluten proteins in breast milk: implications for the development of celiac disease

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This study was supported by the Celiac Disease Consortium, an Innovative Cluster approved by the Netherlands Genomics Initiative and partially funded by the Dutch Government (BSIK03009)

ABSTRACT

Aims: Celiac disease (CD) is a multifactorial disease with a strong genetic association and is caused by a T cell mediated immune response to gluten. Although much is known about the molecular mechanism, the trigger for the onset of CD is still unclear. A correlation between a reduced risk of developing CD and breastfeeding has been shown. The preventive mechanism however, remains unclear. In this study we test the hypothesis that T cell stimulatory epitopes originating from dietary gluten appear in the human breast milk.
Method: Breast milk of 23 mothers on a normal diet (mean gluten intake: 17 g/day) and 13 mothers on a strict gluten-free diet was collected in the period from one week until eight months after delivery. The presence of gluten proteins was studied using monoclonal antibody based competition assays specific for the T cell stimulatory epitopes of gliadin (Glia-α9, Glia-α20, Glia-γ1) and glutenin (Glt-156 and High Molecular Weight (HMW).
Results: T cell stimulatory epitopes of both gliadin and glutenin (Glia-α9 and HMW glutenin) were detected in the breast milk of mothers on a normal diet. Correlation studies revealed that the gluten intake was not correlated with the level of the Glia-α9 T cell stimulatory epitope detected.
Conclusion: Infants are exposed to small levels of gluten via breast milk. It is tempting to assume that these low levels of gluten are responsible for the induction of oral tolerance to gluten.

INTRODUCTION

Celiac disease (CD) is an inflammatory intestinal disorder caused by immune responses induced by dietary gluten proteins (1-3). It is a multi factorial disease with a strong HLA association. Approximately 95% of celiac disease patients are HLA-DQ2 (α1*0501, β1*0202), whereas the remainder is usually HLA-DQ8 (α1*0301, β1*0302).
With a prevalence of approximately 1 in 100-200, CD is the most common food induced enteropathy in the western world. A wide range of variable symptoms are associated with CD, including abdominal pain, diarrhea, constipation, vomiting, osteoporosis, growth retardation, and migraine. Many patients, however, have only very mild or no apparent clinical symptoms and are never diagnosed properly. At present, the only possible treatment for CD patients is a strict life-long gluten-free diet (GFD).
Although feasible, a GFD is complicated by the widespread use of gluten in the food industry and as an additive to many products that are not normally associated with gluten or wheat, like medication. Moreover, a strict GFD causes a severe restriction in the patients' social life (4).
Gluten molecules are the storage proteins of wheat and can be subdivided in the gliadin and glutenin protein families, both involved in CD. The proteins have a high proline content (5) and as a result, gluten proteins are poorly degraded by enzymes of the gastrointestinal tract (6). In the small intestine of CD patients, the partially degraded gluten proteins are modified by the activity of the enzyme tissue transglutaminase (tTG) (7-9). This so called deamidation introduces a negative charge in gluten peptides which facilitates their binding to the disease predisposing HLA-DQ2/8 molecules and facilitates efficient presentation of gluten peptides to CD4+ T cells of the immune system (7-14).
The T cell response against gluten is specific for CD patients since no evidence of T cell mediated reactivity against dietary gluten has been reported in normal, non-celiac, mucosa. The gluten reactive T cells have a Th0/Th1 phenotype and usually release the proinflammatory cytokine IFN-γ (15). Although at least 50 T cell stimulatory epitopes in gluten proteins have been identified, a unique 33-mer peptide of α-gliadin seems to be the most immunogenic (6,16). This 33-mer harbours six in part overlapping epitopes and it is resistant to the enzymatic degradation by gastric, pancreatic and brush border enzymes.
Although much is known about the molecular mechanism underlying CD, little is known about the onset and possibilities to prevent the disease. Since only a minority of the genetically predisposed individuals actually develop CD, a threshold of tolerance was suggested (17). This threshold is influenced by both gene dose (18) and gluten exposure (19). A large repertoire of abundant immunogenic gluten peptides in the diet, together with a high copy number of HLA-DQ2, thus may favor the breaking of oral tolerance. In present day practice, gluten is introduced into the diet of infants at the age of 6-7 months

(20). As there is no restriction in the amount of gluten given, gluten intake at the age of 12 months is between 6 and 9 grams daily (21), while gluten-specific T cells of CD patients are known to respond to microgram amounts. The sudden introduction of grams of gluten may thus play an important role in the breaking of oral tolerance. Another factor influencing the threshold or breaking of tolerance is breastfeeding. Recent studies have shown that breastfeeding offers protection against the development of CD (19,22). Breastfeeding at the time of gluten introduction and ongoing breastfeeding while gluten is already being consumed were associated with a reduced risk of development of CD. The exact preventive mechanism of breastfeeding on the development of CD, however, remains unclear. A tentative explanation might be that small amounts of gluten in breast milk promote the induction of low dose oral tolerance against gluten.
In this study, the presence of gluten in breast milk of mothers on a normal gluten-containing diet (ND), was investigated and compared to a control group of mothers on a GFD.

MATERIALS AND METHODS

Subjects

In 2005 lactating women on a ND were contacted at random at one Child Health Care Center (Nieuw Vennep, the Netherlands). In the year 2004 year, the Child Health Care Centers were attended by 91% of the families with infants in the Netherlands (23). Lactating mothers on a GFD were contacted through a call in 'Glutenvrij' a periodical distributed among members of the Dutch Celiac Disease Society (NCV), and a call on the website of the NCV.

Samples

Breast milk of women on a ND and of women on a GFD was collected longitudinally. Both groups of participants were asked to collect three samples of breast milk (morning, afternoon and evening) once a month. The samples were stored at home in labeled tubes in the freezer. After collection, the samples were transported to the laboratory, thawed, subdivided into small portions and stored at -70°C. Before analysis, one portion of each sample was thawed and the whey fraction was obtained by centrifugation at 14,000 rpm for 30 minutes at room temperature, after which the fat was removed.

Competition assays for the quantitative detection of T cell stimulatory epitopes of gluten proteins

Competition assays were performed as described earlier (24-26). For quantification of the gliadin assays, a standard curve was made by the Prolamine Working Group gliadin standard (27) in a concentration range of 10 µg/ml-10 ng/ml. The assays specific for the

detection of T cell stimulatory epitopes of LMW glutenin were calibrated using a 25-mer synthetic peptide as a standard that contains the Glt-156 epitope (14). The HMW-glutenin specific assay was calibrated using a chymotrypsin digest of purified HMW-glutenin proteins (kindly provided by P. Shewry, Rothamsted Research, Hampenden, United Kingdom). Both glutenin standards were used in a concentration range from 1 μg/ml-2 ng/ml.

Food record and gluten calculation

The mothers on a ND recorded a food record on three consecutive days preceding the day of breast milk collection. The last day of recording coincided with the day of breast milk collection. The amounts of food consumed were recorded in household measures and the name of the manufacturer of the products used was precisely written down. To determine the gluten intake, the vegetable protein content of the gluten-containing products was calculated according to the Dutch Food Composition Table (28). Since there are no analyses on gluten content of products, this was calculated by multiplying the grams of vegetable protein of the gluten-containing food by 0.8 as described by Overbeek *et al.*, (29) and by linking a food composition table spreadsheet to food consumption data using MS Access 2000. The products that may contain gluten, but with missing brand information or with a rounded number of zero grams protein in the food composition table, were defined by us as 'risk products'. As an assumption for the gluten content in those risk products an amount of 20 mg gluten per 100 g food product was used, which is the maximum of the Codex Alimentarius norm (30) for gluten-free products.

Data analysis

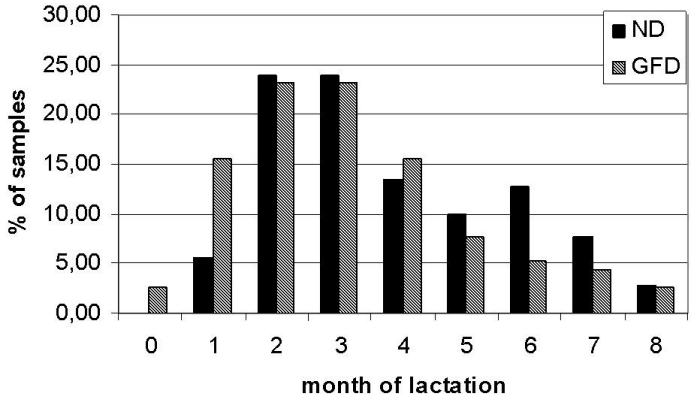
The data obtained by the competition assays and the commercial gliadin ELISA were imported in the scientific graphing and statistics program Graph Pad Prism version 4.02 (GraphPad Software, Inc. San Diego CA, USA). The significance of the differences detected between the level of gluten epitopes in the breast milk of mothers on a ND *vs* those on a GFD were assessed with a 2-sided unpaired t-test. $P<0.05$ was considered to indicate a significant difference. The Glia-α9 epitope is part of the degradation resistant 33-mer of alpha gliadin (16), and therefore the most likely to be detected in the breast milk as compared to Glia-α20, Glia-γ1 and HMW glt. Correlations between grams of gluten intake of the mother and Glia-α9 concentrations in breast milk were carried out using SPSS version 14.0 for Windows and checked by the Pearson correlation test. $P<0.05$ was considered to indicate a significant correlation.

RESULTS

Subjects

Twenty-three mothers on a ND (mean age 33±5 y) and 13 mothers on a GFD (mean age 34±4 y) responded and joined the study. Twelve of the 13 mothers on a GFD were CD patients diagnosed by small bowel biopsy. One mother showed clinical symptoms of CD and went on a GFD without being diagnosed.

Figure 1: Distribution of breast milk samples collected at different months of lactation. Breast milk samples were collected longitudinally at different stages of lactation of mothers on a normal diet (ND, n=131) and mothers on a gluten-free diet (GFD, n=116). In both groups, most samples were obtained from month 2 and 3 of the lactation period.



Breast milk samples

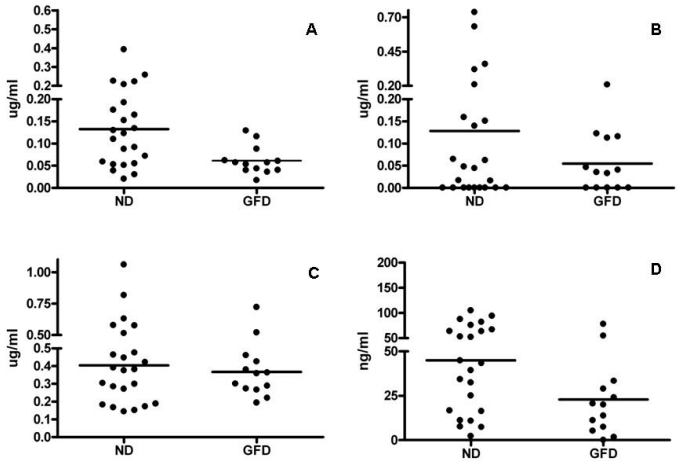
Breast milk samples were collected from the first week after delivery up to eight months of lactation. A total of 131 samples of mothers on a ND and 116 samples of mothers on a GFD were obtained. The sample distribution over the various months of lactation was comparable for both groups with the highest number of samples obtained in months 2 and 3 of lactation (Figure 1).

The presence of gliadin- and glutenin-derived T cell stimulatory epitopes in breast milk

The presence of T cell stimulatory epitopes of gluten proteins known to be involved in CD (both from gliadin and glutenin) was determined in the whey fractions of the samples using mAb-based competition assays. In both groups of samples, ND *vs* GFD, T cell

stimulatory epitopes were detected in the assays specific for the Glia-α9, Glia-α20, Glia-γ1 and HMW-glutenin T cell stimulatory epitopes (Figure 2).

Figure 2: Level of T cell stimulatory epitopes of gliadin and glutenin in breast milk.
Whey fraction of breast milk of mothers on a normal diet (ND) and mothers on a gluten-free diet (GFD) were measured in the mAb-based competition assays specific for T cell stimulatory epitopes of gliadin and glutenin. Mean level per mother of (A) the Glia-α9, (B) the Glia-α20, (C) the Glia-γ1 and (D) the HMW glutenin-derived T cell stimulatory epitope.



For the LMW glutenin-derived T cell stimulatory epitopes no results were obtained since the LMW specific competition assay was not suited to measure the presence of the T cell stimulatory epitopes in breast milk. T cell stimulatory epitopes Glia-α9 and HMW glutenin present in the samples from mothers on a ND were significantly increased ($P<0.05$) compared to the levels in the samples of mothers on a GFD. The differences between the levels of the T cell stimulatory Glia-α20 and Glia-γ1 epitopes were higher in women on a ND, but did not reach significance (Table 1).

Table 1: Levels of T cell stimulatory epitopes of gliadin and glutenin in breast milk of mothers on a normal diet versus mothers on a gluten-free diet.

Epitope	Normal diet (n=131)		Gluten-free diet (n=116)		
	mean	SEM	mean	SEM	*P*
Glia-α9 (μg/ml)	0.1324	0.0187	0.0615	0.0088	0.0135
Glia-α20 (μg/ml)	0.1285	0.0425	0.0549	0.0181	0.5410
Glia-γ1 (μg/ml)	0.4038	0.0471	0.3669	0.0394	0.7922
HMW glt (ng/ml)	44.92	6.388	22.92	6.162	0.0351

SEM = standard error of the mean; p indicates a two sided p value for the difference between gliadin and glutenin levels detected in breast milk of mothers on a normal diet compared to mothers on a gluten-free diet (unpaired t test); $P<0.05$ indicate a significant difference between the compared mean values.

Gluten intake of mothers on a normal, gluten-containing diet

Of the 23 mothers on a ND 22 kept a 3-day food record preceding the day of breast milk collection. In total, 48 food records were collected and the mean gluten intake was 17±3.8 g/day. The median consumption of gluten from risk products was 1.5 mg/day which is less than 0.01% of the total gluten intake. Because of this small percentage, the gluten intake from risk products was not taken into account in further analysis.

No correlation between gluten intake and level of Glia-α9 detected in breast milk of mothers on a normal diet

The correlation between gluten intake and the level of gluten epitopes detected in the breast milk of mothers on a ND was studied using the mean levels detected for the degradation resistant Glia-α9 epitope. This epitope is part of the degradation resistant 33-mer of alpha gliadin (16), and therefore the most likely to be detected in the breast milk. We checked the correlation between the gluten intake of the mothers on a ND and the level of Glia-α9. No correlations were found between the mean level of Glia-α9 epitope detected and the amount of gluten consumed in the three or two days preceding the day of breast milk collection ($r= -0.142$, $P=0.37$; $r= -0.131$, $P=0.42$, respectively). In addition, no correlation was detected between the mean level of the Glia-α9 epitope and the gluten intake during day 2 or day 3 ($r= -0.104$, $P=0.52$; $r= -0.109$, $P=0.50$, respectively).

DISCUSSION

In this study, breast milk samples were analyzed by antibody based assays specific for the detection of T cell stimulatory gluten peptides, for the presence of dietary gluten-derived peptides involved in the development of CD. Increased levels of peptides of both gliadin and glutenin could be shown in breast milk of mothers on a ND compared to those in

the breast milk of mothers on a GFD. The detected level of Glia-α9 however, did not correlate with the gluten intake of the mothers on a ND. Our result indicates that breast-fed children are exposed to low amounts of gluten-derived T cell stimulatory peptides involved in CD, even before gluten is introduced into the infants' diet.

The presence of gluten proteins in breast milk has been reported previously (31,32). The mean level of gliadin in breast milk of 178 ng/ml (range: 5-2000 ng/ml) reported in previous studies (31,32) correlates well with the mean levels of T cell stimulatory epitopes of gliadin detected in our study (Glia-α9 mean level 132 ng/ml, range 20.2-392 ng/ml; Glia-α20 mean level 129 ng/ml, range 0-737 ng/ml and Glia-γ1 mean level 404 ng/ml, range 143.7-1059 ng/ml).

Moreover, with the recently developed competition assay specific for the T cell stimulatory epitope of HMW glutenin (25), we could show that next to gliadin HMW glutenin also appears in breast milk (HMW mean level 44.92 ng/ml, range 2.23-104.8 ng/ml). Regarding the presence of LMW glutenin-derived T cell stimulatory epitopes in breast milk, nothing is known. The assay specific for detection of the LMW-derived T cell stimulatory epitope of LMW glutenin (25) was not suitable for detection of this epitope in breast milk (this study).

Gliadin given to mothers on a gluten restricted diet, appeared in the breast milk between 2-4 hours after gliadin intake (31). Similar to our study, basal levels of gliadin were detectable even before intake of gliadin (31). From that study it is not clear however, whether the level of gliadin detected in the breast milk correlated with the gluten intake of the mothers. The basal level detected both in our study and in the study reported previously (31), might be explained by cross reactivity of the gluten specific antibodies used for detection and human proteins that have some similarity with gluten sequences. For example, recently, cross reactivity between human anti-gliadin antibodies and a prolin glutamin rich neuronal protein, Synapsin I, has been described (33). Analyses of the human database for the minimal epitopes detected by our antibodies used for the competition experiments, did not reveal any human proteins that might be recognized by our antibodies (result not shown). On the other hand, we did not have detailed information on the strictness of the diet of mothers on a GFD and it is possible that the GFD diet was not kept strictly, either intended or unintended. Future experiments should be aimed at what proteins are detected by gluten specific assays in the breast milk of mothers on a strict GFD.

Upon ingestion, gluten proteins are digested by enzymes of the gastrointestinal tract including pepsin, trypsin, chymotrypsin and brush border enzymes. However, because of the high proline content of the gluten proteins, digestion is not complete and both of α-gliadin (33-mer containing 6 overlapping T cell stimulatory epitopes including the Glia-α9 epitope detected in this study) and of γ-gliadin, degradation resistant peptides have been

described (6,16). Those peptides contain the T cell stimulatory epitopes known to be involved in CD.
Moreover, in a recent study in which gluten degradation in the gastrointestinal system was mimicked in a gastrointestinal model, it was shown that also the HMW glutenin proteins are relatively resistant to degradation, probably even more resistant than gliadin proteins (26).
It has been suggested that the incomplete degradation of gluten in combination with the binding properties of the gluten peptides to HLA-DQ2 and HLA-DQ8 molecules, especially after deamidation by tTG, are the main cause of CD. Until now however, it is not known how those degradation resistant peptides cross the epithelial barrier and reach the lamina propria where, after endocytosis by dendritic cells, they are presented to the immune system. Analysis of the peptides detected in the breast milk in the near future might reveal in which form gluten crosses the epithelial barrier and enters the human body.
The presence of gliadin- and glutenin-derived peptides in breast milk of mothers indicates that infants are exposed to small amounts of gluten through the breast milk before the introduction of gluten into the infants' diet, which normally is advised from the age of 6 months. It is tempting to assume that those low amounts of gluten peptides induce oral tolerance to gluten as is described for other antigens (34-36).
Lactating mammary glands are part of the integrated mucosal immune system and milk antibodies reflect antigenic stimulation of the mucosal immune system in the gut and in the airways. Secretory IgA from breast milk exhibits antibody specificities for an array of both intestinal and respiratory common pathogens (37) and dietary proteins like cows milk proteins and gluten (38,39). Until the infant develops its own secretory IgA and IgM producing B cell blasts and plasma cells, it is dependent on the maternal secretory antibodies present in breast milk.
Next to antibodies, other various dietary antigens are present in breast milk; however, dietary restriction during pregnancy and breastfeeding has shown no conclusive effect on the development of atopic diseases in the child (40,41). These antigens stimulate the maturation of the infants' mucosal immune system (41) and under hyporeactive or immunosuppressive conditions, such as low antigen dose and/or presence of down regulatory cytokines as IL-10 and TGF-β (42), activation of the immune system might be skewed towards a Th2 or tolerogenic phenotype.
CD is a disease with a strong genetic association. HLA-DR3, DQ2 positive individuals have a five times higher risk of developing CD than HLA-DR3, DQ2 heterozygous individuals and a more than 11 times higher risk than people with other HLA haplotypes (18). However, since most HLA-DR3, DQ2 positive individuals do not develop CD, it is generally assumed that gluten induces oral tolerance. Recently, dietary gluten specific, CD4+, IL-10 and TGF-β producing regulatory T cells have been described. The cells are

present in the mucosa and inhibit pathogenic gluten reactive T cells (43). It would be very interesting to know to which peptides those regulatory T cells respond and whether those peptides are present in breast milk and involved in the induction of oral tolerance against CD.

In conclusion, in the present study we show that low amounts of T cell stimulatory epitopes of gluten, both from gliadin and glutenin, are present in breast milk of mothers on a ND. Since oral tolerance to food antigens is induced early in childhood, in the period infants are breast-fed, these peptides might be involved in the induction of gluten tolerance. Future experiments should be aimed at the characterization and identification of the gluten peptides present in breast milk.

This knowledge will give some new insights in the way gluten tolerance is induced and will help us to generate novel strategies aimed at the prevention of the onset of CD.

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CHAPTER 3

Food questionnaire for assessment of infant gluten consumption

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Clin Nutr 2007;26:264-71

ABSTRACT

Background: In light of the possibly preventive role of timing and amount of gluten in celiac disease, it would be helpful to have a questionnaire to assess the gluten intake in infants.
Aims: Development and validation of a food questionnaire to assess gluten consumption in healthy infants aged 0-12 months (FQ-gluten).
Methods: A food frequency questionnaire, previously developed for the Generation R study, was adapted for the assessment of gluten intake. The results of a 2-day food record (FR) were compared with the results of this FQ-gluten.
Results: Eighty-seven parents filled in the FR and the FQ-gluten. The number of children who consume gluten and who are breast-fed is higher, reported in the FQ-gluten. The amount of gluten is comparable from the age of 3 up to 10 months, but at 11 and 12 months a higher gluten intake is reported using the FR, probably due to a larger variety of food products not detectable by the FQ-gluten. However, there is a high agreement in the food groups (Cohens' Kappa = 0.6-0.8).
Conclusions: This new, short, standardized, validated and easy to use FQ-gluten may be a useful instrument to assess gluten intake in infants, both at the individual and at the population level. The use of this method by investigators in other countries provides the opportunity for a better comparison of the results of gluten consumption in (co-operative) studies throughout different countries.

INTRODUCTION

Celiac disease (CD) is a lifelong disorder caused by intolerance for gluten, and CD is treated with a gluten-free diet. Breastfeeding (BF) and weaning influence the development of the gastro-intestinal tract and it is possible that gradual introduction of antigens will lead to the development of oral tolerance (1-3). It is also likely that the response of the immune system to gluten is modified by BF (4,5).
The occurrence and disappearance of 'epidemics' of gluten intolerance after changes in Swedish infant feeding, during the 1980s and 1990s respectively, suggests that early feeding may be important in this respect. The analysis of the Swedish 'experiment in nature' has shown that ongoing BF during the period of gradual introduction of gluten-containing foods into the infant's diet, significantly reduces the risk of gluten intolerance (6,7).
Several studies on gluten consumption and BF have been performed in different countries (7-13), but the methods used to assess gluten intake mostly were time consuming and differed from each other. To our knowledge, there is no information on gluten introduction and gluten intake in the Netherlands.
As it is impossible to know precisely what a free living individual eats, there are various methods available for dietary assessment as an estimation of the intake, all of which have their advantages and disadvantages with regard to adequacy and work load (14,15). The food record (FR) assesses the intake recorded on the specific days on which the food and drinks are filled in; considering the intake on these days as a reflection of what someone normally eats. The food questionnaire (FQ) estimates how frequently certain foods are eaten during a specific period in time and only gives information on those foods or nutrients relevant to a specific question. Therefore, the FR may be the more accurate method, whereas the FQ may be the more representative, making it arguable which one is best to reflect true dietary intake (14). The FQ is an approach often used in epidemiological studies and is less time consuming than the FR (15).
The aim of this study was to develop and validate an FQ for the assessment of gluten consumption (FQ-gluten) in children aged 0-12 months.

SUBJECTS AND METHODS

Subjects

From February until July 2004, 192 consecutive parents of children aged 0-12 months who attended 4 Child Health Care Centres in the south-west part of the Netherlands were asked to participate in this study. In 2004 the Child Health Care Centres were attended by 91% of the infants in the Netherlands (16).

Exclusion criteria were: 1) gestational age less then 36 weeks, 2) mental retardation or oral-motor dysfunction, 3) diagnosed food allergy, 4) impossibility of oral food intake, and 5) parents without enough knowledge of the Dutch language.

METHODS

Development of the FQ-gluten

As a basis for our new instrument to assess gluten consumption, we used the FQ designed for a prospective cohort study in Rotterdam, the Netherlands; the Generation R study (17).

The Generation R study is a prospective population-based cohort study from foetal life until young adulthood. The study is designed to identify early environmental and genetic causes for growth, development and health in childhood and adulthood. For the Generation R study, 3 age specific FQs (0-2, 3-6 and 7-12 months of age) were developed by means of a standardized method in cooperation with the division of Human Nutrition and Epidemiology of the Wageningen University and Research Centre (18).

The FQs collect information on family characteristics and actual food intake, and, in retrospect, on the start and cessation of BF, the age at first introduction of food groups (e.g. fruits and meats), and on the intake of allergens and nutrients. However, they do not contain the whole spectrum of food products necessary to assess gluten intake.

In order to develop the FQ-gluten, we added gluten-containing food products according to the database of a recent food consumption study among young children aged 9 – 18 months (19) and according to the Dutch Food composition table (20), such as a variety of components for breakfast and the warm meal, flavoured milk products, ready-to-eat infant meals and porridges. The brand names of these products available in the Netherlands were derived from the list of food products (21). The FQ-gluten for children aged 7-12 months is the most extended FQ and comprises 68 items on food intake and BF (Table 1).

Table 1. Food frequency questionnaire for Dutch children at the age of 7 – 12 months of age.

1. This questionnaire is filled in by:	□ Mother □ Father □ Both □ Other
2. Date of filling in the questionnaire	……../ ……../ ……
3. Date of birth of your child	……../ ……../ ……
4. Sex	□ Boy / □ Girl
5. Order of birth	□ 1st □ 2nd □ 3rd □ 4th or later

6. Did you ever feed your child by breastfeeding
□ No, go to question number 9
□ Yes

7. What age was your child when you stopped breastfeeding it?
□ I still breast-feed
□ Younger than 1 month
□ Between 1 and 2 months
□ Between 2 and 3 months
□ Between 3 and 4 months
□ Between 4 and 5 months
□ Between 5 and 6 months
□ Between 6 and 7 months
□ Between 7 and 8 months
□ Between 8 and 9 months
□ Between 9 and 10 months
□ Between 10 and 11 months
□ Older than 11 months

8. How many times do you breast-feed at this moment?
□ 1 – 2 times a day
□ 2 – 3 times a day
□ 3 – 5 times a day
□ 5 – 7 times a day
□ More than 7 times a day

9. Do you feed your child formula feeding?
□ No, go to question number 11
□ Yes

10. What kind of formula feeding do you give your child?
□ Normal for the age
□ For a younger age
□ For an older age
□ Adapted to food allergy

11. Do you feed your child porridge?
□ No, go to question number 13
□ Yes, namely,
□ Bambix or Brinta or Molenaar
□ Nutrix, Biobim junior
□ Oatmeal porridge
□ Semolina

12. How much porridge do you feed your child per day?
□ If given by bottle:
□ Less than ½ bottle
□ ½ - 1 bottle
□ 1-1½ bottle
□ 1½ - 2 bottles
□ More than 2 bottles

□ If given by spoon:
□ Less than ½ plate
□ ½ - 1 plate
□ 1 – 1½ plate
□ 1½ - 2 plates
□ More than 2 plates

13. Do you add one of the following products to your child's food (e.g. mixed with fruit, etc.)
□ Baby biscuits (Bambix, Liga 2nd step, Liga 'big and strong'), rusk
□ Wheat flour
□ None of the above mentioned

14. When you use ready-to-feed meals for your child, what brand do you normally use?

□ I do not use ready-to-feed meals
□ Akwarius baby meals for 7 months
□ Olvarit meal for 8, 15 or 18 months
□ Olvarit 'Wereldreis' for 12 or 17 months
□ Zonnatura meal for 8 or 12 months
□ None of the above mentioned

15. When you use ready-to-feed fruit for your child what brand do you normally use?

□ I do not use ready-to-feed fruit
□ Zonnatura for 8 or 12 months
□ Olvarit fruit for 8 months with biscuit
□ Zonnatura summer fruit with grains
□ None of the above mentioned

16. With what frequency do you give your child the following food products?

Food product	Never	Less than once a week	1-3 times a week	4-6 times a week	Once a day	Twice a day	3 times or more a day
Bread (1 slice=35 g)							
French bread / Baguette (1 slice=15 g)							
Currant bread (1 slice=35g)							
Rusk / crisp bread							
Honey-cake (1 slice=20g)							
Cracker/mazoth							
Multigrain rice waffle							
Yoghurt or other milk product with flavour (150 ml)							
Bambix or Brinta or Molenaar porridge (150ml)							
Oatmeal porridge (150ml)							
Semolina porridge (150ml)							
Baby biscuit: Bambix, Liga 2nd step, Liga 'big and strong'							
"Lange vinger" biscuit							
Other sweet biscuits							
Soup stick							
Cake (1 slice=30 g)							
Pastry (1 piece=85 g)							
Ready-to-feed fruit							
Ready-to-feed warm meal							
Pasta: macaroni, spaghetti etc. (1 portion = 50g)							
Bulgur / couscous (1 portion=50g)							
Multigrain rice (1 portion= 50g)							
Pancake							
Fritters (1 portion = 10)							
Pizza (1/8= 50g)							
Crumbed products (meat, fish, chicken, cheese) (1 portion = 75g)							
Vegetarian burgers and balls (1 portion=75g)							
Wheat flour based sauces (1 spoon=25g)							

17. Please note for the following products at what age you child first received them

Food product	Never given	< 3 months	3 - 6 months	6 - 9 months	> 9 months
Bread					
French bread / baguette					
Currant bread					
Rusk / crisp bread					
Honey-cake					
Cracker/mazoth					
Multigrain rice waffle					
Yoghurt or other milk product with flavour					
Bambix or Brinta or Molenaar porridge					
Oatmeal porridge					
Semolina porridge					
Baby biscuit: Bambix, Liga 2nd step, Liga 'big and strong'					
"Lange vinger" biscuit					
Other sweet biscuits					
Soup stick					
Cake					
Pastry					
Ready-to-feed fruit					
Ready-to-feed warm meal					
Pasta: macaroni, spaghetti etc.					
Bulgur					
Couscous					
Multigrain rice					
Pancake					
Fritters					
Pizza					
Crumbed products (meat, fish, chicken, cheese)					
Vegetarian burgers and balls					
Wheat flour based sauces					

18. Does your child use medicine or vitamins at the moment? □ No □ Yes, namely:…….

Validation of the FQ-gluten

To validate the FQ-gluten, we asked the parents to fill in the new FQ-gluten and a 2-day FR (i.e. one weekday and one weekend day) of their child's food intake in household measures and to precisely note the name of the manufacturer of the product used. A 2-day FR is an accepted method used in food consumption studies (19, 22).

Assessing gluten amount

We considered food products containing wheat, rye and barley as gluten-containing. Since there is no information on the gluten content of food products, we used the method of Overbeek et al. (23) to calculate the content of gluten. Following this method, we multiplied the grams of gluten-containing protein according to the Dutch Food composition table, by 0.8 (20).

As 'risk products' we defined those products known to possibly contain gluten, but for which the exact amount of gluten could not be calculated due to either missing brand information or a rounded number of zero grams protein in the food composition table. We used the Codex Alimentarius norm (20 mg gluten per 100 g food product) to make an assumption about the gluten content of these risk products (24).

Statistical analysis

All analyses were carried out using the software of the Statistical Package for the Social Sciences release 10 (1999, SPSS Inc. Chicago IL, USA). Microsoft Access version 2000 (2000, Microsoft Office) was used to assess the gluten intake by connecting files with the calculated gluten content of food products to the individual intake.

The cross-sectional data on the amounts of gluten consumption were derived from the FQ-gluten and the FR. Per child, the mean percentages of gluten from different food products were calculated. The mean percentages between FR and FQ were compared using the paired *t*-test. Agreement was assessed by the Bland-Altman plot and by calculating limits of agreement, defined as the mean difference±2SD. The Cohen's Kappa was used to test agreement between categorical variables. *P*-values <0.05 were considered significant.

RESULTS

Of the parents invited for the study, 87 (45%) agreed to participate and filled in the FQ-gluten and the FR. Fifty-nine percent (n=51) of the children were girls; 11 in the age category of 0-2 months, 17 aged 3-6 months and 23 aged 7-12 months.

Validation of the FQ-gluten

The comparison of the frequency of gluten consumption and BF, assessed by the FQ-gluten and by the FR is presented in Table 2. The FQ-gluten detected more children with gluten consumption (3 children) and BF (3 children), than the FR. The difference in the assessment of gluten consumption in the age categories of 3 to 6 months was caused by 3 infants aged 4, 5 and 6 months, who did not consume gluten according to the FR, but who consumed baby biscuits 1 or 2 times per week according to the FQ-gluten.

Table 2. Comparison of the frequency of gluten consumption (GC) and breastfeeding (BF) assessed by the food questionnaire gluten (FQ-gluten) and by the food record (FR).

		GC		BF		BF and GC	
Age (months)	Children *(n)*	FQ-gluten *(n)*	FR *(n)*	FQ-gluten *(n)*	FR *(n)*	FQ-gluten *(n)*	FR *(n)*
0-2	13	0	0	7	6	0	0
3-6	34	6	3	18	17	2	1
7-12	40	38	38	6	5	6	5
Total	87	44	41	31	28	8	6

Table 3. Comparison of the origins of the gluten intake in the 44 Dutch children, of whom gluten consumption was indicated, assessed by the food record (FR) and the food questionnaire for gluten (FQ-gluten).

Age (m)	Food product	FR Mean % of gluten intake	FQ-gluten Mean % of gluten intake
3-6	Porridge	60	40
	Bread	30	41
	Baby biscuits	10	13
	Other[a]	--	6
7-12	Porridge	44	37
	Bread	42	38
	Baby biscuits	12*	20
	Other[b]	1*	5

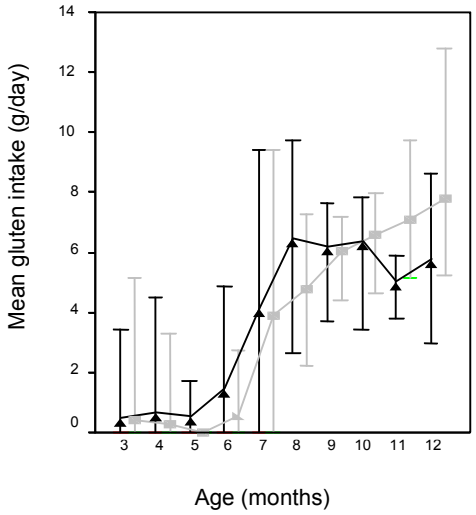
[a]Pasta or biscuit; [b]Pancake, pasta or breakfast components; *$p<0.05$ between FR and FQ-gluten.

The origin of the gluten intake, as reported by the parents, is presented in Table 3. Both instruments reported consumption of similar gluten-containing products (Cohen's kappa for porridge (k=0.7), bread (k=0.8) and biscuits (k=0.6)) and for BF (k=0.8).
All of the gluten-containing products that were reported in the food of the children aged 3-6 months by using the FR, were contained in the FQ-gluten for this age category. However, for children aged 7-12 months, 95% of the gluten delivering products reported in the FR, were contained in the FQ-gluten. The explanation was that one of the baby biscuits often used, two of the cookies and two breakfast components were not specified in the FQ-gluten.

The contribution of the 'risk products' to the total gluten consumption was 0.65% (34 mg; 0-117 mg) as assessed by the FQ-gluten, and 0.21% (10 mg; 0-63 mg) as assessed by the FR ($P<0.0001$). These 'risk products' were consumed by children in the age of 3-12 months and they consisted of ready-to-eat fruit and vegetable mixes, and flavoured milk products (e.g. fruit yoghurt).

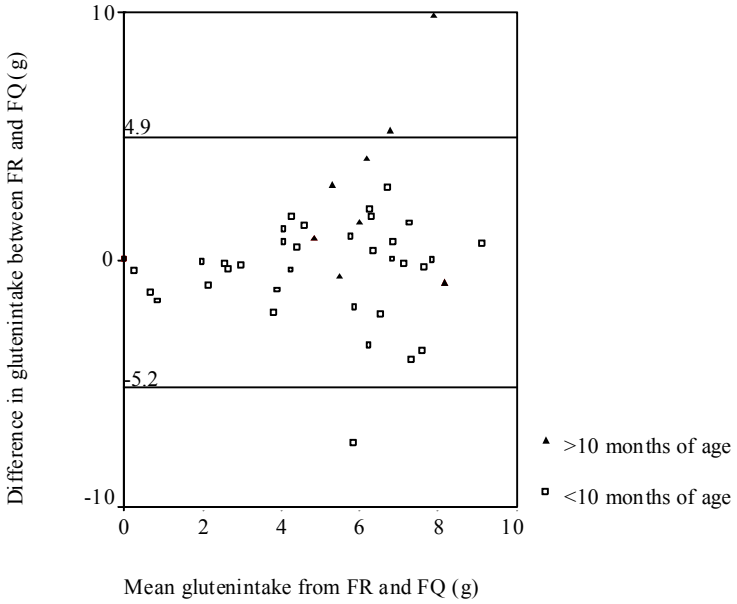
The comparison of the amount of gluten consumed by the children, calculated from the reports of the parents using the 2 instruments, is shown in Fig. 1. The assessment of the gluten intake was similar until the age of 10 months ($P=0.7$), but the FR reported a higher gluten intake for older children ($P=0.07$). The absolute median difference in the assessment of gluten consumption by the FR or the FQ-gluten was 1.1 g (*r*: 0.022-9.8).

Figure 1. Mean gluten consumption (g/day) and range calculated from the food questionnaires (FQ-gluten;▲) and the food record (FR;■).



The Bland-Altman plot with limits of agreement is presented in Fig. 2. The SD of the differences was 2.5 g, which means that the differences in the gluten intake using the 2 methods are high in some individual cases.

Figure 2. Bland-Altman plot of the gluten intake according to the food questionnaire (FQ-gluten) and to the food record (from the age of 3-12 months).



DISCUSSION

To our knowledge, we are the first to develop an FQ to assess the gluten consumption in infants; the FQ-gluten. We have validated this FQ-gluten by using the 2-day FR as a reference.

We have found that the FQ-gluten, compared to the frequently used FR method, detects a larger number of children consuming gluten and receiving BF, that it shows a high agreement in the food groups consumed, and that it provides similar data concerning the amount of gluten up to the age of 10 months.

A possible explanation for the detection of a larger number of children with gluten consumption and BF we found in the FQ-gluten may be that a 7-day method (FQ-gluten) is compared to a 2-day method (FR). The 7-day method detects the consumption of foods that are used once or a few times a week, while the 2-day FR only assesses the consumption on the 2 days. Would we have compared the results of the FQ-gluten to a 7-day FR, probably the results would be in higher agreement. However, a 2-day FR is an accepted method used in food consumption studies (19,22), is of less burden on the

parents than a 7-day FR and it gives good enough information on the variety of the food products these young children use.
The mean amount of gluten consumption assessed with the FQ-gluten in the children older than 10 months was less than the one assessed with the FR. This is probably due to the fact that, instead of 100%, only 95% of the gluten-containing products was contained in the FQ-gluten for 7-12 months and that this causes a gap in the total amount assessed with the FQ-gluten. For future use, the FQ-gluten can easily be improved by adding these missing food products.
Concerning the 'risk products', we found a limited contribution to the total gluten intake (0.21-0.65%). However, these products can introduce small amounts of gluten into the infant's diet and therefore need to be listed in order to detect (the first) gluten consumption.
It has been suggested that BF at the time of gluten introduction, and even that ongoing BF while gluten is already being consumed, plays a preventive role in the development of CD (7). A 52% reduction in the risk of developing CD was found for infants who started with gluten-containing food while they were being breast-fed, compared to the ones who were not being breast-fed at that time.
An important recent American study was published of a cohort of 1560 children who had an increased risk of developing CD or type 1 diabetes, as defined by possessing either HLA-DR3 or -DR4 alleles, or having a first-degree relative with type 1 diabetes, derived from the DAISY project (Diabetes Autoimmunity Study in the Young). At a mean follow-up of 4.8 years, the authors concluded that: 1) there is a 'window of opportunity' in the introduction of gluten into the diet when the child is aged between 4 and 6 months with regard to the risk of developing CD, and that 2) the contribution of BF was to be disregarded in this respect (13). However, the authors did not make specific attempts to calculate the gluten amount ingested by the children or to correlate this important early nutrition event with the presence or absence of BF.
This year, a systematic review and a meta-analysis of observational studies which were published between 1966 and June 2004 and which examined the association between BF and the development of CD, has been published (25). The authors concluded that BF may offer protection against the development of CD. BF during the introduction of dietary gluten, and increasing duration of BF, were associated with a reduced risk of developing CD. It is, however, not clear from the primary studies whether BF delays the onset of symptoms or provides a permanent protection against the disease.
Long-term prospective cohort studies on BF and gluten intake may shed light on the importance of the quantity of exposure to gluten in early life with regard to the development of CD (26).
One problem in this respect is that, until now, there were no validated instruments to quantify the gluten intake by young infants. The FQ-gluten presented here may be a

useful instrument for this purpose. As the FQ-gluten we developed is based on the Dutch eating pattern and contains specific Dutch brand names it can only be used for the Dutch population. On the other hand, this FQ-gluten can be adapted by investigators in other countries to the eating patterns and food products used by young children in their country. A possible way for other investigators to adapt an FQ-gluten to their own food habits is to get information of the food products and brand names used by their children from food consumption studies performed in their nations. If such studies are not available, the information may be obtained by a basis enquiry among young children using a FR. From these results an FQ-gluten can be adapted and validated.
In conclusion; this new, short, standardized, validated and easy to use FQ-gluten detects more children consuming gluten than the FR and may be a useful instrument to assess gluten intake in infants, both at the individual and at the population level. The use of this method by investigators in different countries will provide the opportunity for a better comparison of the results of gluten consumption in (co-operative) studies throughout different countries.

ACKNOWLEDGMENTS

Erica G. Hopman and Jessica C. Kiefte- de Jong developed the FQ-gluten, collected and analysed the data of the study. Erica Hopman also wrote the manuscript. Saskia le Cessie assisted in the analysis of the data, Henriëtte A. Moll, Jacqueline C. Witteman and Sacha E. Bleeker contributed to the development of the FQ used in the Generation R study, M. Luisa Mearin proposed the idea for the study. They all critically read the manuscript.
We thank Mrs. N.A.P. van der Herberg-van de Wetering, pediatric-dietitian at the Department of Dietetics, Leiden University Medical Centre, for her input on infants' nutrition, and Dr. P.J.M. Weijs of the Amsterdam School of Nutrition and Dietetics for his assistance in Microsoft Access. We also thank Mrs. C. Aarsen of the Dutch Nutrition Centre for providing information on the Dutch recommendations on BF and gluten introduction, and of course Drs. J. van Goor, Drs. C. Visée, Drs. C. Helders, Drs. M. de Bond and Drs. S. Grolman from the Child Health Care Centres at the Rijn-Duin-Bollenstreek, for the distribution of the food records and questionnaires.

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CHAPTER 4

Nutritional management of the gluten-free diet in young people with celiac disease in the Netherlands

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J Pediatr Gastroenterol Nutr 2006;43:102-8

This work is financially supported by the Board of Directors of the LUMC and Nutricia B.V., the Netherlands and through the Fund for Nutritional Research, LUMC, the Netherlands

ABSTRACT

Background: For young people with celiac disease, adherence to the gluten-free diet may be difficult to achieve and gluten restriction may lead to insufficient nutrient intake and unbalanced food intake resulting in overweight. In the Netherlands, no nutritional information is available. Therefore, we evaluated the nutritional management and nutritional state in young celiac patients.
Methods: The Dutch Celiac Society invited all its members aged 12 to 25 years to complete a food record and a questionnaire. Nutrient intakes were compared with the recommendations and the intake in the general population. Total immunoglobulin A, endomysial antibody, tissue transglutaminase and IgA gliadin were determined and height and weight were assessed.
Results: Strict dietary compliance was reported by 75%. The fiber and iron intakes were significantly lower, and the saturated fat intake significantly higher than recommended, but comparable with the general population. Most of the patients (61%) found the diet easy to follow. Regular medical controls were reported by 86%, but regular dietary controls by only 7% of the patients. Mean and SD scores for height and body mass index were -0.3±1.1 and -0.3±0.8, respectively.
Conclusions: The dietary compliance in this group is high, the nutritional state is adequate, but the nutrient intake is not. Better medical and dietary support is necessary to prevent long-term complications and to achieve an ongoing satisfying management in this group of young patients with a chronic disorder.

INTRODUCTION

The treatment of celiac disease (CD) consists in a life long strict gluten-free diet (GFD). Nonadherence to the GFD may lead to complications such as diarrhoea, abdominal pain, anaemia, osteoporosis, infertility and cancer (1).
For many patients, adherence to the diet may be difficult to achieve (2-5). This seems to be particularly true among adolescent celiacs. In Europe, the compliance with the GFD by young people varies between 52% and 81% (6-11).
In addition, adherence to a GFD may have negative nutritional consequences (12,13). Mariani et al., (11) reported that 72% of the Italian adolescents with CD adhering strictly to the diet were overweight and consumed an unbalanced diet rich in fat and protein, poor in carbohydrate and deficient in calcium, iron and fiber.
In the Netherlands, there is no information on the nutritional intake and dietary compliance by young people with CD. Therefore, we evaluated the nutritional management of the GFD and the nutritional state in celiac patients aged 12 to 25 years, an age group with an expected poor compliance.

MATERIALS AND METHODS

In January 2000, the Dutch Celiac Society invited all its 395 members in the age category 12 to 25 years to participate in the study. Inclusion criteria were diagnosis of CD based on at least one small-intestinal biopsy showing histological abnormalities characteristic of CD (14) and a good knowledge of the Dutch language. Nonresponders (age and sex known) were sent a reminder after 2 months.
We used anonymous information from the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA) to check the total number of CD patients in the Netherlands aged 12 to 25 years at the start of the study. The reported diagnosis of CD of the responders was cross-checked by means of the information provided by PALGA. Only confirmed CD patients were included in the study.
Nonparticipants were asked some additional questions concerning body weight, height and educational level.
A 34-item questionnaire was composed in collaboration with the Dutch Nutrition Centre. This questionnaire was based on a 2-hour focus group interview with 10 CD patients in the target age category. Under the direction of a trained facilitator (MH), the focus group members were encouraged to express their perceptions and opinions about living with CD and adhering to a GFD. The questionnaire was pretested individually by 4 celiac patients (one aged 12 years, two aged 14 years and one aged 19 years) in presence of the investigator (EGDH).

Patients were asked to fill in a 3-day food record (two weekdays and one weekend day) in household measures and to precisely note the name of the manufacturer of the product used. The food records were analysed for total energy and macronutrient intake, saturated fat, vitamins B1, B2, B6, iron, calcium and fiber intake, according to the Dutch Food Composition Table 1996, (15) using the computerised nutrition calculating program (BECEL 5, the Netherlands).

The data were compared with both the Dutch (DRDA) (16) and American recommendations (ARDA) (17,18) and the General Dutch Population (GDP) (19) for which the total group had to be divided in the age categories less than 13, 13 to 16, 16 to 19, 19 to 22 and 22 to 25 years. For comparison, the sex distribution in the different age categories was taken into account.

Because there are no data on gluten content of food products, the gluten intake reported by the patients in their questionnaires was approximately estimated using the information in the Draft Revised Standard for gluten-free foods of the Codex Alimentarius Commission from the year 2000, (20) that stated that the total content of gluten in wheat-starch based gluten-free products should not exceed 200 ppm (20 mg gluten per 100 g). For the gluten-containing products, we used the estimation method from Overbeek et al., (21) that consists on multiplying the grams of vegetable protein by 0.8. For products of which is known that they may contain gluten, but the amount of gluten could not be estimated because of a rounded number of zero grams protein in the food composition table, the 20 mg gluten per 100 g was used for calculations.

Participants had blood punctures at either our hospital or at their family doctor's. All determinations were done at the laboratory for clinical immunology of the Free University Hospital, Amsterdam. Patients with a total immunoglobulin A (IgA) concentration less than 0.06 g/L were considered as IgA deficient. The serum titers of IgA gliadin (AGA) and tissue transglutaminase (tTGA) antibodies were measured using enzyme-linked immunosorbent assay techniques (22,23). Serum IgA endomysium antibodies (EMA) were detected by indirect immunofluorescence on monkey oesophagus (22).

We also estimated the gluten intake from the food record from 15 randomly selected patients: 6 with positive EMA and/or tTGA and 9 without these antibodies.

Patients performed the measurement of their own height and weight at home according to standardized instructions and of 26 patients visiting the LUMC for blood puncture the measurements were repeated by one of us (EGDH). Height was measured using a wall-mounted stadiometer. Height and body mass index (BMI) were expressed as SD scores (SDS) (24).

All analyses were carried out using the software of the Statistical Package for the Social Sciences release 10 (1999, SPSS Inc, Chicago IL). The Student *t* test was used to compare the characteristics of the participating and nonparticipating patients. We checked whether

the 95% confidence interval of the energy and nutrient intakes contained the values of the DRDA and the ARDA and the values of the intakes of the GDP. For differences between medians, the Mann-Whitney *U* test was performed. The reliability of the self-reported height and weight was assessed by paired *t* test and Bland-Altman analysis by comparing these measurements with the ones assessed at the LUMC. Percentages were compared using the Chi-square test. We divided the total group into the age categories of 12 to 16 years and 17 to 25 years.

ETHICAL CONSIDERATIONS

The study was approved by the Medical Ethics Committee of the LUMC.

RESULTS

Figure 1 shows the patient flow and participation rates of the patients invited for the study. The 395 members of the Celiac Society represented 61% of the patients in this age category in the Netherlands as checked through PALGA. The responders were significantly younger compared with the nonresponders: 16.9±4.3 years versus 18.1±3.8 years (P=0.005), and there were more female responders in the age category of 17 to 25 years (74% versus 57%; P=0.049). There were no significant differences between participants and nonparticipants concerning sex distribution, mean age and height SDS, but the nonparticipants had a significantly lower BMI SDS (-0.9±0.7; P=0.016). Without the 33 participants and 3 nonparticipants still attending primary school, 50% of the nonparticipants had a low educational level compared with 38% of the participants, but this difference did not reach statistical significance. The characteristics of the participating patients are shown in Table 1. Of the participants, 8% had an associated disease. Dividing the total group into the age categories of 12 to 16 years (n=80) and 17 to 25 years (n=52), there were significant differences in the median age at diagnosis (2.8 years [0.8-14.7] versus 7.3 years [0.3-23.6; P=0.003]), and the mean duration of the GFD (8.5±3.9 years versus 11.2±8.2 years; P=0.03).
The anthropometric measurements of the 26 patients (20%) visiting the outpatient department were higher than the ones they performed at home: 1.3 cm (-1.2 – 3.8; P<0.0001) for height and 1.6 kg (-1.9 – 5.1; P<0.0001) for weight, resulting in a higher BMI (19.1±2.2 versus 18.7±2.2; P<0.05).Eighty-four percent of the patients (111) returned the completed food record. The nutritional data from all the patients were analysed and as an example, the data of the patients aged 13 to 16 years are presented in Table 2.

Figure 1. Patient flow and participation rates.

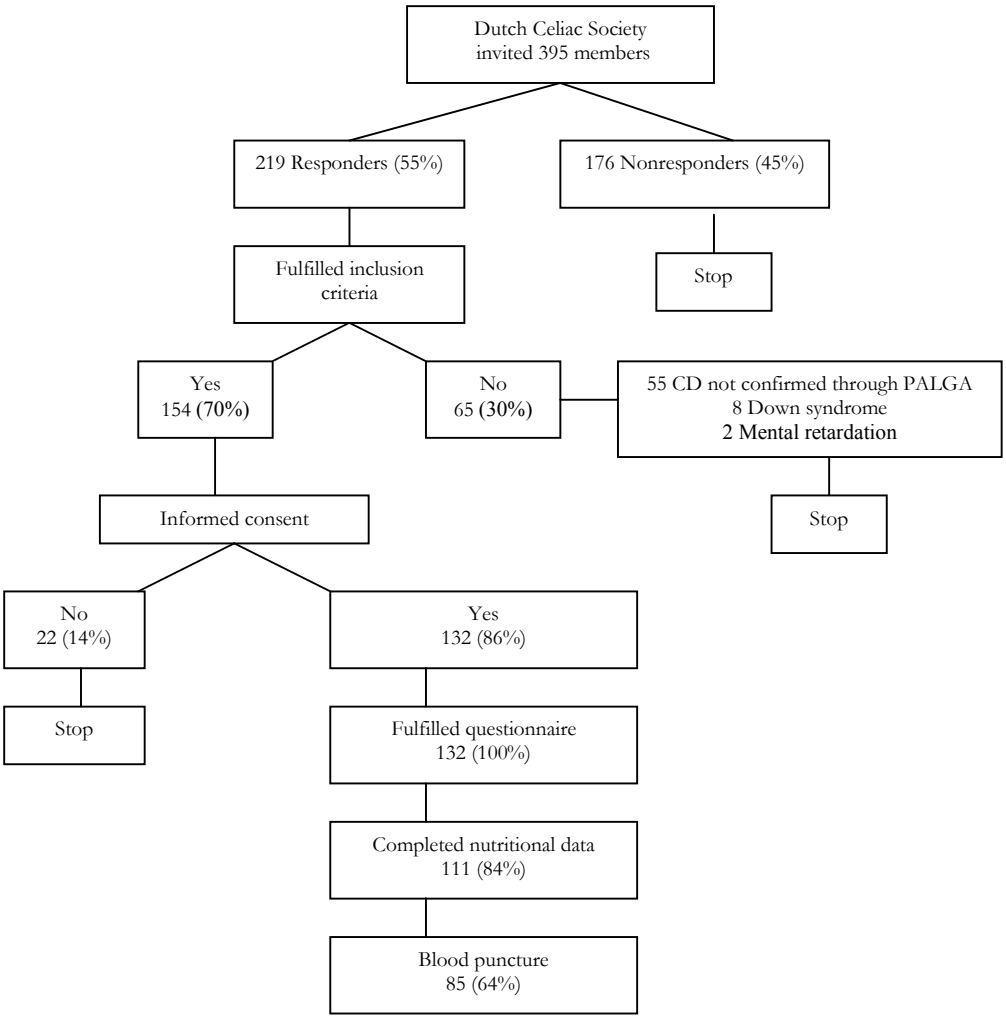

Table 1. Characteristics of 132 Dutch patients with celiac disease aged 12 to 25 years.

Females, n (%)	87 (66)
Age, y (mean±SD)	16.6±4.4
Age at diagnosis, y median (range)	4.3 (0.3-23.6)
Duration of GFD, y (mean±SD)	9.6±6.0
Height, SDS (mean±SD)	-0.3±1.1
BMI, SDS (mean±SD)	-0.3±0.8
Associated diseases, n (%)	
Thyroid dysfunction	5 (4)
IDDM	2 (2)
Thyroid dysfunction + IDDM	2 (2)
Ulcerative Colitis	1 (1)
Psychiatric Disorder	1 (1)
Low education*, n (%)	50 (38)

*With a maximum level of (senior) secondary vocational education.
IDDM, insulin-dependent diabetes mellitus.

Table 2. Nutritional intakes of 37 patients with celiac disease aged 13 to 16 years, presented as means *(95% CI)*, compared with the intakes in the same age category by the GDP, the DRDA and the ARDA.

	Celiac patients	GDP	DRDA	ARDA§
Energy (kcal)	2264 (2097-2432)*	2280	2486	2320
Protein (%)	12 (11.7-12.8)†	14	10-15	10-30
Fat (%)	34 (32-36)	36	30-35	25-35
Saturated fat (%)	12 (11.6-13.2)*,†	14	Max. 10	¶
CH (%)	54 (52-56)†	51	55	45-65
Fiber (g)	17 (15-19)*,†‡	20	31	28
Vit. B1 (mg)	1.0 (0.9-1.1)‡	1.1	1.0	1.2
Vit. B2 (mg)	1.7 (1.5-1.9)*,†‡	1.4	1.4	1.4
Vit. B6 (mg)	1.5 (1.4-1.7)*	1.5	1.3	1.5
Calcium (mg)	1082 (960-1204)‡	960	780-1080	1300
Iron (mg)	10 (9-11)*,‡	10	13	14

*P<0.05 CD patients compared with DRDA; †P<0.05 CD patients compared with GDP; ‡P<0.05 CD patients compared with ARDA.
§For the ARDA, the age category of 11 to 14 years was used for the RDA of energy, vitamins and iron and that of 9 to 13 years for the RDA of protein, total fat, saturated fat, carbohydrates, fiber and calcium;
¶ Indicates as low as possible; %, energy percentage; CH, carbohydrates.

CD patients of all age categories had an intake of fiber and iron significantly lower than both the RDAs and an energy percentage saturated fat significantly higher than the DRDA. In addition, the intake of calcium by the patients younger than 19 years did not reach the ARDA, which are higher than the DRDA for all ages. Concerning the B vitamins, the intake in all ages reached or exceeded the RDAs.
Compared with the GDP, a significantly lower intake was found in the patients aged 13 to 16 years and 22 to 25 years for protein and saturated fat, in the patients younger than 19 for fiber, and in the patients aged 16 to 19 years for iron. Only the patients in the oldest age category (22-25 years) had a significantly higher calcium intake than the GDP (tables presented on the internet).
Most of the patients (64%) used vitamin- and mineral-enriched gluten-free food products, and 35% of these patients also used vitamin and mineral supplementation. Of the patients who did not use enriched food products, 47% used vitamin and mineral supplementation, which is significantly higher than in the GDP (17%) ($P<0.0001$). Reasons for using supplementation were "just as an addition to the GFD" (17%), "in case of tiredness" (11%), "concern about having less resistance" or "prevention of becoming ill" (19%) and "advised by the doctor or the dietician" (6%). Comparing the intake of those using enriched gluten-free products and those who do not use them, the latter had a significant lower intake of B vitamins, calcium, fiber and iron (table presented on the internet). However, the intake of B vitamins and calcium by the last group still reached the DRDA but not the ARDA for vitamin B1 and calcium. In addition, patients with CD consuming enriched products still not reach the RDAs for fiber and iron and the ARDA for calcium.
Seventy-five percent of the patients reported a strict adherence to the GFD, of whom 10% added that it might be possible that they consume gluten only by accident. Occasional consumption of gluten-containing food was admitted by 23% of the patients (estimated median gluten intake: 153 mg/d [2-6382 mg]). Two patients (2%) did not adhere to the GFD. Most of the patients (61%) found that the GFD was easy to follow. From the 85 patients who had serologic antibodies determination, 2 (2%) were found to be IgA deficient. The patients who did not have blood puncture were significantly older (18.0±4.1 years versus 15.8±4.3 years, $P=0.006$), had a lower educational level 38% versus 21%, $P=0.034$), less often completed the food record (66% versus 94%, $P<0.0001$) but were not different in self-reported strictness in following the GFD (74.5% versus 75.3%) compared with the ones who did have blood punctures. Positieve EMA and tTGA were found in 7% to 17% of the patients (6/83-14/83). There was no difference in the self-reported compliance between the patients who did and who did not have detectable antibodies in serum, neither was there any difference between the group categories aged 12 to16 years and aged 17 to 25 years. There was no difference in the percentage of patients with high serum antibodies between the 2 groups (17% versus

15%) (Table 3). However, considering the duration of the diet, a higher percentage of the patients after a GFD for shorter period (less than 5 years) had high serum antibodies (33% versus 14%; *P*=0.058).

Table 3. IgA serum antibodies in 83 patients aged 12 to 25 years with celiac disease, reporting to follow a strict GFD or a GFD with gluten consumption from time to time.

Positive IgA serum antibodies	GFD n=63 (%)	GC n=20 (%)
AGA	0	0
EMA	9 (14)	3 (15)
tTGA	6 (10)	0
At least one positive antibody (AGA, EMA or tTGA)	11 (18)	3 (15)
EMA and/or tTGA	10 (16)	3 (15)
No positive antibodies	52 (83)	17 (85)

GC, GFD with gluten consumption from time to time.

The gluten intake estimated from the food records were compared with the self-reported adherence to the GFD. The median daily gluten intake of the 6 patients with high serum antibodies was 29 mg (17-40 mg), whereas that of the 9 patients with CD without antibodies was 47 mg (8-1394 mg). This is however much lower than the intake in the GDP, which is approximately 10 to 15 g/d. Seven of the 15 patients admitted that they consumed gluten-containing food from time to time, but only one of them had high antibodies. Three patients with the highest gluten intake stated that they sometimes got complaints after gluten ingestion (table presented on the internet).

The most frequent sources of gluten were candies (53%), chocolates or crisps (47%) and fast food (31%). This last product group was significantly more often consumed by the older patients (43% versus 18%; *P*<0.05). The gluten-containing food products were mostly consumed at special occasions (60%) or at home (49%). A high percentage of the patients (65%) reported symptoms after gluten consumption, especially abdominal pain (83%), diarrhoea (73%) and lassitude (52%).

Reported strategies to find out whether or not a food product was suitable for the GFD were "reading the ingredients on the food label" (94%), followed by "checking the list of gluten-free products" (72%). A less reported strategy was to "just eat it and see whether it gives problems" (6%), but this was used significantly more often by the older group (12% versus 3%; *P*<0.05). As expected, "asking my parents" was a strategy mostly used by the younger group (66% versus 15%; *P*<0.01).

The most frequent answer to the question of "what the long-term consequences of not adhering to the GFD can be" was cancer: in the first place, gastrointestinal cancer (46%), particularly among the older group of patients (65% versus 33%; $P<0.01$), but also other types of cancer (15%).
Most of the patients reported regular medical controls (86%; median duration 8.4 years; 0-23 years), with a significantly higher frequency in the younger group (95% versus 71%; $P<0.0001$). The physicians consulted usually were paediatricians or internists (61%), and only 38% reported controls by the (pediatric-) gastroenterologist. Sixty-two percent of the patients undergoing regular medical controls thought that it was important to be controlled, an opinion shared by 32% of the patients without medical controls.
Only 7% of the patients had regular dietary controls by a dietician. A great number of the patients (47%) reported that the GFD is instructed during a single visit. However, 16% thinks that it is important to have these dietary controls (11% in the younger and 23% in the older group). Of the patients who have regular dietetic control, 33% think that the controls are important. About 84% think their knowledge about CD and the GFD is sufficient.

DISCUSSION

In this study among young patients with CD in the Netherlands, we have found a self-reported compliance with the GFD of 75%, which is within the range of the one found by other European investigators (6-11). The mean duration of the GFD was significantly longer in the oldest group, although the age at diagnosis was significantly higher compared with the younger group, indicating a trend of earlier recognition of CD by the Dutch pediatricians (25,26).
Determination of celiac antibodies in serum has been reported as a reliable way to monitor the compliance with the GFD (27). Interestingly, we did not find a correlation between the self-reported compliance with the diet and the results of the celiac antibodies in serum. Neither did we find a relation between the estimated amount of gluten consumed and the presence of antibodies, but presumable, there is a role for the duration of the GFD. One possible explanation is that our patients overestimated their gluten intake. It is also possible that the determination of the antibodies in serum is not an adequate method to detect adherence to the GFD, both in adults and in adolescents, as has been suggested by others (28-31). Another reason for a lack of rise in AGA upon gluten intake could be the fact that the diagnosis of CD was established before AGA had developed, so treatment was started before an immunological memory for AGA production had been formed, and no up-regulation of AGA levels occurred upon gluten consumption.

The high compliance in our group of patients may be explained by the fact that all of them were members of the Dutch Celiac Society, and they may have a higher awareness of the importance of adhering to the GFD. It is also possible that especially the most compliant patients were willing to participate. However, our rates of response (55%) and participation (42%) are within the range of those in other studies among celiac patients (2,32). It is also possible that the higher level of education, which may have influenced their willingness to fill in the questionnaire and the food record, may have influenced their compliance with the diet as well. On the other hand, the CD diagnosis of 51% of the nonparticipants was not confirmed, so it is also possible that an important number of them did not participate because they did not have CD.

In contrast to the report from Italy (11), we found a good nutritional state among the young people with CD, both males and females, although we used the home measurements for height and weight for analyses, which were significantly lower than the ones performed at the outpatient department.

The data on the nutrient intakes of the patients were compared with the DRDA and the ARDA. A problem was that the age categories of the ARDA were not similar to those of the DRDA, so the most suitable age category of the ARDA had to be chosen to make a comparison. However, this did not change the evaluation of the adequacy of the nutrient intake, except for the patients up to 19 years with respect to the calcium intake, the ARDA of which is higher than the DRDA.

We have found that the nutritional intake of young people with CD in the Netherlands is comparable with their peer groups in the GDP, which means a higher saturated fat intake and a lower fiber and iron intake than is recommended. In some age categories, however, the intakes of protein and saturated fat are lower than in the GDP, but still higher than the RDA. However, for fiber and iron, the intakes are lower than in the GDP, which means much more deficient with regard to the RDA. In these cases, adhering to the GFD does have consequences for the adequacy of the nutritional intake, as has been shown before (11,12).

Approximately 45% of the patients reported an intake for saturated fat over 125% of the RDA. Seventy percent and 65% reported intakes, for respectively, fiber and iron less than 75% of the RDA. Because we did not measure the fat and iron levels of the celiac patients in serum, we do not know whether their self-reported inadequate intakes also had biological consequences for them.

Compared with the general population, a significantly higher percentage of the celiac patients used vitamin and mineral supplementation, either by enriched food products or tablets or by both. In almost all cases, this supplementation was taken on their own initiative. Apparently, the patients themselves thought that CD or the GFD formed risk factors for nutrient deficiencies.

Most young people with CD thought that avoiding cancer was the most important reason to adhere to the GFD. It has been found that when patients with CD adhere to a GFD for 5 consecutive years or more, their risk of malignancy is not increased compared with that of the general population (33,34). On the other hand, over the last years it has become clear that, although CD patients have a higher risk of developing cancer than the general population, the risk is much lower than previously presumed (35-37).
At present, the GFD is the only effective treatment for CD, and it is prudent to recommend strict adherence to the diet to all celiac patients. However, the fear of developing malignancy is not necessarily the most important reason for advising a strict diet to CD patients. Physicians should, arguably, mainly stress the advantages of the diet with regard to the prevention of other complications of CD, such as osteoporosis (38) and autoimmune disorders (39). They should also point out the relation between adherence to the GFD and improvement of fertility and birth outcomes (40-42). These facts support the necessity of regular medical and dietary controls, subscribed by the patients themselves but not brought into practice.
In conclusion, we have found that the dietary compliance of young people with CD is high in the Netherlands, and that their nutritional state is adequate. Their nutritional intake is inadequate, albeit similar to the one of the GDP. A suitable medical and dietary treatment is necessary, with special attention to the intakes of saturated fat, fiber and iron to prevent long-term complications and to guarantee ongoing satisfying management of CD and the GFD in this group of young patients with a chronic disorder.

ACKNOWLEDGMENTS AND AFFILIATIONS

The authors thank Drs. M. Hekman from the Dutch Nutrition Centre for her help in composing the assessment questionnaire and Prof. J.M. Wit for fruitful discussions and for critically reading the manuscript.
This work was performed in cooperation with the Dutch Celiac Society.

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CHAPTER 5

Tef in the diet of celiac patients in the Netherlands

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Scand J Gastroenterol 2008;43:277-282

This work was supported by the Celiac Disease Consortium, an innovative cluster approved by the Netherlands Genomics Initiative and partially funded by a Dutch government grant (BSIK03009) in cooperation with the Dutch Celiac Disease Society

ABSTRACT

Objective: Celiac disease (CD) is a multifactorial disease with a strong genetic association. It is caused by a T-cell-mediated immune response to wheat gluten. The treatment is a strict gluten-free diet (GFD). The purpose of this study was to investigate whether the naturally gluten-free cereal *Eragrostis tef* (tef) is associated with health problems when used by CD patients.
Material and methods: In March 2006, all 7990 members of the Dutch Celiac Disease Society were invited to complete a questionnaire on tef use and the development of symptoms after tef consumption. Respondents and their family members willing to participate were sent an extended questionnaire on the use of commercially available tef products and on the character of their subsequent symptoms.
Results: Thirty-six percent responded to the first questionnaire of whom 53% consumed tef and 15% reported complaints. For the second questionnaire, out of the 1828 participants willing to complete it, 1545 had biopsy proven CD (median duration GFD: 6.5 y (range: 0-66.5 years)). Of these, 66% used tef (median duration 1.4 y (range: 0.1-5 years)) and 17% reported symptoms after consumption. The percentage for symptoms was significantly lower than that in patients without tef consumption reporting on their regular GFD (17% versus 61%; p=0.0001).
Conclusions: Tef is frequently used by Dutch CD patients and a wide majority can consume tef without experiencing any clinical symptoms. CD patients using tef reported a significant reduction in symptoms, possibly related to a reduction in gluten intake or to an increase in fiber intake. Hence, tef can be a valuable addition to the GFD of CD patients.

INTRODUCTION

Celiac disease (CD) is a permanent intolerance to gluten, the storage protein of wheat. With a prevalence of approximately 1:100-200, CD is the most common food-related enteropathy (1-4). Gluten causes inflammation in the small bowel of CD patients resulting in (sub-)total villous atrophy. Clinical presentation varies from a complete lack of symptoms to frank malabsorption. The treatment for the disease is a lifelong gluten-free diet (GFD). GFD heals the intestinal mucosa, improves symptoms, and protects from the development of complications such as osteoporosis, infertility, and malignancies of the gastrointestinal tract (5-7).

Although from a medical perspective a GFD is a safe and methodical treatment, it is quite a burden from the perspective of the celiac patient. The intake of gluten is hard to avoid in Western society, since wheat is the most commonly used cereal in staple food and is also widely used within the food industry. A GFD may thus have a negative impact on patients' social life (8,9) as well as on quality of life (10,11). Special gluten-free products have a lower palatability and nutritional value (such as fiber content), and their availability is limited. Therefore, the addition of gluten-free cereals to the pool of currently available ones is most welcome.

In the past few years, researchers have studied the use of oats in the GFD. Oats have been described as safe, and diversify the diet for most adults as well as children with CD (12-16). However, uncontaminated oats are difficult to obtain and some CD patients still develop clinical symptoms and mucosal damage after oats consumption (17,18).

Another alternative cereal for a GFD may be tef (*Eragrostis tef)*. Tef is only very distantly related to cereals like wheat, barley, rye, and oats, and lacks gluten-like prolamins that cause problems for CD patients (19). Tef is a cereal from Ethiopia that is used as an ingredient for a staple food called *injera*, a kind of pancake. Tef flour can be used for the same purposes as wheat flour, and its nutritional value is similar (20,21). Compared with the available gluten-free products, tef has a higher vitamin and fiber content, and therefore may be a valuable supplement to a GFD.

In 2002, tef was introduced to the gluten-free market in the Netherlands and became a component of the Dutch celiac food package. Although tef is demonstrated to be gluten-free in the laboratory, symptoms after tef consumption were reported by CD patients to the Dutch Celiac Disease Society (NCV). We therefore investigated the use of tef among Dutch CD patients and whether tef is associated with clinical symptoms.

MATERIALS AND METHODS

Patients

We investigated the use of tef and the development of symptoms after tef consumption among all members of the NCV and their relatives through a two-step postal questionnaire. The mastery of the Dutch language was required for participation.
Step 1. To study the percentage of CD patients using tef, a 10 item questionnaire on patient characteristics, diagnosis, tef use, and symptoms, and informed consent to participate in the second questionnaire were sent to all members by regular mail. Family members using tef, either with or without CD, could state if they were also willing to complete a second questionnaire.
Step 2. The second questionnaire contained 31 items on patient characteristics, diagnosis, and tef use, with detailed questions on previous symptoms on a regular GFD (without tef) and on symptoms after tef consumption, and on the use of commercially available tef products suitable for a GFD.

Statistical analysis

All analyses were carried out using SPSS version 14.0 for Windows (SPSS Inc., Chicago Ill., USA). Discrete variables were compared using the chi-square test. Continuous variables were compared using the Mann-Whitney U-test because of skewed distribution. The Wilcoxon signed-rank test was used to compare the percentage of symptoms before and after introduction of tef into the diet.

Ethics

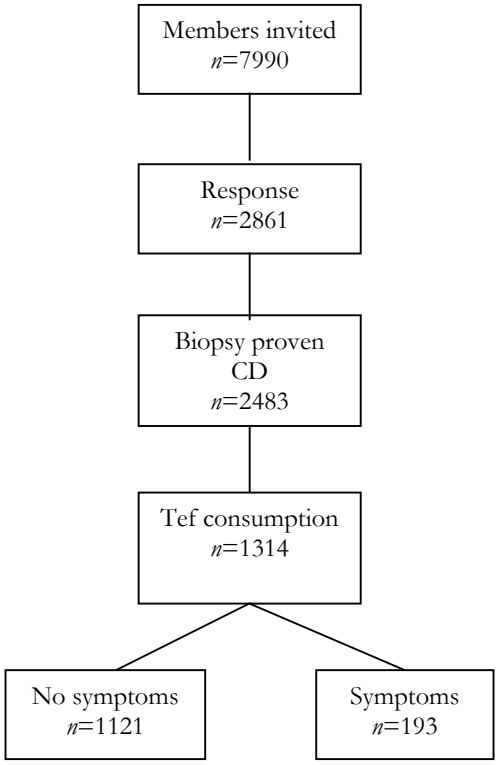
The study protocol was discussed with the Medical Ethics Committee of the Leiden University Medical Center. Informed consent was not required.

RESULTS

Questionnaire 1

Out of 7990 members of the NCV invited, 2861 responded to the first questionnaire (Figure 1). Ninety-nine percent followed a GFD for biopsy-proven CD (87%), non-biopsy proven CD (7%), dermatitis herpetiformis (2%) and for other diagnoses (3%: food allergy, inflammatory bowel disease, gastrointestinal symptoms of unknown etiology). For further analyses, only data from biopsy-proven CD patients were used. Of these, 53% consumed tef and 15% of tef users reported complaints after consumption.

Figure 1. Flow chart of the members of the Dutch Celiac Disease Society for questionnaire 1.



Questionnaire 2

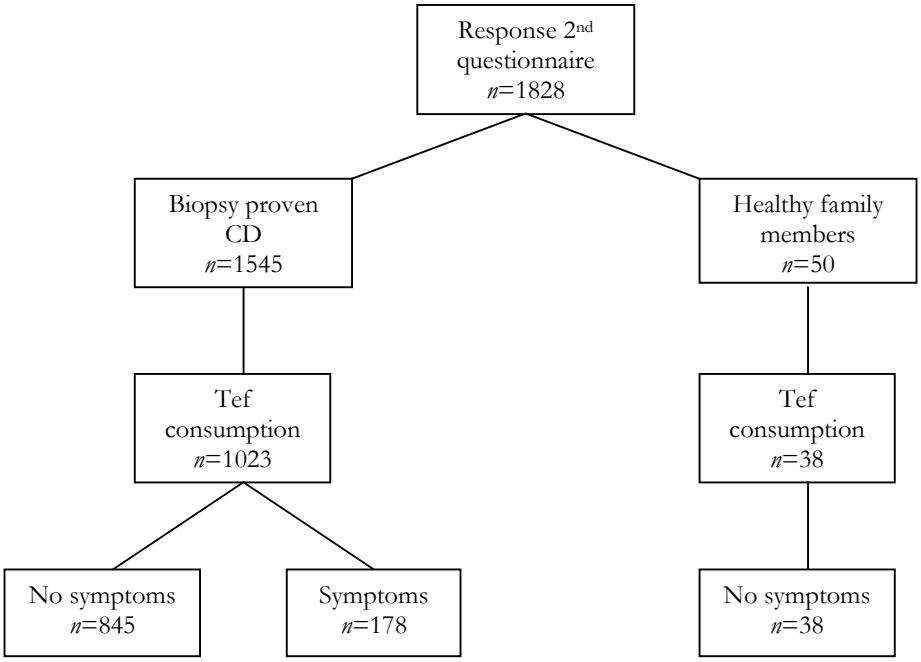
The second questionnaire was filled in by 1828 patients and healthy family members (Figure 2). Of these, 1545 had biopsy-proven CD with a median duration on GFD of 6.5 years (range: 0-66.5 years). Of the non-celiacs, 50 were healthy family members and 233 had various conditions, mostly non-biopsy-proven CD. Further analyses were done in biopsy-proven CD patients and healthy family members.

Celiac patients using tef versus healthy family members using tef

Tef was used, now or in the past, by 66% of biopsy-proven CD patients and 76% of healthy family members. The median age (50 versus 45 years; p=0.26) and median duration of tef consumption (1.4 versus 0.5 years; p=0.22) were comparable between CD patients and healthy family members, but there were usually significantly more often female CD patients (71% versus 45%, p=0.001). Furthermore, CD patients reported

significantly more symptoms after tef consumption than healthy family members (17% versus 0%; p=0.014).

Figure 2. Flow chart of the responders to the second questionnaire.



Celiac patients on a GFD with tef versus celiac patients on a GFD who never used tef

Thirty-four percent of CD patients never used tef in their GFD; 61% of these patients reported having symptoms while on their GFD. This percentage was comparable with the percentage of symptoms reported by tef users before tef was introduced into their GFD (61% versus 58%; p=0.42). However, a significant reduction of symptoms from 58% to 17% (p=0.005) was reported after adding tef to the GFD.

Compared with CD patients who never used tef, tef users reported significantly fewer symptoms (17% versus 61%, p=0.0001) and a significantly shorter duration on GFD (6.2 versus 7.5 years, p=0.001). Details on the reported symptoms are presented in Table 1.

Table 1. Distribution of symptoms reported by biopsy-proven celiac patients before and after tef consumption and on regular gluten-free diet.

	With tef consumption (n=1023)		Without tef consumption (n=522)
Symptom (%)	Before introduction of tef in GFD	After introduction of tef in GFD	On GFD
Abdominal pain	20	10*,#	23
Bloated feeling	18	7*,#	18
Diarrhoea	17	8*,#	20
Lassitude	30	5*,#	33
Constipation	14	2*,#	16
Nausea	7	3*,#	9
Anorexia	4	2*,#	5
Depression	4	1*	6
Aphthous ulcers	8	0.7*,#	9
Muscle weakness	8	0.6*,#	8
Migraine	5	0.4*,#	6
Vomiting	2§	0.4*	3
Weight loss	4	0.4*	6
Epilepsy	0.2	0*	0.8

Abbreviation: GFD=gluten-free diet;
*p<0.05 between symptoms after introduction of tef in a GFD and on a GFD; #=p<0.05 between symptoms before and after introduction of tef in a GFD; §=p<0.05 between symptoms before introduction of tef in a GFD and on a GFD.

All symptoms were significantly less frequently reported by patients after tef consumption compared with patients who never used tef in their GFD. When comparing symptoms before and after the introduction of tef into the GFD, there was significantly less reporting of all symptoms by patients after tef consumption, with the exception of depression, vomiting, weight loss, and epilepsy.

Celiac patients with symptoms after tef use versus celiac patients without symptoms after tef use

Not all patients who introduced tef completely recovered from symptoms: 12% of tef users still reported symptoms. An additional 5% of tef users reported symptoms after tef consumption that they did not have before they introduced tef into their GFD. Patients who reported symptoms after introduction of tef were significantly older (median 55 versus 48 years, p=0.02), had a significantly shorter median duration of tef consumption (0.5 versus 1.5 years, p=0.008), and reported having significantly more symptoms on their

regular GFD (before tef introduction) compared with tef users without symptoms after tef consumption (70 versus 55%, p=0.0001).
Thirteen CD patients with tef in their diet reported medical investigations following their symptoms. In five cases, duodenal biopsies were taken (1 Marsh 0, 1 Marsh 2, 1 Marsh 3a, 1 suspected irritable bowel syndrome, and 1 unknown result). In one case, dietetic consultation was sought, and in the other cases, symptoms were treated by advising that tef consumption should be stopped (n=5), by prescribing hormonal cream because of dermatitis herpetiformis (n=1), or by prescribing laxatives for constipation (n=1).

Celiac patients who continued tef use versus celiac patients who discontinued tef use
Of the CD patients who introduced tef into their GFD, 16% discontinued tef use because of: physical complaints (40%), unpalatable taste (49%) or baking quality (9%), difficulty in obtaining tef (7%), or high costs (10%), with some patients reporting more than one reason. Patients who discontinued tef consumption reported significantly more symptoms before and after tef was added to their GFD, compared with patients still using tef (64% versus 55%; p=0.043 and 44% versus 9%; p=0.0001, respectively).

Tef products
From the 13 commercially available tef products, two brown bread mixes were used significantly more often by CD patients without symptoms than by CD patients with symptoms after tef use. In contrast, no products were consumed more often by CD patients with symptoms compared with CD patients without symptoms after tef use (data not shown). Fifty-seven percent of tef users (42 patients with and 393 patients without symptoms after tef use) reported their daily amount of tef products used. Both the patients with and those without symptoms after tef use consumed an equal median amount of 71 g of tef products per day (p=0.85).

DISCUSSION

We carried out the first inventory study on tef use by CD patients among members of the Dutch Celiac Disease Society. Our results show that tef is frequently used in the GFD of Dutch CD patients and that patients using tef reported fewer symptoms after adding tef to the GFD. The percentage of celiac patients who reported symptoms on a regular GFD (either before tef use or among those who never used tef) was fairly high in our study population.
Tef has been commercially available in our country for a few years and has already been accepted in the GFD by two-thirds of the participants. The main difference we found between celiac patients who used tef and those who did not was that tef users were on a GFD for a significantly shorter period. This emphasizes the need for new gluten-free

cereals, especially for those patients with many symptoms who are willing to try alternatives to improve their symptoms before adhering to a more fixed dietary pattern. After introduction of tef, patients reported a significant reduction in symptoms. This finding suggests a beneficial effect of tef on self-reported symptoms of celiac patients. Although our study was not designed to investigate cause-effect relationships, it is tempting to speculate on the nature of the self-reported reduction in symptoms by tef users.

Sixty percent of participating CD patients continued to have symptoms while on GFD, a phenomenon also described by others (22-24). These symptoms may be related to CD in combination with extreme sensitivity to trace amounts of gluten or to intentional or unintentional lapses in the GFD, but our study was not designed to distinguish between underlying factors for these symptoms. Furthermore, it is known that some treated CD patients never fully recover (25). The reduction in symptoms after introduction of tef, however, strongly suggests that dietary factors account for these symptoms.

Patients who used tef were on a GFD for a shorter period of time, but long enough to expect their CD to be well-treated. To study cause-effect relationships between CD-related symptoms and diet-related symptoms, randomized clinical trials will be necessary, although it will be difficult to perform these properly blinded.

Whether tef is likely to have a beneficial effect in the diet of celiac patients might be attributed to differences in gluten content and/or differences in fiber content. Introducing tef into the GFD probably means that patients have replaced their usual gluten-free products for tef. When patients replace wheat starch-containing products, there is a reduction in the intake of trace amounts of gluten, which may exert an influence on the reduction of their reported symptoms.

Furthermore, tef has high fiber content, while a regular GFD is deficient in fiber (26-29). CD patients may need to adapt to the relatively high fiber intake while consuming tef, which may first lead to symptoms in the intestine (30). A gradual introduction and prolonged use of tef in the diet of CD patients may reduce this problem. This adaptation may be reflected by our finding that patients without symptoms after tef use have a significantly longer duration of tef consumption, and thus may have had a longer period to get used to the higher fiber intake.

Symptoms reported after tef use by patients who did not report symptoms after their regular GFD may be related to high fiber content, but also to gluten contamination. Laboratory testing of tef-based food products aimed at the Dutch gluten-free market showed that some products were clearly contaminated with gluten-containing cereals (E.H.A. Dekking, pers. comm.). However, it is not known whether these contaminated products were consumed by our participants.

Introduction of new gluten-free cereals in the market must be accompanied by strict isolation of the entire production process from gluten-containing cereals. Previous experiences with oats have shown how difficult that can be (31,32).
Two patients reported to have crypt hyperplasia or villous atrophy when investigated for an increase of symptoms after tef consumption. Both followed the GFD for more than 15 years and were considered experienced in following the diet properly, but they also reported having symptoms when making mistakes in the diet. It is not known whether dietary lapses, gluten-contamination of the tef products, or development of refractory CD were responsible for these histological findings. Follow-up was not available.
Owing to the response rate (36%), it is a possibility that CD patients with many symptoms and CD patients who are using tef are overrepresented in this study. This would lead to overestimation of the percentage of CD patients using tef in our country and overestimation of self-reported symptoms in CD patients on a regular GFD. However, even if all non-participants did not use tef and had no symptoms on a regular GFD at all, we could still show that a significant proportion of Dutch CD patients are using tef, and that a significant reduction of symptoms is clearly associated with the use of tef.
In conclusion, we found that tef is frequently used without any clinical problems by a large group of CD patients in our country. CD patients using tef reported a significant reduction in symptoms, possibly related to a reduction in gluten intake or to an increase in fiber intake. Tef seems to be a valuable addition to the gluten-free diet of many CD patients.

ACKNOWLEDGEMENTS

We thank Prof. J.M. Wit, MD, PhD, emeritus professor of Paediatrics for critical reading of the manuscript.

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CHAPTER 6

Dietary compliance and health-related quality of life in patients with celiac disease

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Submitted

Funding: Fund 'Doelmatigheidsonderzoek', Leiden University Medical Center, projectcode P03.106

ABSTRACT

Objective: Celiac disease is treated with a lifelong gluten-free diet. The aim of our study was to investigate whether dietary (non-) compliance is associated with health-related quality of life (HRQoL) of celiac patients.
Patients and methods: Patients from our hospital, known with celiac disease for more than 10 years, were invited to participate in a study on possible gluten tolerance. HRQoL was assesses by the SF-36, symptoms by the Gastrointestinal Symptom Rating Scale and dietary compliance by a food frequency questionnaire. HRQoL of celiac patients was compared with that of the general population.
Results: Fifty-three biopsy-confirmed celiac patients were divided into three groups according to gluten consumption: gluten-free diet (n=33), gluten transgression (<10 g gluten/day; n=8), and normal gluten-containing diet (>10 g gluten/day; n=12). Compared with the general population, celiac patients scored significantly worse on general health perception but significantly better on bodily pain and limitations due to physical problems. The results of the Gastrointestinal Symptom Rating Scale and the SF-36 were similar in all three dietary groups.
Conclusions: Although adhering to the gluten-free diet strictly is important to prevent future complications, patients with partial or non-adherence report similar HRQoL compared with patients with strict adherence in this group of adult celiac patients.

INTRODUCTION

Gluten in the diet of celiac disease (CD) patients causes histological alterations in their small bowel that may lead to disturbances in nutrient absorption, to symptoms such as diarrhea and abdominal pain, or to extra-intestinal complications such as osteoporosis, infertility, and cancer (1). The treatment of CD consists of a lifelong gluten-free diet (GFD) to heal the duodenal musoca, improve symptoms, and protect from development of complications (2,3), although in some cases histological remission is incomplete (4). Treatment with a GFD has in general a positive effect on the patients' medical condition (1), but results from studies on the impact of treatment on their emotional and psychological well-being differ in outcome. The psychological well-being and health-related quality of life (HRQoL) of treated CD patients have been reported to be similar to healthy controls, both in children (5) and in adults (6,7). On the other hand, a negative impact of CD on social life and a lower HRQoL in CD patients have also been described (8-11). Furthermore, gender differences seem to play a role in the HRQoL of CD patients, in particular being a disadvantage for women (6,7,12).
The dietary compliance of CD patients has frequently been studied (13,14) and compliance with the GFD may be one of the factors associated with HRQoL (7,9). It is known that HRQoL improves once newly diagnosed CD patients are treated with a GFD (15,16). Some patients, however, stop following the GFD and remain consuming gluten for a long time with good clinical tolerance to the challenge (17,18). In our study on possible development of tolerance to gluten among adult CD patients, we found that 23% of them did not adhere to the GFD (18). The aim of the present study was to investigate whether the HRQoL was associated with dietary (non-) compliance in the group of CD patients recruited for that study.

METHODS

Adult CD patients known to have CD for at least 10 years and diagnosed at the Leiden University Medical Center (LUMC) were invited to participate.

Dietary compliance

Dietary information was obtained by a questionnaire in which the patients were asked to register their degree of compliance with the diet as strict, partial, or none. The consumption of gluten-containing products was checked by a food frequency questionnaire. The food frequency questionnaire for this study was developed according to the database of the Dutch Food consumption study, held in 1998 (19), and according to the Dutch Food composition table (20). Gluten intake was estimated by multiplying the grams of vegetable protein from gluten-containing cereals by 0.8 (21). Based on

gluten intake, three groups were distinguished: 1) gluten-free diet (GFD); 2) gluten transgression (GT: < 10 g gluten/day), and 3) normal gluten-containing diet (GCD: >10 g gluten/day) (22).

Assessment of the health-related quality of life

The SF-36 health survey was used to assess the general HRQoL (23). It is composed of 36 questions and standardized response choices, organized into eight multi-item scales: physical functioning, role limitations due to physical health problems, bodily pain, general health perception, vitality, social functioning, role limitations due to emotional problems, and general mental health.

Comparison with the general population

Data from a random, nationwide sample in the Netherlands (1742 respondents, 56% male, age 16-94 years), were used as a reference to compare the results of the SF-36 (23).

Assessment of symptoms

Specific gastrointestinal symptoms were evaluated according to the Gastrointestinal Symptom Rating Scale (GSRS), which rates gastrointestinal symptoms from 1 'no discomfort at all' to 7 'very severe discomfort' (24).

Statistics

Statistics were performed by means of SPSS for Windows version 14.0 (SPSS Inc., Chicago, Ill., USA). To compare patient characteristics and results, the Kruskall Wallis and Mann-Whitney U tests were used for skewed distribution and the ANOVA and Student T-test for normal distribution. Numbers of patients were compared using the Fisher's Exact test. P-values <0.05 were considered significant. The internal consistency of the SF-36 and the GSRS was evaluated using Cronbach's alpha, and 0.7 or higher was considered to represent adequate reliability. For better comparison, the results of all the items of the SF-36 were recoded in the same direction and transformed into a scale of 0 to 100. Results of the SF-36 and the GSRS are presented as means (standard deviation). By using the data from the general population (23), we calculated expected values of the items of the SF-36, based on the distribution of age, gender and the total number of chronic health conditions of our sample. Mean scale scores of the SF-36 and the GSRS were corrected for group differences in age, gender and presence of chronic health conditions by multivariate regression analyses. Selective IgA deficiency was not considered to play a role in the HRQoL and thus was not corrected for. 95% Confidence intervals were used to compare the results of the SF-36 with the general population.

ETHICS

The study protocol was approved by the Medical Ethics Committee of the LUMC.

RESULTS

Of the 77 CD patients eligible to be invited for participation in the original study on possible development of tolerance to gluten (18), 66 gave informed consent. In 13 of them CD could not be confirmed by revision of the diagnostic biopsy, and only the remaining 53 patients with biopsy-confirmed CD participated in the study. Their characteristics, grouped according to their dietary habits, are presented in Table 1.

Table 1. Clinical characteristics of 53 patients with biopsy-confirmed celiac disease according to their dietary habits.

Median (range)	Gluten-free diet (GFD) *(n=33)*	Diet with gluten transgression (GT) *(n=8)*	Gluten-containing diet (GCD) *(n=12)*	Overall p-value
Sex (m)	7	5	4	0.07*
Age (y)	57 (21-77)	26 (22-66)	30 (25-53)	0.001*#
Age at diagnosis (y)	24 (1-65)	4 (0.9-32)	2 (0.7-15)	0.001*#
Duration of diagnosis (y)	25 (12-52)	24 (17-34)	28 (16-44)	0.48
Duration of actual gluten consumption (y)	0	8 (2-34)	18 (1-32)	0.001*#
Gluten intake (g/day)	0	0.9 (0.004-3)	15 (10-24)	0.001*#$
Associated chronic health conditon n (%)	10 (30%)	2 (25%)	5 (42%)	0.70
BMI, kg/m^2 (mean ± SD)	24.6 ± 3.6	22.8 ± 2.5	22.4 ± 3.3	0.20

Gluten-free diet = no gluten/day; gluten transgression = < 10 g gluten/day; gluten-containing diet = >10 g gluten/day; *= significance between GFD and GT; #= significance between GFD and GCD; $=significance between GT and GCD.

An associated chronic health condition was reported 27 times (by 32% of the patients). Some patients had more than one associated disease. Divided over the three diet groups, there were patients with selective IgA deficiency (n=5), hypothyroidism (n=4), dermatitis herpetiformis (n=3), diabetes mellitus type 1 (n=2), rheumatoid arthritis (n=2), Sjögren syndrome (n=2), cancer (n=2), secondary hyperparathyroidism (n=1), ulcerative colitis (n=1), multiple sclerosis (n=1), systemic lupus erythematosis (n=1), scleroderma (n=1), fertility problems (n=1) and miscarriages (n=1). There were no significant differences in

the distribution of patients with an associated chronic health conditon between the three groups (Table 1).

Dietary compliance

The self-reported strict compliance with the GFD was 62% (33/53). Twelve patients (23%) reported to consume a normal GCD, and eight reported to consume low amounts of gluten (GT). The duration of CD was comparable in all three groups. However, the patients consuming gluten (GCD or GT) were diagnosed with CD at a significant younger age and are presently significantly younger than the ones strictly adhering to a GFD (Table 1).

SF-36

The internal consistency of the SF-36 subscales ranged from 0.77 to 0.95. Results of the SF-36 are presented in Table 2 and 3.

Comparing the HRQoL of the three groups of CD patients with different dietary compliance, significantly better scores were found in the patients consuming gluten: better general mental health in patients with GT and less bodily pain in patients with a GCD. These differences, however, disappeared when the comparisons were corrected for age, gender and presence of associated chronic health conditions (Table 2).

Table 2. Mean ± standard deviation scores of the SF-36 in 53 patients with biopsy-confirmed celiac disease according to their dietary regimen.

	Gluten-free diet (n=33)	Diet with gluten transgression (n=8)	Gluten-containing diet (n=12)	Overall p-value*
General health perception	59.9 ± 24.3	60.6 ± 21.5	70.8 ± 24.9	0.21
General mental health	75.4 ± 18.5	90.5 ± 9.8	75.0 ± 17.0	0.41
Vitality	61.7 ± 20.3	74.4 ± 18.6	71.3 ± 16.7	0.96
Social functioning	80.7 ± 26.3	92.2 ± 22.1	87.5 ± 22.6	0.92
Physical functioning	85.3 ± 23.3	85.0 ± 34.6	94.6 ± 6.9	0.11
Bodily pain	80.7 ± 20.4	93.8 ± 7.4	94.6 ± 12.5	0.72
Limitations due to physical problems	85.8 ± 33.3	87.5 ± 35.4	93.8 ± 11.3	0.21
Limitatons due to emotional problems	83.3 ± 32.5	100.0 ± 0	88.9 ± 21.7	0.67

*p-value when corrected for group differences in age, gender and associated chronic health conditions.

Overall, corrected for age and gender (Table 3), CD patients reported significantly worse general health perception but significantly better HRQoL for the domains bodily pain and limitations due to physical problems compared with the general population. Upon that, when corrected for the presence or absence of chronic health conditions CD patients showed significantly higher scores for the domains physical functioning and limitations due to emotional problems (Table 3).

Table 3. Mean (95% CI) scores of the SF-36 in 53 patients with biopsy-confirmed celiac disease (CD) compared with results from the general population (GP; n=1742), corrected for the distribution of age, gender and chronic health conditions of our sample.

	CD patients	GP-age	GP-gender	GP-chronic health conditions
General health perception	62.5 (55.8-69.1)	71.3*	70.4*	65.0
General mental health	77.6 (72.7-82.6)	77.2	75.3	73.7
Vitality	65.8 (60.3-71.2)	68.8	66.7	64.4
Social functioning	84.0 (77.1-90.8)	84.6	83.2	80.0
Physical functioning	87.5 (81.0-94.0)	84.8	82.0	77.6*
Bodily pain	86.1 (80.9-91.3)	75.9*	73.5*	69.0*
Limitations due to physical problems	88.0 (79.6-96.4)	78.5*	75.3*	68.0*
Limitatons due to emotional problems	87.3 (79.5-95.2)	83.2	80.6	78.0*

GP; Aaronson *et al.*[23] ; *=significant different from the corrected general population.

GSRS

The internal consistency of the GSRS subdimensions ranged from 0.64 to 0.92. Results of the GSRS are presented in Table 4. No significant differences were found in the mean scores of the GSRS between the different dietary groups, corrected for age, gender or associated chronic health conditions.

Table 4. Means ± standard deviation scores of the Gastrointestinal Symptom Rating Scale (GSRS) in the 53 patients with biopsy-confirmed celiac disease according to their dietary regimen.

	Gluten-free diet (n=33)	Diet with gluten transgression (n=8)	Gluten-containing diet (n=12)	Overall p-value*
Abdominal pain	1.6 ± 0.8	1.6 ± 0.7	1.8 ± 1.5	0.92
Indigestion	2.1 ± 0.9	2.5 ± 1.1	2.0 ± 1.2	0.47
Diarrhea	2.0 ± 1.7	2.1 ± 1.5	1.5 ± 0.7	0.23
Constipation	2.6 ± 1.5	1.9 ± 1.3	1.9 ± 1.6	0.38

*p-value when corrected for group differences in age, gender and associated chronic health conditions.

DISCUSSION

In this study among CD patients with and without adherence to the GFD, we have found a similar HRQoL in patients with different dietary compliance. Our findings are in agreement with earlier reporting in Swedish patients (12), but are in contrast with the better HRQoL found in Italian patients with a better dietary compliance (9). In the latter study, however, patients had a shorter duration of CD (e.g. 2 years) possibly explaining their better HRQoL.

One of the factors associated with HRQoL in CD patients may be the presence of symptoms (16). Considering the gastrointestinal symptoms scored by GSRS, we did not find differences between the three groups of patients with different dietary compliance. This similarity of the GSRS scores may partly explain the similarity in HRQoL between the three dietary groups. On the other hand it is possible that the GSRS is not sensitive enough since it was not specifically designed to study symptoms in CD patients, although in earlier studies, the instrument discriminated symptom scores between male and female CD patients (12,25). Another possibility is that the presence of symptoms in CD patients is not associated with dietary compliance and that patients adhering to a strict GFD for several years may continue to have gastrointestinal symptoms (12,25,26).

The risk of CD related co-morbidity or potential complications are other factors associated with HRQoL (16) and can be measured with the item 'I expect my health to deteriorate' as part of the general health perception domain of the SF-36. In our study, we did not find significant differences in this domain between the three dietary groups. Furthermore, we were able to compare CD patients with associated chronic health conditions already present with CD patients without these and found comparable SF-36 scores (data not shown).

The HRQoL might also be associated with factors not related to CD. It is known that, concerning the results of the SF-36, in general older respondents score significantly lower than younger respondents and women have lower scores than men (23). In our study, the patients adhering to the GFD tended to have lower HRQoL scores than the other groups, but this apparent difference can be explained because the group was older and contained a higher percentage of females.

When comparing HRQoL of the CD patients in general with the reference population, celiac patients showed worse general health perception, confirming earlier studies (9,10,12), but they had better scores concerning the physical domains. Presumably, CD patients, once diagnosed and treated, experience their new-found vitality and health as a relief, as has been reported by patients 10 years after diagnosis (27), which may positively influence the interpretation of the questions of the SF-36.

The interest within the medical community in measuring QoL is increasing (7-12,28-30). It has been argued that what matters in HRQoL is the way patients feel about their functioning, not their functioning itself (31). The subjective assessment of physical, mental, and social dimensions of well-being and social functioning can be performed by generic or disease-specific instruments. In this study, we have used the SF-36. Being a generic instrument for QoL measurement, the SF-36 can be used to measure the impact of treatment on HRQoL but not to measure the disease-specific QoL aspects such as, in the case of CD, the possible social and financial restrictions due to the GFD. Therefore, the use of both generic- and disease-specific instruments is recommended to assess the HRQoL (32). Recently, disease-specific instruments to assess HRQoL in celiac patients have been developed and validated, both for adults (CDQ; 33) and for children (CDDUX; 11).

In light of upcoming developments for new treatment strategies for CD (34-37), the comparison of HRQoL of celiac patients with current and alternative treatment will be necessary in the future to get information on which treatment under which circumstances will be most appreciated. The disease-specific instruments to assess HRQoL in CD, may be valuable tools for the caregivers to get information on the HRQoL of adult and pediatric CD patients aiming to ameliorate their treatment.

Limitations of our study were that the CD patients were originally invited to participate in a study on the possible development of tolerance to gluten (18), and that it was performed among a limited number of patients all diagnosed at the same university hospital. Therefore, further studies are needed to confirm our results in larger samples and in a more general group of CD patients.

In conclusion, the HRQoL of the chronic ill patient is an important factor in his treatment. Equally important is the prevention of co-morbidity and complications of CD patients. We and others have found that strict adherence to the GFD does not have negative consequences for the HRQoL, hence, considering the importance of prevention

of co-morbidity and complications of CD patients, achieved by strict adherence to the GFD, the GFD should be advised to all the CD patients.

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CHAPTER 7

Gluten tolerance in adult patients with celiac disease 20 years after diagnosis?

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Eur J Gastroenterol Hepatol 2008;20:423-9

Funding: Fund 'Doelmatigheidsonderzoek', Leiden University Medical Center, projectcode P03.106

ABSTRACT

Background & objective: Celiac disease (CD) is believed to be a permanent intolerance to gluten. A number of patients, however, discontinue the gluten-free diet (GFD) without developing symptoms or signs. The aim of our study was to investigate whether CD patients are capable of developing tolerance to gluten.
Methods: All 77 adult patients from our hospital known to have biopsy-proven CD for more than 10 years were invited to participate. We investigated symptoms, gluten consumption, antibodies for CD and other autoimmunity, human leukocyte antigen (HLA)-typing, bone mineral density, and performed small bowel biopsies. Tolerance was defined as no immunological or histological signs of CD while consuming gluten.
Results: Sixty-six patients accepted participation, but after review of the diagnostic biospies 53 were found to have true CD. Twenty-three percent of patients had a gluten-containing diet (GCD), 15% admitted gluten transgression (GT) and 62% followed the GFD. Patients on a GFD had significantly more osteoporosis. Normal small bowel mucosa was found in four of eight on GCD and in four of four with GT. Two patients were considered to have developed tolerance to gluten. One of them was HLA-DQ2/DQ8 negative.
Conclusion: Development of tolerance to gluten seems possible in some patients with CD. Further follow-up will show whether this tolerance is permanent or only a long-term return to latency. This feature may be associated with genetic characteristics, especially with HLA genotypes that differ from DQ2 or DQ8. More insight into the mechanisms of the development of gluten tolerance may help to distinguish those CD patients that might not require life-long GFD.

INTRODUCTION

Celiac disease (CD) is a chronic disorder caused by an inflammatory T-cell response to the gluten proteins present in wheat. Gluten causes villous atrophy of the small intestine in CD patients, which may lead to nutrient malabsorption, causing a broad spectrum of symptoms. Furthermore, CD may also be asymptomatic.
CD is considered to be a permanent disorder and, the advised treatment is life-long adherence to a gluten-free diet (GFD). GFD improves the health status of CD patients (1,2) and protects them from development of complications (3,4), but is frequently experienced as a burden on social life and on quality of life (5-7), and compliance may pose a problem (5,6,8-10). It is well known that a number of CD patients stop following the GFD after a time of treatment without developing symptoms or signs of disease. Most of them will develop CD intestinal lesions after different periods of gluten consumption, but some seem to tolerate gluten for a long time (11-13). The aim of the present study was to investigate whether there are CD patients who may develop tolerance to gluten, and if so, to explore their genetic, immunological and clinical characteristics.

METHODS

In this follow-up study, we collected all patients aged 20-80 years from the files of the medical administration of the Leiden University Medical Center who had undergone a small bowel biopsy between 1975 and 1994 and met the diagnostic criteria for CD. These were clinical signs of CD and at least one biopsy of the small intestine, showing the characteristic appearance of CD (villous atrophy, crypt hyperplasia, inflammatory infiltration) during gluten consumption, and clinical and/or histological recovery, once treated with a GFD (14).
The patients were traced using the national telephone registry or information from the municipal authority of the last known address of the patient. Informed consent to participate was asked for by letter. Non-responders were sent a reminder after 2 months and, in case of no response, were contacted by telephone after 6 months.

Food questionnaire

Patients were asked to register their degree of compliance with the diet as strict, partial (gluten transgression; GT: 0-10 g gluten/day) or none (gluten-containing diet; GCD: >10 g gluten/day). From the patients with partial or nonadherence to the diet, consumption of gluten-containing products was checked by a food frequency questionnaire. Gluten intake was estimated by multiplying the grams of vegetable protein from gluten-containing cereals by 0.8 (15).

Assessment of symptoms

Specific gastrointestinal symptoms were evaluated according to the Gastrointestinal Symptom Rating Scale (GSRS) (2,16,17) which rates symptoms from 1 'no discomfort at all' to 7 'very severe discomfort'. Furthermore, 27 items on associated diseases and other CD-related symptoms such as vomiting, anorexia, weight loss, aphthous ulcers, lassitude, anaemia, alopecia, muscular cramp and weakness, erythema nodosum, osteoarthropathy, dental enamel defects and peripheral neuropathy were asked and also scored from 1 to 7.

Anthropometry

Patients were instructed to perform three measurements of their own height and weight at home following written instructions and the average was used for further analyses.

Blood tests

Participants were asked to have blood puncture. Human leukocyte antigen (HLA)-typing was performed at our hospital (Professor F.H.J. Claas), and serum ferritine, folic acid, vitamin B12, 25(OH)vitamin D and calcium were measured according to routine testing. Titers of serum immunoglobulin (Ig)-A against gliadin (AGA), tissue transglutaminase (tTGA) and endomysium (EMA) were measured, respectively, using enzym-linked immunosorbent assay techniques and indirect immunofluorescence on monkey oesophagus (BME von Blomberg) (18,19). In case of IgA deficiency (total IgA concentration <0.06 g/l), serum IgG-AGA and IgG-tTGA were measured.
To check the development of other autoimmune phenomena, the following parameters were measured in serum (MR Batstra, BME von Blomberg): thyroperoxidase (TPO) and thyroglobulin antibodies, by haemagglutination (Thymune-M and Thymune-T; Remel Europe, Ltd, Kent, UK), thyroid stimulating hormone receptor (TSH-R) antibodies by radioimmunoassay (TRAK assay; BRAHMS diagnostica GmbH, Berlin, Germany), glutamic acid decarboxylase antibodies (anti-GAD), insulinoma-associated protein-2 antibodies (IA2) by radioimmuno-assay (RSR Ltd, Cardiff, UK) and islet cell antibodies (ICA) by indirect immuno-fluorescence on human bloodgroup O pancreas.

Bone mineral density

Bone mineral density (BMD) of both right and left femural neck and the lumbar spine were assessed by dual-energy X-ray absorptiometry (DEXA). Osteopenia was defined as BMD of –1 to –2.5 SD and osteoporosis as BMD of less than -2.5 SD.

Histology

Duodenal biopsies were offered to all patients consuming gluten (GT, GCD), and to patients with positive celiac antibodies or decreased BMD while following a GFD. Biopsies were performed according to routine procedures. All study and diagnostic

biopsies were reviewed by the same pathologist (H.M.) who was unaware of the clinical situation of the patient and classified according to the modified Marsh criteria (20,21).

Tolerance

Tolerance to gluten was defined as no immunological or histological signs of CD while the patient was consuming a GCD for more than 2 years.

Statistical analysis

All analyses were carried out using SPSS 11.5. Results with normal distribution are presented as means with standard deviations and with skewed distribution as medians with ranges. To compare patient characteristics and results, the Kruskall Wallis and Mann-Whitney *U*- tests were used for skewed distribution and the analyses of variance for normal distribution. Percentages and numbers of patients were compared using the Fisher's Exact test. *P*-values less than 0.05 were considered as significant.

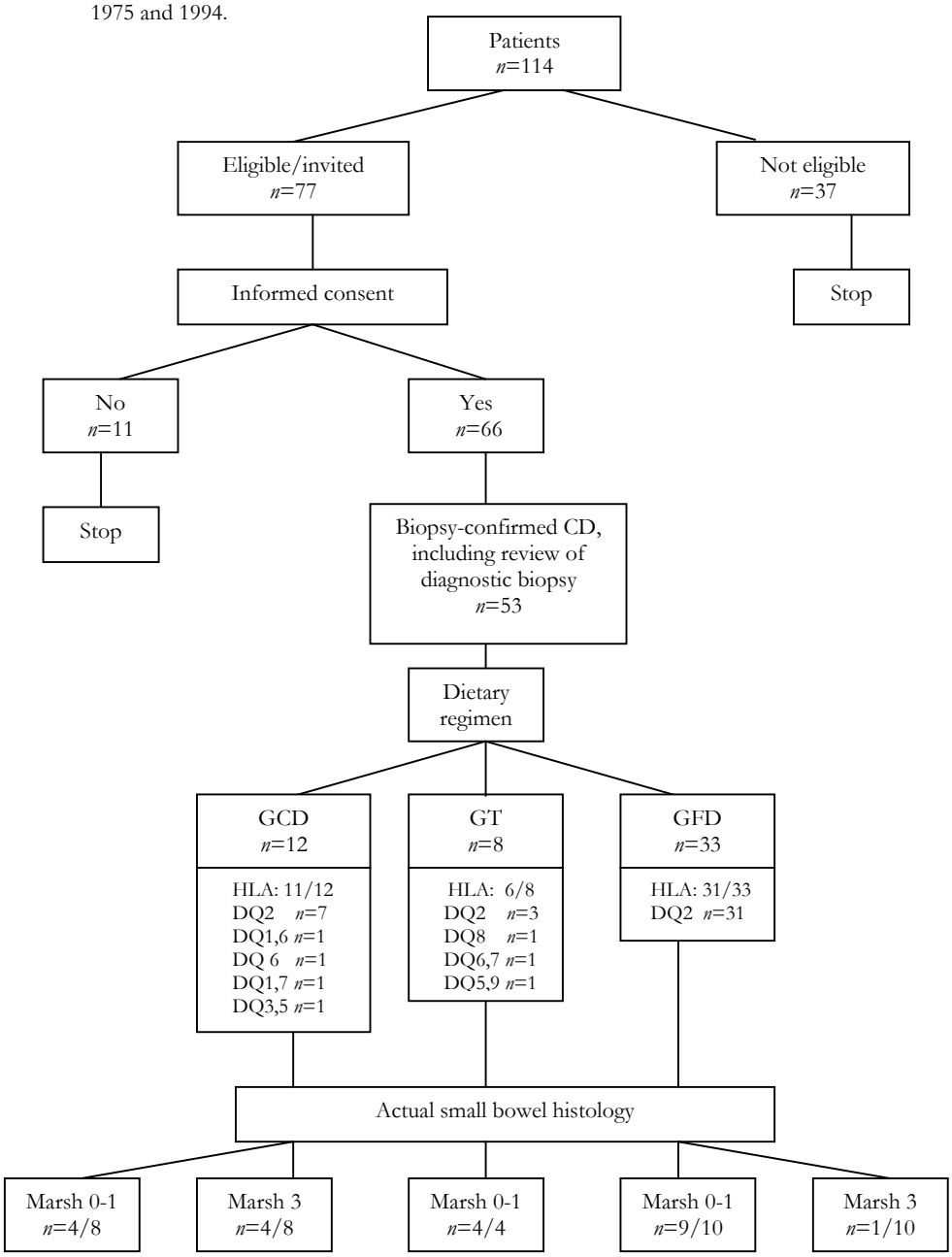
Ethics

The study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center.

RESULTS

The patient flow chart is presented in Fig. 1. Of the 114 patients with villous atrophy, 37 were not eligible to be invited. Reasons for ineligibility were death (n=18), diagnoses different from CD (n=8: lactose intolerance, tropical sprue, isolated short stature, chronic nonspecific diarrhea or giardiasis), severe psychiatric or social problems (n=2) and psychomotor retardation (n=1). In addition, eight could not be invited due to unknown address, emigration or failure to contact them by telephone.

Figure 1. Flow chart of all the patients aged 20-80 years known in the files of the medical administration of the Leiden University Medical Center, with a small bowel biopsy with villous atrophy between 1975 and 1994.



GCD, gluten-containing diet; GFD, gluten-free diet; GT, gluten transgression; HLA, human leukocyte antigen.

Of the 77 patients invited (67%), 66 agreed to participate (86%). Through review of the diagnostic biospies 53 patients were found to have true CD and only these patients were taken into account in this study. Characteristics of the patients are presented in Table 1, grouped according to their dietary habits. Patients on GCD and those with GT were diagnosed with CD at a significantly younger age (median: 2 years; range: 0.7-32) than patients who strictly adhered to the GFD. The duration of CD was, however, comparable in all three groups.

Table 1. Clinical characteristics of 53 patients with biopsy-confirmed celiac disease according to their dietary habits.

Median (range)	GCD (*n*=12)	GT (*n*=8)	GFD (*n*=33)	Overall *P*-value
Sex (m)	4	5	7	0.07[b]
Age (y)	30 (25-53)	26 (22-66)	57 (21-77)	0.0001[a,b]
Age at diagnosis (y)	2 (0.7-15)	4 (0.9-32)	24 (1-65)	0.0001[a,b]
Duration of CD (y)	28 (16-44)	24 (17-34)	25 (12-52)	0.48
Duration of GFD (y)	6 (1-28)	14 (0.2-23)	24 (8-36)	0.0001[a,b]
Duration of actual gluten consumption (y)	18 (1-32)	8 (2-34)	0	0.0001[a,b]
Gluten intake (g/day)	15 (10-24)	0.9 (0.004-3)	0	0.0001[a,b,c]
BMI, kg/m^2 (mean ± SD)	22.4 ± 3.3	22.8 ± 2.5	24.6 ± 3.6	0.20

GCD, gluten-containing diet (>10 g gluten/day); GFD, gluten-free diet (0 g gluten/day); GT, gluten transgression (0 – 10 g gluten/day); [a]Significance between GCD and GFD; [b]Significance between GT and GFD; [c]Significance between GCD and GT.

Food questionnaire

All participating patients completed the questionnaire on gluten consumption. A strict compliance with the GFD was self-reported by 62% of the patients. Twelve patients (23%) reported a GCD with a median gluten intake of 15 g/day for a median of 18 years, which was, of course, significantly higher than the gluten intake (0.9 g/day) of the eight patients with GT (Table 1). The reasons for restarting gluten consumption were: 'according to the doctor CD was cured' (*n*=4) or 'no complaints after gluten ingestion' (*n*=8).

Health status

No differences were found in the results of the Gastrointestinal Symptom Rating Scale and in the other CD-related symptoms among patients in the 3 groups (Table 2).

Table 2. Comparison of the Gastrointestinal Symptom Rating Scale scores and other CD-related symptoms in patients with biopsy-confirmed celiac disease according to their dietary habits.

GSRS symptom (mean±SD)	GCD (*n*=12)	GT (*n*=8)	GFD (*n*=33)	Overall *P*-value
Abdominal pain	1.8±1.5	1.6±0.7	1.6±0.8	0.93
Indigestion	2.0±1.2	2.5±1.1	2.1±0.9	0.63
Diarrhea	1.5±0.7	2.1±1.5	2.0±1.7	0.51
Constipation	1.9±1.6	1.9±1.3	2.6±1.5	0.26
Other CD-related symptoms	1.4±0.4	1.4±0.2	1.5±0.4	0.52

CD, celiac disease; GCD, gluten-containing diet (>10 g gluten/day); GFD, gluten-free diet (0 g gluten/day); GSRS, Gastrointestinal Symptom Rating Scale (with scores from 1 'no discomfort at all' to 7 'very severe discomfort'); GT, gluten transgression (0 – 10 g gluten/day).
CD-related symptoms=vomiting, anorexia, weight loss, aphthous ulcers, lassitude, anaemia, alopecia, muscular cramp and weakness, erythema nodosum, osteoarthropathy,
dental enamel defects, peripheral neuropathy.

A significantly higher number of patients following a GFD reported osteoporosis: 13 compared to none of the patients in the other two diet groups (*P*=0.008). In addition, two new cases of osteoporosis were found during the study in patients following a GFD, but none were found in the other groups. The present median age (61 vs. 40 years, *P*=0.001) and median age at diagnosis (32 vs. 11 years, *P*=0.001) of patients on a GFD with osteoporosis was significantly higher compared with the ones on a GFD without osteoporosis. No difference was, however, observed in the sex distribution among those with and without osteoporosis (*P*=0.41). In two cases, osteoporosis was the presenting symptom of CD. Osteopenia was found to be equally distributed throughout the three dietary groups: in two of 10 (20%) patients on a GCD, in four of six (67%) with GT and in six of 30 (20%) on a GFD (NS).
An associated disease was reported for 27 times (by 32% of the patients). Some patients had more than one associated disease. Divided over the three diet groups, there were five patients with selective IgA deficiency, three with dermatitis herpetiformis, three with hypothyroidism, two with diabetes mellitus type 1, two with rheumatoid arthritis, one with secondary hyperparathyroidism, two with Sjögren syndrome, two with cancer, one with ulcerative colitis, one with multiple sclerosis, one with auto-immune hypothyroidism, one with systemic lupus erythematosis, one with scleroderma and one with fertility problems and miscarriages. No significant differences exist in the distribution of patients with an associated disease among the three groups.

Blood tests

Fourty-six of the 53 participating patients (87%) were consented to blood puncture. No deficiencies were found in any of the patients. Five patients were IgA deficient: one of them (GCD) had IgG-AGA and another one (GFD) had IgG-tTGA. IgA positivity for CD antibodies was found in four of 41 patients (10%) (Table 3). A significant difference was found in the number of patients with positive IgA-EMA among the three dietary groups (P=0.043).

HLA-typing was performed in 48 patients: 41 (85%) were HLA-DQ2 and one (2%) was HLA-DQ8 (Fig. 1).

Table 3. Positivity for (auto)antibodies in 41 non-IgA-deficient patients with biopsy-confirmed celiac disease, according to their dietary habits.

Antibody	GCD (n=9)	GT (n=6)	GFD (n=26)	Overall P-value
(n)				
IgA-AGA	1	0	1	0.60
IgA-EMA	2	1	0	0.043
IgA-tTGA	1	0	1	0.60
TPO	1	1	2	0.77
ICA	0	0	1	1.00
Anti-GAD	1	1	2	0.77
IA-2	0	0	2	1.00

AGA, antigliadin antibody; anti-GAD, glutamic acid decarboxylase antibodies; EMA, anti endomysium antibody; GCD, gluten-containing diet (>10 g gluten/day); GFD, gluten-free diet (0 g gluten/day); GT, gluten transgression (0 – 10 g gluten/day); IA2, insulinoma-associated protein-2 antibodies; ICA, islet cell antibodies; TPO, thyroperoxidase antibodies; tTGA, tissue transglutaminase antibody;

Histology of the small bowel mucosa

A small bowel biopsy was offered to 41 patients and 22 patients consented. A normal small bowel mucosa (Marsh 0-1) was found in four of eight patients on a GCD, in all four patients with GT and in nine of 10 patients adhering to a GFD. Marsh 3a-c lesions, suggestive of active CD, were found in four patients on a GCD and in one following a GFD (Fig. 1). The patient on a GFD with Marsh 3a lesion is now being studied for possible refractory CD.

CD patients with possible tolerance to gluten

Four patients were considered as possibly tolerant to gluten. None of them had serum antibodies for CD or other (auto) antibodies and none of them had osteoporosis.

The first patient is a 31-year-old man, with HLA-DQ1/DQ6, diagnosed with CD at the age of 2.7 years. He presented with vomiting, chronic diarrhoea, abdominal pain and weight loss. The patient's small bowel biopsy showed Marsh 2 lesions. The symptoms improved on a GFD. A gluten challenge was performed at the age of 6 years: he developed high IgA-AGA (at that time measurement of EMA or tTGA was not available) and the small bowel biopsy showed Marsh 3a lesions. At present, after 13 months of gluten consumption he has Marsh 1 lesions. This short period of gluten consumption, however, is not enough to consider him tolerant to gluten.

The second patient is a 37-year-old, HLA-DQ2 positive, woman diagnosed with CD at the age of 1 year. She presented with growth retardation, weight loss, distended abdomen, lassitude and chronic diarrhoea with steatorrhoe (78% fat absorption). Small bowel histology showed total villous atrophy, Marsh 3c. After GFD, her symptoms, fat absorption (93%) and small bowel mucosa improved significantly. A small bowel biopsy after gluten challenge at the age of 3 years showed again Marsh 3c lesions. At present, after consuming gluten for more than 21 years, the patient has Marsh 1 lesions. The patient reported fertility problems and miscarriages and has recently been diagnosed with a basal cell carcinoma of the skin, she, therefore can not be considered tolerant to gluten.

Patient 3 is a 25-year-old woman, with HLA-DQ1/DQ2, diagnosed at 2.3 years of age. She presented with chronic diarrhoea. Her small bowel biopsy showed Marsh 3a lesions. After GFD, both her symptoms and her small bowel biopsy improved: a control biopsy at the age of 7 years was normal (Marsh 0). After that she has been eating gluten, but control biopsies taken after 2, 3, 7, 10 and 18 years of gluten consumption showed normal or almost normal mucosa without significant increase of IEL: Marsh 0-1 lesions, with IEL counts of 10-30/100 epithelial cells. The patient's mother and brother also have CD. She may be considered as having developed tolerance to gluten.

Patient 4 is a 32-year-old man, with HLA-DQ3/DQ5, diagnosed with CD at the age of 2.2 years. He presented with vomiting, chronic diarrhoea and distended abdomen. His small bowel biopsy showed Marsh 3a lesions. The symptoms and small bowel mucosa improved after GFD. Gluten challenge resulted in growth retardation, lassitude and diarrhoea, and his small bowel mucosa showed Marsh 2 lesions at the age of 9 years. After reintroduction of the GFD, the clinical symptoms improved again. The patient has now been consuming gluten for more than 22 years and has Marsh 0-1 lesions. The only alteration found in this study was osteopenia so we consider him to have developed tolerance to gluten.

DISCUSSION

In this study, we have found two patients with biopsy-confirmed CD who show no signs of active CD after a mean gluten consumption period of 20 years. These observations suggest that the development of gluten tolerance may be possible in CD patients.

The intestinal immune system has several arms of defence aimed at avoiding systemic and peripheral inflammatory immune responses. This can occur by activation of regulatory T cells to tolerate innocuous antigens, such as food proteins. This hyporesponsiveness to antigens in the intestine is a phenomenon termed 'oral tolerance' (22-24). The immunopathological origin of CD, however, may be explained by poorly developed intestinal tolerance against gluten leading to disrupted proximal gut homeostasis in genetically susceptible individuals (25).

In this study, we defined tolerance to gluten as no immunological or histological signs of CD while the patient was consuming gluten for more than 2 years. Two years is a generally accepted, although not proven, period in which histological relapse will occur on gluten consumption, but much longer durations have been described (26-28). The two patients who may be considered tolerant both consume gluten for a considerable longer period: 18 and 22 years, respectively. It is arguable whether the term of tolerance is appropriate to describe our two patients, as it is known that CD patients may relapse after a long period of apparent tolerance (28) and the term 'return to latency' has been used before in this context. Only further follow-up will make clear whether our patients are permanently tolerant to gluten. We consider them now as possibly tolerant and indeed have returned to latency for a (very) long period of time.

It is arguable whether the absence of clinical symptoms should be included in the concept of possible tolerance. Our results show that the frequency of symptoms in CD patients within the three groups with different gluten consumption was comparable (Table 2). In other words, patients adhering to a strict GFD also had gastrointestinal and other CD-related complaints. This confirms that the presence of health complaints is not an indicator of the histological status (29,30).

In contrast to expectations, we found osteoporosis only in patients on a GFD. This may be explained by their old age (61 years) and high age at diagnosis (32 years), an age before which formation of bone peak mass is reached in healthy situations. Furthermore, some patients started to adhere to the GFD with a delay of 6 years after CD diagnosis. It is well known that GFD improves reduced bone mineral density frequently present in newly diagnosed adult CD patients, although in contrast to children in adult patients it may not normalize (31-32).

The development of gluten tolerance in CD patients is controversial and the main question with regard to our patients is whether they ever had CD in the first place. For that reason, together with their medical files, the diagnostic small bowel biopsies were

revised by one experienced pathologist (H.M.). In 13 patients CD could not be confirmed, but in the ones who developed tolerance to gluten CD was confirmed in this way. This interobserver variation in the histopathological diagnosis of CD has also been found by others (33,34) and, when in doubt, an experienced gastrointestinal pathologist should be consulted (Dr. J.W. Meijer, personal communication). Fourty-two of the 48 patients for whom HLA-typing was available were HLA-DQ2 and/or -DQ8 positive. The other six patients had various HLA-typings of whom two (HLA-DQ6/DQ7 and HLA-DQ3/DQ5) were found to express a functional homolog of the DQA1*0501 chain of HLA-DQ2, thus one of the two chains of the disease associated HLA-DQ2 dimer. The presence of half of the HLA-DQ2 chain in HLA-DQ2/DQ8 negative CD patients has been described earlier (35). CD is associated with HLA-DQ2 and -DQ8 (36-38) and the chance of having CD without these haplotypes is very low, although not absent (37,39,40). The other four non-HLA-DQ2 or non-HLA-DQ8 patients (HLA-DQ6/DQ7, HLA-DQ6, HLA-DQ5/DQ9, and HLA-DQ1/DQ7) are exceptions among the CD population, but they were diagnosed with CD according to the accepted ESPGHAN criteria (14), two of them including deterioration of the small bowel mucosa after gluten challenge (41), so we consider them as CD patients.

CD patients who tolerate gluten have been reported by a French group of well-known CD researchers (11-13,28). Recently they have described eight adult CD patients without clinical symptoms or no immunological or histological signs of CD while consuming gluten for a median period of 14 years. One of the reasons to consider that CD is a permanent disorder is the result of a large study in young children showing that 95% of them had histological alterations characteristic of CD after gluten challenge (42). It is possible that gluten sensitivity, however, may decrease or increase during different periods of life. Adult CD patients, who had been a few years on a GFD during childhood and now consume gluten, show a less severe clinical picture than observed in newly diagnosed adult CD patients, suggesting some patients develop resistance to gluten after a period of GFD (43). Indeed our two patients who returned to latency were both diagnosed during childhood.

In conclusion, in this follow-up study we have found two CD patients who returned to latency and have possibly developed tolerance to gluten. This phenomenon may be associated with specific genetic characteristics, especially with HLA genotypes, as we have found that one of the CD patients who returned to latency has HLA-typing other than DQ2 or DQ8. The factors and mechanisms that play a role in the development of tolerance in CD patients are unclear and more studies are needed to unravel this phenomenon to allow us to identify these exceptional CD patients that may not require life-long GFD. Further follow-up is, however, needed to investigate whether these patients remain at this level and confirm whether CD can be transient. Meanwhile, CD patients should be aware of the potential risk they are at for complications and the

possibility of relapse when they have gluten consumption and therefore, regular dietary and medical follow-up remains necessary (3,4,28).

ACKNOWLEDGEMENTS

The authors thank Mrs E.M.C. Kooy-Winkelaar for her work on the duodenal tissue and Professor J.M. Wit and Dr. S. le Cessie for helpful discussions and their critical reading of the manuscript.

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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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Summary

Chapter 1 contains a general introduction on celiac disease and its treatment and a description of the aims and outline of the thesis. Celiac disease is the result of a sensitivity to dietary gluten in genetically predisposed individuals. The diagnosis of celiac disease is based on characteristic histological alterations of the small bowel mucosa during gluten consumption and clear clinical remission on avoidance of gluten. The treatment of celiac disease consists of a life-long, gluten-free diet to heal the duodenal mucosa, improve symptoms and protect from development of complications. The aims of this thesis were to measure some of the environmental factors (e.g. breastfeeding and gluten introduction) considered to play a role in the prevention of celiac disease and in the development of oral tolerance. Furthermore, to explore the relationship of celiac patients with gluten and the gluten-free diet at different ages, their ability to develop gluten tolerance and the impact of the gluten-free diet on health-related quality of life.

Breastfeeding has been shown to prevent, or at least delay the development of celiac disease. Breast milk contains many immunologic factors that stimulate the infant's immune system, but its exact role in the prevention of celiac disease is not known. Furthermore, breast milk contains small amounts of food antigens, like gluten peptides that may contribute to tolerance induction. In chapter 2 we describe the results of a study on the presence of T cell stimulatory epitopes originating from dietary gluten in the breast milk of 23 mothers on a normal diet and of 13 mothers on a gluten-free diet. T cell stimulatory epitopes of both gliadin and glutenin were detected in breast milk but no correlation with the gluten intake of the mother was found. We conclude that infants are exposed to small levels of gluten through breast milk. These small levels may be one of the factors responsible for the induction of oral tolerance to gluten.

Another possible factor in the prevention of celiac disease or the development of oral tolerance is the timing and amount of gluten introduced into the infants' diet. An easy and reliable instrument that can be used to assess the gluten intake in young infants was lacking until now. In chapter 3 we describe the development and validation of a food questionnaire to assess gluten intake in young infants. Eighty-seven parents of healthy infants aged 0-12 months completed the newly developed food questionnaire and a 2-day food record as a reference. We found that up to the age of 10 months the gluten intake assessed with the food questionnaire was comparable with that of the food record, but in the older children it was higher when assessed with the food record. This difference was probably caused by a greater variety of food products in the older children that could not be detected by our food questionnaire: all of the gluten-containing products that were reported in the food of the children aged 3-6 months by using the food record, were contained in the food questionnaire for this age category, but for children aged 7-12 months this was the case for 95% of the gluten delivering products reported in the food

record. For future use among the older children, the food questionnaire can easily be improved. We conclude that this new, short, standardized, validated and easy to use food questionnaire may be a useful instrument to assess gluten intake in infants. After necessary translation, adaptation and validation the food questionnaire can be used to assess gluten intake of young infants across countries and can be used in collaborative studies.

In chapter 4 we present the results of a study on the nutritional state and the management of the gluten-free diet by young Dutch celiacs. Of all 395 celiac patients aged 12 to 25 years who were members of the Dutch Celiac Society, 219 responded to the invitation letter and 132 gave informed consent to participate. Strict dietary compliance was reported by 75% of them, which was comparable with the percentage of dietary compliance in adolescents in other European countries. The young celiac patients had a higher consumption of saturated fat and a lower consumption of fiber and iron than recommended, but similar to the general population. Most of these young patients (61%) found the diet easy to follow. Eighty six percent of the patients reported regular medical controls, but only 7% had regular dietary controls. The mean standard deviation score for body mass index was -0.3±0.8. We conclude that the dietary compliance in this group is high, and that the nutritional state is adequate but the nutrient intake has to be improved. We believe that adequate medical and dietary support will be necessary for this group of young celiac patients to maintain this self-reported good management of the gluten-free diet and to prevent long-term complications.

Gluten-free food products usually have a lower nutritional value and thus patients adhering strictly to a gluten-free diet are at risk for nutrient deficiencies. Furthermore, the compliance with the gluten-free diet may, among others, depend on the variability and possibilities within the gluten-free food package. We wanted to assess whether the naturally gluten-free cereal *Eragrostis tef* (tef), having a nutritional value comparable with that of wheat, is associated with health problems when used by celiac patients. The results of this study can be found in chapter 5. All 7990 members of the Dutch Celiac Disease Society were invited to complete a 2-step questionnaire on tef use and the development of symptoms after tef consumption. Thirty-six percent responded to the first questionnaire of whom 53% consumed tef and 15% reported complaints. For the second more detailed questionnaire on the use of commercially available tef products suitable for a gluten-free diet, on previous symptoms during regular gluten-free diet without tef and on symptoms after tef consumption, 1828 were willing to complete it. 1545 had biopsy proven celiac disease, 66% of them used tef and 17% reported symptoms after tef consumption. This percentage of symptoms was significantly lower than patients without tef consumption reported in their regular gluten-free diet (17%

versus 61%; p=0.0001). We conclude that tef is frequently used by Dutch celiac patients and a wide majority can consume tef without clinical symptoms. Celiac patients using tef reported a significant reduction in symptoms, possibly related to a reduction in gluten intake or to an increase in fiber intake. We believe that tef can be a valuable addition to the gluten-free diet of celiac patients.

Chapter 6 contains the results of the study on whether health-related quality of life of celiac patients assessed with the SF-36 is associated with dietary compliance. This study was performed among the patients participating in the study described in chapter 7. We found that compared with the general population celiac patients scored significantly worse on general health perception, but significantly better on bodily pain and limitations due to physical problems. We conclude that strict adherence to a gluten-free diet is not associated with a lower health-related quality of life of celiac patients.

Celiac disease is believed to be a permanent intolerance to gluten. However, a number of patients discontinue the gluten-free diet without developing symptoms or signs. The aim of our study described in chapter 7 was to investigate whether celiac patients are capable of developing tolerance to gluten. We defined tolerance as no immunological or histological signs of celiac disease while consuming gluten. We found that of the 53 patients from our hospital known to have biopsy confirmed celiac disease for more than 10 years, 12 (23%) consumed a gluten-containing diet, 8 (15%) admitted gluten transgression and 33 (62%) followed a gluten-free diet. Twenty-two patients consented to small bowel biopsy. A normal small bowel mucosa (Marsh 0-1) was found in 4 of 8 patients on a gluten-containing diet, in all 4 patients with gluten transgression and in 9 of 10 patients adhering to a gluten-free diet. Marsh 3a-c lesions, suggestive of active celiac disease, were found in 4 patients on a gluten-containing diet and in 1 following a gluten-free diet. The patient on a gluten-free diet with Marsh 3a lesion is now being studied for possible refractory celiac disease. In contrast to expectations, we found osteoporosis only in the patients on a gluten-free diet, possibly explained by their significant older age and significant higher age at diagnosis or by the fact that some patients started to adhere to the gluten-free diet with a delay of 6 years after diagnosis of celiac disease.
From the 53 patients, 2 were considered to have developed tolerance to gluten. One of them was HLA-DQ2/DQ8 negative. We conclude that development of tolerance to gluten is possible in some exceptional patients with celiac disease. However, further follow-up will be necessary to find out whether this tolerance is permanent or only a long-term return to latency. This feature may be associated with genetic characteristics, especially with HLA genotypes that differ from DQ2 or DQ8. More insight into the mechanisms of the development of gluten tolerance may help to distinguish those celiac patients that might not require life-long treatment with a gluten-free diet.

Chapter 8 contains the general discussion and the conclusions of this thesis. In an effort to develop methods to prevent celiac disease, we have measured some of the environmental factors that are considered to play a role in the development or prevention of celiac disease. We have measured the presence of gliadin and glutenin in breastfeeding, and we have developed an instrument to assess the gluten intake in young infants. Both methods are applied in a recently started prospective collaborative European study on breastfeeding and gluten intake in newborns from high-risk families which explores the possibilities of primary prevention of celiac disease. The information resulting from that study may possibly lead to changes in the actual European guidelines for infant feeding, concerning the introduction of gluten after 6 months of age.

In the literature there is an ongoing discussion about the question whether celiac disease is a permanent condition, or can return to latency. We made an attempt to find patients who have become tolerant to gluten. We found 2 of these exceptional patients and found that one of them had HLA typing different from HLA-DQ2/DQ8, suggesting that genetic factors may play a role in the development of tolerance. However, the underlying mechanisms leading to prevention or development of celiac disease, or leading to tolerance to gluten once celiac disease has been diagnosed are complex and need to be further studied.
In the near future more patients will be diagnosed with celiac disease and professionals involved in their treatment have to be prepared to take care of the increasing number of patients. Therefore, new methods for treating and supporting this increasing number of patients need to be explored. Combined consultation of the doctor and the dietitian and the use of facilities like telephone and the internet in supporting the patients are ways to provide efficient and adequate support.

The only available treatment of celiac disease is adherence to the gluten-free diet. Attention to the adequacy of the nutrient intake within the gluten-free diet is necessary to prevent celiac patients from health risks. However, new possibilities in the treatment are promising and may decrease the burden of the treatment and positively influence the nutrient intake and the health-related quality of life. This may prevent the patients from cessation with the gluten-free diet, and thus protect them from the potential risk of complications.

Samenvatting

Hoofdstuk 1 bevat een algemene inleiding over coeliakie en de behandeling en een beschrijving van het doel van dit proefschrift. Coeliakie komt tot expressie na gluten-inname bij genetisch gepredisponeerde individuen. De diagnose coeliakie wordt gesteld op aanwezigheid van karakteristieke beschadigingen van de mucosa van de dunnedarm na gluten consumptie en klinisch herstel na een dieet vrij van gluten. De behandeling van coeliakie bestaat uit een levenslang glutenvrij dieet met als doel het herstellen van het dunnedarm slijmvlies, het verminderen van symptomen en het beschermen voor de ontwikkeling van complicaties. Het doel van dit proefschrift was omgevingsfactoren meten waarvan verondersteld wordt dat zij een rol spelen bij de preventie van coeliakie en het ontwikkelen van orale tolerantie, zoals borstvoeding en de introductie van gluten. Vervolgens stelden we ons ten doel om de relatie te onderzoeken die coeliakie patiënten hebben met gluten en het glutenvrije dieet op verschillende leeftijden, om te bestuderen of het mogelijk is tolerantie voor gluten te ontwikkelen en om de impact die het glutenvrije dieet heeft op gezondheid gerelateerde kwaliteit van leven in kaart te brengen.

Borstvoeding lijkt de ontwikkeling van coeliakie te kunnen voorkomen of tenminste uit te stellen. Moedermelk bevat vele immunologische factoren die het immuunsysteem van de zuigeling stimuleren, maar de precieze rol bij de preventie van coeliakie is niet bekend. Daarnaast bevat moedermelk kleine hoeveelheden antigenen afkomstig van voeding, zoals gluten peptiden die mogelijk een bijdrage leveren aan de inductie van tolerantie. In hoofdstuk 2 beschrijven we de resultaten van een studie naar de aanwezigheid van T cel stimulatoire epitopen afkomstig van gluten uit de voeding in de moedermelk van 23 moeders met een glutenbevattend dieet en van 13 moeders met een glutenvrij dieet. T cel stimulatoire epitopen van zowel gliadine als glutenine konden worden aangetoond in de moedermelk maar een correlatie met de gluten-inname van de moeder werd niet gevonden. We concluderen dat zuigelingen blootgesteld worden aan kleine hoeveelheden gluten via de moedermelk. Deze kleine hoeveelheden gluten zouden deels verantwoordelijk kunnen zijn voor de inductie van orale tolerantie voor gluten.
Een andere mogelijke factor in de preventie van coeliakie of de ontwikkeling van orale tolerantie is het tijdstip waarop en de hoeveelheid waarin gluten geïntroduceerd wordt in het dieet van de zuigeling. Een eenvoudig en betrouwbaar instrument welke gebruikt kan worden om de gluten-inname te meten was tot op heden niet beschikbaar. In hoofdstuk 3 beschrijven we de ontwikkeling en validatie van een voedselvragenlijst om gluten-inname te meten bij jonge kinderen. Achtenzeventig ouders van een gezonde zuigeling van 0 tot 12 maanden hebben de nieuw ontwikkelde voedselvragenlijst en een 2-daags voedseldagboek als referentie ingevuld. We hebben gevonden dat tot de leeftijd van 10 maanden de gluten-inname, gemeten met de vragenlijst, vergelijkbaar was met die van het dagboek, maar dat de inname bij de oudere kinderen hoger was dan die gemeten met het dagboek. Dit verschil werd mogelijk veroorzaakt door een grotere diversiteit aan

voedingsmiddelen welke gebruikt werd door oudere kinderen, waarvoor de voedselvragenlijst niet gevoelig genoeg was: alle glutenbevattende voedingsmiddelen die in de voedseldagboeken werden gerapporteerd in de voeding van de kinderen van 3 tot 6 maanden waren opgenomen in de voedselvragenlijst voor deze leeftijdscategorie, maar voor de kinderen in de leeftijd van 7 tot 12 maanden was dat het geval in 95% van de glutenbevattende voedingsmiddelen. Voor toekomstig gebruik bij de oudere kinderen is de voedselvragenlijst eenvoudig te verbeteren. We concluderen dat deze nieuwe, korte, gestandaardiseerde, gevalideerde en eenvoudig te gebruiken voedselvragenlijst een bruikbaar instrument zou kunnen zijn om de gluten-inname van jonge kinderen te meten. Deze voedselvragenlijst zou na noodzakelijke vertaling, aanpassing en validatie gebruikt kunnen worden om de voedselinname van jonge kinderen te meten in verschillende landen en zou gebruikt kunnen worden in gezamenlijke studies.

In hoofdstuk 4 presenteren we de resultaten van het onderzoek naar de voedingstoestand van jonge Nederlandse coeliakie patiënten en hoe zij omgaan met het glutenvrije dieet. Van alle 395 coeliakie patiënten van 12 tot 25 jaar die lid zijn van de Nederlandse Coeliakie Vereniging, hebben 219 van hen op de uitnodigingsbrief geantwoord en 132 gaven toestemming voor deelname. Vijfenzeventig procent van hen rapporteerde het glutenvrije dieet strikt te volgen, wat vergelijkbaar is met het percentage dieettrouw gevonden bij de adolescenten in andere Europese landen. De jonge coeliakie patiënten hadden een hogere consumptie van verzadigd vet en een lagere consumptie van vezel en ijzer vergeleken met de aanbeveling, maar deze was vergelijkbaar met die van de algemene bevolking. De meeste van deze jonge patiënten (61%) vonden het volgen van het glutenvrije dieet gemakkelijk. Achtenzestig procent van de patiënten rapporteerde regelmatig medische controles te hebben, maar slechts 7% rapporteerde regelmatig dieetcontroles te hebben door de diëtist. De gemiddelde standaard deviatie score voor de body mass index was –0.3±0.8. We concluderen dat de dieettrouw bij deze groep jongeren hoog is, de voedingstoestand voldoende is, maar de voedingsinname verbeterd zou moeten worden. We geloven dat goede medische- en dieetbegeleiding noodzakelijk is voor deze groep jonge coelakie patiënten, om de zelfgerapporteerde goede dieettrouw voort te zetten.

Glutenvrije voedingsmiddelen hebben vaak een lagere voedingswaarde, zodat patiënten die een glutenvrij dieet moeten volgen een risico lopen op voedingsdeficiënties. Daarnaast kan de dieettrouw onder andere afhangen van de diversiteit en de mogelijkheden binnen het glutenvrije voedselpakket. We hebben bekeken of het van nature glutenvrije graan *Eragrostis tef* (teff), dat een voedingswaarde heeft vergelijkbaar met tarwe, geassocieerd is met gezondheidsproblemen als het wordt gebruikt door coeliakie patiënten. De resultaten van deze studie zijn te vinden in hoofdstuk 5. Alle 7990

leden van de Nederlandse Coeliakie Vereniging werden uitgenodigd om een 2-ledige vragenlijst in te vullen over het gebruik van teff en de ontwikkeling van klachten na teff consumptie. Zesendertig procent reageerde op de eerste vragenlijst. Drieënvijftig procent gebruikte teff en 15% van hen rapporteerde klachten. De tweede, meer gedetailleerde vragenlijst over het gebruik van teff produkten die geschikt zijn voor het glutenvrije dieet, en over klachten tijdens het reguliere glutenvrije dieet zonder teff en na teff consumptie, werd ingevuld door 1828 personen. Vijftienhonderd vijfenveertig van hen hadden met biopsie bevestigde coeliakie, 66% van hen gebruikte teff en 17% van hen rapporteerde symptomen na teff gebruik. Dit percentage klachten was significant lager dan het percentage klachten dat werd gerapporteerd door patiënten die geen teff gebruiken (17% versus 61%; p=0.0001). We concluderen dat teff frequent wordt gebruikt door Nederlandse coeliakie patiënten en dat een grote meerderheid teff kan gebruiken zonder klinische symptomen. Coeliakie patiënten die teff gebruiken rapporteerden significant minder klachten, mogelijk gerelateerd aan een lagere gluteninname of aan een hogere inname van voedingsvezel. We denken dat teff een waardevolle aanvulling kan zijn op het glutenvrije dieet van patiënten met coeliakie.

Hoofdstuk 6 beschrijft de resultaten van de studie waarin onderzocht is of de gezondheid gerelateerde kwaliteit van leven van coeliakie patiënten, gemeten met de SF-36, geassocieerd is met de mate van dieettrouw. Deze studie is verricht bij de patiënten die deelnamen aan het onderzoek beschreven in hoofdstuk 7. We vonden dat de coeliakie patiënten, vergeleken met de algemene bevolking, significant slechter scoorden wat betreft algemene gezondheidsperceptie, maar significant beter met betrekking tot lichamelijke pijn en beperkingen door fysieke problemen. We concluderen dat strikte dieettrouw niet is geassocieerd met een slechtere gezondheid gerelateerde kwaliteit van leven van coeliakie patiënten.

Coeliakie wordt beschouwd als een permanente intolerantie voor gluten. Een aantal patiënten stopt echter met een glutenvrij dieet zonder het ontwikkelen van symptomen of klachten. Het doel van de studie beschreven in hoofdstuk 7 was te onderzoeken of patiënten met coeliakie tolerantie voor gluten kunnen ontwikkelen. We definieerden tolerantie als geen immunologische of histologische tekenen van coeliakie gedurende gluten consumptie. We hebben gevonden dat van de 53 patiënten die langer dan 10 jaar bij ons in het ziekenhuis bekend waren met door biopsie bevestigde coeliakie, 12 (23%) een glutenbevattend dieet gebruikten, 8 (15%) af en toe gluten gebruikten en 33 (62%) een strikt glutenvrij dieet volgden. Tweeëntwintig patiënten gaven toestemming voor een dunnedarm biopsie. Een normale dunnedarm mucosa (Marsh 0-1) werd gevonden bij 4 van de 8 patiënten met een normale glutenbevattende voeding, bij alle 4 de patiënten die af en toe gluten gebruiken en bij 9 van de 10 patiënten die zich strikt aan een glutenvrij

dieet houden. Marsh 3a-c, mogelijk wijzend op actieve coeliakie, werd gevonden bij 4 patiënten die een normale glutenbevattende voeding gebruiken en bij 1 patiënt op een strikt glutenvrij dieet. Deze laatste patiënt wordt nu verder onderzocht voor mogelijke refractaire coeliakie. In tegenstelling tot wat men zou verwachten hebben we alleen bij mensen die zich strikt aan een glutenvrij dieet houden osteoporose gevonden. Mogelijk is dit te verklaren door het feit dat patiënten die een strikt glutenvrij dieet volgen significant ouder waren en ook significant ouder waren ten tijde van het stellen van de diagnose coeliakie en mogelijk door het feit dat sommige patiënten pas 6 jaar na het stellen van de diagnose coeliakie zijn gestart met een glutenvrij dieet.
Van de 53 patiënten leken 2 patiënten een tolerantie voor gluten te hebben ontwikkeld. Een van hen was HLA-DQ2/DQ8 negatief. We concluderen dat ontwikkeling van tolerantie voor gluten mogelijk lijkt bij uitzonderlijke patiënten met coeliakie. Verder vervolg van deze patiënten is noodzakelijk om te bevestigen dat deze ontwikkelde tolerantie permanent is of om aan te tonen dat dit alleen een langdurige terugkeer naar een latente coeliakie is geweest. Het kunnen ontwikkelen van tolerantie voor gluten zou geassocieerd kunnen zijn met genetische factoren, voornamelijk met HLA genotypen die afwijken van DQ2 of DQ8. Meer inzicht in de mechanismen van de ontwikkeling van tolerantie voor gluten zou kunnen bijdragen aan het onderscheiden van die specifieke coeliakie patiënten die een levenslange behandeling met een glutenvrij dieet niet nodig hebben.

Hoofdstuk 8 bevat de algemene discussie en de conclusies van dit proefschrift. In een poging om methoden te ontwikkelen om coeliakie te voorkomen, hebben we omgevingsfactoren gemeten waarvan verwacht wordt dat zij een rol spelen in de ontwikkeling en preventie van coeliakie. We hebben de aanwezigheid van gliadine en glutenine gemeten in borstvoeding en we hebben een instrument ontwikkeld om de gluten-inname van jonge kinderen te meten. Beide meetmethoden worden nu toegepast in een recent gestarte prospectieve Europese studie waarin de mogelijkheden van primaire preventie van coeliakie worden onderzocht. In deze studie worden borstvoeding en gluten-inname bepaald bij pasgeborenen binnen hoog risico families. De informatie uit deze studie kan mogelijk leiden tot aanpassing van de huidige Europese richtlijnen voor kindervoeding voor wat betreft de introductie van gluten na de leeftijd van 6 maanden.

In de literatuur bestaat discussie over de vraag of coeliakie altijd blijvend is, of kan overgaan in een latente coeliakie. We hebben een poging gedaan om coeliakie patiënten te vinden die tolerant zijn geworden voor gluten. We hebben 2 van deze uitzonderlijke patiënten gevonden. Een van hen had een HLA-typering anders dan HLA-DQ2/DQ8, wat suggereert dat genetische factoren mogelijk een rol spelen in de ontwikkeling van

tolerantie voor gluten. Echter, de onderliggende mechanismen die leiden tot preventie van de ontwikkeling van coeliakie of tot de ontwikkeling van tolerantie voor gluten als coeliakie eenmaal is gediagnosticeerd, zijn complex en dienen verder te worden onderzocht.

In de toekomst zullen meer mensen met coeliakie worden gediagnosticeerd en de behandelaars dienen te zijn voorbereid op het stijgend aantal patiënten. Daarom is het zinvol de toepassing van nieuwe manieren van behandeling en begeleiding te onderzoeken. Gecombineerde spreekuren met de dokter en de diëtist en het gebruik van mogelijkheden als de telefoon en het internet bij het begeleiden van de patiënten zijn manieren om te voorzien in efficiënte and adequate ondersteuning.

De enig mogelijke behandeling van coeliakie is het volgen van een glutenvrij dieet. Aandacht voor een volwaardige inname van voedingsstoffen binnen het glutenvrije dieet is noodzakelijk om te voorkomen dat coeliakie patiënten gezondheidsrisico's lopen. Echter, nieuwe mogelijkheden in de behandeling zijn veelbelovend en kunnen mogelijk de last van de huidige behandeling verminderen en de voedingsstoffeninname en de gezondheid gerelateerde kwaliteit van leven positief beïnvloeden. Dit zou mogelijk kunnen voorkomen dat patiënten stoppen met het volgen van een glutenvrij dieet en hen daarmee beschermen voor het risico op complicaties.

Curriculum vitae

De auteur van dit proefschrift werd geboren op 20 juni 1965 te Den Burg op Texel. In 1983 behaalde zij het eindexamen VWO aan de Rijks Scholen Gemeenschap Texel. In 1988 is zij gestart met de opleiding Voeding en Diëtetiek aan de Hogeschool van Amsterdam waar zij in 1992 is afgestudeerd als diëtist. Datzelfde jaar is zij begonnen bij de Dienst Diëtetiek (hoofd: Mw. M. 't Hart-Eerdmans, thans ad interim Mw. M.J.G. van Ham) van het Leids Universitair Medisch Centrum (voorheen Academisch Ziekenhuis Leiden). Zij heeft haar medewerking verleend aan onderzoeksprojecten van A.C. Toornvliet (gepromoveerd 1998) en N.I. Weijl (gepromoveerd 2001). Vanaf 1994 is de samenwerking op het gebied van onderzoek gestart met de afdeling kindergeneeskunde (Mw. dr. M.L. Mearin, hoofd: Prof. dr. J.M. Wit, later drs. R.A. Holl, thans Prof. dr. H.A. Delemarre-van de Waal), eerst bij kinderen met het syndroom van Down en daarna bij kinderen die een beenmergtransplantatie ondergaan. Vanaf 2000 heeft zij gewerkt aan de onderzoeksprojecten die in dit proefschrift zijn opgenomen. Daarnaast is zij werkzaam als diëtist op de interne poliklinieken.